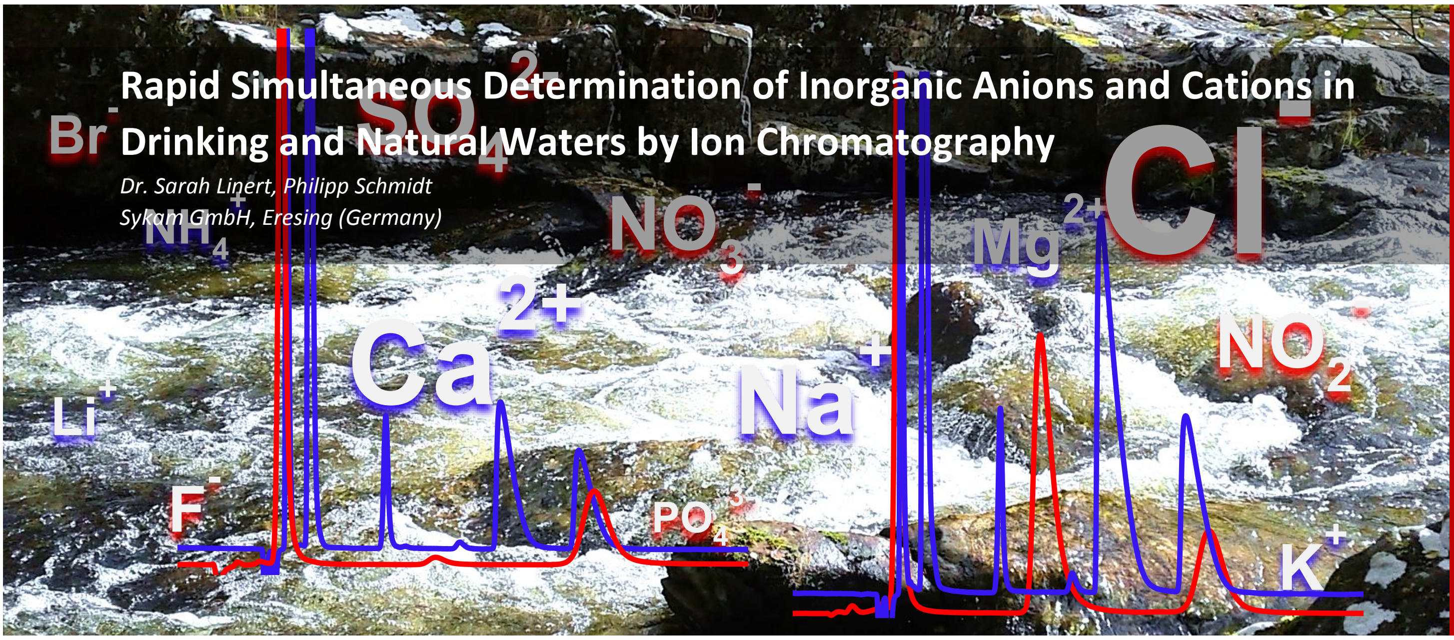

Rapid Simultaneous Determination of Inorganic Anions and Cations in Drinking and Natural Waters by Ion Chromatography

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Sykam Application Note 04

Introduction

The impact humans have on the planet is ever growing. Whether climate change or pollution, the consequences for the environment, caused by continuing industrial growth, are undeniable. With an increasing population, the need to monitor our negative influence on the planet grows, both for protection of the environment from pollution and to prevent possible health hazards. As one of our most precious resources, drinking water requires special attention. Therefore, many countries around the globe have implemented regulations to ensure the quality of water, both to protect the ecosystem and the population.

Amongst these are the European Union's Drinking Water Directive (DWD) 98/83[1] (1998, latest version 2020/2184 published in December 2020)[2] and the Safe Drinking Water Act (SDWA) established in the United States in 1974.[3] Both these regulations aim to secure the quality of drinking water for human consumption, to protect human health from adverse effects caused by the contamination of water. Likewise, the Water Framework Directive (2000/60/EC) of the EU, forms the basis to preserve surface, transitional, coastal and groundwaters in order to maintain and enhance the status of aquatic ecosystems,[4] while in the U.S., the Clean Water Act (CWA) aims to reduce the amount of pollutants released into the environment.[5]

These pollutants are sorted into primary and secondary contaminants and are regulated in the U.S. National Primary and Secondary Drinking Water Standard (NPDWS/NSDWS)[6] and the EU DWD[2] by defining Maximum Contaminant Levels (MCL). In the U.S., fluoride, nitrite and nitrate are considered primary contaminants, while chloride and sulfate are categorized as secondary contaminants.[6] In the EU, these inorganic ions are also regulated by the Drinking Water Directive.[2] Additionally, naturally occurring common cations in drinking water are also regulated, and reported by most suppliers in the EU, in contrast to the U.S., where they are not considered primary contaminants.[2,6] In the EU, magnesium and calcium are measured to determine the hardness of water. Similarly to the U.S. and the EU, most parts of the world have passed their own regulations to protect and monitor drinking and natural waters, which are often based on the aforementioned regulations.

Many national and international standardization organizations have defined regulations and methods for the analysis of drinking and natural waters, amongst them the International Organization for Standardization (ISO), the American Society for Testing and Materials (ASTM), the American Water Works Association (AWWA) and the United States Environmental Protection Agency (EPA).

Ion chromatography (IC) has been validated and defined as the standard method for monitoring the quantity of ionic species in drinking and natural waters by these organizations. As such, a number of regulatory IC methods for the analysis of ions in drinking and natural waters have been published worldwide. The methods for the determination of anions include the EN ISO 10304-1[7] (Technical Committee ISO/TC 147 "Water quality"), the EPA 300.1[8] or the ASTM D4327.[9] Likewise, the methods for analysis of cations using IC are described in ASTM D6919[10] and EN ISO 14911.[11] These allow the use of IC for the determination of alkaline and alkaline earth metals alongside ammonium in a single run.[10,11]

The above-mentioned methods define quality control parameters and measuring conditions, but also allow the use of optional columns and corresponding, different chromatographic conditions to improve the separations or lower the costs of measurement.[7-9] A major factor of measurement cost, is the run time of a method. With growing population and industry around the world, more and more samples need to be measured to ensure the continued quality of drinking and natural waters. Shorter run times not only allow more samples to be measured faster, but also consume less reagents and electricity to do so and reduce the stress put on analytical equipment increasing their lifespan.

The method described in this application note “Sykam AN04” was developed precisely to fulfill the need for fast, yet reliable analysis of drinking and natural waters. It describes the rapid, simultaneous determination of inorganic anions and cations in water samples by IC using suppressed conductivity measurement for anion determination and direct conductivity measurement for cation determination. The method was validated under consideration of the requirements described in the abovementioned methods for anion and cation analysis and therefore provides an economical way to meet the testing requirements of EN ISO 10304-1:2009-07,[7] EPA 300.1, Revision 1.0[8] and ASTM D4327-17[9] as well as ASTM D6919[10] and EN ISO 14911[11] using a Sykam S153 Dual IC system. The columns Sykam A09 (75 x 2.6 mm) and Sykam C01 (125 x 4.6 mm) were used for the method validation and the validation results are discussed within this application note.

Equipment

Application note Sykam AN04 is designed for the use of a Sykam S153-AG Dual IC system with dual-channel anion and cation detection, consisting of the following components:

- S150 Ion Chromatography Module including column oven, dual-channel conductivity detector and chemical anion suppressor module
- S1130 Quaternary Gradient Pump (PEEK) including 4-channel degasser
- S1130 Isocratic Pump (PEEK) including 1-channel degasser
- S5300 Automatic Sample Injector with S6165 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) or alternative data processing software.

The use of a second S1130 isocratic pump instead of an S1130 gradient pump is also possible.

Reagents and Standards

All reagents for the preparation of eluents 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 of comparable or better purity can be used.

- 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)
- 2,6-Pyridinedicarboxylic acid ($C_7H_5NO_4$, 99%), Sigma-Aldrich (M0375)
- Maleic acid ($C_4H_4O_4$, ReagentPlus®, ≥99% (HPLC)), Sigma-Aldrich (P63808)
- Fluoride standard solution (traceable to SRM from NIST, NaF in H_2O, 1000 mg/L F, Certipur®), Merck (1.19814)
- Chloride standard solution (traceable to SRM from NIST, NaCl in H_2O, 1000 mg/L Cl, Certipur®), Merck (1.19897)
- Nitrite standard solution (traceable to SRM from NIST, $NaNO_2$ in H_2O, 1000 mg/L NO_2, Certipur®), Merck (1.19899)
- Bromide standard solution (traceable to SRM from NIST, NaBr in H_2O, 1000 mg/L Br, Certipur®), Merck (1.19896)
- Nitrate standard solution (traceable to SRM from NIST, $NaNO_3$ in H_2O, 1000 mg/L NO_3, Certipur®), Merck (1.19811)
- Phosphate standard solution (traceable to SRM from NIST, KH_2PO_4 in H_2O, 1000 mg/L PO_4, Certipur®), Merck (1.19898)
- Sulfate standard solution (traceable to SRM from NIST, Na_2SO_4 in H_2O, 1000 mg/L SO_4, Certipur®), Merck (1.19813)
- Lithium standard solution (traceable to SRM from NIST, $LiNO_3$ in 0.5 mol/L HNO_3, 1000 mg/L Li, Certipur®), Merck (1.70223)
- Sodium standard solution (traceable to SRM from NIST, $NaNO_3$ in H_2O, 1000 mg/L Na, Certipur®), Merck (1.19507)
- Ammonium standard solution (traceable to SRM from NIST, NH_4Cl in H_2O, 1000 mg/L NH_4, Certipur®), Merck (1.19812)
- Potassium standard solution (traceable to SRM from NIST, KNO_3 in 0.5 mol/L HNO_3, 1000 mg/L K, Certipur®), Merck (1.70230)
- Calcium standard solution (traceable to SRM from NIST, $Ca(NO_3)_2$ in 0.5 mol/L HNO_3, 1000 mg/L Ca, Certipur®), Merck (1.19778)
- Magnesium standard solution (traceable to SRM from NIST, $Mg(NO_3)_2$ in 0.5 mol/L HNO_3, 1000 mg/L Mg, Certipur®), Merck (1.19788)

If the 1000 mg/L standard solutions are freshly prepared, we recommend the use of the following analytical or ACS reagent grade chemicals. Alternatively, other chemicals of comparable or better purity can be used. If possible, anhydrous salts should be used for the preparation of standard solutions, since they can be dried in a vacuum oven if necessary. We recommend the use of sodium or potassium salts for the preparation of anion standard solutions, while chlorides or nitrates are the preferred salts for preparation of cation standard solutions.

- 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)
- Lithium nitrate ($LiNO_3$, anhydrous, extra pure, SLR), Fisher Chemical (10568620)
- Ammonium chloride (NH_4Cl, Suprapur®), Merck (1.01143)
- Potassium nitrate (KNO_3, ACS reagent, ≥99.0%), Sigma-Aldrich (221295)
- Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4\,H_2O$, for analysis EMSURE, ACS, ISO, Reag. Ph Eur), Merck (1.02121)
- Magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6\,H_2O$, ACS reagent, 99%), Sigma-Aldrich (237175)

Samples

For the method validation, six water samples were analyzed (Table 1).

Table 1. List of analyzed samples.

No.	Name
1	Drinking Water (Türkheim)
2	Drinking Water (Milbertshofen)
3	Mineral Water 1
4	Mineral Water 2
5	Lake Water (Wörthsee)
6	Stream Water (Seefeld)

All six water samples were collected in southern Germany in the northern foothills of the Alps, where lime containing soil is the prevailing geological formation. The two drinking water samples were collected as tap water from two different municipal water suppliers in Türkheim and Milbertshofen. The two mineral water samples were commercially available mineral waters from companies, that obtain their water from natural ground water sources. The lake water was collected from the Wörthsee and the stream water was collected from a stream in the Schloßpark of Seefeld. All samples were collected in 1000 mL glass or PE-bottles with a screw cap and stored at 4 °C immediately after collection.

Chromatographic Conditions

Method Validation Anions

Columns:	Sykam A09 (75 x 2.6 mm), Analytical column Sykam AGC-05 (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:	9 min
Temperature:	35 °C
Injection Volume:	50 µL (full loop)
Detection:	Suppressed Conductivity, Sykam Chemical Anion Suppressor
Suppressor Speed:	80 rpm
Backpressure:	151 bar (2190 psi)
Base Conductivity:	19 µS/cm
Noise:	<5 nS/cm

Method Validation Cations

Columns:	Sykam C01 (125 x 4.6 mm), Analytical column Sykam CGC-01 (20 x 3.0 mm), Guard column
Eluent:	2.0 mM 2,6-Pyridine-dicarboxylic acid, 2.8 mM Maleic acid
Flow Rate:	1.0 mL/min
Run Time:	9 min
Temperature:	35 °C
Injection Volume:	10 µL (full loop)
Detection:	Non-suppressed Conductivity (Inverted Signal)
Backpressure:	58 bar (840 psi)
Base Conductivity:	890 µS/cm
Noise:	<50 nS/cm

All settings listed here are part of the chromatographic method and can be saved in the "Method Setup" window of the Clarity software. Since cation detection is non-suppressed, the cation signal needs to be inverted by clicking the box "Reverse Signal" in the "Acquisition" Tab of the "Method Setup" window to obtain positive peaks for integration.

To achieve reproducible results, only full loop injections were performed. In case of the dual analysis, both injection loops are connected in series, which means that an injection volume of at least 100 µL needs to be programmed in the sequence to fill both loops with 50% excess.

Preparation of Solutions and Reagents

Standard Stock Solutions (1000 mg/L)

The standard stock solutions of the seven standard anions fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulfate, and the six standard cations lithium, sodium, ammonium, potassium, calcium and magnesium can either be purchased from commercial sources as indicated in section "Reagents and Standards" or prepared from the respective salts, 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. Should only a few, and not all, of the standard ions need to be analyzed, only the respective standard solutions of these ions are needed. The 1000 mg/L standard stock solutions prepared from the salts are stable for at least one month.

Table 2. Amounts of salts needed for preparation of 1000 mL 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
Lithium	Lithium nitrate ($LiNO_3$)	9.933
Sodium	Sodium nitrate ($NaNO_3$)	3.697
Ammonium	Ammonium chloride (NH_4Cl)	2.965
Potassium	Potassium nitrate (KNO_3)	2.586
Calcium	Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4\ H_2O$)	5.892
Magnesium	Magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6\ H_2O$)	10.547

Working Standard Solutions

All single and mixed 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, 5, 2 and 1 µg/L of each anion is prepared from 10 mg/L single standard solutions for the preliminary estimation of the method detection limit. For the estimation of the method detection limits of the cations a series of mixed standards at concentrations of 500, 200, 100, 50, 20, 10, 5, 2 and 1 µg/L of each cation is prepared from 10 mg/L single standard solutions.

Table 3 gives the concentrations of the standards prepared to calculate the method detection limits (MDL_S) as well as the concentrations of the Quality Control Samples (QCS), which are analyzed to determine retention time stability and peak area precision of the system.

The determination of the linear calibration range of the seven standard anions covering the expected concentrations of the drinking and natural water samples is achieved by injecting the following twelve concentration levels of a mixed anion standard: 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 50, 75 and 100 mg/L (0.01 – 20 mg/L for fluoride). All concentration levels are prepared from the 1000 mg/L standard stock solutions.

For the determination of the linear calibration range of the six standard cations, covering the expected concentrations of the drinking and natural water samples, the following twelve concentration levels of a mixed cation standard were injected: 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 50, 75 and 100 mg/L (0.01 – 20 mg/L for lithium). All concentration levels are prepared from the 1000 mg/L standard stock solutions.

Table 3. Concentrations of MDL_S, QCS and 100% linearity standard.

Analyte	MDL_S Calculation Standard Anions (µg/L)	QCS for Precision Anions (mg/L)	Maximum Standard Concentration Anions (mg/L)
Fluoride	50	2	20
Chloride	10	10	100
Nitrite	20	2	100
Bromide	50	2	100
Nitrate	50	20	100
Phosphate	150	2	100
Sulfate	100	30	100
Analyte	**MDL_S Calculation Standard Cations (µg/L)**	**QCS for Precision Cations (mg/L)**	**Maximum Standard Concentration Cations (mg/L)**
Lithium	75	2	20
Sodium	250	15	100
Ammonium	250	5	100
Potassium	750	10	100
Calcium	750	40	100
Magnesium	500	20	100

To assess the performance of the chromatographic system, External Control Samples (ECS) are 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

The eluents are prepared from eluent stock solutions with concentrations of 1.00 mol/L.

For the sodium carbonate stock solution 53.00 g of Na_2CO_3 are dissolved in 400 mL of deionized water in a 500 mL volumetric flask. The solution is mixed thoroughly until completely clear and the flask is filled up to the mark once the solution has reached room temperature.

For the sodium thiocyanate stock solution 4.054 g of NaSCN are dissolved in 40 mL of deionized water in a 50 mL volumetric flask in the same manner, and the flask is filled to the mark.

For the chromatographic system discussed here, the following eluents are prepared:

Anions (Sykam A09): 3.5 mM Na_2CO_3, 25 µM NaSCN – 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 are added to 2000 mL of deionized water. The solution is mixed thoroughly and filtered over a 0.45 µm regenerated cellulose filter disc. The eluent is transferred to the S7150 Reagent Organizer and the container is pressurize with inert gas (optional).

Cations (Sykam C07): 2.0 mM 2,6-pyridine-dicarboxylic acid, 2.8 mM maleic acid – 668.4 mg of 2,6-pyridinedicarboxylic acid and 650.0 mg of maleic acid are added to 2000 mL of deionized water. The solution is mixed thoroughly until the solid is dissolved completely. Then it is filtered over a 0.45 µm regenerated cellulose filter disc. The eluent is transferred to the S7150 Reagent Organizer and the container is pressurize with inert gas (optional).

Preparation of the IC system

If new columns are used, install the columns and condition them according to the procedure described in the respective column data sheet, before slowly increasing the flow rate to the desired value of 1.2 mL/min (A09) and 1.0 mL/min (C01). To achieve reproducible results, the system and especially the chemical suppressor unit, have to warm up and 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 <5.0 nS/cm for the anion channel and <50 nS/cm for the cation channel. 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 (0.05 mL/min), so that the system can readily be used for the following analyses. The suppressor speed can be reduced to 20 rpm overnight. Make sure, that the deionized water for washing of the automatic sample injector is sufficiently filled 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.

Sample Preparation

All drinking and natural water samples were degassed under vacuum for approx. 10 minutes prior to analysis. In the laboratory the samples were divided into an initial sample and a laboratory duplicate. The duplicate sample was treated identical to the initial sample to control the precision of laboratory procedures. Samples must 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, which may change the analyzed 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),[8] as well as ISO EN 10304-1:2009-07[7] and ASTM D4327-17[9] ensuring that all requirements of these methods regarding quality control parameters are met. As defined by the methods, optional columns and corresponding, different chromatographic conditions may be used to improve separation or lower the cost of measurement. Here, the Sykam A09 column together with the Sykam S153-AG Dual IC module under the chromatographic conditions listed above were used for the validation. The following quality control parameters were tested:

- Method detection limits MDL_S and MDL_B[12]
- Linearity (Linear Calibration Range, LCR),[13]
- 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 duplicates
- Assessment of analyte recovery by injection of Laboratory Fortified Matrices (LFM) for each sample.

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

The Sykam A09 is a high-capacity anion exchange column based on trimethylammonium functionalized PS-DVB, with a particle size of 5 µm. Figure 1 shows the separation of a low-mg/L inorganic anion standard using the Sykam A09.

Column: Sykam A09 (75 x 2.6 mm) and Sykam AGC-05 (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, 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

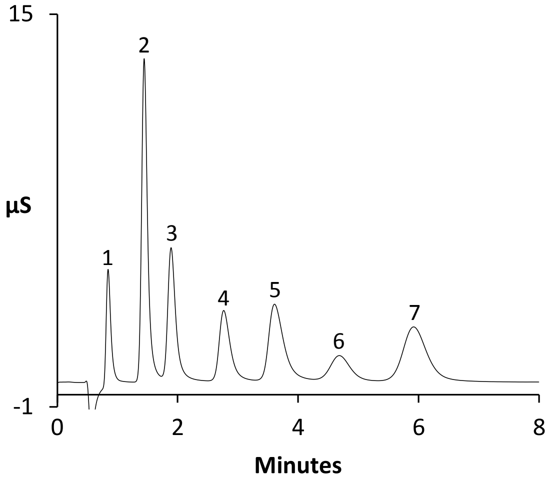

Figure 1. Separation of the 5% calibration standard for anions.

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.[7] The column exhibits excellent peak symmetry, which allows exact integration of peak areas, as all peaks are baseline-separated.

Table 4. Column performance parameters (5% calibration standard) of Sykam A09 and Sykam C01 at the chromatographic conditions listed above.

Analyte	Retention time (min)	Resolution R	Peak Symmetry
Fluoride	0.84	–	1.76
Chloride	1.44	3.79	1.36
Nitrite	1.89	2.21	1.44
Bromide	2.76	3.14	1.45
Nitrate	3.61	2.20	1.52
Phosphate	4.68	2.09	1.16
Sulfate	5.92	1.89	1.25
Lithium	2.54	–	1.19
Sodium	2.93	3.54	1.30
Ammonium	3.20	2.20	1.39
Potassium	3.96	4.50	1.74
Calcium	4.78	3.20	1.57
Magnesium	5.81	2.98	1.84

The method validation for the determination of cations in drinking and natural waters was performed according to the regulations listed in ASTM D6919[10] and EN ISO 14911.[11] The Sykam C01 is a weak cation exchange column based on polybutadiene-maleic acid coated spherical silica with a particle size of 5 µm. Figure 2 shows the separation of an inorganic cation standard using the Sykam C01.

Column: Sykam C01 (125 x 4.6 mm) and Sykam CGC-01 (20 x 3.0 mm)
Eluent: 2.0 mM 2,6-Pyridine-dicarboxylic acid, 2.8 mM Maleic acid
Flow Rate: 1.0 mL/min
Inj. Vol.: 10 µL
Detection: Non-suppressed Conductivity
Peaks: 1. Lithium 1.0 mg/L
2. Sodium 5.0
3. Ammonium 5.0
4. Potassium 5.0
5. Calcium 5.0
6. Magnesium 5.0

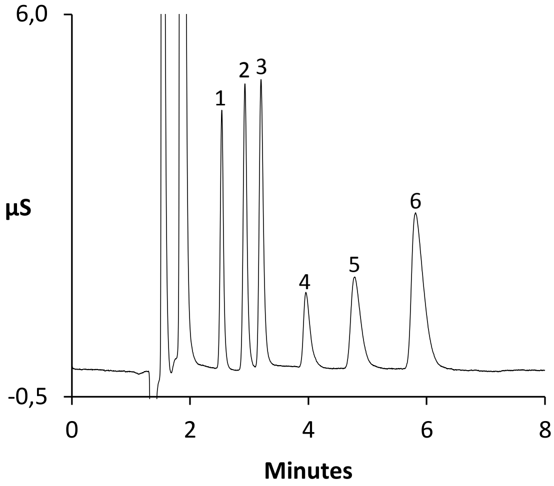

Figure 2. Separation of the 5% calibration standard for cations.

Method Detection Limits MDL_S and MDL_B

First the method detection limits based on sample injection (MDL_S) were estimated. For this purpose, eight mixed anion standards with the following concentrations were injected: 200, 100, 50, 20, 10, 5, 2 and 1 µg/L for each anion. The same procedure was used to determine the MDL_S of the cations, injecting nine mixed cation standards with concentrations of 500, 200, 100, 50, 20, 10, 5, 2 and 1 µg/L per cation. For each ion 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 of the MDL_S-Calculation standards (Table 3). The MDL_S for each ion was determined by performing seven replicate injections of the MDL_S-Calculation standards for anions and cations respectively. The MDL_S was then calculated as $MDL_S = t \times SD$, where t is the Student´s value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t = 3.142613 for seven replicates) and SD is the standard deviation of the replicate analysis of the MDL_S-Calculation standard for each ion. The thus obtained method detection limits are listed in Tables 5 and 6 for the anions and cations 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 ions and is calculated as: $MDL_B = X + t \times SD$, where X is the mean of the LRB results.[12] t is again 3.142613 for seven replicates and SD is the standard deviation of the replicate analysis of the LRB. In this case, seven replicates of the LRB were analyzed with the result that none of the inorganic ions were found in any of the LRB injections.

Linearity (LCR)

To assess the linear calibration range for each ion, mixed anion standards and mixed cation standards at twelve calibration levels each were injected: 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 50, 75 and 100 mg/L. For fluoride and lithium, the calibration range was determined to 0.01–20 mg/L, which is sufficiently high, since both these ions are usually contained in trace amounts of <1.0 mg/L in most drinking and natural water samples. For quantification of the samples and the fortified samples, the calibration range was limited to its linear range for each ion. Therefore, only the calibration levels, that were found to be in a linear range, were used to assess the calibration curve individually for each ion. Figure 3 shows the linear calibration plots for all ions. Calibration points, which are outside the linear range, were omitted and are displayed in red. Nitrite exhibits a linear range of up to 50 mg/L, whereas bromide, nitrate, phosphate and sulfate were calibrated up to a maximum calibration level of 20 mg/L. Fluoride was only calibrated up to 10 mg/L. Sodium and ammonium exhibit a linear calibration range of 0.05–75 mg/L. At concentrations higher than 75 mg/L of both these cations, sodium and ammonium are not resolved sufficiently anymore, which causes too low responses. Magnesium was calibrated in a concentration range of 0.05–100 mg/L. The calibration range of potassium and calcium covers the range of 0.1–100 mg/L. For lithium a small concentration range of 0.01–20 mg/L was used. In tables 5 and 6 the linear calibration ranges, as well as the correlation factors r^2 of the linear fits for all ions are listed. All linear fits exhibit correlation factors of $r^2 \geq 0.999$ and are therefore considered very accurate.

Table 5. Linearity, MDL_S, retention time and peak area precision for the standard anions.

Analyte	Calibration range (mg/L)	Linearity (r^2)	Calculated MDL_S (µg/L)	Retention Time Precision (RSD, %)	Peak Area Precision (RSD, %)
Fluoride	0.01 – 10	1.0000	2.7	0.62	0.29
Chloride	0.05 – 20	0.9990	2.3	0.33	0.18
Nitrite	0.05 – 50	1.0000	2.1	0.22	0.22
Bromide	0.05 – 20	0.9996	4.3	0.17	0.35
Nitrate	0.05 – 20	0.9995	8.8	0.16	0.23
Phosphate	0.05 – 20	0.9995	21.3	0.13	0.97
Sulfate	0.05 – 20	0.9997	17.5	0.10	0.79

Table 6. Linearity, MDL_S, retention time and peak area precision for the standard cations.

Analyte	Calibration range (mg/L)	Linearity (r^2)	Calculated MDL_S (µg/L)	Retention Time Precision (RSD, %)	Peak Area Precision (RSD, %)
Lithium	0.01 – 20	1.0000	7.5	0.13	0.59
Sodium	0.05 – 75	1.0000	21.7	0.11	0.60
Ammonium	0.05 – 75	1.0000	12.2	0.11	0.81
Potassium	0.1 – 100	1.0000	25.4	0.09	1.36
Calcium	0.1 – 100	1.0000	78.1	0.09	0.77
Magnesium	0.05 – 100	1.0000	31.0	0.09	0.43

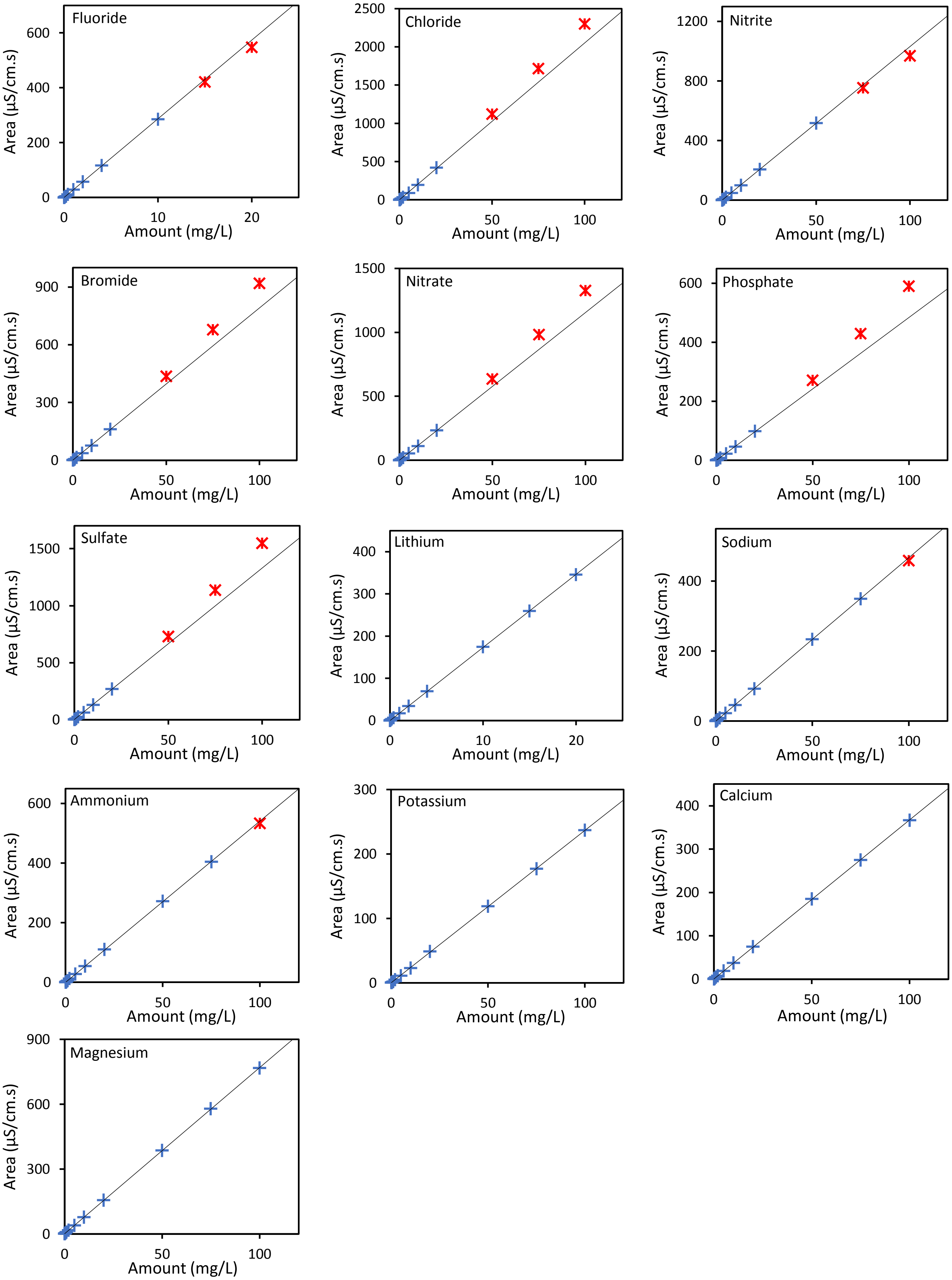

Figure 3. Calibration plots of the standard anions and cations indicating the LCR used for analysis of the samples.

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 concentration of each individual inorganic ion in the QCS is within the same range as found in the field samples. The concentrations are listed 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 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 all investigated ions, illustrating the high precision reached with the Sykam S153-AG Dual IC system using the Sykam A09 and Sykam C01 columns. If no new calibration is performed prior to analysis, the calibration standards and acceptable instrument performance are verified by the preparation and analysis of a QCS. In this case, a new calibration was performed and the instrument performance was verified by analysis of an External Calibration Standard (ECS). The ECS is prepared from 1000 mg/L single ion standard stock solutions, which are prepared from a different source than the standard stock solutions used for the calibration. The QCS, however is prepared from the same standard stock solutions that were used for the preparation of the calibration standards. If used for verification of instrument performance, the acceptance range for the Relative Percent Differences (RPD) between the ECS and the QCS is ±15%.[8] In table 7 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 are listed. The determined differences are well within the specified deviation range of ±15%.[8]

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

Analyte	RPD (%)
Fluoride	3.2
Chloride	0.7
Nitrite	2.1
Bromide	1.1
Nitrate	-0.9
Phosphate	3.7
Sulfate	0.0
Lithium	1.3
Sodium	-1.3
Ammonium	2.8
Potassium	-3.4
Calcium	-2.1
Magnesium	5.0

Sample Analysis

All samples (Table 1) were analyzed twice, undiluted after filtration, each as initial sample and laboratory duplicate. The nitrate and calcium contents of the drinking water sample from Türkheim exceeded the highest calibration level. Therefore, the sample was diluted in a 1:1 ratio with deionized water and the analysis was repeated with the diluted sample to obtain valid results. Tables 10 and 11 give an overview of the analysis results for the six drinking and natural water samples, listing the mean value of the duplicate analysis and their Relative Percent Difference (RPD). The amounts of ions given for the drinking water sample from Türkheim were obtained from the diluted sample and multiplied by two to calculate the total content in the undiluted sample.

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

Analyte	MRL (mg/L)
Fluoride	0.01
Chloride	0.05
Nitrite	0.05
Bromide	0.05
Nitrate	0.05
Phosphate	0.05
Sulfate	0.05
Lithium	0.01
Sodium	0.05
Ammonium	0.05
Potassium	0.1
Calcium	0.1
Magnesium	0.05

Nitrite was detected in none of the six drinking and natural water samples, while bromide, was found only at very low concentrations below the Minimum Reporting Level (MRL). The MRL is defined as the lowest concentration, at which an analyte can be quantified. The MRL is higher or equal to the lowest calibration concentration and higher than the MDL.[8] Table 8 gives an overview of the MRLs for each ion based on the applied linear calibration range and the calculated MDLs.

Phosphate was only found in mineral water 2 in small amounts of 0.11 mg/L. Fluoride was found in all samples in low contents of 0.051–0.16 mg/L. Nitrate was found at a relatively high concentration of 24.9 mg/L in drinking water sample 1, while significantly lower amounts ranging from 0.61–6.3 mg/L were found in the other samples. The concentrations of sulfate (9.0–14.8 mg/L) and chloride (10.3–15.5 mg/L) were in a similar range for all six water samples.

Lithium and ammonium were not detected in most of the drinking and natural water samples and only at low concentrations near or below the MRL in the other samples. Potassium was only found at low concentrations of 1.1–3.1 mg/L. The contents of sodium range from 6.6–16.8 mg/L, and magnesium was found in varying amounts of 3.7–28.2 mg/L. The detected concentrations of calcium cover a wide range of 41.9 mg/L to 112.2 mg/L.

Table 9 summarizes the measurement results for mineral waters 1 and 2. The values are compared to the supplier's analysis results printed on the bottle label of each mineral water. For both mineral water samples, the determined values correspond well with the analysis results given by the suppliers as shown in table 9.

Column: Sykam A09 (75 x 2.6 mm) and Sykam AGC-05 (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, Sykam Chemical Anion Suppressor (80 rpm)
Peaks: 1. Fluoride 0.051 mg/L
2. Chloride 12.5
3. Bromide <MRL
4. Nitrate 6.3
5. Sulfate 12.2

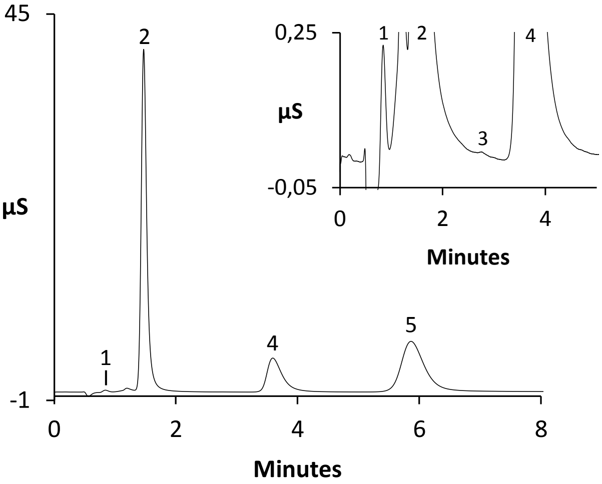

Figure 4. Determination of anions in drinking water (Milbertshofen).

Column: Sykam C01 (125 x 4.6 mm) and Sykam CGC-01 (20 x 3.0 mm)
Eluent: 2.0 mM 2,6-Pyridine-dicarboxylic acid, 2.8 mM Maleic acid
Flow Rate: 1.0 mL/min
Inj. Vol.: 10 µL
Detection: Non-suppressed Conductivity
Peaks: 1. Sodium 4.9 mg/L
2. Potassium 1.6
3. Calcium 56.1
4. Magnesium 12.8

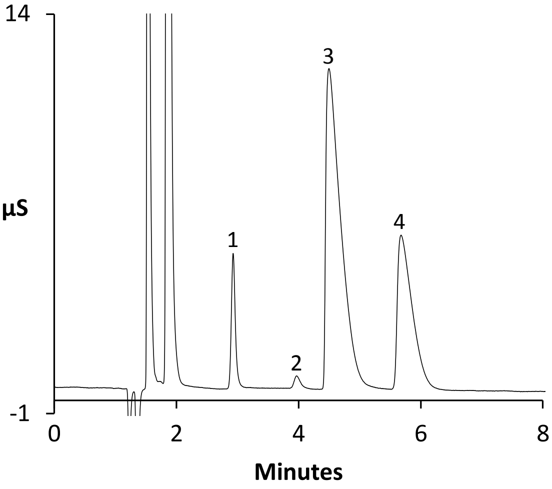

Figure 5. Determination of cations in diluted (1:1) drinking water (Türkheim).

Column: Sykam A09 (75 x 2.6 mm) and Sykam AGC-05 (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, Sykam Chemical Anion Suppressor (80 rpm)
Peaks: 1. Fluoride 0.053 mg/L
2. Chloride 10.9
3. Bromide <MRL
4. Nitrate 3.2
5. Sulfate 14.8

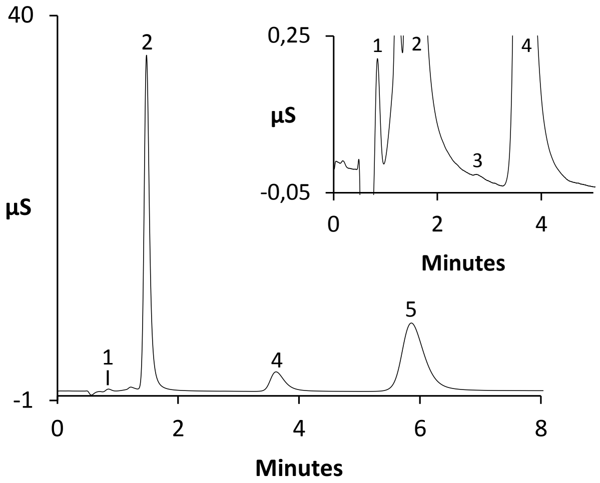

Figure 6. Determination of anions in mineral water 1.

Column: Sykam C01 (125 x 4.6 mm) and Sykam CGC-01 (20 x 3.0 mm)
Eluent: 2.0 mM 2,6-Pyridine-dicarboxylic acid, 2.8 mM Maleic acid
Flow Rate: 1.0 mL/min
Inj. Vol.: 10 µL
Detection: Non-suppressed Conductivity
Peaks: 1. Sodium 8.4 mg/L
2. Ammonium 0.079
3. Potassium 1.5
4. Calcium 41.9
5. Magnesium 14.7

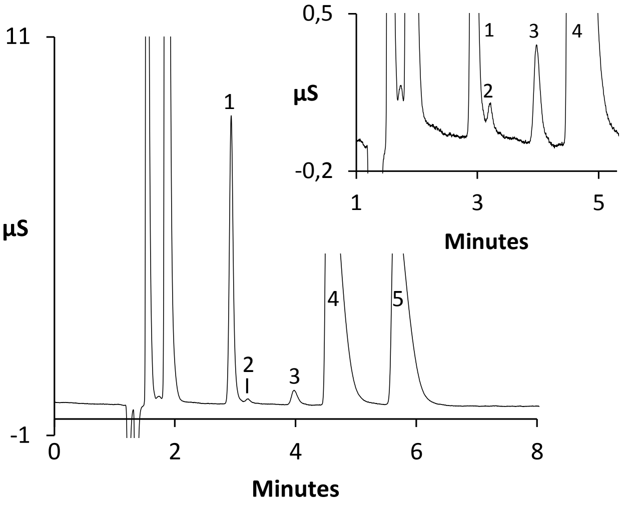

Figure 7. Determination of cations in lake water (Wörthsee).

Table 9. Analysis results during method validation (mg/L) and reported analysis results (mg/L) from mineral water suppliers.

Analyte	Mineral Water 1		Mineral Water 2	
	Method Validation	Supplier	Method Validation	Supplier
Fluoride	0.053	–	0.16	0.17
Chloride	10.9	10	13.5	12
Nitrate	3.2	3.8	0.61	0.7
Sulfate	14.8	14	12.6	10
Sodium	7.0	6.5	16.8	16.4
Potassium	1.1	1	2.1	2.0
Calcium	80.1	80	48.0	47
Magnesium	28.0	26	3.7	3.6

The values found in the laboratory duplicates do not differ significantly from the respective initial samples, which indicates the robustness of the validated method. 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.[8] All duplicate analysis results are found to be well within these limits. The good consistency of both the initial and duplicate samples during the validation, shows that this method is suitable for the analysis of drinking and natural water samples and the reproducibility of the generated results is very high.

Table 10. Analysis results (mg/L) and RPD (%) of duplicate analyses of standard anions.

Analyte	Drinking Water Türkheim	Drinking Water Milbertshofen	Mineral Water 1
Fluoride	0.11 (0.0)	0.051 (-1.9)	0.053 (1.9)
Chloride	10.3 (-0.2)	12.5 (0.3)	10.9 (0.3)
Nitrite	n.d.	n.d.	n.d.
Bromide	<MRL	<MRL	<MRL
Nitrate	24.9 (-0.1)	6.3 (0.0)	3.2 (-0.2)
Phosphate	n.d.	n.d.	n.d.
Sulfate	9.0 (0.0)	12.2 (0.6)	14.8 (0.0)

Analyte	Mineral Water 2	Lake Water Wörthsee	Stream Water Seefeld
Fluoride	0.16 (-0.6)	0.058 (0.0)	0.083 (1.2)
Chloride	13.5 (0.0)	15.5 (-0.7)	12.0 (0.2)
Nitrite	n.d.	n.d.	n.d.
Bromide	<MRL	<MRL	<MRL
Nitrate	0.61 (0.5)	0.74 (1.4)	4.1 (0.8)
Phosphate	0.11 (12.0)	n.d.	n.d.
Sulfate	12.6 (0.0)	9.9 (-0.6)	11.8 (1.3)

Table 11. Analysis results (mg/L) and RPD (%) of duplicate analyses of standard cations.

Analyte	Drinking Water Türkheim	Drinking Water Milbertshofen	Mineral Water 1
Lithium	n.d.	n.d.	<MRL
Sodium	9.9 (-0.2)	6.6 (-1.8)	7.0 (0.4)
Ammonium	n.d.	<MRL	n.d.
Potassium	3.1 (-6.3)	1.4 (8.1)	1.1 (7.2)
Calcium	112.2 (0.1)	82.1 (-0.4)	80.1 (-0.1)
Magnesium	25.5 (-0.3)	22.4 (1.0)	28.0 (0.6)

Analyte	Mineral Water 2	Lake Water Wörthsee	Stream Water Seefeld
Lithium	<MRL	n.d.	n.d.
Sodium	16.8 (-0.4)	8.4 (-0.2)	11.4 (0.4)
Ammonium	n.d.	0.079 (8.9)	<MRL
Potassium	2.1 (8.5)	1.5 (7.0)	1.5 (-6.6)
Calcium	48.0 (0.0)	41.9 (-0.5)	82.5 (0.5)
Magnesium	3.7 (0.2)	14.7 (-0.6)	28.2 (0.6)

To confirm that the calibration was still valid, initial, continuing and end calibration check standards were injected before the first sample injection, during the sequence after ten injections each, and at the end of the sequence. During the validation, the 5% anion calibration standard and the 5% cation calibration standard were employed as check standards. The EPA 300.1 requirement for deviation of the check standard is ±15%.[8] During the validation, the check standards did not differ by more than 3.9% from the corresponding anion calibration standard and 14.3% from the corresponding cation calibration standard respectively. In most cases, the difference was less than 2% for anions and less than 5% for cations. For evaluation, the peak areas of each ion in the calibration standard and the corresponding check standard were directly compared to each other.

Analyte Recovery in Fortified Water Samples

The performance of methods used for environmental analysis are typically validated by single- and multi-operator precision and bias studies on fortified samples (Laboratory Fortified Matrix, LFM). Tables 13 and 14 show the obtained recovery results for single-operator data for the standard inorganic ions, spiked into the drinking and natural water samples. 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 that are found in the unfortified sample for the respective ions. Generally, 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 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, that are not detected or detected below the MRL, is 5x MRL.

Additionally, a Laboratory Blank is fortified at the highest amount added to one of the LFMs for each ion respectively (Laboratory Fortified Blank, LFB). An LFB is prepared for both anions and cations. Table 12 summarizes the recoveries found in the Laboratory Fortified Blanks.

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

Analyte	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	96.2
Chloride	6	90.5
Nitrite	2	95.5
Bromide	2	90.4
Nitrate	7	80.8
Phosphate	5	87.6
Sulfate	10	98.0
Lithium	0.2	96.5
Sodium	17	98.7
Ammonium	2	114.7
Potassium	3	94.8
Calcium	49	96.5
Magnesium	29	96.3

Table 13. Recovery Data from fortified Drinking and Natural Water Samples for standard anions.

Analyte	Drinking Water Türkheim (Diluted 1:1)		Drinking Water Milbertshofen		Mineral Water 1	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	99.4	0.5	95.9	0.5	90.0
Chloride	6	104.0	–	–	–	–
Nitrite	2	91.6	2	91.4	2	85.8
Bromide	2	90.5	2	90.9	2	84.3
Nitrate	–	–	7	84.2	4	79.1
Phosphate	5	83.8	5	82.9	5	92.9
Sulfate	5	99.4	–	–	–	–

Analyte	Mineral Water 2		Lake Water Wörthsee		Stream Water Seefeld	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Fluoride	0.5	106.3	0.5	99.6	0.5	91.3
Chloride	–	–	–	–	–	–
Nitrite	2	91.4	2	87.7	2	92.4
Bromide	2	92.9	2	89.8	2	90.3
Nitrate	2	80.6	2	79.6	5	81.3
Phosphate	5	95.0	5	91.6	5	86.4
Sulfate	–	–	10	103.9	–	–

Table 14. Recovery Data from fortified Drinking and Natural Water Samples for standard cations.

Analyte	Drinking Water Türkheim (Diluted 1:1)		Drinking Water Milbertshofen		Mineral Water 1	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Lithium	0.2	99.5	0.2	100.0	0.2	90.6
Sodium	5	98.3	7	93.4	9	89.8
Ammonium	2	94.8	2	91.4	2	88.6
Potassium	2	93.7	2	83.2	2	90.9
Calcium	–	–	–	–	–	–
Magnesium	13	99.1	23	96.9	28	91.6
Analyte	**Mineral Water 2**		**Lake Water Wörthsee**		**Stream Water Seefeld**	
	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)	Amount added (mg/L)	Recovery (%)
Lithium	0.2	98.3	0.2	96.0	0.2	101.0
Sodium	17	99.2	9	97.0	12	98.1
Ammonium	2	108.1	2	101.4	2	105.6
Potassium	3	88.8	2	86.6	2	95.3
Calcium	49	97.4	42	94.0	–	–
Magnesium	4	108.5	15	97.2	29	97.9

The specification for the recoveries given in EPA Method 300.1 is ±25% for the LFMs and LFB, or ±15% for the LFB, if the fortification level of the LFB is >10x MRL.[8] The LFB shows recovery results of 80–98% for anions, which, apart from the nitrate recovery, is within the specified range. For cations the recoveries in the LFB range from 96–115%. The recoveries of anions for the LFM range from 79–107%, while for cations recoveries of 83–109% are found. The recoveries for all determined ions are within the range of ±25%.

Column: Sykam A09 (75 x 2.6 mm) and Sykam AGC-05 (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, Sykam Chemical Anion Suppressor (80 rpm)

Peaks:		
1. Fluoride	0.083	0.54 mg/L
2. Chloride	12.0	11.8
3. Nitrite	-	1.8
4. Bromide	<MRL	1.8
5. Nitrate	4.1	8.1
6. Phosphate	-	4.3
7. Sulfate	11.8	11.8

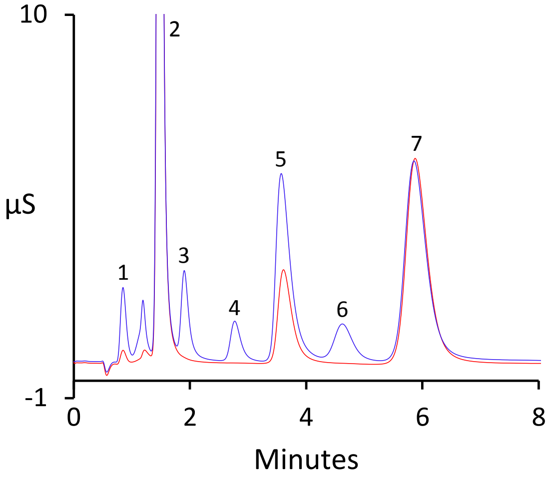

Figure 8. Determination of anions in spiked (blue) and unspiked (red) water samples of stream water (Seefeld).

Column: Sykam C01 (125 x 4.6 mm) and Sykam CGC-01 (20 x 3.0 mm)
Eluent: 2.0 2,6-Pyridine-dicarboxylic acid, 2.8 mM Maleic acid
Flow Rate: 1.0 mL/min
Inj. Vol.: 10 µL
Detection: Non-suppressed Conductivity

Peaks:		
1. Lithium	<MRL	0.20 mg/L
2. Sodium	16.8	32.4
3. Ammonium	-	2.2
4. Potassium	2.1	4.6
5. Calcium	48.0	92.1
6. Magnesium	3.7	7.8

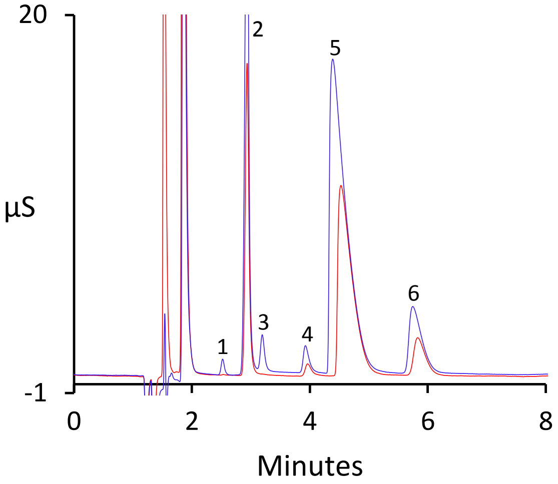

Figure 9. Determination of cations in spiked (blue) and unspiked (red) water samples of mineral water 2.

Summary

The Sykam A09 and C01 columns provide suitable performance for the rapid, simultaneous determination of standard inorganic anions and cations in natural and drinking waters, using a method with a runtime of only 9 minutes. This method complies with U.S. EPA Method 300.1, ASTM D4327 and EN ISO 10304-1 for anions and ASTM D6919 and EN ISO 14911 for cations, as was shown by a full validation in regards to all specifications given in the mentioned guidelines concerning the determination of MDLs, resolution, linearity and precision. Good to very good recovery data for the fortified sample matrices as well as for the fortified blank were obtained under the described chromatographic conditions, especially for cations. For cation analysis, the linearity range of the Sykam C01 column was determined over more than two orders of magnitude, making it possible to analyze samples of up to 100 mg/L of potassium, magnesium or calcium without the need for sample dilution. Despite the short runtime, the Sykam A09 and C01 columns show high resolution of all examined standard ions. This makes them suitable for the analyses of complex matrices or samples with large concentration differences between closely eluting peak pairs. The only exception is in cases, where small amounts of ammonium need to be analyzed alongside high levels of sodium. For this we recommend the use of a longer method, such as the one described in Sykam AN03, using the Sykam C05 column. Additionally, the detection of nitrite alongside large concentrations of chloride may be improved by the use of a UV/Vis detector.

References

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[6] www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations (accessed January 18, 2023).

[7] EN ISO 10304-1:2009: Water quality – Determination of dissolved anions by liquid chromatography of ions – Part 1: Determination of bromide, chloride, fluoride, nitrate, nitrite, phosphate and sulfate.

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[11] EN ISO 14911:1999: Determination of dissolved Li^+, Na^+, NH_4^+, K^+, Mn^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} using ion chromatography – Method for water and waste water

[12] 40 CFR Appendix -B-to-Part-136 – Definition and Procedure for the Determination of the Method Detection Limit – Revision 2.

[13] DIN 38402-51 – German standard methods for the examination of water, waste water and sludge – General information (group A) – Part 51: Calibration of analytical methods – Linear calibration (A 51).

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