Determination of Inorganic Anions in Drinking and Natural Waters by Ion Chromatography using Electrochemical Suppression

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Sykam Application Note 07

Introduction

Supplying the human population with clean drinking water is one of the most important responsibilities of the public in every part of our world. Therefore, the monitoring and protection of natural water resources is of uppermost importance nowadays and in future to retain the basis of our existence. In the last decades, great efforts have been made by the governments of most of the industrialized countries to enforce regulations, which ensure a responsible handling of our natural water resources. For instance, the European Union has established the Drinking Water Directive (DWD) 98/83/EC[1] in 1998, which has been regularly updated until today with its latest version 2020/2184[2] published in December 2020. The objectives of this directive are to protect human health from the adverse effects of any contamination of water intended for human consumption. The respective federal law in the United States is the Safe Drinking Water Act[3] (SDWA), which ensures the integrity and safety of drinking waters. Also for the protection of natural water resources, regulations have been established, which in the EU is the Water Framework Directive 2000/60/EC[4] enforced in October 2000, the purpose of which it is to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater to prevent further deterioration and protect and enhance the status of aquatic ecosystems, or the U.S. Clean Water Act (CWA) to reduce the discharge of pollutants into natural waters. Also in other parts of the world, similar health and environmental standards have been established often by adopting the aforementioned regulations, which have been implemented by the EU or the U.S.

Ion Chromatography (IC) has been established one of the most commonly used analytical techniques for the determination of ionic contaminants in drinking and natural waters and is the standard method, that has been validated and published by many national and international standard organizations like ISO (International Organization for Standardization), ASTM (American Society for Testing and Materials), AWWA (American Water Works Association) or the U.S. EPA (Environmental Protection Agency of the U.S.). The content of ionic contaminants in drinking and natural waters is regulated as Maximum Contaminant Level (MCL) under the EU Drinking Water Directive (DWD)[1][2] and needs to be steadily monitored. In the U.S., the MCL for ionic contaminants, that reveal potential health effects arising from their ingestion in drinking water, like fluoride, nitrite and nitrate, are specified in the National Primary Drinking Water Standard.[5] Other common inorganic anions such as chloride and sulfate are considered secondary contaminants and are regulated under the U.S. National Secondary Drinking Water Standard. For the analysis of anions in drinking and natural waters, a considerable number of regulatory IC methods have been published worldwide, including the EN ISO 10304-1[6] (Technical Committee ISO/TC 147 “Water quality”), the EPA 300.1[7] or the ASTM D4327[8]. In this Application Note “Sykam AN07”, the determination of inorganic anions in drinking and natural waters by IC using suppressed conductivity measurement with electrochemical suppression is described. In contrast to the application note Sykam AN01, this procedure utilizes the S150+ Ion Chromatography Module, which is working with an electrochemical self-regenerating suppressor using electrolytic eluent regeneration. The testing requirements of the EPA 300.1, Revision 1.0[7] as well as EN ISO 10304-1:2009-07[6] and ASTM D4327-17[8] 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. Two columns, Sykam A07 (150 x 2.6 mm) and Sykam A08 (125 x 2.6 mm) were used for the method validation and the validation results are discussed and directly compared to each other.

Equipment

The application note Sykam AN07 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 a column oven, one-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)

SYKAM CHROMATOGRAPHY

- 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 an S1130 isocratic pump (S151-A+) instead of a S1130 gradient pump is also possible as well as the use of a S6120 manual injection valve (S151-M+, S151-G+) instead of an 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 thiocyanate (NaSCN, ACS reagent, ≥98.0%), Sigma-Aldrich (251410)
- 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)
- Bromide standard solution 1000 mg/L (traceable to SRM from NIST NaBr in H_2O 1000 mg/L Br Certipur®), Merck (1.19896)
- 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.

- 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 bromide (NaBr, ACS reagent, ≥99.0%), Sigma-Aldrich (310506)
- 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, eight water samples were analyzed using the two different columns Sykam A07 and Sykam A08 (Table 1).

Table 1. List of samples analyzed.

No.	Name
1	Drinking Water (Augsburg)
2	Drinking Water (Riesen (Steingaden))
3	Mineral Water (VIO, Coca-Cola)
4	Mineral Water (Adelholzener)
5	River Water (Donau)
6	River Water (Illach)
7	Mineral Water (RAPP)
8	Mineral Water (St. Leonhard)

The drinking water samples as well as the river water samples were collected in Southern Germany in the northern foothills of the Alps, where lime containing soil is the prevailing geological formation. The drinking water samples 1 and 2 were collected as tap water from one municipal water supplier in Augsburg and a local spring in Riesen (Steingaden) respectively. The two mineral water samples 3 and 4 are commercially available mineral waters from an international company and a local drinking water distributor. The river water samples were collected from the Donau river in Donauwörth and the comparatively small Illach river near Steingaden respectively. The two mineral water samples 7 and 8 were analyzed for direct comparison of the performance of the IC system S151+ to the previous version S151 regarding baseline noise, method detection limits and precision. The performance of the S151 IC system was discussed in application note AN01. All samples were collected in duplicate, whereas the duplicate sample was collected under the exact same conditions as the initial sample. The samples were collected in 100 mL PE-bottles with screw cap and stored at 4 °C immediately after collection.

Chromatographic Conditions

Method Validation 1 (Sykam A07)

Columns:	Sykam A07 (150 x 2.6 mm), Analytical column Sykam AGC-04 (20 x 2.6 mm), Guard column
Eluent:	4.0 mM Na_2CO_3, 25 µM NaSCN
Flow Rate:	1.0 mL/min
Run Time:	20 min
Temperature:	60 °C
Injection Volume:	50 µL (full loop)
Detection:	Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor
Suppressor Current:	40 mA
Backpressure:	51 bar (740 psi)
Base Conductivity:	19 µS/cm
Noise:	<2 nS/cm

Method Validation 2 (Sykam A08)

Columns:	Sykam A08 (125 x 2.6 mm), Analytical column Sykam AGC-04 (20 x 2.6 mm), Guard column
Eluent:	3.5 mM Na_2CO_3, 25 µM NaSCN
Flow Rate:	1.2 mL/min
Run Time:	14 min
Temperature:	60 °C
Injection Volume:	50 µL (full loop)
Detection:	Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor
Suppressor Current:	50 mA
Backpressure:	69 bar (1000 psi)
Base Conductivity:	21 µS/cm
Noise:	<5 nS/cm

Preparation of Solutions and Reagents

Anion Standard Stock Solutions (1000 mg/L)

The standard stock solutions for the seven standard anions fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulfate 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. The amounts needed for the preparation of 1000 mL of each standard stock solution are given in Table 2. If not all of the seven standard 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)
Fluoride	Sodium fluoride (NaF)	2.210
Chloride	Sodium chloride (NaCl)	1.648
Nitrite	Sodium nitrite ($NaNO_2$)	1.500
Bromide	Sodium bromide (NaBr)	1.288
Nitrate	Sodium nitrate ($NaNO_3$)	1.371
Phosphate	Potassium dihydrogen phosphate (KH_2PO_4)	1.433
Sulfate	Sodium sulfate (Na_2SO_4)	1.479

Working Standard Solutions

All single anion 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. A series of mixed standards at concentrations of 200, 100, 50, 20, 10 and 5 µg/L of each anion is prepared from 10 mg/L single standard solutions for the preliminary estimation of the method detection limit. Additionally, a mixed anion standard for the calculation of the method detection limit MDL_S is prepared from the 10 mg/L single standard solutions. Table 3 gives the concentrations of the mixed anion standards prepared to calculate the method detection limits (MDL_S) as well as the concentrations of the Quality Control Sample (QCS), which is analyzed to determine retention time stability and peak area precision of the instrument.

Table 3. Concentrations of the MDL_S and QCS standards for both method validations.

Analyte	MDL_S Calculation Standard (µg/L)		QCS for Precision (mg/L)	
	A07	A08	A07	A08
Fluoride	10	10	2	2
Chloride	10	10	10	10
Nitrite	50	75	2	2
Bromide	50	75	2	2
Nitrate	50	75	20	20
Phosphate	250	250	2	2
Sulfate	100	150	30	30

The determination of the linear calibration range covering the expected concentrations of the drinking and natural water samples is achieved by injecting ten levels of calibration of a mixed anion standard at the following concentrations: 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 50 and 100 mg/L for chloride, nitrite, bromide, nitrate, phosphate and sulfate as well as 0.02, 0.04, 0.1, 0.2, 0.4, 1.0, 2.0, 4.0, 10 and 20 mg/L for fluoride. All concentration levels were prepared from the 1000 mg/L standard stock solutions by diluting the respective volumes to 100 mL with deionized 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.

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 thiocyanate stock solution dissolve 4.054 g of NaSCN in 40 mL of deionized water in a 50 mL volumetric flask in the same manner by filling up the flask to the mark and shaking it vigorously. For the two chromatographic systems discussed here, the following eluents are prepared:

Sykam A07: 4.0 mM Na_2CO_3, 25 µM NaSCN – Add 8.0 mL of the 1.0 mol/L Na_2CO_3 stock solution as well as 50 µL of the 1.0 mol/L NaSCN 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).

Sykam A08: 3.5 mM Na_2CO_3, 25 µM NaSCN – Add 7.0 mL of the 1.0 mol/L Na_2CO_3 stock solution as well as 50 µL of the 1.0 mol/L NaSCN 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 the desired temperature (60 °C). Then switch to eluent and slowly increase the flow rate to the desired value, which is 1.0 mL/min for the Sykam A07 and 1.2 mL/min for the Sykam A08. Switch on the current of the electrochemical suppressor. For a faster equilibration, a higher suppressor current (150 mA) can be applied for 2 hours at the desired flow rate of 1.0 mL/min or 1.2 mL/min respectively. Ideally, the system is be equilibrated overnight at the conditions, that are used for the analytical procedure regarding flow rate and suppressor current. The baseline noise of the equilibrated system should be <5 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, which is 1.0 mL/min for the Sykam A07 and 1.2 mL/min for the Sykam A08.

Sample Preparation

All drinking and natural 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. To prevent nitrification and denitrification in the samples by microbial action, changing the analyzed content of nitrite and nitrate, the samples have to be stored at 4 °C and analyzed within 24 hours after collection.

Results and Discussion

The method validation for the determination of inorganic anions in drinking and natural waters was performed according to EPA method 300.1 (Revision 01)[7], so that all requirements of this method as well as ISO EN 10304-1:2009-07[6] and ASTM D4327-17[8] regarding quality control parameters are met. The methods define, that also optional columns and correspondingly different chromatographic conditions may be used to improve the separations or lower the costs of measurement. Here, the two columns Sykam A07 and Sykam A08 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 $\mathrm{MDL_S}$ and $\mathrm{MDL_B}$[9], Linearity (Linear Calibration Range, LCR)[10], 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 field 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. The Sykam A07 (150 x 2.6 mm) and Sykam A08 (125 x 2.6 mm) are both based on trimethylammonium functionalized PS-DVB with a particle size of 10 µm. The columns exhibit a high capacity, whereas the Sykam A07 is recommended for analyses, where all seven standard anions have to be determined, since the standard anions are separated with a higher resolution compared to the Sykam A08. Especially for quantification of the fast eluting analyte fluoride or for the separation of neighboring peak pairs with a large difference in concentration, as it is often found e.g. for chloride and nitrite, the Sykam A07 is the column of choice. The method using the Sykam A07 described here, reveals a high signal-to-noise ratio, making it suitable for trace analyses at concentrations in the sub-ppm-range down to the single digit-ppb-range, depending on the analyte, as indicated by the method detection limits listed in Table 5. If the focus of analysis is on quantification of the ubiquitous found anions chloride, nitrate and sulfate, in case of the absence of anions like nitrite, bromide or phosphate or if the expected concentration range is higher than 1 ppm for any of the analytes, the analyst can save on time and consumables if using the Sykam A08 with its lower retention times for the inorganic anions found in drinking and natural waters.

Table 4. Column performance parameters of Sykam A07 and Sykam A08 at the chromatographic conditions listed above (5 mg/L mixed anion standard).

Analyte	Retention time (min)		Resolution R		Peak Symmetry	
	Sykam A07	Sykam A08	Sykam A07	Sykam A08	Sykam A07	Sykam A08
Fluoride	1.42	1.12	-	-	1.37	1.72
Chloride	2.83	2.27	6.98	5.59	1.14	1.34
Nitrite	3.94	3.03	3.55	3.05	1.24	1.40
Bromide	5.83	4.25	4.07	3.60	1.24	1.40
Nitrate	8.03	5.65	3.24	2.85	1.35	1.49
Phosphate	10.97	8.54	2.76	2.64	1.09	1.17
Sulfate	14.79	11.47	2.58	2.14	1.12	1.22

Column: Sykam A07 (150 x 2.6 mm) and Sykam AGC-04 (20 x 2.6 mm)
Eluent: 4.0 mM Na_2CO_3, 25 µM NaSCN
Flow Rate: 1.0 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (40 mA)

Peaks:	A	B
1. Fluoride	1.0 mg/L	10 µg/L
2. Chloride	5.0	10
3. Nitrite	5.0	50
4. Bromide	5.0	50
5. Nitrate	5.0	50
6. Phosphate	5.0	250
7. Sulfate	5.0	100

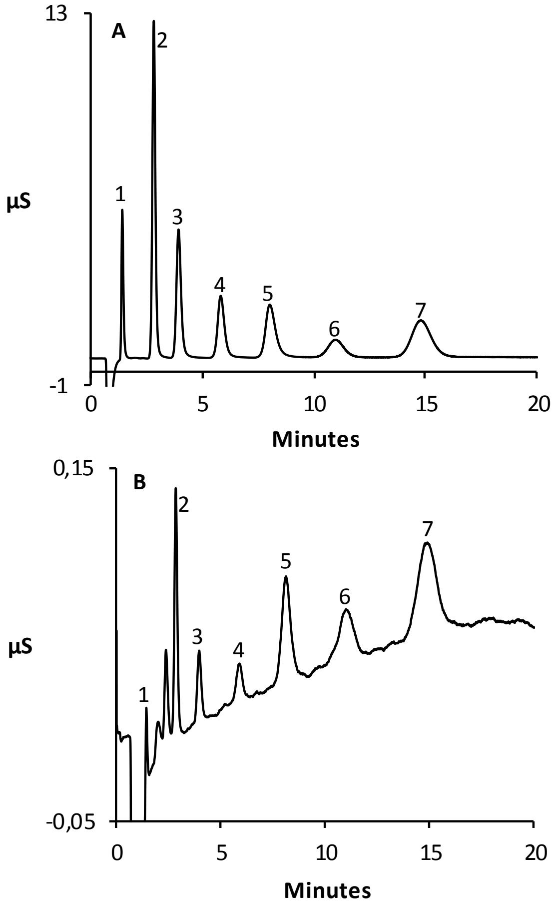

Figure 1. Separation of A) a 5.0 mg/L inorganic anion standard (Fluoride 1.0 mg/L) and B) the MDL_S Calculation Standard using a Sykam A07.

For both columns, the peak resolution is R >1.3 for all standard anions (Table 4), as required in the method performance section of ISO EN 10304-1[6]. Both columns exhibit excellent peak symmetry allowing for exact integration of the peak areas, since all peaks are baseline-separated. Figures 1 and 2 show the separation of a low-mg/L inorganic anion standard using the Sykam A07 and Sykam A08 respectively as well as the corresponding chromatogram of the MDL_S-Calculation Standard for the Sykam A07.

Method Detection Limits MDL_S and MDL_B

First, the method detection limit based on sample injection (MDL_S) was estimated by the injection of six mixed anion standards with the following concentrations: 200, 100, 50, 20, 10 and 5 µg/L. For each anion, the concentration, at which the signal-to-noise ratio lies between 3 and 5 was determined or extrapolated.

Column: Sykam A08 (125 x 2.6 mm) and Sykam AGC-04 (20 x 2.6 mm)

Eluent: 3.5 mM Na_2CO_3, 25 µM NaSCN
Flow Rate: 1.2 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (50 mA)

Peaks:	
1. Fluoride	1.0 mg/L
2. Chloride	5.0
3. Nitrite	5.0
4. Bromide	5.0
5. Nitrate	5.0
6. Phosphate	5.0
7. Sulfate	5.0

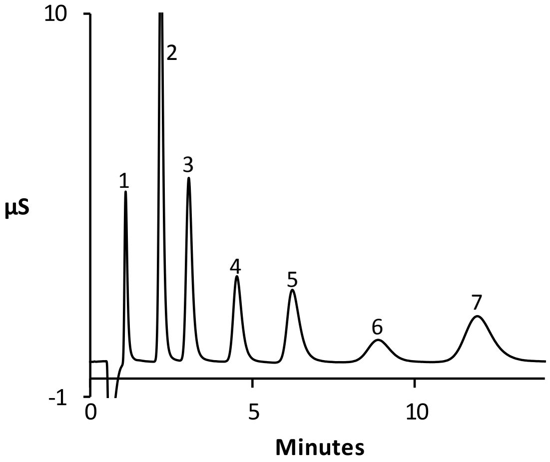

Figure 2. Separation of a 5.0 mg/L inorganic anion standard (Fluoride 1.0 mg/L) using a Sykam A08.

The estimated method detection limits were multiplied by a factor of 5 to obtain the final concentrations of the MDL_S-Calculation standard (Table 3). 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) x (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. The so obtained method detection limits are listed in Tables 5 and 6 for the Sykam A07 and Sykam A08 respectively. 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. In this case, seven replicates of the LRB were analyzed with the result, that chloride was found at a mean level of 3.8 µg/L (Sykam A07) and 21.1 µg/L (Sykam A08) respectively. For chloride, the method detection limit MDL_S does not apply and the MDL_B is calculated for chloride instead: MDL_B = X + (t) x (SD), where X is the mean of the LRB results[9]. t is again 3.142613 for seven replicates and SD is the standard deviation of the replicate analysis of the LRB. The MDL_B for chloride was calculated to 9.0 µg/L for the Sykam A07 and 35.2 µg/L for the Sykam A08. The large difference between the two validations could be traced back to a change of the ion exchange cartridge in the laboratory between the two validation processes. None of the other inorganic anions was found in any of the LRB injections.

Linearity (LCR)

To assess the linear calibration range for each anion, mixed anion standards at ten calibration levels were injected: 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 50 and 100 mg/L (0.02 – 20 mg/L for fluoride). For quantification of the samples and the fortified samples, the calibration range was limited to its linear range individually for each anion. Therefor only the calibration levels that were found to be in a linear range were used to assess the calibration curve for the respective anion. Figure 3 shows the linear calibration plots for all anions in both validations using the Sykam A07 and Sykam A08. Calibration points, which are outside the linear range were omitted and are displayed in red. Chloride, nitrite, nitrate and sulfate were found to exhibit a large linear calibration range of 0.1 – 50 mg/L and 0.1 – 100 mg/L respectively during the validation using the Sykam A07, whereas bromide and phosphate did not exhibit a linear response at the highest concentrations. Since the contents of fluoride, bromide and phosphate in drinking and natural water samples are expected to be in the low single digit mg/L or sub-mg/L range, smaller concentration ranges of 0.02 – 20 (fluoride) or 0.1 – 20 mg/L (bromide, phosphate) were used for their respective determinations. For the validation using the Sykam A08, the LCR for all anions except fluoride was set to 0.1 – 50 mg/L or 0.1 – 100 mg/L respectively. Again, fluoride was calibrated at 0.02 – 20 mg/L. For phosphate, the first calibration point at 0.1 mg/L was omitted since its MDL_S was determined to be 179.8 mg/L. Tables 5 and 6 show the linear calibration range as well as the correlation factors r^2 of the linear fit for all anions in both validations. All linear fits exhibit correlation factors of r^2 >0.99 and are considered very accurate.

Table 5. Linearity, MDL_S, retention time and peak area precision for Sykam A07.

Analyte	Calibration range (mg/L)	Linearity (r^2)	Calculated MDL_S (µg/L)	Retention Time Precision (RSD, %)	Peak Area Precision (RSD, %)
Fluoride	0.02 – 20	0.9999	1.2	0.44	0.45
Chloride	0.1 – 100	0.9994	0.5*	0.24	0.59
Nitrite	0.1 – 100	1.0000	11.9	0.17	1.04
Bromide	0.1 – 20	0.9999	9.7	0.14	0.65
Nitrate	0.1 – 50	0.9995	7.5	0.10	0.53
Phosphate	0.1 – 20	0.9999	62.4	0.12	0.97
Sulfate	0.1 – 50	0.9998	19.9	0.08	0.64

*Value not valid, MDL_B is used instead

Table 6. Linearity, MDL_S, retention time and peak area precision for Sykam A08.

Analyte	Calibration range (mg/L)	Linearity (r^2)	Calculated MDL_S (µg/L)	Retention Time Precision (RSD, %)	Peak Area Precision (RSD, %)
Fluoride	0.02 – 20	0.9999	1.1	0.59	0.29
Chloride	0.1 – 100	0.9996	1.7*	0.30	0.28
Nitrite	0.1 – 100	0.9999	12.4	0.23	0.83
Bromide	0.1 – 50	0.9998	44.1	0.16	0.95
Nitrate	0.1 – 50	0.9996	50.8	0.12	0.43
Phosphate	0.2 – 50	0.9998	179.8	0.19	8.23
Sulfate	0.1 – 50	0.9999	68.6	0.09	0.23

*Value not valid, MDL_B is used instead

Precision (QCS) and Instrument Performance (ECS)

Retention time and peak area precision was determined from seven (Sykam A07) and six (Sykam A08) replicate injections of a Quality Control Standard (QCS). The concentration of the single inorganic anions in the QCS ideally is very similar to those found in the field samples and is displayed in Table 3. Tables 5 and 6 show the results of the Relative Standard Deviation (RSD) of the retention time and peak area after seven (or six) replicate injections of the QCS. The RSD of the retention time is well below 1.0% and the RSD of the peak area was found to be less than 2.0% for almost all investigated anions, illustrating the high precision reached with the Sykam S151-AG+ IC system using the Sykam A07 or Sykam A08 columns. Only the peak area precision of the phosphate peak using the Sykam A08 exhibits a larger value (8.23%) which most probably can be traced back to baseline fluctuations influencing the integration of the phosphate peak at one of the replicate injections. 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. 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 7 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 well within the specified deviation range of ±15%.

Table 7. Relative Percent Differences (RPD) of QCS and ECS.

Analyte	Sykam A07 – RPD (%)	Sykam A08 – RPD (%)
Fluoride	+1.4	-2.6
Chloride	-0.8	-1.6
Nitrite	-0.3	-4.8
Bromide	-1.7	-5.5
Nitrate	-0.4	-1.0
Phosphate	+0.2	-7.8
Sulfate	-0.4	-1.1

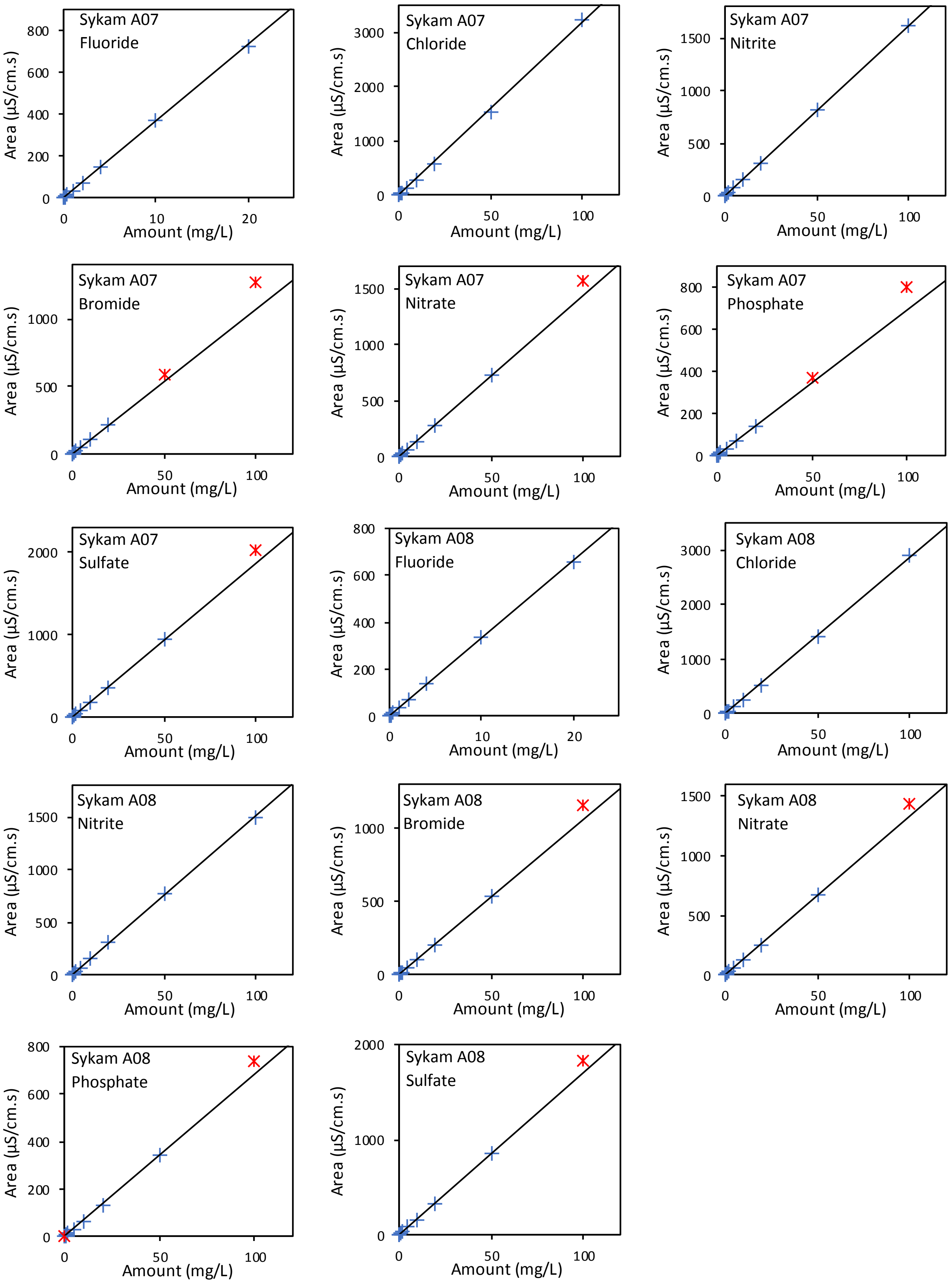

Figure 3. Calibration plots of the seven standard anions indicating the LCR used for calibration of the samples.

Sample Analysis

Eight samples were analyzed undiluted after filtration on the Sykam A07 each as field duplicates, whereas also six of them were analyzed using the Sykam A08. Interestingly, fluoride was found in all of the samples, however in very small 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 8 gives an overview of the MRLs for each anion in both validations based on the applied linear calibration range and the calculated MDLs.

Table 8. Minimum Reporting Levels based on the LCR and MDL.

Analyte	Sykam A07 – MRL (mg/L)	Sykam A08 – MRL (mg/L)
Fluoride	0.02	0.02
Chloride	0.10	0.10
Nitrite	0.10	0.10
Bromide	0.10	0.10
Nitrate	0.10	0.10
Phosphate	0.10	0.20
Sulfate	0.10	0.10

Chloride, nitrate and sulfate concentrations in the samples reach from the low single-digit mg/L-range up to roughly 30 mg/L, however the values found in the field duplicates do not differ significantly from the respective initial samples indicating the robustness of the validated methods.

Column: Sykam A07 (150 x 2.6 mm) and Sykam AGC-04 (20 x 2.6 mm)
Eluent: 4.0 mM Na_2CO_3, 25 µM NaSCN
Flow Rate: 1.0 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (40 mA)

Peaks:	
1. Fluoride	0.14 mg/L
2. Chloride	18.6
3. Bromide	0.04 (< MRL)
4. Nitrate	0.31
5. Sulfate	23.1

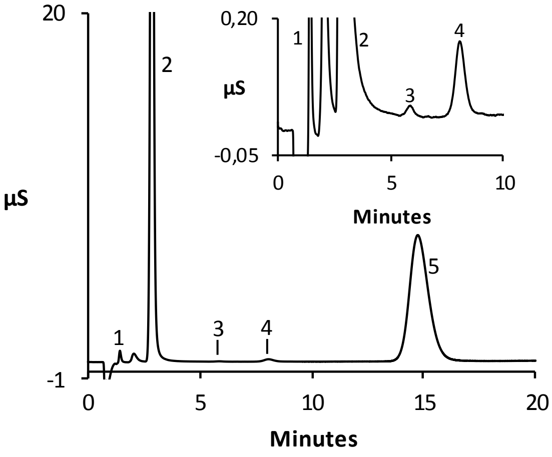

Figure 4. Determination of anions in mineral water (VIO) using the Sykam A07.

Figures 4 – 7 exemplarily show typical chromatograms obtained from the examined drinking and natural waters using the Sykam A07 and Sykam A08 respectively. Although the samples for both validations were collected on different days, the comparison of both validated methods shows no significant differences in the values found for each examined inorganic anion implying that both methods can be applied equivalently. 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[7]. All duplicate analysis results are found to be well within the declared limits.

Column: Sykam A07 (150 x 2.6 mm) and Sykam AGC-04 (20 x 2.6 mm)
Eluent: 4.0 mM Na_2CO_3, 25 µM NaSCN
Flow Rate: 1.0 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (40 mA)

Peaks:	A	B
1. Fluoride	0.07 mg/L	0.10 mg/L
2. Chloride	4.0	29.2
3. Nitrite	-	0.02 (< MRL)
4. Bromide	-	0.01 (< MRL)
5. Nitrate	2.9	9.3
6. Phosphate	-	0.08 (< MRL)
7. Sulfate	3.1	29.7

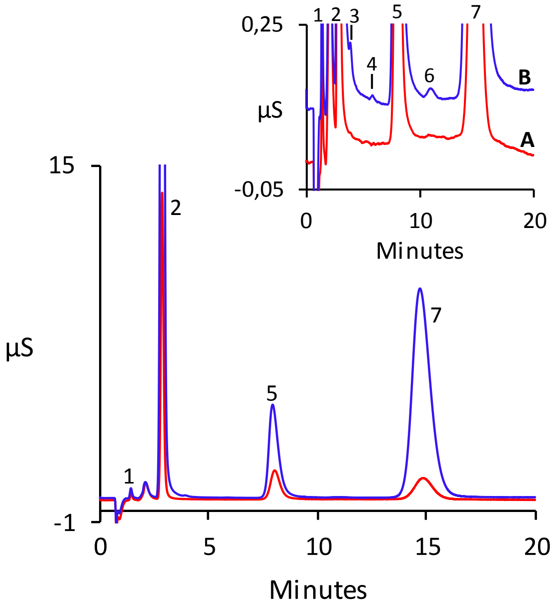

Figure 5. Determination of anions in river water (Illach (A) and Donau (B)) using the Sykam A07.

The good consistency of both, the initial and duplicate samples within each validation as well as the good consistency of the values of both validations compared to each other show, that both, the Sykam A07 and A08 are suitable columns for the analysis of drinking and natural water samples and the reproducibility of the generated results is very high. Table 9 gives an overview of the analysis results for the eight drinking and natural water samples, whereas the mean value of the duplicate analysis and their Relative Percent Difference (RPD) is displayed.

Table 9. Analysis results (mg/L) and RPD (%) of duplicate analyses of both validations.

Analyte	Drinking Water Augsburg		Drinking Water Riesen (Steingaden)		Mineral Water VIO		Mineral Water Adelholzener	
	A07	A08	A07	A08	A07	A08	A07	A08
Fluoride	0.11 (0.2)	0.11 (3.8)	0.09 (0.1)	0.07 (-1.3)	0.14 (-0.8)	0.15 (-0.7)	0.19 (0.8)	0.17 (-1.0)
Chloride	6.0 (-0.7)	5.9 (-0.8)	2.5 (-0.4)	2.5 (0.0)	18.6 (-0.5)	18.1 (0.1)	16.3 (0.1)	15.7 (-0.7)
Nitrite	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bromide	< MRL	n.d.	< MRL	n.d.	< MRL	< MRL	< MRL	n.d.
Nitrate	4.7 (-0.7)	4.5 (-3.7)	3.8 (0.3)	3.6 (-0.6)	0.31 (0.9)	0.29 (0.8)	< MRL	n.d.
Phosphate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfate	21.5 (0.4)	20.5 (0.7)	3.7 (-2.0)	3.5 (-0.7)	23.1 (-0.2)	22.1 (-0.6)	29.3 (-0.2)	27.7 (-1.1)

Analyte	River Water Donau		River Water Illach		Mineral Water RAPP		Mineral Water St. Leonhard	
	A07	A08	A07	A08	A07	A08	A07	A08
Fluoride	0.10 (-0.1)	0.08 (-2,7)	0.07 (-0.3)	0.06 (0.1)	0.04 (-0.5)	-	0.06 (1.2)	-
Chloride	29.2 (-0.2)	24.0 (-0.5)	4.0 (0.1)	4.8 (0.2)	0.41 (-1.1)	-	16.6 (-0.5)	-
Nitrite	< MRL	< MRL	n.d.	n.d.	n.d.	-	n.d.	-
Bromide	< MRL	< MRL	n.d.	n.d.	n.d.	-	< MRL	-
Nitrate	9.3 (-0.3)	9.1 (1.0)	2.9 (2.4)	3.3 (6.4)	< MRL	-	1.8 (0.3)	-
Phosphate	< MRL	< MRL	n.d.	n.d.	n.d.	-	n.d.	-
Sulfate	29.7 (-0.5)	24.1 (-0.5)	3.1 (-0.6)	3.1 (-1.8)	10.8 (-1.0)	-	6.2 (-1.2)	-

Column:	Sykam A08 (125 x 2.6 mm) and Sykam AGC-04 (20 x 2.6 mm)	
Eluent:	3.5 mM Na_2CO_3, 25 µM NaSCN	
Flow Rate:	1.2 mL/min	
Inj. Vol.:	50 µL	
Detection:	Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (50 mA)	
Peaks:	1. Fluoride	0.07 mg/L
	2. Chloride	2.5
	3. Nitrate	3.6
	4. Sulfate	3.5

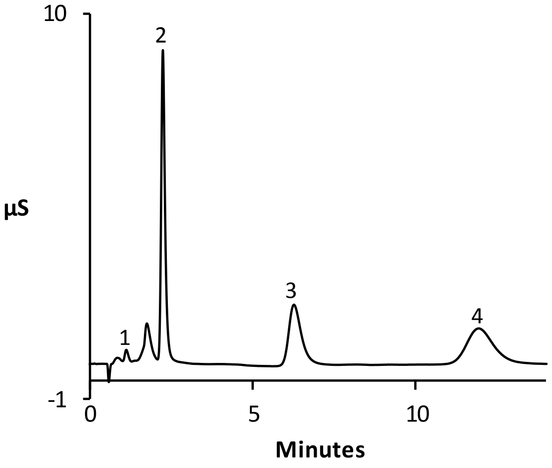

Figure 6. Determination of anions in drinking water (Riesen) using the Sykam A08.

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 concentration of the 5% calibration standard was used as check standard. The EPA 300.1 requirement for the deviation of the check standard is ±15%[7]. During the first validation, the check standards did not differ by more than 9.2% from the corresponding calibration standard, whereas during the second validation, the maximum deviation is 4.0%. In most cases, the difference was less than 2%. 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.

Column:	Sykam A08 (125 x 2.6 mm) and Sykam AGC-04 (20 x 2.6 mm)	
Eluent:	3.5 mM Na_2CO_3, 25 µM NaSCN	
Flow Rate:	1.2 mL/min	
Inj. Vol.:	50 µL	
Detection:	Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (50 mA)	
Peaks:	1. Fluoride	0.17 mg/L
	2. Chloride	15.7
	3. Sulfate	27.7

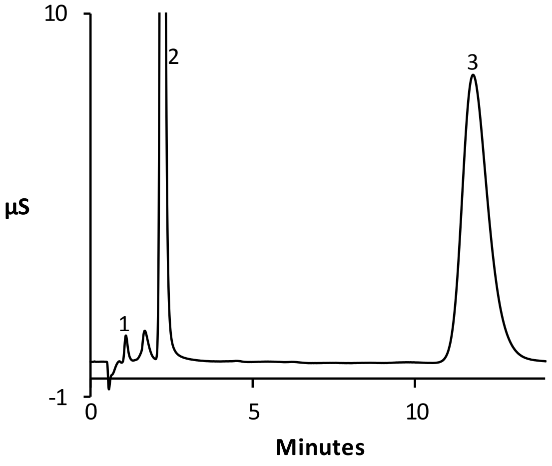

Figure 7. Determination of anions in mineral water (Adelholzener) using the Sykam A08

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 10 and 11 show the obtained recovery results for single-operator data using the Sykam A07 and A08 columns for the standard inorganic anions spiked into drinking and natural water samples with different matrices. The samples were spiked with the standard stock solutions used for the calibration at approximately the same levels as specified in the EPA Method 300.1. The method specifies, that the samples have to be fortified ideally at the same concentrations, which are found in the unfortified sample for the respective anions. Figure 8 shows a typical example for a fortified drinking water sample (from Augsburg) directly compared to the unfortified sample. The fortified concentration, as indicated in EPA 300.1, should not exceed a level five times as high as the concentration of the unfortified sample and not be less than the analyzed concentration in the unfortified sample, which implies, that recovery data of samples with analyte contents of more than 50% of the maximum calibration concentration are not collected. The minimum fortification level for analytes, which are not detected or detected below the MRL is 5x MRL. Additionally, a Laboratory Blank is fortified at the highest concentration of each anion found throughout the samples analyzed during the validation (Laboratory Fortified Blank, LFB). Table 12 summarizes the recovery data found in the Laboratory Fortified Blank for both validations.

Table 12. Recovery Data of the Laboratory Fortified Blank (LFB).

Analyte	LFB – Sykam A07		LFB – Sykam A08	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	101.7	0.5	101.0
Chloride	30	99.2	25	93.1
Nitrite	2	100.7	2	86.3
Bromide	2	99.2	2	96.0
Nitrate	10	98.9	10	93.3
Phosphate	5	102.3	5	91.6
Sulfate	30	105.2	30	99.9

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[7]. The LFM results indicate, that for all matrices, recoveries were within the acceptable range of ±25%. The LFB shows recovery results of 86-105%, which is also within the specified range. The found recoveries of sulfate for the VIO and Adelholzener mineral Water as well as the Donau river water using the Sykam A07 and for the Adelholzener mineral water using the Sykam A08 are above the highest calibration level for sulfate of 50 mg/L and hence, should not be discussed.

Table 10. Recovery Data from fortified Drinking and Natural Water Samples using the Sykam A07.

Analyte	Drinking Water Augsburg		Drinking Water Riesen (Steingaden)		Mineral Water VIO		Mineral Water Adelholzer	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	99.8	0.5	97.5	0.5	102.9	0.5	100.8
Chloride	7	99.7	3	92.0	20	109.1	20	108.3
Nitrite	2	97.1	2	93.9	2	106.7	2	104.2
Bromide	2	99.8	2	100.1	2	99.5	2	100.0
Nitrate	5	100.0	4	101.5	2	92.2	2	92.2
Phosphate	5	103.7	5	104.5	5	104.1	5	104.8
Sulfate	25	114.6	4	102.2	25	(114.6)*	30	(116.7)*

Analyte	River Water Donau		River Water Illach		Mineral Water RAPP		Mineral Water St. Leonhard	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	100.3	0.5	100.9	0.5	100.6	0.5	99.5
Chloride	30	110.7	5	97.6	1	96.2	20	108.7
Nitrite	2	89.3	2	97.1	2	92.9	2	103.6
Bromide	2	99.4	2	100.6	2	96.7	2	99.3
Nitrate	10	106.5	3	100.4	2	91.3	2	98.2
Phosphate	5	103.4	5	105.8	5	103.5	5	104.5
Sulfate	30	(117.8)*	4	102.8	11	108.3	7	108.0

*Concentration of fortified sample is above the highest calibration level

Table 11. Recovery Data from fortified Drinking and Natural Water Samples using the Sykam A08.

Analyte	Drinking Water Augsburg		Drinking Water Riesen (Steingaden)		Mineral Water VIO	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	95.6	0.5	92.3	0.5	97.7
Chloride	6	94.9	3	86.6	20	101.7
Nitrite	2	88.0	2	88.9	2	84.3
Bromide	2	94.6	2	86.3	2	93.4
Nitrate	5	96.1	4	91.9	2	90.3
Phosphate	5	87.6	5	91.3	5	92.5
Sulfate	25	107.5	4	99.9	25	108.0

Analyte	Mineral Water Adelholzener		River Water Donau		River Water Illach	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	96.3	0.5	95.4	0.5	94.4
Chloride	20	100.7	25	102.3	5	93.1
Nitrite	2	83.9	2	84.3	2	86.3
Bromide	2	94.0	10	94.0	4	96.0
Nitrate	2	93.5	2	99.4	3	93.3
Phosphate	5	90.1	5	91.6	5	91.6
Sulfate	30	(108.9)*	25	107.3	4	99.9

*Concentration of fortified sample is above the highest calibration level

Column: Sykam A07 (150 x 2.6 mm) and Sykam AGC-04 (20 x 2.6 mm)
Eluent: 4.0 mM Na_2CO_3, 25 µM NaSCN
Flow Rate: 1.0 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Electrochemical Self-Regenerating Anion Suppressor (40 mA)
Peaks:

	A	B
1. Fluoride	0.11 mg/L	0.61 mg/L
2. Chloride	6.0	12.7
3. Nitrite	–	1.9
4. Bromide	0.01 (< MRL)	2.0
5. Nitrate	4.7	9.5
6. Phosphate	–	5.2
7. Sulfate	21.5	49.1

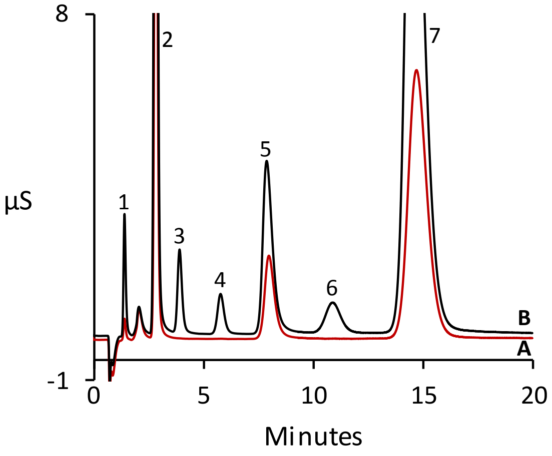

Figure 8. Determination of anions in unspiked (A) and spiked (B) drinking water samples from Augsburg using a Sykam A07.

Column: Sykam A07 (150 x 2.6 mm) and Sykam AGC-04 (20 x 2.6 mm)
Eluent: 4.0 mM Na_2CO_3, 25 µM NaSCN
Flow Rate: 1.0 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Sykam Chemical Anion Suppressor (80 rpm, **A**) and Electrochemical Self-Regenerating Anion Suppressor (40 mA, **B**)
Peaks:

	A	B
1. Fluoride	0.05 mg/L	0.06 mg/L
2. Chloride	16.0	16.6
3. Bromide	–	0.009 (< MRL)
4. Nitrate	1.7	1.8
5. Sulfate	6.1	6.2

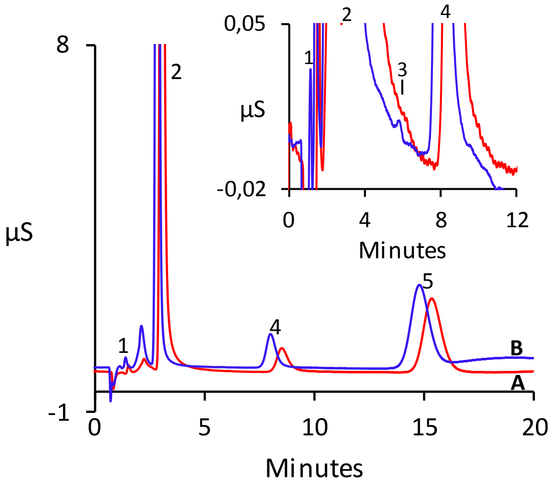

Figure 9. Determination of anions in mineral water samples (St. Leonhard) using a Sykam A07 with the Sykam chemical anion suppressor (A) and the electrochemical self-regenerating anion suppressor (B).

Comparison of chemical and electrochemical suppression

The suppression technique used in the S150+ series of Sykam IC instruments is based on electrochemical suppression and has different advantages compared to the previously employed chemical suppression technique of the former S150 series. First of all, electrochemical suppressors are self-regenerating and therefore can be operated without any maintenance steps like regeneration, since the protons, which are necessary for the cation exchange in the eluent are generated by electrolysis of water molecules, that are obtained from the eluent stream itself. In case of using the Sykam A07 at a flow rate of 1.0 mL/min, electrochemical suppression generates less baseline noise (<2 nS/cm) as compared to the chemical suppression (<5 nS/cm) allowing for trace level analysis of analytes like bromide or nitrite, which in commonly provided drinking water as well as in natural water samples are present at very low concentrations in the lower µg/L-range. In Figure 9, two chromatograms of the analysis of a commercially available drinking water sample (St. Leonhard) are displayed, one obtained using chemical suppression (A) and the second one obtained using electrochemical suppression technique (B). Chromatogram B reveals higher signals together with a lower baseline noise resulting in an improved signal-to-noise ratio for any of the analytes, which in case of the very low concentrated bromide ion (9 µg/L) results in reliable detection of this analyte when using electrochemical suppression. In chromatogram A, using chemical suppression, the bromide ion could not be detected at this concentration level.

Summary

The Sykam A07 and A08 columns both provide suitable performance for the determination of inorganic anions in drinking and natural waters, as outlined in U.S. EPA Method 300.1, ASTM D4327 and ISO EN 10304-1. This was shown by full method validations 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 A07 and A08 under the described chromatographic conditions. Both columns exhibit high capacities, as the linearity range was determined over more than two orders of magnitude, making it possible to analyze samples containing up to 100 mg/L of chloride or other inorganic anions without a time-consuming dilution step.

The Sykam A07 reveals high resolution for all examined standard anions, making it suitable for the analyses of complex matrices, large concentration differences between closely eluting peak pairs (e.g. chloride and nitrite) or if the focus is on the analysis of all seven standard anions. If very low values in the sub-mg/L-range for any of the examined inorganic anions of interest is expected in the field samples, the Sykam A07 using the designated chromatographic conditions should be employed. The Sykam A08 is a faster eluting column with shorter retention times for all anions, which is advantageous for time saving analyses, especially, if the focus is on selectively analyzing the ubiquitously found anions chloride, nitrate and sulfate, which naturally occur in higher concentrations.

References

[1] Amtsblatt der Europäischen Gemeinschaften, Richtlinie 98/83/EG des Rates vom 03. November 1998 über die Qualität von Wasser für den menschlichen Gebrauch.

[2] Directive (EU) 2020/2184 of the European Parliament and of the Council of 16th December 2020 on the quality of water intended for human consumption (recast).

[3] Safe Drinking Water Act (Title XIV of the Public Health Service Act, Chapter 373 of the 78th Congress).

[4] Richtlinie 2000/60/EG des Europäischen Parlaments und des Rates vom 23. Oktober 2000 zur Schaffung eines Ordnungsrahmens für Maßnahmen der Gemeinschaft im Bereich der Wasserpolitik.

[5] www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations.

[6] EN ISO 10304-1:2009: Wasserbeschaffenheit – Bestimmung von gelösten Anionen mittels Flüssigkeits-Ionenchromatographie – Teil 1: Bestimmung von Bromid, Chlorid, Fluorid, Nitrat, Nitrit, Phosphat und Sulfat.

[7] Method 300.1 – Determination of Inorganic Anions in Drinking Water by Ion Chromatography, Revision 1.0, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OHIO 45268.

[8] ASTM International: Designation D4327-17, Standard Test Method for Anions in Water by Suppressed Ion Chromatography.

[9] 40 CFR Appendix -B-to-Part-136 – Definition and Procedure for the Determination of the Method Detection Limit – Revision 2.

[10] DIN 38402-51 – Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung – Allgemeine Angaben (Gruppe A) – Teil 51: Kalibrierung von Analyseverfahren – Lineare Kalibrierfunktion (A51).