

Column manual



Metrosep C 4 (6.1050.XX0)

Manual

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1 General information

This separation column is the standard column for cation analysis. With the Metrosep C 4, not only standard cations but also a large amount of amines as well as transition metals can be determined.

1.1 Ordering information

Table 1 4-mm columns

Order number	Designation
6.1050.450	Metrosep C 4 - 50/4.0
6.1050.410	Metrosep C 4 - 100/4.0
6.1050.420	Metrosep C 4 - 150/4.0
6.1050.430	Metrosep C 4 - 250/4.0

Table 2 2-mm columns

Order number	Designation
6.1050.210	Metrosep C 4 - 100/2.0
6.1050.220	Metrosep C 4 - 150/2.0
6.1050.230	Metrosep C 4 - 250/2.0

Table 3 Guard columns

Order number	Designation
6.1050.500	Metrosep C 4 Guard/4.0
6.1050.510	Metrosep C 4 S-Guard/4.0
6.1050.530	Metrosep C 4 S-Guard - 50/4.0
6.1050.600	Metrosep C 4 Guard/2.0
6.1050.610	Metrosep C 4 S-Guard/2.0



1.2 Technical specifications

Column material Silica gel with carboxyl groups

Particle size 5 µm

Measurements

Order number	Measurements
6.1050.450	50 x 4.0 mm
6.1050.410	100 x 4.0 mm
6.1050.420	150 x 4.0 mm
6.1050.430	250 x 2.0 mm
6.1050.210	100 x 2.0 mm
6.1050.220	150 x 2.0 mm
6.1050.230	250 x 2.0 mm

pH range 2 to 7

Temperature range 20 to 60 °C

Recommended standard temperature 25 °C

Maximum pressure

4 mm	25 MPa (250 bar)
2 mm	25 MPa (250 bar)

Flow rate

Order number	Recommended flow rate	Maximum flow rate
6.1050.450	0.9 mL/min	2.0 mL/min
6.1050.410	0.9 mL/min	2.0 mL/min
6.1050.420	0.9 mL/min	2.0 mL/min
6.1050.430	0.9 mL/min	2.0 mL/min
6.1050.210	0.2 mL/min	0.6 mL/min
6.1050.220	0.2 mL/min	0.6 mL/min
6.1050.230	0.2 mL/min	0.6 mL/min

Standard eluent 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid

Permitted organic additives

In the eluent 0 - 100% acetone and acetonitrile (no alcohol)

In the sample matrix 0 - 100% acetone, acetonitrile and alcohols

<i>Capacity</i>	Order number	Capacity
	6.1050.450	5
	6.1050.410	10
	6.1050.420	15
	6.1050.430	25
	6.1050.210	3
	6.1050.220	4
	6.1050.230	6

Preparation 1. Use a flow gradient to set the column to the standard flow within 2 minutes.
2. Then wait until the baseline is given.

Storage Store the column in standard eluent or ultrapure water.

Typical pressure For columns without a guard column under standard conditions

Order number	Typical pressure
6.1050.450	3.3 ± 2 MPa
6.1050.410	4.9 ± 2 MPa
6.1050.420	7.7 ± 2 MPa
6.1050.430	10.9 ± 2 MPa
6.1050.210	3.2 ± 2 MPa
6.1050.220	5.2 ± 2 MPa
6.1050.230	6.9 ± 2 MPa

Column housing Smart column with a chip, called an iColumn, made of PEEK

Application Determination of standard cations, alkaline metals and alkaline earth metals as well as amines and transition metals in aqueous media.



2 Key aspects of working with separation columns

<i>Storage</i>	Rinse the column with ultrapure water. Once the backpressure in your ion chromatograph has dissipated, remove the column at ambient temperature. Seal the column at both ends using the original stoppers (6.2744.060). Keep them refrigerated at 4 to 8 °C, if possible.
<i>Bacterial growth</i>	<p>Bacterial growth significantly affects chromatography and ruins separation columns. A vast array of problems in chromatography are caused by the growth of algae, bacteria and fungi.</p> <p>In order to prevent bacterial growth, always use fresh eluents, rinsing solutions and regeneration solutions. Do not use any solutions that have not been used for a prolonged period. We recommend cleaning all vessels as follows before filling them:</p> <ol style="list-style-type: none">1. Thoroughly rinse with ultrapure, UV-treated water (> 18.2 MΩ).2. Swirl an acetone-water mixture around in the vessel.3. Rinse again with ultrapure water. <p>If you notice the growth of bacteria or algae despite these precautionary measures, add 5% acetonitrile or acetone to the eluent. Only do this if you are <i>not using a membrane suppressor</i>. Membrane suppressors can be destroyed by organic solvents. The Metrohm Suppressor Modules ("MSM", "MSM-HC" and "MSM-LC") are 100% solvent-resistant.</p>
<i>Chemical quality</i>	All chemicals must have at least a quality of p.a. or puriss. Standard solutions must be intended specifically for ion chromatography.
<i>Chemical stress</i>	Even though specifications may indicate that separation phases do cover a large pH range, this does not mean they are chemically inert. Separation columns last longest when subjected to constant chemical conditions. Never allow a column to dry out and ensure the column is sealed well at all times.
<i>Eluent bottles</i>	The eluents are usually placed directly on the IC system in special eluent bottles. The bottles must feature an adsorber tube in order to prevent moisture and carbon dioxide from getting into the eluent. Normally, the adsorber tube is filled with molecular sieve or - for sodium hydroxide and carbonate eluents - with soda lime (a weak CO ₂ adsorber).
<i>Degassing the eluent</i>	In order to prevent bubbles from forming, we recommend degassing the produced eluent before using it in your IC system. To degas the eluent, create a vacuum for approximately ten minutes using a water-jet pump or

an oil pump. Alternatively, use an ultrasonic bath or work with an eluent degasser.

Filter

Problems that occur in IC systems are usually related to particles. These particles can be introduced from the following sources:

- Bacterial growth
- Unfiltered eluents
- The sample
- The rinsing solution and/or regeneration solution

Minimize this risk by using an aspiration filter (6.2821.090), inline filter (6.2821.120) and guard columns. The filters are part of the basic equipment for Metrohm ion chromatographs and are included in the scope of delivery. We also recommend changing the filters regularly.

Filtering the eluent

All eluents have to be microfiltered (0.45 µm) immediately before use.

Mechanical stress

Mechanical loads on the column should be avoided. For example, the column impacting a hard surface can cause a break or gap in the column packing (separation phase material). This affects the chromatography results. The column would be irreparably damaged as a result.

Particles

All solutions, samples, regeneration solutions, water and eluents must be free of particles. Particles clog separation columns over time (column pressure increases). Be especially conscious of ensuring that there are no particles present when producing eluents. The eluent continuously flows through the column at a rate of 500 to 1000 mL per workday compared to about 0.5 mL of the sample solution. Filter or dialyze your sample automatically with one of the Metrohm Inline Sample Preparation techniques (MISP).

Sample preparation cartridges

Sample preparation cartridges are used to prepare critical samples that must not be injected directly into the separation column. They perform tasks such as removing organic contaminants or neutralizing heavily alkaline or acidic samples. Sample preparation cartridges are consumables that generally cannot be regenerated. Sample preparation cartridges do not replace the guard columns, which should always be used with each separation column. Metrohm Inline Sample Preparation techniques (MISP) can be used as an alternative to sample preparation cartridges.

Pulsation absorber

We recommend always using a pulsation absorber (6.2620.150). Polymethacrylate columns and polyvinyl alcohol columns in particular must be protected from the brief pressure surges that inevitably occur when switching the valves.

Regenerating separation columns

If separation columns are operated with clean eluents and filled with samples free of particles, you can expect the column to have a long service



life. This means regenerating the column is not required and, after a multitude of injections, no longer possible.

If the pressure in the column increases unexpectedly despite this or if the separating efficiency decreases, the regeneration steps specified for every column can be carried out. Generally, it is important to keep in mind that the regeneration takes place outside the analytical line. Connect the separation column to the pump directly. Route the regeneration solution through the column directly into a waste container. Before reinstalling the separation column, it must be properly rinsed with fresh eluent.

Shutting down the ion chromatograph

If you will not be working with the ion chromatograph for a prolonged period (> 1 week), we recommend removing the separation column and sealing it with the stoppers provided. Rinse the ion chromatograph, including all three suppressor chambers, with methanol/water (1:4). Store the separation column in the medium indicated on the column leaflet and, ideally, at a temperature between 4 and 8 °C if not specified otherwise.

When you return the instrument to operation, rinse the ion chromatograph with fresh eluent. Bring the separation column back to ambient temperature before you install it. Then increase the temperature if necessary.

Fun

Ion chromatography should be fun and should not stress you out. Metrohm puts all its work into ensuring you can work reliably with your IC systems with minimal maintenance, servicing and costs. Metrosep separation columns embody the attributes of quality, long service life and excellent results.

Environmental protection

A significant advantage of ion chromatography is that most of the work involves aqueous media. As a result, the chemicals used in ion chromatography are largely non-toxic and do not impact the environment. However, if you are working with acids, bases, organic solvents or heavy metal standards, dispose of them properly after use.

Guard columns

Guard columns are used to protect separation columns. We strongly recommend their use. They normally contain the same stationary phase also used in the separation columns. However, the quantity is significantly reduced to avoid impacting the chromatography. Guard columns remove critical contaminants that could react with column material; they also effectively remove particles and bacterial contaminants. Replace the guard column in the following cases:

- If the backpressure in the system increases.
- If the chromatography results deteriorate.

Guard columns are available for all Metrosep separation columns.



We recommend replacing the guard column three to four times during the service life of the analytical column.

Water quality

Aqueous media are mostly used in work involving ion chromatography. This means that water quality is a critical factor for good chromatography. If the water quality is inadequate, the results will be as well. In addition, there is a risk of damaging instruments and separation columns when using water with inadequate quality. The ultrapure water being used should have a specific resistance greater than 18.2 M Ω ·cm and should be free of particles. Therefore, we recommend filtering the water using a 0.45- μ m filter and treating it with UV light. Modern ultrapure water systems for laboratory use ensure this level of water quality (Type I).



3 Eluent production

We recommend selecting a high level of purity for chemicals for both standard production and eluent production.

3.1 Chemicals

Recommended chemicals

- Nitric acid, HNO₃, 2 mol/L
Sigma Aldrich order number: 35278
- Dipicolinic acid, C₇H₅NO₄, 99%
Sigma Aldrich order number: 63808
- Ultrapure water of type I (see ASTM D1193)
Resistance > 18.2 MΩ·cm (25 °C)
TOC < 10 µg/L

3.2 Production of standard eluent

Proceed as follows to produce 2 L of standard eluent with 1.7 mmol/L of nitric acid and 0.7 mmol/L of dipicolinic acid:

Producing 2 L of standard eluent

- 1
 - Pre-rinse the eluent bottle with ultrapure water several times.
 - Set out 1.8 L of ultrapure water.
- 2 If the eluent is not degassed using an eluent degasser:
 - Degas the ultrapure water for the eluent using a vacuum pump. This prevents problems with air bubbles in the high-pressure pump.
- 3 Measure the following quantity of chemicals:
 - Nitric acid: 1.7 mL
 - Dipicolinic acid: 234 mg
- 4
 - Pipette 1.7 mL nitric acid into the eluent bottle.
 - Put approx. 100 mL ultrapure water in a beaker.
 - Add 234 mg dipicolinic acid into the beaker.
 - Heat the beaker for approx. 1 minute in the microwave at full potential.
If no microwave is available, heat the suspension to 80 °C.

- Afterwards, swirl the beaker until the dipicolinic acid has dissolved.
- Pour the solution into the eluent bottle.
- Fill the eluent bottle with ultrapure water to 2 L.

This eluent (1.7 mmol/L of nitric acid, 0.7 mmol/L of dipicolinic acid) can be used to achieve background conductivity of approx. 700 $\mu\text{S}/\text{cm}$. The noise is typically less than 2 nS/cm.



4 Start-up

4.1 Connecting and rinsing the guard column

Guard columns protect separation columns and significantly increase their service life. The guard columns available from Metrohm are either actual guard columns or guard column cartridges used together with a cartridge holder. The process of installing a guard column cartridge into the corresponding holder is described in the guard column leaflet.



NOTICE

Metrohm recommends always working with guard columns. They protect the separation columns and can be replaced regularly as needed.



NOTICE

Information regarding which guard column is suitable for your separation column can be found in the **Metrohm Column Program** (which is available from your Metrohm representative), the leaflet provided along with your separation column or the product information about the separation column at <http://www.metrohm.com> (Ion Chromatography product area), or it can be obtained directly from your representative.



CAUTION

New guard columns are filled with a solution and sealed with stoppers or caps on both sides.

Before inserting the guard column, ensure that this solution can be mixed with the eluent being used (follow the information provided by the manufacturer).

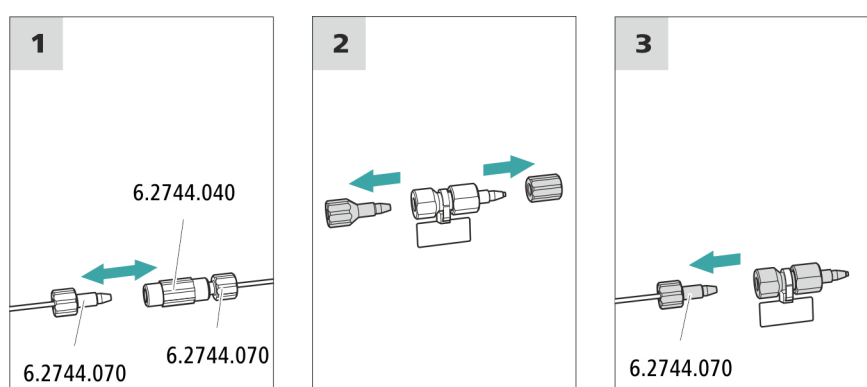
**NOTICE**

The guard column may not be connected until after the instrument has already been put into operation once. The guard column and the separation column have to be replaced by a coupling (6.2744.040) until then.

Accessories

For this step, you need the following accessories:

- Guard column (suitable for separation column)

Connecting the guard column**1 Removing the coupling**

Remove the coupling (6.2744.040) installed between the column inlet capillary and the column outlet capillary for the initial start-up.

2 Preparing the guard column

- Remove the stopper and the sealing cap from the guard column.

3 Connecting the guard column**CAUTION**

When inserting the guard column, ensure that it is inserted correctly based on the marked flow direction (if specified).

- Fasten the inlet of the guard column to the column inlet capillary using a short pressure screw (6.2744.070).



- If the guard column is connected to the separation column using a connection capillary, fasten this connection capillary to the guard column outlet with a pressure screw.

Rinsing the guard column

1 Rinsing the guard column

- Place a beaker under the guard column's outlet.
- Start manual control in MagIC Net and select the high-pressure pump: **Manual ▶ Manual control ▶ Pump**
 - **Flow: in accordance with column leaflet**
 - **On**
- Rinse the guard column with eluent for approx. 5 minutes.
- Stop the high-pressure pump in the manual control in MagIC Net again: **Off**.

4.2 Connecting the separation column

The smart separation column (iColumn) is the heart of ion chromatographic analysis. It separates the different components according to their interactions with the column. Metrohm separation columns are equipped with a chip where their technical specifications and history (start-up, operating hours, injections etc) are stored.



NOTICE

Information regarding which separation column is suitable for your application can be found in the **Metrohm Column Program**, the product information for the separation column or it can be obtained through your representative.

You can find product information for your separation column at <http://www.metrohm.com> in the Ion Chromatography product area.

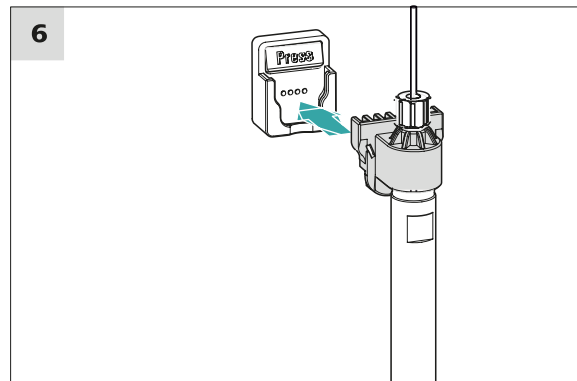
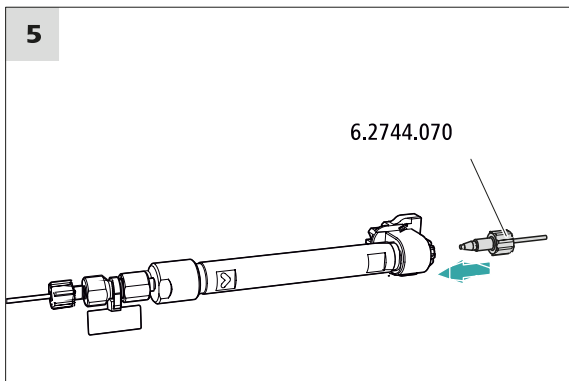
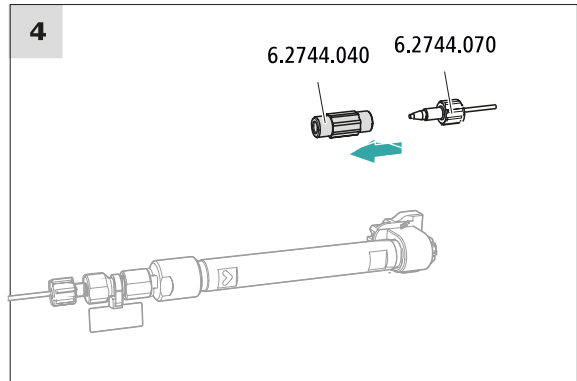
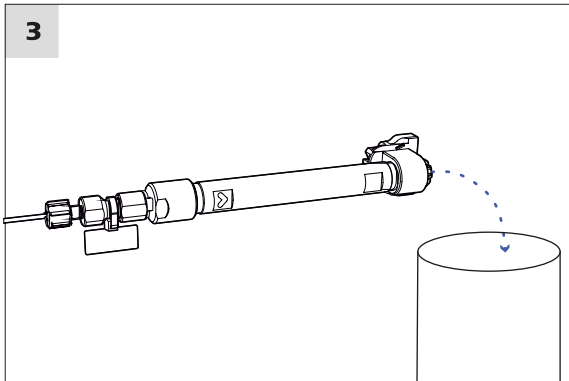
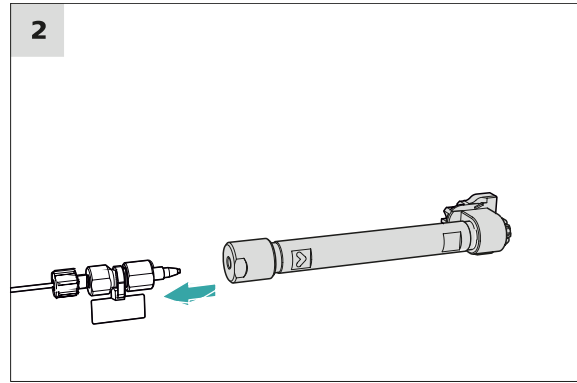
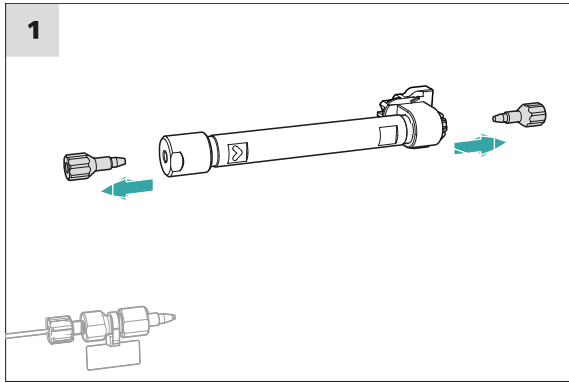
A test chromatogram and a leaflet accompanies every column. Detailed information on special IC applications can be found in the corresponding "**Application Bulletins**" or "**Application Notes**". You can find these online at <http://www.metrohm.com> in the Applications area or request them from your responsible Metrohm representative free of charge.

**CAUTION**

New separation columns are filled with a solution and sealed with stoppers on both sides. Before inserting the column, ensure that this solution can be mixed with the eluent being used (follow the information provided by the manufacturer).

**NOTICE**

Connect the separation column only after the initial start-up of the instrument. Until that point, insert a coupling (6.2744.040) instead of the guard column and separation column.



Connecting the separation column

1 Removing the stoppers

- Remove the stoppers from the separation column.

2 Installing the inlet of the separation column



CAUTION

When inserting the column, ensure that it is inserted correctly based on the marked flow direction.

There are three options:

- Attach the column inlet directly onto the guard column or,
- if the guard column is connected to the separation column using a connection capillary: Connect the column inlet to the guard column outlet capillary using a PEEK pressure screw (6.2744.070) or,
- if no guard column is used (not recommended): Connect the column inlet capillary to the inlet of the separation column using a short pressure screw (6.2744.070).

3 Rinsing the separation column

- Place a beaker under the outlet of the separation column.
- Start manual control in MagIC Net and select the high-pressure pump: **Manual ▶ Manual control ▶ Pump**
 - **Flow:** Increase gradually up to the flow rate recommended in the column leaflet.
 - **On**
- Rinse the separation column with eluent for approx. 10 minutes.
- Stop the high-pressure pump in the manual control in MagIC Net again: **Off**.

4 Removing the coupling

- Remove the coupling (6.2744.040) from the column outlet capillary.

5 Installing the outlet of the separation column

- Fasten the column outlet capillary to the column outlet using a short PEEK pressure screw (6.2744.070).

6 Inserting the separation column

- Insert the separation column with the chip into the column holder until you hear it snap in place.

The separation column is now detected by MagIC Net.

- Visually inspect whether all capillaries and their connections from the high-pressure pump to the detector are leak-tight. If eluent is leaking out anywhere, tighten the corresponding pressure screw further, or loosen the pressure screw, check the end of the capillary and shorten it using the capillary cutter if necessary and retighten the pressure screw.

4 Conditioning the system

Continue rinsing the system with eluent until the desired stability level for the baseline has been attained .

The instrument is now ready for measuring samples.

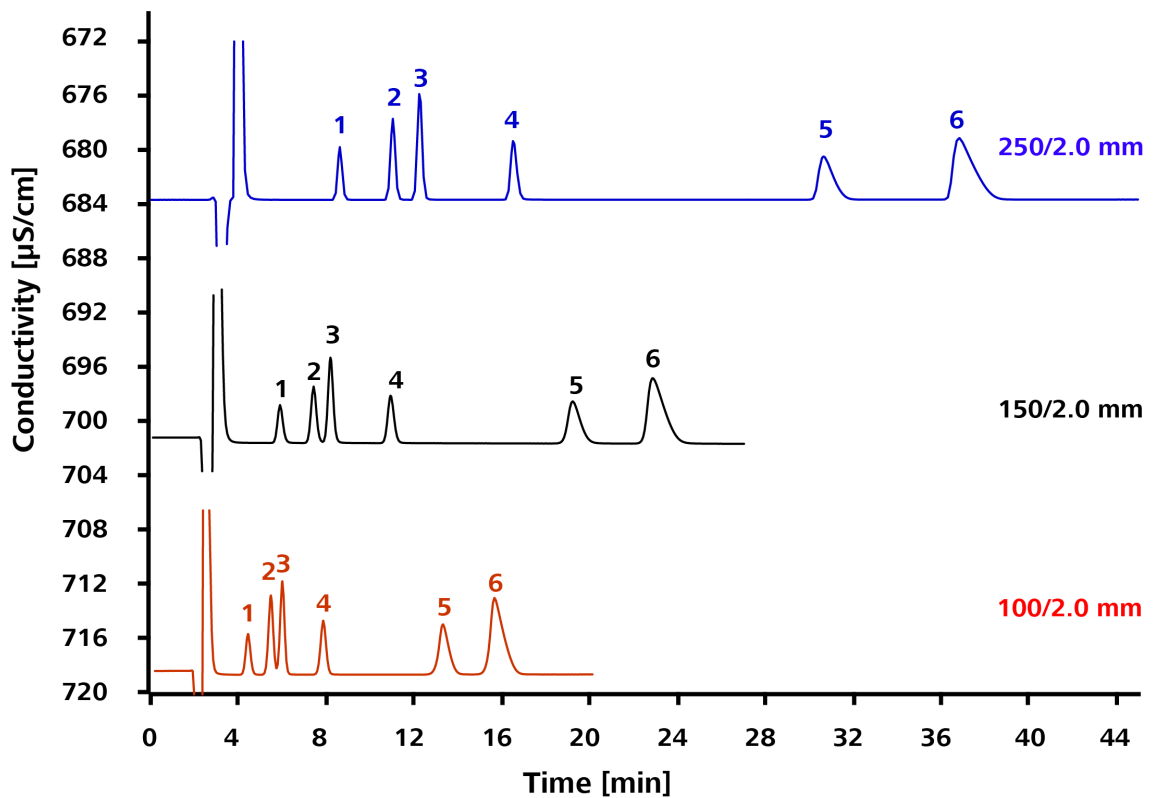


5 Applications

5.1 Standard chromatogram

2-mm columns

Sample preparation: –
Detection: Conductivity
Suppression: –
Temperature: 25 °C
Loop: 10 µL
Flow rate: 0.2 mL/min
Eluent: 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid

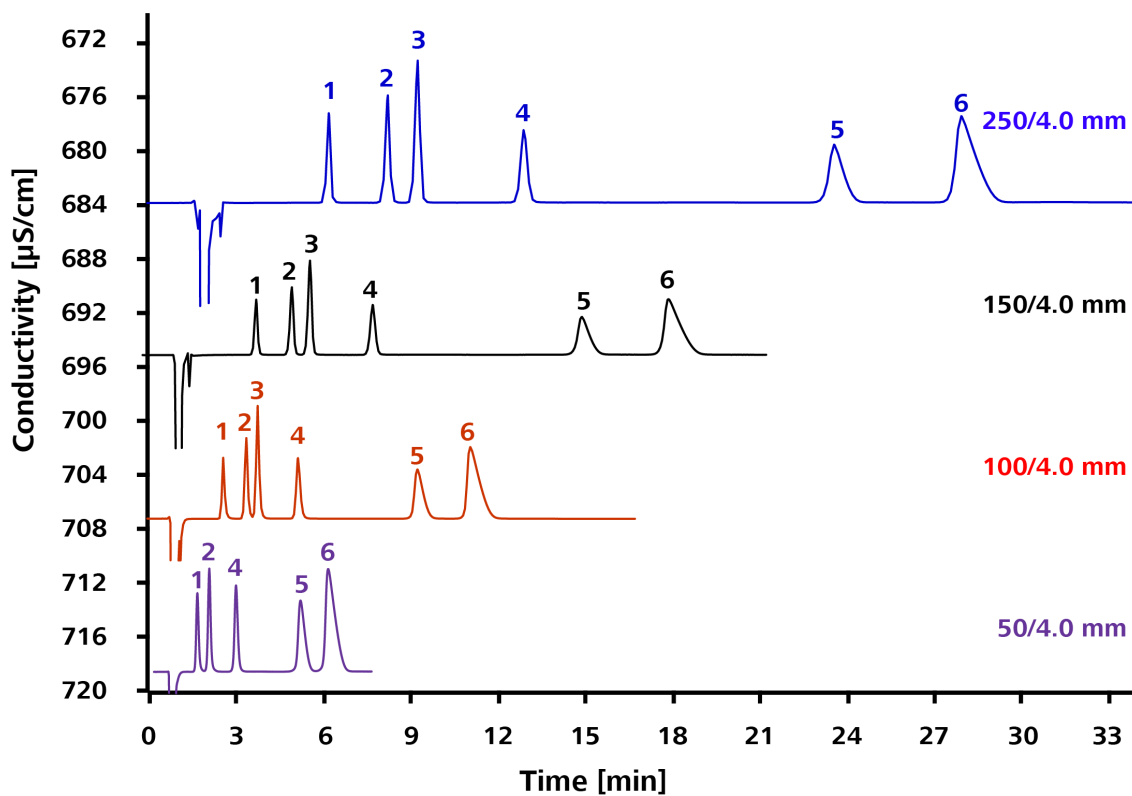


Metrosep C 4 - XX0/2.0		mg/L
1	Lithium	1

	Metrosep C 4 - XX0/2.0	mg/L
2	Sodium	5
3	Ammonium	5
4	Potassium	10
5	Calcium	10
6	Magnesium	10

4-mm columns

Sample preparation: –
Detection: Conductivity
Suppression: –
Temperature: 25 °C
Loop: 10 µL
Flow rate: 0.9 mL/min
Eluent: 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid





	Metrosep C 4 - XX0/4.0	mg/L
1	Lithium	1
2	Sodium	5
3	Ammonium	5
4	Potassium	10
5	Calcium	10
6	Magnesium	10

5.2 Effects of temperature

Column: Metrosep C 4 - 150/4.0

Sample preparation: –

Detection: Conductivity

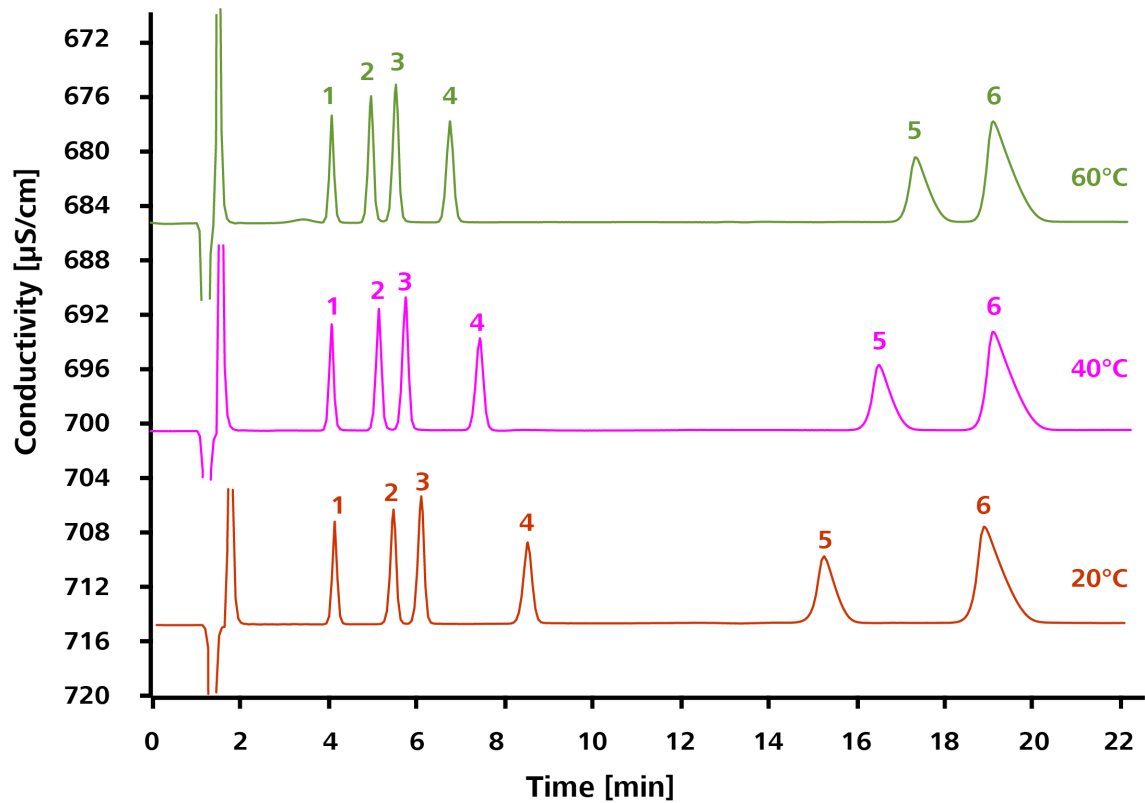
Suppression: –

Temperature: 20 to 60 °C

Loop: 10 µL

Flow rate: 0.9 mL/min

Eluent: 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid



	Metrosep C 4 - 150/4.0	mg/L
1	Lithium	1
2	Sodium	5
3	Ammonium	5
4	Potassium	10
5	Calcium	10
6	Magnesium	10

An increase in temperature results in longer retention times of the polyvalent ions such as calcium and magnesium. In the case of calcium, the complexation with dipicolinic acid is weakened with higher temperature. The retention time is thus increased more in comparison with magnesium. The other cations such as lithium, sodium, ammonium and potassium elute a bit earlier.



5.3 Eluent flow rate variation

Column: Metrosep C4 - 150/4.0

Sample preparation: –

Detection: Conductivity

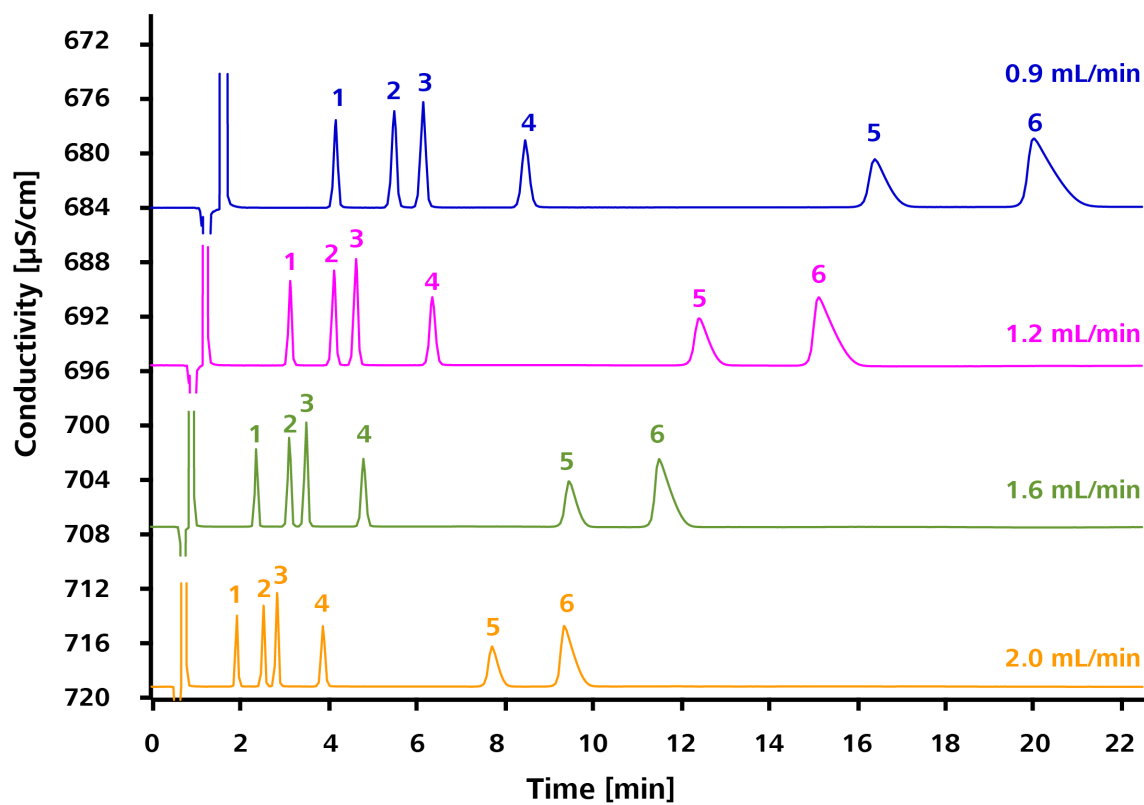
Suppression: –

Temperature: 25 °C

Loop: 10 µL

Flow rate: 0.9 up to 2.0 mL/min

Eluent: 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid



	Metrosep C 4 - 150/4.0	mg/L
1	Lithium	1
2	Sodium	5
3	Ammonium	5

	Metrosep C 4 - 150/4.0	mg/L
4	Potassium	10
5	Calcium	10
6	Magnesium	10

All cations elute faster when the flow rate is higher.

5.4 Variation of the eluent

5.4.1 Variation of the nitric acid concentration

Influence on standard cations

Column: Metrosep C4 - 150/4.0

Sample preparation: –

Detection: Conductivity

Suppression: –

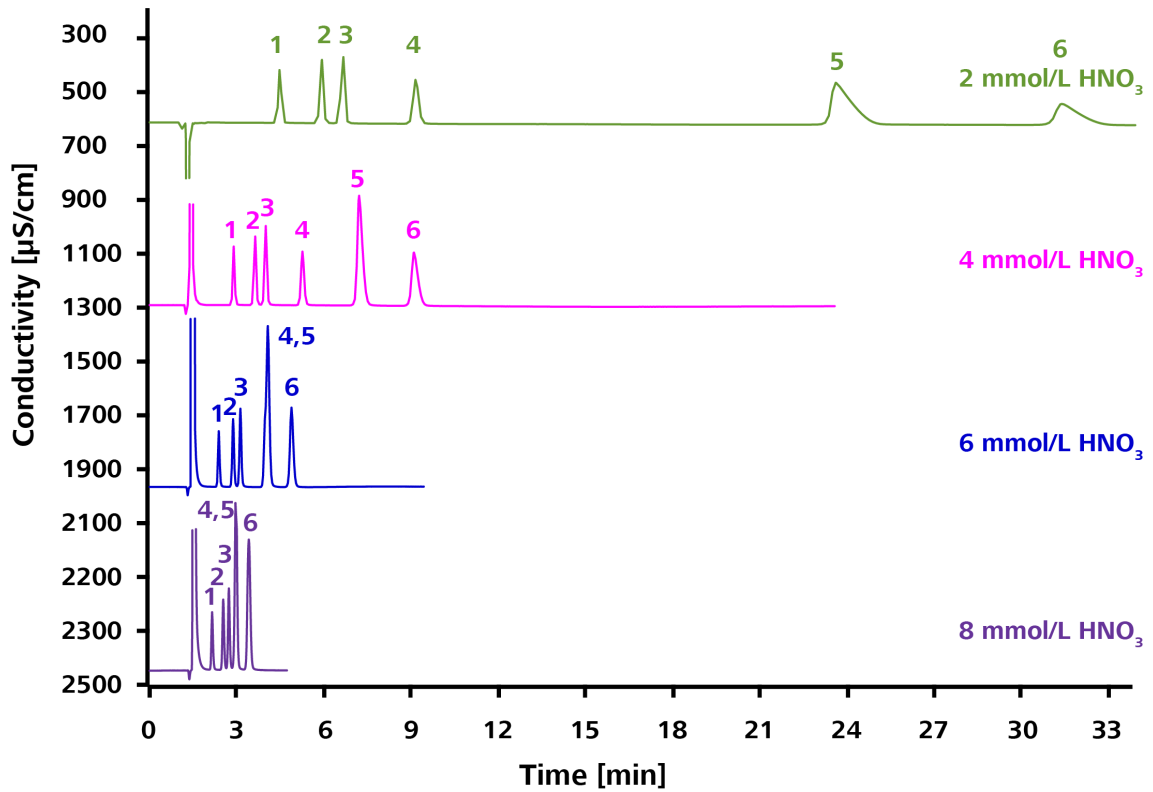
Temperature: 25 °C

Loop: 10 µL

Flow rate: 0.9 mL/min

Eluent:

- A) 2 mmol/L nitric acid
- B) 4 mmol/L nitric acid
- C) 6 mmol/L nitric acid
- D) 8 mmol/L nitric acid



	Metrosep C 4 - 150/4.0	mg/L
1	Lithium	1
2	Sodium	5
3	Ammonium	5
4	Potassium	10
5	Calcium	10
6	Magnesium	10

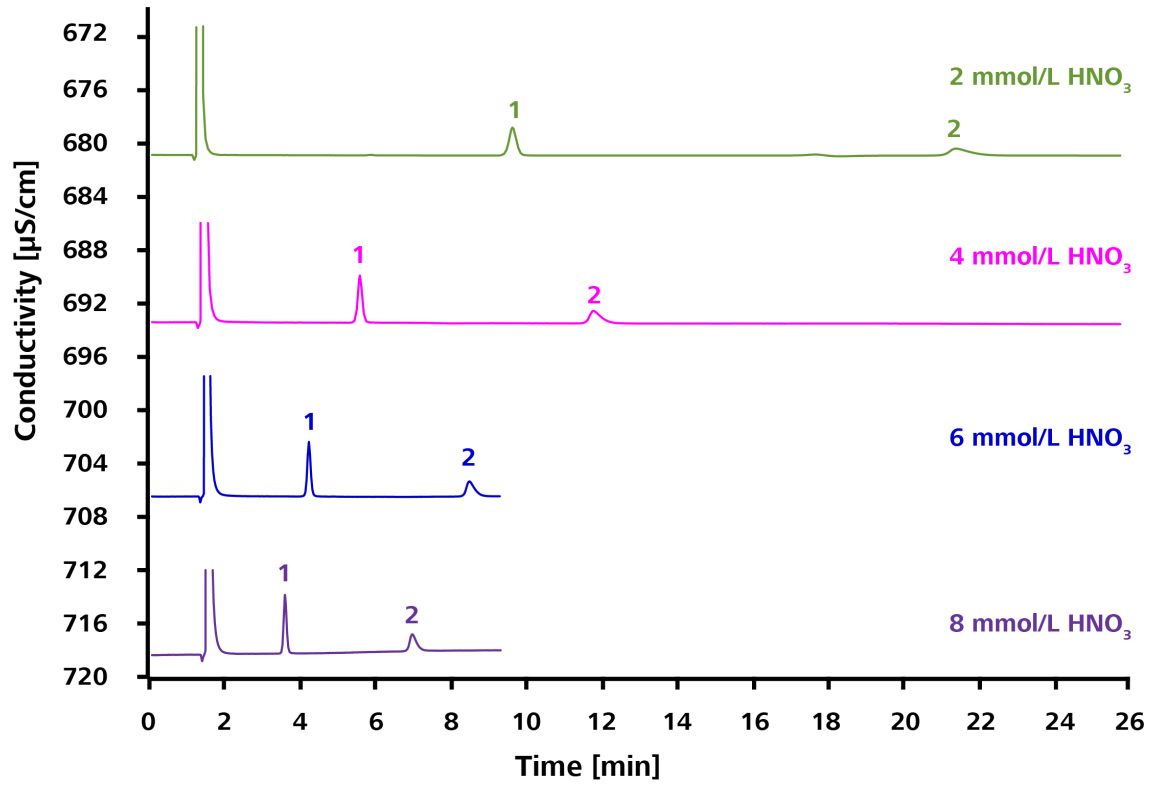
Increasing the nitric acid concentration accelerates all standard cations. The divalent cations are accelerated disproportionately.

Influence on amines

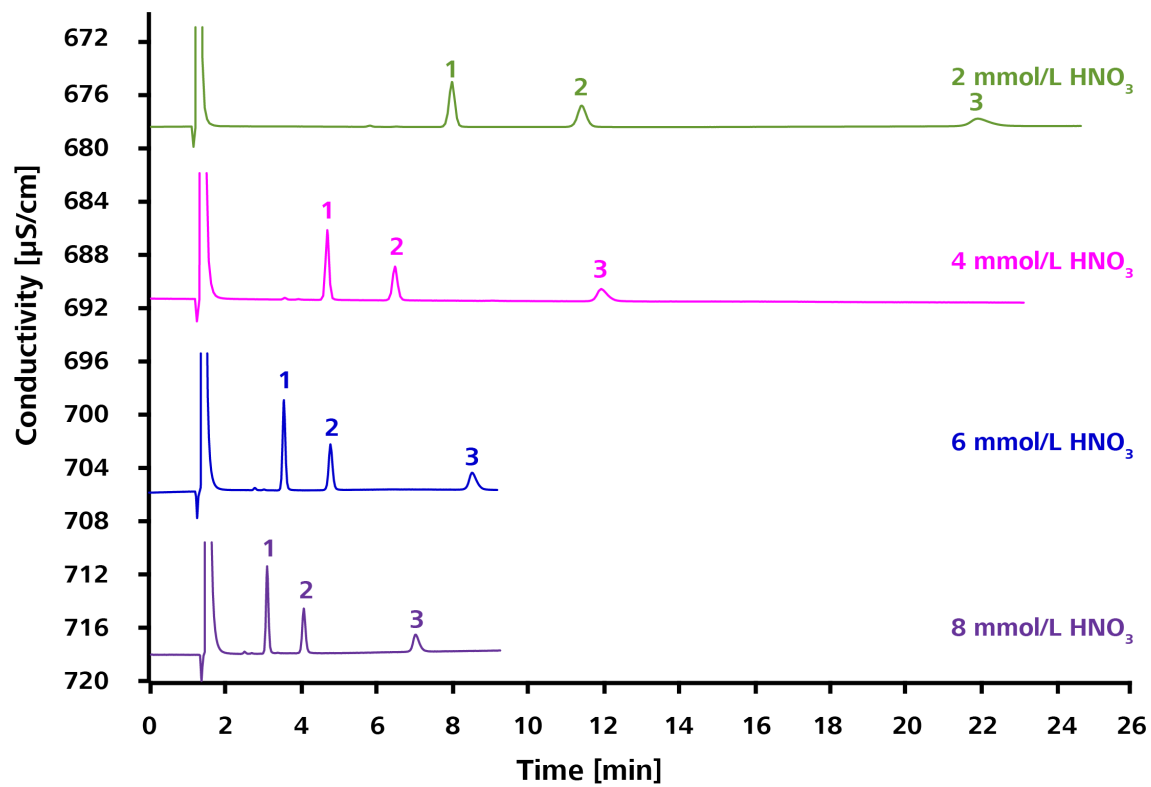
- Column: Metrosep C4 - 150/4.0
- Sample preparation: –
- Detection: Conductivity
- Suppression: –
- Temperature: 25 °C
- Loop: 10 µL

Flow rate: 0.9 mL/min

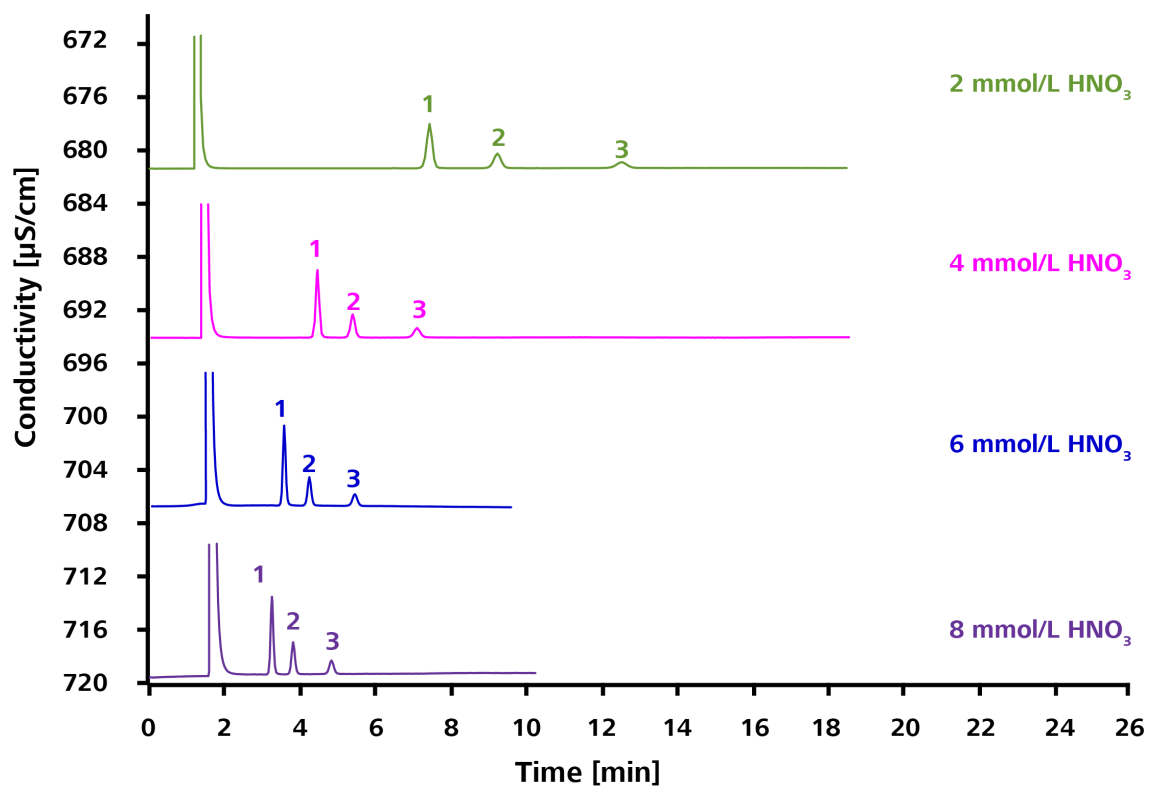
Eluent:
 A) 2 mmol/L nitric acid
 B) 4 mmol/L nitric acid
 C) 6 mmol/L nitric acid
 D) 8 mmol/L nitric acid



Metrosep C 4 - 150/4.0	mg/L
1 Monoethylamine	5
2 Diethylamine	5



	Metrosep C 4 - 150/4.0	mg/L
1	Monomethylamine	5
2	Dimethylamine	5
3	Trimethylamine	5



Metrosep C 4 - 150/4.0	mg/L
1 Monoethanolamine	5
2 Diethanolamine	5
3 Triethanolamine	5

Increasing the nitric acid concentration shortens the retention time of the amines.

5.4.2 Variation of the dipicolinic acid concentration

Column: Metrosep C4 - 150/4.0

Sample preparation: –

Detection: Conductivity

Suppression: –

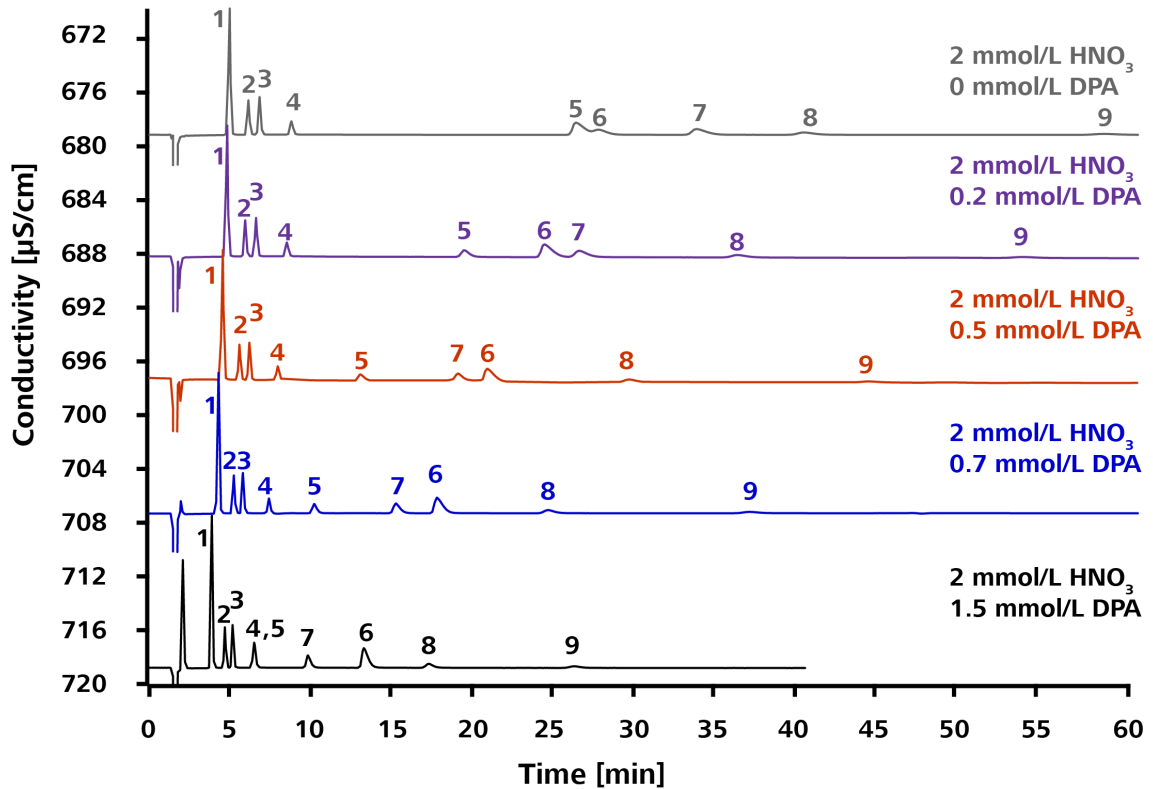
Temperature: 25 °C

Loop: 10 µL

Flow rate: 0.9 mL/min

Eluent: A) 2 mmol/L nitric acid, 0 mmol/L dipicolinic acid

- B) 2 mmol/L nitric acid, 0.2 mmol/L dipicolinic acid
 C) 2 mmol/L nitric acid, 0.5 mmol/L dipicolinic acid
 D) 2 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid
 E) 2 mmol/L nitric acid, 1.5 mmol/L dipicolinic acid



	Metrosep C 4 - 150/4.0	mg/L
1	Lithium	5
2	Sodium	5
3	Ammonium	5
4	Potassium	5
5	Manganese	5
6	Magnesium	5
7	Calcium	5
8	Strontium	5
9	Barium	5

The addition of dipicolinic acid to the nitric acid influences the retention of manganese, strontium, barium and calcium through complexation. Increasing the dipicolinic acid concentration accelerates these cations. The best separation is reached with 0.7 mmol/L dipicolinic acid. From 0.5

mol/L dipicolinic acid, calcium elutes before magnesium. Manganese and potassium co-elute at a dipicolinic acid concentration of 1.5 mmol/L.

5.4.3 Variation of the oxalic acid concentration

Column: Metrosep C4 - 150/4.0

Sample preparation: –

Detection: Conductivity

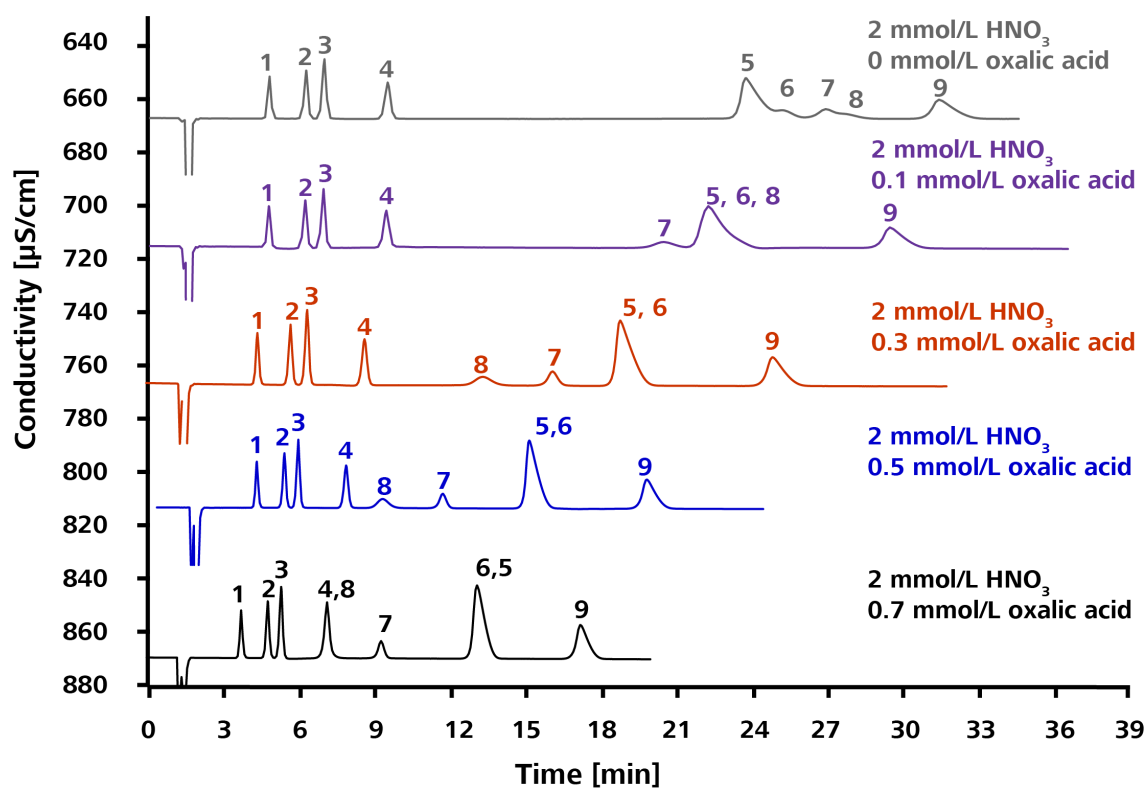
Suppression: –

Temperature: 25 °C

Loop: 10 µL

Flow rate: 0.9 mL/min

Eluent:
 A) 2 mmol/L nitric acid, 0 mmol/L oxalic acid
 B) 2 mmol/L nitric acid, 0.1 mmol/L oxalic acid
 C) 2 mmol/L nitric acid, 0.3 mmol/L oxalic acid
 D) 2 mmol/L nitric acid, 0.5 mmol/L oxalic acid
 E) 2 mmol/L nitric acid, 0.7 mmol/L oxalic acid

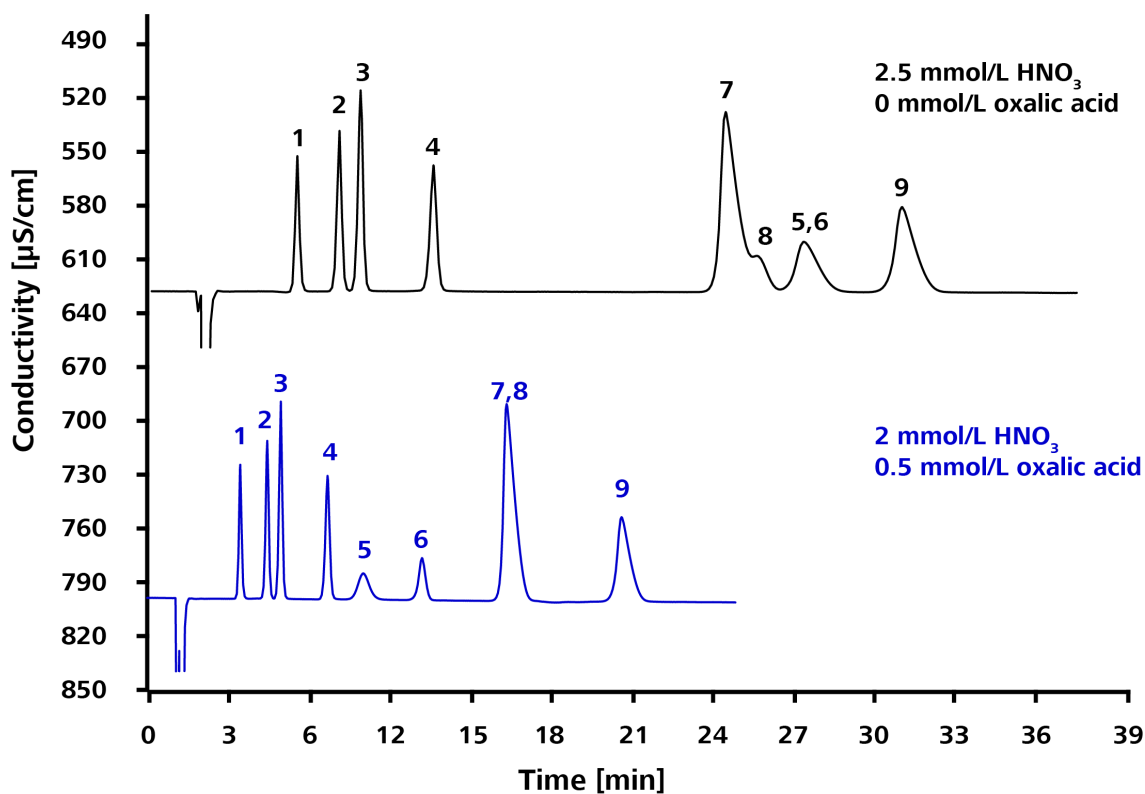




	Metrosep C 4 - 150/4.0	mg/L
1	Lithium	1
2	Sodium	5
3	Ammonium	5
4	Potassium	10
5	Magnesium	10
6	Manganese	5
7	Zinc	5
8	Nickel	5
9	Calcium	10

The addition of oxalic acid to the nitric acid influences the retention of nickel and zinc through complexation. Increasing the oxalic acid concentration will accelerate these cations. Manganese and zinc co-elute at 0.1 mmol/L oxalic acid with magnesium. At 0.7 mmol/L oxalic acid, nickel co-elutes with potassium.

Comparison of 2.5 mmol/L nitric acid with 2.0 mmol/L nitric acid with 0.5 mmol/L oxalic acid



	Metrosep C 4 - 150/4.0	mg/L
1	Lithium	1

	Metrosep C 4 - 150/4.0	mg/L
2	Sodium	5
3	Ammonium	5
4	Potassium	10
5	Nickel	5
6	Zinc	5
7	Magnesium	10
8	Manganese	5
9	Calcium	10

By adding oxalic acid, nickel and zinc is complexed and elutes faster than without oxalic acid. Manganese co-elutes with magnesium at 2.5 mmol/L nitric acid. Nickel co-elutes with zinc at 2 mmol/L nitric acid, 0.5 mmol/L oxalic acid.

5.5 Variation of organic modifiers

5.5.1 Variation of the acetone concentration

Column: Metrosep C4 - 150/4.0

Sample preparation: –

Detection: Conductivity

Suppression: –

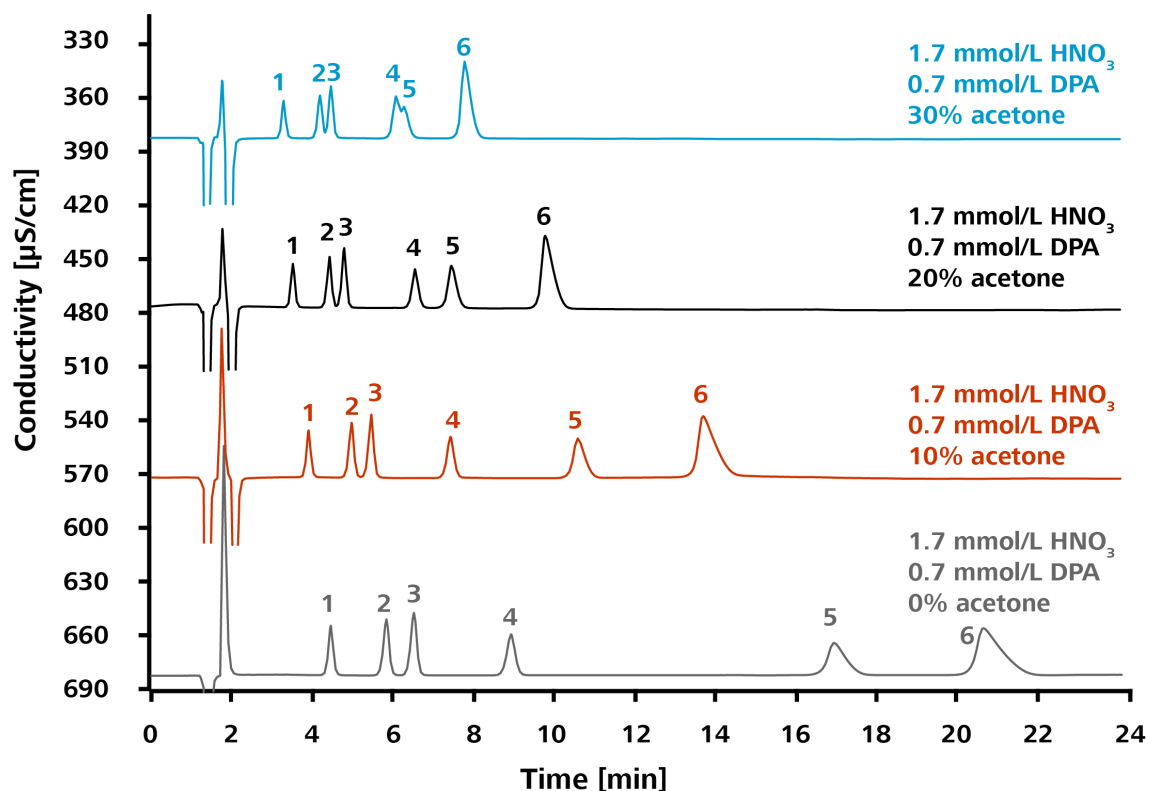
Temperature: 25 °C

Loop: 10 µL

Flow rate: 0.9 mL/min

Eluent:

- A) 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid, 0% acetone
- B) 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid, 10% acetone
- C) 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid, 20% acetone
- D) 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid, 30% acetone



	Metrosep C 4 - 150/4.0	mg/L
1	Lithium	1
2	Sodium	5
3	Ammonium	5
4	Potassium	10
5	Calcium	10
6	Magnesium	10

By adding acetone to the eluent, the retention time of all cations is shortened, whereat the effect is more visible in divalent cations. From 30% acetone in the eluent, potassium and calcium co-elute. The background conductivity is also reduced by increasing the solvent content. A quantifiable determination is still possible at 20% acetone. The pressure increases more by adding acetone than by adding acetonitrile.

5.5.2 Variation of the acetonitrile concentration

Column: Metrosep C4 - 150/4.0

Sample preparation: –

Detection: Conductivity

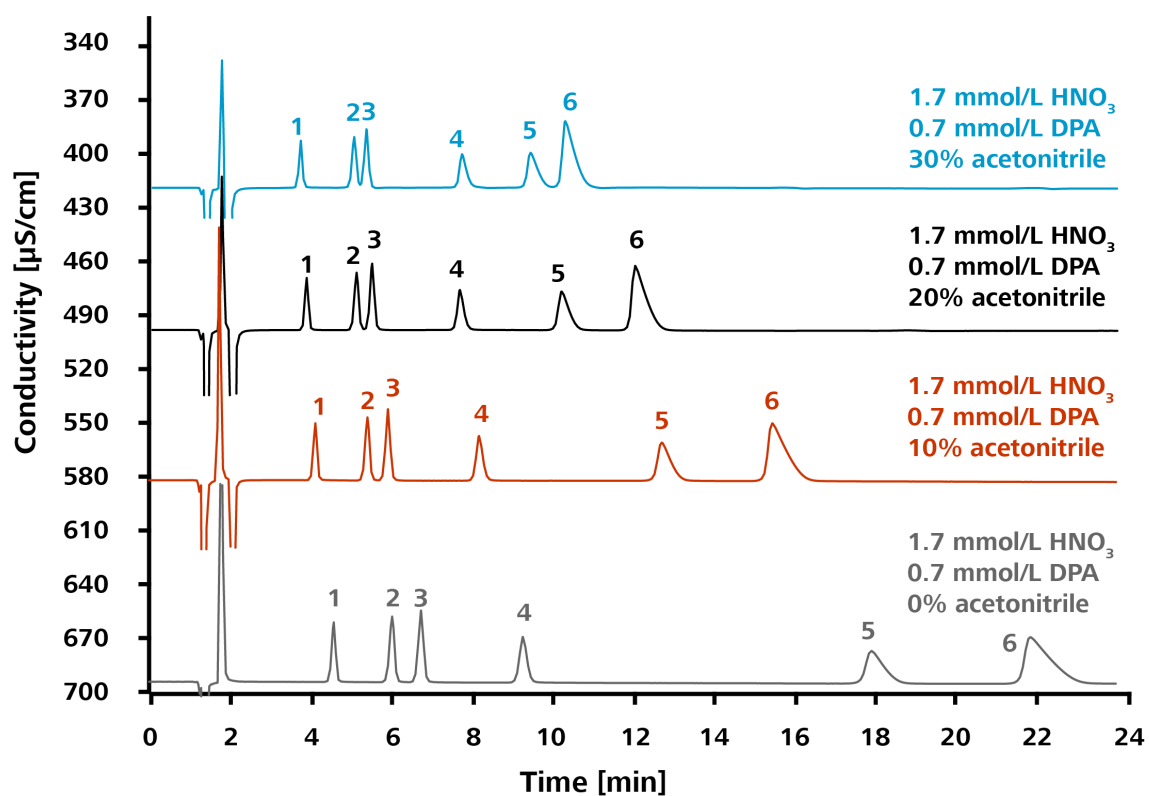
Suppression: –

Temperature: 25 °C

Loop: 10 µL

Flow rate: 0.9 mL/min

Eluent:
 A) 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid, 0% acetonitrile
 B) 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid, 10% acetonitrile
 C) 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid, 20% acetonitrile
 D) 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid, 30% acetonitrile



	Metrosep C 4 - 150/4.0	mg/L
1	Lithium	1
2	Sodium	5
3	Ammonium	5
4	Potassium	10
5	Calcium	10
6	Magnesium	10

By adding acetonitrile to the eluent, the retention time of all cations is shortened, whereas the effect is more visible in divalent cations. A quanti-



fiable determination is still possible at 30% acetonitrile. The pressure increases gradually as acetonitrile content increases.

5.6 Trace analysis in nuclear power plants

Column: Metrosep C4 - 250/2.0

Sample preparation: Inline Preconcentration of a sample with 3.3 mg/L of lithium hydroxide and 2 g/L of boric acid (MiPCT-ME)

Detection: Conductivity

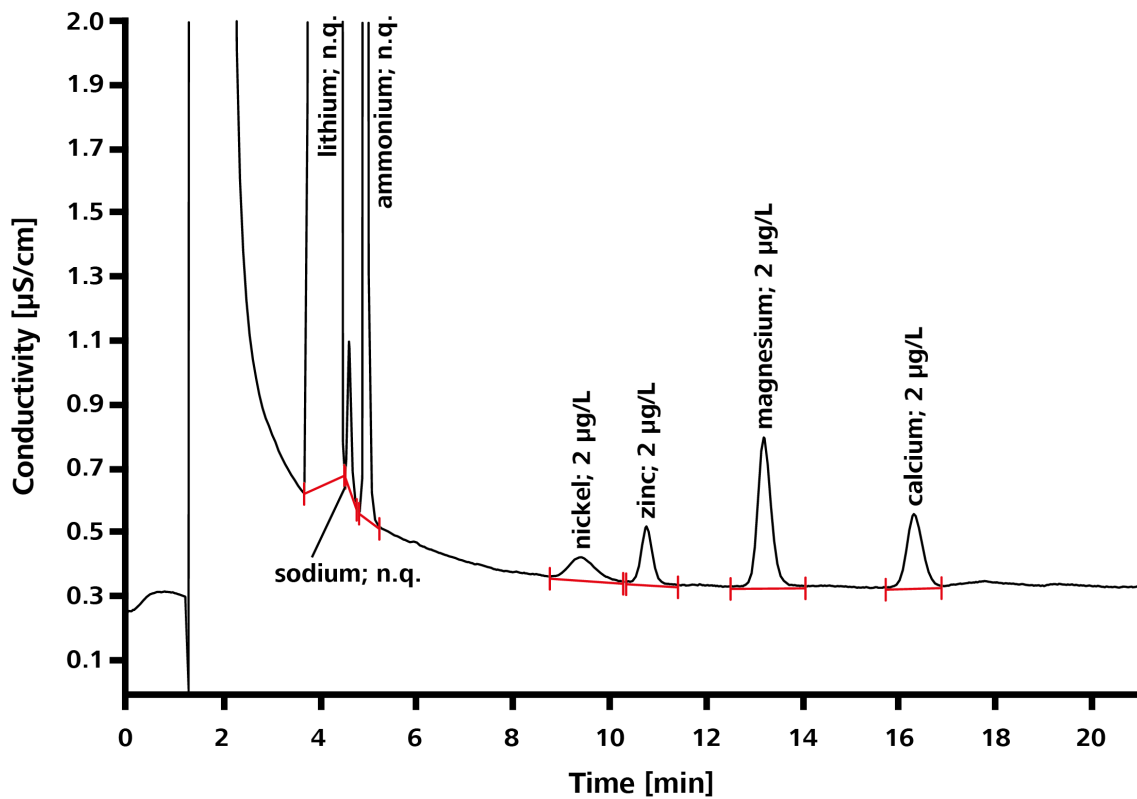
Suppression: MCS

Temperature: 32 °C

Loop: 1,000 µL with preconcentration on Metrosep A PCC 2 HC/4.0

Flow rate: 0.4 mL/min

Eluent: 2.5 mmol/L nitric acid, 0.5 mmol/L oxalic acid



5.7 Fast determination of standard cations in tap water

Column: Metrosep C4 - 100/2.0

Sample preparation: –

Detection: Conductivity

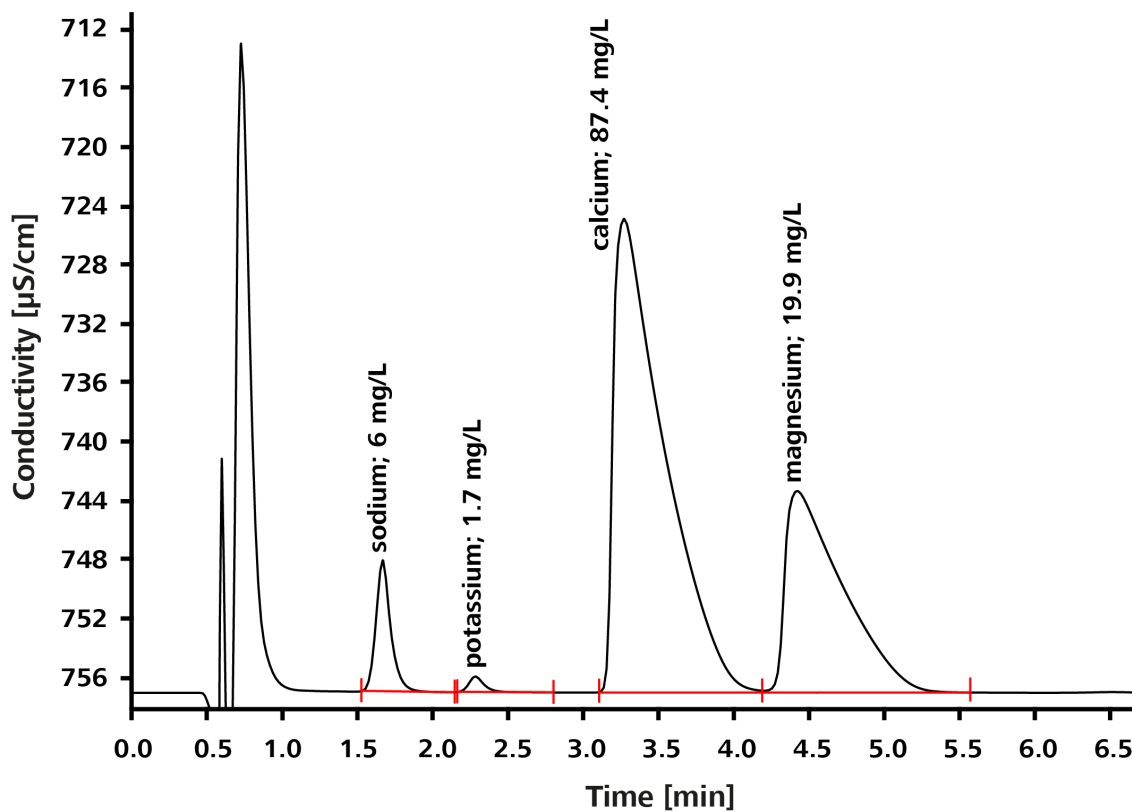
Suppression: –

Temperature: 30 °C

Loop: 10 µL

Flow rate: 0.5 mL/min

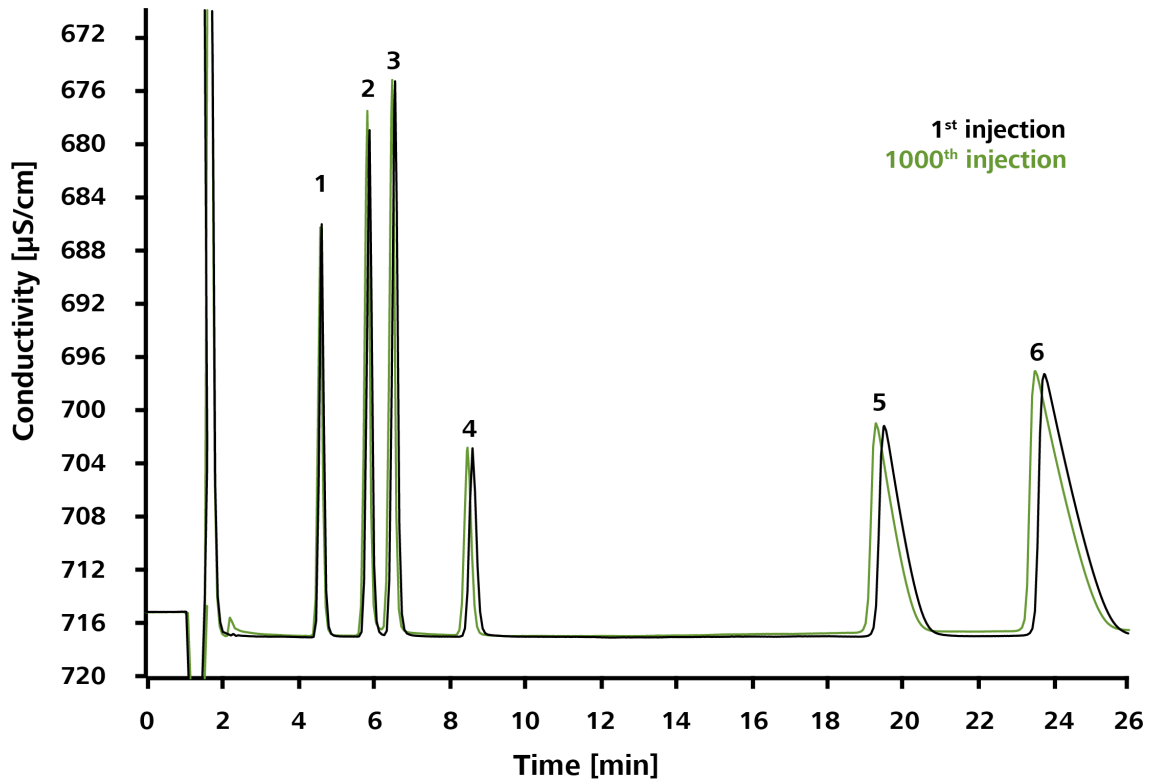
Eluent: 2.0 mmol/L nitric acid, 1.2 mmol/L dipicolinic acid





5.8 Measurement stability in alcohol determination

Column: Metrosep C4 - 150/4.0
 Sample preparation: –
 Detection: Conductivity
 Suppression: –
 Temperature: 30 °C
 Loop: 10 µL
 Flow rate: 0.9 mL/min
 Eluent: 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid



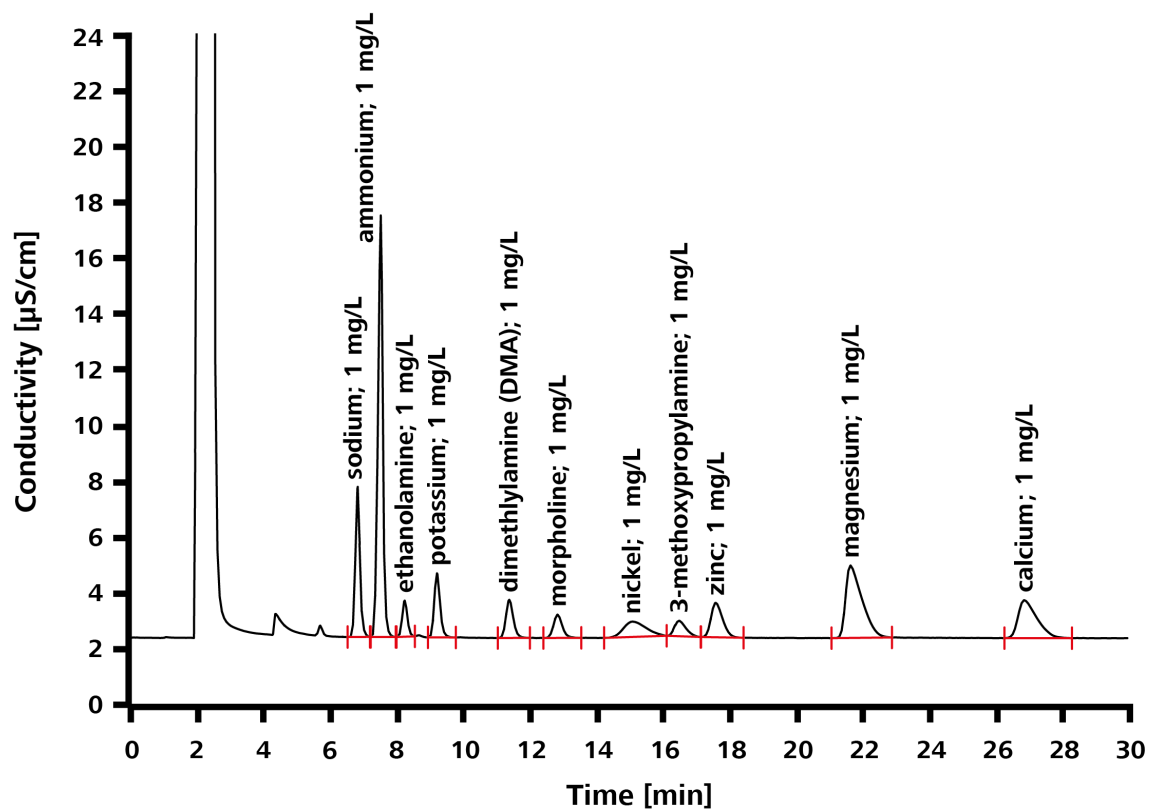
	Metrosep C 4 - 150/4.0	mg/L
1	Lithium	1
2	Sodium	5
3	Ammonium	5

	Metrosep C 4 - 150/4.0	mg/L
4	Potassium	10
5	Calcium	10
6	Magnesium	10

Vodka samples (37.5%) were injected continuously. After every 10th injection a cation standard was measured to document possible changes. The overlay above shows the cation standard before and after 1,000 injections.

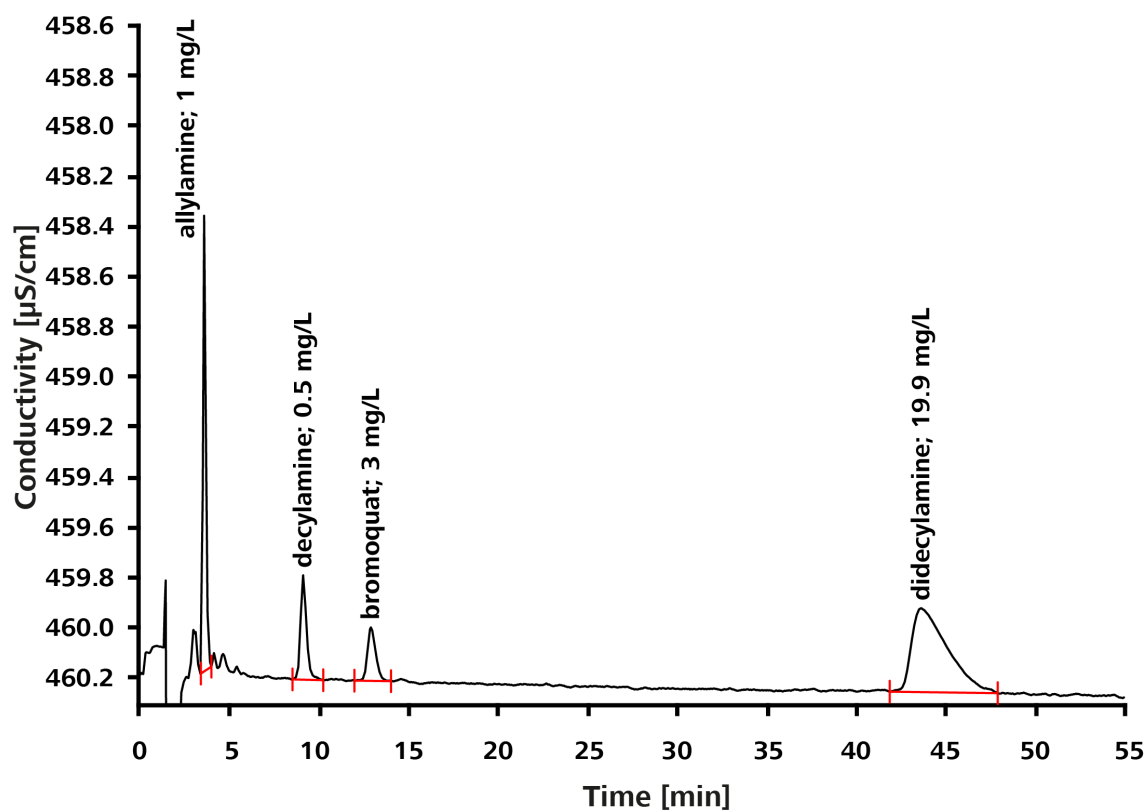
5.9 Amine determination in addition to standard cations

<i>Column:</i>	Metrosep C4 - 250/4.0
<i>Sample preparation:</i>	–
<i>Detection:</i>	Conductivity
<i>Suppression:</i>	–
<i>Temperature:</i>	32 °C
<i>Loop:</i>	100 µL
<i>Flow rate:</i>	0.9 mL/min
<i>Eluent:</i>	2.5 mmol/L nitric acid, 0.5 mmol/L oxalic acid



5.10 Determination of amines in Colesevelam HCl

Column:	Metrosep C4 - 150/4.0
Sample preparation:	–
Detection:	Conductivity
Suppression:	–
Temperature:	25 °C
Loop:	100 µL
Flow rate:	1.0 mL/min
Eluent:	2.5 mmol/L nitric acid, 40% acetone



5.11 Determination of cations in beer

Column: Metrosep C4 - 150/4.0

Sample preparation: Filtration with membrane filter (sample size 0.45 µm), acidification with 2 mmol/L nitric acid, dilution 1:10

Detection: Conductivity

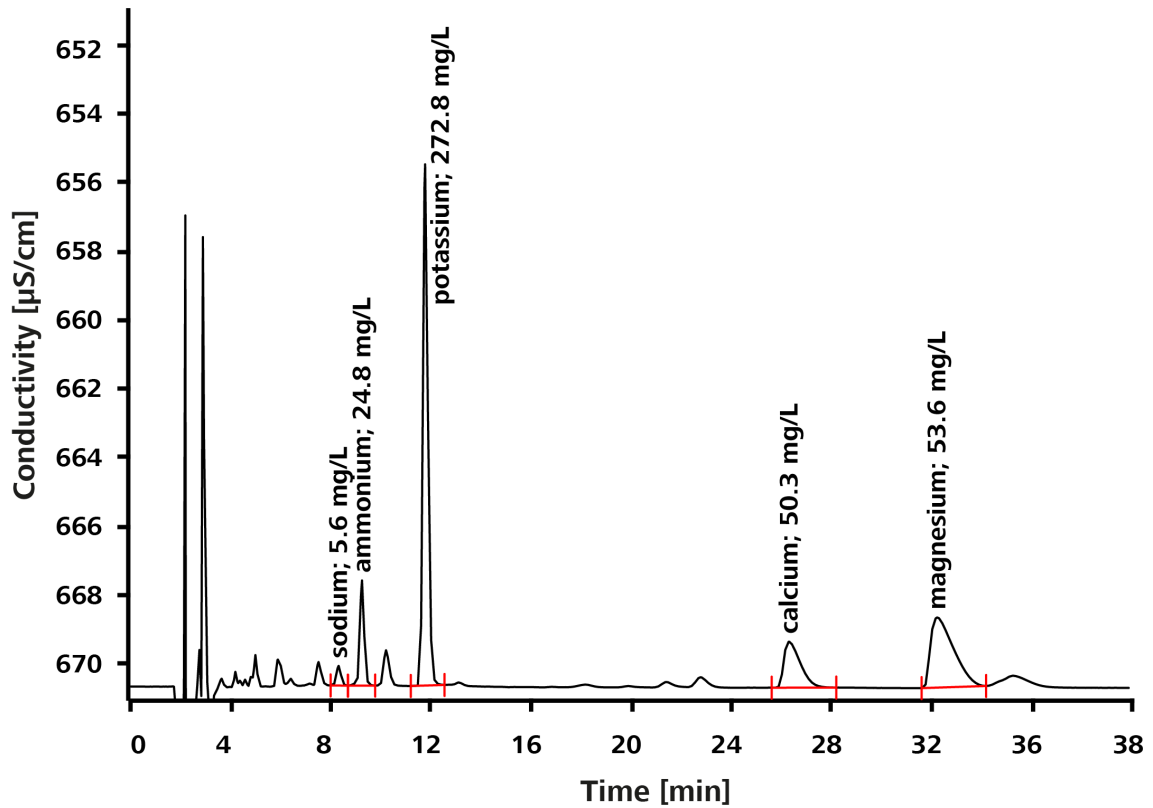
Suppression: –

Temperature: 30 °C

Loop: 20 µL

Flow rate: 1.0 mL/min

Eluent: 1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid



Beer contains a lot of natural ingredients. That is why there are a lot of non-quantified peaks in the chromatogram.

5.12 Determination of piperazine and N-Methyldiethanolamine in washing solutions

Column: Metrosep C4 - 150/4.0

Sample preparation: Dilution 1:20

Detection: Conductivity

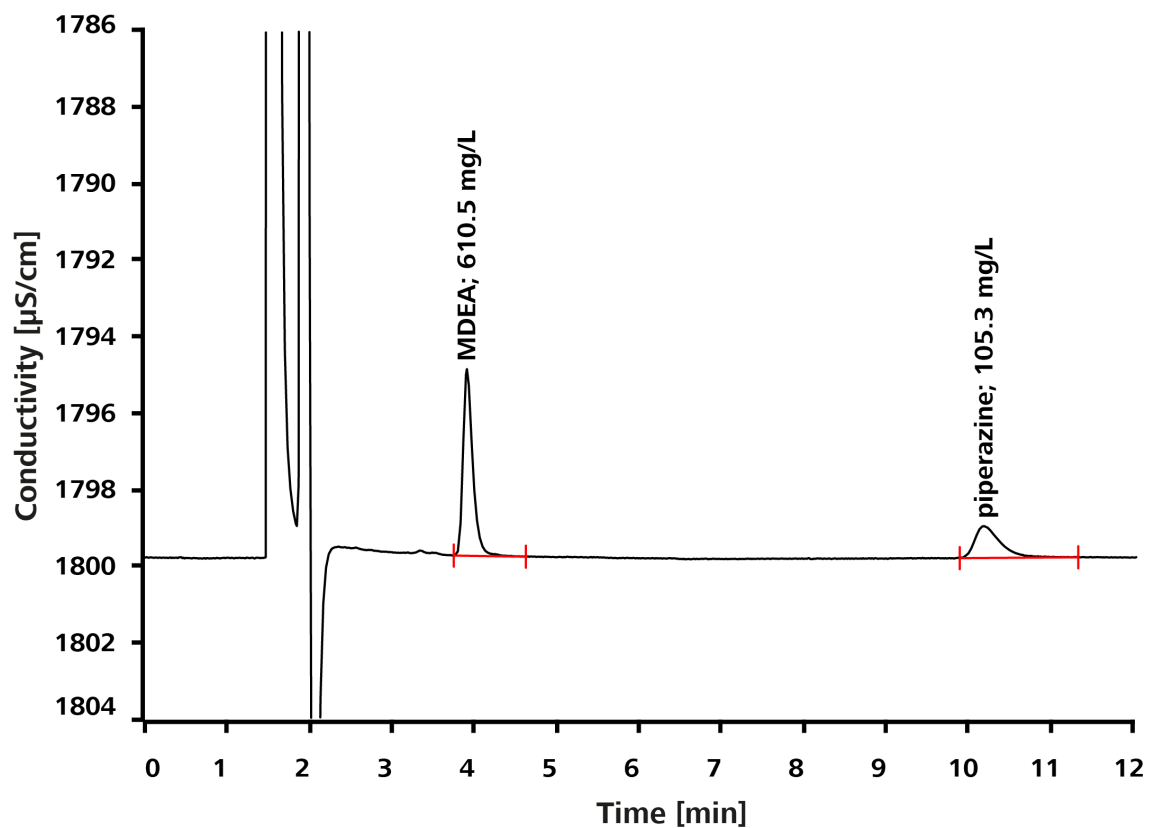
Suppression: –

Temperature: 30 °C

Loop: 10 µL

Flow rate: 0.9 mL/min

Eluent: 6.0 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid, 5% acetone



5.13 Determination of cations in fertilizer

Column: Metrosep C4 - 150/4.0

Sample preparation: Inline Ultrafiltration, dilution 1:198.9

Detection: Conductivity

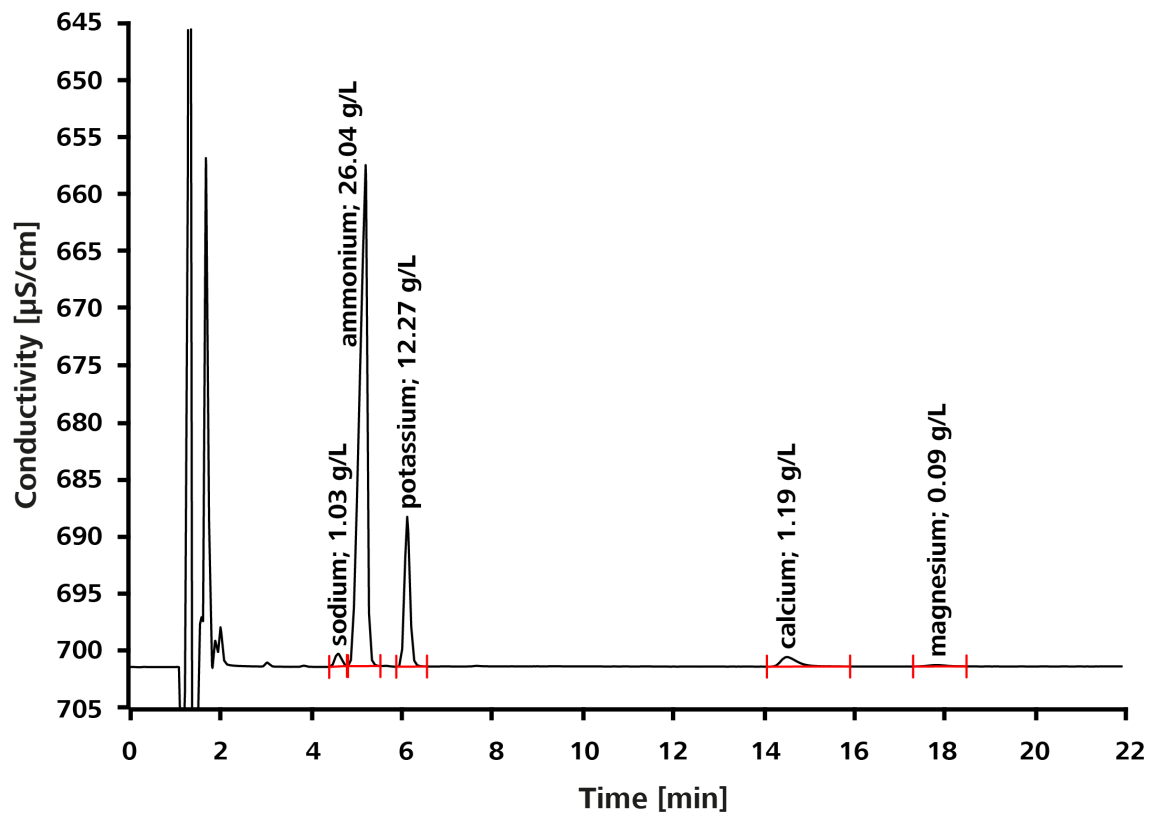
Suppression: –

Temperature: 30 °C

Loop: 10 μL

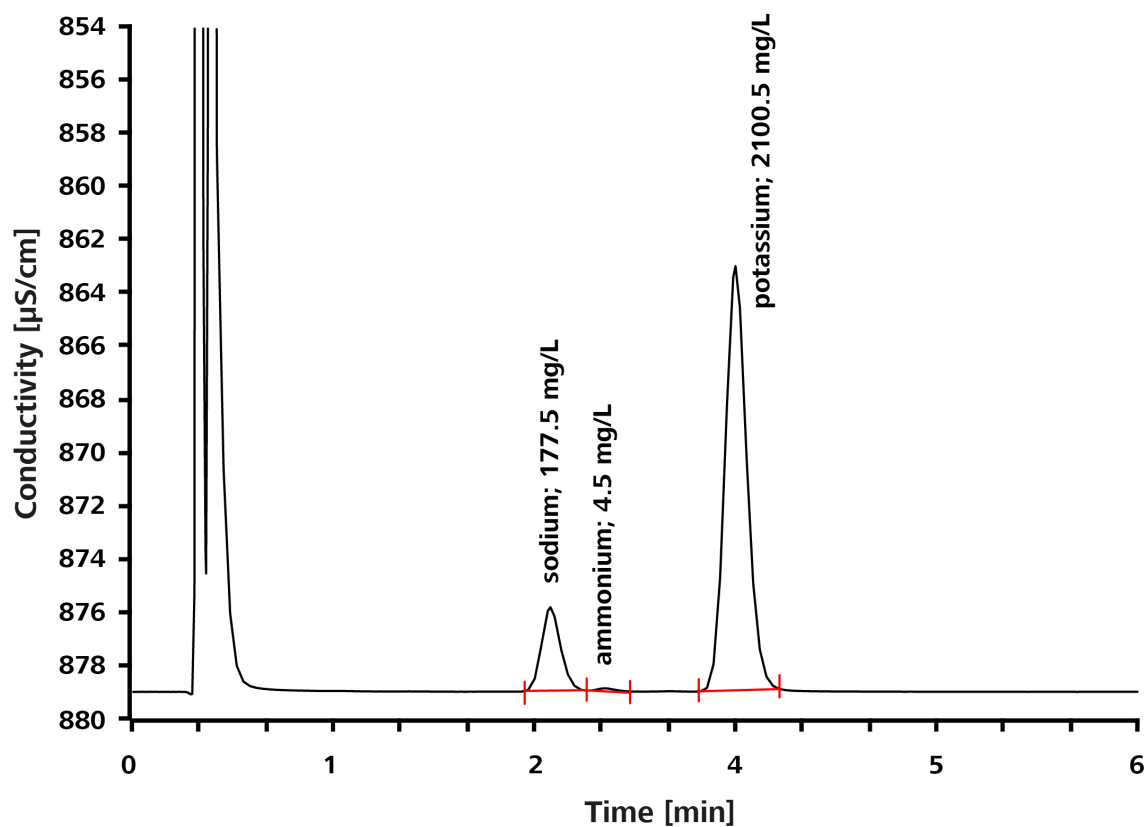
Flow rate: 0.9 mL/min

Eluent: 1.7 mmol/L nitric acid, 1.0 mmol/L dipicolinic acid



5.14 Nutrient analysis of a cell culture

<i>Column:</i>	Metrosep C4 - 150/2.0
<i>Sample preparation:</i>	Inline Dilution (1:100), Inline Ultrafiltration
<i>Detection:</i>	Conductivity
<i>Suppression:</i>	–
<i>Temperature:</i>	45 °C
<i>Loop:</i>	5 µL
<i>Flow rate:</i>	0.4 mL/min
<i>Eluent:</i>	1.7 mmol/L nitric acid, 0.7 mmol/L dipicolinic acid



Direct measurement of the "Pseudomonas putida" cell culture.

5.15 Determination of cations with IC-MS and conductivity detection

Column: Metrosep C4 - 150/2.0

Sample preparation: –

Detection: Conductivity, MS

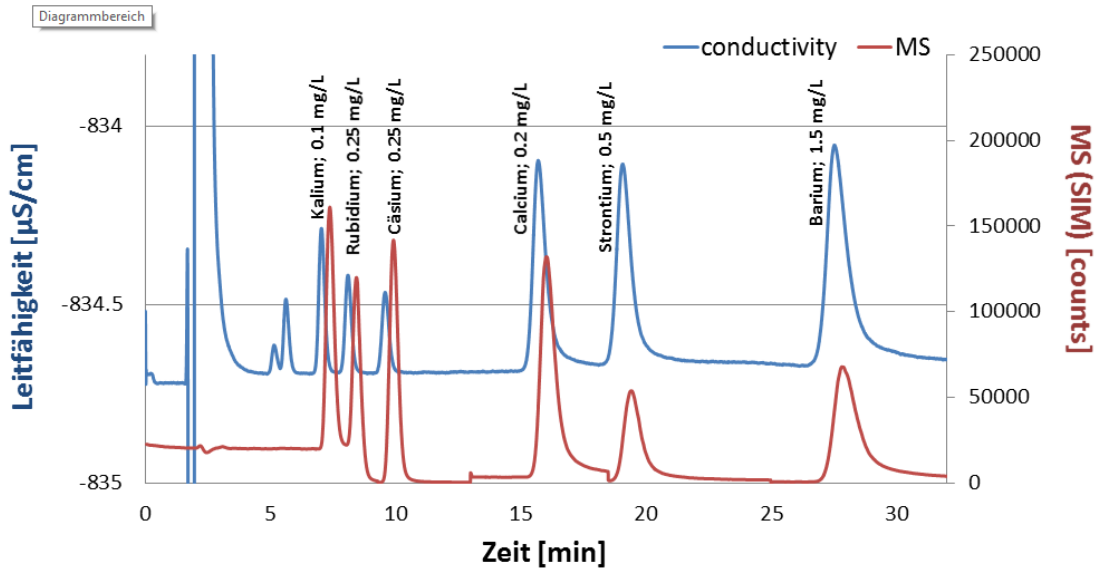
Suppression: –

Temperature: 30 °C

Loop: IC: 30 μL with MiPT
MS: 50 μL with MiPT

Flow rate: 0.2 mL/min

Eluent: 3 mmol/L oxalic acid



6 Troubleshooting

6.1 Regeneration



CAUTION

Do not regenerate the column as a preventive measure!

Each regeneration process causes stress on the separation column and reduces its service life *see "Regenerating separation columns", page 5.*

Problem

- The backpressure increases.
- Double peaks occur.
- Tailing effects occur.
- The retention times become shorter.
- The resolution deteriorates.

Correction

Regenerating the separation column

Start by replacing the guard column if the above problems occur. Only regenerate the separation column as described below if this measure does not help.

1 Disconnecting the separation column from the IC system

Disconnect the separation column outlet from the detector inlet.

2 Regenerating the separation column

Depending on the type of contamination, regenerate the separation column as follows:

- Contamination with organic components (*see table 4, page 46*).
- Contamination with inorganic components (*see table 5, page 46*).



Table 4 Contamination with organic components

	Rinse with	Duration [min]	Flow rate [mL/min]	
			2-mm columns	4-mm columns
1	Ultrapure water, opposite to flow direction	60	0.2	0.9
2	Acetonitrile/water (40/60), opposite to flow direction	60	0.2	0.9
3	Ultrapure water	60	0.2	0.9

Table 5 Contamination with inorganic components

	Rinse with	Duration [min]	Flow rate [mL/min]	
			2-mm columns	4-mm columns
1	Ultrapure water, opposite to flow direction	30	0.2	0.9
2	10 mmol/L nitric acid and 4 mmol/L dipicolinic acid, opposite to flow direction	60	0.2	0.9
3	Ultrapure water, opposite to flow direction	30	0.2	0.9

6.2 Decreasing resolution / peak shapes

Problem

The resolution of peaks deteriorates or peak shapes are asymmetrical.

Causes and prevention

Causes

The separation column has been overloaded.

Prevention/correction

The separation column can be overloaded by factors such as a high salt content in the sample matrix.

- Dilute the sample.
- Inject less sample.



Causes	Prevention/correction
There are dead volumes in the IC system.	<ul style="list-style-type: none"> ▪ Check that all of the capillaries have a diameter of ≤ 0.25 mm (6.1831.010). If not, replace the larger capillaries. ▪ Check that all of the capillaries have been installed correctly. The step-by-step installation process is shown in the IC Maintenance multimedia guide.

6.3 Unstable retention times

Problem

The retention times are unstable.

Causes and prevention

Causes	Prevention/correction
Air bubbles in the eluent	<p>Air bubbles make the eluent flow rate unstable. Backpressure is one indicator of an unstable flow rate. Backpressure should remain stable within ± 0.1 MPa.</p> <ul style="list-style-type: none"> ▪ Deaerate the high-pressure pump. ▪ Use the eluent degasser.

6.4 Unknown peaks

Problem

The chromatogram contains wide, unknown peaks.

Causes and prevention

Causes	Prevention/correction
Analytes eluting late	<p>Some wider, unknown peaks can be the result of sample components eluting late. In these cases, this is the result of the previous injection.</p> <ul style="list-style-type: none"> ▪ Extend the chromatogram duration.



6.5 Increasing backpressure

Problem

The backpressure increases.

Causes and prevention

Causes	Prevention/correction
Particles on the guard column	<ul style="list-style-type: none"> ▪ Replace the guard column.
Particles on the separation column	<p>Rinse the separation column in the direction opposite to the flow direction.</p> <ul style="list-style-type: none"> ▪ Hold the column outlet in a beaker. ▪ Rinse the separation column for approximately 1 h. ▪ Install the separation column back in the flow direction.
Particles in the sample	<ul style="list-style-type: none"> ▪ Sample preparation, e.g. removing particles through Inline Ultrafiltration.

7 Literature

We recommend the following literature for more detailed information:

- Application Note C-119 Bethanechol chloride and calcium in tablets
- Application Note C-120 Bethanechol chloride and HPTA (2-hydroxypropyl-trimethyl ammonium chloride) besides sodium and calcium
- Application Note C-124 Ethanolamines besides standard cations
- Application Note C-125 Methylamines besides standard cations including cesium
- Application Note C-126 Methylamines and ethanolamines
- Application Note C-129 Nine cations on the Metrosep C 4 – 150 column
- Application Note C-132 Traces of lithium, sodium and ammonium besides ethanolamine
- Application Note U-52: Transition metal cations applying UV/VIS detection after post-column reaction with PAR (4-(2-pyridylazo)resorcinol)
- Application Note C-135 Cations in drinking water using Metrosep C 4 - 150/4.0 column according to ISO 14911
- Application Note C-147 Fast analysis of cations in tap water using Metrosep C 4 - 100/2.0
- Application Note C-148 Metrohm Inline Dilution Technique – get dilution factors up to 10,000 with two intelligent dilution steps
- Application Note C-150 Fast IC: separation of standard cations in eleven minutes
- Application Note C-151 Fast IC: separation of standard cations in five minutes
- Application Note C-152 Fast IC: separation of ethanolamines in 2.5 minutes
- Application Note C-153 Fast IC: separation of methylamines in four minutes
- Application Note C-157: Sodium and potassium in a polyol sample applying Inline Matrix Elimination
- Application Note C-159 Cation traces on the Metrosep C 4 - 250/2.0 column after Inline Preconcentration with matrix elimination
- Application Note C-160 Calcium in Bayer caustic soda
- Application Note C-167 Quality test of an automatic and direct 1:2000 dilution applying Inline Dilution Technique
- Application Note C-170 Separation of N-methyldiethanolamine and piperazine besides standard cations
- Application Note C-171 N-methyldiethanolamine and piperazine in scrubber solution - Hörler, M.; van der Kruijs, S. Improved cation separation thanks to a new column material, Poster



- Characterization and source apportionment of organic aerosol using offline aerosol mass spectrometry; Daellenbach, K.R.; Bozzetti, C.; Krepelova, A.; Canonaco, F.; Wolf, R.; Zotter, P.; Fermo, P.; Crippa, M.; Slowik, J.G.; Sosedova, Y.; Zhang, Y.; Huang, R.J.; Poulain, L.; Szidat, S.; Baltensperger, U.; Prevot, A.S.H.; El Haddad, I., Atmospheric Environment Techniques Discussions; p. 8599-8644, 08/2015
- Monograph: Analysis of water samples and water constituents with Metrohm instruments, p. 73 ff (8.038.5003)
- Column catalog, 8.000.5194

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