Monograph

Ion Chromatography

2nd Edition

Dr. Helwig Schäfer
Dr. Markus Läubli
Dr. Roland Dörig

Metrohm

Ion Chromatography

Theory
Columns and Eluents

Helwig Schäfer
Markus Läubli
Roland Dörig

Metrohm Monograph

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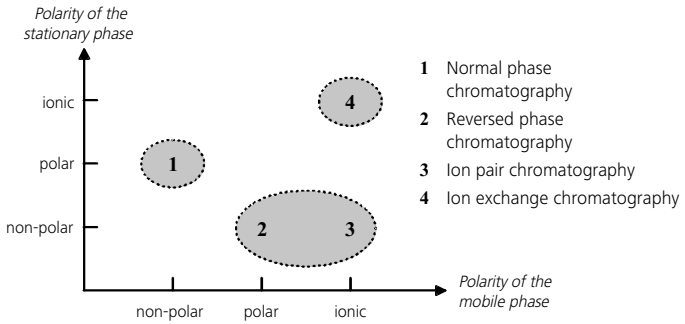
1 Theory of Ion Chromatography

1.1 Introduction

The term «chromatography» is the general name for a wide range of physicochemical separation processes in which the components to be separated are distributed between a stationary and a mobile phase. The classification of the various types of chromatography depends on the state of aggregation of these two phases:

Mobile phase	*Stationary phase*	
	liquid	*solid*
gaseous	GLC	GSC
liquid	LLC	LSC (HPLC)

Since the introduction of high pressure or high performance liquid chromatography (HPLC) at the end of the sixties, liquid chromatography has developed into one of the most comprehensive and important methods of modern instrumental analysis. Based on the polarity of the stationary and mobile phases, a distinction is made between the following methods:



Ion chromatography was first introduced in 1975 and within a short time has developed into an independent analytical technique that today encompasses all HPLC methods to determine inorganic or organic ions as well as polar substances. The combination of ion exchange columns and conductivity detection continues to represent the most important type of ion chromatography, and two different techniques are used in practice.

In the technique with chemical suppression, the background conductivity is suppressed chemically. In contrast, the direct chromatographic technique (non-suppressed ion chromatography) employs eluents with salts of organic acids in low concentration on ion exchangers of very low capacity to achieve a relatively low background conductivity, which can be suppressed directly by electronic means. The following sections deal with both techniques that have also been implemented in the actual Metrohm IC systems.

1.2 Ion exchange as a separation mechanism

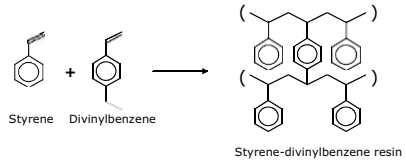
The vast majority of ion chromatographic separations occur by ion exchange on stationary phases with charged functional groups. The corresponding counter ions are located in the vicinity of the functional groups and can be exchanged with other ions of the same charge in the mobile phase. For every ion, the exchange process is characterized by a corresponding ion exchange equilibrium, which determines the distribution between the mobile and stationary phase, for example in the case of an anion A^-:

$$E^-_{stat} + A^-_{mob} \leftrightarrow A^-_{stat} + E^-_{mob}$$

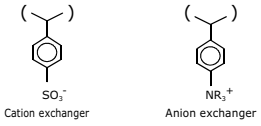
$$K_A = \frac{[A^-]_{stat} \times [E^-]_{mob}}{[A^-]_{mob} \times [E^-]_{stat}}$$

K_A: equilibrium constant
A: sample anion
E: eluent anion (counter ion)

The various ionic components of a sample can thus be separated based on their different affinities for the stationary phase of the ion exchanger (different equilibrium constants *K*). The most important group of ion exchangers is that of the organic materials based on synthetic resins. A copolymer of styrene and divinylbenzene is frequently used as the support.



Cation exchangers are obtained by subsequent sulfonation of this styrene- divinylbenzene resin, anion exchangers by chloromethylation followed by amination.

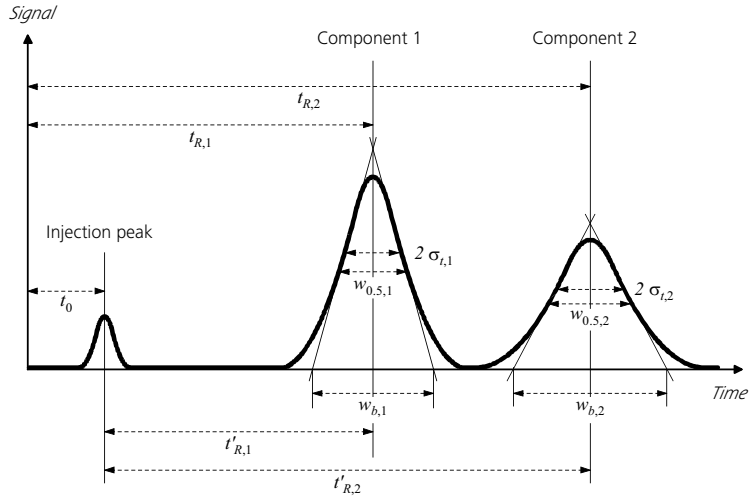


In addition to these resins, other polymers and silica gels with chemically bound phases are also employed. Whereas the classical ion exchange is performed on macroporous particles of a high exchange capacity with a size of 75…250 µm in the batch or column process, in modern ion chromatography, ion exchange materials of low capacity with particle sizes of 3…10 µm are employed. This makes it possible to separate and detect anions, cations, carbohydrates, and polar substances efficiently and rapidly with eluents of low concentration.

1.3 Chromatographic characteristics

1.3.1 Retention time and peak width

The elution curve (signal vs time) following a chromatographic separation is called a **chromatogram**. It generally has the following appearance:



The time parameters of the resulting bands (peaks) characterize the chromatogram and are illustrated in the Figure:

t_0	**Dead time**	time needed by the mobile phase to flow through the separation system
t_R	**Retention time**	time needed by an injected substance until its concentration maximum appears at the end of the separation system
t'_R	**Net retention time**	= retention time t_R – dead time t_0
σ_t	**Standard deviation**	half peak width at the inflection points
$w_{0.5}$	**Peak width at half height**	= 2.354 σ_t
w_b	**Peak base width**	= 4 σ_t

The time parameters t_0, t_R and t'_R can be converted into the dead volume V_0, *retention volume* V_R and *net retention volume* V'_R using the constant flow rate. If symmetric elution profiles are found, the shape of the chromatographic peaks can be described with sufficient accuracy by a Gaussian curve. The width of such a Gaussian-shaped peak is determined from the chromatogram as standard deviation σ_t, width at half height $w_{0.5}$ or base width w_b.

1.3.2 Capacity factor k'

The **retention time t_R** is the qualitative information of a chromatogram. It is constant for a given component provided the chromatographic conditions remain unchanged (column, mobile phase, temperature, etc.). For the characterization of a substance, it is more convenient to quote the capacity factor *k'* since, in contrast to the retention times, this is dependent neither on the flow of the eluent nor on the column length:

$$k' = \frac{t'_R}{t_0} = \frac{t_R - t_0}{t_0} = \frac{t_R}{t_0} - 1$$

Low values of *k'* signify that the corresponding ions are eluted near the injection peak and the separation is consequently very poor. Capacity factors between 1 and 5 are optimum in practice; larger *k'* values lead only to peak broadening, lower detection sensitivity and long analysis times.

1.3.3 Selectivity α

Two substances are separated only if they have different *k'* values. A measure of the separation efficiency of a chromatographic system is the ***selectivity α*** (also known as relative retention):

$$\alpha = \frac{k'_2}{k'_1} = \frac{t_{R,2} - t_0}{t_{R,1} - t_0} \qquad (k'_2 > k'_1)$$

1.3.4 Plate number N

An additional useful quantity to characterize a separation system is the **plate number *N*** (number of theoretical plates). A theoretical plate is defined as that zone of a separation system within which a thermodynamic equilibrium is established between the mean concentration of a component in the stationary phase and its mean concentration in the mobile phase. If a Gaussian peak shape can be assumed, the plate number *N* for peaks with a relatively large retention time can be calculated as follows with the aid of the parameters retention time and peak width read off from the chromatogram:

$$N = \left(\frac{t_R}{\sigma_t}\right)^2 = 5.54\,\left(\frac{t_R}{w_{0.5}}\right)^2 = 16\,\left(\frac{t_R}{w_b}\right)^2$$

1.3.5 Resolution R

A measure of the quality of the separation actually found in practice is the **resolution *R*** between neighboring peaks:

$$R = \frac{2\left(t_{R,2} - t_{R,1}\right)}{w_{b,1} + w_{b,2}} = \frac{1.177\left(t_{R,2} - t_{R,1}\right)}{w_{0.5,1} + w_{0.5,2}}$$

If the peak base width $w_{b,1}$ and $w_{b,2}$ are approximately the same, the resolution R signifies the number of times the peak width w_b can be fitted into the distance between the peak maxima. At a resolution of R = 0.5, two maxima can still be perceived separately. For quantitative analysis, a minimum resolution of R = 1.5 is desirable; greater values of the resolution lead only to unnecessarily long analysis times.

The resolution R is dependent on the parameters k_2' (capacity factor of the later eluted substance), selectivity α and plate number N of the column:

$$R = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k_2'}{1 + k_2'}$$

There are thus three possibilities to improve the resolution of two peaks:

- **Altering the *k'* values:**
  The capacity factors of the individual components are dependent essentially on the elution strength of the mobile phase, i.e. changing the concentration and ionic strength of the eluent gives rise to other k' values. Here, however, the capacity of the column and frequently also the detector are limiting factors.

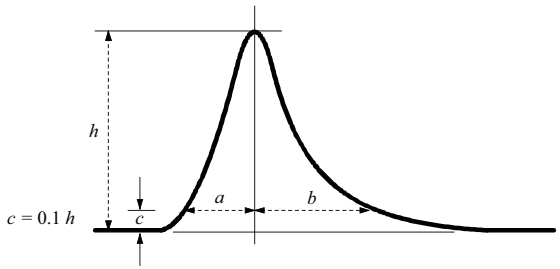
- **Increasing the plate number** *N:*
  The more plates a column possesses, the greater the resolution, but the retention times are also longer thereby increasing the analysis time. The plate number increases with increasing efficiency and length of the column. With packing materials of particle size 10 µm or greater, the plate number increases with decreasing flow.

- **Increasing the selectivity *α*:**
  The most effective possibility to improve the resolution involves increasing the relative retention ***α*** by using a different column better suited to the separation problem or by changing the composition of the eluent. However, this is sometimes extremely difficult or very time intensive.

If a given column and one particular eluent have to be used, the plate number N is the controlling influence with regard to the resolution and hence the quality of the chromatogram. For a given column length, the plate number N depends only on the quality of the packing material and the packing itself.

1.3.6 Asymmetry factor T

The elution of chromatographic signals as Gaussian peaks is often not achieved in practice. An asymmetric peak shape, known as tailing, is often found.



The peak asymmetry is quantified by the **asymmetry factor** (tailing factor) ***T*** with a and b being determined at 10% peak height:

$$T = \frac{b}{a}$$

For trouble-free evaluation of the area of a peak, T must be < 2.5, above this, the end of the peak can be recognized only with difficulty. Tailing can have many causes:

- **Dead volume**
  Dead volumes between injector and detector lead to peak broadening and tailing. The asymmetry is more pronounced with peaks eluted earlier than with those eluted later, and the tailing increases with the flow.

- **Column overloading**
  If too much substance has been injected, i.e. the maximum loading of the column has been exceeded, broadened peaks with severe tailing are obtained. Overloading is recognized by a lowering of the capacity factor by more than 10%.

- **Chemical effects**
  The dominant separation mechanism is adversely influenced by another mechanism, e.g. adsorption phenomena in ion-exchange chromatography. The lower the flow, the more evident this tailing.

1.4 Ion chromatographic separations

The chromatographic parameters retention time, peak shape, dead time and selectivity are the results of the whole chromatographic system, i.e. alterations to the column, the eluent or the flow rate inevitably lead to alterations of the chromatographic parameters. This is why test chromatograms are normally included with the separation columns to demonstrate that the separation behavior described can be achieved under exactly defined conditions.

The following sections contain several useful information about the optimization of the column, eluent and flow rate parameters with reference to analytical problems which may be encountered.

1.4.1 Dependence of the separation on the column material

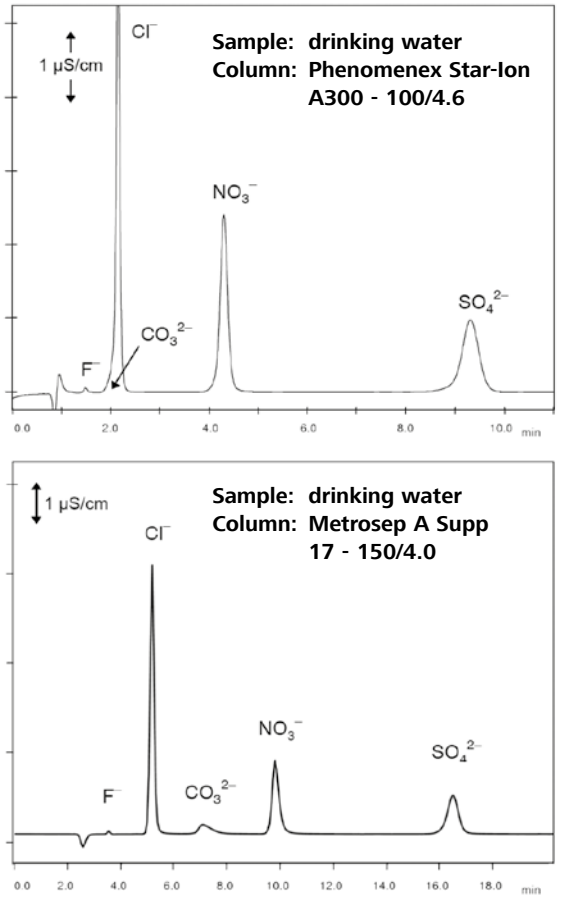
The separation behavior of an ion exchange column is basically determined by the ion exchanger groups bound to the support material (under otherwise identical chromatographic conditions). However, interactions of the analytes with the support material also occur: these are considerably stronger for organic ions than for inorganic ions. For this reason, it is a good idea to use columns with different support materials to solve an analytical problem.

The following are the main support materials used today for separation columns:

- polystyrene/divinylbenzene copolymer
- polymethacrylate
- polyvinyl alcohol
- coated silica gel

The use of suitable combinations of support materials and ion-exchanger groups allows optimal conditions to be provided for special separation problems. Even interferences such as those caused by the eluent or, for example, by the carbonate content in water samples can be avoided by the selection of a suitable column as is shown in the following illustrations (Figure 2a and 2b). Whereas with the first column the carbonate peak and the chloride peak overlap, on column 2 a separation of carbonate and chloride is obtained.

These effects are far stronger in the separation of organic anions. In such cases it is also a good idea to test separation columns with different combinations of support materials and ion-exchanger groups.



1.4.2 Influence of the eluent composition on the separation

During the separation of ions by means of ion-exchanger columns small ions are eluted ahead of larger ions and monovalent ions ahead of di- and trivalent ions; i.e. for anions the following sequence is obtained: fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate, arsenate, selenate, etc. When carbonate eluents are used (pH 9…10; mixture of Na_2CO_3 and $NaHCO_3$) the ions are eluted in the same sequence. If the pH is shifted towards higher values then dissociation of phosphate and arsenate occurs: HPO_4^{2-} to PO_4^{3-} and $HAsO_4^{2-}$ to AsO_4^{3-}. There is thus an increase in the retention time which means that by increasing the Na_2CO_3 share of the eluent both phosphate and arsenate can be shifted in ion chromatograms so that, for example, phosphate is only eluted when sulfate has already been eluted.

In principle, retention times can be shifted by the addition of ligands to the eluent for the determination of cations. The more selective the ligand, the less the retention times of the

other cations are influenced. This effect is used in particular for the separation of the complex-forming metals, the transition metals.

However, ligands can also be used to obtain better separation of alkali metal ions. The addition of crown-ether, for example, leads to a slightly better separation of sodium, ammonium and potassium, as can be seen in the following illustrations. Potassium is primarily influenced as this ion fits perfectly into the cavity of 18-crown-6. Especially in amine determinations, this allows to move potassium away from short-chain amines.

The complexation of potassium with 18-crown-6 does not change the charge. The complex has a charge of +1 but is larger than potassium itself. Therefore, the complex is eluted at later retention times. With higher crown-ether concentrations the elution takes longer according the rate of complexed potassium.

a) Example of the influence of 18-crown-6 to the retention of potassium.

Table 1 Parameters – crown-ether

Column	6.1050.420 Metrosep C 6 - 150/4.0
Eluent	a) 1.7 mmol/L nitric acid / 0.7 mmol/L dipicolinic acid b) 1.7 mmol/L nitric acid / 0.7 mmol/L dipicolinic acid / 0.1 mmol/L crown-ether Conductivity approx. 630 µS/cm

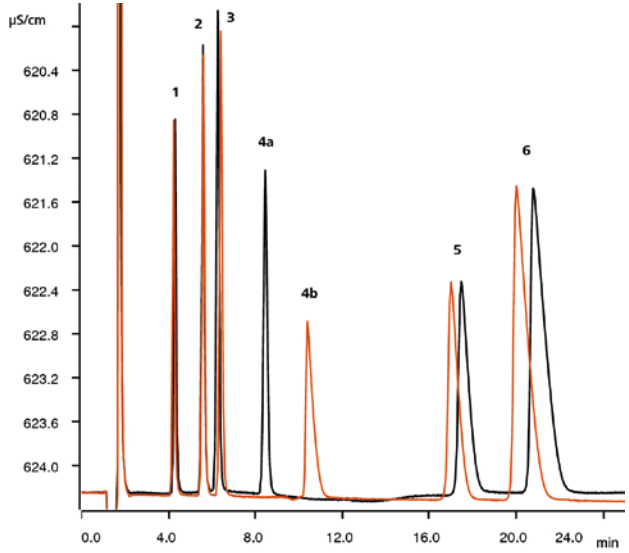


Figure 3: Overlay of a chromatogram without crown-ether (black) and one with crown-ether (red); 1 lithium, 2 sodium, 3 ammonium, 4 potassium, 5 calcium, 6 magnesium

Table 2 Components – crown-ether

Peak	Component	T_R [min]	T_R [min] (18-crown-6)	Conc. [mg/L]
1	Lithium	4.31	4.25	10
2	Sodium	5.60	5.61	10
3	Ammonium	6.28	6.42	10
4	Potassium	8.46	10.39	10
5	Calcium	17.47	17.00	10
6	Magnesium	20.78	20.00	10

b) Example of the influence of dipicolinic acid to calcium and zinc.

In case of dipicolinic acid (2,6-pyridine dicarboxylic acid) a different effect is achieved. Dipicolinic acid complexes many divalent cations. Typically, neutral complexes are built. Therefore, divalent cations are eluted at earlier retention times when dipicolinic acid is present in the eluent. The amount of acceleration depends on the complex building constant of the respective cation complex. The example shows the effect to magnesium, calcium, and zinc.

The transition metal zinc is much stronger complexed with dipicolinic acid then calcium or magnesium. As a result it is much stronger influenced by already a low concentration of the complexing agent. In chromatogram b) it is already eluted ahead of lithium and totally complexed and eluted in the injection peak in c). Calcium is weakly complex but stronger than magnesium. In chromatogram b) the resolution of magnesium and calcium is reduced. While with eluent c) calcium is already eluted ahead of magnesium. This is applied to reduce the run time of determinations analyzing calcium and magnesium besides alkali metal cations.

Table 3 Parameters – Dipicolinic acid

Column	6.1050.420 Metrosep C 6 - 150/4.0
Eluent	a) 1.7 mmol/L nitric acid Conductivity approx. 500 µS/cm b) 1.7 mmol/L nitric acid / 0.1 mmol/L dipicolinic acid Conductivity approx. 527 µS/cm c) 1.7 mmol/L nitric acid / 0.7 mmol/L dipicolinic acid Conductivity approx. 650 µS/cm

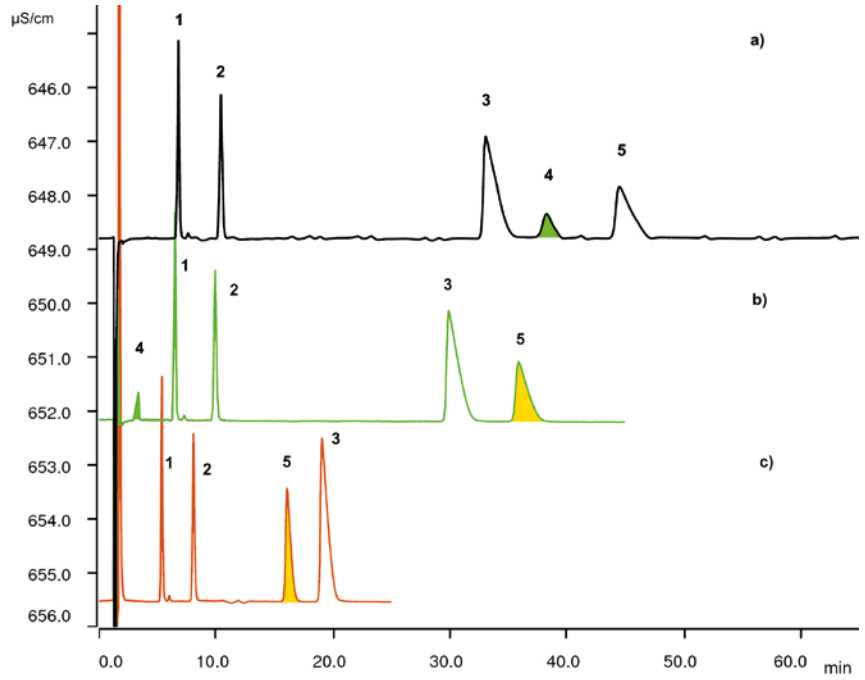


Figure 4: Chromatograms with different amounts of dipicolinic acid in the eluent: a) 0 mol/L, b) 0.1 mmol/L, c) 0.7 mmol/L

Table 4 Components – dipicolinic acid

Peak	Component	T_R [min] (a)	T_R [min] (b)	T_R [min] (c)	Conc. [mg/L]
1	Sodium	6.79	6.50	5.39	10
2	Potassium	10.42	9.93	8.08	10
3	Magnesium	33.05	29.90	19.05	10
4	Zinc	38.24	3.38	–	10
5	Calcium	44.48	35.87	16.08	10

1.4.3 Interferences in the chromatogram: system peak

Ionic eluents are normally used in ion chromatography (*see section 1.2*); these compete with the analyte ions at the ion exchanger. For these eluent anions, e.g. carbonate, phthalate or benzoate, the same chromatographic conditions apply as for all other ions, i.e. there is also a defined retention time for the eluent anions in each chromatographic system.

When the sample is injected into the chromatographic system the eluent flow is interrupted by the sample (water with low analyte contents), i.e. there will be a negative amount of eluent ions compared with the eluent concentration injected. This must lead to a negative peak in the ion chromatogram with the retention time of the eluent anion. This dip must not be confused with the water peak, which is obtained at the start of the chromatogram (dead time).

Such peaks which can be allocated to the eluent anions are actually obtained; these are generally known as system peaks. These system peaks not only appear when the eluent is diluted, but also for each chemical reaction of the eluent ions with the sample. Examples of this are alterations to the pH and thus the shift in the dissociation equilibrium or reactions of metal ions with the eluent ion (e.g. Ca^{2+} with CO_3^{2-}).

The system peaks are most apparent in direct ion chromatography (non-suppressed) as here measurements are made at a high conductivity level and small differences in the ionic strength of the eluent are extremely noticeable.

In contrast, in ion chromatography with suppression measurements are carried out at a low conductivity level and the system peak is thus considerably smaller. Previously the carbonate system peak has been deliberately ignored as it often lies in the area of the chloride peak. Especially, on polystyrene/divinylbenzene columns which were often used and all samples that contain sufficient chloride to obscure the carbonate peak. Since acrylate columns have been used it has been clearly seen that the carbonate system peak is present and that this presence can lead to false results (see Figure 2a).

In particular, in the analysis of natural waters with varying carbonate contents the total of carbonate + chloride has usually been determined as chloride.

1.5 Conductivity detection

1.5.1 General

As a universal method to determine ionic components, conductivity measurement (conductometry) occupies a central position in ion chromatography. Conductometry is defined as the ability of electrolyte solutions in an electric field applied between two electrodes to transport current by ion migration. The relationship between the applied voltage *U* and the current *I* is given by Ohm's law:

$$R = \frac{U}{I} \qquad [\,S\,]$$

U: voltage [V]
I: current [A]

The reciprocal of the ohmic resistance *R* is the conductance *G*, which has the unit Siemens:

$$G = \frac{1}{R} \qquad [\,S\,]$$

The conductance of an electrolyte solution depends on the electrode surface *A* and the interelectrode distance l. The usual measured variable in conductometry is thus the **conductivity** κ:

$$\kappa = G \times K_c \qquad [\,S\ cm^{-}$$

$$K_c = \frac{l}{A} \qquad [\,cm^{-1}\,]$$

The quotient *l/A* is known as the **cell constant** K_c, this can usually not be calculated directly but is determined with calibration solutions. The dependence of the electrical conductivity κ on the type and concentration of the dissolved ion can be described as follows:

$$\kappa = \frac{\Lambda_\infty \times c \times z}{1000}$$

Λ: equivalent conductivity [S cm² mol⁻¹]
c (eq): equivalent amount-of-substance concentration [mol/1000 cm³]; *c(eq)* = *c* × *z*
c: amount-of-substance concentration [mol/1000 cm³]
z: valency

For multi-ion solutions, the individual ion concentrations need to be taken into account. The total conductivity of a solution is the sum of all single ion conductivity:

$$\kappa = \sum_{i=1}^{n} \frac{(\Lambda_{\infty,i} \cdot c_i \cdot z_i)}{1000}$$

The conductivity thus increases with increasing electrolyte concentration. This linear relation holds only for dilute solutions, however, since the equivalent conductivity Λ is itself dependent on concentration in accordance with Kohlrausch's law:

$$\kappa = \Lambda_\infty - A\sqrt{c(eq)}$$

Λ_∞: equivalent conductivity in an infinitely dilute solution
A: constant

The ionic conductivities of anions and cations are usually between 35…80 S cm² mol⁻¹, the only exceptions being H^+ and OH^- ions on account of their very high mobilities of 350 and 198 S cm² mol⁻¹, respectively. With the aid of the tabulated ionic conductivities, the conductivity of a pure solution for dilute, aqueous electrolyte solutions can be calculated in advance with considerable accuracy.

In addition to the ionic species and ionic concentration, the temperature and polarity of the solvent also influence the electrical conductivity. The temperature dependence of 2…10 %/°C is very pronounced.

Equivalent ionic conductivities for infinite dilution in aqueous solutions at 25 °C

Table 5 Equivalent conductivities of ions

Anions	Λ^{-}_{∞} **[S cm² mol^{-1}]**	**Cations**	Λ^{+}_{∞} **[S cm² mol^{-1}]**
OH^-	198	H^+	350
F^-	54	Li^+	39
Cl^-	76	Na^+	50
Br^-	78	K^+	74
I^-	77	NH_4^+	73
NO_2^-	72	½ Mg^{2+}	53
NO_3^-	71	½ Ca^{2+}	60
HCO_3^-	45	½ Sr^{2+}	59
½ CO_3^{2-}	72	½ Ba^{2+}	64
$H_2PO_4^-$	33	½ Zn^{2+}	53
½ HPO_4^{2-}	57	½ Hg^{2+}	53
1/3 PO_4^{3-}	69	½ Cu^{2+}	55
½ SO_4^{2-}	80	½ Pb^{2+}	71
SCN^-	66	½ Co^{2+}	53
Acetate	41	1/3 Fe^{3+}	70
½ Phthalate	38	$N(Et)_4^+$	33
Propionate	36		
Benzoate	32		
Salicylate	30		

1.5.2 Direct ion chromatography

In ion chromatography without suppression (direct ion chromatography), very high demands are made on the conductivity detector since the background conductivity which must be compensated is large in comparison with the measuring signal. Of prime importance for the measurement is the constancy of the background. Since the conductivity is, as mentioned above, greatly dependent on the temperature, an extremely good temperature constancy of the eluent in the conductivity measuring cell is required (≤ 0.001 °C).

The **sensitivity** of the conductivity measurement depends on the difference between the equivalent ionic conductivities of sample ion (a) and eluent ion (e):

$$\Delta\kappa \propto c \cdot (\Lambda_a + \Lambda_e)$$

$\Lambda_a > \Lambda_e$: positive peak
$\Lambda_a = \Lambda_e$: no peak
$\Lambda_a < \Lambda_e$ negative peak

In anion determinations, salts of phthalic, salicylic or benzoic acid are usually employed since these possess a low equivalent ionic conductivity (see Table 5 on page 22). If now an anion with a higher equivalent ionic conductivity appears in the detector cell, the conductivity increases and a positive peak is obtained. On the other hand, negative peaks appear if the equivalent ionic conductivity of the sample ion is lower than that of the eluent ion.

An example of this is phosphate that, for example, in 2 mmol/L phthalic acid at pH = 5.0, is present as $H_2PO_4^-$. Since Λ^{-}_{∞} ($H_2PO_4^-$) = 33 is lower than Λ^{-}_{∞} (phthalate) = 38, an insensitive negative peak is obtained under these conditions (corrective measure: higher pH since Λ^{-}_{∞} ($H_2PO_4^{2-}$) = 57). The sensitivity Δκ for anions is in general 0.1…0.5 µS/cm per 1 mg/L (= 1 ppm).

With alkali and alkaline earth metal cations, chromatography is generally performed with dilute acids such as 2 mmol/L HNO_3 as eluent. Since the proton has an exceptionally high equivalent ionic conductivity, as a result of its special migration mechanism, the conductivity sinks drastically as soon as other cations replace the H+ ions. Generally, negative peaks are obtained which are very sensitive owing to the high Δκ value (in general 1…10 µS/cm per 1 mg/L).

The lower limit of the sensitivity depends on the detector noise, which lies between 0.1…10 nS/cm. This noise is determined essentially by the quality of the high pressure pump and the background conductivity. If the detection limit is defined as the signal height at a signal/noise ratio S/N = 3, it has the value 2…50 µg/L for anions and 1…10 µg/L for cations.

1.5.3 Ion chromatography with suppression

1.5.3.1 Chemical suppression for anions

In suppressed ion chromatography the background conductivity is removed by the suppression reactions. In the following sensitivity estimations, the background conductivity is neglected. As the background is chemically removed the measured conductivity is proportional to the sum of the equivalent conductivities of the sample ion and the introduced counter ion as shown below:

$$\Delta\kappa \propto c \cdot \left(\Lambda_{sample\ ion} + \Lambda_{counter\ ion}\right)$$

Chemical suppression of anion chromatography is based on the use of the salts of weakly dissociating acids (e.g. $NaHCO_3$) as eluents. These eluents can be eliminated to a large extent by cation exchange in a post-column reaction according to the following equation.

$$Na^+ + HCO_3^- \xrightarrow[-Na^+]{+H^+} H_2CO_3$$

The carbonic acid formed as a result of cation exchange is very weakly dissociated and thus exhibits a very low residual conductivity. The sample anions undergo a corresponding reaction, shown here with the chloride anion as an example:

$$Na^+ + Cl^- \xrightarrow[-Na^+]{+H^+} HCl$$

NaCl is converted to the corresponding free acid by the suppression; this has a considerably higher conductivity than the original salt. Of course, this only applies for stronger acids; for medium-strong and weak acids the increase in sensitivity is correspondingly lower. The signal to be measured is thus the sum of Λ^-_∞ (Cl^-) and Λ^-_∞ (H^+) against a low background conductivity.

For reasons which up to now are not completely clear the calibration function of this system is not linear with the concentration. This means that for calibration a considerably higher expenditure is required.

The eluent after chemical suppression contains carbonic acid, which is present in an equilibrium of hydrogen carbonate – carbonic acid – dissolved CO_2.

$$HCO_3^- + H^+ \leftrightarrows H_2CO_3 \rightleftarrows CO_2 + H_2O$$

The dissociated carbonic acid yields in a background conductivity of approx. 15 µS/cm. This level works well for most application.

During a peak the pH changes due to the increasing concentration of H^+, hence e.g. chloride is eluted as HCl. This pH change induces a decreasing baseline as the hydrogen carbonate – carbonic acid equilibrium is pushed towards carbonic acid. The effect is schematically shown in Figure 5. The calculated baseline is the straight line, but the real baseline shows a small negative peak. The negative peak area is not taken into account for the quantification of the respective component. This increases the deviation from linearity of the calibration curve. In most cases it is therefore recommended to apply quadratic calibration.

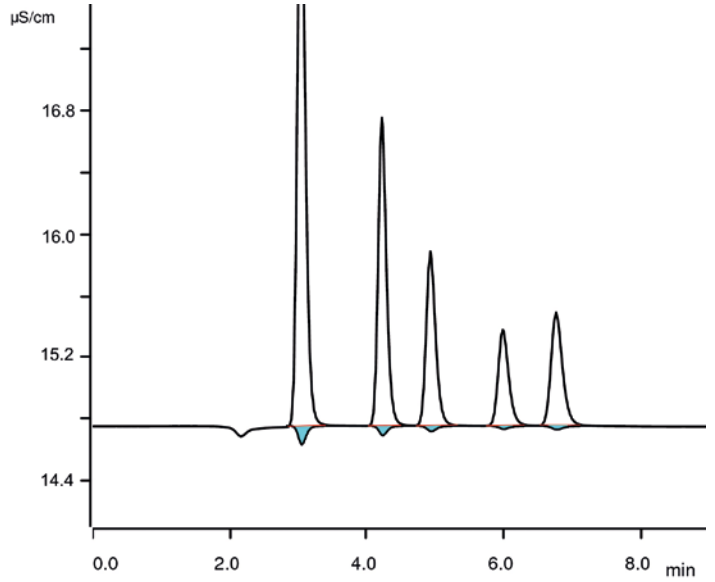


Figure 5: Chromatogram with chemical suppression. The blue area is not taken in to account in the quantification. Negative peaks: real baseline due to pH change

1.5.3.2 Sequential suppression for anions

Sequential suppression stands for the combination of chemical suppression and CO_2-Suppression. The Metrohm CO_2-Suppressor removes CO_2 from the eluent after chemical suppression and before detection. This shifts the equilibrium carbonic acid/CO_2 towards dissolved CO_2. Applying sequential suppression reduces the background conductivity to < 1 µS/cm this corresponds very much to that of ultrapure water.

As an effect of sequential suppression the water dip as well as the system peak (carbonate peak) is reduced dramatically. The first allows easier integration of early eluting peaks (see Figure 6). The latter reduces the interference and disturbance of peaks of interest. There are no negative baseline peaks and the linearity is improved. Nevertheless, it is still recommended to apply quadratic curve fit for a calibration range of more than 1 order of magnitude.

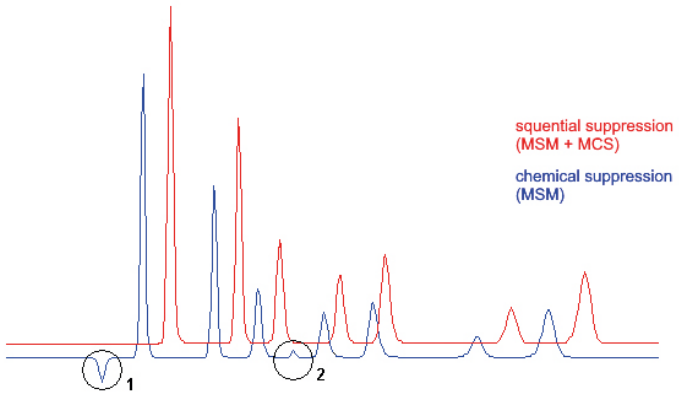


Figure 6: Overlay of a chromatogram of standard anions with chemical suppression (MSM, blue) and a chromatogram of the same standard applying sequential suppression (MSM+ MCS, red). The water dip (1, injection peak) and the system peak (2, carbonate peak) are no longer available with sequential suppression.

1.5.3.3 Chemical suppression for cations

Chemical cation suppression (mainly performed on membrane-based suppressors) is based on the use of a strong acid eluents (e.g. nitric acid). The eluent anions are removed by ion exchange in a post-column reaction according to the following equation.

$$H^+ + NO_3^- \xrightarrow[-NO_3^-]{+OH^-} H_2O$$

Water (H2O) is formed which exhibits a very low residual conductivity. The sample cation undergoes the respective reaction, described here for sodium.

$$Na^+ + NO_3^- \xrightarrow[-NO_3^-]{+OH^-} NaOH$$

Sodium nitrate is converted to sodium hydroxide by suppression; this has a higher conductivity than the original salt. This of course for strong bases, for medium to weak bases the signal increase is lower. In the alkaline medium of such peaks, solubility of multivalent cations often is compromised and no peaks can be found at all. Compared to non-suppressed cation analysis the signal after suppression is typically smaller. However, for trace analysis this method has an advantage due to the higher baseline noise of non-suppressed.

1.5.3.4 Sequential suppression for cations

Metrohm applies sequential suppression for suppressed cation chromatography. In this technique the chemical suppression step is the conversion of the eluent into carbonic acid according to the equation below.

$$H^+ + NO_3^- \xrightarrow[-NO_3^-]{+HCO_3^-} H^+ + HCO_3^- \leftrightarrows H_2CO_3 \rightleftarrows CO_2 + H_2O$$

Applying the CO_2-degasser as in sequential anion suppression converts the eluent to water as well. For the sample cation the respective equation is shown below.

$$Na^+ + NO_3^- \xrightarrow[-NO_3^-]{+HCO_3^-} Na^+ + HCO_3^-$$

The cations are converted to their hydrogen carbonate form. Within the peak, the pH is close to neutral or slightly acid which increases the solubility of divalent cations.

CO_2-suppression has a very high efficiency. Nevertheless, there is a small amount of hydrogen carbonate remaining. To avoid loss of sensitivity in the lowest concentrations, a small amount of e.g. rubidium nitrate is added to the eluent. The remaining hydrogen carbonate is eluted as rubidium hydrogen carbonate. This provides stable background conductivity independent of whether analytes are present or not.

The reactions that take place in the Metrohm CO_2 Suppressor (MCS) are given below (Figure 7).

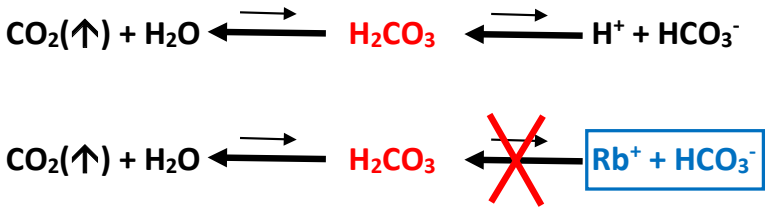


Figure 7: Reactions of CO_2 suppression in sequential cation chromatography

With sequential cation suppression, the residual conductivity is below 0.1 µS/cm. This allows detection limits below 1 µg/L.

Due to the simplicity of a non-suppressed cation system, it is recommended to apply suppressed cation chromatography for trace analysis only.

1.5.4 Comparison in the sensitivities of conductivity detection with and without chemical suppression

For **anions** the relationships mentioned above can be used to determine the following sensitivities for conductivity detection with electronic and chemical suppression:

Anion	**Sensitivity with electronic background suppression** $(\Lambda^-_{sample}) - (\Lambda^-_{phthalate})$	**Sensitivity with chemical suppression** $(\Lambda^-_{sample}) + (\Lambda^+_H)$
Fluoride	16	404
Chloride	38	426
Nitrate	33	421
½ Sulfate	42	430
Acetate	3	391

Table 6: Sensitivity for anions without and with suppression

According to these calculations the sensitivity for anions is approximately 10 times higher than without suppression.

For **cations** the sensitivity with direct conductivity detection is already higher than with chemical suppression. The hydroxides of ammonium and the doubly charged cations also show a lower dissociation. Thus, chemical suppression has no advantages for cation chromatography in the range of approx. 0.05 mg/L and above.

Cation	**Sensitivity with electronic background suppression** $(\Lambda^+_{sample}) - (\Lambda^+_H)$	**Sensitivity with chemical suppression** $(\Lambda^+_{sample}) + (\Lambda^-_{OH})$
Lithium	– 311	237
Sodium	– 300	248
Potassium	– 276	272
Ammonium	– 277	271
½ Magnesium	– 397	251
½ Calcium	– 290	258

Table 7: Sensitivity for cations without and with suppression

1.5.5 Linearity of the calibration curve

An important parameter for analytical determination methods is the linearity of the calibration. If chemical suppression is used slightly curved calibration curves are usually obtained. In such cases the calibration values must be located as close as possible to the contents of the sample in order to obtain the most accurate results. In contrast, calibration curves obtained without chemical suppression remain linear for several orders of magnitude. An example of the differing linearity is shown in the following illustration.

Calibration curves for nitrate with and without chemical suppression

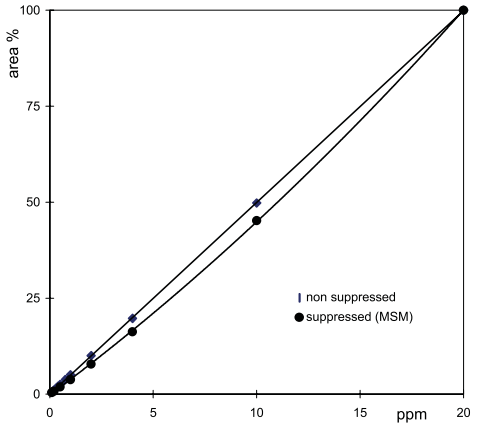


Figure 8 Nitrate calibration curves with and without suppression

These calibration curves indicate the slight non-linearity of suppressed ion chromatography. Chemically suppression exhibits larger effect than sequential suppression. Quantifications with suppression require therefore multipoint calibrations. Typically, a quadratic curve fit is recommended. If large calibration ranges (> 1…2 orders of magnitude) it recommended to apply high/low calibration, were the range is split into two or more sections. In this way, the deviation from linearity is smaller for each section and more accurate results are achieved.

1.6 Optical detection

1.6.1 General

In ion chromatography four different types of optical detection modes have been used up till now:

- **Direct UV-VIS detection**
  Direct UV-VIS detection is used as an addition to conductivity detection, e.g. for the determination of ions which absorb strongly in the UV range (nitrite, nitrate, organic anions) in the presence of high concentrations of inorganic ions (chloride, phosphate, sulfate), which either have no UV absorption or very little.

- **Indirect UV-VIS detection**
  Indirect UV-VIS detection finds universal application in direct ion chromatography for use with eluents with high UV absorption (e.g. phthalate buffers). Ions with no or very low UV activity show negative peaks and ions with a larger UV activity than the eluent show positive peaks.

- **UV-VIS detection with post-column derivatization**
  UV-VIS detection is partly used coupled with post-column derivatization for the detection of transitional metals (iron, nickel, copper, manganese, zinc...).

- **Fluorescence detection**
  In some special applications fluorescence detection is used for the highly sensitive detection of fluorescent compounds.

1.6.2 Direct UV-VIS detection

In direct UV-VIS detection the advantage of having different chromophore groups and thus very different detection sensitivities for the ions is used. The main area of application lies in the UV range, in which almost all organic ions absorb. The more chromophore groups (NO_2, NO_3, double bonds, phenyl, ketone, ...) which a molecule contains, the better the detection sensitivity.

The maximum absorption of the most important functional groups is as follows:

Functional group	$\Lambda_{max.}$ in nm
Amino	195
Aldehyde	210
Carboxyl	200
Ester	205
Ethylene	190
Ketone	195
Nitro	310
Phenyl	200

Table 8: Absorption maximum of functional groups

The very different detection sensitivity of the inorganic ions can be used with advantage for matrix reduction, particularly if the analytical samples have a high salt content. Chloride, sulfate and phosphate exhibit only very low UV absorption so that, for example, very low nitrite concentrations can be determined in the presence of large amounts of chloride.

In addition the use of a UV detector allows the determination of organic acids which coelute with sulfate as in this case only the organic acids, and not however the sulfate, are detected.

1.6.3 Indirect UV-VIS detection

In indirect UV-VIS detection an eluent with as high an absorption as possible is used so that the analytes reduce the detector signal. As already shown in section *1.5.2,* the eluent concentration in the region of a peak is reduced by the amount of the analyte concentration. This means that at the same UV absorption of analyte and eluent no detector signal will be received while lower analyte absorption will result in a negative signal and higher analyte absorption a positive signal.

Typical eluents for indirect UV-VIS detection are phthalate, benzoate, salicylate, various sulfonates and many others. The following table gives the optimum wavelengths and some detection limits for this type of detection:

Eluent	$\Lambda_{max.}$ in nm	Detection limit (mg/L)
Phthalate	300	0.1 – 1
Benzoate	254	0.1 – 1
Citrate	290	0.2 – 2

Table 9: Absortion maximum of eluents and the respective typical detection limits

1.6.4 UV-VIS detection with post-column derivatization

Post-column derivatization should only be used if no other possibility exists; in principle all chemical or mechanical manipulations of any sort should be avoided after the separation as such reactions always result in a deterioration in the separation efficiency and in particular of the peak shape.

One of the main uses of post-column derivatization are the determination of bromate, chromate and transition metals in ion chromatography. If direct conductivity detection is no longer possible (due to low response, too little separation, matrix effects) then a photochemical reagent and auxiliary solutions are added to the eluent stream after the separation column. After passing through a reaction spiral the optical absorption of the reaction product is measured.

1.6.5 Fluorescence detection

Direct fluorescence detection has virtually no applications in ion chromatography as the analytes are inactive with the exception of a few metal complexes with fluorescent ligands.

On the other hand, there are several eluents used in IC which are suitable for use in indirect fluorescence detection (e.g. salicylate).

1.6.6 UV-VIS multi-wavelength detection

The use of multi-wavelength detectors such as diode arrays or "fast scanning" detectors requires a considerably larger expenditure for the necessary calculations (PC programs) but produces far better interpretations for problematical analyses.

In particular for samples with peaks whose allocation is unknown certain statements about the structure of the ions under investigation can be made from the UV or VIS spectra obtained.

1.7 Electrochemical detection

Electrochemical detection has occasionally been used with a lot of success. A pre-requirement is that the ions to be determined must be able to be either reduced or oxidized. This includes many organic compounds, the transitional metals and anions such as nitrite, nitrate, halides, oxohalides, pseudohalides, sulfide, sulfite, etc.

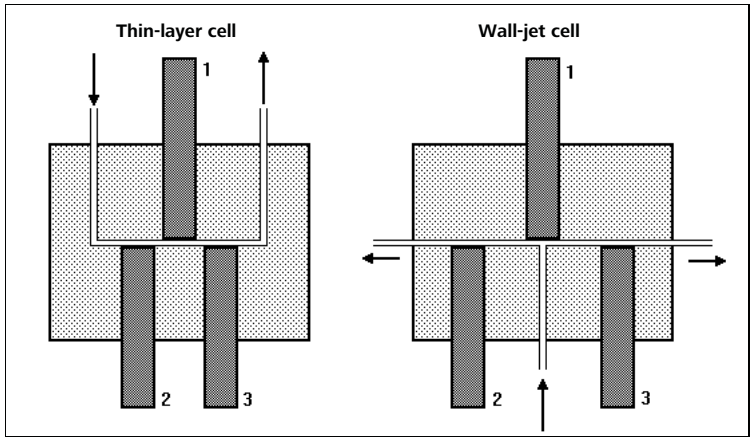
There are four different techniques:

Amperometry	Measurement of the current at a constant potential
Coulometry	Measurement of the current at a constant potential but with 100% conversion of the analyte
Voltammetry	Measurement of the current against potential in a defined range of potential
Pulsed amperometry	Measurement of the current at constant potential pulses

Amperometric (including pulsed) and coulometric detectors are most frequently used. The cells normally contain three electrodes:

- the **working electrode,** where the electrochemical reaction occurs,
- the **reference electrode** for currentless potential measurement and
- the **auxiliary electrode** as opposite pole to the working electrode.

There are two different types of cell, the thin-layer cell and the wall-jet cell, which are shown schematically in the following illustration.



1 Working electrode (WE)
2 Reference electrode (RE)
3 Auxiliary electrode (AE)

Figure 9: Schematic setup of a thin-layer and a wall-jet cell

The three electrodes are located in very different positions in various types of thin-layer cell. In the sequence in the direction of eluent flow all possible combinations can be found, e.g. WE first, then RE and finally AE or RE first, then WE followed by AE.

In the eluent flow is directed vertically onto the working electrode. Often the eluent flow is guided though the auxiliary electrode to the WE and the reference electrode is placed at the cell outlet.

The wall-jet cells are to be preferred to the thin-layer cells because of their greater sensitivity; however, after the WE there is a stronger mixing effect and turbulence in the eluent which is a disadvantage if a second detector has to be included.

For the working electrodes mainly carbon (graphite or glassy carbon), gold, platinum, silver and very occasionally mercury (voltammetric detector cells) are used as electrode materials. When selecting the electrode material it must be remembered that every material is also electrochemically reduced and oxidized, i.e. each material provides a different potential window. These application ranges depend to a great extent on pH, solvent, type of ion and ionic concentration of the electrolyte. In the negative potential range, the measuring range is limited by the electrolytic production of hydrogen from the protons of the solution and in the positive potential range by the electrochemical dissolution of the electrode material. The dissolution potential of the electrode depends very strongly on the anion of the electrolyte and on complex formers in particular. Approximate ranges for the various materials are given below:

Working electrode	**Potential range E**	
	acidic (pH = 1)	alkaline (pH = 13)
Ag	–0.55…+0.40 V	–1.20…+0.10 V
Au	–0.35…+1.10 V	–0.90…+0.75 V
Pt	–0.30…+1.20 V	–0.90…+0.65 V
Glassy carbon	–0.80…+1.30 V	–1.50…+0.60 V
Cu	–0.50…–0.05 V	–1.20…–0.35 V
CuO*	–	–0.40…+1.00 V

* generated by potential pulses

Table 10: Potential range (E) with Ag/AgCl/KCl sat. reference electrode

Working electrode	Potential range E	
	acidic (pH = 1)	alkaline (pH = 13)
Ag	–1.25…–0.30 V	–1.20…+0.10 V
Au	–1.05…+0.40 V	–0.90…+0.75 V
Pt	–1.00…+0.50 V	–0.90…+0.65 V
Glassy carbon	–1.50…+0.60 V	–1.50…+0.60 V
Cu	–1.20…–0.50 V	–1.20…–0.35 V
CuO*	–	–0.40…+1.00 V

* generated by potential pulses

Table 11: Potential range (E) with Pd/PdO reference electrode

1.8 Multiple detection

The use of several detectors during a chromatographic analysis may be appropriate for complicated analytical problems. For example, it is a good idea to use a UV/VIS detector or an amperometric detector together with the conductivity detector for the determination of very small amounts of nitrite in the presence of large amounts of chloride, as can be seen from the following example of its determination in water sample. Larger concentrations of chloride start interfering with the nitrite peak. This will make the quantification difficult or even impossible with conductivity detection. In the UV chromatogram, chloride does not interfere and enables perfect quantification.

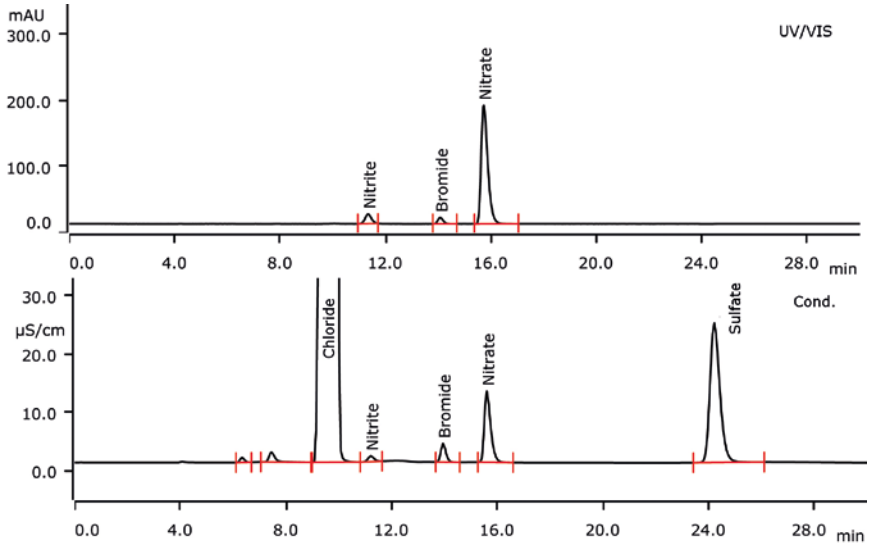


Figure 10 Serial detection of anions in a water sample. Top chromatogram (UV/VIS detection), bottom chromatogram (conductivity detection)

The combination of conductivity and amperometric detection is also used for nitrite besides high chloride. Both combinations are increasing the selectivity and sensitivity of components besides high concentrations of interfering components that are not active in the respective detector.

1.9 Suppressors

All suppressors have basically the task of improving the detection of the analyte. In this respect, a differentiation must be made between two effects or criteria in the suppression reaction:

- The high conductivity of the eluent is to be reduced to as low a level as possible.
- In addition, the counter ions of the analyte ions should be converted to a single ionic species with a higher equivalent conductivity.

1.9.1 Suppressor reactions

The basic reaction of such a suppressor in the anionic range has already been described in section 1.5.3. In the anion analysis sector when the normal sodium carbonate/hydrogen carbonate electrolytes are used the suppressor exchanges the sodium ions against protons and this carbonate ion leads to the formation of poorly dissociated carbonic acid. This reaction leads to a reduction in the conductivity of the electrolyte (first criterion).

$$Na^+ + HCO_3^- \xrightarrow[-Na^+]{+H^+} H_2CO_3$$

In addition all cations in the sample are converted to protons. As the proton has the largest equivalent conductivity of all cations this reaction provides a better detection sensitivity (second criterion).

$$Na^+ + Cl^- \xrightarrow[-Na^+]{+H^+} HCl$$

Careful attention must be given to both criteria when assessing whether the use of a suppressor makes sense. Such considerations lead to the result that in the anionic range the use of suppressors is indicated if carbonate eluents can be used.

On the other hand only the first criterion is fulfilled for the use of suppressors in cationic analysis (reduction of the eluent conductivity), but not the second. Then in this case, for example, the suppressor reactions are as follows if nitric acid is used as the eluent and sodium chloride is present as analyte:

Eluent: $$H^+ + NO_3^- \xrightarrow[-NO_3^-]{+OH^-} H_2O$$

Analyte: $$Na^+ + NO_3^- \xrightarrow[-NO_3^-]{+OH^-} NaOH$$

It follows that in direct ion chromatography the analyte ion Na+ is measured against H^+, i.e. a negative peak will be obtained (difference $\kappa\{Na^+\} - \kappa\{H^+\} = -300$ µS). In contrast in the suppressor reaction the sodium ion is measured against water and all analyte anions will be converted to hydroxide ions (sum of $\kappa\{OH^-$ $\kappa\{Na^+\} + \} = 248$ µS). From these figures it can be seen that normally the use of suppressors is only required when very low concentrations need to be determined. LOD of non-suppressed cation chromatography is around 50…100 µg/L. This is given due to a typical baseline noise of typically > 1 nS/cm. Applying sequential suppression with hydrogen carbonate as counter ion, the baseline noise is reduced to below 0.1 nS /cm. this enables detection limits of cations down to less than 1 µg/L with direct injection.

1.9.2 Construction of suppressors

In the initial phases of ion chromatography, ion exchanger columns were used as suppressors. After several hours of operation, the columns were exhausted and had to be regenerated. A disadvantage of these columns was, apart from the necessary regeneration, a shift in the water peak which depends on the degree to which the column was exhausted.

At the moment, both continuously and discontinuously regenerated suppressors are applied.

All the continuously regenerated suppressors contain ion-exchange membranes and work on the counter-current principle.

The membrane suppressors are constructed according to the sandwich principle. The eluent and analyte ions flow through a film between two membranes and the regeneration solution in spaces above and below these membranes. The so-called electrolytically regenerated suppressor is applying the same setup. But the suppressing ion (H^+ or OH^-) is generated electrolytically. The chemistry of suppression is exactly the same for chemically and electrolytically regenerated suppression. H^+ and OH^- are replacing the respective eluent counter ions. The term electrolytic suppression is therefore rather misleading as the electrolytic process only generates the required ions.

The advantage of these membrane suppressors is their continuous regeneration, however their pressure sensitivity and the diffusion of regenerator ions into the eluent are disadvantages.

In the discontinuously regenerated suppressors, which can also be operated completely automatically, small ion exchanger columns or microchannels packed with ion exchangers (packed bed suppressor, (MSM)) are used. In this procedure, the suppressor compartments are exchanged automatically after one or a few chromatograms. The advantages of these suppressors are their high-pressure stability and their purity.

1.10 Evaluation and calibration

1.10.1 Evaluation of peak area

For a chromatogram recorded using a conductivity detector, the area under a substance peak is directly proportional to the amount of substance. To determine the peak areas, today electronic integrators or evaluation programs incorporating special algorithms for peak smoothing, peak recording and consideration of baseline drift are used almost without exception.

Provided the start and end of the peak can be identified reasonably accurate, the peak area evaluation provides very good results for medium to high concentrations. The peak area determination must particularly be employed in cases where the capacity factors k' change (e.g. through matrix effects). The area calculation can be problematic when the peak overlap is extensive, with peaks exhibiting severe tailing and with perceptible detector noise.

1.10.2 Evaluation of peak height

When the peak shape is constant, the peak height (the distance between the baseline and the peak maximum) is a quantity which is proportional to the peak area and which can also be used to evaluate chromatograms. The peak height determination is easily performed manually and is thus the method of choice if the chromatograms are only plotted on a recorder.

A condition for the applicability of the height evaluation is constant k' values. For reasons of linearity, it is suitable only for low to medium sample concentrations. The linear range is larger for components eluted later than for those eluted rapidly. With excessive peak overlap or a noisy baseline, the peak height evaluation can be superior to that of the peak area.

1.10.3 Calibration with external standard

The direct comparison of the signal magnitude (peak area or peak height) in an unknown sample with that of a standard solution of the same substance is by far the most frequently employed method of calibration in ion chromatography. It requires the injection of constant volumes under invariable chromatographic conditions. The substance to be analyzed is injected as a standard solution of about the same concentration as that in the sample. This can also be carried out repeatedly at different concentrations (one-point, two-point, multipoint calibration). In a one-point calibration, the sample concentration found is calculated as follows:

$$c_a = S_a \times \frac{c_{st}}{S_{st}}$$

c_a : concentraion in analysis sample
c_{st} : concentration of standard solution
S_a : signal of sample
S_{st} : signal of standard

With two-point and multipoint calibrations, the calibration function specific to the substance is first plotted. Frequently, this function can be described by a calibration line obtained from the measured points with the aid of a linear regression analysis:

$$S_{st} = S_0 + m \times c_{st}$$

S_0: intercept of the calibration line
m: slope of the calibration line

In this case, the concentration in the analysis sample is calculated from the formula

$$c_a = \frac{S_a - S_0}{m}$$

For multi-point calibrations, linear regression or polynomial curve fit may be applied. For all these calibration modes, the signal of the sample under investigation should lie between that of the lowest and the highest calibration standard since the calibration line is defined only within the concentration range investigated.

1.10.4 Calibration with internal standard

To take into account errors in the sample pretreatment (e.g. dispensing errors) or to determine recovery yields, the calibration with a second substance added to both the external standard and the sample can be used. This component, called an internal standard, should be eluted as near as possible to the substance to be analyzed yet be completely resolved and have a similar concentration, response and chemical structure.

Since the sensitivities of the internal standard and the substance under investigation are usually not the same, the correction factor F_x must first be determined with ultrapure calibration solutions. Here, a solution of known concentration of the substance of interest (c_x) and the internal standard (c_{is}) analyzed. F_x is obtained from the measured signal heights S_{is} and S_x:

$$F_x = \frac{S_{is} \times C_x}{S_x \times C_{is}}$$

If the correction factor F_x can be assumed constant in the concentration range of interest, the concentration c_a of a sample, to which the internal standard has also been added, can be calculated as follows:

$$c_a = \frac{S_a}{S_{is}} \times c_{is} \times F_x$$

1.10.5 Calibration by standard addition

The standard addition method is used in ion chromatography primarily when matrix problems occur. The sample solution is spiked with a known quantity of the substance to be determined. The signals of the untreated sample and the spiked sample solution are measured; the chromatographic conditions must be identical. The standard addition can be performed once, twice or several times.

In the simplest case with one standard addition, the unknown sample concentration c_a is calculated with the aid of the known, added concentration difference Δc and the measured signal increase ΔS:

$$c_a = \frac{S_a}{} \times \frac{\Delta_c}{\Delta_S}$$

With several standard additions, the sample concentration is calculated by linear regression analysis.

The advantage of the standard addition method lies in its greater reliability since calibration in the sample is performed under actual matrix conditions. Problems are recognized rapidly by non-linear standard addition or shifts in peak height. Long-term changes in temperature, pressure, etc. are taken into account by the continual updating of the calibration and have no influence on the measurement results. However, this reliability costs analysis time as calibration must be performed with every sample and not just periodically as with the external standard method.

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2 Columns and eluents

2.1 General hints

2.1.1 Eluents

For the preparation of the eluents one should use chemicals of a purity degree of at least «p.a.». For dilution use ultrapure water only.

Fresh eluents should always be **microfiltered** (0.2 µm filter) and **degassed** (with N_2, He or vacuum). For alkaline eluents and eluents with low buffering capacity one should preferably use **CO_2 absorbers.**

The supply vessel containing the eluent must be closed to avoid excessive evaporation. This is primarily important with eluents containing organic solvents (e.g. acetone), the evaporation of which can lead to drifts in the long term. If work is performed in a very sensitive range, even if one drop of condensate falls back in the eluent this can cause a noticeable change in the background conductivity.

Influence of various parameters on anion columns

- *Concentration:* An increase in the concentration usually leads to shorter retention times and quicker separation, but also to a higher background conductivity.
- *pH:* pH alterations lead to shifts in the dissociation equilibrium and thus to changes in the retention times.
- *Organic modifiers:* Addition of an organic solvent (e.g. methanol, acetone, acetonitrile) to aqueous eluents has generally little influence to the retention time of non-polarizable ions (fluoride, chloride, and sodium, calcium, etc.). Polarizable and less hydrophilic ions (iodide, thiocyanate, and organic ammonium cations, etc.) are typically eluted earlier with organic modifier.

Eluent change

When the eluent is changed, it must be ensured that no precipitates can be formed. Solutions used in direct succession must therefore be miscible. If the system has to be rinsed with an organic solution, several solvents with increasing or decreasing lipophilic character may possibly have to be used (e.g. water ↔ acetone ↔ chloroform).

2.1.2 Protection of separation columns

To protect the column against particles, which could have an adverse influence on the separation efficiency, it is recommended to apply microfiltration (0.2 µm filter) to samples and eluent and to siphon the eluent through the aspiration filter.

To avoid contamination by abrasive particles arising from piston seals of the IC Pump, it is strongly recommended to install an Inline filter 2 µm between the pump and the injector.

The use of guard columns serves to protect the actual separating columns and increase their service life appreciably.

Always store the separation columns closed when not in use According to the manufacturer's specifications.

2.1.3 Column regeneration

If the separation properties of the column have deteriorated, it can be regenerated in accordance with the column manufacturer's specifications.

In the case of separating columns with carrier material based on silica, ***no alkaline solutions*** *may be used for regeneration, otherwise the columns could be damaged.*

2.2 Metrosep A Supp 4 - 250/4.0 (6.1006.430, non-suppressed and suppressed anion chromatography)

2.2.1 Column specifications

Substrate:	Polyvinyl alcohol with quaternary ammonium groups
Column dimensions:	250 x 4.0 mm
Column body:	PEEK
Standard flow:	1.0 mL/min
Maximum flow:	2.0 mL/min
Maximum pressure:	12 MPa
Particle size:	9 µm
Organic modifier:	0…100% (particularly acetone, acetonitrile, methanol)
pH range:	3…12
Temperature range:	20…60 °C
Capacity:	37 µmol (Cl^-)
Storage:	In eluent
Regeneration:	**Contamination with hydrophilic ions:** a) Rinse with ultrapure water (15 min at 0.5 mL/min) b) Rinse with 10x concentrated eluent (60 min at 0.5 mL/min) c) Rinse with ultrapure water (15 min at 0.5 mL/min) d) Rinse with eluent (60 min at 0.5 mL/min) **Contamination with lipophilic ions:** a) Rinse with ultrapure water (15 min at 0.5 mL/min) b) Rinse with 5% acetonitrile (10 min at 0.5 mL/min) c) Rinse with 100% acetonitrile (60 min at 0.5 mL/min) d) Rinse with 50% acetonitrile (10 min at 0.5 mL/min) e) Rinse with ultrapure water (30 min at 0.5 mL/min) f) Rinse with eluent (60 min at 0.5 mL/min)

2.2.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column

Inline filter 2 µm	6.2821.120
Spare filters for inline filter	6.2821.130
Metrosep A Supp 4 Guard/4.0	6.01021.500
Metrosep A Supp 4 S-Guard/4.0	6.01021.510

- For more information on this column as well as different diameters available check the Column Finder on www.metrohm.com.

2.2.3 Eluents

2.2.3.1 Phthalic acid eluent (standard eluent, non-suppressed)

Phthalic acid	1660 mg/2 L	5.0 mmol/L
Acetonitrile	40 mL/2 L	2%
TRIS		pH = 4.4

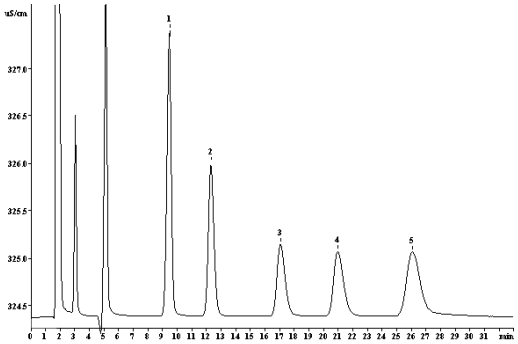

Figure 11: Standard chromatogram on Metrosep A Supp 4 - 250/4.0 – non-suppressed

Phthalate eluent, standard					Conc. (mg/L)
1	Fluoride	25.0	5	Nitrate	25.0
2	Chloride	25.0	6	Phosphate	25.0
3	Nitrite	25.0	7	Sulfate	25.0
4	Bromide	25.0			

2.2.3.2 Carbonate eluent (standard eluent, suppressed)

Sodium hydrogen carbonate	286 mg/2 L	1.7 mmol/L
Sodium carbonate	382 mg/2 L	1.8 mmol/L

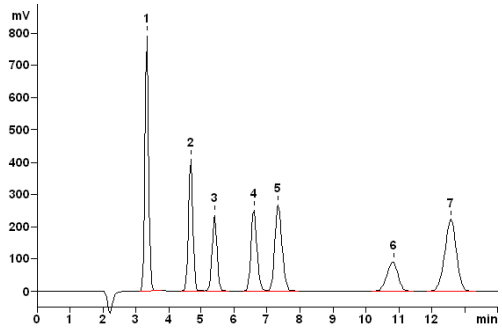

Figure 12: Standard chromatogram on Metrosep A Supp 4 - 250/4.0 – suppressed

Carbonate eluent, standard					Conc. (mg/L)
1	Fluoride	5.00	5	Nitrate	10.00
2	Chloride	5.00	6	Phosphate	10.00
3	Nitrite	5.00	7	Sulfate	10.00
4	Bromide	10.00			

2.3 Metrosep A Supp 5 - 150/4.0 (6.1006.520, suppressed anion chromatography)

2.3.1 Column specifications

Substrate: Polyvinyl alcohol with quaternary ammonium groups
Column dimensions: 150 x 4.0 mm
Column body: PEEK
Standard flow: 0.7 mL/min
Maximum flow: 0.8 mL/min
Maximum pressure: 15 MPa
Particle size: 5 µm
Organic modifier: 0…100% (particularly acetone, acetonitrile, methanol)
pH range: 3…12
Temperature range: 20…60 °C
Capacity: 52 µmol (Cl^-)
Storage: In eluent
Regeneration:

Contamination with hydrophilic ions:
a) Rinse with ultrapure water (25 min at 0.3 mL/min)
b) Rinse with 10x concentrated standard eluent (100 min at 0.3 mL/min)
c) Rinse with ultrapure water (25 min at 0.3 mL/min)
d) Rinse with eluent (100 min at 0.3 mL/min)

Contamination with lipophilic ions:
a) Rinse with ultrapure water (25 min at 0.3 mL/min)
b) Rinse with 5% acetonitrile (20 min at 0.3 mL/min)
c) Rinse with 100% acetonitrile (60 min at 0.3 mL/min)
d) Rinse with 50% acetonitrile (10 min at 0.3 mL/min)
e) Rinse with ultrapure water (50 min at 0.3 mL/min)
f) Rinse with eluent (100 min at 0.3 mL/min)

With shifted system peak (regeneration method with column oven):
a) Rinse with concentrated eluent 1 mol/L Na_2CO_3 (25 min at 0.4 mL/min)
b) Maintain for 10…12 h at 45…50 °C (without rinsing)
c) Rinse with standard eluent (at least 40 min at 0.4 mL/min)

2.3.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column

Inline filter 2 µm	6.2821.120
Spare filters for inline filter	6.2821.130
Metrosep A Supp 5 Guard/4.0	6.1006.500
Metrosep A Supp 5 S-Guard/4.0	6.1006.540

- For more information on this column as well as different column lengths and diameters available check the Column Finder on www.metrohm.com.

2.3.3 Eluent

2.3.3.1 Carbonate eluent (standard eluent)

Sodium hydrogen carbonate	168 mg/2 L	1.0 mmol/L
Sodium carbonate	678 mg/2 L	3.2 mmol/L

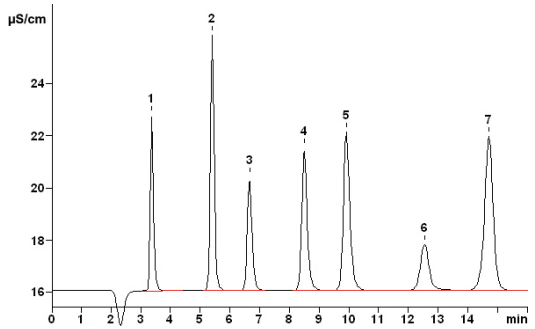

Figure 13: Standard chromatogram on Metrosep A Supp 5 - 150/4.0

Carbonate eluent, standard					Conc. (mg/L)
1	Fluoride	2.00	5	Nitrate	10.00
2	Chloride	5.00	6	Phosphate	10.00
3	Nitrite	5.00	7	Sulfate	10.00
4	Bromide	10.00			

2.4 Metrosep A Supp 7 - 250/4.0 (6.1006.630, suppressed anion chromatography)

2.4.1 Column specifications

Substrate: Polyvinyl alcohol with quaternary ammonium groups
Column dimensions: 250 x 4.0 mm
Column body: PEEK
Standard flow: 0.7 mL/min
Maximum flow: 1.0 mL/min
Maximum pressure: 15 MPa
Particle size: 5 µm
Organic modifier: 0…100% (particularly acetone, acetonitrile, methanol)
pH range: 3…12
Temperature range: 20…60 °C
Capacity: 110 µmol (Cl^-)
Storage: In the eluent at max. 8 °C
Regeneration: **Contamination with low-valency hydrophilic ions:**
a) Rinse with ultrapure water (25 min at 0.3 mL/min)
b) Rinse with 10x concentrated eluent (100 min at 0.3 mL/min)
c) Rinse with ultrapure water (25 min at 0.3 mL/min)
d) Rinse with eluent (100 min at 0.3 mL/min)

Contaminations with high-valency hydrophobic ions and organic contaminations:
a) Rinse with ultrapure water (25 min at 0.3 mL/min)
b) Rinse with 100% acetonitrile (20 min at 0.3 mL/min)
c) Rinse with ultrapure water (25 min at 0.3 mL/min)
d) Rinse with 10x concentrated eluent (100 min at 0.3 mL/min)
e) Rinse with ultrapure water (25 min at 0.3 mL/min)
f) Rinse with eluent (100 min at 0.3 mL/min)

2.4.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column

Inline filter 2 µm	6.2821.120
Spare filters for inline filter	6.2821.130
Metrosep RP 3 Guard HC/4.0	6.1011.040
Metrosep RP 2 Guard/3.5	6.1011.030
Replacement filters for RP 2 Guard/3.5 (10 pcs.)	6.1011.130
Metrosep A Supp 5 Guard/4.0	6.1006.500
Metrosep A Supp 5 S-Guard/4.0	6.1006.540
Metrosep A Supp 16 Gurad/4.0	6.1031.500
Metrosep A Supp 16 S-Guard/4.0	6.1031.510

- For more information on this column as well as different column lengths and diameters available check the Column Finder on www.metrohm.com.

2.4.3 Eluents

2.4.3.1 Carbonate eluent (standard eluent)

Sodium carbonate	763 mg/2 L	3.6 mmol/L
Column temperature	45 °C	

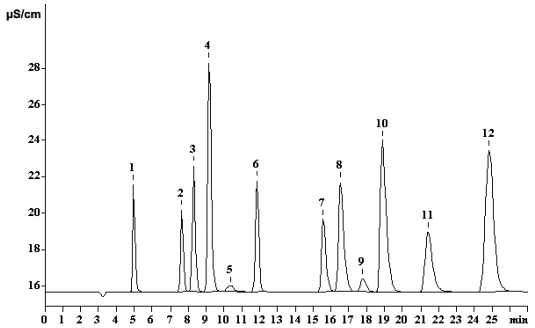

Figure 14: Standard chromatogram on Metrosep A Supp 7 - 250/4.0

Carbonate eluent, standard					Conc. (mg/L)
1	Fluoride	2.00	7	Bromide	10.00
2	Chlorite	10.00	8	Chlorate	20.00
3	Bromate	20.00	9	DCA	5.00
4	Chloride	3.00	10	Nitrate	10.00
5	System peak	-	11	Phosphate	20.00
6	Nitrite	10.00	12	Sulfate	15.00

2.5 Metrosep A Supp 16 - 150/4.0 (6.1031.420, suppressed anion chromatography)

2.5.1 Column specifications

Substrate: Polystyrene/divinylbenzene copolymer with quaternary ammonium groups
Column dimensions: 150 x 4.0 mm
Column body: PEEK
Standard flow: 0.8 mL/min
Maximum flow: 1.2 mL/min
Maximum pressure: 20 MPa
Particle size: 4.6 µm
Organic modifier: 0…10%
pH range: 0…14
Temperature range: 10…70 °C
Capacity: 125 µmol (Cl^-)
Storage: In eluent
Regeneration:
a) Rinse the column overnight (12 h) with standard eluent at a low flow rate (0.4 mL/min).
b) Rinse the column with one third of the standard flow in the opposite direction for 2 h with 15 mmol/L Na_2CO_3 and then for 2 h with ultrapure water.

Eluent change: When installing or changing to eluents which have an organic modifier to avoid high backpressure, adjust the flow in small increments from 0.4 mL/min to match standard conditions within one hour while maintaining the direction of flow.

2.5.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column

Inline filter 2 µm	6.2821.120
Spare filters for inline filter	6.2821.130
Metrosep A Supp 16 Guard/4.0	6.1031.500
Metrosep A Supp 16 S-Guard/4.0	6.1031.510

- For more information on this column as well as different column lengths and diameters available check the Column Finder on www.metrohm.com.

2.5.3 Eluent

2.5.3.1 Carbonate/hydroxide eluent (standard eluent)

Sodium carbonate	1590 mg/2 L	7.5 mmol/L
Sodium hydroxide (c = 0.25 mol/L)	6.0 mL/2 L	0.75 mmol/L
Column temperature	45 °C	

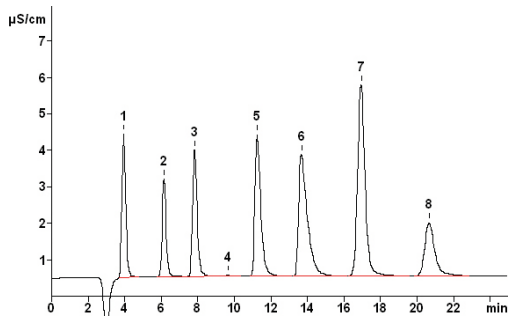

Figure 15: Standard chromatogram on Metrosep A Supp 16 - 150/4.0

Carbonate/hydroxide eluent, standard					Conc. (mg/L)
1	Fluoride	2.00	5	Nitrate	10.00
2	Chloride	5.00	6	Phosphate	10.00
3	Nitrite	5.00	7	Sulfate	10.00
4	Bromide	10.00			

2.6 Metrosep A Supp 17 - 150/4.0 (6.01032.420, suppressed anion chromatography)

2.6.1 Column specifications

Substrate: Polystyrene/divinylbenzene copolymer with quaternary ammonium groups
Column dimensions: 150 x 4.0 mm
Column body: PEEK
Standard flow: 0.6 mL/min
Maximum flow: 1.4 mL/min
Maximum pressure: 18 MPa
Particle size: 5.0 µm
Organic modifier: 0…10% methanol, 0…40% acetone or acetonitrile
pH range: 0…14
Temperature range: 10…70 °C
Capacity: 65 µmol (Cl^-)
Storage: In eluent
Preparation: Rinse the column with eluent for 2…3 h
Regeneration: **Inorganic contamination**
1. Rinse with ultrapure water (20 min at 0.3 mL/min)
2. Rinse with 10x concentrated standard eluent (120 min at 0.3 mL/min)
3. Rinse with ultrapure water (20 min at 0.3 mL/min)
4. Rinse with standard eluent (120 min at 0.3 mL/min)

Organic contamination
1. Rinse with 70% methanol (16 h at 0.3 mL/min)
2. Rinse with standard eluent (120 min at 0.3 mL/min)

2.6.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column
- Inline filter 2 µm 6.2821.120
- Spare filters for inline filter 6.2821.130
- Metrosep A Supp 17 Guard/4.0 6.01032.500
- Metrosep A Supp 17 S-Guard/4.0 6.01032.510
- For more information on this column as well as different column lengths and diameters available check the Column Finder on www.metrohm.com.

2.6.3 Eluent

2.6.3.1 Carbonate eluent (standard eluent)

Sodium hydrogen carbonate	33.6 mg/2 L	0.2 mmol/L
Sodium carbonate	1060 mg/2 L	5.0 mmol/L

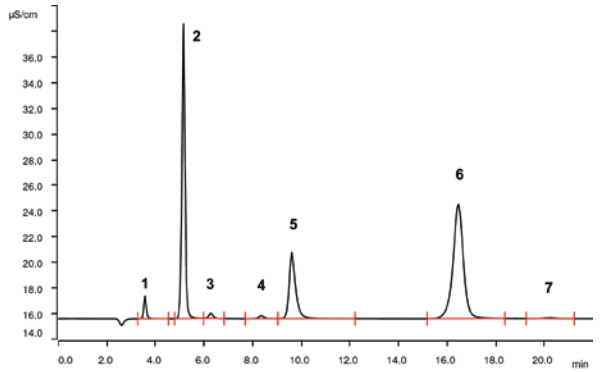

Figure 16: Standard chromatogram on Metrosep A Supp 17 - 150/4.0

Carbonate/hydroxide eluent, standard					Conc. (mg/L)
1	Fluoride	1.25	5	Nitrate	25.00
2	Chloride	25.00	6	Sulfate	50.00
3	Nitrite	1.25	7	Phosphate	1.25
4	Bromide	1.25			

2.7 Metrosep A Supp 10 - 100/4.0 (6.1020.010, suppressed anion chromatography

2.7.1 Column specifications

Substrate: Polystyrene/divinylbenzene copolymer with quaternary ammonium groups
Column dimensions: 100 x 4.0 mm
Column body: PEEK
Standard flow: 1.0 mL/min
Maximum flow: 2.0 mL/min
Maximum pressure: 25 MPa
Particle size: 4.6 µm
Organic modifier: 0…100%
pH range: 0…14
Temperature range: 10…70 °C
Capacity: 37 µmol (Cl^-)
Storage: In eluent
Regeneration: **General:**
Rinse with 50 mL of a 0.05 mol/L solution of Na_4EDTA at a flow rate of 0.5 mL/min. Then rinse with 0.1 mol/L NaOH at 0.5 mL/min for 1 h.

Organic contaminants:
Rinse with 70% methanol at 1.0 mL/min for 12 h. The addition of 1% acetic acid may be useful.

2.7.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column
- Inline filter 2 µm 6.2821.120
- Spare filters for inline filter 6.2821.130
- Metrosep A Supp 10 Guard/4.0 6.1020.500
- Metrosep A Supp 10 S-Guard/4.0 6.1020.510
- Metrosep A Supp 10 Guard HC/4.0 6.1020.520
- For more information on this column as well as different column lengths and diameters available check the Column Finder on www.metrohm.com.

2.7.3 Eluent

2.7.3.1 Carbonate eluent (standard eluent)

Sodium hydrogen carbonate	840 mg/2 L	5.0 mmol/L
Sodium carbonate	1060 mg/2 L	5.0 mmol/L
Column temperature	45 °C	

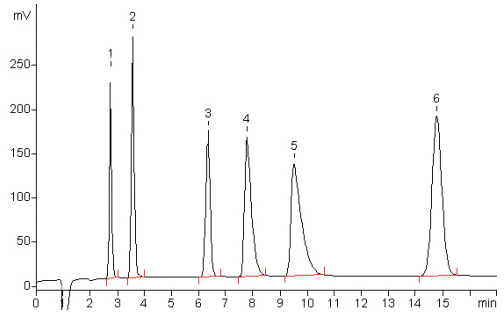

Figure 17: Standard chromatogram on Metrosep A Supp 10 - 100/4.0

Carbonate/hydroxide eluent, standard					Conc. (mg/L)
1	System peak	-	5	Bromide	10.00
2	Chloride	5.00	6	Nitrate	10.00
3	Nitrite	5.00	7	Sulfate	10.00
4	Phosphate	10.00			

2.8 Metrosep A Supp 18 - 250/4.0 (6.01033.430, suppressed anion chromatography)

2.8.1 Column specifications

Substrate: Polyvinyl alcohol with quarternary ammonium groups
Column dimensions: 250 x 4.0 mm
Column body: PEEK
Standard flow: 0.5 mL/min
Maximum flow: 0.65 mL/min
Maximum pressure: 22 MPa
Particle size: 3.5 µm
Organic modifier: 0…100% (particularly acetone, acetonitrile, methanol)
pH range: 3…13
Temperature range: 10…70 °C
Capacity: 85 µmol (Cl^-)
Storage: Store the column in standard eluent (23.0 mmol/L potassium hydroxide) at 4…8 °C.
Regeneration:

Organic contamination
Regenerate the column in the direction opposite to the flow at a flow rate of 0.4 mL/min with the following solutions in succession:
a. 1 h with ultrapure water
b. 2 h with acetonitrile-water mixture (50:50)
c. 1 h with ultrapure water

Inorganic contamination
Regenerate the column in the direction opposite to the flow at a flow rate of 0.4 mL/min for 2 h with 50 mmol/L potassium hydroxide.

2.8.2 General Remarks

- Sample solutions should always be filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column
- Inline filter 2 µm 6.2821.120
- Spare filters for inline filter 6.2821.130
- Metrosep RP 3 Guard HC/4.0 6.1011.040
- Metrosep RP 2 Guard/3.5 6.1011.030
- Replacement filters for RP 2 Guard/3.5 (10 pcs.) 6.1011.130
- Metrosep A Supp 18 Guard/4.0 6.01033.500
- For more information on this column check the Column Finder on www.metrohm.com.

2.8.3 Eluents

2.8.3.1 Potassium hydroxide (standard eluent)

Potassium hydroxide	13…52 mmol/L Gradient)

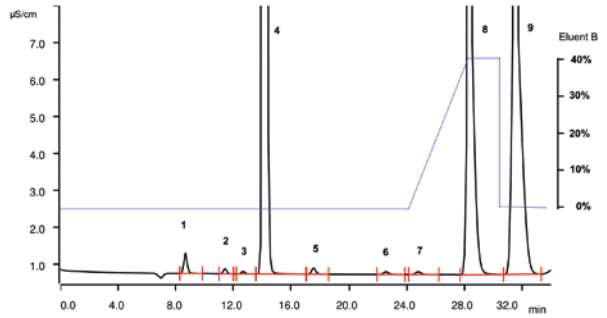

Figure 18: Standard chromatogram on Metrosep A Supp 18 - 250/4.0

KOH eluent, standard					Conc. (mg/L)
1	Fluoride	20.00	6	Chlorate	20.00
2	Chlorite	20.00	7	Bromide	20.00
3	Bromate	20.00	8	Nitrate	2000.00
4	Chloride	2000.00	9	Sulfate	2000.00
5	Nitrite	20.00			

2.9 Metrosep C 6 - 150/4.0 (6.1051.420, non-suppressed cation chromatography)

2.9.1 Column specifications

Substrate: Polybutadienemaleic acid on a silica gel base / spherical silica gel
Column dimensions: 150 x 4.0 mm
Column body: PEEK
Standard flow: 0.9 mL/min
Maximum flow: 2.5 mL/min
Maximum pressure: 20 MPa
Particle size: 5 µm
Organic modifier: **Eluent**
0…100% acetone and acetonitrile (no alcohols)
Sample
0…100% acetone, acetonitrile, and alcohols
pH range: 2…7
Temperature range: 20…60 °C
Capacity: 30 µmol (K^+)
Storage: In eluent at 10…22 °C
Regeneration: **Organic contamination**
Rinse the column in the opposite flow direction at a flow rate of 0.9 mL/min for 1 h with ultrapure water, then for 1 h with acetonitrile/water (40/60).

Inorganic contamination
Rinse the column in the opposite flow direction with 10 mmol/L HNO_3 + 4 mmol/L dipicolinic acid for 1 h at a flow rate of 0.9 mL/min

2.9.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column
- Inline filter 2 µm 6.2821.120
- Spare filters for inline filter 6.2821.130
- Metrosep C 6 Guard/4.0 6.1051.500
- Metrosep C 6 S-Guard/4.0 6.1051.510
- For more information on this column as well as different column lengths and diameters available check the Column Finder on www.metrohm.com.

2.9.3 Eluent

2.9.3.1 Nitric acid/dipicolinic acid eluent (standard eluent, non-suppressed)

Nitric acid (c = 1 mol/L)	3.4 mL/2 L	1.7 mmol/L
Dipicolinic acid	568 mg/2 L	1.7 mmol/L

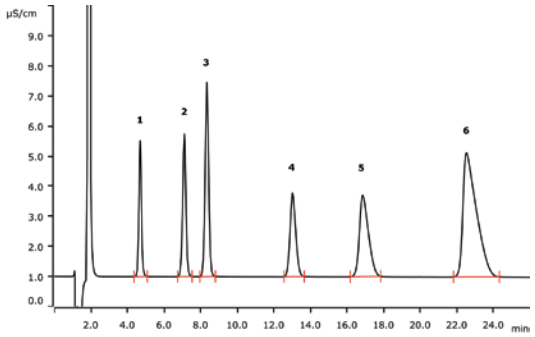

Figure 19: Standard chromatogram on Metrosep C 6 - 150/4.0

Nitric acid/dipicolinic acid eluent, standard					Conc. (mg/L)
1	Lithium	1.00	4	Potassium	10.00
2	Sodium	5.00	5	Calcium	10.00
3	Ammonium	5.00	6	Magnesium	10.00

2.10 Metrosep C Supp 2 - 150/4.0 (6.01053.420, suppressed cation chromatography)

2.10.1 Column specifications

Substrate: Polystyrene/divinylbenzene copolymer with carboxyl groups
Column dimensions: 150 x 4.0 mm
Column body: PEEK
Standard flow: 1.0 mL/min
Maximum flow: 3.1 mL/min
Maximum pressure: 25 MPa
Particle size: 5 µm
Organic modifier: **Eluent**
0…100% acetone and acetonitrile (no alcohol)
Sample
0…100% acetone, acetonitrile and alcohols
pH range: **Eluent**
0…12
Sample
0…14
Temperature range: 10…60 °C
Standard temperature 40 °C
Capacity: 35 µmol (K^+)
Storage: In standard eluent at ambient temperature
Preparation: Rinse the column with eluent for 3 h.
Regeneration: **Note:**
Ensure that the maximum pressure is never exceeded during regeneration. If the pressure becomes too high, reduce the flow rate.

Organic contaminations:
Regenerate the column in the direction opposite to the flow at a flow rate of 1.0 mL/min with the following solutions in succession:
1. 1 h with ultrapure water
2. 1 h with acetonitrile-water mixture (40:60)
3. 1 h with ultrapure water

Inorganic contaminations:
Regenerate the column in the direction opposite to the flow at a flow rate of 1.0 mL/min for 1 h with 50 mmol/L nitric acid.

2.10.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column
- Inline filter 2 µm 6.2821.120
- Spare filters for inline filter 6.2821.130
- Metrosep C Supp 2 Guard/4.0 6.01053.500
- For more information on this column as well as different column lengths and diameters available check the Column Finder on www.metrohm.com.

2.10.3 Eluents

2.10.3.1 Nitric acid eluent (standard eluent, sequentially suppressed)

Nitric acid (c = 1 mol/L)	10 mL/2 L	5.0 mmol/L

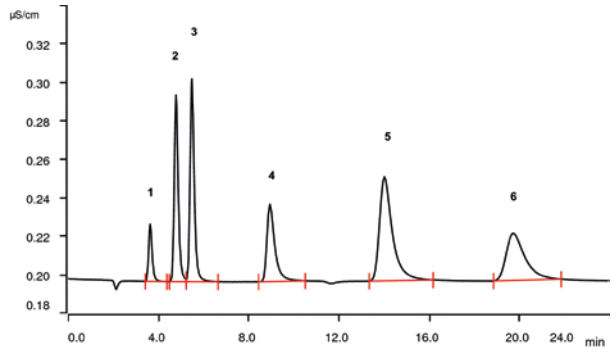

Figure 20: Standard chromatogram on Metrosep C Supp 2 - 150/4.0

Nitric acid eluent, standard					Conc. (mg/L)
1	Lithium	1.00	6	Potassium	10.00
2	Sodium	5.00	7	Calcium	10.00
3	Ammonium	5.00	8	Magnesium	10.00

2.11 Metrosep Organic Acids - 250/7.8 (6.1005.200, ion-exclusion chromatography)

2.11.1 Column specifications

Substrate: Polystyrene/divinylbenzene copolymer with sulfonic acid groups
Column dimensions: 250 x 7.8 mm
Column body: Stainless steel
Standard flow: 0.5 mL/min
Maximum flow: 0.6 mL/min
Maximum pressure: 7 MPa
Particle size: 9 µm
Organic modifier: 0…20%
pH range: 1…13
Temperature range: 5…90 °C
Capacity: 35 µmol (K^+)
Storage: For short periods (days) in the eluent, for longer periods (weeks) in ultrapure water. The column can be stored in a refrigerator at no colder than +4 °C.
Regeneration: Rinse the column in the opposite direction with 20% acetonitrile in 0.01 mol/L H2SO4 at a flow rate of 0.1 mL/min for 4 hours at 65 °C.

Contaminations with metals:
If retention times are shortened: Rinse the column in the opposite direction with approx. 30 mL 0.1 mol/L H_2SO_4 at a flow rate of 0.1 mL/min.

Organic contaminants:
Rinse the column in the opposite direction with approx. 30 mL 0.01 mol/L H_2SO_4/acetonitrile (80/20) at a flowrate of 0.1 mL/min.

2.11.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column
- Inline filter 2 µm 6.2821.120
- Spare filters for inline filter 6.2821.130
- Metrosep Organic Acids Guard/4.6 6.1005.250
- For more information on this column as well as different column lengths available check the Column Finder on www.metrohm.com.

2.11.3 Eluent

2.11.3.1 Sulfuric acid eluent (standard eluent, inverse suppression)

Sulfuric acid (c = 2 mol/L)	0.5 mL/2 L	0.5 mmol/L
Acetone	300 mL/2 L	15%

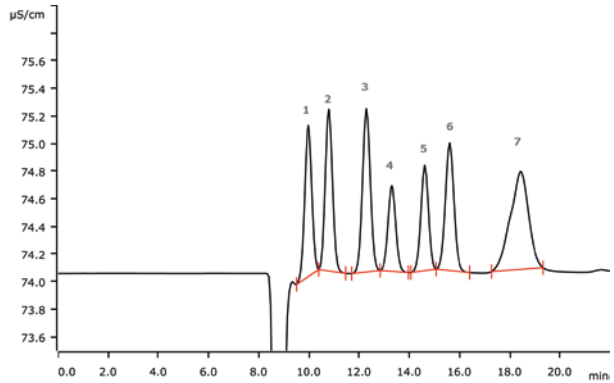

Figure 21: Standard chromatogram on Metrosep Organic Acids - 250/7.8

Sulfuric acid eluent, standard					Conc. (mg/L)
1	Tartrate	10.00	5	Formate	5.00
2	Malate	10.00	6	Acetate	10.00
3	Succinate	10.00	7	System peak	-
4	Lactate	10.00			

2.12 Metrosep Carb 2 - 150/4.0 (6.1090.420)

2.12.1 Column specifications

Substrate: Styrene/divinylbenzene copolymer with quaternary ammonium groups
Column dimensions: 150 x 4.0 mm
Column body: PEEK
Standard flow: 0.5 mL/min
Maximum flow: 1.2 mL/min
Maximum pressure: 20 MPa
Particle size: 5.0 µm
Organic modifier: **Eluent**
0…50% acetonitrile or methanol
Sample
0…100% acetone, acetonitrile or methanol
pH range: 0…14
Temperature range: 20…60 °C
Capacity: 35 µmol (K^+)
Storage: In standard eluent
Note: 1. Use a flow ramp to establish the standard flow in the column within 5 min.
2. Rinse the column for 2 h at 30 °C with the desired eluent
Regeneration: **Organic contamination:**
Rinse the column in the flow direction with standard eluent in 50% acetonitrile at a flow rate of 0.5 mL/min for 3 h.

Inorganic contamination:
Rinse the column in the flow direction with a mixture of 100 mmol/L sodium hydroxide and 500 mmol/L sodium acetate at a flow rate of 0.5 mL/min for at least 5 h.

After regeneration, rinse the column with standard eluent for at least 5 h.

2.12.2 General Remarks

- Sample solutions should always filtered through 0.2 µm filter or Metrohm Inline Ultrafiltration should be applied.
- For column preservation it is recommended to use inline filters and a guard column
- Inline filter 2 µm 6.2821.120
- Spare filters for inline filter 6.2821.130
- Metrosep Carb 2 Guard/4.0 6.1090.500
- Metrosep Carb 2 S-Guard/4.0 6.1090.510
- For more information on this column as well as different column lengths and diameters available check the Column Finder on www.metrohm.com.

2.12.3 Eluents

2.12.3.1 Hydroxide/acetate eluent (standard eluent)

Sodium hydroxide (c = 20 mol/L)	10 mL/2 L	100 mmol/L
Sodium acetate	1640.7 mg/2 L	10.0 mmol/L

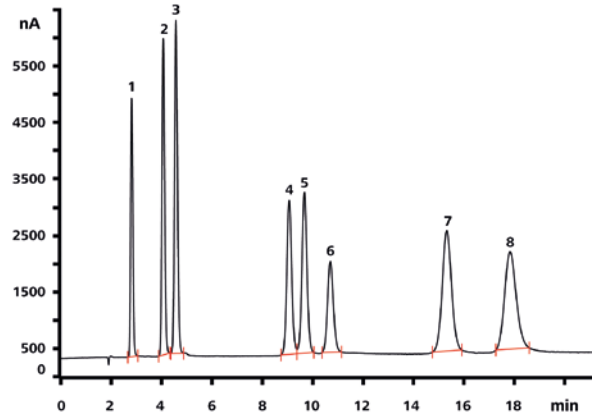

Figure 22: Standard chromatogram on Metrosep Carb 2 - 150/4.0

Hydroxide/acetate eluent, standard					Conc. (mg/L)
1	Inositol	2.50	5	Xylose	5.00
2	Arabitol	5.00	6	Fructose	5.00
3	Sorbitol	5.00	7	Lactose	10.00
4	Glucose	5.00	8	Sucrose	15.00

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