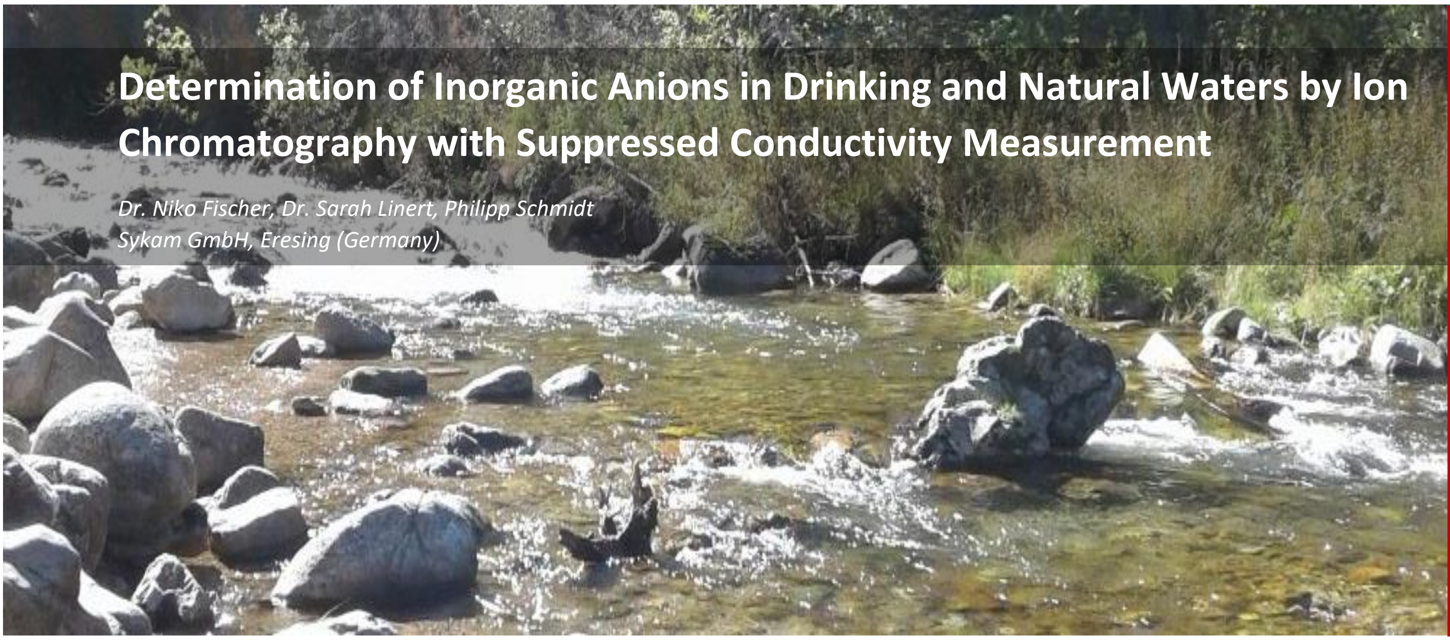

Determination of Inorganic Anions in Drinking and Natural Waters by Ion Chromatography with Suppressed Conductivity Measurement

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Introduction

A secured supply of clean drinking water as well as the protection of natural waters from pollutants, arising from industrial and other human sources, is one of the most challenging tasks for humankind today and in the future. A lack of clean drinking water due to exponentially increasing consumption and contamination of natural water resources, together with climate change related water shortages have attracted considerable attention within the last decades, not only in immediately affected countries, but all over the world. In the European Union, the quality of drinking water is regulated by the Drinking Water Directive (DWD) 98/83/EC[1] from 1998 with its latest version 2020/2184[2] published in December 2020. The objective of this directive is to ensure the safety of water intended for human consumption. In the United States, water quality is legislated by the Safe Drinking Water Act[3] (SDWA), which is meant to ensure the integrity and safety of drinking waters. For the protection of natural water resources, the European Union established the Water Framework Directive 2000/60/EC[4] in October 2000, whose purpose is to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater from further deterioration and protect and enhance the status of aquatic ecosystems. The respective primary federal law in the United States is the Clean Water Act (CWA), which aims to reduce the discharge of pollutants into natural waters. In many parts of the world, similar health and environmental standards have been established, often by adopting the aforementioned EU or U.S. regulations.

Ion Chromatography (IC) has been established as one of the most commonly used analytical techniques for the determination of ionic species in drinking and natural waters and is the standard method, that has been validated and published by many national and international standardization organizations like the ISO (International Organization for Standardization), ASTM (American Society for Testing and Materials) or AWWA (American Water Works Association) and also the U.S. EPA (Environmental Protection Agency). In the EU Drinking Water Directive (DWD)[1, 2] and the U.S. National Primary and Secondary Drinking Water Standards (NPDWS/NSDWS)[5] the content of anions in drinking and natural waters is regulated by Maximum Contaminant Levels (MCL). A considerable number of regulatory IC methods have been published worldwide to monitor these levels, including the EN ISO 10304-1[6] (Technical Committee ISO/TC 147 “Water quality”), the EPA 300.1[7] or the ASTM D4327.[8]

This application note “Sykam AN01” describes the determination of inorganic anions in drinking and natural waters by IC using suppressed conductivity detection. The herein described method was validated under consideration of the requirements described in EPA Method 300.1[7] and therefore provides an economical way to meet the testing requirements of EPA 300.1, Revision 1.0[7] as well as EN ISO 10304-1:2009-07[6] and ASTM D4327-17[8] using a Sykam S151 IC system. 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 below.

Equipment

Application note Sykam AN01 was designed for single channel anion detection using a Sykam S151-AG IC system consisting of the following components:

- S150 Ion Chromatography Module, including a column oven, one-channel conductivity detector and chemical anion suppressor module
- S1130 Quaternary Gradient Pump (PEEK), including a 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 an 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 an S5300 Automatic Sample Injector. Alternatively, the dual channel S153 IC system can be used for this application, by running the system in single channel mode for anion detection.

Reagents and Standards

All reagents for eluent preparation and standards 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, 0.05 µS/cm conductivity (18 MΩ/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 the respective 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 analyzed samples.

No.	Name
1	Drinking Water (Steingaden)
2	Drinking Water (Eresing)
3	Mineral Water 1
4	Mineral Water 2
5	River Water (Lech)
6	Lake Water (Deutensee)
7	Stream Water (Staltannen)
8	Fish Pond Water (Pitzling)

All drinking and natural water samples were collected at locations in rural areas of Southern Germany in the northern foothills of the Alps, where lime containing soil is the prevailing geological formation. The drinking water samples were collected as tap water from two different municipal water suppliers in Steingaden and Eresing. The river water was collected from the Lech River near Lechbruck and the lake water was collected from the Deutensee, which is a moor lake with relatively high concentrations of humic acids. The stream water was collected from a stream near Staltannen and the last natural water sample from a fish pond populated with samlet near Pitzling. Additionally, two commercially available mineral water samples from companies, which obtain their water from natural ground water sources, were analyzed. All samples were collected as initial sample and duplicate, with the duplicate samples collected under the same conditions as the initial samples. The samples were collected in 100 mL PE-bottles with a screw cap and stored at 4 °C immediately after collection.

Chromatographic Conditions

Method Validation 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, Sykam Chemical Anion Suppressor
Suppressor Speed:	80 rpm
Backpressure:	51 bar (740 psi)
Base Conductivity:	22 µS/cm
Noise:	<5 nS/cm

Method Validation 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:	1.5 mL/min
Run Time:	13 min
Temperature:	60 °C
Injection Volume:	50 µL (Full Loop)
Detection:	Suppressed Conductivity, Sykam Chemical Anion Suppressor
Suppressor Speed:	80 rpm
Backpressure:	73 bar (1060 psi)
Base Conductivity:	22 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, if commercial standard stock solutions are not available, can be prepared from the respective salts as indicated above. The amounts needed for the preparation of 1000 mL of each standard stock solution are given in Table 2. If one or more of the seven standard anions do not 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 and mixed anion standard solutions at concentrations lower than 1000 mg/L are prepared from the 1000 mg/L 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. Using the results of this estimation, 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 lists the concentrations of the 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 used during the validation.

Table 3. Concentrations of the MDL_S calculation standard and QCS 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	50	2	2
Bromide	70	100	2	2
Nitrate	80	50	20	20
Phosphate	200	250	2	2
Sulfate	100	75	30	30

The determination of the linear calibration range, covering the expected concentrations of the anions in drinking and natural water samples, is achieved by injecting ten levels 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. Since fluoride in drinking and natural water samples is usually only present at low concentrations, the amount of fluoride in the calibration standards was reduced to 0.02, 0.04, 0.1, 0.2, 0.4, 1.0, 2.0, 4.0, 10 and 20 mg/L respectively for the method validation of the A08 to improve accuracy. All concentration levels are prepared from the 1000 mg/L standard stock solutions. 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 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 all solids are dissolved and fill up the flask to the mark, after 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, fill up the flask to the mark and shake it vigorously. For the two chromatographic methods 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, especially the chemical suppressor unit, has to be equilibrated. For equilibration, switch on the column oven and the suppressor at the respective conditions.

Apply the desired flow rate until the baseline has stabilized. The baseline noise should be <5 nS/cm. If the system has not been in use for several days or longer, let it equilibrate for 3 hours, if the system is used on a daily basis, leave the column oven, as well as the suppressor unit, switched on overnight and apply a low flow rate (0.1 mL/min), so that the system can be readily used for subsequent analyses. The suppressor speed can be reduced to 20 rpm overnight. 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 each injection. For more detailed instructions, refer to the corresponding installation, maintenance and operating manuals of the instruments. If using a new column, install the column and condition it according to the procedure described in the corresponding column data sheet.

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 activity, leading to changes in the content of nitrite and nitrate, the samples must 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. These methods state that optional columns and corresponding different chromatographic conditions may also be used to improve separation or lower measurement costs. For the validations, the two columns Sykam A07 and Sykam A08, together with the Sykam S151-AG IC system were used under the chromatographic conditions listed above, testing all required quality control parameters including:

- Determination of method detection limits MDL_S and MDL_B[9]
- Linearity (Linear Calibration Range, LCR)[10]
- Precision by repeated injection of a Quality Control Sample (QCS)
- Accuracy of calibration standards and instrument performance by injection of an External Control Sample (ECS)
- Laboratory performance by injection of a Laboratory Reagent Blank (LRB) and Laboratory Fortified Blank (LFB)
- Analysis of duplicates
- Assessment of analyte recovery by injection of Laboratory Fortified Matrices (LFM) for each sample

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

The column material of the Sykam A07 (150 x 2.6 mm) as well as the Sykam A08 (125 x 2.6 mm) is based on trimethylammonium functionalized PS-DVB with a particle size of 10 µm. Due to the high capacity of these columns, they are both suitable for the analysis of all seven standard anions in one run. As the A07 has a higher resolution than the Sykam A08, it is the column of choice for the quantification of the fast-eluting analyte fluoride and for the separation of neighboring peak pairs with a large difference in concentration, as, for example, is often found for chloride and nitrite. The method using the Sykam A07 described here has a high signal-to-noise ratio, making it suitable for trace analyses at concentrations in the sub-mg/L range down to the single-digit µg/L 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 ubiquitously found anions chloride, nitrate and sulfate, or in case of the absence of anions like nitrite, bromide or phosphate, the analyst can save on time and consumables by using the Sykam A08 with its shorter retention times.

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)

Peaks:	1. Fluoride	5.0 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

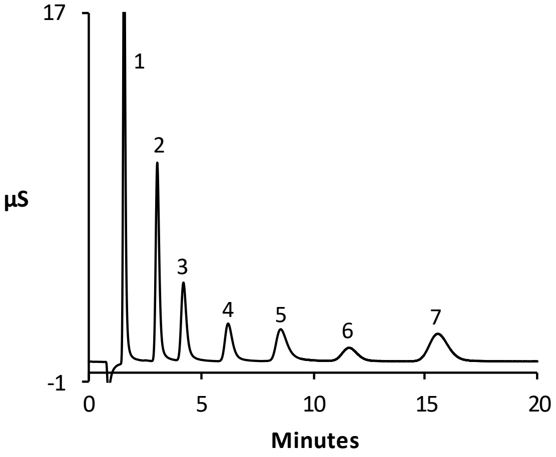

Figure 1. Separation of a 5.0 mg/L inorganic anion standard using the Sykam A07.

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 or Sykam A08 respectively.

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	1.60	0.97	-	-	1.44	1.40
Chloride	3.14	1.93	6.75	6.40	1.34	1.17
Nitrite	4.37	2.69	3.53	3.27	1.52	1.28
Bromide	6.45	4.05	4.07	3.92	1.49	1.21
Nitrate	8.91	5.58	3.28	3.01	1.55	1.32
Phosphate	11.89	7.77	2.95	2.94	1.16	1.06
Sulfate	16.09	10.16	3.31	2.44	1.19	1.09

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: 1.5 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Sykam Chemical Anion Suppressor (80 rpm)
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

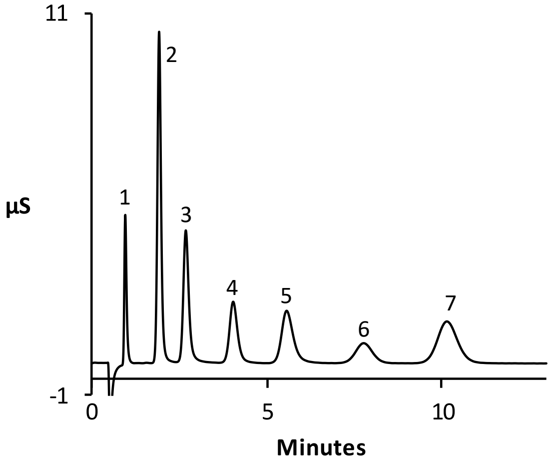

Figure 2. Separation of a 5.0 mg/L inorganic anion standard (Fluoride 1.0 mg/L) using the Sykam A08.

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 concentrations of 200, 100, 50, 20, 10 and 5 µg/L of each anion. For each analyte, the concentration, at which the signal-to-noise ratio is between 3 and 5 was determined or extrapolated. The estimated method detection limits were multiplied by a factor of 5 to obtain the final concentrations used for 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 $MDL_S = t \times SD$, 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 the respective anion. The thus 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 for anions, which give a numerical result in the injections of the Laboratory Reagent Blank (LRB). In this case, seven replicates of the LRB were analyzed with the result, that chloride was found at a mean level of 7.6 µg/L (Sykam A07) and 9.9 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: $MDL_B = X + t \times SD$, 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 9.8 µg/L for the Sykam A07 and 13.6 µg/L for the Sykam A08. None of the other anions were 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. For quantification of the samples and the fortified samples, the calibration range was limited to its linear range individually for each anion. Therefore, only the calibration levels that were found to be within 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, whereas fluoride, bromide and phosphate did not exhibit a linear response at high 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 0.1–10 or 0.1–20 mg/L were used for their respective determinations. For phosphate, the first calibration point at 0.1 mg/L was omitted. For the validation using the Sykam A08 fluoride was calibrated at 0.02–20 mg/L. Tables 5 and 6 list 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.

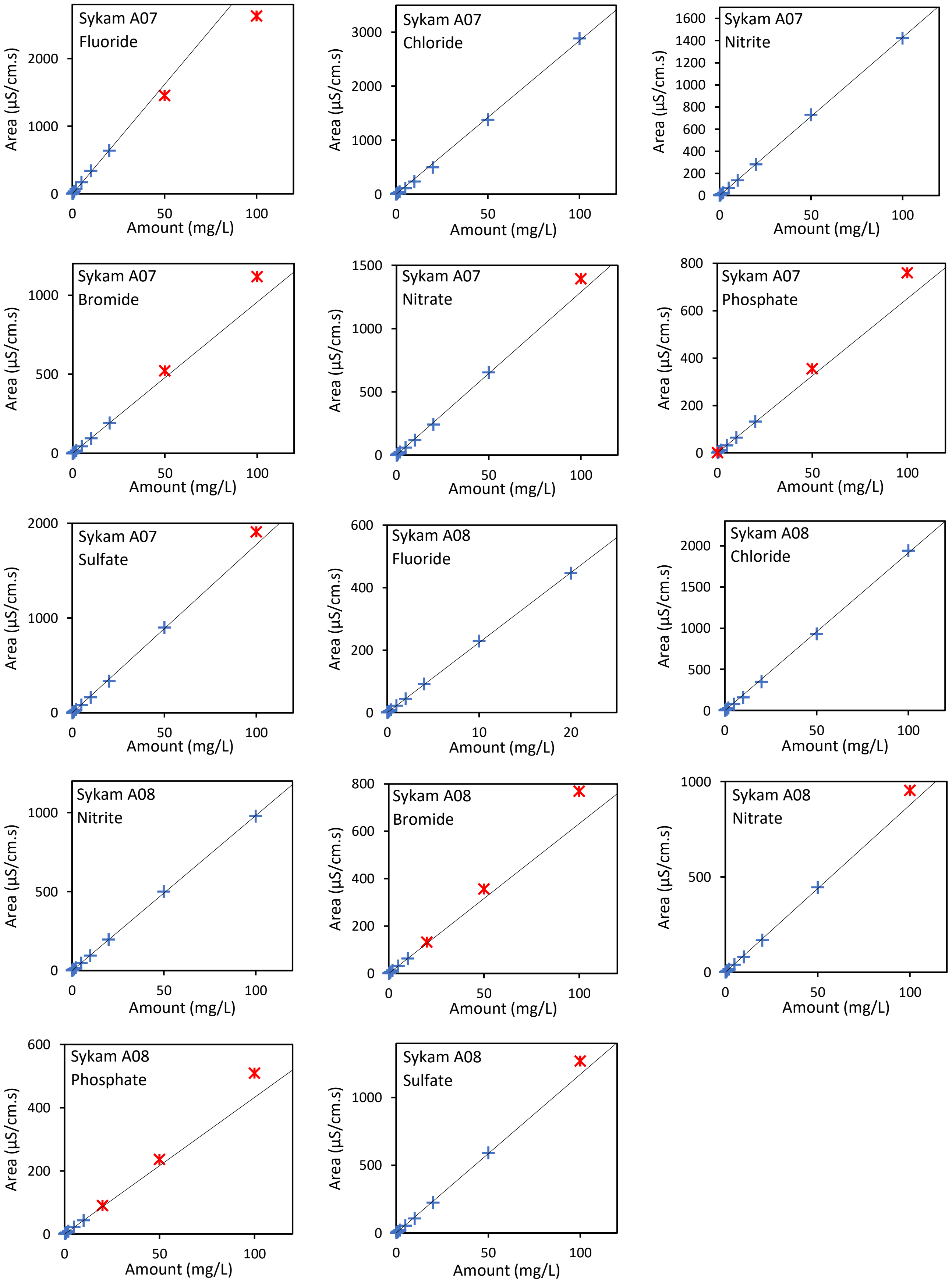

Figure 3. Calibration plots of the seven standard anions indicating the LCR used for analysis of the samples.

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	0.1 – 20	0.9994	1.2	0.47	0.56
Chloride	0.1 – 100	0.9995	1.6*	0.25	0.67
Nitrite	0.1 – 100	0.9999	6.1	0.19	0.81
Bromide	0.1 – 20	0.9999	18.5	0.16	0.85
Nitrate	0.1 – 50	0.9995	34.2	0.14	0.64
Phosphate	0.2 – 20	0.9999	57.6	0.20	1.15
Sulfate	0.1 – 50	0.9996	44.8	0.11	0.56

* 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	1.7	0.55	0.68
Chloride	0.1 – 100	0.9996	2.5*	0.29	0.76
Nitrite	0.1 – 100	0.9999	12.6	0.24	1.08
Bromide	0.1 – 10	1.0000	22.3	0.17	0.80
Nitrate	0.1 – 50	0.9996	33.2	0.12	0.65
Phosphate	0.2 – 10	1.0000	191.8	0.19	1.85
Sulfate	0.1 – 50	0.9997	36.9	0.11	0.72

* Value not valid, MDL_B is used instead

Precision (QCS) and Instrument Performance (ECS)

Retention time and peak area precision were determined from seven replicate injections of a Quality Control Standard (QCS). Ideally the concentrations of the single inorganic anions in the QCS are very similar to those found in the field samples. The concentrations of the QCS are listed in Table 3. Tables 5 and 6 list the Relative Standard Deviation (RSD) of the retention times and peak areas of seven replicate injections of the QCS.

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

Analyte	Sykam A07 – RPD (%)	Sykam A08 – RPD (%)
Fluoride	+3.5	+1.7
Chloride	+2.1	+0.1
Nitrite	-1.5	-0.3
Bromide	-0.2	-3.1
Nitrate	+0.5	-1.1
Phosphate	+3.4	+5.7
Sulfate	+1.0	-0.6

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 all investigated anions, illustrating the high precision achieved with the Sykam S151 IC system using the Sykam A07 or Sykam A08 columns.

If no new calibration is performed prior to analysis, the quality of the calibration standards and acceptable instrument performance is verified by the preparation and analysis of a QCS. In this case, a new calibration was performed and instrument performance was verified by 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 calibration. The QCS, however is prepared from the same standard stock solutions that were used for the preparation of the calibration standards. If the QCS is used for the verification of instrument performance, the acceptance range is ±15%. This value also applies for the Relative Percent Difference (RPD) of the ECS in comparison to the QCS.[7] Table 7 lists the RPD of the ECS, prepared from the external source stock solutions, and the QCS, prepared from the stock solutions used for calibration. The determined differences are well within the specified deviation range of ±15%.

Sample Analysis

All eight samples were analyzed undiluted after filtration on both columns, each as initial sample and field duplicate. Table 9 gives an overview of the analysis results for the eight drinking and natural water samples, listing the mean value of the initial sample and duplicate analysis and their RPDs.

Phosphate was not found in any of the samples, and nitrite and bromide were detected in some of the samples in very small amounts below the Minimum Reporting Level (MRL). The MRL is defined as the lowest concentration, at which an analyte can be quantified. In this case it is a concentration that is both higher than the MDL and higher or equal to the lowest calibration point. 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. Fluoride was found in all of the samples in very low concentrations near or below the MRL. Chloride, nitrate and sulfate concentrations in the samples reached from a low single-digit-mg/L-range up to more than 40 mg/L.

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

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

The values found in the field duplicates are very similar to the initial samples, indicating the robustness of the validated methods. 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 these 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, Sykam Chemical Anion Suppressor (80 rpm)
Peaks: 1. Fluoride 0.05 mg/L (< MRL)
2. Chloride 4.1
3. Nitrate 5.9
4. Sulfate 5.4

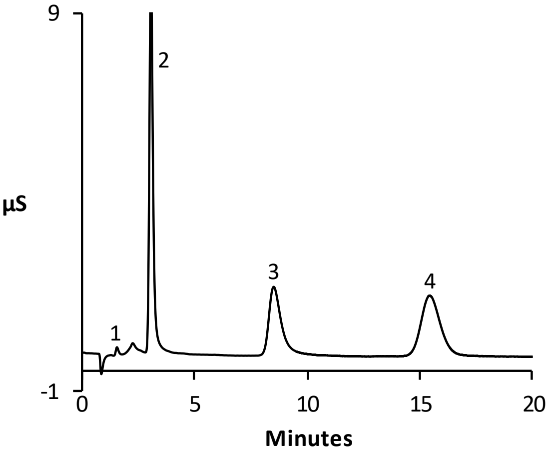

Figure 4. Determination of anions in drinking water (Steingaden) using the Sykam A07.

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)
Peaks: 1. Fluoride 0.08 mg/L (< MRL)
2. Chloride 0.42
3. Nitrate 0.05 (< MRL)
4. Sulfate 10.0

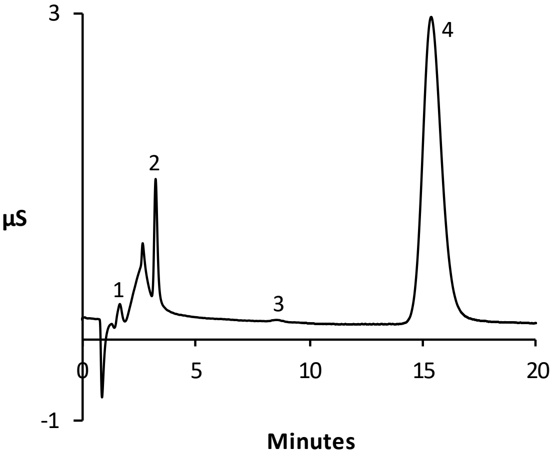

Figure 5. Determination of anions in mineral water 1 using the Sykam A07.

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: 1.5 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Sykam Chemical Anion Suppressor (80 rpm)
Peaks: 1. Fluoride 0.09 mg/L
2. Chloride 2.7
3. Nitrite 0.005 (< MRL)
4. Nitrate 0.99
5. Sulfate 19.0

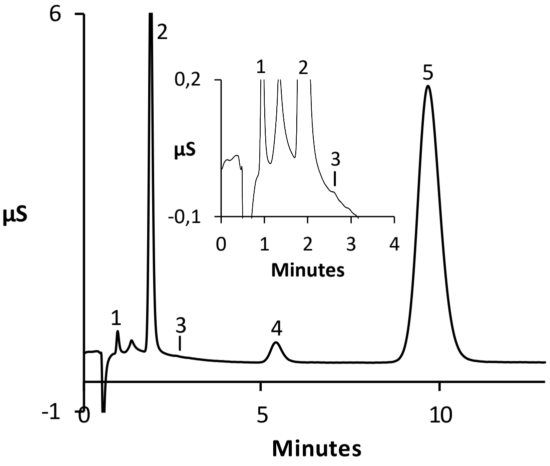

Figure 6. Determination of anions in river water (Lech) using the Sykam A08.

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: 1.5 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Sykam Chemical Anion Suppressor (80 rpm)
Peaks: 1. Fluoride 0.10 mg/L
2. Chloride 4.1
3. Nitrite 0.015 (< MRL)
4. Nitrate 41.9
5. Sulfate 12.5

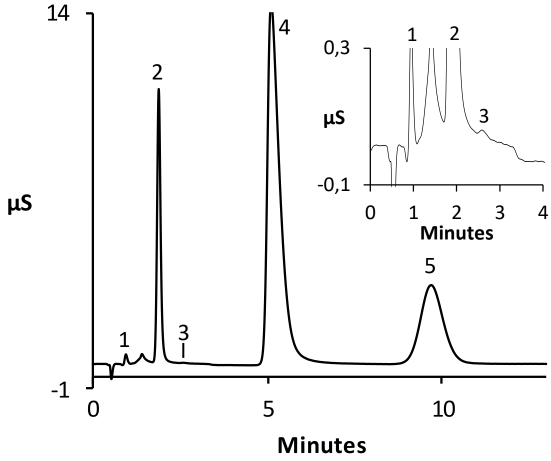

Figure 7. Determination of anions in fish pond water (Pitzling) using the Sykam A08.

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

Analyte	Drinking Water Steingaden		Drinking Water Eresing		Mineral Water 1		Mineral Water 2	
	A07	**A08**	**A07**	**A08**	**A07**	**A08**	**A07**	**A08**
Fluoride	< MRL	0.06 (-5.4)	< MRL	0.07 (3.5)	< MRL	0.04 (7.5)	< MRL	0.05 (-1.8)
Chloride	4.1 (-1.3)	4.2 (0.4)	16.8 (-1.2)	16.8 (-0.7)	0.42 (0.1)	0.40 (1.2)	16.0 (-0.7)	16.0 (-0.3)
Nitrite	n.d.	n.d.	n.d.	< MRL	n.d.	n.d.	n.d.	n.d.
Bromide	n.d.	n.d.	< MRL	< MRL	n.d.	n.d.	n.d.	n.d.
Nitrate	5.9 (-3.3)	5.6 (0.8)	26.2 (0.8)	25.6 (-0.2)	< MRL	< MRL	1.7 (0.2)	1.6 (-0.2)
Phosphate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfate	5.4 (4.2)	5.2 (1.4)	11.5 (-0.1)	11.5 (-0.4)	10.0 (0.6)	10.5 (0.2)	6.1 (-0.3)	5.9 (-0.2)
Analyte	**River Water Lech**		**Lake Water Deutensee**		**Stream Water Staltannen**		**Fish Pond Water Pitzling**	
	A07	**A08**	**A07**	**A08**	**A07**	**A08**	**A07**	**A08**
Fluoride	< MRL	0.09 (0.1)	< MRL	0.07 (2.1)	< MRL	0.07 (0.5)	< MRL	0.10 (-0.2)
Chloride	2.6 (3.5)	2.7 (0.2)	1.7 (2.4)	1.5 (0.1)	0.87 (3.7)	0.97 (-0.4)	4.4 (1.9)	4.1 (-0.2)
Nitrite	< MRL	< MRL	n.d.	< MRL	n.d.	n.d.	< MRL	< MRL
Bromide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< MRL	n.d.
Nitrate	1.3 (-1.4)	0.99 (-3.5)	< MRL	0.26 (8.0)	1.9 (0.3)	2.2 (-1.8)	27.5 (0.5)	41.9 (-0.3)
Phosphate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfate	20.1 (0.2)	19.0 (-0.9)	< MRL	0.40 (2.3)	1.6 (4.6)	1.6 (0.0)	10.9 (0.2)	12.5 (-0.3)

The comparison of both validations results to each other shows very good consistency, even though the samples were collected on different days. The only exceptions are the nitrate and sulfate content of sample 8, which can be traced back to heavy rainfalls washing out the surrounding soil between the two sampling dates. 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, with very high reproducibility of the generated results.

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. For the validation with the Sykam A07, the 100% calibration standard was used as check standard, while for the Sykam A08 the 5% calibration standard was used. In both cases, the EPA 300.1 threshold for deviation from the check standard is ±15%.[7] During the validations, the check standards did not differ by more than 4.3% from the corresponding calibration standard. In most cases, the difference was less than 2%. For evaluation, the areas under the peak 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 is typically validated through single- and multi-operator precision and bias studies on fortified samples (Laboratory Fortified Matrix, LFM). Tables 10 and 11 list 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 of different matrices. The samples were spiked with the standard stock solutions used for the calibration at approximately the same levels as specified in EPA Method 300.1. The method specifies, that the samples have to be fortified at ideally the same concentrations, which were found in the unfortified sample for the respective anions. However, the fortified concentration should not exceed a level five times as high as the concentration of the unfortified sample and not be less than the detected concentration in the unfortified sample. This means that recovery data of samples with analyte contents of more than 50% of the maximum calibration concentration are not collected, as spiked samples would exceed the maximum calibration concentration and no longer be accurate. The minimum fortification level for analytes, which are not detected or detected below the MRL, is 5x MRL.

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

Analyte	Drinking Water Steingaden		Drinking Water Eresing		Mineral Water 1		Mineral Water 2	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	98.5	0.5	91.3	0.5	92.1	0.5	90.9
Chloride	5	93.2	20	99.1	0.5	100.2	20	99.8
Nitrite	2	88.2	2	78.0	2	90.7	2	78.9
Bromide	2	94.0	2	88.4	2	95.4	2	89.8
Nitrate	6	87.1	25	98.3	1	90.0	2	89.6
Phosphate	5	63.5	5	84.6	5	95.3	5	94.9
Sulfate	6	94.0	12	96.3	10	98.2	6	94.5
Analyte	**River Water Lech**		**Lake Water Deutensee**		**Stream Water Staltannen**		**Fish Pond Water Pitzling**	
	Amount added (mg/L)	**Recovery (%)**	**Amount added (mg/L)**	**Recovery (%)**	**Amount added (mg/L)**	**Recovery (%)**	**Amount added (mg/L)**	**Recovery (%)**
Fluoride	0.5	93.7	0.5	95.7	0.5	91.1	0.5	87.3
Chloride	3	83.1	2	83.0	1	77.5	5	85.6
Nitrite	2	88.9	2	90.4	2	93.5	2	84.5
Bromide	2	92.5	2	93.5	2	93.7	2	88.4
Nitrate	2	86.9	1	89.0	2	86.4	30	99.2
Phosphate	5	96.7	5	96.2	5	92.8	5	84.3
Sulfate	20	100.1	1	103.8	2	88.8	12	94.2

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

Analyte	Drinking Water Steingaden		Drinking Water Eresing		Mineral Water 1		Mineral Water 2	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	94.5	0.5	95.3	0.5	100.3	0.5	107.5
Chloride	5	90.6	20	100.1	1	97.0	20	102.0
Nitrite	2	93.8	2	97.8	2	91.0	2	101.7
Bromide	2	99.9	2	91.6	2	97.0	2	100.2
Nitrate	6	94.0	25	104.6	2	87.5	2	93.9
Phosphate	5	86.1	5	89.7	5	98.5	5	97.2
Sulfate	5	94.4	15	99.5	10	96.3	6	100.0
Analyte	**River Water Lech**		**Lake Water Deutensee**		**Stream Water Staltannen**		**Fish Pond Water Pitzling**	
	Amount added (mg/L)	**Recovery (%)**	**Amount added (mg/L)**	**Recovery (%)**	**Amount added (mg/L)**	**Recovery (%)**	**Amount added (mg/L)**	**Recovery (%)**
Fluoride	0.5	97.2	0.5	98.2	0.5	95.0	0.5	95.2
Chloride	3	86.0	2	84.4	1	82.1	5	89.1
Nitrite	2	91.8	2	92.5	2	91.9	2	90.4
Bromide	2	98.0	2	97.7	2	97.0	2	102.3
Nitrate	2	92.2	2	90.2	3	86.4	(40)	(108.6)
Phosphate	5	99.4	5	96.2	5	95.3	5	87.9
Sulfate	20	102.8	2	91.7	2	95.7	15	98.8

Additionally, a Laboratory Blank is fortified at approximately the highest concentration of each anion found in the samples analyzed during the validation (Laboratory Fortified Blank, LFB). Table 12 summarizes the recoveries 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	116.8	0.5	98.5
Chloride	20	92.3	20	93.2
Nitrite	2	89.6	2	103.0
Bromide	2	98.6	2	96.5
Nitrate	30	97.5	40	100.5
Phosphate	5	89.6	5	89.2
Sulfate	20	96.8	20	96.0

The specification for the recoveries given in EPA Method 300.1 is ±25% for the LFM and LFB, or ±15% for the LFB, if the fortification level of the LFB is >10x MRL.[7] The LFM and LFB results indicate, that for almost all matrices, the recoveries were within the acceptable range of ±25%. The LFB shows recovery results of 90-117%, which, apart from fluoride for the A07, is within the limit of ±15% recovery. As the fortification level of Fluoride is bellow 10x MRL however, a limit of ±25% applies and it is therefore also within the specified range. The only exception for low recovery is the phosphate value found for the Steingaden drinking water. The result was verified by a duplicate analysis, so that a chromatographic error leading to wrong analysis results can be excluded.

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)

Peaks:	1. Fluoride	0.06 (< MRL)	0.53 mg/L
	2. Chloride	1.7	3.2
	3. Nitrite	–	1.8
	4. Bromide	–	1.9
	5. Nitrate	0.05 (< MRL)	0.9
	6. Phosphate	–	4.8
	7. Sulfate	0.06 (< MRL)	1.1

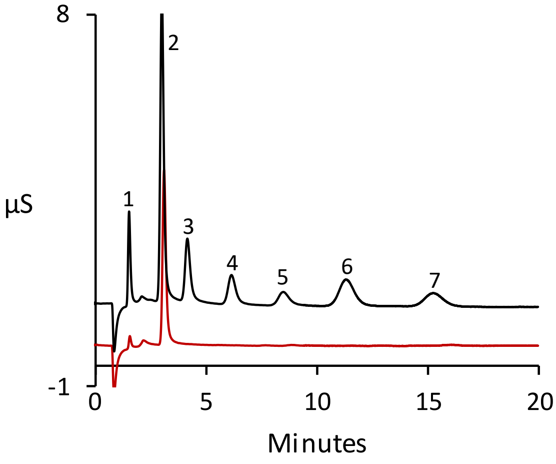

Figure 8. Determination of anions in spiked (black) and unspiked (red) water samples of Deutensee using the Sykam A07.

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: 1.5 mL/min
Inj. Vol.: 50 µL
Detection: Suppressed Conductivity, Sykam Chemical Anion Suppressor (80 rpm)

Peaks:	1. Fluoride	0.05	0.59 mg/L
	2. Chloride	16.0	34.8
	3. Nitrite	–	2.0
	4. Bromide	–	2.0
	5. Nitrate	1.6	3.4
	6. Phosphate	–	4.9
	7. Sulfate	5.9	11.3

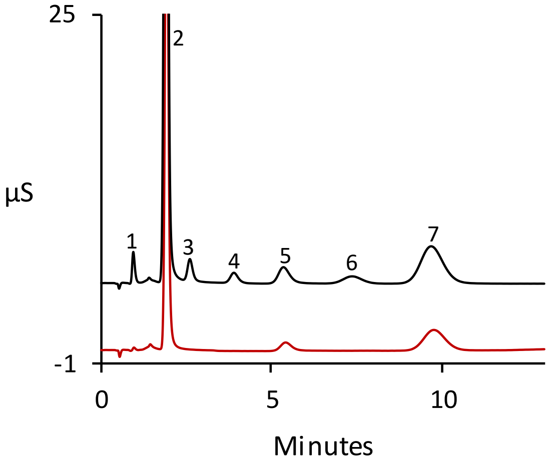

Figure 9. Determination of anions in spiked (black) and unspiked (red) water samples of mineral water 2 using the Sykam A08.

This indicates that the low phosphate recovery found here might be based on matrix effects. Also, the lake water from Deutensee shows lower recoveries for all anions compared to the other natural water samples, which could be caused by a relatively high content of humic acids, since the lake is fed with water from the surrounding moorlands. The recovery of nitrate in the fish pond water sample was determined, but should not be evaluated, as the concentration of the fortified sample (around 80 mg/L) is above the highest calibration point of 50 mg/L.

Summary

The Sykam A07 and A08 column 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 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 A07 as well as the Sykam 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 of up to 100 mg/L of chloride or other inorganic anions without a time-consuming dilution step.

The Sykam A07 shows a high resolution for all examined standard anions, making it suitable for the analyses of complex matrices, even if closely eluting peak pairs (e.g. chloride and nitrite) with large concentration differences are determined or if the focus is on the analysis of all seven standard anions. The Sykam A08 is a column with shorter retention times for all anions, which is advantageous for time saving analyses, especially if the focus is on the analysis of only a few selected anions like chloride, nitrate and sulfate.

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).

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