Monograph

Practical Ion Chromatography

An Introduction

Metrohm

Practical Ion Chromatography

An Introduction
3rd edition

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2 Introduction

Examining things that do not reveal themselves directly is always a challenge. The reasons for this vary from simple curiosity to the necessity of survival. There are many different ways of looking behind the curtains. The simplest way is to use the human senses: listening, touching, smelling, tasting and seeing. In earlier days the alchemists liked to use these five senses. This is why today acids taste acidic and bromine has its name derived from «bromos», Greek for fetid. To the naked eye chromium appears to be colored, as «chroma» from a historical linguistic point of view is the same as color. The alchemists also expressed their feelings with insults such as «you kobold» for cobalt, the presence of which caused our ancestors great difficulty in the production of iron.

Many things cannot be seen directly. They are too intimately mixed with other components or the human senses are not capable of identifying them. This is the moment when analysis comes into play. From an undefined mixture of components, it is able to extract precise information that cannot be obtained by the human senses alone.

Although the organism is full of them, the human senses cannot experience them directly – we are talking about ions, those charged atoms or molecules that are an integral part of virtually all living and dead matter. Ions are responsible for the transfer of information along the nerves, for ensuring that digestion takes place, for making sure that the blood pressure is correct and that there is sufficient oxygen in the blood. Ions bring salt into the sea, control your thirst and ionic constituents are used as food by all living things – from bacteria up to human beings.

A knowledge about the type and number of ions to be found in the environment helps us to understand biochemical and ecological relationships. If the ionic concentrations in a foodstuff is known then these provide us with information about whether the food is safe to eat or not. There are many different ways of determining ions qualitatively (by their type) and quantitatively (by their amount). Each piece of information is important. One method used for obtaining this information is ion chromatography. Chromatography basically means «writing with color». In traditional analysis this means the separation of substances according to their color and their determination by visual observation. Although not all ions are characterized by visible colors the term has been retained, but other methods of determination are used today.

Ion chromatography is one member of this large family of chromatographic methods. It can be used – to put it very simply – to determine all ions that carry one or two charges. In the past ion chromatography or «IC» used to be a very expensive method but today it is much more favorably priced. This is why it has developed into a universal and powerful analytical tool that is easy to use.

This «Practical Ion Chromatography» will show that IC is not just an abstract analytical form, but that it can provide rapid answers to common daily problems such as: Is the drinking water suitable for feeding babies? How much nitrate is there in the spinach? Why does the washing machine scale up? Does the wastewater cause environmental pollution? As accurate practical analytical work is almost impossible without a theoretical background, this monograph also contains detailed information in a separate theoretical section.

«Practical Ion Chromatography» is intended not only to provide you with a knowledge of the basic principles of IC but also to provide you with an overview of general chromatographic principles. And chromatography can do an awful lot: It satisfies scientific curiosity and ensures healthy survival in a polluted environment.

3 Theoretical section

3.1 The history and importance of ion chromatography

The beginnings of ion chromatography (IC) or, more exactly, ion exchange chromatography go back to the middle of the previous century. Between 1935 and 1950 knowledge about ion exchangers and their applications was considerably extended by the «Manhattan Project». In the fifties and sixties theoretical models for understanding the phenomenon of ion exchange and of ion chromatography, which is based on this, were worked out. Continuous detectors were used in the seventies; this allowed the jump from low-pressure to high-pressure or high-performance chromatography to be completed.

ca. 1850	Soil as an ion exchanger for Mg^{2+}, Ca^{2+} and NH_4^+	Thomson & Way	LC
1935	Sulfonated and aminated condensation polymers (phenol/formaldehyde)	Adams, Holmes	
1942	Sulfonated PS/DVB resins as cation exchangers (Manhattan-Projekt)	d'Alelio	
1947	Aminated PS/DVB resins as anion exchangers	McBurney	
1953	Ion exclusion chromatography	Wheaton, Baumann	
1957	Macroporous ion exchangers	Corte, Meyer, Kunin et al.	
1959	Basic theoretical principles	Helfferich	
1967–70	Pellicular ion exchangers	Horvath, Kirkland	
1975	Ion exchange chromatography with conductivity detection using a «stripper»	Small, Stevens, Baumann	HPLC
1979	Conductivity detection without a «stripper»	Gjerde, Fritz, Schmuckler	
1976-80	Ion pair chromatography	Waters, Bidlingmeier, Horvath et al.	

Table 1 History of ion exchange and ion chromatography, the analytical technique based on ion exchange

The term «ion chromatography» was coined in 1975 with the introduction of detection by conductivity combined with a chemical reduction in conductivity by Small, Stevens and Baumann; it was subsequently used as a trade name for marketing purposes for a long time. In the meantime the abbreviated term ion chromatography has become established as the superordinate term for the ion exchange, ion exclusion and ion pair chromatography methods included under high-performance liquid chromatography (HPLC) [1]. IC today is dominant in the determination of anions while the atomic spectrometry methods, commonly used for the determination of cations, are hardly useful for determining the electronegative anion formers of the fifth to seventh main groups of the periodic system.
The most important field of application today for anion chromatography is the routine investigation of aqueous systems; this is of vital importance in the analysis of drinking water [2, 3, 4].

IC is also used for the analysis of the element species in anionic elements or complexes; this is mainly for solving environmentally relevant problems. The third important field of application for anion chromatography is ultratrace analysis in ultrapure process chemicals required chiefly in the semiconductor industry.

Today the ion exchangers normally used for HPLC consist of spherical polymer particles with a diameter of about 5 to 15 µm. Various methods are used to attach so-called anchor groups to the surface of the polymer; these are used as spacers between the basic polymer and real functional groups. These normally consist of quaternary ammonium ions which are chemically attached to the anchor groups. The total number of functional groups is known as the exchange capacity; this is a basic characteristic of ion exchangers.

Commercial packing materials for anion chromatography are of a low-capacity nature with exchange capacities of 50 to 100 µmol per separating column. The reason for this is the dominant application of conductivity detection, which is all the more sensitive the lower the inherent conductivity of the elution system. Low-capacity anion exchangers allow to use very dilute aqueous solutions of NaOH or carbonate buffers, whose inherent conductivity can even be additionally reduced by chemical suppression [2, 4].

In anion chromatography it is chiefly functional groups of Type I (trimethylammonium, TMA) and Type II (dimethylethanolammonium, DMEA) which are used today. As the real interaction between the stationary phase and the analyte anions takes place at the functional group this means that its structure has a decisive influence on the selective behavior of packing materials. According to current knowledge the polarity of the functional groups, which can be controlled by the number of hydroxyethyl residues ($–CH_2CH_2OH$) on the quaternary nitrogen, is of particular importance [2, 4].

The term ion chromatography includes all separations of ionic species within HPLC with on-line detection and is therefore largely independent of apparative limitations [5]. IC has developed into the method of choice, above all in anion analysis, thanks to the wide range of separating columns, elution systems and detectors which are now available. The reason for this is that only a few separation processes exist for anions; these are hardly suitable for practical use. Gravimetric and volumetric methods are limited by their sensitivity and their selectivity. Even the meteoric development of gas chromatography from 1965 onwards did not have any great advantages for anions, as the non-volatile ions needed to be derivatized first and the sensitivity did not meet the demands placed on trace analysis today [6]. For the analysis of cations powerful atomic spectrometry alternatives to IC exist, e.g. ICP-AES/MS, so that the value of cation chromatography is considerably less when compared with anion chromatography. However, cation chromatography has achieved a certain importance in the analysis of alkali and alkaline earth metals and in the determination of ammonium-nitrogen (drinking water analysis). In the speciation of ionic compounds IC in combination with element-specific detectors is indispensable. A good overview of the applications of IC in various sectors is given in the works of Haddad et al. and Weiss [2, 4].

3.2 Theory of chromatography

3.2.1 Chromatography divisions and terminology

Chromatography is a physicochemical method for separating mixtures of substances. The separation effect is based on repeated distribution between two phases; one phase is stationary while the second, mobile phase moves in a particular direction [7, 8]. Chromatography techniques are divided into the physical states of the two participating phases:

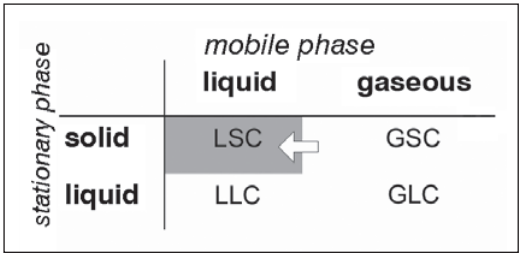


Figure 1
Division of chromatography methods according to the physical states of the stationary and mobile phases

A further differentiation of chromatography methods can be made according to the basic processes which occur during separation, such as adsorption or distribution, or according to the type of procedure carried out (column or planar chromatography) [9].

Retention parameters

If a mixture of substances is subjected to chromatographic separation then a distribution equilibrium is formed between the mobile and stationary phases for each individual component. The substances can only be separated successfully when the **distribution coefficients D** of the components differ sufficiently from one another. D is defined as the ratio of the concentrations of a substance A between the stationary (Index $_S$) and the mobile phases (Index $_M$):

$$D_A = \frac{[A]_S}{[A]_M} \qquad (1)$$

Accordingly, substances with a larger distribution coefficient D will be held back (retained) more strongly than those with a smaller D. The chromatographic separation procedure is shown in the form of a chromatogram in which a detector signal is recorded as a function of the elution volume of the mobile phase or the time. This means that it corresponds to a concentration or mass profile as a function of time. The detector signal should be proportional to the concentration of an analyte at the end of the migration path [8]. As shown in equation 2, the residence time or **gross retention time t_R** of a substance on the stationary phase is obtained by adding the **net retention time t_S,** which corresponds to the actual residence time on the migration path, and the flow time of the mobile phase without any interaction, **the dead time t_M**:

$$t_R = t_S + t_M \qquad (2)$$

Owing to the formation of channels, diffusion processes or irregularities in the equilibrium achieved between the mobile and stationary phases some analytes may pass through the

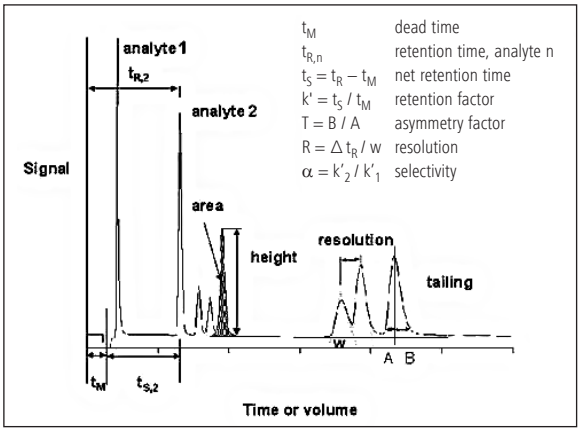


Figure 2
Elution chromatogram of a separation by ion chromatography with indication of the most important quantities

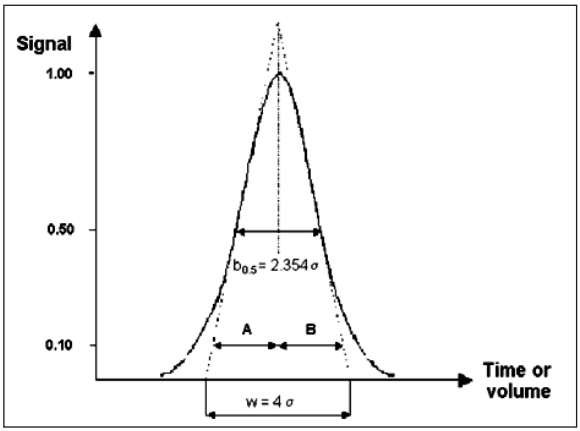


Figure 3
Gaussian distribution with the most important quantities

stationary phase more slowly or more quickly than is to be expected from the net retention time t_S. This means that a chromatogram does not consist of an infinite number of narrow signals, but ideally of Gaussian peaks (see Figure 2).

As a result of diffusion processes, which increase in importance as the residence time on the stationary phase increases, the width of a substance peak increases as the retention time increases. This phenomenon is characteristic of all chromatography methods.

As already mentioned, under ideal circumstances a peak in a chromatogram shows Gaussian distribution. Figure 3 shows an example of Gaussian distribution.

The width at half the peak height is known as the half-width $b_{0,5}$ and corresponds to the 2.354-fold variance V of the distribution. The base width w is defined as the distance between the points of intersection of the slope tangents with the y axis, which is the same as the fourfold variance of the Gauss function. Both quantities are a measure of the performance of a chromatography separating column and, with an ideal peak shape, can be used for calculating the number of plates.

Variations from the ideal peak form can be described by a so-called **asymmetry factor T**. This is defined as the ratio of the distances A and B between the central verticals and the slopes of the distribution at 10% of their height (see Figures 2 and 3) and can be calculated to:

$$T = \frac{B}{A} \qquad (3)$$

For Gaussian peaks T = 1. A variation towards a larger T value is known as tailing, towards a smaller one as fronting. In practice the intention is to achieve an asymmetry factor from T = 0.9 to 1.1.

Retention factor, selectivity and resolution

As the gross retention time t_R depends largely on the chromatographic conditions it is only under defined conditions that it is characteristic for a particular substance and can therefore be used for qualitative identification. A dimensionless quantity is introduced, **the retention factor k';** this allows comparisons to be made between different chromatographic systems. It provides information about how much longer a substance remains on the migration path than in the mobile phase [8]. The retention factor is mathematically defined as the product of the distribution coefficient D and the phase volume ratio V_S/V_M between the stationary and mobile phase or as the ratio of the net retention time t_S to the dead time t_M. A calculation over the length L of the migration path and the speed u of the mobile phase is also possible (Equation 4).

$$k' = D \cdot \frac{V_S}{V_M} = \frac{t_S}{t_M} = \frac{u \cdot t_R}{L} - 1 \qquad (4)$$

At small values of k' a substance elutes close to the dead time or at the dead volume of the chromatography system; this means that separation is poor. If k' is very large this means that, although the separation is good, there is a long residence time on the migration path and the peak becomes wider. Ideally the retention factor should be between 2 and 5.

Two substances will only be adequately separated if their retention factors differ from each other sufficiently. The **selectivity** α, also known as the relative separating factor, is a measure of the separability of two substances and is defined as follows:

$$\alpha = \frac{t_{R2} - t_M}{t_{R1} - t_M} = \frac{t_{S2}}{t_{S1}} = \frac{k'_2}{k'_1} \quad k'_2 > k'_1 \qquad (5)$$

If two substances cannot be separated then $\alpha = 1$ and coelution occurs. The larger the value of α, the better the separation. However, as α increases the time required for the separation also increases, so that in practice selectivity values of $\alpha = 1.5$ are aimed for [10].

The selectivity does not describe the quality of the separation process. The **resolution R,** however, does not just take the relative positions of the peaks into account, but also their half-widths $b_{0.5}$ or base widths w, as can be seen in Equation 6.

$$R = \frac{t_{R2} - t_{R1}}{\frac{(w_1 - w_2)}{2}} = \frac{2 \cdot \Delta t_R}{w_1 + w_2} = 1.177 \cdot \frac{t_{R2} - t_{R1}}{b_{(0.5)1} + b_{(0.5)2}} \qquad (6)$$

If the difference between the retention times of two peaks is large in relation to their base widths or half-widths then the resolution is good. In the case of an ideal peak symmetry two

substances can still be identified with R = 0.5. For qualitative separation R should be 1 (4σ-separation), for quantification a resolution of R = 1.2 to 1.5 is aimed for [25]. Resolutions of R ≥ 2 (8σ-separation) are to be avoided because of the long analysis times involved.

3.2.2 Theoretical concepts for describing the chromatography process

The theoretical separation stages model

The theoretical separation stages model is derived from the distillation process and is used to describe chromatographic separations [11]. It divides the stationary phase into single sections, the theoretical separation stages or plates, on which in principle a completely reversible and infinitely quick equilibrium between the mobile and stationary phases is achieved exactly and instantaneously. The performance (efficiency) of a chromatography system is therefore characterized by as high a number of theoretical separation stages as possible.

The **number of theoretical plates N** can be determined directly from the chromatogram by using the variance σ, the base width w or the half-width $b_{0,5}$ and is calculated as follows [12]:

$$N = 16 \left(\frac{t_R}{w}\right)^2 = 8 \cdot \ln(2) \cdot \left(\frac{t_R}{b_{0,5}}\right)^2 = \left(\frac{t_R}{\sigma}\right)^2 \qquad (7)$$

Instead of the number of theoretical plates the height equivalent to a theoretical plate HETP can also be used to describe the separating performance.

$$HETP = \frac{L}{N} = \frac{\sigma^2}{L} = \frac{L}{8 \cdot \ln(2)} \cdot \left(\frac{b_{0.5}}{t_R}\right)^2 = \frac{L}{16} \cdot \left(\frac{w}{t_R}\right)^2 \qquad (8)$$

From Equations 5 to 8 it can be seen that a stationary phase with a very large number of theoretical plates can still separate two substances even if their retention factors hardly differ at all, i.e. if the selectivity is close to 1. The equations also allow the calculation of the number of theoretical plates which are necessary to solve a separation problem.

The theoretical separation stages model can be used to explain the occurrence of Gaussian signals in chromatography if it is assumed that, because of flow and diffusion processes, only a finitely quick and incomplete equilibrium is achieved between the mobile and stationary phases. This results in a peak broadening process as a narrow substance zone at the start of the migration path clearly becomes broader as the residence time on the stationary phase increases.

The calculation of the number of theoretical plates according to Equation 7 assumes that the peak shape is ideal; however, this rarely occurs in reality. With asymmetric peak shapes the calculation must be carried out according to the momental method [13]. Equation 9 includes the asymmetry factor T and produces approximate values which make sense.

$$N = 41{,}7 \frac{\left[\frac{t_R}{b_{0,5}}\right]^2}{T+1{,}25} \qquad (9)$$

A **number of effective plates n**, which represents the actual separation performance more closely than the number of theoretical plates N, is corrected by the retention factor k' and is obtained from:

$$n = N \cdot \left(\frac{k'}{k'+1}\right)^2 \quad (10)$$

The dynamic theory (van Deemter theory)

The decisive weakness of the theoretical separation stages model is that distillation and chromatography are based on two fundamentally different physicochemical processes. Also no assumptions are made about the influence of important parameters which are experimentally accessible and which do not affect the type or quality of the stationary phase itself [14, 15]. These could be:

- flow rate of the mobile phase
- particle diameter in the stationary phase
- thickness of surface films on the packing material

In addition, quantities such as the diffusion coefficients in the mobile and stationary phases, the temperature or the detector volume in liquid chromatography are very important for the separation performance.

The dynamic theory developed by van Deemter is, in principle, an extension of the theoretical separation stages model which takes non-ideal limiting conditions into account [16]. The following assumptions are made:

- no spontaneous and unhindered achievement of equilibria
- delayed mass transport in the stationary and mobile phases
- no homogeneous mobile phase flow rate over the whole cross-section of the column
- occurrence of scatter diffusion and channel formation in the stationary phase
- longitudinal diffusion irrespective of the mobile phase speed and directly proportional to the residence time on the migration path

The relationship between the dynamic effects mentioned above and the height equivalent to a theoretical plate is given by the van Deemter equation.

$$HETP = A + \frac{B}{u} + C \cdot u \quad (11)$$

The three terms A, B and C depend in different ways upon the flow rate u of the mobile phase. The terms A and B describe the whole mass transport through the stationary phase; term C is determined by interference to the achievement of the equilibrium between the mobile and stationary phases.

Term A describes the eddy diffusion, which can be regarded as being a cause of peak broadening owing to a multi-pathway effect. This term is also known as the packing factor and is independent of the linear flow rate u of the mobile phase, at least in a first approximation. The following relationship applies to term A:

$$A = 2 \cdot \lambda \cdot d_p \quad (12)$$

In Equation 12, d_p is the average particle diameter in the stationary phase, λ describes the statistical irregularity of the packing; this should be as homogeneous as possible and consist of uniform particles.

Term B describes the longitudinal diffusion in or against the direction of flow of the mobile phase. It is of particular interest when capillary columns are used in gas chromatography (GC), as the diffusion coefficients in gases are higher by four to five powers of ten than in liquids. B is calculated as being the product of the diffusion coefficient D_M in the mobile phase and the labyrinth factor γ, which describes the porosity of the stationary phase.

$$B = 2 \cdot \gamma \cdot D_M \tag{13}$$

As the importance of diffusion decreases as the flow rate of the mobile phase increases this means that B is inversely proportional to u.

Term C is known as the mass transfer term. The delayed mass transfer between the mobile and stationary phases usually has the greatest influence on peak broadening. Interference in achieving equilibrium between the mobile and stationary phases increases as u increases, which is why there is a direct proportionality to the linear flow rate. The delays in mass transfer result from the very small diffusion coefficient D_S in the stationary phase compared to the mobile phase; this is why analytes which are actually resident in the pores of the stationary phase remain behind the peak maximum as it moves on together with the mobile phase. The term C can be considerably reduced by short diffusion paths and quick transfer processes. This can mainly be achieved by localizing the pores on the surface so that only a few extend into the interior of the stationary phase. The mass transfer term C is calculated as follows:

$$C = \frac{16 \cdot k'}{\pi \cdot (1 + k')} \cdot \frac{{d_p}^2}{D_S} \tag{14}$$

Graphical representation of the van Deemter equation shows a hyperbolic curve, from whose minimum value the flow rate u_{opt} for the minimum plate height (maximum number of plates) can be determined (Figure 4).

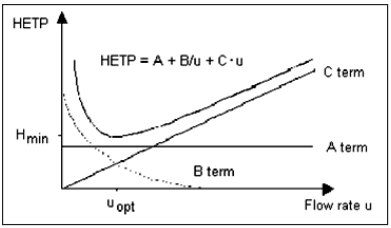


Figure 4
Representation of the individual terms of the van Deemter theory with the resulting van Deemter curve showing the optimum flow rate u_{opt}.

Even the dynamic theory is based on ideal requirements. In reality the three terms A, B and C are only independent of one another in a first approximation, with there being an additional influence of the flow rate u on the eddy diffusion (Term A). Term C can be differentiated by using the terms C_M and C_S, which describe the mass transfer in the mobile phase (C_M) and to the stationary phase and back (C_S). This is why the original van Deemter equation has been modified for numerous applications in HPLC, GC and TLC (thin-layer chromatography) [17, 18].

Modern liquid chromatography (LC)

Liquid chromatography (LC) is to be regarded as the generic term for numerous modern liquid chromatography separation methods. It can be used for a wide range of different substances, is characterized by its excellent analytical performance and is probably the most important separation method used in modern analytical chemistry [3]. Liquid chromatography also includes ion chromatography (IC).

HPLC is a logical further development of the classical liquid chromatography. In classical LC, introduced by Tswett in 1906, glass columns with a diameter of 1 to 5 cm and a length of up to 500 cm were used; these were filled with separation phases with particle sizes of 150 to 200 µm. Even separations of simple mixtures of substances could often take several hours with an average separation performance. As a result of the understanding of the chromatography process which was later developed (see Equation 11) it became clear that increased performance could only be achieved by a dramatic reduction of the particle diameter in the stationary phase; however, this placed completely new demands on the equipment used for chromatography.

Since about 1970 special and powerful instrument technology has become available which is able to overcome the high counterpressures of 10 to 50 MPa which occur when packing materials with a particle diameter of 3 to 10 µm and separating columns of 125 to 250 mm length x 4 mm inner diameter are used.
As a result of the dramatic miniaturization HPLC has developed into a purely analytical separating method; in contrast classical LC is today practically only used for preparative purposes. The advantages of HPLC in comparison with classical LC are chiefly:

- excellent chromatographic efficiency
- continuous working process
- on-line detection of the separated substances
- high sensitivity and reproducibility
- utilization of the retention time for qualitative identification of substances
- short analysis times

Irrespective of its field of application, an HPLC system consists chiefly of the components shown in Figure 5: high-performance pump with mobile phase (eluent) storage, injector (sample introduction), separating column and detection system (including derivatization, data acquisition and processing).

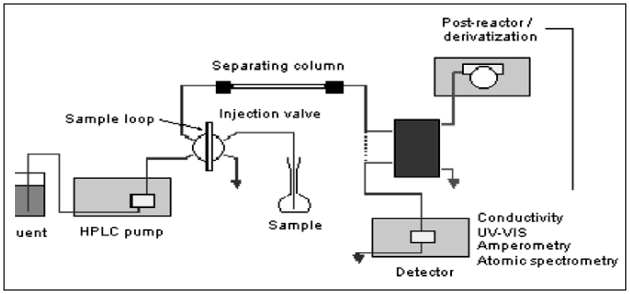


Figure 5
Schematic of HPLC or IC unit setup showing the most important components

Apart from the separating column the pump is the heart of every HPLC system. It must be able to deliver the eluent as uniformly and pulsation-free as possible, even against high counter-pressures. This means that it is also necessary to use a special loop injector for sample introduction. A six-way valve is normally used; this allows to accept a defined volume of the sample in a loop at standard pressure and to transfer it to the HPLC system operating at high pressure. The composition of the mobile phase and the type of separating column must be adapted to the analytical problem to be solved. This also applies to the selection of the detection system. Today all data acquisition and processing is carried out by computer. This basic setup of an HPLC system can be extended virtually at will to solve a particular analytical problem.

Separating principles in LC

HPLC can be differentiated according to the different physicochemical interactions between the substances in a sample and the stationary phase. Although in reality there are usually several different mechanisms responsible for a successful separation [9], a rough classification according to the following separation mechanisms is possible:

- adsorption
- distribution
- size exclusion
- affinity
- ion exchange
- ion pair formation
- ion exclusion

Adsorption chromatography is defined by interfacial reactions, in which liquid or gaseous substances are enriched at a solid phase. Various models are available for providing a qualitative and quantitative description of adsorption processes; here we only refer to the relevant physical chemistry literature [19]. Two different techniques are used. In normal phase chromatography the stationary phase is usually silica gel and therefore considerably more polar than the mobile phase (hydrocarbons). In reversed phase chromatography (RPC) the conditions are exactly the opposite. For practical reasons, which chiefly concern eluent handling, virtually only RPC is used today [3, 9].

In **distribution chromatography** the stationary phase is a liquid which is immiscible with the mobile phase. Separation is based on the differing solubilities of the analytes in the two phases. In an ideal case the Nernst distribution law applies. This separating mechanism plays an important role, particularly in gas chromatography when capillaries coated with separating liquids are used as the stationary phase. Distribution chromatography may also occur in HPLC if silica gels modified with nonpolar hydrocarbons, e.g. so-called octadecyl phases, are used as the separating material.

Size exclusion chromatography (SEC) allows separation according to the molecular size as a result of sieve effects. Silica gels or organic polymer resins with a defined pore structure are used as the stationary phase. Smaller analytes can diffuse into the pores and are retarded. As the molecule size increases any interaction with the pores becomes less likely, until at a particular size molecules are completely excluded and practically elute in the dead volume. SEC is widely used in polymer analysis and bioanalysis.

Affinity chromatography allows the separation of mixtures of substances by selective or specific interactive forces. Highly specific interactions can be observed between antibodies and antigens (key-keyhole principle), as well as with enzymes and their substrates in particular. In practice enzymes or antibodies are chemically immobilized on a stationary phase. If there is a corresponding substrate or antigen in the sample then this is retarded with extreme selectivity. This is why bioaffinity chromatography is indispensable in the active substance analysis sector (pharmacology).

Ion exchange chromatography (IC) together with **ion pair** and **ion exclusion chromatography** are described in detail in the following section.

3.3 Basic principles of ion chromatography (IC)

3.3.1 Terminology and classification in LC

Ion exchange chromatography or ion chromatography (IC) is a subdivision of HPLC. According to IUPAC ion exchange chromatography is defined as follows [7, 8]:

«In ion exchange chromatography separation is based on differences in the ion exchange affinities of the individual analytes. If inorganic ions are separated and can be detected by conductivity detectors or by indirect UV detection then this is also called ion chromatography».

For several reasons this definition is an unhappy choice. The detection technique should be considered separately from the existing separation mechanism. Apart from this, a limitation of the term «ion chromatography» to inorganic ions is difficult to understand as in practice in any given system both organic and inorganic ions can be separated and identified simultaneously.

An older, more general definition is more suitable for defining ion chromatography [20]:

«Ion chromatography includes all rapid liquid chromatography separations of ions in columns coupled on-line with detection and quantification in a flow-through detector.»

This definition characterizes ion chromatography irrespective of the separating mechanism and detection method while at the same time distinguishing it from classical ion exchange. The following separation principles apply in ion chromatography:

- ion exchange
- ion pair formation
- ion exclusion

Chromatography methods are defined by the chief separation mechanism used. Today ion exchange chromatography is simply known as ion chromatography (IC), while ion pair chromatography (IPC) and ion exclusion chromatography (IEC) are regarded as being more specialized applications.

3.3.2 Ion exchange

Ion exchange chromatography (IC) is based on a stoichiometric chemical reaction between ions in a solution and a normally solid substance carrying functional groups which can retain ions as a result of electrostatic forces. In the simplest case in cation chromatography these are sulfonic acid groups, in anion chromatography quaternary ammonium groups. In theory ions with the same charge can be exchanged completely reversibly between the two phases. The process of ion exchange leads to a condition of equilibrium. The side towards which the equilibrium lies depends on the affinity of the participating ions to the functional groups of the stationary phase. Figure 6 is a schematic diagram showing the exchange processes for cations and anions. The analyte ions are marked A, the eluent ions competing with them for the exchange positions with E.

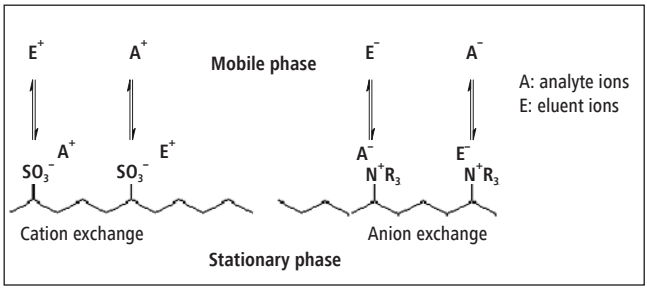


Figure 6
Schematic diagram showing the ion exchange process in ion chromatography.

left: cation exchange
right: anion exchange

Thermodynamic aspects of the ion exchange process

Ion exchangers normally consist of solid phases on whose surface ionic groups are fixed. Because of the condition of electroneutrality there is always an oppositely-charged counter-ion in the vicinity of the functional group. The counter-ion usually originates from the mobile phase and is therefore also known as the eluent ion.

If a sample is added which contains two analyte ions A^- and B^- then these briefly displace the eluent ions E^- and are retained at the fixed charges before they are in turn exchanged for eluent ions. For anion chromatography this results in the following reversible equilibria:

$$\text{Resin} - \text{N}^+\text{R}_3\text{E}^- + \text{A}^- \leftrightharpoons \text{Resin} - \text{N}^+\text{R}_3\text{A}^- + \text{E}^- \quad (15)$$

$$\text{Resin} - \text{N}^+\text{R}_3\text{E}^- + \text{B}^- \leftrightharpoons \text{Resin} - \text{N}^+\text{R}_3\text{B}^- + \text{E}^- \quad (16)$$

The different affinities of A^- and B^- to the functional groups mean that separation is possible. The equilibrium constant K is also known as the selectivity coefficient and is calculated as follows for anion A^-:

$$\text{K}_\text{A} = \frac{[\text{Resin} - \text{N}^+\text{R}_3\text{A}^-] \cdot [\text{E}^-]}{[\text{Resin} - \text{N}^+\text{R}_3\text{E}^-] \cdot [\text{A}^-]} = \frac{[\text{A}^-]_S \cdot [\text{E}^-]_M}{[\text{E}^{--}]_S \cdot [\text{A}^-]_M} \quad (17)$$

If it can be assumed that the concentration of the eluent ions is normally higher than that of the analyte ions by several powers of ten then $[E^-]$ can be regarded as being a constant in the mobile and stationary phases. This means that the distribution coefficient D_A (Equation 1) and the retention factor k'_A (Equation 4) can be calculated. Strictly speaking, such calculations are only permissible if the concentrations in Equation 17 correspond to the activities; however, this is only the case for an infinite dilution [19]. In principle the activities of the ions in the stationary phase are inaccessible [4]. For the most frequently used ion exchangers of low capacity, which can only be used as the mobile phase with very dilute electrolytes, the activities are simply disregarded. Such very coarse approximations are no longer valid for high-capacity (>200 mmol/g) packing materials and concentrated eluents; these show clear variations from the «ideal» behavior.

3.3.3 Ion pair formation

With the aid of ion pair chromatography it is possible to separate the same analytes as in ion exclusion chromatography, but the separation mechanism is completely different. The stationary phases used are completely polar reversed phase materials such as are used in distribution chromatography. A so-called ion pair regent is added to the eluents; this consists of anionic or cationic surfactants such as tetraalkylammonium salts or n-alkylsulfonic acids. Together with the oppositely charged analyte ions the ion pair reagents form an uncharged ion pair, which can be retarded at the stationary phase by hydrophobic interactions. Separation is possible because of the formation constants of the ion pairs and their different degrees of adsorption. Figure 7 shows a simplified static ion exchange model in which it is assumed that interactions with the analytes only occur after adsorption of the ion pair reagent at the stationary phase.

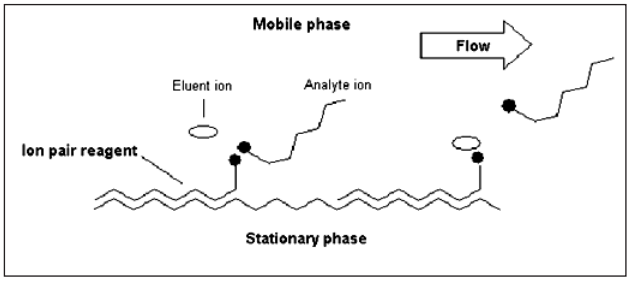


Figure 7
Schematic diagram showing the static ion exchange model in ion pair chromatography (IPC). The separation principle applies to both anions and cations

3.3.4 Ion exclusion

Ion exclusion chromatography (IEC) is mainly used for the separation of weak acids or bases [2, 4]. The greatest importance of IEC is for the determination of weak acids such as carboxylic acids, carbohydrates, phenols or amino acids. Figure 8 shows the separation principle of IEC using an R–COOH carboxylic acid as an example

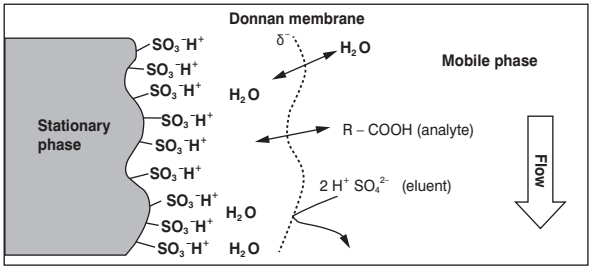


Figure 8
Donnan exclusion as the separation principle in ion exclusion chromatography (IEC)

In IEC a completely sulfonated cation exchanger whose sulfonic acids groups are electrically neutral with protons as counter-ions is frequently used as packing material. In aqueous eluents the functional groups are hydrated. The hydrate shell is limited by an (imaginary) negatively charged membrane (Donnan membrane). It is only passable by uncharged, non-dissociated molecules such as water. Organic carboxylic acids can be separated if strong mineral acids such as sulfuric acid are used as the mobile phase. Due to their low acid constants (pK_A values) the carboxylic acids are almost completely present in a non-dissociated form in strongly acidic eluents. They can pass through the Donnan membrane and be adsorbed at the stationary phase, whereas the sulfate ions of the completely dissociated sulfuric acid are excluded.

Figure 9 shows the typical dependency of the elution volume of an acid on its pK_A value for separation by ion exclusion. Superimposed adsorption (long-chain carboxylic acids, H_2S) and the limits of the practical working range can be clearly recognized. In the final instance carboxylic acids are separated because of their different pK_A values.

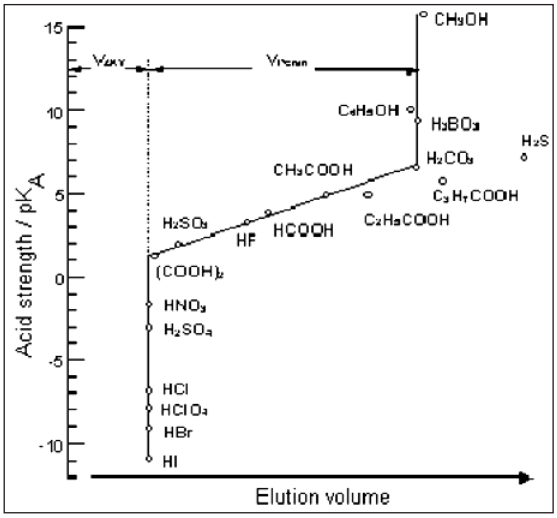


Figure 9
Dependency of the elution volume on the particular pK_A value of the acids in ion exclusion

3.4 Retention models in ion chromatography

In an ideal case the retention of an analyte in ion chromatography would only be determined by its affinity to the functional groups of the ion exchanger. This affinity can be described by the formulation of a chemical reaction, the ion exchange reaction, and can be explained by using the law of mass action.

The retention models described below attempt to make predictions about the retention behavior of participating analytes under particular chromatographic conditions based on the law of mass action. If the resulting models are suitable for explaining the macroscopic observations then with their help it is possible, for example, to optimize an elution system for a particular separation problem.

3.4.1 Retention models in anion chromatography

The following observations initially only involve elution by isoionic displacement as the simplest elution mechanism in ion chromatography. The actual example refers to anion chromatography, but the same considerations apply similarly to cation chromatography. Only when complexing agents are added to the eluents is it necessary to extend the retention model; this is described in the section «Retention model for elution in the presence of complexing agents» (chapter 3.4.2).

Retention model for eluents with one anion

If electroneutrality is assumed then the simplest approach to a retention model for isoionic displacement is one in which a single eluent anion E^{y-} competes with one analyte anion A^{x-} for the functional groups of the stationary phase [4]. The concentration of the eluent anions E^{y-} remains constant with time (isocratic elution).

The exchange sites on a separating column with a capacity Q are occupied by eluent anions E^{y-} at the start of the chromatographic process. If a sample containing the analyte ion A^{x-} is added then the following equilibrium becomes established between the stationary phase (index $_S$) and the mobile phase (index $_M$):

$$y \cdot {A_M}^{x-} + x \cdot {E_S}^{y-} \;\leftrightharpoons\; y \cdot {A_S}^{x-} + x \cdot {E_M}^{y-} \tag{18}$$

According to the law of mass action this equilibrium can be described by a thermodynamic equilibrium constant. If the activities of the participating ions are taken into account then the following thermodynamic equilibrium constant is obtained:

$$K_{A,E} = \frac{[{A_S}^{x-}]^y \cdot [{E_M}^{y-}]^x}{[{A_M}^{x-}]^y \cdot [{E_S}^{y-}]^x} \cdot \frac{\Upsilon^y_{{A_S}^{x-}} \cdot \Upsilon^x_{{E_M}^{y-}}}{\Upsilon^y_{{A_M}^{x-}} \cdot \Upsilon^x_{{E_S}^{y-}}} \tag{19}$$

As the activities of the participating ions cannot be determined in the stationary and mobile phases the activity in the stationary phase is ignored and set as being 1.

If for the analyte anion A^{x-} two known quantities, the distribution coefficient D_A and the retention factor k'_A, are now introduced from chapter 3.2.1,

$$D_A = \frac{[A]_S}{[A]_M} \qquad \text{with} \quad k'_A = D_A \cdot \frac{V_S}{V_M} \tag{20}$$

then, by including these quantities and neglecting the activities, Equation 19 can be converted to:

$$K_{A,E} = \left(k'_A \cdot \frac{V_M}{V_S}\right)^y \cdot \left(\frac{{E_M}^{y-}}{{E_S}^{y-}}\right)^x \tag{21}$$

As the concentration of the eluent ions E is normally higher than that of the analyte anions A^{x-} by several powers of ten, a good approximation can be obtained by assuming that all the functional groups are occupied by E^{y-}. Under this assumption the non-determinable concentration of E^{y-} in the stationary phase can be replaced by the more easily accessible parameters of exchange capacity Q and charge y of the eluent anion:

$$[{E_S}^{y-}] = \frac{Q}{y} \tag{22}$$

This means that Equation 21 can be converted to:

$$K_{A,E} = \left(k'_A \cdot \frac{V_M}{V_S}\right)^y \cdot \left(\frac{Q}{y}\right)^{-x} \cdot [{E_M}^{y-}]^x \tag{23}$$

The retention factor k'_A of the analyte anion A^{x-} can easily be obtained from a chromatogram. Equation 23 is therefore solved for this quantity.

$$k'_A = \frac{V_S}{V_M} \left(K_{A,E}\right)^{\frac{1}{y}} \left(\frac{Q}{y}\right)^{\frac{x}{y}} [{E_M}^{y-}]^{-\frac{x}{y}} \tag{24}$$

This equation is of crucial importance for anion chromatography as it provides a quantitative relationship between the retention factor k'_A and several experimentally accessible parameters such as the concentration of the eluent and the exchange capacity. In practice a logarithmic version of Equation 24 is used for reasons of clarity.

$$\log k'_A = \frac{1}{y} \log K_{A,E} + \frac{x}{y} \log \frac{Q}{y} + \log \Phi - \frac{x}{y} \log [{E_M}^{y-}] \quad \text{mit} \quad \phi = \frac{V_S}{V_M} \tag{25}$$

From Equation 25 it can be seen that:

- Increasing the eluent concentration $[E^{y-}]$ accelerates elution.
  - Larger retention factors result from larger equilibrium constants $K_{A,E}$, higher exchange capacities Q and a larger phase volume ratio Φ.
- Multivalent analytes A^{nx-} are retarded more strongly than monovalent A^{x-},
  - at least as long as the eluent concentration $[E^{y-}]$ is relatively low. This is also known as electroselectivity.

- Multivalent eluents E^{ny-} have a higher elution power than monovalent E^{y-}.
  - The elution of multivalent analytes A^{nx-} is more strongly influenced by increased concentrations of monovalent eluent ions E^{y-} than that of monovalent analytes A^{x-}.

$$k'_A \propto \frac{Q}{[E_M{}^{y-}]} \tag{26}$$

From Equation 26 it can be seen that if the exchange capacity Q is increased then the concentration of the eluent $[E^{y-}]$ must be increased proportionally in order to obtain constant retention factors. This is the reason why low-capacity separation phases are normally used in ion chromatography, as high electrolyte concentrations would make the most important detection method used in ion chromatography, conductivity detection, practically impossible.

In order to optimize separation problems the eluent concentration $[E^{y-}]$ is often varied. If all the other parameters occurring in Equation 25 are kept constant then this can be simplified to:

$$\log k'_A = C_1 - \frac{x}{y} \log [E_M{}^{y-}] \tag{27}$$

A graphical plot of Equation 27 gives a straight line with a slope m = –x/y and an intercept on the axis C_1 which contains the quantities Q, Φ and $K_{A,E}$. If a monoanionic eluent is used then m is also known as the effective charge. Figure 10 shows the result of Equation 27 for various combinations of differently charged eluent and analyte anions.

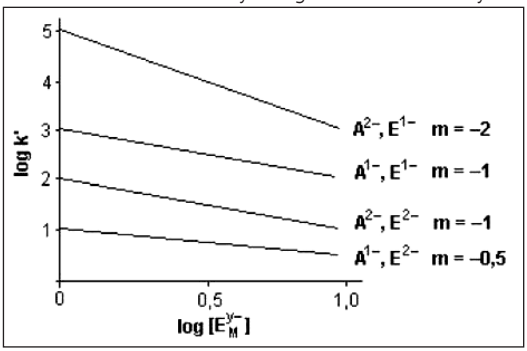


Figure 10
Graphical presentation of Equation 27 for various combinations of differently charged eluent and analyte anions [4]

Equation 27 has been confirmed in numerous publications; however, under the assumption that low-capacity separation materials and dilute eluents have been used.

If the exchange capacity Q is varied while the other parameters remain constant, then Equation 25 can be simplified to:

$$\log k'_A = C_2 + \frac{x}{y} \log \frac{Q}{y} \tag{28}$$

The graphical representation of this equation is similar to Figure 10, but with a positive slope. Chromatographic investigations into the variation of Q have to date only been carried out once for the separation of divalent cations. This has shown that, in contrast to previous assumptions, the retention factor and the selectivity coefficients cannot be regarded as being independent of the exchange capacity. For the optimization of separation problems it is clear that apart from the concentration of the eluent [E^{y-}] the exchange capacity Q is also an important quantity.
The above considerations only apply for one analyte anion. If two different anions A^{x-} and B^{z-} compete for the functional groups then the following applies for the selectivity coefficients $K_{A,B}$:

$$K_{A,B} = \frac{[{A_S}^{x-}]^z \cdot [{B_M}^{z-}]^x}{[{A_M}^{x-}]^z \cdot [{B_S}^{z-}]^x} \tag{29}$$

By taking Equation 20 into account the selectivity $\alpha_{A,B}$ is first obtained,

$$\alpha_{A,B} = \frac{k'_A}{k'_B} = \frac{[{A_S}^{x-}] \cdot [{B_M}^{z-}]}{[{A_M}^{x-}] \cdot [{B_S}^{z-}]} \tag{30}$$

and then, after conversion, Equations 31a and b,

$$\log \alpha_{A,B} = \frac{1}{z} \cdot \log K_{A,B} + \frac{x-z}{z} \log\left(\frac{k'_B \cdot V_M}{V_S}\right) \tag{31}$$

$$\log \alpha_{A,B} = \frac{1}{x} \cdot \log K_{A,B} + \frac{x-z}{z} \log\left(\frac{k'_A \cdot V_M}{V_S}\right) \tag{32}$$

which, for analytes with the same charge (x = z), can be simplified to:

$$\log \alpha_{A,B} = \frac{1}{z} \cdot \log K_{A,B} \qquad \log \alpha_{A,B} = \frac{1}{x} \log K_{A,B} \tag{33}$$

For the selectivity between two similarly charged analyte anions this means:

- It is only a function of the selectivity coefficients $K_{A,B}$ and the charges z and x.
- At constant $K_{A,B}$ the selectivity depends neither on the concentration [E^{y-}] nor on the chemical constitution of the eluent anion (!)

If A and B have different charges then:

- $\alpha_{A,B}$ depends on the retention factor of one of the two analytes.
- The two retention factors k'_A and k'_B are not independent of one another (!)

In Equations 31 to 33 it is particularly interesting that the selectivity between two anions initially depends neither on the chemical constitution nor on the concentration or charge of the eluent anion, providing that the phase volume ratio and the selectivity coefficient are constant. However, in practice an alteration in $\alpha_{A,B}$ can be achieved by a variation of $[E^{y-}]$, as two analytes with the same charge can nevertheless have different chemical properties, e.g. polarizability and hydration; this can result in different affinities to the stationary phase. However, these interactions are not taken into account in the classical derivation.

Retention models for eluents with several anions

The previous observations have referred to elution systems with only a single eluent anion. In practice there are usually several eluting species present, for example in carbonate/hydrogen carbonate buffers or in polybasic acids such as phosphoric acids, whose dissociation and therefore distribution of species depends strongly on the pH.

Even in simple cases in which none of the participating eluent anions are involved in the acid-base equilibrium, the relationship between the retention factor k' and the eluent concentration $[E^-]$ cannot be represented in the form of a simple log-log relationship according to Equation 28. This would only be possible if the concentration or the elution power of the other eluent anions could be ignored; this would then correspond to the retention model for monoanionic eluents.

In the literature several models concerned with polyanionic eluents are described; these are briefly discussed below:

- dominant equilibrium model [21]
- effective charge model [22 – 24]
- multiple eluent species model [25, 26]

If an eluent based on phosphate with $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} (in the following H_2P^-, HP^{2-} and P^{3-}) and the monovalent analyte ion A^- is considered then the following equilibria are formed:

$$A_M^- + H_2P_S^- \leftrightharpoons A_S^- + H_2P_M^- \quad ; \; x_1 \tag{34}$$

$$A_M^- + \frac{1}{2}HP_S^{2-} \leftrightharpoons A_S^- + \frac{1}{2}HP_M^{2-} \quad ; \; x_2 \tag{35}$$

$$A_M^- + \frac{1}{3}P_S^{3-} \leftrightharpoons A_S^- + \frac{1}{3}P_M^{3-} \quad ; \; x_3 \tag{36}$$

Here the quantities x_{1-3} correspond to the shares of the particular reaction in the retention, which is why:

$$x_1 + x_2 + x_3 = 1 \tag{37}$$

Both the dominant equilibrium model and the effective charge model postulate a particular charge for the eluent anion, even though several species are present; this means that the retention model for monoanionic eluents (see above) can be used.

The **dominant equilibrium model** assumes that the equilibrium in Equation 36 is wholly on the right-hand side, as P^{3-} is bound far more strongly to the stationary phase than H_2P^- and HP^{2-} as a result of its higher charge. This means that P^{3-} alone is decisive for the elution so that the charge of the eluent anion is $^-3$. However, in practice this model only achieves a good agreement with multivalent analytes [4].

In the **effective charge model** an effective charge is calculated, taking into account the pH value, from the molar fractions of the possible species H_2P^-, HP^{2-} and P^{3-} [22]. By using these together with the existing concentrations of the eluent species a relationship analogous to Equation 27 can be obtained. However, a requirement for such a type of calculation is that the selectivities of the individual eluent species with respect to the analyte ion A^- do not differ greatly. The effective charge model is chiefly suitable for use with monovalent analytes [4].

In reality the **multiple eluent species model** is most suitable for the description of eluents whose components are chemically derived from one another. The following observations are based on the model of Mongay et al. [27], which is a further development of the work of Jenke and Pagenkopf [25].

Equations 34 to 36 can be used to express the global equilibrium on the separating column (Equation 38). By taking Equation 37 into account, the equilibrium constants $K_{A,P}$ can be defined for the exchange process if the activities are ignored (Equation 39).

$$(x_1 + x_2 + x_3) \cdot A_M^- + \frac{x_1}{1} \cdot H_2P_S^- + \frac{x_2}{2} \cdot HP_S^{2-} + \frac{x_3}{3} \cdot P_S^- \quad \leftrightharpoons$$
$$(x_1 + x_2 + x_3) \cdot A_S^- + \frac{x_1}{1} \cdot H_2P_M^- + \frac{x_2}{2} \cdot HP_M^{2-} + \frac{x_3}{3} \cdot P_M^- \qquad (38)$$

$$K_{A,P} = \frac{[A_S^-] \cdot [H_2P_M^-]^{\frac{x_1}{1}} \cdot [HP_M^{2-}]^{\frac{x_2}{2}} \cdot [P_M^{3-}]^{\frac{x_3}{3}}}{[A_M^-] \cdot [H_2P_S^-]^{\frac{x_1}{1}} \cdot [HP_S^{2-}]^{\frac{x_2}{2}} \cdot [P_S^{3-}]^{\frac{x_3}{3}}} \qquad (39)$$

The further mathematical treatment is carried out as for the derivation of the retention model for monoanionic eluents. The following must chiefly be taken into account:

- the (possible) dissociation of the analyte anion A^-
- the total concentration of the eluent species: $c_P = [H_3P] + [H_2P^-] + [HP^{2-}] + [P^{3-}]$
- the extent of the interactions between the eluent species and the functional groups

The introduction of the retention factor k'_A (Equation 20) and the exchange capacity Q (Equation 22) supplies, after further mathematical conversion, a complicated expression for k'_A [28]; this is given here only in its logarithmic and further simplified form:

$$\log k'_A = C_3 - \left(\frac{x_1}{1} + \frac{x_2}{2} + \frac{x_3}{3}\right) \cdot \log c_P \qquad (40)$$

C_3 is a constant which, similar to Equation 27, contains quantities such as the phase volume ratio, the exchange capacity and the equilibrium constant; c_P is the total of the concentrations of the eluent species. From Equation 40 it can be deduced that the slopes of the straight lines in a double logarithmic plot must always be smaller than those according to the simple retention model for monoanionic eluents (Equation 27), as the total in the brackets is always smaller than 1. It is also clear that the pH value has a decisive influence on the extent to which the log-log relationship is influenced.

For eluent species which are not chemically derived from one another Janoš et al. have provided a model which was developed for describing eluents containing a phosphate buffer and additional perchlorate [29]. This model was derived according to similar considerations to those described above, but in addition an exchange equilibrium must be taken into account for a further monovalent eluent ion. The calculations provide very complicated expressions for the retention factor; these can be dramatically simplified for neutral or acidic eluents. If only a single further monovalent eluent species is present in addition to the perchlorate then Equation 41 is obtained in which x and y represent the contributions of the corresponding equilibrium reactions (x: phosphate buffer, y: perchlorate) to the retention. As in the other models, C_4 is a constant, while the factor a, which is not defined any more closely, is intended to take into account how much more strongly the perchlorate ion is bound at the stationary phase than the phosphate species involved.

$$\log k'_A = C_4 - \left(y + \Sigma \frac{x_i}{i}\right) \cdot \log a - \left(y + \Sigma \frac{x_i}{i}\right) \cdot \log[E^-] \qquad (41)$$

As in Equation 41 the terms within the brackets are always smaller than 1, the slope of the log-log plot is always less than would be expected from the simple retention model. In actual applications the model provides good agreement with the experimental data. However, the form described above cannot be used for alkaline elution systems.

3.4.2 Retention models in cation chromatography

Cation chromatography must be divided into two groups of retention models. One group is concerned with alkali and alkaline earth cations as analytes and only requires one elution system based on isoionic displacement. In this case the stationary phase has carboxylic acid groups as functional groups. In the separation of metal ions with two or more charges the use of a complexing agent is essential; its influence on retention is described below.

Retention model for eluents with one cation

The explanations given in the section «Retention model for eluents with one anion» apply in an analogous way for cation chromatography with elution by isoionic displacement. In practice this is relevant for the determination of alkali and alkaline earth metals, ammonium and short-chain amines. Apart from H^+, organic cations such as 2,3-diaminopropionic acid (DAP) are used as eluent cations in combination with dilute hydrochloric acid. Depending on the set pH of the eluent, DAP is present in the ionic forms (1) and (2) (Figure 11). After suppression the zwitterionic form (3) is obtained, which has no inherent conductivity.

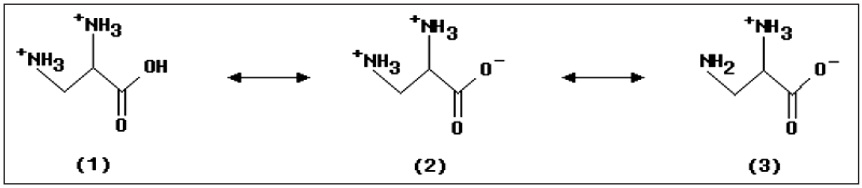


Figure 11 Ionic species of diaminopropionic acid

Retention model for elution in the presence of complexing agent

In cation chromatography eluents which contain a complexing agent in addition to the eluent cation E^{y+} are used for the separation of alkaline earth, transition and heavy metal ions. The complexing agents used are mainly dicarboxylic acids H_2L such as tartaric acid, oxalic acid and pyridinedicarboxylic acid (PDCA) or citric acid. The analytes form complexes of differing stabilities with the anions of the complexing agents HL^- and L^{2-}; their stoichiometries also differ. As a result of the complexing process the effective charge, i.e. the charge of the analyte present over an average period of time, is reduced. As this occurs in accordance with the kinetics of complex formation and the stability constants of the complexes, the differences in selectivity increase and the separation of even similar analytes becomes possible. Apart from ion exchange, complex formation is decisive for the separation of higher-charged metal ions.

$$Me^{x+} + L^{2-} \leftrightharpoons MeL^{x-2} \quad ; K_{MeL} = \frac{[MeL^{x-2}]}{[Me^{x+}][L^{2-}]} \tag{42}$$

$$MeL^{x-2} + L^{2-} \leftrightharpoons MeL_2^{x-4} \quad ; K_{MeL_2} = \frac{[MeL_2^{x-4}]}{[MeL^{x-2}][L^{2-}]} \tag{43}$$

$$Me^{x+} + HL^{-} \leftrightharpoons MeHL^{x-1} \quad ; K_{MeHL} = \frac{[MeHL^{x-1}]}{[Me^{x+}][HL^{-}]} \tag{44}$$

In order to take the influence of the complexing agent on the ion chromatographic separation into account the retention model for isoionic displacement (see section «Retention model for eluents with one anion», chapter 3.4.1) is extended. The α_M value is introduced as a variable which describes the degree of complex formation of the analyte. The fraction α_M of the free analyte ions in the mobile phase is given as

$$\alpha_M = \frac{[Me^{x+}]}{[Me^{x+}]+[MeHL^{x-1}]+[MeL^{x-2}]+[MeL_2^{x-4}]} = \frac{[Me^{x+}]}{[Me']} \tag{45}$$

with [Me'] as the total concentration of the metal ions. The α_M value can be calculated from the complex formation constants, the acid dissociation constant of the carboxylic acid and the pH of the eluent. If complex formation is taken into account then the following is obtained for the distribution coefficient D_{Me}:

$$D_{Me} = \frac{[MeR_x]}{[Me']} = \alpha_M \frac{[MeR_x]}{[Me^{x+}]} \tag{46}$$

If it is assumed that only free analyte ions Me^{x+} interact with the carboxylic acid or sulfonic acid groups and that $c(E^{y+}) >> c(H^+)$, then the following is obtained for Equation 23:

$$K_{Me,E} = \left(\frac{k'_{Me}}{\alpha_M \Phi}\right)^y \left(\frac{Q}{y}\right)^{-x} [M^{y*}]^x \quad (47)$$

In a similar manner to Equation 25 the logarithmic form of Equation 47 is obtained as:

$$\log k'_{Me} = \log \alpha_M + \frac{1}{y} \log K_{Me,E} + \frac{x}{y} \log \frac{Q}{y} + \log \Phi - \frac{x}{y} \log \left[E_M^{y+}\right] \quad (48)$$

If several cationic metal species occur together, e.g. Me^{x+} and $MeHL^{(x-1)+}$, then normally only a single peak is obtained in the chromatogram for the analytes involved. The number of peaks which are obtained depends on the kinetics of the complexing and decomplexing equilibria in the mobile phase. Only one peak is obtained if the complex equilibria are achieved more rapidly in the mobile phase in comparison with the residence time of the complex in the stationary phase. On the other hand, if the complexing process occurs slowly then asymmetric or multiple peaks may occur.

If it is assumed that all metal species present in the mobile phase can interact with the stationary phase then the following is obtained for the experimentally determined retention factor k'_{exp} of the analyte:

$$k'_{exp} = k'_{Me^{x+}} \alpha_{Me^{x+}} + k'_{MeHL^{x-1}} \alpha_{MeHL^{x-1}} + k'_{MeL^{x-2}} \alpha_{Me^{x-2}} \quad (49)$$

Consideration of the dependency of the retention factor on the variables Q, $[E^{y+}]$ as well as α_M requires that the relationship presented in Equation 48 is used as the basis, as the divalent analytes mainly form neutral or anionic complexes with strong complexing agents.

Calculation of α_M values

According to Equation 45, the α_M value is defined as the ratio of the concentration of the free metal ions to the total concentration of the metal ions. The concentrations of the metal species present in the mobile phase can be calculated from the relevant complex formation constants and the acid dissociation constants of the carboxylic acids used.

If tartaric acid is used as the complexing agent in eluents then it is chiefly the neutral MeL 1:1 complexes which are formed with the alkaline earth, transition and heavy metals together with a lesser amount of the hydrogen tartrate complex $MeHL^+$. For tartaric acid eluents the following is obtained for the calculation of the α_M value:

$$\alpha_M = \frac{[Me^{2+}]}{[Me^{2+}] + [MeL] + [MeHL^+]} = \frac{1}{1 + K_{MeL}\, \alpha_L\, c_L + K_{MeHL}\, \alpha_{HL}\, c_L} \quad (50)$$

where C_L is the total concentration of tartaric acid and α_{HL} and α_L are the molar fractions of the acid anions HL^- and L^{2-}.

Apart from 1:1 complexes, some metal ions also form stable MeL_2^{2-} complexes with oxalic acid and/or pyridinedicarboxylic acid , so that α_M can be calculated as follows:

$$\alpha_M = \frac{[Me^{2+}]}{[Me^{2+}]+[MeL]+[MeL_2^{2-}]} = \frac{1}{1+ K_{MeL}\,\alpha_L\,c_L + K_{MeL}\,K_{MeL_2}\,\alpha_L{}^2\,c_L{}^2} \quad (51)$$

Calculation of the acid dissociation

The pH and concentration of the complexing agent in the mobile phase determine the concentration at the ligand and therefore the extent to which the analyte is complexed. An acid with two protons dissociates in two stages:

$$H_2L \leftrightharpoons HL^- + H^+ \qquad ; K_{S_1} = \frac{[HL^-]\,[H^+]}{[H_2L]} \quad (52)$$

$$HL^- \leftrightharpoons L^{2-} + H^+ \qquad ; K_{S_2} = \frac{[L^{2-}]\,[H^+]}{[HL^-]} \quad (53)$$

with the acid constants K_{S1} And K_{S2}. The molar fractions α_{H2L}, α_{HL} and α_L used to calculate the α_M value are obtained from the laws of mass action of the individual deprotonization stages:

$$\alpha_{H_2L} = \frac{[H^+]}{[H_2L]+[HL^-]+[L^{2-}]} = \frac{[H^+]^2}{[H^+]^2+ K_{S_1}\,[H^+]+ K_{S_1}\,K_{S_2}} \quad (54)$$

$$\alpha_{HL} = \frac{[HL^-]}{[H_2L]+[HL^-]+[L^{2-}]} = \frac{K_{S_1}\,[H^+]^2}{[H^+]^2+ K_{S_1}\,[H^+]+ K_{S_1}\,K_{S_2}} \quad (55)$$

$$\alpha_L = \frac{[L^{2-}]}{[H_2L]+[HL^-]+[L^{2-}]} = \frac{K_{S_1}\,K_{S_2}}{[H^+]^2+ K_{S_1}\,[H^+]+ K_{S_1}\,K_{S_2}} \quad (56)$$

3.5 Ion chromatography detection systems

Different methods are used for the detection of substances in the HPLC sector. The selection of a suitable detector must always be made in accordance with the analytical problem to be solved. The demands placed on the detector can be summarized as follows:

- high measuring sensitivity and short response times
- measuring signal proportional to the analyte concentration (large linear range)
- small baseline changes (drift)
- low background noise
- as small a volume as possible to reduce peak broadening

A general differentiation is made between selective and non-selective detectors. Whereas a selective detector responds directly to a property of the analyte, non-selective detectors react

to an alteration to one of the physical properties of the whole elution system caused by the analyte. As the detectors used in ion chromatography do not differ in principle from those used for «conventional» HPLC the most important detection systems will at least be mentioned in this section. The universal and most frequently used IC detector is the conductivity detector.

3.5.1 Electrochemical detection methods

Conductivity detection

Conductivity detection, also known as conductometric detection, is the most important detection mode in the ion chromatography sector. If the number of ion chromatographs which have been sold is taken into account then this share is probably much higher today. Conductivity detection is a non-selective detection principle; in this case both direct and indirect detection determinations are possible. As aqueous electrolytes are frequently used as the mobile phase in ion chromatography, the detector must be able to respond to the relatively small changes in the total conductivity of the eluent caused by the analyte ions. By the use of so-called suppression techniques the inherent conductivity of certain eluents can be dramatically reduced; in the case of strong acid anions it is thus possible to considerably improve the sensitivity.

The conductivity κ is determined technically as the reciprocal of the resistance R which a liquid produces between two electrodes with an area of A at a distance of L.

$$\kappa = \frac{L}{A \cdot R} \tag{57}$$

The equivalent conductivity Λ of a solution can be determined as:

$$\Lambda = \frac{\kappa}{c} \tag{58}$$

The limiting conductivity Λ^{∞} and the variation of conductivity with concentration can be determined by using Equation 59. Constants A and B are empirical substance constants.

$$\Lambda = \Lambda^{\infty} - (A + B\,\Lambda^{\infty})\sqrt{c} \tag{59}$$

The conductivity of an electrolyte is obtained by adding the ionic conductivities Λ^{-}_{Anion} and Λ^{+}_{Cation} together:

$$\kappa = c\,(\Lambda^{-}_{Anion} + \Lambda^{+}_{Cation}) \tag{60}$$

According to Kohlrausch's law, the conductivity of a dilute solution is proportional to the sum of the conductivities of all the ions multiplied by their concentrations.

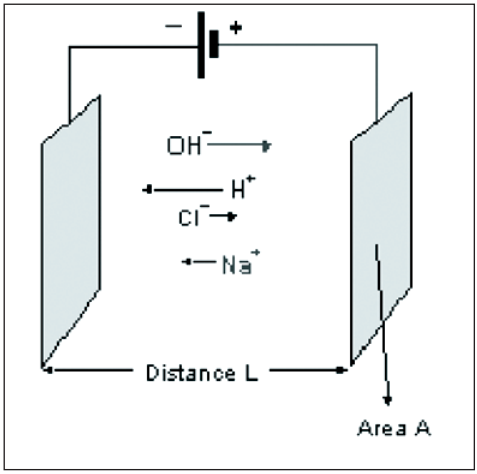


Figure 12 Construction of a conductivity measuring cell

$$\kappa = \frac{\sum \Lambda_i \, c_i}{1000} \tag{61}$$

where κ is the conductivity in S cm^{-1}, Λ the limiting conductivity in S cm^2 (z mol)$^{-1}$ and c the concentration in z mol L^{-1} (z corresponds to the charge on the ion). The factor 1000 comes from the fact that 1 liter is equal to 1000 cm^3.

The change in conductivity caused by the analyte is proportional to its concentration in the eluate,

$$\Delta\kappa = \frac{(\Lambda_S - \Lambda_E)\, c_S}{1000} \tag{62}$$

where S and E stand for the analyte and eluent ion respectively. As in conductivity detection the alteration in conductivity is measured, in anion chromatography only small alterations in conductivity occur at high background conductivities. This means that keeping the background conductivity as low as possible is advantageous.

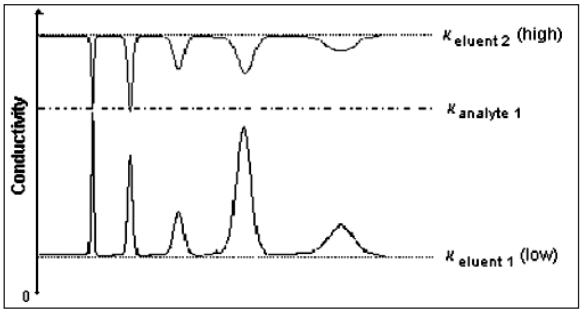


Figure 13
Changes in the eluate conductivity during the separation of a multi-substance mixture by ion chromatography. The plots are for an eluent with high and low conductivity

In ion chromatography the conductivity of an eluate can either be determined directly or after passage through a suppressor. These versions are known as IC without or with chemical suppression. The version which is to be preferred can be determined by carrying out a rough calculation.

If direct conductivity detection is used in anion chromatography then the sensitivity κ_{Peak} of the measurement depends on the difference in the equivalent conductivities of the analyte and eluent anions; with chloride as the analyte and carbonate as the eluent anion the following equations are obtained:

$$\kappa_{Peak} \approx c_{Analyte} (\Lambda^{-}_{Cl^-} - \Lambda^{-}_{CO_3^{2-}}) \approx c_{Analyte} (76 - 72)$$
$$\kappa_{Peak} \approx c_{Analyte} \cdot 4$$

If the eluent is adapted to the requirements of direct conductivity detection then the following sensitivity is obtained by replacing the carbonate eluent by a phthalate eluent:

$$\kappa_{Peak} \approx c_{Analyte} (\Lambda^{-}_{Cl^-} - \Lambda^{-}_{Phthalat}) \approx c_{Analyte} (76 - 38)$$
$$\kappa_{Peak} \approx c_{Analyte} \cdot 38$$

If, on the other hand, the conductivity of the eluent is chemically suppressed (exchange of the eluent cations for H+), the sensitivity depends on the sum of the equivalent conductivities of the analyte anion and the H^+ ion; the following then applies for Cl^- as analyte anion:

$$\kappa_{Peak} \approx c_{Analyte} (\Lambda^{-}_{Cl^-} + \Lambda^{+}_{H+}) \approx c_{Analyte} (76 + 350)$$
$$\kappa_{Peak} \approx c_{Analyte} \cdot 426$$

From this rough calculation it can be seen that, for anions, direct conductivity detection is less sensitive by a factor of 10 than conductivity detection after chemical suppression.

In a similar way the following rough calculation can be made for cation chromatography, with Na^+ as the analyte and H^+ as the eluent cation. In the case of direct conductivity detection (NaCl/HCl) the following equations are obtained for the sensitivity κ_{Peak} of the measurement:

$$\kappa_{Peak} \approx c_{Analyte} (\Lambda^{+}_{Na^+} - \Lambda^{+}_{H+}) \approx c_{Analyte} (50 - 350)$$
$$\kappa_{Peak} \approx c_{Analyte} \cdot (-300)$$

If, on the other hand, the conductivity of the eluent is chemically suppressed (exchange of the eluent anions Cl– for OH–), the following applies:

$$\kappa_{Peak} \approx c_{Analyte} (\Lambda^{+}_{Na+} + \Lambda^{-}_{OH^-}) \approx c_{Analyte} (50 + 198)$$
$$\kappa_{Peak} \approx c_{Analyte} \cdot 248$$

This means that the sensitivity is better for direct conductivity detection of cations than for conductivity detection after chemical suppression.

Table 2 Equivalent conductivity Λ_∞ of several ions

Cations	Λ^+ (S cm² eq⁻¹)	Anions	Λ^- (S cm² eq⁻¹)
H^+	350	OH^-	198
Li^+	39	F^-	54
Na^+	50	Cl^-	76
K^+	74	Br^-	78
NH_4^+	73	I^-	77
½ Mg^{2+}	53	NO_2^-	72
½ Ca^{2+}	60	NO_3^-	71
½ Sr^{2+}	59	HCO_3^-	45
½ Ba^{2+}	64	½ CO_3^{2-}	72
½ Zn^{2+}	52	$H_2PO_4^-$	33
½ Hg^{2+}	53	½ HPO_4^{2-}	57
½ Cu^{2+}	55	$^1/_3$ PO_4^{3-}	69
½ Pb^{2+}	71	½ SO_4^{2-}	80
½ Co^{2+}	53	CN^-	82
$^1/_3$ Fe^{3+}	70	SCN^-	66
$N(Et)_4^+$	33	Acetate	41
		½ Phthalate	38
		Propionate	36
		Benzoate	32
		Salicylate	30
		½ Oxalate	74

Suppressors for ion chromatography can be arranged either as discontinuously working packed-bed suppressors as shown in Figure 14 or as continuously working membrane suppressors. The packed-bed suppressor in the revolver version used by Metrohm has three identical suppressor units; while one unit acts as the suppressor the second is regenerated and the third is rinsed with the detector effluent (STREAM). After an analysis has been carried out the revolver is rotated by 120 degrees and the column which has just been rinsed is used as the suppressor. This makes quasi-continuous work possible.

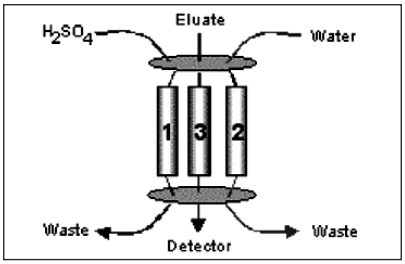


Figure 14
Schematic construction of a packed-bed suppressor for quasi-continuous work

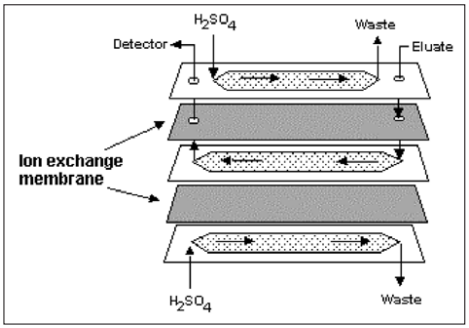


Figure 15
Schematic construction of a continuously working membrane suppressor

The membrane suppressor shown in Figure 15 allows continuous work but, as a result of the use of ion exchange membranes, is susceptible to occupation of the membrane surface; this reduces the suppression capacity and finally causes the suppressor to cease functioning.

Amperometric detection

In principle voltammetric detectors can be used for all compounds which have functional groups which are easily reduced or oxidized. The amperometric detector (applying a steady potential e.g. DC or different potential wave forms e.g. PAD, flexIPAD) is the most important version. In this detector a certain potential is applied between a working electrode and a reference electrode. If an electrochemically active analyte, whose half-wave potential is such that the applied potential causes reduction or oxidation, now passes between the electrodes then a current will flow; this represents the measuring signal. Amperometry is very sensitive; the conversion rate is only about 10%. Apart from a few cations (Fe^{3+}, Co^{2+}) it is chiefly anions such as nitrite, nitrate, thiosulfate as well as halides and pseudohalides which can be determined in the ion analysis sector. The most important applications lie, however, in the analysis of sugars by anion chromatography and in clinical analysis. Owing to its different working principle, the coulometric detector provides a quantitative turnover without, however, there being any increased sensitivity.

Potentiometric detection

In potentiometric detection ion-sensitive electrodes are used, some of which have a very high selectivity. Despite their necessary high degree of miniaturization the sensors must function reliably; this still causes problems in practice. This is the reason why up to now potentiometric detection, in the ion chromatography sector, is limited to a few special applications.

3.5.2 Spectroscopic detection methods

Photometric detection

Because of its extremely wide range of application photometric or UV/VIS detection is the most important detection method used in HPLC, as virtually all organic molecules contain chromophore groups which are able to absorb in the UV or VIS spectrum. A requirement is that the eluent used does not absorb in the range of wavelengths used. With direct detection at the maximum absorption of an analyte UV/VIS detection is practically selective. Substances which either only have a limited absorption or no absorption at all in the particular wavelength range can be determined indirectly by measuring the maximum absorption of the elution system. In the field of inorganic ion analysis UV/VIS detection plays a smaller role. While of the simple

anions only analytes such as nitrate, bromide or iodide absorb, important analytes such as fluoride, sulfate or phosphate can only be measured indirectly [4]. Many cations do not absorb at all, but multivalent and transition metals in particular can be converted in a post-column derivatization with chelate formers such as 4-(2-pyridylazo)-resorcinol (PAR) or Tiron to form colored complexes. Redox-active analytes such as bromate and other oxohalides can be analyzed by UV/VIS detection after undergoing a post-column reaction with an electrochemically active indicator.

Fluorescence detection

Fluorescence detection is very sensitive and is always possible whenever analytes can be excited to fluoresce; this is mainly the case for organic compounds with extended p-electron systems. This means that typical applications are found in the fields of clinical and organic analysis. In connection with ion chromatography, fluorescence detection is used in a few special cases, as only particular ions such as Ce^{3+} are directly accessible and non-fluorescing ions can only be detected after derivatization. It is extremely difficult to develop elution systems for this detection method because of its great susceptibility to interference by contaminants. Furthermore the linear range of the method is relatively small (often less than two powers of ten) owing to self-absorption effects.

Coupling techniques

So-called coupling techniques represent the link-up of a chromatography system with an independent analytical method, usually spectrometry [3]. In recent years these methods have greatly increased in importance. Although the coupling of a gas chromatograph with a mass spectrometer (GC-MS) is well established, coupling HPLC with spectrometric methods causes great technical problems. In classical HPLC, i.e. the analysis of organic compounds, couplings with a mass spectrometer (LC-MS), IR-spectrometer (LC-FTIR) and nuclear magnetic resonance spectrometer (LC-NMR) are available [3]. In particular, powerful atomic spectrometric detectors are used in ion chromatography (IC). Examples are atomic emission and mass spectrometry with inductively coupled plasma (IC-ICP-AES, MS); as a result of their element specificity and sensitivity these provide excellent performance data. This is the reason why, despite their relatively high costs, such systems are used for the analysis of species and in the ultratrace analysis of elements.

Refractometry

Differential refractometry is a further detection method based on an optical method. This detector is also called an RI detector (from refractive index). It is wholly non-specific and is suitable for universal use as the quantity measured is the change in the refractive index of the pure eluent caused by the analyte. However, the great temperature sensitivity of the refractive index means that the method is very susceptible to interference. Provided that the temperature is absolutely stable the method has a linear range of three powers of ten. As simple inorganic ions have an extremely low refractive index they can only be determined indirectly by using eluents to which strongly refracting compounds have been added.

3.6 Stationary phases in ion chromatography

Efficient ion chromatography requires packing materials which are made of very small particles which are as spherical as possible and have a narrow particle size distribution range. Particles with diameters from 2 to 10 µm are used. In addition the packing material should display ion exchange kinetics which are as rapid as possible. Apart from the particle this also determines the performance of ion exchangers.

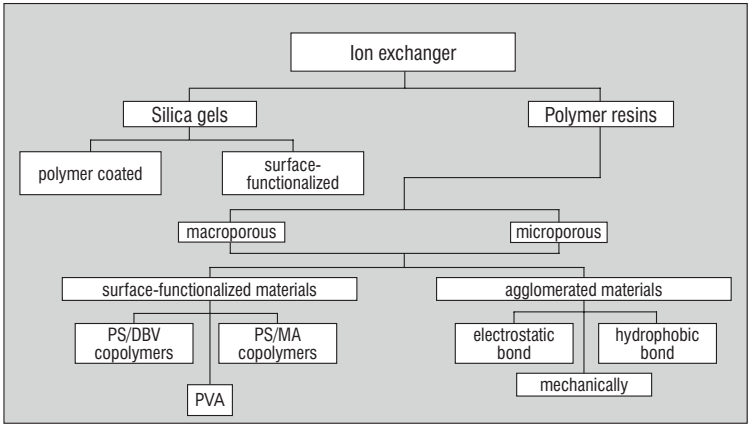
3.6.1 Overview of common stationary phases

A wide range of different organic and inorganic materials is suitable for use in ion chromatography. What they all have in common is that their surfaces bear functional groups which are able to exchange ions. The following classes of substances can be used [4]:

- modified organic polymer resins
- modified silica gels
- inorganic salts (e.g. polyphosphates)
- glasses
- zeolites
- metal oxides (e.g. Al_2O_3)
- cellulose derivatives

Apart from these, systems with a very complex constitution are also possible, such as anion exchangers with functional groups consisting of alkali metal ions bound to a stationary phase by crown ethers. In practice the most important representatives are based on modified organic polymer resins and silica gels. Figure 16 provides an overview of separation materials used in IC:

Figure 16
Commonly used stationary phases in ion chromatography [4]



All stationary phases can be further differentiated according to their type of application (anion or cation chromatography) or the structure of the functional group. The packing materials based on silica gel were originally used for ion chromatography. Although they have a very good separating performance and are mechanically extremely stable, their chemical instability means that they can only be used in the pH range 2 to 7.

From about 1980 onwards ion exchangers became available which were based on organic polymers and could be used in ion chromatography; these were manufactured by modifying commercially available adsorber resins. Today the packing materials used are normally based on either polystyrene-divinylbenzene copolymers (PS-DVB) or methacrylate polymers (MMA).

These two basic copolymer types mainly differ in their polarity. The PS-DVB copolymers are completely nonpolar and represent RP phases whereas the MMA polymers are relatively polar. This situation is an advantage in IC as the more polar separation phases have a lesser tendency to secondary interactions such as adsorption.

The greatest advantage of the organic polymer resins is their great chemical stability throughout the whole pH range. After initial problems had been overcome their chromatographic efficiency is similar to that of most silica gels. However, MMA phases may have a limited mechanical stability, this could limit the length of the separating column used or the maximum possible eluent flow rate.

In ion chromatography today two types of stationary phases are used which differ in principle; surface-functionalized ion exchangers and pellicular ion exchangers. In the first type the functional groups are located directly on the polymer surface or in the pores; the pellicular materials have very small particles (also surface-functionalized) which are bound to larger central particles [4]. The bonding can either be mechanical or result from hydrophobic or electrostatic interactions. Figure 17 shows the arrangement of the two types of packing material using anion exchangers as examples

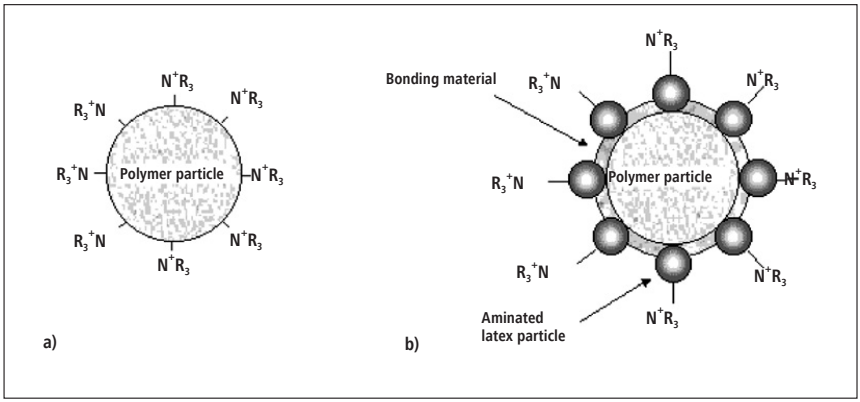


Figure 17 Structure of surface-functionalized (a) and pellicular anion exchangers with mechanical bonding (b)

The pellicular packing materials have a higher chromatographic efficiency as the diffusion paths are kept very short owing to the greater distance of the functional groups from the base material; this results in excellent mass transfer. However, the chemical stability of these separation phases is considerably less than that of the surface-functionalized materials.

3.6.2 Stationary phases for anion chromatography

The functional group used in anion chromatography is normally produced by converting an anchor group with a suitable amine. This produces ammonium ions which are fixed on the polymer surface. Functional groups based on nitrogen are virtually the only ones used for the separation of anions by IC. This is mainly due to their unusually good chemical stability and the almost unlimited number of possible substituents at the nitrogen atom.

The alkyl residues at the positively charged nitrogen can be varied over a wide range. In the simplest case R = H and a primary ammonium ion is formed. However, this can be deprotonated at higher pH values and loose its charge. With this type of packing material the exchange capacity depends on the pH of the eluent which is why they are also known as weakly basic. If the hydrogen atoms are now substituted successively by alkyl groups then first secondary and then tertiary ammonium groups are formed; these can also be deprotonated. Only when all the R groups consist of alkyl groups is the capacity of charge independent of the pH of the eluent and a strongly basic quaternary anion exchanger is obtained. In chromatography a pH-independent capacity is normally the aim so that only completely alkylated materials are used. For special applications such as protein analysis or preconcentration techniques weakly basic materials are also used.

The two most important functional groups in anion chromatography are derived from trimethylamine (TMA) and dimethylethanolamine (DMEA, 2-dimethylaminoethanol). Practically all commercially available separation materials use one of these two groups. TMA groups are also known in the literature as Type I groups, DMEA groups as Type II. Other functional groups with a close relationship to those mentioned above are shown in Figure 18:

In commercial materials, which are usually derived from Type I or II, the exact arrangement of the functional groups is a well-guarded secret [4].

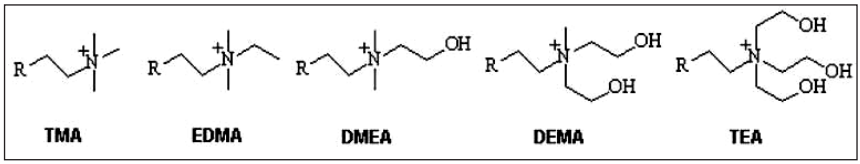


Figure 18

Overview of the important functional groups which are mentioned in this work

*TMA: **Tri**methyl**a**mine (Type I)*
*EDMA: **E**thyl**di**methyl**a**mine*
*DMEA: **Di**methyl**e**thanol**a**mine (Type II)*
*DEMA: **Die**thanol**m**ethyl**a**mine*
*TEA: **Trie**thanol**a**mine*

3.6.3 Stationary phases in cation chromatography

In cation chromatography both materials based on silica gel and polymer-based materials are in use. In contrast to anion chromatography with its usual alkaline eluents the conditions for cation chromatography also allow the use of silica gels.

3.6.4 Cation exchangers based on silica gel

With cation exchangers based on silica gel a differentiation is made between directly functionalized materials and those which are coated by a polymer.

Practically the only reports concerning directly functionalized materials are about strongly acidic exchangers with sulfonic acid groups [2, 4]. These have a good chromatographic efficiency, but are unsuitable for the simultaneous determination of alkali and alkaline earth metals due to the large differences in affinity.

With polymer-coated silica gels, the so-called Schomburg phases, the silicate surface is coated with a «prepolymer» which is then immobilized by cross-linking. By subsequent functionalization various different types of exchanger can be realized. In order to obtain weakly acidic cation exchangers polybutadienemaleic acid (PBDMA) is used, this is then radically cross-linked in-situ [30]. As a result of the thin polymer layer of about 1 to 5 nm [31] the analyte diffusion paths are short so that a high degree of chromatographic efficiency is the result.

A large number of applications for silica-gel-based ion exchangers exist. The simultaneous separation of alkali and alkaline earth metals is one of the most frequent applications; the separation of transition metal and heavy metal ions is also possible. However, several disadvantages prevent the universal use of ion exchangers based on silica gels:

- At pH <2 the bond between the silicon matrix and the functional group becomes increasingly weaker; this leads to a gradual erosion of the functional groups and a loss in capacity.
- At pH >7 the solubility of the silica gel increases considerably; this results in a reduction in the mechanical stability of the packing, particle break-up and a dead volume at the start of the column.

3.6.5 Cation exchangers based on organic polymers

Resins produced by styrene-divinylbenzene copolymerization are mainly used as the matrix. The limitations mentioned for silica gel exchangers do not exist here; cation exchangers based on organic polymers can be used over the whole pH range from 0 to 14 and are also inert to fluoride. Although their pressure resistance is less than that of silica gels it is generally still adequate, with the exception of a few methacrylate resins.

The majority of commercially available cation exchangers use sulfonic acid groups as the functional groups. These can be bound to the aromatic system either directly or via a spacer whose length is variable. Sulfonic acid exchangers with spacers have a considerably better chromatographic efficiency; the spacer length is of secondary importance. They are not suitable for the simultaneous determination of alkali and alkaline earth metals due to the large differences in affinity.

3.6.6 Pellicular cation exchangers

As well as the directly functionalized resins pellicular exchangers are also available. They have a double layer arrangement as it is not possible to present a completely aminated substrate particle. This is why a fully sulfonated substrate particle is first surrounded by a layer of ami-

nated and then by a layer of sulfonated latex particles. Fixing takes place via electrostatic and van der Waals interactions. The relatively large diameter of the substrate particles (10 - 30 µm) results in a comparatively low counterpressure. The small diameter (20 - 250 nm) of the latex particles allows short diffusion paths with correspondingly rapid exchange processes and therefore a high separating column efficiency. The disadvantage of pellicular materials is their sensitivity to organic solvents [32] and to mobile phases with a high ionic strength as in this case the latex particles are gradually displaced.

The simultaneous determination of alkali and alkaline earth metals is possible by using a modified version in which the aminated layer is covalently bound. However, the differences in selectivity between potassium, magnesium and calcium are unnecessarily large so that relatively long analysis times are required. This can be traced back to the strongly acidic sulfonic acid groups which are used.

3.6.7 Stationary phases in ion exclusion chromatography

The choice of stationary phases for IEC is very limited. The formation of the Donnan membrane by dissociated functional groups is important for the separation process. Their number should be relatively high. In addition the packing material should avoid analyte adsorption on the stationary phase as much as possible. As the analytes are mainly from the carboxylic acid and sugar groups, as polar a surface as possible is the aim. Practically no demands are placed on the kinetics of the ion exchange reaction as the separation is based on an exclusion mechanism. Low cross-linked PS/DVB polymers are almost the only ones used; these are completely sulfonated.

3.6.8 The meaning of the capacity of ion exchangers

Apart from the sort of matrix and the type of functional group, the exchange capacity Q is the decisive quantity for characterizing ion exchangers. It provides information about the number of places which are available for ion exchange.
The exchange capacity is normally given in grams of dry packing material, either in microequivalents (µeq/g) or micromols (µmol/g) [2]. Other information is also normally given [33]. For analytical purposes ion exchangers can be divided roughly according to their capacity into:

- low-capacity materials: $Q < 100$ µmol/g
- medium-capacity materials: $100 < Q < 200$ µmol/g
- high-capacity materials: $Q > 200$ µmol/g

The separation phases used in classical ion exchangers are all high-capacity types with 3 to 5 mmol/g [4].
The above definition of the capacity is based on a complete equilibrium being attained between the stationary and mobile phases; this is why it is also known as the static capacity. In contrast, the dynamic (effective) capacity is understood to be the number of functional groups which are actually available during a chromatographic process. It is always smaller than the static capacity [2, 4].

The exchange capacity can be determined in different ways [33]:

- by titration
- by elemental analysis
- by determining retention times

All the methods provide different values for Q for the same material. Volumetric methods are most frequently used in practice. For anion exchangers a packed separating column or a defined amount of resin is loaded, e.g. with chloride solution. After the residual chloride has been washed out the column or resin can be eluted with nitrate. The amount of chloride eluted, which corresponds to the static exchange capacity under equilibrium conditions, can be titrated against $AgNO_3$ solution and quantified.

The pH dependency of ion exchangers can usually be neglected. With cation exchangers the capacity of the weakly acidic carboxylic acid groups (R–COOH) is only given at higher pH values (deprotonation), while the strongly acidic sulfonic acid groups ($R–SO_3H$) are always completely deprotonated and provide a pH-independent capacity.

In combination with the retention models (chapter 3.4) the importance of Q for the selection of the elution and detection systems becomes obvious. Universal conductivity detection was practically impossible to realize with eluents with a high ionic strength, no matter which special technique was used. This is the reason why, since the introduction of ion chromatography in 1975, virtually only low-capacity separating columns have been used. Up to now high-capacity ion exchangers have only been described in IC applications in combination with detection techniques which are not so strongly limited by the elution system, such as UV/VIS detection [2, 4].

While low-capacity separating materials are very suitable for the analysis of samples with a low ionic strength and therefore a low matrix load, at higher ionic strengths they rapidly reach their limits. Overload effects occur because of their low number of functional groups; these result in deformed peaks and a dramatic drop in efficiency. Problems also occur if the analytes are present in very different concentrations; this is almost always the case in ultratrace analysis.

3.7 Eluents in ion chromatography

As in all separations by liquid chromatography, also in ion chromatography the mobile phase is the easiest parameter to alter if one wishes to influence the separation. In contrast, the separating column and the detection system are in most cases predefined.

The choice of a suitable elution system can be made by using a wide range of criteria. In anion chromatography the following parameters must be taken into account, among others [4]:

- compatibility with the detection method
- chemical nature and concentration of the eluent ion
- pH
- buffer capacity
- organic solvent content (modifiers)

In the relevant literature [2, 4, 5] the discussion about the mobile phases mainly takes place from the point of view of whether they are suitable for IC without or with chemical suppression. Even though the focal point of the subject of this work is the exchange capacity Q of the separating column, the two techniques will also be considered. As in the previous section, the observations are mainly limited to anion chromatography.

3.7.1 Anion chromatography

Ion chromatography without chemical suppression

Ion chromatography without chemical suppression was established in ion chromatography by Gjerde and colleagues in 1979 [33]. The outlet of a separating column is directly connected to a detector; this corresponds to the classical arrangement of an HPLC system. This technique is also called «non-suppressed ion chromatography». The eluent containing the analytes leaves the separating column and is not altered chemically. The number of possible elution systems with different chemical characteristics is almost unlimited. Apart from the separation problem itself, only the compatibility of the eluent with the detector needs to be taken into account. For example, if direct UV detection is to be used then the eluent must not absorb in the relevant spectral range. In non-suppressed IC no limitations are placed on the detection system so that in principle all the usual HPLC detectors could be used.

For low-capacity anion exchangers (Q < 100 µmol/separating column) a whole range of eluents with different chemical characteristics are available for non-suppressed IC. The concentration of the eluents is normally in the lower mmol/L range or even below. Among other things, the following substance classes can be used [4]:

- aromatic carboxylic acids
- aliphatic carboxylic acids
- sulfonic acids
- alkali hydroxides
- inorganic acids such as H_2SO_4, HCl or H_3PO_4

In special cases complexing agents such as EDTA or borate-mannitol complexes can also be used.

The most important class of compounds are the aromatic carboxylic acids and their salts. The representatives which are most frequently used are shown in Figure 19

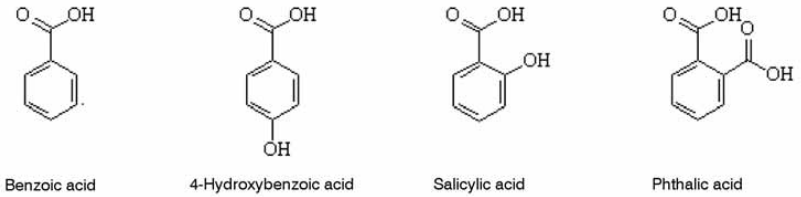


Figure 19 Structures of the most important aromatic carboxylic acids used in non-suppressed IC

This class of substances is used so frequently because the solutions of the acids and their salts have a very high elution power combined with a relatively small inherent conductivity, so that they can be used for direct conductivity detection. With polybasic acids the charge and therefore the elution power can be controlled via the pH which, however, must be maintained very accurately. Aromatic carboxylic acids have a high absorption in the UV spectrum so that they can be used advantageously in indirect UV/VIS detection. For aromatic sulfonic acids such as p-toluene sulfonic acid the same statements apply in principle; these are always present in a deprotonated state and therefore possess no buffer capacity. Their elution power can only be controlled via the concentration.

Aliphatic carboxylic acids such as oxalic acid and citric acid have a high inherent conductivity but are UV-transparent, so that they can be used for direct UV/VIS detection. This also applies to aliphatic sulfonic acids, of which methanesulfonic acid is the one which is most frequently used. The higher homologues of both classes of compounds with their long hydrocarbon chains and the low equivalent conductivities with which this is associated offer the possibility of being used for direct conductivity detection [2, 4].

Alkali hydroxides have only a limited use in non-suppressed IC as the OH^- ion has a very low affinity to quaternary ammonium groups. As a result high eluent concentrations are required even at low capacities so that at the best only indirect conductivity detection can be used. Typical applications are the determination of weak acid anions which are not dissociated at lower pH (silicate, cyanide etc.). In contrast, UV/VIS detection is easily possible, although the basically UV-transparent hydroxide ion has a distinct absorption below 220 nm at higher concentrations.

If inorganic acids or their salts are used then their virtually complete dissociation and associated high conductivity means that only photometric detectors can be used. If phosphoric acid or phosphates are used then the buffer capacity and the elution power can be controlled via the pH of the eluent.

Most of the eluents mentioned here also allow the use of other detection systems such as amperometry, fluorimetry or coupling techniques. If you wish to use the elution systems mentioned above with these detectors please refer to the relevant literature [2, 4, 5].

Suppressor technique

The suppressor technique is the detection technique originally used when IC was introduced [1]. In contrast to non-suppressed IC, this method uses only conductivity detection. In the suppressor technique a so-called suppressor is inserted between the separating column and the detector, which is why this technique is also known as «suppressed ion chromatography» or «ion chromatography with chemical suppression» [2, 4]. Both the eluent and the analytes are chemically modified in the suppressor; this is of particular importance with respect to the subsequent conductivity detection. The suppressor has the task of reducing the inherent conductivity of the eluent and, if possible, of increasing the detectability of the analytes. The principle of chemical suppression is shown in Equations 63 and 64 for an anion chromatography application. An $NaHCO_3$ eluent is used, the chloride ion is the analyte. Suppression is carried out with a strongly acidic H^+-form cation exchanger.

$$R\text{-}SO_3^- H^+ + Na^+ + HCO_3^- \rightleftharpoons R\text{-}SO_3^- Na^+ + H_2O + CO_2 \quad (63)$$

$$R\text{-}SO_3^- H^+ + Na^+ + Cl^- \rightleftharpoons R\text{-}SO_3^- Na^+ + H^+ + Cl^- \quad (64)$$

In the simplest case the suppressor unit consists of a column inserted after the separating column, which is where the older term «two-column technique» originates. The eluent sodium hydrogen carbonate is neutralized according to Equation 63, as the sodium ions are replaced by protons. This dramatically lowers the inherent conductivity of the eluent. The analyte Cl^- itself is not altered (Equation 64), but its counter-ion Na^+ is exchanged for H^+, which has a considerably larger equivalent conductivity [19]. As the detector records the sum of the conductivities of the analyte and counter-ion as the signal, the two reactions which take place result in a clearly improved sensitivity.

The columns with H^+-form cation exchangers originally used as suppressors made a considerable contribution to broadening the peaks due to their large dead volume. In addition they could only be operated discontinuously, as the cation exchanger had to be regenerated periodically. In contrast to this, modern packed-bed suppressors such as the Metrohm Suppressor Module guarantee excellent performance and allow quasi-continuous work. Continuously operating membrane suppressors are also used; in these the regenerant, usually dilute sulfuric acid, and the eluent are led past each other in a counterflow principle. Compared to packed-bed suppressors, membrane suppressors are, however, considerably more susceptible to interferences and less resistant (pressure, organic solvents).

Despite its advantages the suppressor technique also has several crucial disadvantages. In practice only those eluents which are based on alkali hydroxides or carbonates can be successfully suppressed in anion chromatography. Anions of weaker acids, e.g. acetate or fluoride, are almost completely protonated after the suppression reaction, so that their detectability is much lower when compared with non-suppressed IC. High-valency cations must be removed before the analysis as they form insoluble hydroxides which precipitate on the separating column and can block it.

As has already been made clear, the suppressor technique is synonymous with chemical suppression of the eluent and the use of direct conductivity detection [2, 4]. This technique is unusually widespread in anion chromatography, chiefly because in many cases it is more sensitive than the direct conductivity detection used in non-suppressed IC. The inherent conductivity of the classical eluents for non-suppressed IC (e.g. 2 mmol/L phthalate, pH = 8) is about 200 μS/cm. For suppressible eluents it is usually lower by at least one order of magnitude.
Chemical suppression of the inherent conductivity is only possible for a few eluents. These are solutions of [4]:

- alkali hydroxides
- alkali carbonates and hydrogen carbonates
- borates (e.g. $B_4O_7^{2-}$)
- amino acids

In practice, of the eluents mentioned above only the alkali hydroxides and carbonate buffers are of any great importance, which means that the choice of potential mobile phases is very limited.
The hydroxide ion is an extremely weak eluent ion, so that even with low-capacity separating materials work must be carried out at high concentrations above 50 mmol/L. If very polar functional groups are used then the relative elution power of the OH^- ion can be considerably

increased by utilizing the hydroxide selectivity. With OH^- eluents manipulation of the retention times or selectivities can only be carried out via the concentration.

The use of alkali carbonates and hydrogen carbonates permits considerably more flexible elution systems. Both species, HCO_3^- and CO_3^{2-}, are present as carbonic acid H_2CO_3 after suppression; this is only dissociated to a very small extent (*. Hydrogen carbonate has an even lower elution power than hydroxide, whereas carbonate is a relatively strong eluent. Both anions are normally used together; this results in an eluent with a buffer effect whose elution power can be easily controlled via the concentrations of the two components and the ratio of their concentrations. Because of the charges on the eluent species the selectivity for monovalent and multivalent analytes can be influenced very selectively. The concentration ratio of the two eluent ions can also be adjusted very accurately via the pH, this is why the pH range used for HCO_3^-/CO_3^{2-} eluents is between 8 and 11. Just as for OH^- eluents, elution can be accelerated by the use of stationary phases with polar functional groups.

Both elution systems can only be used successfully with surface-functionalized anion exchangers when either the matrix (methacrylate copolymers) or the functional group has a high polarity. For separating columns based on PS-DVB very poor peak symmetries and long retention times have been observed for weak analytes (nitrate, bromide) even when polar functional groups are used. With pellicular materials these effects are not so marked, which explains their exceptionally widespread use in the suppressor technique [2, 4].

(Carbonic acid may be further reduced by applying a CO_2 Suppressor. This CO_2 suppressor reduces CO2 and therefore carbonic acid to almost zero.*

3.7.2 Cation chromatography

3.7.2.1 Cation chromatography of alkali, alkaline earth and ammonium ions with conductivity detection

In the separation of alkali metal and ammonium ions as well as short-chain aliphatic amines on sulfonated separation phases by ion chromatography, mineral acids such as HCl or HNO_3 are mainly used as eluents [4]. The acid concentration of the eluent depends on the type and capacity of the cation exchanger used and is a few mmol/L. Divalent cations such as the alkaline earth metals cannot be eluted with mineral acids as they have a distinctly higher affinity to the stationary phase and a dramatic increase in the acid concentration would make detection too insensitive or mean that suppression could no longer be guaranteed. As an alternative an organic base such as ethylenediamine can be used for the separation of alkaline earth ions. At low pH values this is protonated and is present as a divalent cation.

The simultaneous determination of alkali and alkaline earth cations on strongly acidic cation exchangers is mainly carried out with eluents which contain hydrochloric acid and 2,3-diaminopropionic acid [2]. By varying the pH it is possible to alter the degree of protonation of the amino groups and therefore the elution power of the 2,3-diaminopropionic acid (see section «Retention model for eluents with one cation», chapter 3.4.2).

In ion chromatography systems without suppression, weak organic acids can also be used as eluents in addition to the mineral acids. Organic acids used are e.g. oxalic acid, citric acid and

tartaric acid. Complexing agents such as 2,6-pyridinedicarboxylic acid (dipicolinic acid) and the crown ether 18-crown-6 are used to selectively influence the analysis times of individual cations.

There are two different types of conductivity detection. If the background conductivity of the eluents is high, e.g. with dilute mineral acids because of the high conductivity of the H+ ions, then direct conductivity detection is possible with the analytes having a clearly lower conductivity than the eluent. This means that negative peaks are produced which, however, can be converted into a normal chromatogram by reversing the polarity of the detector or by simple inversion. Non-suppressed cation chromatography shows typically the same or an even higher response than cations after suppression (e.g. NaOH). For trace cation analysis suppression is advantageous due to the lower detection limits for the cations.

3.7.2.2 Cation chromatography of transition metal and alkaline earth ions with post-column derivatization and photometric detection

Monovalent cations such as H^+ or Na^+ are unsuitable for use as eluents for the separation of transition and heavy metal ions as the selectivity coefficients of the analytes hardly differ at the same charge number. However, separation can be realized by the introduction of a secondary equilibrium. This is done by using complex-forming carboxylic acids (see Figure 20) such as citric acid, oxalic acid and tartaric acid as eluents. Together with the metal ions these form neutral or anionic complexes (see chapter 3.4).
By complexing the metal cations the effective charge density of the analytes is reduced. In addition the different complex forming constants of the individual metal ions increase the selectivity of the separation. The elution mechanism is a result of the isoionic displacement by the counter-ion (pushing effect) and complex formation (pulling effect) by the complex-forming ligands [4].

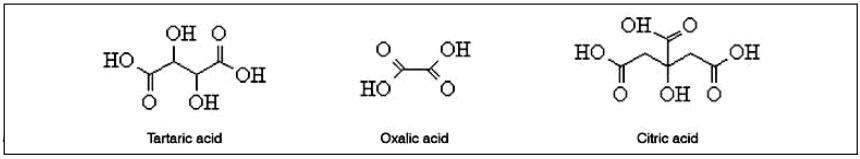


Figure 21 shows the equilibria between the analyte Me^{2+}, the complexing agent L^{2-} and the counter-ions E^+ which participate in the elution mechanism.

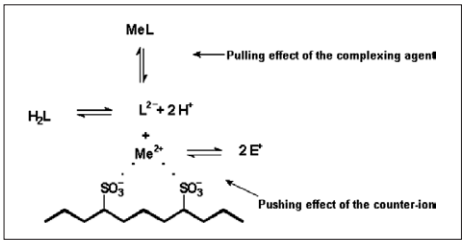


Figure 21 Schematic diagram of the equilibria participating in the exchange process [4]

The extent of the isoionic displacement by the counter-ions E^+ is determined by the affinity of the cation to the stationary phase. With monovalent counter-ions the cation with the greater affinity to the stationary phase has a stronger eluting effect. The influence of the complexing agent can be varied by altering the pH and concentration of the eluent. In addition the elution power can be influenced by the use of several complexing agents as well as by using a divalent cation as the counter-ion.

Whereas both types of conductivity detection are suitable for the detection of alkali and alkaline earth metals, transition and heavy metal ions can only be determined by direct conductivity measurement without suppressor. If the suppressor technique were to be used then these metals would be converted into (mostly) insoluble hydroxides by the suppression reaction. Direct conductivity detection is only possible when eluents with a low background conductivity are used together with low-capacity cation exchangers.

This is why post-column derivatization with the formation of photometrically detectable metal complexes is mainly used for the detection of transition and heavy metal ions. After leaving the separating column the eluate is mixed with a metallochromic reagent in a post-reactor; this reacts with the analytes to form colored metal complexes which can be detected photometrically.
A wide range of azo-dyes can be used as the coloring agents [2, 4]; these react with a large number of metal cations. Transition metals and lanthanoides react with 4-(2-pyridylazo)-resorcinol (PAR) (Figure 22) to form colored complexes. Lanthanoides and actinoides can be detected by using 2,7-bis(2-arsenophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid (Arsenazo III). Pyrocatechol-3,5-disulfonic acid (Tiron) is suitable for the post-column derivatization of aluminum.

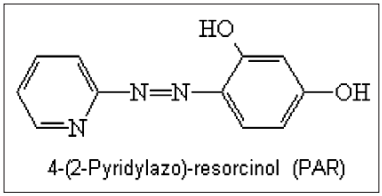


Figure 22
Structure of the metal indicator PAR

PAR is mainly used for the detection of transition and heavy metal ions. With Fe, Co, Ni, Cu, Zn, Mn, Pb and Cd PAR forms colored complexes; these absorb at wavelengths from 490 to 520 nm with absorption coefficients up to 104 L mol^{-1} cm^{-1} and can be detected photometrically. The sensitivity of the method is based on the fact that the extinction coefficients of the metal-PAR complexes are large compared with the absorption coefficient of the reagent at the wavelengths used.

3.7.2.3 Ion exclusion chromatography

In IEC the choice of the elution agent is, like the selection of the packing material, not very exciting. The range extends from pure water up to dilute mineral acids. When selecting the most suitable system it is also necessary to take the detection system into account. The most frequently used

detection methods are photometric and conductivity detection. With photometric detection dilute sulfuric or perchloric acid are often used because of their absolute UV transparency. When applying conductivity detection, dilute sulfuric acid is the eluent of choice as it yields good chromatograms with a minimum of equipment (no suppressor required). As the separated acids (medium weak to weak) are only partially dissociated in the acidic eluent, the conductometric response is limited. It gets lower with increasing pK_a. To increase this response inverse suppression can be applied. An anion suppressor is used, but in the opposite way as in anion chromatography. The suppressor in the lithium or sodium form replaces H^+ by the respective cation and regeneration is done with the respective cation solution (e.g. LiCl). The background is reduced to a neutral pH and the dissociation and therefore the response of the acids is improved.

The concentration of the acid in the eluent determines the degree of dissociation of the analytes and therefore also their retention. In general the retention times decrease as the acid concentration increases. The peak shape is also influenced by the acid concentration.

4 Practical section

The practical section of this textbook presents experiments which provide a detailed introduction to the world of ion chromatography. A start is made with experiments covering the theory of ion chromatography (chapter 4.2); the second part (chapter 4.3) continues with the determination of anions and the third and final part (chapter 4.4) deals with the determination of organic and inorganic cations.

In principle, the experiments can be carried out on any ion chromatograph. However, the equipment level of the instrument should still fulfill the following requirements:

- low-pulsation double-piston pump, if possible working without an external nitrogen or helium supply
- flow control meeting column requirements
- electric injection valve
- possibility of connecting various sample loops and preconcentration columns
- ion chromatography with and without chemical suppression
- temperature-stabilized conductivity detector, if possible better than +/- 0.001 °C
- thermally and electronically shielded housing
- anion and cation operation
- PC control is recommended

All actual as well as older IC systems from Metrohm fulfill the above requirements as a matter of course. However, the Eco IC is specially designed for training and teaching and is therefore recommended for carrying out the experiments described below.

Figure 23 The Metrohm Eco IC: specially developed for training and teaching

4.1 Information about the practical work

***B**acterial growth*

In order to prevent bacterial growth, the eluent, rinsing and regenerating solutions should always be made up freshly and not used over a longer period of time. If bacterial or algal growth should nevertheless occur then 5% methanol or acetone can be added to the eluent. If membrane suppressors are used this is not possible as these could be destroyed by organic

solvents. However, the Metrohm Suppressor Module «MSM» is 100% solvent-resistant.

***C**ation analysis*

In each analysis carried out the sample must be acidified (pH 2.5 - 3.5) with nitric acid (approx.100 µL 2 mol/L HNO_3 to 100 mL sample), as otherwise no reproducible results will be obtained for divalent cations.

***C**hemical grade*

All the chemicals used should have at least the grades p.a. (analytical grade) or puriss. (extra pure). Any standards used must be specially suitable for ion chromatography; for example, sodium salts should be dissolved in water, not in acid.

***C**ontamination sources*

All solutions, samples, regenerating solutions, the water and the eluents used should be free of particles as these will block the separating columns in the course of time (rise of column pressure). This is especially important when preparing the eluents as these flow through the column continuously (500 to 1000 mL per working day, whereas the sample throughput is approximately 0.5 mL).

***E**luent degassing*

In order to avoid bubble formation it is recommended that the water used for preparing the eluent is degassed before the reagents are added to it. This can be done by applying a vacuum from a water-jet or vacuum pump for about 10 minutes or by using an ultrasonic bath.

***E**nvironmental protection*

One of the great advantages of ion chromatography is that usually aqueous media are used. This is why the chemicals used in ion chromatography are to a large extent non-toxic and do not pollute the environment. However, if acids, bases, organic solvents or heavy metal standards are used then care should be taken that these are disposed of properly after use.

***I**nstrument shut-down*

If the ion chromatograph is not to be used for a longer period of time (>1 week) then the separating column should be removed and the ion chromatograph rinsed with methanol/water (1:4). Care should be taken that all three chambers of the suppressor are rinsed.

***S**afety information*

Protective goggles, protective clothing and, if necessary, protective gloves should be worn when carrying out all the experiments. Be sure to observe the safety information given for the (see the respective MSDS).

***S**electing a column*

Most of the experiments described are carried out on very favorably priced columns – Metrosep A Supp 17 - 150/4.0 for anions and Metrosep C 4 - 150/4.0 for cations. These columns provide adequate separation for most of the experiments described. Of course, the Metrosep product range includes separating columns which have a considerably better perfor-

Storing the separating columns

- **Metrosep A Supp 17 - 150/4.0 (6.01032.420)**

The column is stored in the eluent.

Figure 24
Metrosep A Supp 17 - 150/4.0 (6.01032.420)

- **Metrosep C 4 - 150/4.0 (6.1050.420)**

The column is stored in eluent or ultrapure water.

Figure 25
Metrosep C 4 - 150/4.0 (6.1050.420)

- **Metrosep A Supp 4 - 250/4.0 (6.1006.430)**

The column is stored in the eluent.

Figure 24
Metrosep A Supp 4 - 250/4.0 (6.1006.430)

• **Metrosep C 6 - 150/4.0 (6.1051.420)**
The column is stored in eluent or ultrapure water.

• **Nucleosil 5SA - 125/4.0 (6.1007.000)**
For short-term storage (days) the column is stored in the eluent, for long-term storage (weeks) in methanol/water (1:4).

• **Metrosep Organic Acids column (6.1005.200)**
For short-term storage (days) the column is stored in the eluent, for long-term storage (weeks) in ultrapure water.

• **Separating columns from other manufacturers**
Please refer to the manufacturer's instructions.

***W**ater quality*
In ion chromatography mainly aqueous media are used. This is why the water quality is crucial for good chromatography results. If the water quality is unsatisfactory then it is certain that the results will also be unsatisfactory. In addition there is the danger of damaging the instrument and separating column. The ultrapure water should have a resistivity of minimum 18.2 MΩ (25 °C) and be particle-free. It is recommended that the water is filtered through a 0.2 µm filter.

4.2 Experiments covering the theory of ion chromatography

4.2.1 Experiment 1 – Ion chromatography with and without chemical suppression

The equivalent conductivity Λ_∞ is always obtained by adding together the equivalent conductivities of all anions Λ_∞^- and cations Λ_∞^+ in the solution:

$$\Lambda_\infty = \Lambda_\infty^+ + \Lambda_\infty^-$$

In principle the conductivity increases as the concentration of the electrolyte or ions increases. A linear relationship only exists for dilute solutions, as Λ depends on the concentration (Kohlrausch's law). The values found in tables (see section «Conductivity detection») apply for Λ_∞ – equivalent conductivity in infinitely dilute solutions.

The size of the equivalent conductivity depends to a great extent on the temperature (±2%/ °C). In particular, for measurements without chemical suppression, i.e. mostly against a high background, errors caused by a change in temperature are extremely noticeable. This is why temperature fluctuations within the detector should not be larger than 0.01 °C.

In ion chromatography without chemical suppression, in which the background conductivity is suppressed electronically, the eluent should have as low a conductivity as possible. The salts of weak acids such as phthalic acid, salicylic acid and benzoic acid come into question here.
A value measured without chemical suppression depends on the difference in the equivalent conductivities of the sample ion and the eluent ion:

$$\Delta\Lambda \sim (\Lambda_S - \Lambda_E)$$

Negative peaks always occur when the conductivity of the sample ion is lower than that of the eluent ion. An example of this is the phosphate anion. At pH = 5 it is mainly present as $H_2PO_4^-$. As the equivalent conductivity Λ_∞ of the $H_2PO_4^-$ with 33 S*cm^2/mol is lower than the equivalent conductivity Λ_∞ of the phthalate with 38 S*cm^2/mol a small negative peak is obtained. This can be remedied by selecting a higher pH, at which the phosphate is present as HPO_4^{2-} with an equivalent conductivity Λ_∞ of 57 S*cm^2/mol.

Ion chromatography with chemical suppression means that the background conductivity is reduced chemically. An eluent with a high conductivity is converted in a post-column reaction – the suppression reaction – into an eluent with a low conductivity.

In anion analysis this is carried out by using the salts of weak acids, e.g. HCO_3^-, CO_3^{2-} and BO_3^{3-}, in the eluent. All the cations in the eluent and in the sample are exchanged for H^+ in the suppressor. Weakly dissociated acids are formed from the eluent according to the following reaction:

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

The resulting carbonic acid is mainly present as $CO_2 + H_2O$. As a result the residual conductivity is very low. As the counter-ions of the anions to be determined are also exchanged for H^+ in the suppressor the following equation can be formulated:

$$Na^+ + Cl^- \xrightarrow{+H^+ -Na^+} H^+ + Cl^-$$

Instead of the Na^+ and Cl^- ions which were originally present in the sample the considerably higher equivalent conductivity of H^+ and Cl^- is now measured and this also against a lower background conductivity. Theoretically a signal can be expected which is larger by a factor of ten than for a measurement without chemical suppression. However, in practice only an increase in sensitivity in the range of a factor of 2 to 4 is obtained.

In contrast to the linear calibration function obtained when working without chemical suppression, when chemical suppression is used a quadratic calibration function is obtained; this means that a multi-point calibration is necessary for its calculation. The linear working range is considerably smaller (approx. 1/20 to 1/50) than for IC without chemical suppression.

Learning content

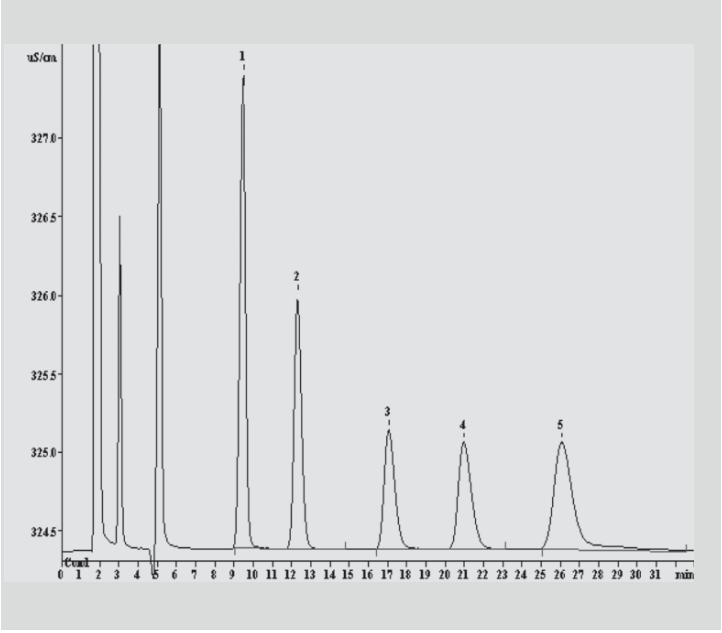
- Principles of an IC system setup
- Differences between working with and without chemical suppression
- Determination of the different sensitivities of the two methods

Experiment 1a – Measurement without chemical suppression
Table 3 Parameters – Experiment 1a

Column	6.1006.430 Metrosep A Supp 4 - 250/4.0(*
Eluent	5.0 mmol/L phthalic acid / 2% acetone, pH 4.4 tris (hydroxymethyl) aminomethane, TRIS Conductivity approx. 324 µS/cm
Sample	Standard (fluoride, chloride, nitrite, bromide, nitrate, sulfate)
Flow	1.0 mL/min
Pressure	5.5 MPa
Analysis time	33 min
Loop	20 µL
Polarity	+

Footnote: (The Metrosep A Supp 17 - 150/4.0 is not suited for non-suppressed applications. Therefore, the Metrosep A Supp 4 - 250/4.0 is applied for these measurements.*

Eluent preparation
Dissolve 830 mg phthalic acid in 20 mL acetone and a little water and make up to 1 L. Adjust the pH to 4.4 by adding approx. 1 g TRIS (solid). Before addition of the reagents degas the water used by applying a water-jet pump vacuum for 10 min or by using an ultrasonic bath.



Chromatogram 1 Standard solution – without chemical suppression

Table 4 Components – Experiment 1a

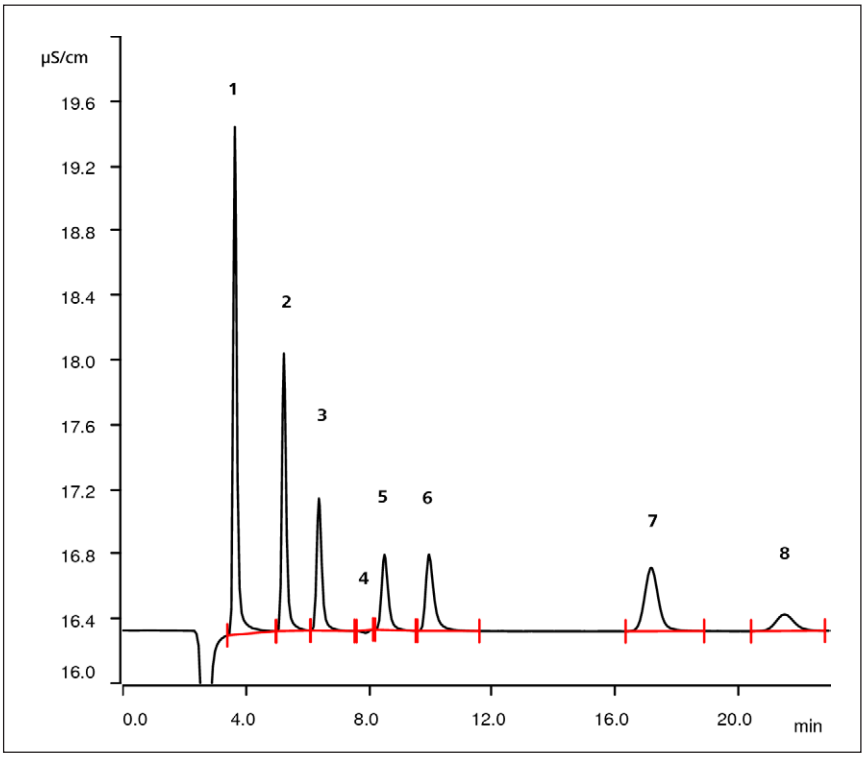
Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Chloride	9.5	25	4834	61.48
2	Nitrite	12.3	25	4709	43.67
3	Bromide	17.1	25	4272	29.83
4	Nitrate	21.0	25	4174	33.44
5	Sulfate	26.1	25	2636	52.62

Experiment 1b – Measurement with chemical suppression

Table 5 Parameters – Experiment 1b

Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	5.0 mmol/L Na2CO3 / 0.2 mmol/L NaHCO3 Conductivity after chemical suppression approx. 16 µS/cm
Sample	Standard (fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate)
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	20 min
Loop	20 µL
Suppressor	Regenerant: 100 mmol/L H_2SO_4, STREAM
Polarity	+

Eluent preparation
Dissolve 529.9 mg sodium carbonate (anhydrous) and 16.8 mg sodium hydrogen carbonate in 1 L ultrapure water.



Chromatogram 2 Standard solution – with chemical suppression

Table 6 Components – Experiment 1b

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.7	1	4990	0.455
2	Chloride	5.2	1	7997	0.278
3	Nitrite	6.4	1	7419	0.164
4	System peak				
5	Bromide	8.5	1	8074	0.117
6	Nitrate	10.0	1	7504	0.150
7	Sulfate	17.2	1	7454	0.204
8	Phosphate	21.5	1	6186	0.072

4.2.2 Experiment 2 – Capacity of separating columns

Cut-off peaks, shark-fin-shaped peaks, altered retention times for the same ions, peaks with tailing or fronting – all these can have a common cause: overloading the column.

Each column has a finite number of ion exchange sites. Before the sample is injected these are completely occupied by eluent ions. When the sample is injected, ion exchange starts to take place: Eluent ions are exchanged for sample ions and sample ions for eluent ions. As the ion species differ in their bonding constants they elute from the column at different speeds. The required result is the separation of the mixture of substances and therefore the chromatogram.

This process only functions perfectly if the number of exchange sites is substantially higher than the number of bonding places required by the sample. For example, a column with a capacity of 1 meq (milliequivalent) can bind a maximum of 1 mmol monovalent ions.

A second effect which has a negative influence on the separation is the fact that in principle each ion can act as an eluent ion. If the column is overloaded then many eluent ions are replaced by sample ions. This results in incalculable alterations to the column equilibria and the separation becomes worse.

The capacity of a column can be determined by completely loading it with chloride ions. After a rinsing step with demineralized water the chloride ions are eluted with a carbonate eluent and then quantified by either ion chromatography or argentometric titration.

In the following experiment the chloride concentration is increased until the column is overloaded. Some effects associated with overloading the column are described below:

➔ the retention times of the following ions become shorter,

➔ the peak of the dominant ion is cut off,

➔ the peak of the dominant ion tails,

➔ the number of theoretical plates (TP) becomes smaller,

➔ the area/height ratio becomes worse (at constant area, the peak height decreases) and

➔ the peak symmetry becomes poorer.

The peak symmetry also becomes poorer with blocked column frits, increased dead volumes and as a result of absorption effects on the column material.

Learning content

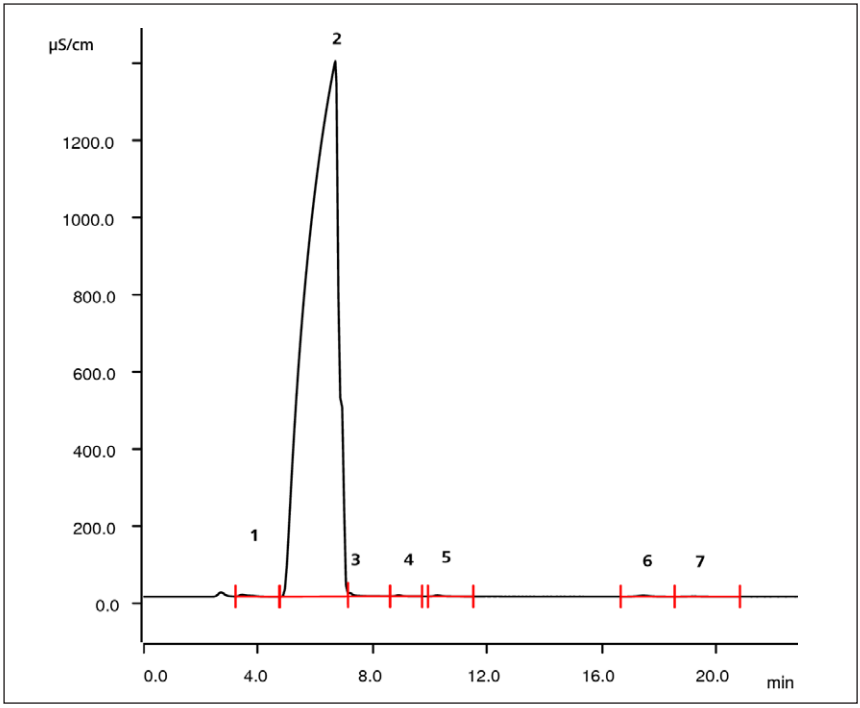
- Explanation of the following chromatographic parameters: retention time, resolution, area, number of plates, symmetry and area/height ratio
- Influence of a dominant main component on components with a much smaller cocentration: alterations to the above-mentioned parameters

Table 7 Parameters – Experiment 2

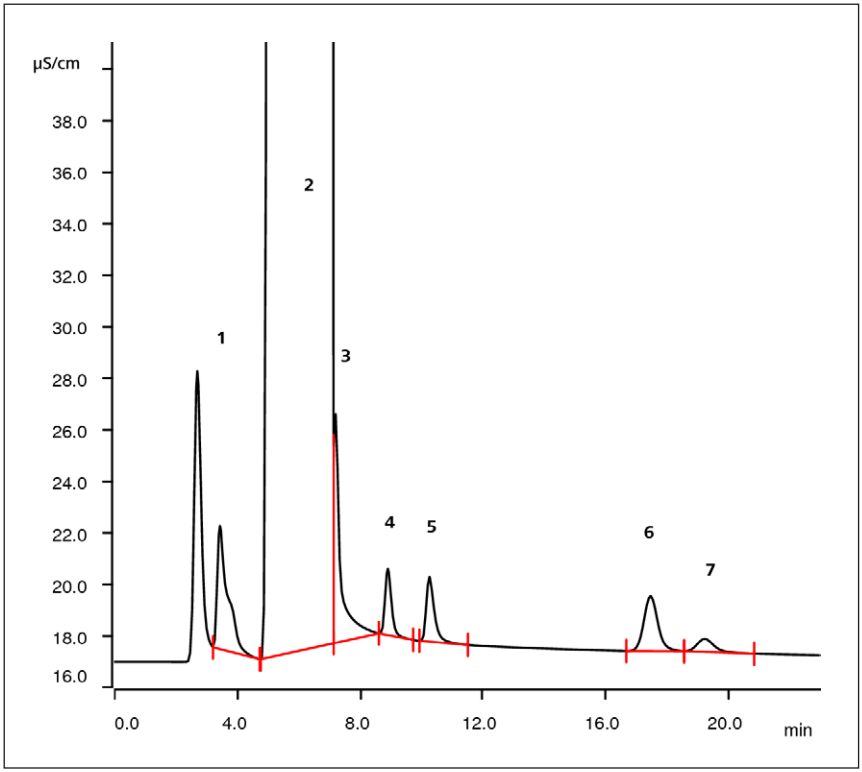
Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$ Conductivity after chemical suppression approx. 16 µS/cm
Sample	Standard (fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate) + NaCl (approx. 6.25 g/L)
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	23 min
Loop	20 µL
Suppressor	Regenerant: 100 mmol/L H_2SO_4, STREAM
Polarity	+

Eluent preparation

Dissolve 529.9 mg sodium carbonate (anhydrous) and 16.8 mg sodium hydrogen carbonate in 1 L ultrapure water.



Chromatogram 3 Standard solution with a very high chloride concentration



Chromatogram 4 Standard solution with a very high chloride concentration; enlarged part of Chromatogram 3

Remarks

Sodium chloride is weighed in. The retention times of the peaks as well as the values for the number of plates and area may vary depending on the quantity of NaCl weighed in.

The huge chloride peak interferes with the evaluation of the following peaks. Correct identification of the individual peaks is only possible by spiking the sample solution with the respective anions.

4.2.3 Experiment 3 – Selectivity of separating columns

If the strength of the eluent is plotted against the logarithm of the retention times of mono- and divalent ions then a straight line is obtained. The slope of this line is greater for divalent ions than for monovalent ions. The increase in the elution strength of the eluent therefore has a greater influence on the retention time of the divalent ions than on the retention time of monovalent ions.

The influence can be observed on the sulfate ion. As the eluent concentration increases the elution of sulfate is accelerated more than that of the monovalent ions.

In general divalent eluent ions are stronger eluents than monovalent ones as they can form two bonds with the stationary phase and therefore enter into stronger interactions with it.
At the same molarity, sodium hydroxide has a higher pH than carbonate/hydrogen carbonate. The elution power of the OH^- ion is lower compared to that of the carbonate as the hydroxide ion does not interact as strongly with the stationary phase.
The retention time of the phosphate ion depends strongly on the pH value. At high pH values the equilibrium is displaced from HPO_4^{2-} towards PO_4^{3-}. If sodium hydroxide is added to a sodium carbonate eluent, this results in the retention time of the phosphate being increased while the retention times of all other ions are shortened.

Learning content

- Comparison of eluents with monovalent and divalent anions
- Comparison of sodium hydroxide and carbonate/hydrogen carbonate eluents
- Influence of the eluent pH on the retention of phosphate

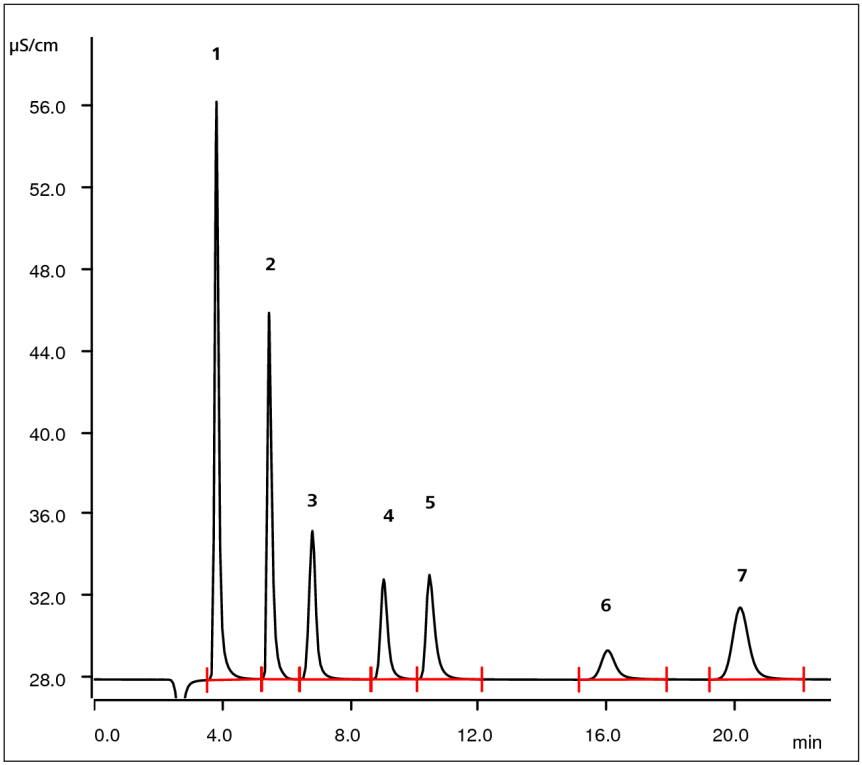
Table 8 Parameters – Experiments 3a to 3f

Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	a) 12.0 mmol/L Na_2CO_3 / 3.0 mmol/L $NaHCO_3$ / pH = 9.54 b) 9.0 mmol/L Na_2CO_3 / 6.0 mmol/L $NaHCO_3$ / pH = 9.94 c) 5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$ / pH = 10.75 d) 4.0 mmol/L Na_2CO_3 / 1.0 mmol/L NaOH / pH = 11.07 e) 1.0 mmol/L Na_2CO_3 / 4.0 mmol/L NaOH / pH = 11.48 f) 1.0 mmol/L Na_2CO_3 / 20 mmol/L NaOH / pH = 12.05
Sample	Standard (fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate)
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	a) 23 min b) 14 min c) 23 min d) 32 min e) 48 min f) 35 min
Loop	20 µL
Suppressor	Regenerant: 100 mmol/L H_2SO_4, STREAM
Polarity	+

Experiment 3a – Eluent: 12.0 mmol/L Na_2CO_3 / 3.0 mmol/L $NaHCO_3$

Eluent preparation

Dissolve 1271.9 mg sodium carbonate (anhydrous) and 252.0 mg sodium hydrogen carbonate in 1 L ultrapure water.



Chromatogram 5 Standard solution – Eluent: 12.0 mmol/L Na_2CO_3 / 3.0 mmol/L $NaHCO_3$

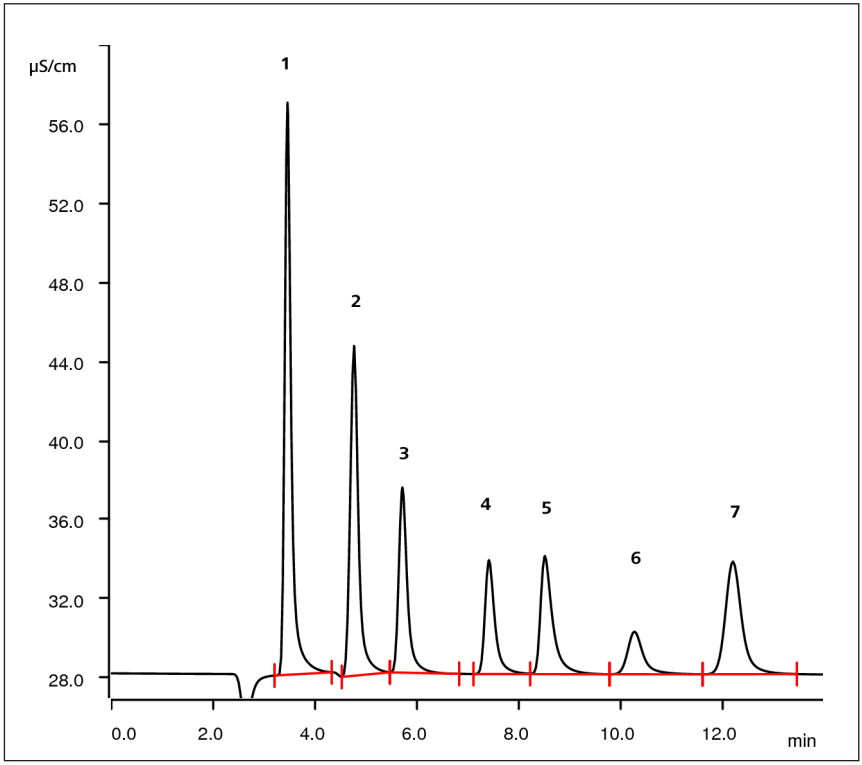
Table 9 Components – Experiment 3a

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.8	10	4562	4.486
2	Chloride	5.5	10	7097	3.164
3	Nitrite	6.8	10	5128	1.870
4	Bromide	9.0	10	8086	1.319
5	Nitrate	10.5	10	7594	1.661
6	Sulfate	16.0	10	6289	0.782
7	Phosphate	20.2	10	7571	2.140

Experiment 3b – Eluent: 9.0 mmol/L Na_2CO_3 / 6.0 mmol/L $NaHCO_3$

Eluent preparation

Dissolve 953.9 mg sodium carbonate (anhydrous) and 504.0 mg sodium hydrogen carbonate in 1 L ultrapure water.



Chromatogram 6 Standard solution – Eluent: 9.0 mmol/L Na_2CO_3 / 6.0 mmol/L $NaHCO_3$

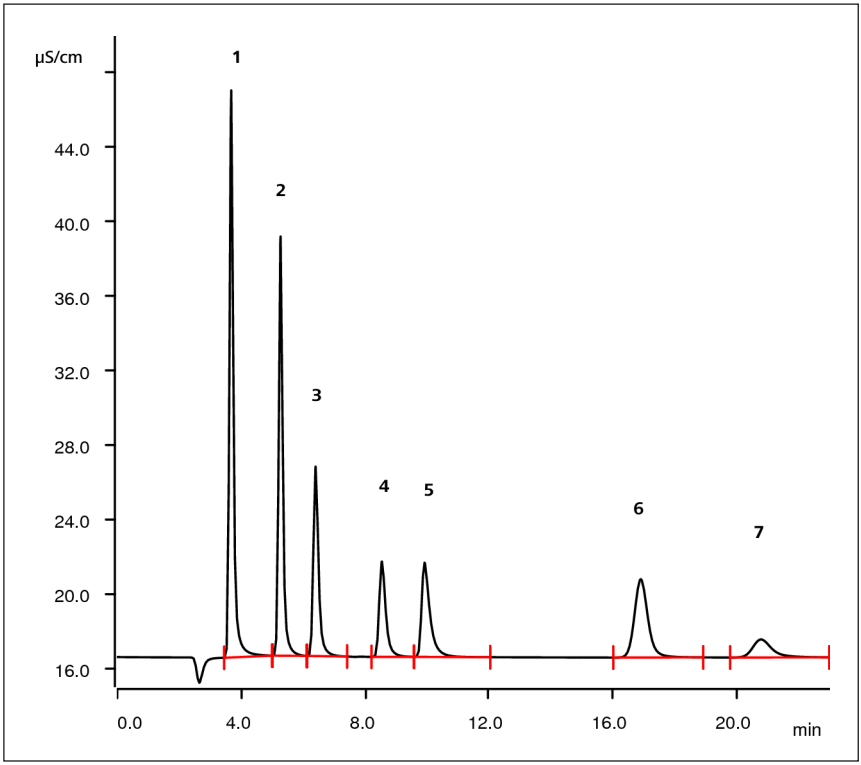
Table 10 Components – Experiment 3b

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.5	10	4118	4.390
2	Chloride	4.8	10	4639	3.174
3	Nitrite	5.7	10	6270	1.850
4	Bromide	7.4	10	7867	1.319
5	Nitrate	8.5	10	7179	1.662
6	Sulfate	10.3	10	6172	0.778
7	Phosphate	12.2	10	7462	2.170

Experiment 3c – Eluent: 5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$

Eluent preparation

Dissolve 529.9 mg sodium carbonate (anhydrous) and 16.8 mg sodium hydrogen carbonate in 1 L ultrapure water.



Chromatogram 7 Standard solution – Eluent: 5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$

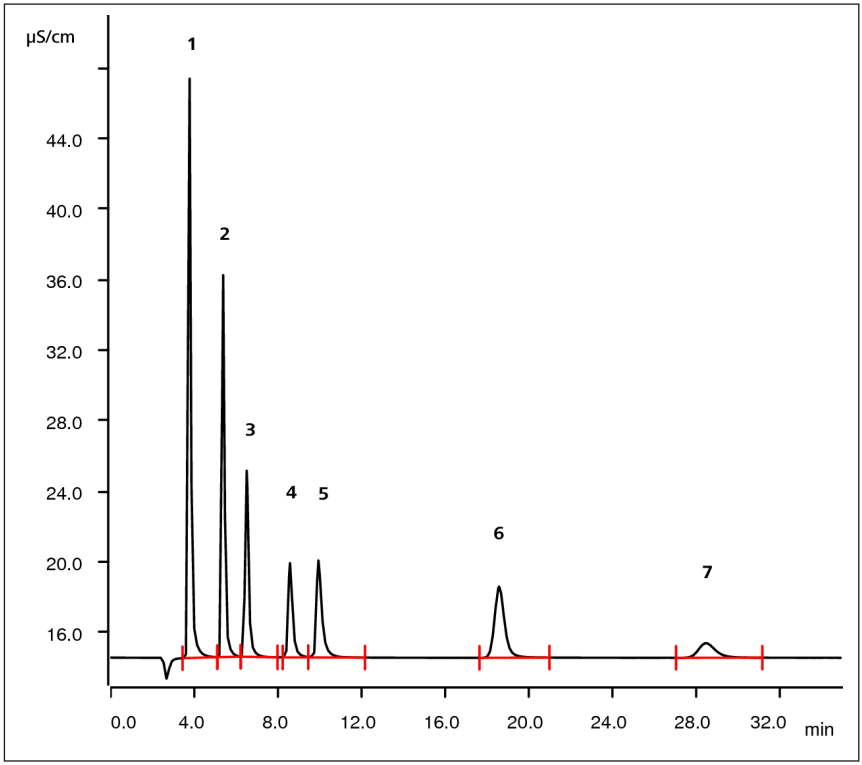
Table 11 Components – Experiment 3c

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.7	10	4537	4.641
2	Chloride	5.3	10	8591	3.476
3	Nitrite	6.4	10	7620	2.023
4	Bromide	8.6	10	8471	1.285
5	Nitrate	9.9	10	7552	1.598
6	Sulfate	16.9	10	7716	2.128
7	Phosphate	20.8	10	5888	0.716

Experiment 3d – Eluent: 4.0 mmol/L Na_2CO_3 / 1.0 mmol/L NaOH

Eluent preparation

Dissolve 424 mg sodium carbonate (anhydrous) and 100 µL 30% NaOH in 1 L ultrapure water.



Chromatogram 8 Standard solution - Eluent: 4.0 mmol/L Na_2CO_3 / 1.0 mmol/L NaOH

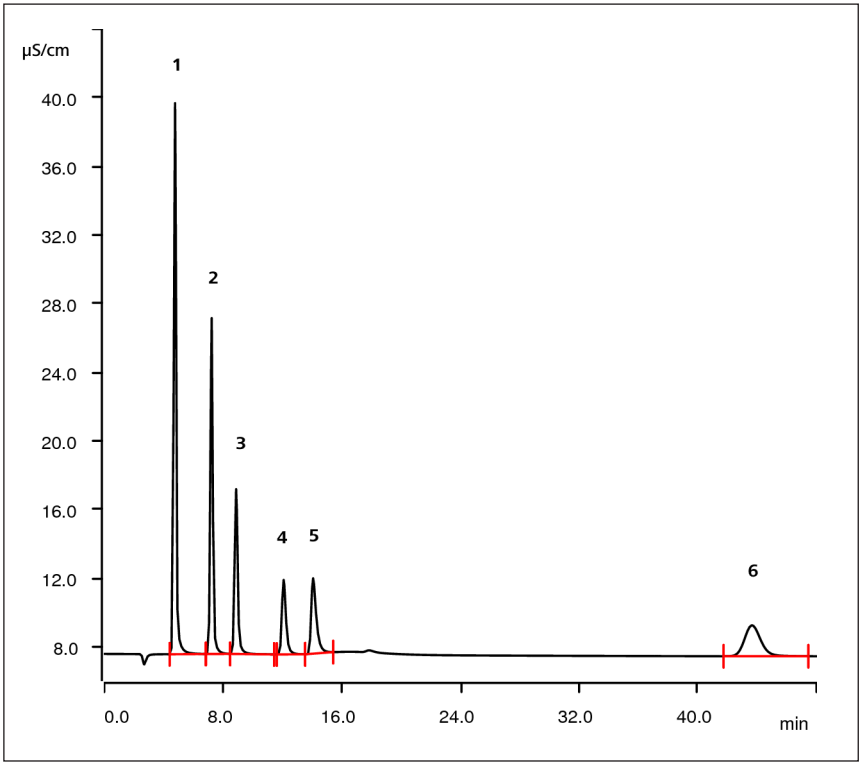
Table 12 Components – Experiment 3d

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.78	10	4429	5.201
2	Chloride	5.38	10	8573	3.441
3	Nitrite	6.51	10	7732	2.139
4	Bromide	8.58	10	8715	1.345
5	Nitrate	9.94	10	7973	1.728
6	Sulfate	18.57	10	7759	2.254
7	Phosphate	28.46	10	6018	0.800

Experiment 3e – Eluent: 1.0 mmol/L Na_2CO_3 / 4.0 mmol/L NaOH

Eluent preparation

Dissolve 106 mg sodium carbonate (anhydrous) and 400 µL 30% NaOH in 1 L ultrapure water.



Chromatogram 9 Standard solution – Eluent: 1.0 mmol/L Na_2CO_3 / 4.0 mmol/L NaOH

Table 13 Components – Experiment 3e

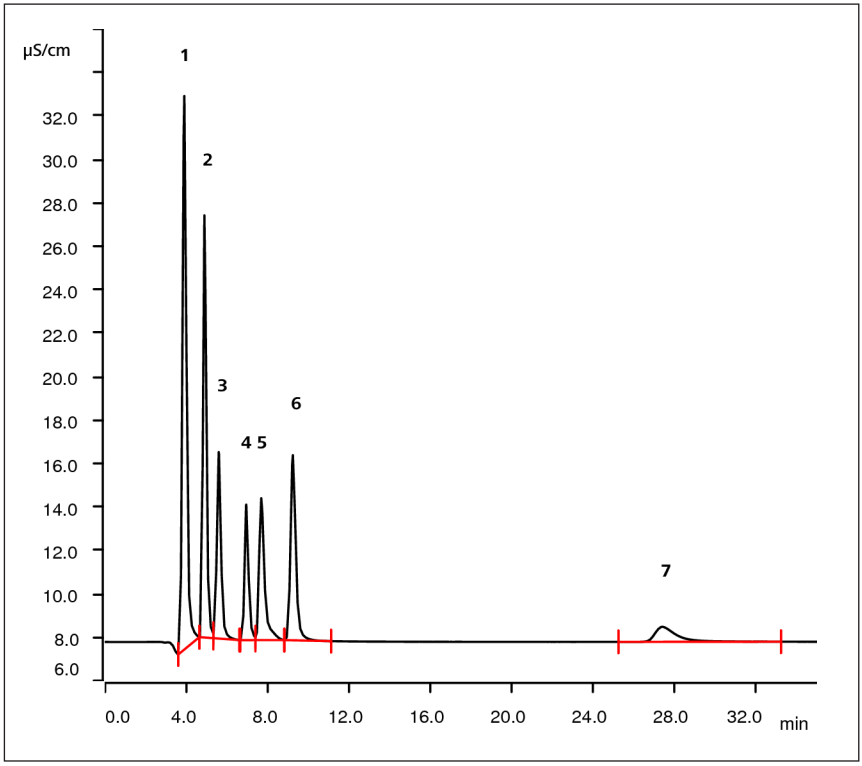
Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	4.8	10	4593	6.036
2	Chloride	7.2	10	9854	3.817
3	Nitrite	8.9	10	9469	2.374
4	Bromide	12.1	10	9712	1.422
5	Nitrate	14.1	10	8672	1.780
6	Sulfate	43.6	10	7930	2.285

Phosphate elutes very late due to the week and alkanine eluent and is not shown in the chromatogram.

Experiment 3f – Eluent: 1.0 mmol/L Na_2CO_3 / 20.0 mmol/L NaOH

Eluent preparation

Dissolve 106 mg sodium carbonate (anhydrous) and 2.0 mL 30% NaOH in 1 L ultrapure water.



Chromatogram 10 Standard solution – Eluent: 1.0 mmol/L Na_2CO_3 / 20.0 mmol/L NaOH

Table 14 Components – Experiment 3f

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.9	10	1831	5.988
2	Chloride	4.9	10	4258	3.747
3	Nitrite	5.6	10	3649	2.085
4	Bromide	6.9	10	6068	1.451
5	Nitrate	7.7	10	5048	2.018
6	Sulfate	9.2	10	6663	2.582
7	Phosphate	27.4	10	3888	0.879

4.2.4 Experiment 4 – Calibration, detection limits and determination limits in ion chromatography

Important parameters for analytical determination methods are the linear range, detection limit and determination limit. The mathematical methods for these are laid down in standards, e.g. in DIN 32645.

If chromatograms are recorded with a conductivity detector then the peak area is usually used for evaluation. The peak area is proportional to the amount of substance. If the peak area is plotted against the concentration then the calibration function is obtained. It is linear for measurements without chemical suppression. As a first approximation it is a quadratic function for measurements with chemical suppression. Evaluation programs calculate the calibration functions automatically. Evaluation using peak height is used preferentially for strongly tailing peaks or for insufficiently separated peaks with strongly differing area/height ratios as in those cases the area-based evaluation leads to large errors.

The detection limit is the minimum concentration of an analyte which can still be detected with known statistical certainty. The theoretically smallest concentration which can just be distinguished from a blank value is calculated.

There are two methods for calculating the detection limit:

Blank value method

The blank sample should be a sample which does not contain the ion to be determined but produces a signal at the same location as the sample ion. The repeated measurement of a blank sample gives for a concentration «0» (x value) measured values (y values) whose mode (average maximal frequency) is known as the blank value. By using the calibration curve the maximum y value is assigned to a concentration value on the x axis; this is the detection limit.

Calibration curve method

This method is used when it is not possible to determine a blank value because the ion under investigation cannot be detected in the sample. In the calibration curve method multiple measurements are carried out on different concentrations of the ion. A range of confidence is then obtained from the standard deviation. In this way, the concentration «0» corresponds to a particular y interval. The calibration function is used to assign the y interval to a concentration interval whose maximum value is the detection limit.

Signal to noise ratio

The signal/noise ratio is often used to determine the detection limit. For example, the detection limit is defined as being the analyte concentration at which the measuring signal is 3 times, 5 times or 7 times the baseline noise.

The determination limit is reached when the measuring error undercuts a particular value when compared with the analytical value, e.g. 1/3. Only then will a numerical value be given in the analytical report, as otherwise the measuring error is regarded as being too large in comparison with the analytical value. As an estimation it can be said that the determination limit is higher than the detection limit by a factor of three.

Learning content

- What does calibration mean?
- Comparison of a single-point and a multi-point calibration – estimation of error
- Determining the system noise
- Estimating the detection limit

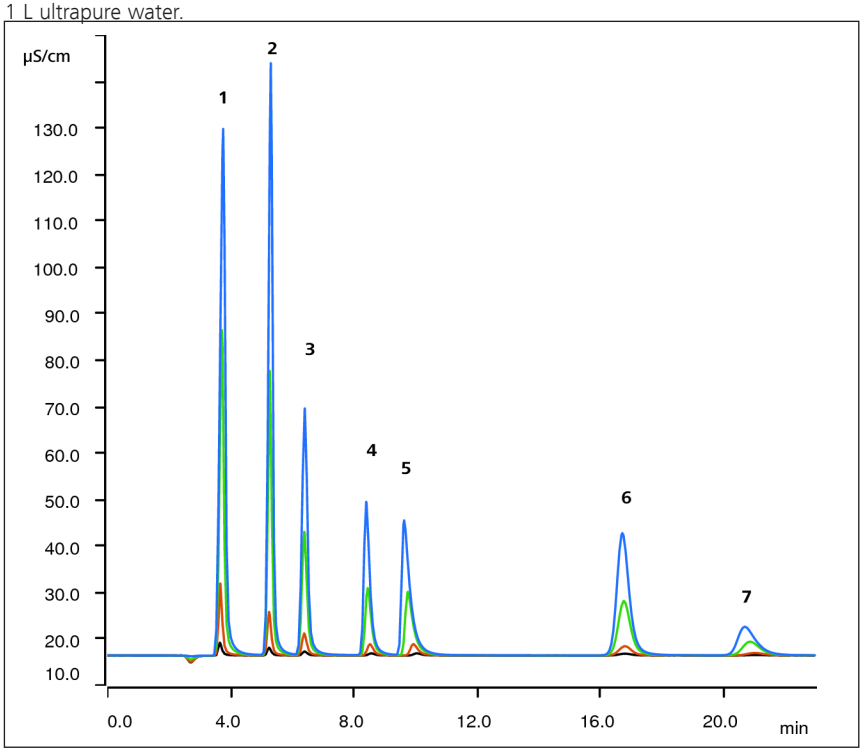
Experiment 4a – Determining anions with chemical suppression

Table 15 Components – Experiment 4a

Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$ Conductivity after chemical suppression approx. 16 µS/cm
Sample	Standard (fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate)
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	18 min
Loop	20 µL
Suppressor	Regenerant: 100 mmol/L H_2SO_4, STREAM
Polarity	+

Eluent preparation

Dissolve 529.9 mg sodium carbonate (anhydrous) and 16.8 mg sodium hydrogen carbonate in 1 L ultrapure water.



Chromatogram 11 Overlay – Standard solutions with different concentrations – with chemical suppression

Table 16 Components – Experiment 4a

Peak	Component	t_R [min]	Conc. [mg/L]			
			Level 1	Level 2	Level 3	Level 4
1	Fluoride	3.9	1	5	25	50
2	Chloride	4.9	1	5	25	50
3	Nitrite	5.6	1	5	25	50
4	Bromide	6.9	1	5	25	50
5	Nitrate	7.7	1	5	25	50
6	Sulfate	9.2	1	5	25	50
7	Phosphate	27.4	1	5	25	50

Experiment 4b – Determining anions without chemical suppression

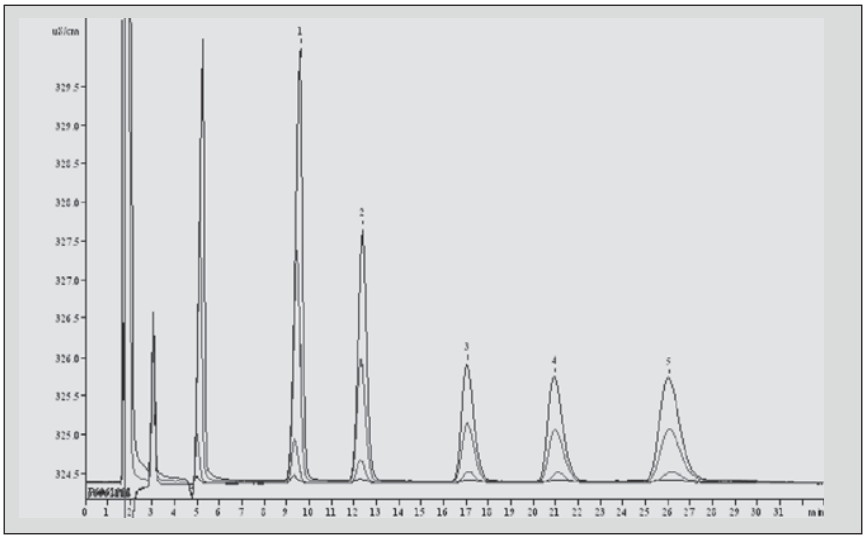
Table 17 Parameters – Experiment 4b

Column	6.1006.430 Metrosep A Supp 4 - 250/4.0(*
Eluent	5 mmol/L phthalic acid + 2% acetone, pH = 4.4 (TRIS) Conductivity approx. 324 µS/cm
Sample	Standard (fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate)
Flow	1.0 mL/min
Pressure	5.5 MPa
Analysis time	33 min
Loop	20 µL
Polarity	+

(* The Metrosep A Supp 17 - 150/4.0 is not suited for non-suppressed applications. Therefore the Metrosep A Supp 4 - 250/4.0 is applied for these measurements.

Eluent preparation

Dissolve 830 mg phthalic acid in 20 mL acetone and a small amount of water and make up to 1 L. Adjust the pH to 4.4 by adding approx. 0.6 g TRIS (solid).



Chromatogram 12 Overlay – Standard solutions with different concentrations – without chemical suppression

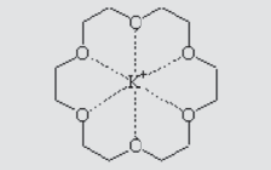
Table 18 Components – Experiment 4b

Peak	Peak	t_R [min]	Conc [mg/L]			
			Level 1	Level 2	Level 3	Level 4
1	Chloride	9.45	1	5	25	50
2	Nitrite	12.30	1	5	25	50
3	Bromide	17.06	1	5	25	50
4	Nitrate	20.96	1	5	25	50
5	Sulfate	26.06	1	5	25	50

4.2.5 Experiment 5 – Altering the selectivity with the aid of crown ethers (18-Crown-6)

The retention times of cations can be altered by adding complexing agents to the eluents. The complexing agent acts as a ligand, the analyte cation is included as the central metal ion. The more selective a ligand with respect to a central metal ion, the stronger the influence on the retention time. In an ideal case the retention times of the other cations will only alter slightly.

Complexing agents are used to obtain better separation of alkali metal ions. The addition of the crown ether 18-Crown-6 to the eluent leads to a better separation of Na^+, NH_4^+ and K^+. For example, the crown ether can be added to the eluent to improve the separation between Na^+ and NH_4^+ when traces of NH_4^+ have to be determined in natural waters. The increase in the retention time of K^+ is particularly dramatic. This can be explained by the formation of the complex from K^+ and 18-crown-6-ether (18-Crown-6). K^+ fits exactly in the «cage» of the ether. It is complexed via the electron pairs of the oxygen atoms. After complexation, a considerably larger molecule with the same charge is separated. This means that the retention time of the potassium is increased as a result of steric hindrance.



The name 18-Crown-6 indicates that the ring system consists of 18 atoms of which 6 are oxygen atoms. Crown ethers not only play an important role in ion chromatography, but are also used as the ion-selective phase in potassium electrodes.

Figure 26 Potassium 18-Crown-6 complex

Learning content

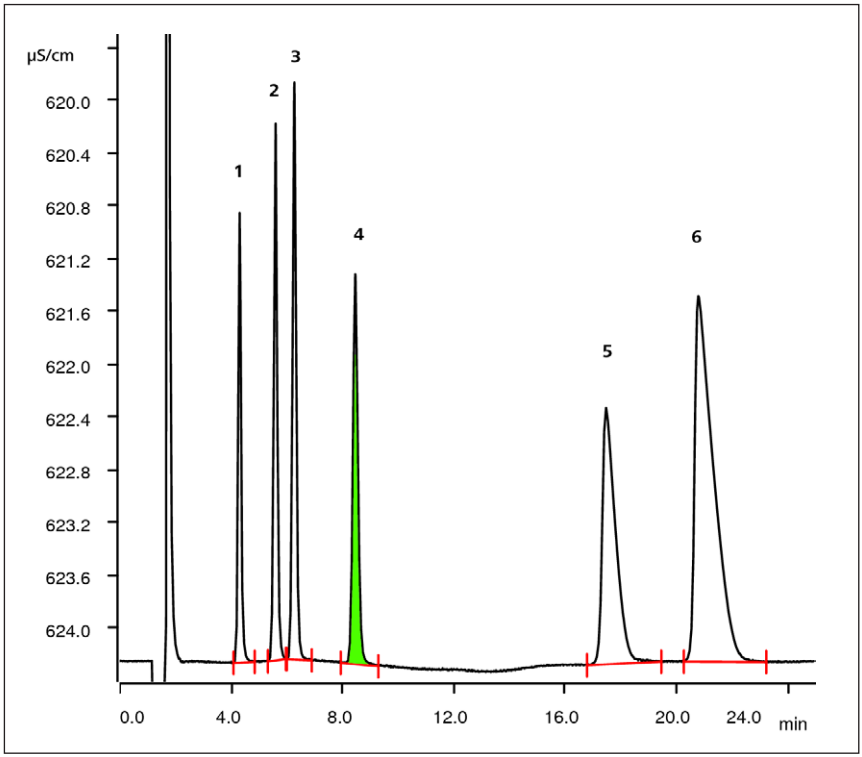
- Effect of a very selective complexing agent on the retention times
- Explanation of the effect in comparison with Experiment 6

Table 19 Parameters – Experiments 5a and 5b

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 18-crown-6 Conductivity approx. 630 µS/cm
Sample	Standard (lithium, sodium, ammonium, potassium, calcium, magnesium in 2 mmol/L HNO_3)
Flow	0.9 mL/min
Pressure	6.6 MPa
Analysis time	25 min
Loop	10 µL
Polarity	-

Eluent preparation

Dissolve 1.7 mL nitric acid (c = 1 mol/L) and 117 mg dipicolinic acid in 100 mL ultrapure water under heating and then make up to 1 L with ultrapure water.



Chromatogram 13 Standard solution – Eluent without crown ether

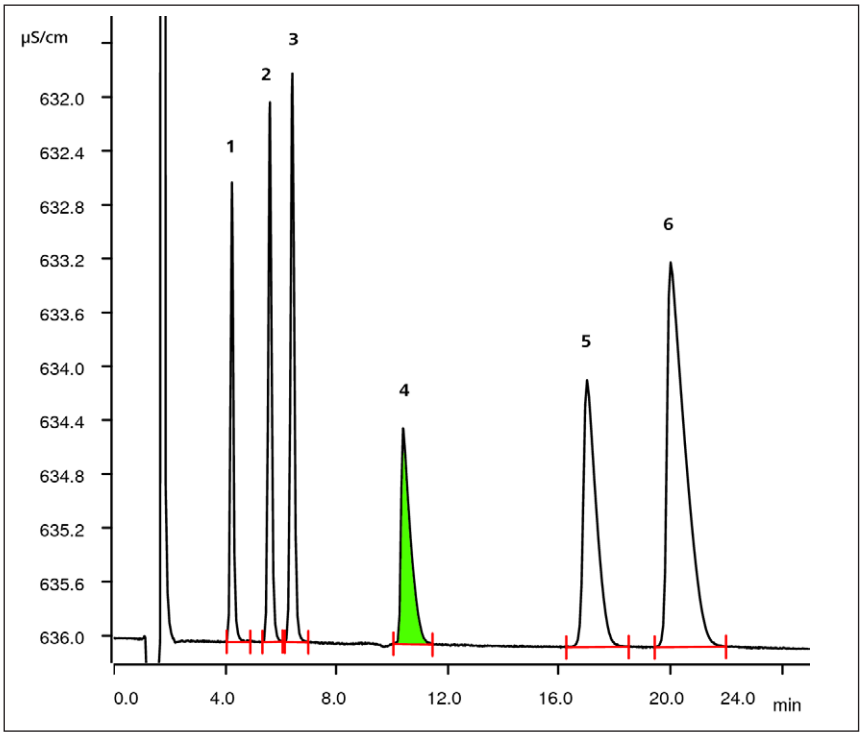
Table 20 Components – Experiment 5a

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Lithium	4.3	10	8543	0.413
2	Sodium	5.6	10	10008	0.584
3	Ammonium	6.3	10	10950	0.673
4	Potassium	8.5	10	10567	0.630
5	Calcium	17.5	10	6320	1.078
6	Magnesium	20.8	10	4340	2.154

Experiment 5b – Eluent with crown ether

Eluent preparation

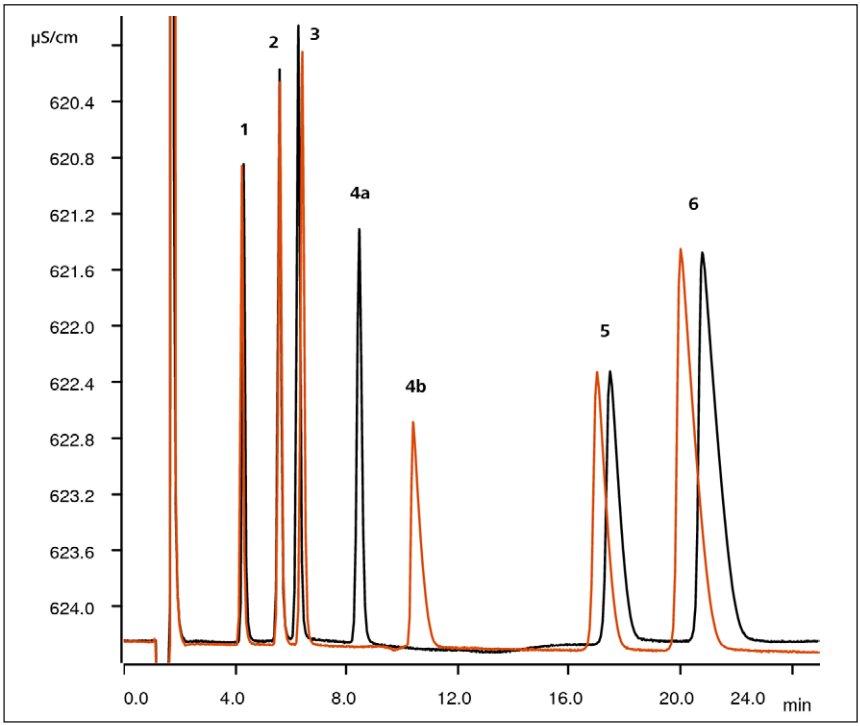
Dissolve 1.7 mL nitric acid (c = 1 mol/L) and 117 mg dipicolinic acid in 100 mL ultrapure water under heating, add 52.8 mg 18-crown-6 and then make up to 1 L with ultrapure water.



Chromatogram 14 Standard solution – Eluent with crown ether

Table 21 Components – Experiment 5b

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Lithium	4.3	10	8448	0.414
2	Sodium	5.6	10	9941	0.582
3	Ammonium	6.4	10	10831	0.670
4	Potassium	10.4	10	4926	0.616
5	Calcium	17.0	10	6285	1.059
6	Magnesium	20.0	10	4425	2.123



Chromatogram 15 Overlay of chromatograms 13 (black) and 14 (red)

4.2.6 Experiment 6 – Altering the selectivity by using complexing agents

For the determination of magnesium, sodium and potassium ions in the presence of zinc and calcium ions, the ability of zinc and calcium ions to form complexes with dipicolinic acid (DPA, 2,6-pyridinedicarboxylic acid) is utilized.

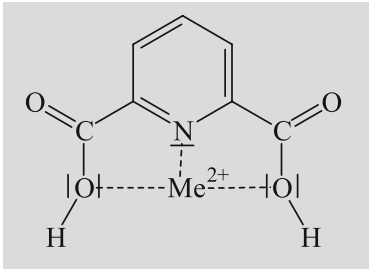


Figure 27 Der Me^{2+}-dipicolinic acid complex MeH_2DPA

The complex formation constant resulting from the reaction is different for each metal.

$$Me^{2+} + (H_2DPA) \longleftrightarrow [Me(H_2DPA)]^{2+}$$

Depending on the pH, the following complexes can be formed (increasing removal of H^+ as the pH increases):

$$[Me(H_2DPA)]^{2+} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} [Me(HDPA)]^{+} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} [Me(DPA)]^{0}$$

acidic conditions weakly acidic conditions alkaline conditions

This means that, depending on the pH, the complex formed has either a double positive charge, a single positive charge, or is uncharged.

The primary separation criterion on a cation exchanger column is the charge of the ions to be separated. Uncharged complexes are not retarded whereas complexes with a threefold positive charge are bound very strongly. As a result of the complex formation constant and the set pH the complex has a certain average equilibrium charge. This equilibrium charge determines the retention time. This is the reason why divalent metal ions can be accelerated by the addition of dipicolinic acid within a particular pH range.

There is no influence on the retention times of monovalent metal ions, which do not form a complex with dipicolinic acid.

Learning content

- Influence of the complex formation constant on the retention time – comparison of zinc and calcium
- Behavior of other transition metal ions
- Influence of the pH value on the total charge of the complex
- Explanation of the effect in comparison with Experiment 5

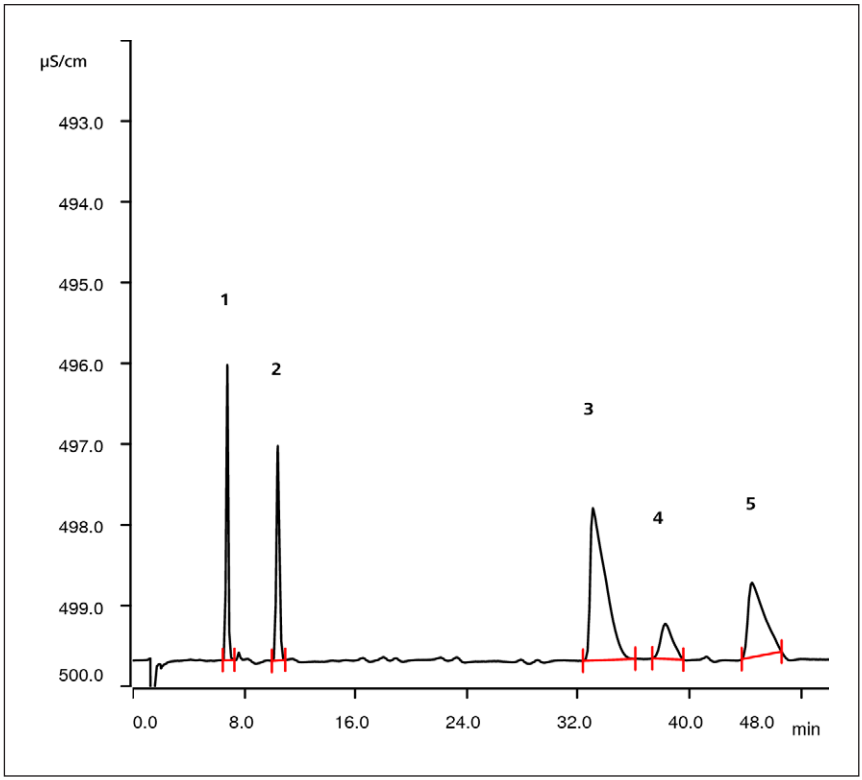
Table 22 Parameters – Experiments 6a to 6d

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 / 0.01 mmol/L dipicolinic acid, Conductivity approx. 515 µS/cm c)1.7 mmol/L nitric acid / 0.1 mmol/L dipicolinic acid, Conductivity approx. 527 µS/cm d)1.7 mmol/L nitric acid / 0.7 mmol/L dipicolinic acid, Conductivity approx. 650 µS/cm
Sample	Standard sodium, potassium, calcium, magnesium, zinc in 2 mmol/L HNO3)
Flow	0.9 mL/min
Pressure	6.6 MPa
Analysis time	a) 50 min b) 45 min c) 45 min d) 22 min
Loop	10 µL
Polarity	-

Experiment 6a – Eluent: 1.7 mmol/L nitric acid

Eluent preparation

Dissolve 1.7 mL nitric acid (c = 1 mol/L) in 1 L with ultrapure water.



Chromatogram 16 Standard solution – Eluent: 1.7 mmol/L nitric acid

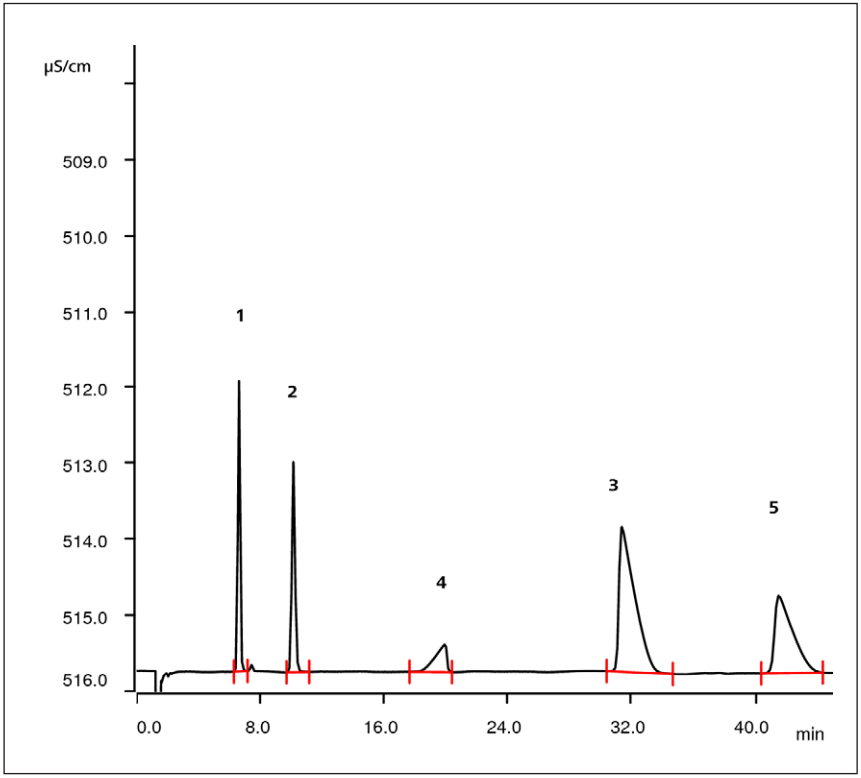
Table 23 Components – Experiment 6a

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Sodium	6.8	10	10723	0.625
2	Potassium	10.4	10	11045	0.674
3	Magnesium	33.1	10	4232	2.351
4	Zinc	38.2	10	9189	0.432
5	Calcium	44.5	10	6765	1.230

Experiment 6b – Eluent: 1.7 mmol/L nitric acid + 0.01 mmol/L dipicolinic acid

Eluent preparation

Dissolve 1.7 mL nitric acid (c = 1 mol/L) and 1.7 mg dipicolinic acid under heating in 100 mL ultrapure water and make up to 1L.



Chromatogram 17 Standard solution – Eluent: 1.7 mmol/L nitric acid + 0.01 mmol/L dipicolinic acid

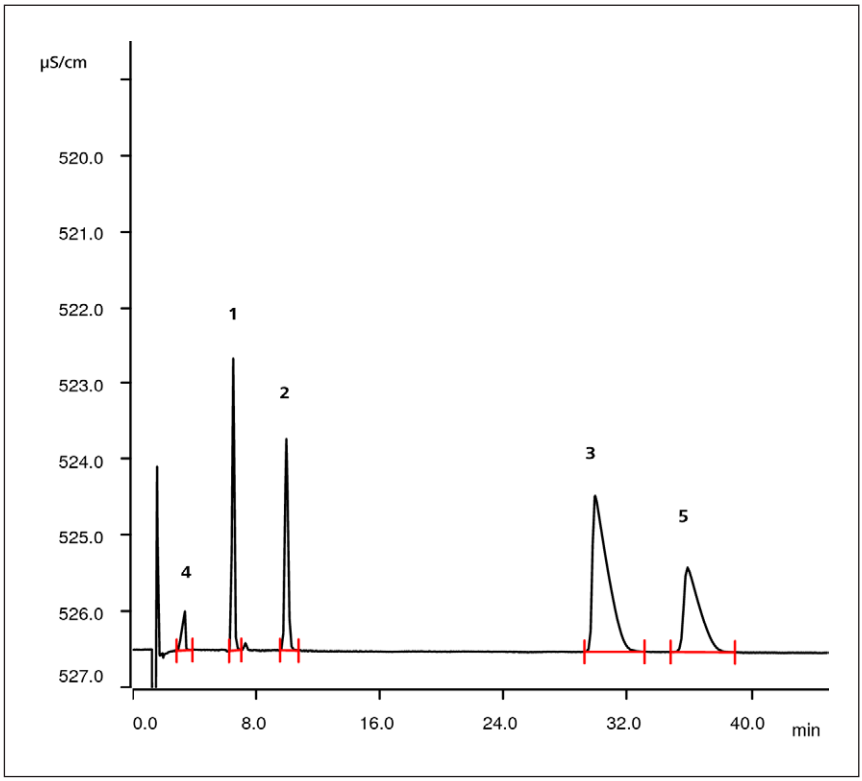
Table 24 Components – Experiment 6b

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Sodium	6.6	10	11421	0.618
2	Potassium	10.1	10	11680	0.670
3	Magnesium	31.4	10	4533	2.234
4	Zinc	19.9	10	3027	0.322
5	Calcium	41.5	10	5924	1.379

Experiment 6c – Eluent: 1.5 mmol/L nitric acid + 0.1 mmol/L dipicolinic acid

Eluent preparation

Dissolve 1.7 mL nitric acid (c = 1 mol/L) and 16.7 mg dipicolinic acid under heating in 100 mL ultrapure water and make up to 1L.



Chromatogram 18 Standard solution – Eluent: 1.7 mmol/L nitric acid + 0.1 mmol/L dipicolinic acid

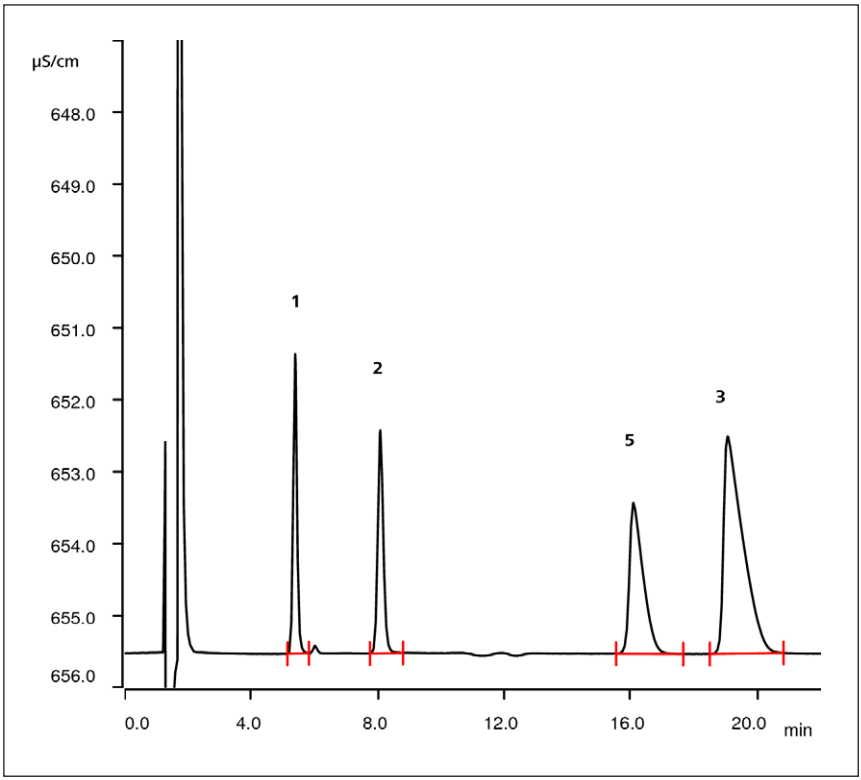
Table 25 Components – Experiment 6d

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Sodium	6.5	10	11308	0.615
2	Potassium	9.9	10	11596	0.664
3	Magnesium	29.9	10	4430	2.326
4	Zinc	3.4	10	1017	0.135
5	Calcium	35.9	10	5938	1.315

Experiment 6d – Eluent: 1.7 mmol/L nitric acid + 0.7 mmol/L dipicolinic acid

Eluent preparation

Dissolve 1.7 mL nitric acid (c = 1 mol/L) and 117 mg dipicolinic acid under heating in 100 mL ultrapure water and make up to 1L.



Chromatogram 19 Standard solution – Eluent: 1.5 mmol/L nitric acid + 0.7 mmol/L dipicolinic acid

Table 26 Components – Experiment 6d

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Sodium	5.4	10	9829	0.582
2	Potassium	8.1	10	10458	0.629
3	Magnesium	19.1	10	4326	2.120
4	Zinc	---	10		
5	Calcium	16.1	10	6355	1.049

Remarks
All solutions must be stored in plastic vessels. For a correct determination of sodium all contact with glass must be avoided. The pH of the standard and sample solutions must be between 2.5 and 3.5.
After changing the eluent, allow the system to run until the baseline is constant.
Chromatograms 19: Zinc is complexed by the dipicolinic acid and elutes in the injection peak.

4.3 Experiments for determining anions

4.3.1 Experiment 7 – Anions in drinking water

Drinking water is our most important foodstuff. It is obtained mainly from ground water and surface water. Surface waters include water from lakes and reservoirs, bank filtrate, groundwater enriched with surface water and river water.

According to DIN 2000, drinking water must satisfy the following requirements: It should be colorless, clear, cool and free from foreign odor and taste. If possible it should be naturally free from pathogens and substances that are hazardous to health. It should not contain too many salts, particularly hardness components, iron, manganese, as well as organic substances (peat and humic substances).It should not cause corrosion. The amount available should be sufficient to supply all the requirements of the population to be supplied with it.

Depending on the degree of pollution, various methods are used for the treatment of drinking water.

Screening, removes coarse soil and larger particles.

Sand filters, for filtration; biodegradation processes also occur in the sand and help with purification.

Active charcoal filters adsorb dissolved organic substances, e.g. pesticides.

Removal of iron and manganese by the oxidation of Fe(II) and Mn(II); this process would otherwise take place in the drinking water pipeline and result in brown turbidity or flocs in the drinking water.

Disinfection is always necessary when the water is not free of pathogens. Chlorine, ozone, chlorine dioxide and UV irradiation are all used.

Preventive chlorination before release into the drinking water pipeline in order to prevent growth of microorganisms on the way to the consumer.

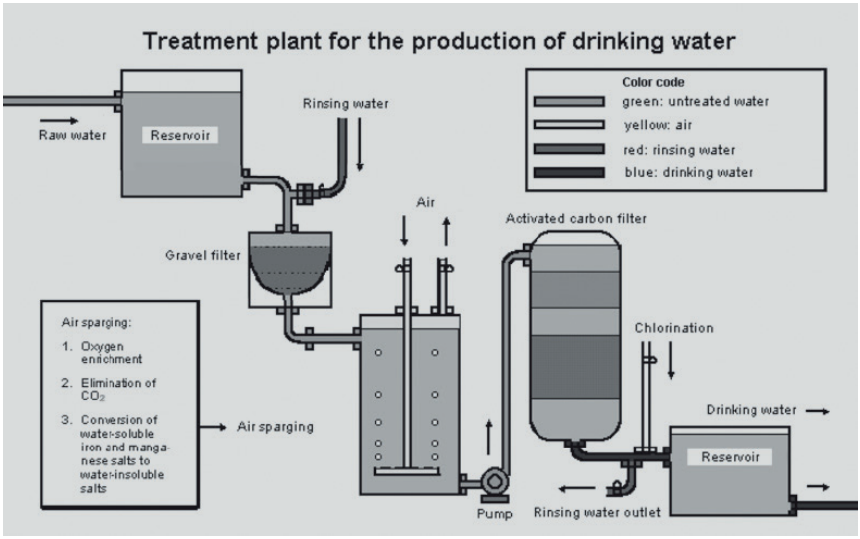


Figure 28 Drinking water treatment – diagram by Thomas Seilnacht, Tuttling

Learning content

- Investigation of the «Number 1» foodstuff
- Checking the information given on mineral water bottles

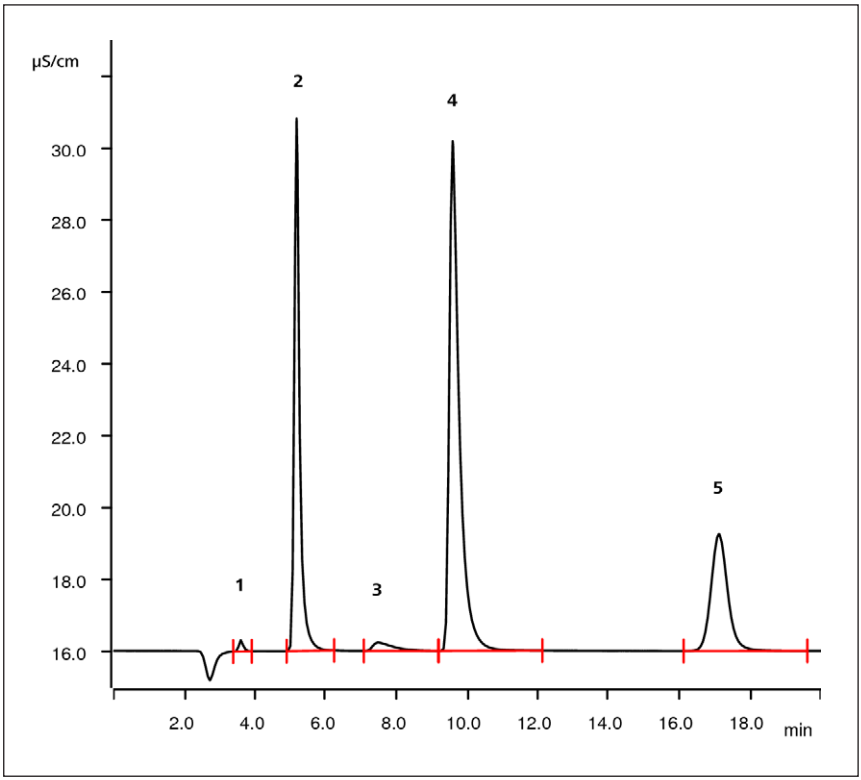
Table 27 Limiting values for drinking water (Federal Republic of Germany)

Fluoride	1.5 mg/L as F^-	Chloride	250 mg/L
Nitrate	50 mg/L as NO_3^-	Sulfate	250 mg/L
Nitrite	0.1 mg/L as NO_2^-		

Table 28 Parameters – Experiments 7

Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$ Conductivity after chemical suppression approx. 16 µS/cm
Sample	Standard (fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate)
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	20 min
Loop	20 µL
Suppressor	Regenerant: 100 mmol/L H_2SO4, STREAM
Polarity	+

Eluent preparation
Dissolve 530 mg sodium carbonate (anhydrous) and 16.8 mg sodium hydrogen carbonate in 1 L ultrapure water.

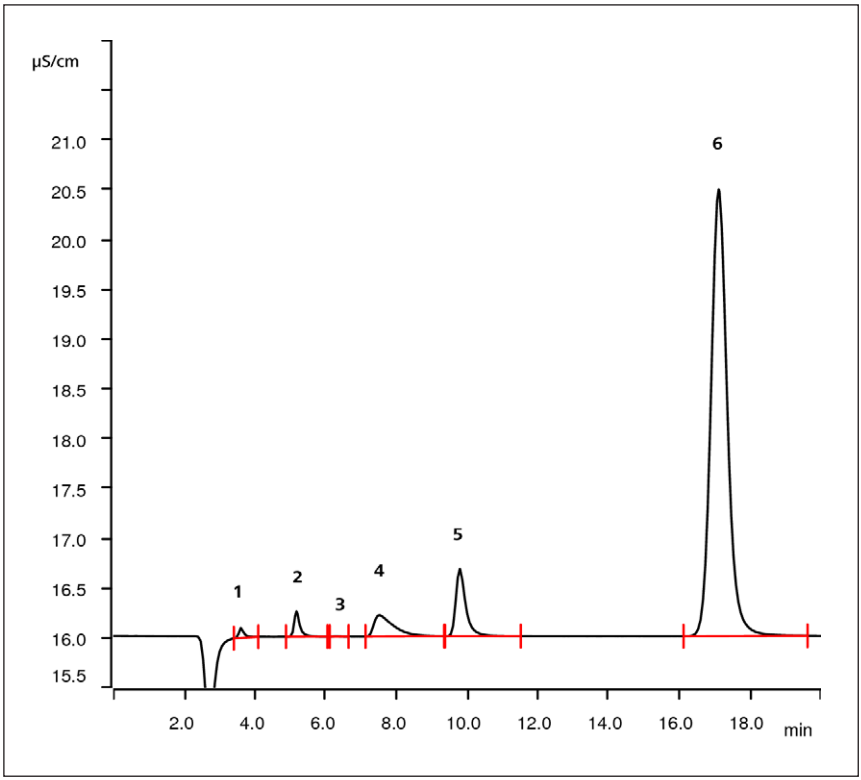


Chromatogram 20 Tap water

Table 29 Components – Experiment 7 – Tap water

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.6	n.q.	4003	0.045
2	Chloride	5.2	7.9	7074	2.601
3	System peak	7.5			
4	Nitrate	9.6	26.9	6702	4.641
5	Sulfate	17.1	7.8	6740	1.767

n.q.: not quantified

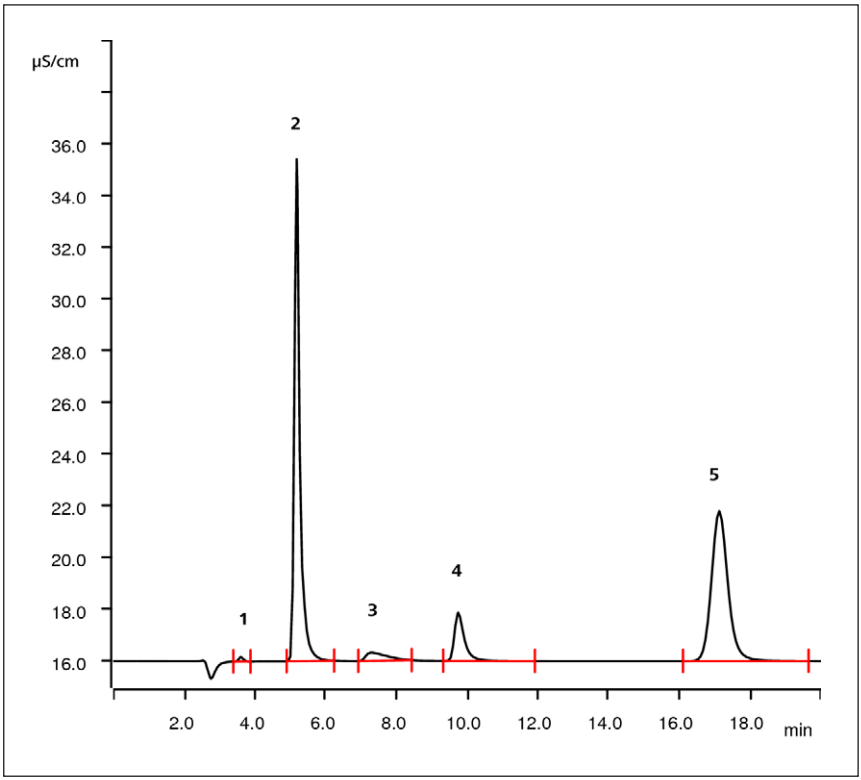


Chromatogram 21 Alpine surface water

Table 30 Components – Experiment 7 – Alpine surface water

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.6	n.q.	3487	0.017
2	Chloride	5.2	0.9	6113	0.048
3	Nitrite	6.3	n.q.	6205	0.001
4	System peak	7.5			
5	Nitrate	9.8	1.4	6753	0.220
6	Sulfate	17.1	10.5	6939	2.409

n.q.: not quantified



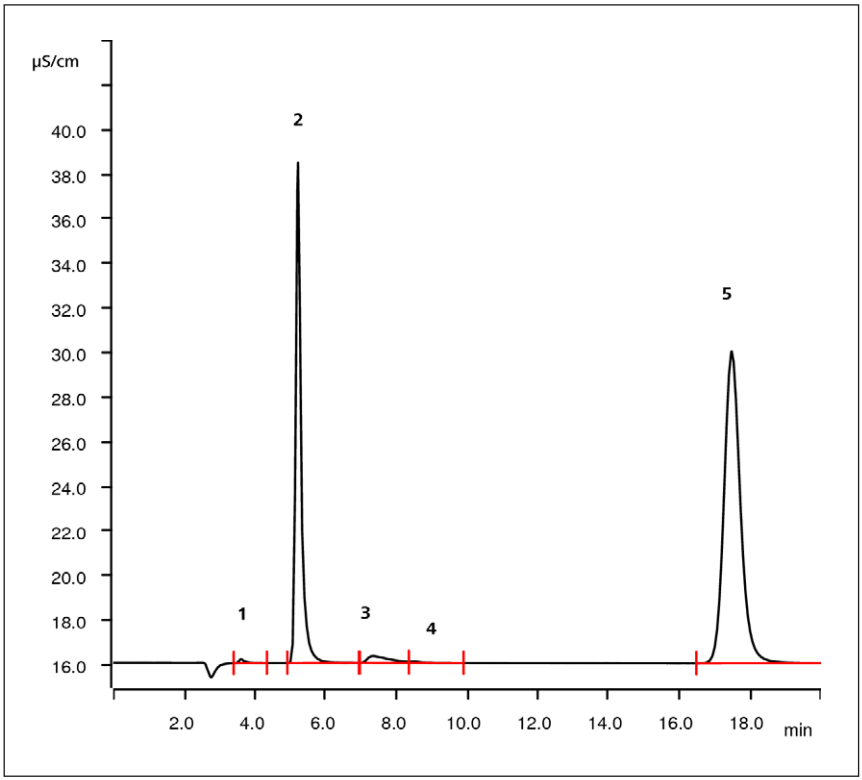
Chromatogram 22 Mineral water

Table 31 Components – Experiment 7 – Mineral water degassed

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.6	n.q.	3760	0.028
2	Chloride	5.2	10.2	7125	3.469
3	System peak	7.3			
4	Nitrate	9.8	3.7	6733	0.607
5	Sulfate	17.1	13.5	6857	3.132

n.q.: not quantified

Remarks
Mineral water containing carbon dioxide must be degassed before the measurement – why?



Chromatogram 23 Healing water

Table 32 Components – Experiment 7 – Healing water (1:10 diluted)

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.6	n.q.	3315	0.035
2	Chloride	5.2	115.5	7161	0.950
3	System peak	7.3			
4	Bromide	8.5	n.q.	1991	0.022
5	Sulfate	17.3	318.7	7217	7.513

n.q.: not quantified

4.3.2 Experiment 8 – Anions in ethanol and spirits (liquor)

The determination of anions in organic solvents is possible, although at the start of the chromatograms pronounced system peaks often occur. Analyses of rum and vodka are used as examples.

In some cases, however, the sample matrix (e.g. ethanol) may greatly interfere with the determination, leading to badly shaped peaks that cannot be evaluated. It is then necessary to remove the matrix by rinsing the preconcentration column with ultrapure water (matrix elimination). In this way good chromatograms are obtained.

Inline Sample Preparation is not in the focus of this monograph. For further information visit www.metrohm.com. Typically ethanol and aqueous mixtures of ethanol can be injected directly. An alternative method to remove organic matter from aqueous solutions is to use a RP (reversed phase) sample preparation column.

Learning content

- Analysis of foodstuffs
- Influence of the matrix on chromatography

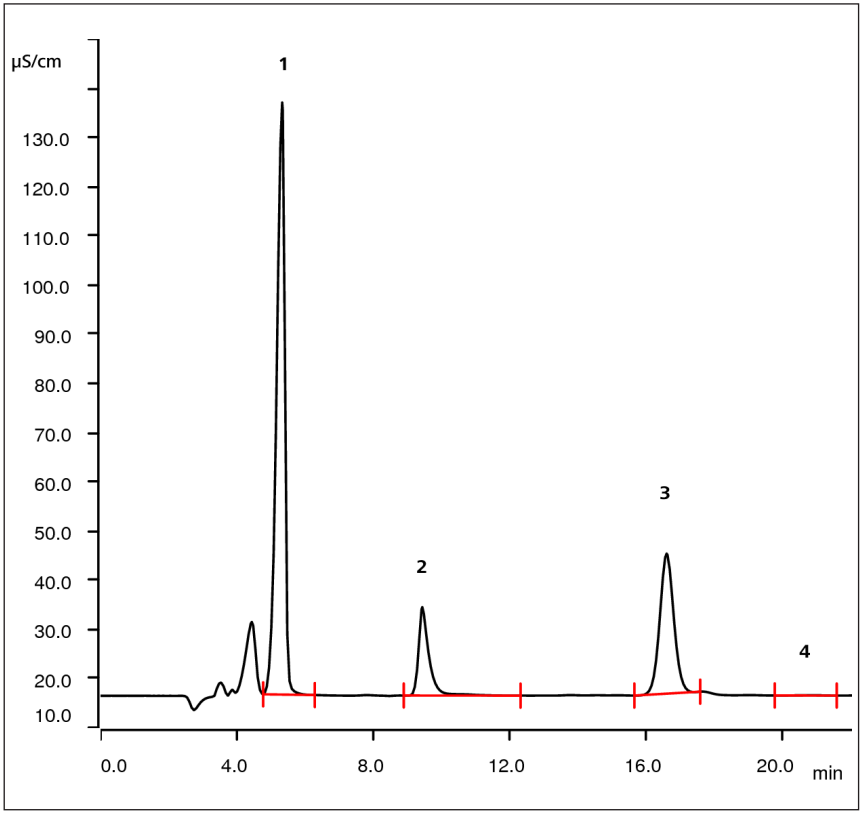
Table 33 Parameters – Experiments 8a and 8b

Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$ Conductivity after chemical suppression approx. 16 µS/cm
Sample	a) Rum b) Vodka
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	22 min
Loop	100 µL
Suppressor	Regenerant: 100 mmol/L H_2SO_4, STREAM
Polarity	+

Eluent preparation

Dissolve 530 mg sodium carbonate (anhydrous) and 16.8 mg sodium hydrogen carbonate in 1 L ultrapure water.

Experiment 8a – Determination of anions in Rum



Chromatogram 24 Analysis of rum

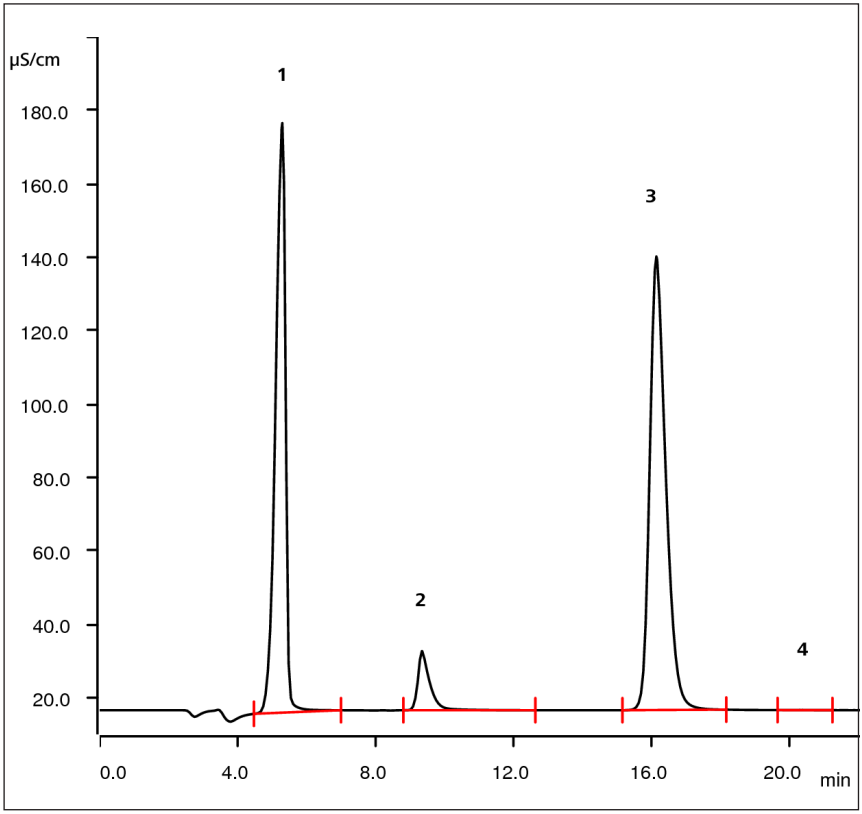
Table 34 Components – Experiment 8a – Rum

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Chloride	5.3	8.8	2423	33.68
2	Nitrate	9.4	0.2	5667	6.324
3	Sulfate	16.6	2.7	7814	13.56
4	Oxalate	20.8	n.q.	3867	0.055

n.q.: not quantified

Remarks
Peaks eluted earlier than chloride are not identified.

Experiment 8b – Determination of anions in Vodka



Chromatogram 25 Analysis of vodka

Table 35 Components – Experiment 8b – Vodka

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Chloride	5.3	14.0	1701	53.88
2	Nitrate	9.3	0.2	4121	6.379
3	Sulfate	16.1	13.5	5691	66.49
4	Oxalate	20.3	n.q.	6226	0.017

n.q.: not quantified

4.3.3 Experiment 9 – Anions in iceberg lettuce

Nitrate enters the human body via drinking water, but also by eating vegetables, salad, etc. The WHO (**W**orld **H**ealth **O**rganization) recommends that the daily nitrate consumption should not exceed 220 mg per person. In Germany the average nitrate consumption is about 130 mg per person per day. Approx. 5% of this originates from meat and sausages; the remainder is split about 50:50 between drinking water and vegetables.

Nitrate can have an adverse effect on human health in several different ways. Nitrate itself is relatively non-toxic. Only when larger amounts are consumed can this lead to inflammation of the gastro-intestinal tract. However, under certain conditions, particularly in the presence of bacteria such as are present in the human mouth, nitrate is reduced to nitrite by the enzyme nitrate reductase:

$$NO_3^- \xrightarrow{\text{enzyme}} NO_2^-$$

Nitrite is able to convert hemoglobin, the red blood colorant, to methemoglobin (Fe^{2+} is oxidized to Fe^{3+}). In contrast to hemoglobin, methemoglobin cannot transport oxygen in the blood. In adult human beings this damage can be remedied by metabolic reactions. However, the metabolism of babies up to 5 months old is not yet able to do this. This results in a lack of oxygen in the baby's blood and the skin turns bluish. This **cyanosis** or **methemoglobinemia** can sometimes result in death.
In the acidic surroundings of the stomach nitrite can react with various secondary amines (two H-atoms of the ammonium molecule are replaced by alkyl residues), which enter the human body via medicines and foodstuffs, to form **nitrosamines:**

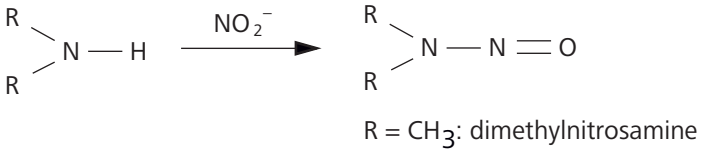


Figure 29 *Formation of nitrosamines from nitrite and secondary amines*

Nitrosamines are among the most carcinogenic substances known. They are formed within the organism from non-carcinogenic compounds.

Learning content

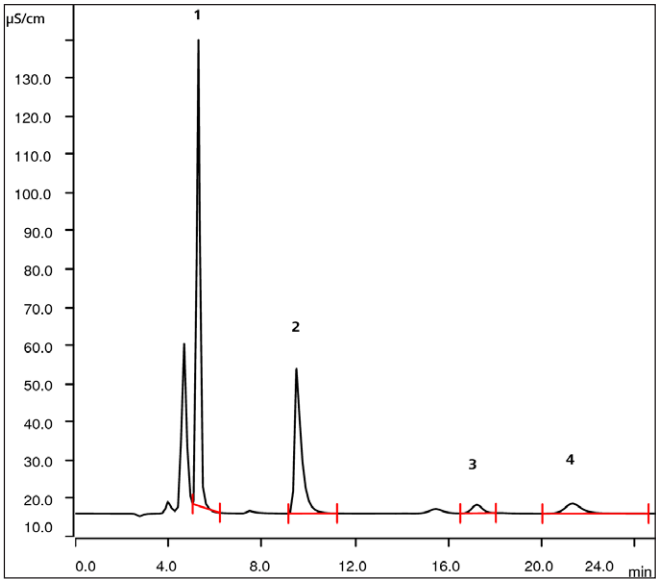
- Analysis of foodstuffs
- Checking foodstuffs for nitrite and nitrate in particular

Table 36 Parameters – Experiment 9

Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$ Conductivity after chemical suppression approx. 16 µS/cm
Sample	Iceberg lettuce
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	25 min
Loop	20 µL
Suppressor	Regenerant: 100 mmol/L H_2SO_4, STREAM
Polarity	+

Eluent preparation
Dissolve 530 mg sodium carbonate (anhydrous) and 16.8 mg sodium hydrogen carbonate in 1 L ultrapure water.

Sample preparation
Chop up lettuce, disperse, add ultrapure water (ratio 1:100) and filter.



Chromatogram 26 Analysis of iceberg lettuce (dilution 1:100)

Table 37 Components – Experiment 9 – Iceberg lettuce

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Chloride	5.3	525	6867	19.96
2	Nitrate	9.5	721	6027	12.94
3	Sulfate	17.2	52.4	7173	1.14
4	Phosphate	21.3	265	4524	2.26

4.3.4 Experiment 10 – Phosphoric acid in cola drinks

Coca-Cola is a non-alcoholic beverage which contains carbon dioxide and caffeine. It was first made in 1885 by the American pharmacist Pemberton. Among its ingredients are extracts of cola nuts, bitter oranges, locust beans, ginger essence, 12% sugar, 0.28% phosphoric acid (pH = 2.7), caramel color and carbon dioxide. The caffeine content is 16 mg/100 mL.

Many cola drinks are available on the market (Pepsi Cola, Club Cola, River Cola, Afri Cola, etc.); the composition of these may differ from Coca-Cola.

The determination of the phosphoric acid content in the cola drinks is of special significance as the local cola bottlers get the concentrate and the bottling is controlled via the phosphate content, requiring a highly accurate determination.

Phosphoric acid is a tribasic acid (pK_{S1}= 2,161, pK_{S2}= 7,207, pK_{S3}= 12,325). The amounts of the different species vary with the pH as can be seen in the following diagram:

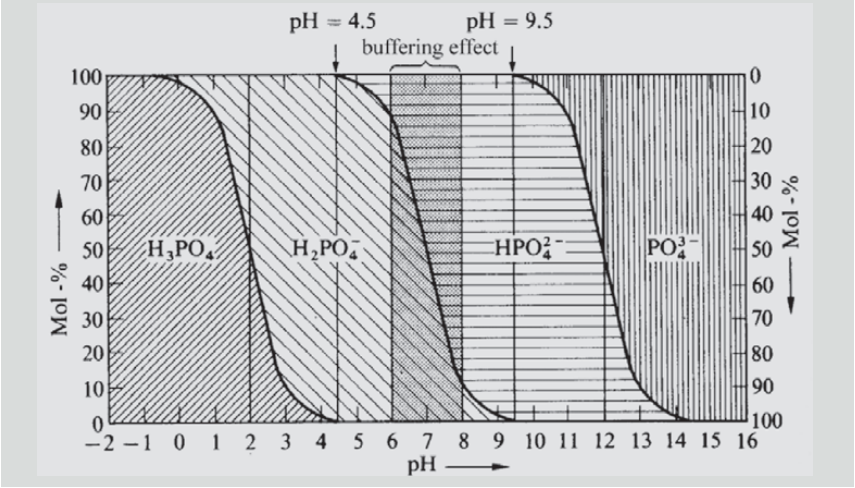


Figure 30 Buffering effect of phosphoric acid in the pH range 6-8

A mixture of primary and secondary phosphate is frequently used as a buffer solution which buffers in the pH range 6-8 (90% $H_2PO_4^-$ + 10% HPO_4^{2-} to 10% $H_2PO_4^-$ + 90% HPO_4^{2-}).

See also the Henderson-Hasselbalch equation (buffer equation):

$$pH = pK_S + \lg \frac{c(A^-)}{c(HA)}$$

Learning content

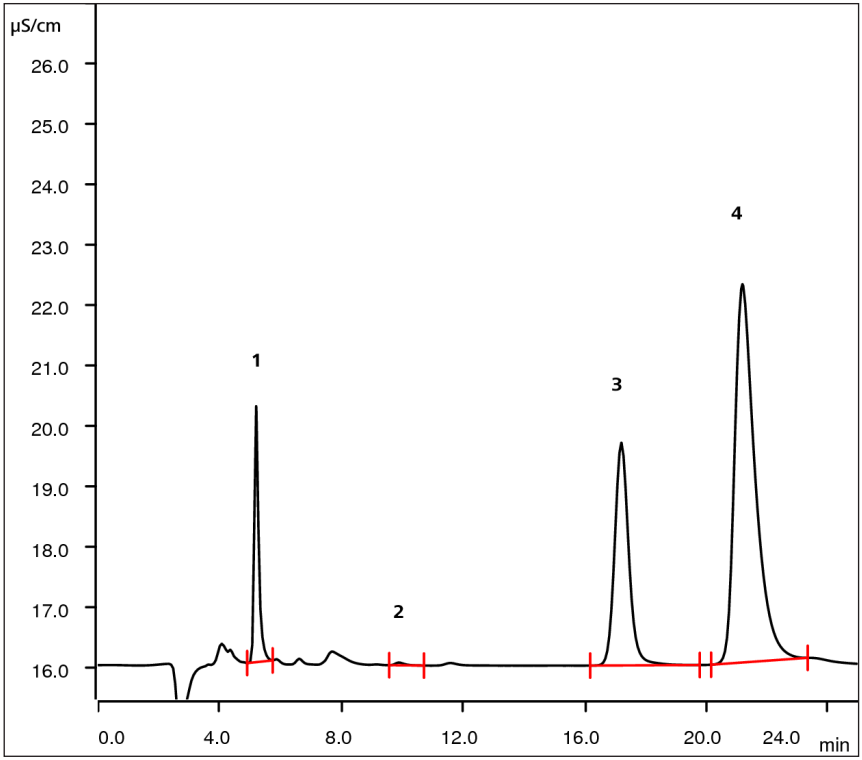
- Analysis of foodstuffs
- Phosphoric acid chemistry
- Influence of the pH on chromatographic separation
- Statistics: reproducibility

Table 38 Parameters – Experiment 10a to 10c

Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$ Conductivity after chemical suppression approx. 16 µS/cm
Sample	Iceberg lettuce
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	25 min
Loop	20 µL
Suppressor	Regenerant: 100 mmol/L H_2SO_4, STREAM
Polarity	+

Eluent preparation
Dissolve 530 mg sodium carbonate (anhydrous) and 16.8 mg sodium hydrogen carbonate in 1 L ultrapure water.

Experiment 10a – Analysis of Cola drink



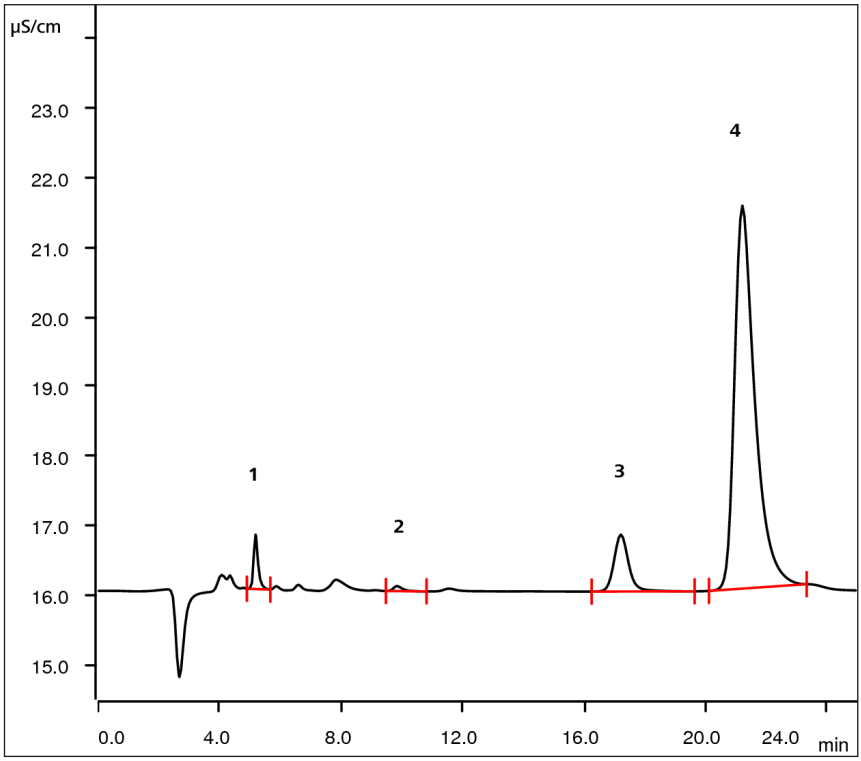
Chromatogram 27 Analysis of Cola drink (dilution 1:10)

Table 39 Components – Experiment 10a – Cola drink

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Chloride	5.2	27.1	7114	0.715
2	Nitrate	9.9	n.q.	5497	0.016
3	Sulfate	17.2	89.3	7045	1.979
4	Phosphate	21.2	551	5038	4.856

n.q.: not quantified

Experiment 10b – Analysis of low-calory Cola drink



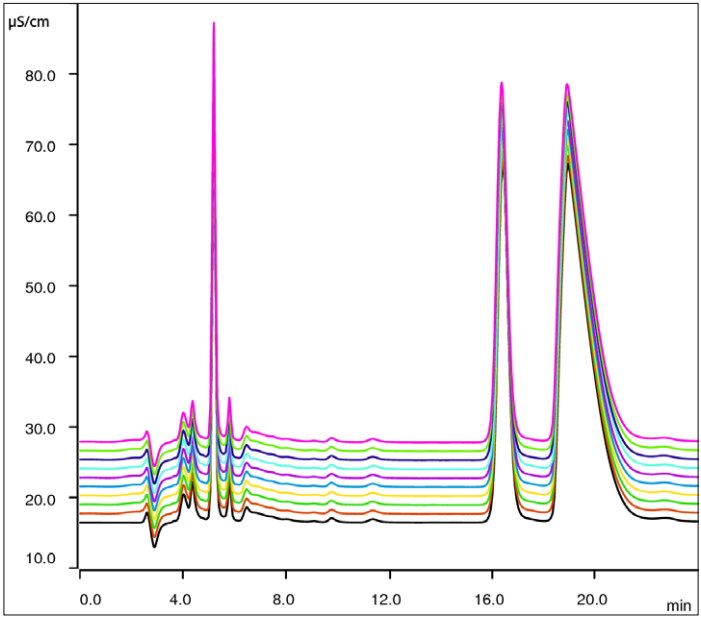
Chromatogram 28 Analysis of low-calory Cola drink (dilution 1:10)

Table 40 Components – Experiment 10b – low-calory Cola drink

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Chloride	5.2	11.1	6706	0.135
2	Nitrate	9.9	2.8	5718	0.025
3	Sulfate	17.2	21.7	6684	0.457
4	Phosphate	21.2	487.6	5208	4.267

n.q.: not quantified

Experiment 10c – Reproducibility of the phosphate determinations in Cola drink



Chromatogram 29 Overlay of 10 measurements (undiluted sample)

Table 41 Reproducibility of measurements

Chromatogram	Area [µS/cm*min]	Phosphate conc.[mg/L]
1	65.55	567.6
2	65.37	565.2
3	65.69	567.7
4	65.65	567.4
5	65.55	566.6
6	65.23	564.1
7	65.26	564.3
8	65.87	569.1
9	65.49	566.1
10	65.56	566.7

Relative standard deviation < 0.3%

Remarks
The cola drinks must be degassed.

4.3.5 Experiment 11 – Organic acids in wine

According to the German wine law, wine is a product which is only obtained by the complete or partial alcoholic fermentation of fresh or mashed grapes or from grape juice. Grape juice contains about 12-25% carbohydrates (glucose, fructose) and 0.9-1.5% acids; the most important are L-(+) tartaric acid (2R, 3R) and malic acid, but citric acid, ketoglutaric acid, succinic acid and lactic acid may also be present.

An important criterion for assessing the grape juice is the Oechsle degree (Oe°); the higher it is the more sugar the juice contains. It indicates the number of grams by which 1 L juice at 20 °C is heavier than 1 L distilled water. For example, juice with a density of 1.115 kg/L (115 g more than 1 L water) has 115 Oe°. From the degrees Oechsle a simple calculation permits determination of the sugar and alcohol content. 1.7 g sugar produce 1 mL (0.794 g) ethanol. At an alcohol volume fraction of 12-15% fermentation comes to a standstill as the yeasts are killed off by the alcohol they have produced.

The aroma of the wine is made up of 600 to 800 components: hydrocarbons, alcohols, aldehydes, ketones, acids, esters, lactones, ethers, phenols and many more.

Of analytical interest are the 2R, 3R-tartaric acid, malic acid, citric acid, lactic acid and succinic acid. The total acid content (calculated as tartaric acid) is usually between 5.5 and 8.5 g/L. Acetic acid, propionic acid, higher fatty acids and abnormal amounts of lactic acid occur in «spoiled» wine and are chiefly produced by microorganisms.

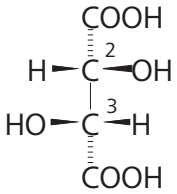


Figure 31 L-(+)-tartaric acid, (2R, 3R)-form

Alcoholic fermentation takes place according to the following equation:

$$C_6H_{12}O_6 + 2\,ADP + 2\,P \xrightarrow{\text{yeast}} 2\,C_2H_5OH + 2\,CO_2 + 2\,ATP$$

Abbreviations: ADP = adenosine diphosphate, ATP = adenosine triphosphate, P = phosphate

The weak organic acids are determined by ion exclusion chromatography (see chapters 3.3.4, 3.6.7 and 3.7.2.3).

Table 42 *Acid content (mg/L) of German wines (mean value from 4 or 2 individual analyses; German denominations have been retained)*

			Riesling				Müller-Thurgau			
	Typical high-quality wine	Portugieser Rotwein	Kabinet	Spätlese	Auslese	Beerenauslese, Eiswein	Silvaner Kabinet	Spätlese	Auslese	Beeren-auslese
Oxalic acid	15,7	13,0	11,0	45,8	16,0	21,7	16,7	40,9	15,4	19,5
Succinic acid	194,0	260,1	205,8	282,1	230,2	149,2	243,8	215,1	264,9	269,2
Fumaric acid	44,2	31,9	24,6	28,3	20,8	36,0	20,9	51,8	27,0	40,6
Glutaric acid	3,7	1,9	4,5	2,6	2,3	7,0	2,6	2,2	1,9	6,2
c- or t- Aconitic acid	2,1	1,9	+	5,5	4,9	6,4	1,4	2,5	1,6	4,5
Glycolic acid	1,8	1,3	+	+	1,4	6,9	3,7	3,4	1,7	5,8
D- + L-Lactic acid	1789	2364	319,8	159,8	151,0	2141	793	221,0	208,1	2096
Anglyceric acid	43,5	62,6	50,1	39,5	59,0	53,0	49,6	87,0	68,8	49,9
Triglyceric acid	±	±	±	±	±	±	±	±	±	±
3-M-2,3-DHBS	±	±	±	±	±	±	±	±	±	±
Malic acid	3971	5080	3850	3871	4235	2251	2893	3163	3410	2897
Tartaric acid	1586	994	1428	1431	817	1251	1280	1014	805	887
Citramalic acid	94,5	53,7	63,0	18,6	46,8	48,2	31,3	33,8	24,2	41,0
α-Hydroxyglutaric acid	24,9	27,5	18,2	24,5	28,4	20,6	18,8	15,3	26,9	22,2
Citric acid	138,9	150,2	241,1	222,8	298,6	287,3	200,7	221,7	240,2	335,1
Glyoxylic acid	+	1,0	+	+	4,0	6,1	2,9	1,8	1,1	3,1
Pyruvic acid	20,6	29,8	17,8	12,4	16,8	8,6	17,6	22,7	18,6	33,0
α-Ketoglutaric acid	41,1	45,7	38,6	36,8	55,7	35,6	41,0	33,7	40,3	27,5
Gluconic acid	59,0	357,2	54,5	95,9	452,1	337,2	53,9	131,1	373,9	540
Mucic acid	–	+	–	–	4,1	20,4	–	+	4,5	11,2
Glucuronic acid	2,1	3,4	3,8	5,8	7,9	14,5	1,7	6,0	7,6	10,2
Galacturonic acid	11,1	11,7	14,1	19,7	22,0	43,4	10,0	17,3	23,2	34,3
L-Ascorbic acid	12,9	9,0	13,7	10,1	9,8	9,6	12,0	10,4	9,7	8,5
Total acids	7977	9491	6349	6302	6474	6744	5685	5284	5565	7333

Remarks
– = not detectable
+ = traces
± = present in traces (approx. 5 mg/L), not evaluated
3-M-2,3-DHBS = 3-methyl-2,3-dihydroxybutyric acid

Learning content

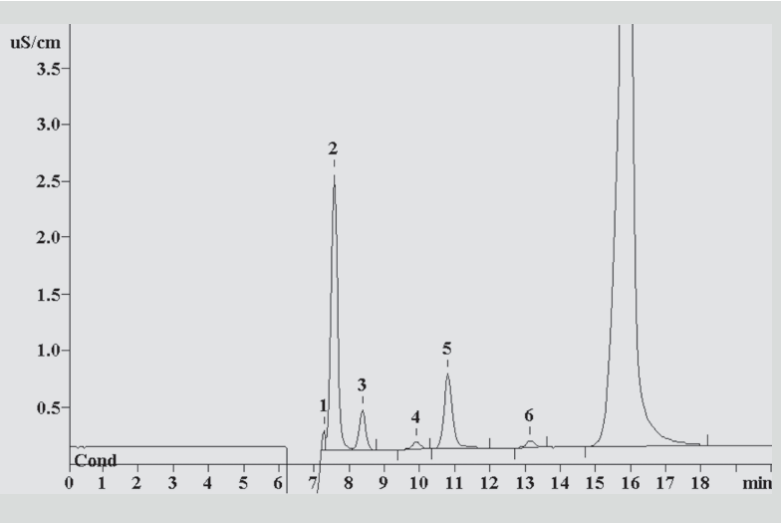
- Analysis of foodstuffs
- Ion exclusion chromatography
- Which ions can be determined by ion exclusion chromatography?

Table 43 Parameters – Experiments 11a and 11b

Column	6.1005.200 Metrosep Organic Acids-250/7.8
Eluent	0.5 mmol/L H_2SO_4 / acetone (85:15)
Sample	White wine, red wine
Flow	0.5 mL/min
Pressure	3 MPa
Analysis time	20 min
Loop	20 µL
Polarity	+

Eluent preparation
Mix together 0,5 mmol H_2SO_4 (850 mL) and acetone (150 mL).

Sample preparation
Dilute wine sample 1:100 and filter through a 0.45 µm filter.

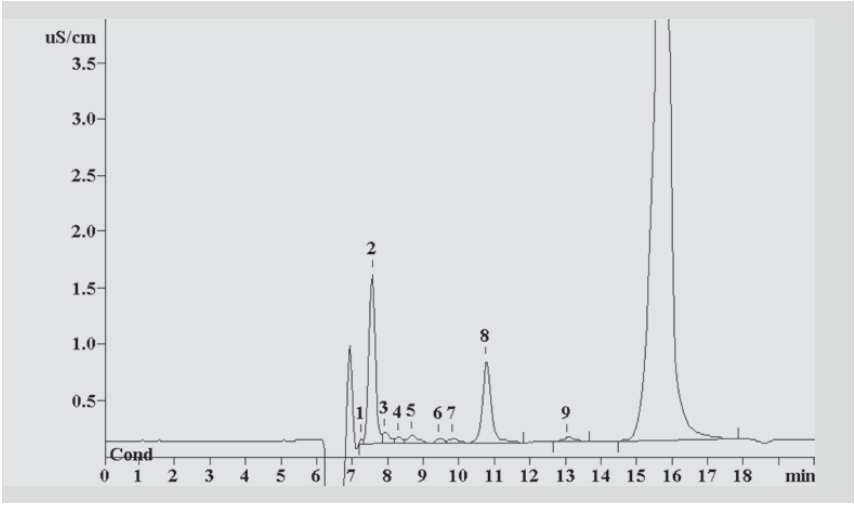


Chromatogram 30 Standard solution for the determination of organic acids in wine

Table 44 Components – Experiments 11a and 11b – Standard

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
1	Citrate	7.2	5	26200	1.1
2	Tartrate	7.5	20	7990	31
3	Malate	8.3	5	9210	4.5
4	Succinate	9.9	5	7140	1.2
5	Lactate	10.8	20	8830	11
6	Acetate	13.1	10	11500	1.2
	System peak	15.9			

Experiment 11a – Determination of organic acids in white wine



Chromatogram 31 Determination of organic acids in white wine (dilution 1:100)

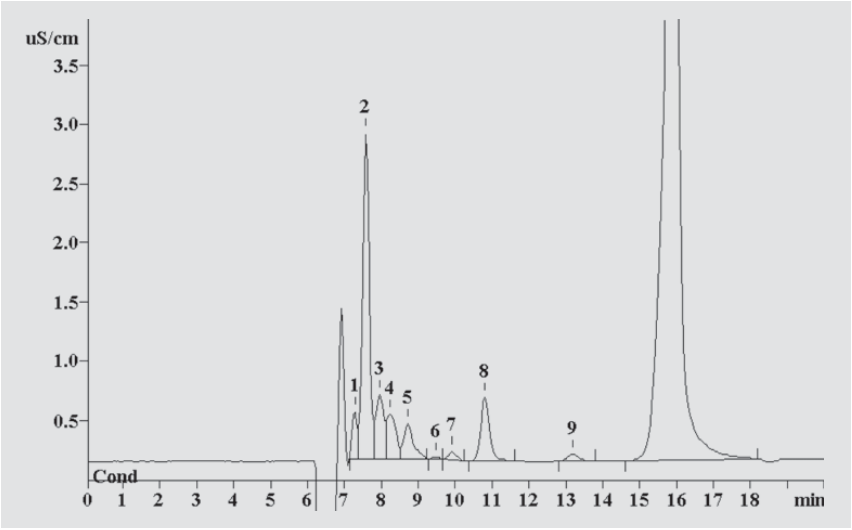
Table 45 Components – Experiment 11a

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
1	Citrate	7.2			
2	Tartrate	7.5	1210	8430	19
4	Malate	8.2			
7	Succinate	9.8	350	5660	0.8
8	Lactate	10.7	2190	8820	13
9	Acetate	13.0	710	5200	0.8
	System peak	15.8			

Remarks

Peaks 3, 5 and 6 were not evaluated.

Experiment 11b – Determination of organic acids in red wine



Chromatogram 32 Determination of organic acids in red wine (dilution 1:100)

Table 45 Components – Experiment 11b

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
1	Citrate	7.2			
2	Tartrate	7.5	2230	8300	34
4	Malate	8.2			
7	Succinate	9.9	410	10500	1.0
8	Lactate	10.8	1560	9570	8.9
9	Acetate	13.1	1010	7950	1.1
	System peak	15.9			

Remarks

Peaks 3, 5 and 6 were not evaluated; citrate and malate could not be quantified correctly.

4.3.6 Experiment 2 – Contaminants in boric acid – determination of chloride and sulfate in borax solutions

Borax (disodium tetraborate $Na_2B4O_7 \cdot 10\ H2O$, $Na_2[B_4O_5(OH)_4] \cdot 8\ H_2O$) has the following structure:

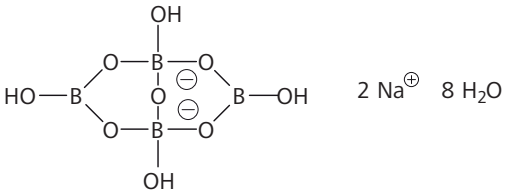


Figure 32 «Borax» (disodium tetraborate, $Na_2B_4O_7 \cdot 10\ H_2O$, $Na_2[B_4O_5(OH)_4]\cdot 8\ H_2O$)

When it melts borax can dissolve many metal oxides with the formation of characteristic colors. These «borax pearls» are well-known in practical inorganic chemistry. Borax is also used in the manufacture of glass, pottery glazes, porcelain and as a flux in brazing. At 100 °C borax loses five water molecules and becomes the pentahydrate «jeweler's borax». If a soldering flux is added to it then surface contaminants – mainly oxide layers – are destroyed. These would otherwise affect the formation of an alloy between the solder (itself an alloy of silver, copper and tin) and the basic material.

Sodium tetraborate (borax) solutions are used in the internal cooling circuit of nuclear power plants as neutron absorbers. The purity of the material is extremely important as traces of chloride and sulfate cause corrosion in the piping, which must be avoided by all means.

Boric acid H_3BO_3 or $B(OH)_3$ is a very weak monobasic acid whose pK_A value of 9.25 corresponds approximately to that of HCN. Boric acid is not an H^+ donor (Brønsted acid definition), but rather an OH^- acceptor (Lewis acid definition):

$$B(OH)_3 + HOH \rightleftharpoons H^+ + B(OH)_4^-$$

Ion chromatography without chemical suppression uses an acidic eluent with pH = 4.1 for the determination of the contaminants in borate. At this pH borate is present almost entirely as boric acid $B(OH)_3$. In contrast to the negatively charged anions, boric acid does not interact with the stationary phase of the separating column. This means that it elutes in the dead volume.

However, with chemical suppression the weakly alkaline carbonate/hydrogen carbonate eluent is used instead. Although it is now possible to separate borate as an anion, the detection does not function. The cause of this can be found in the suppressor, which exchanges all the Na^+ ions for H^+ ions. As a result, the weak boric acid is formed.

The large amount of boric acid disturbs the chromatogram, making the determination of the anions almost impossible. Matrix elimination is the best possibility to solve the problem.

Learning content

- Strong and weak acids

Determination of anions in boric acid
Experiment 12a – Measurement without chemical suppression

Table 46 Parameters – Experiment 12

Column	6.1006.430 Metrosep A Supp 4 - 250/4.0
Eluent	5 mmol/L phthalic acid, 2% acetone; pH = 4.1 Conductivity approx. 293 µS/cm
Sample	0.8% aqueous boric acid solution spiked with 2 ppm chloride and nitrate
Flow	1.0 mL/min
Pressure	5.0 MPa
Analysis time	38 min
Loop	100 µL
Polarity	–

Eluent preparation
Dissolve 821 mg phthalic acid in 20 mL acetone and a small volume of water and make up to 1 L. Adjust the pH to 4.1 by adding approx. 0.5 g TRIS (solid).

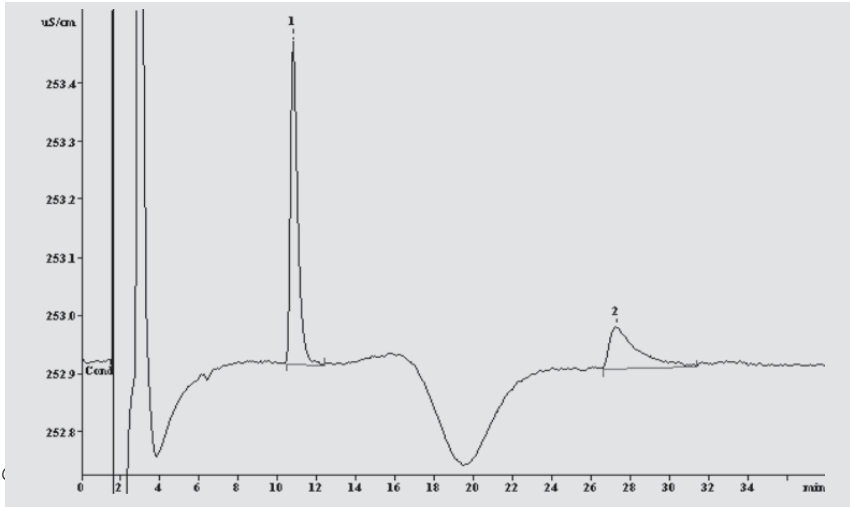


Table 47 Components – Experiment 12

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µScm*s]
1	Chloride	10.8	2.0	4327	14.23
2	Nitrate	27.3	2.0	1669	7.48

4.3.7 Experiment 14 – Anions in wastewater

In biological treatment plants wastewater is mainly purified with the help of bacteria. Organic substances are oxidized to a large extent with the consumption of oxygen:

$$\text{Organic substances} + O_2 \xrightarrow{\text{bacteria}} CO_2 + H_2O + \text{cell mass}$$

Apart from the oxidation of organic substances, ammonium can also be oxidized to nitrate (nitrification) in suitably designed plants:

$$NH_4^+ + 2\,O_2 \xrightarrow{\text{bacteria}} NO_3^- + H_2O + 2\,H^+$$

In oxygen-free basins bacteria utilize the nitrate oxygen for the oxidation of organic substances (denitrification):

$$\text{Organic substances} + 2\,NO_3^- \xrightarrow{\text{bacteria}} 2\,CO_2 + 2\,OH^- + 2\,H_2O + N_2\uparrow$$

Phosphate – a plant nutrient like NH_4^+ and NO_3^- – can be precipitated, e.g. by the addition of Fe_3^+ salt solutions. Apart from the so-called sum parameters – biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC) – which are a measure of the organic load of the wastewater or water in general, the determination of NH_4^+, NO_3^- and PO_4^{3-} is also important. Cl^- and SO_4^{2-} are only determined in special cases.

In communal treatment plants with more than 100'000 so-called population equivalents (1 population equivalent is the sewage flow per head) the following limits have been laid down in Germany:

BSB	15 mg/L
CSB	75 mg/L
NH_4-N	10 mg/L*
N_{tot}	18 mg/L* (sum of NH_4-N, NO_2-N, NO_3-N)
PO_4-P_{tot}	1 mg/L

* Only applies at a wastewater temperature ≥ 12 °C, as nitrification is greatly influenced by the temperature.

NH_4-N, NO_2-N, NO_3-N means that the values refer to the nitrogen contained in the respective ions; the same applies for PO_4-P.

Learning content

- Environmental analysis
- Sample preparation techniques
- Analysis of mixtures of substances with large differences in concentration – dynamic concentration range

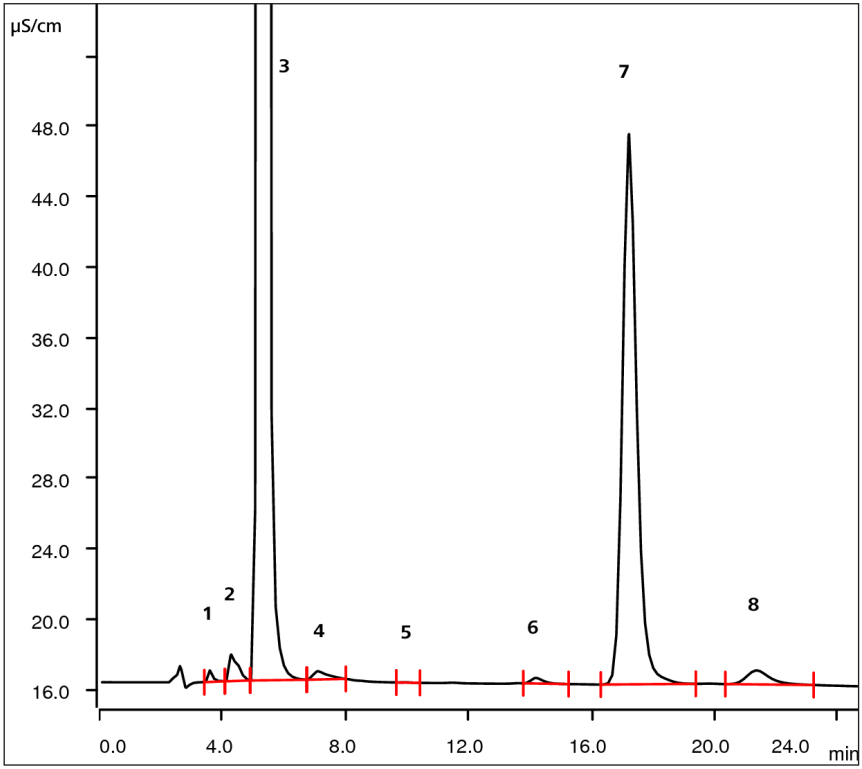
Table 48 Parameters – Experiments 13a and 13b

Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$ Conductivity after chemical suppression approx. 16 µS/cm
Sample	a) wastewater treatment plant inflow b) wastewater treatment plant outflow
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	25 min
Loop	20 µL
Suppressor	Regenerant: 100 mmol/L H_2SO_4, STREAM
Polarity	+

Eluent preparation
Dissolve 530 mg sodium carbonate (anhydrous) and 16.8 mg sodium hydrogen carbonate in 1 L ultrapure water.

Sample preparation
Filtering of the samples is absolutely essential. Use one-way filters with a pore size of 0.2 µm or less. Even seemingly clear solutions can contain very fine particles that damage the column.

Experiment 13a – Treatment plant inflow



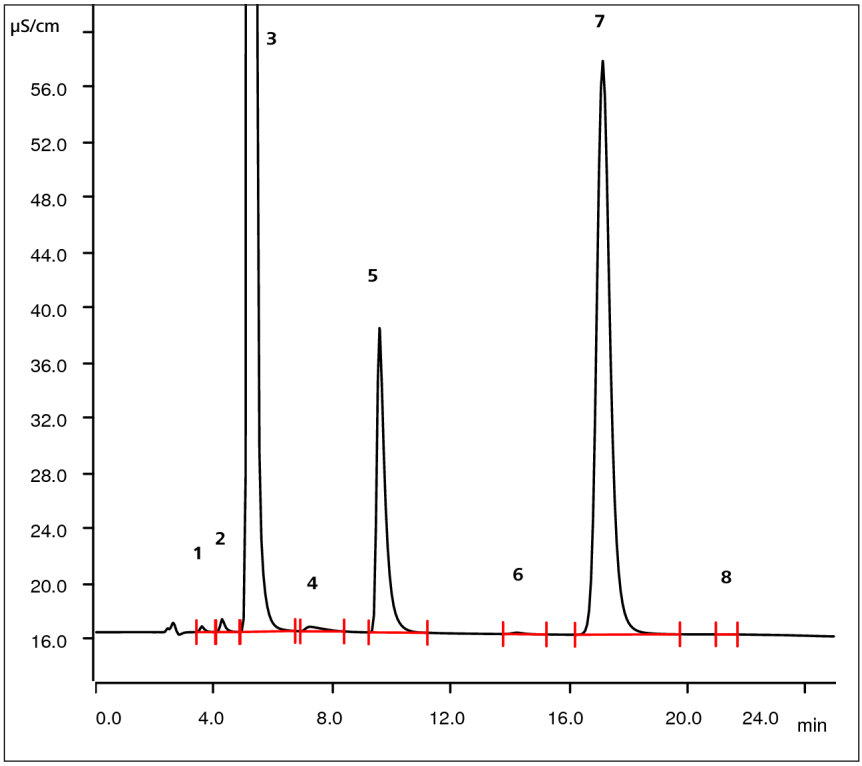
Chromatogram 33 Analysis of the treatment plant inflow

Table 49 Components – Experiment 13a – Treatment plant inflow

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.6	0.1	2954	0.119
2	unknown	4.3			
3	Chloride	5.4	170.4	2681	75.36
4	System peak	7.1			
5	Nitrate	10.0	n.q.		
6	Unknown	14.2			
7	Sulfate	17.2	65.9	7091	16.81
8	Phosphate	21.4	8.7	4097	0.705

n.q.: not quantified

Experiment 14b – Treatment plant outflow



Chromatogram 34 Analysis of the treatment plant outflow

Table 50 Components – Experiment 13b – Treatment plant outflow

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Fluoride	3.6	< 0.1	3040	0.032
2	unknown	4.3			
3	Chloride	5.4	141.2	3228	60.33
4	System peak	7.2			
5	Nitrate	9.6	41.6	6478	7.266
6	Unknown	14.2			
7	Sulfate	17.1	84.2	6978	22.31
8	Phosphate	21.2	n.q.		

n.q.: not quantified

4.3.8 Experiment 14 – Anions in white and brown sugar

Sugars are organic compounds with a semiacetal-forming carbonyl group and several hydroxyl groups. In general use «sugar» normally refers to the disaccharide saccharose

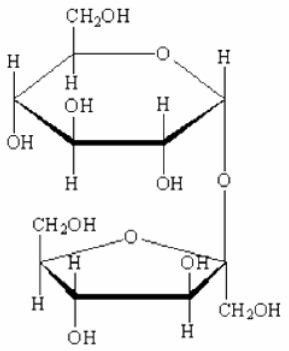


Figure 33 The disaccharide saccharose

In the manufacture of sugar from sugar beet, the beet are first washed and chopped up; they are then extracted with water in so-called diffusion units in counterflow. The soluble constituents – sugar, salts, acids, proteins, pectins – are dissolved. The major share of the non-sugar constituents is precipitated by the addition of burnt lime (CaO). Carbon dioxide (CO_2) is then used to precipitate the excess calcium hydroxide as $CaCO_3$. After filtration the sugar solution is concentrated in multi-stage evaporators to form «thick juice», filtered again and concentrated further until the sugar separates as «white massecuite». The sugar is separated in centrifuges and, after purification (recrystallization), snow-white crystal sugar with a purity of 99.95% is obtained. The centrifuge run-off of the last stage is a brown-colored syrup – molasses.

Brown sugar is less intensively purified and may be colored by the addition of molasses. Apart from organic contaminants, it contains alkali and alkaline earth ions as well as anions; it also has a different taste to white crystal sugar.

The organic contaminants can be separated with an RP (reversed phase) column before the anions are determined.

Learning content

- Analysis of foodstuffs
- Process control
- Checking foodstuffs containing large amounts of sugar, e.g. honey

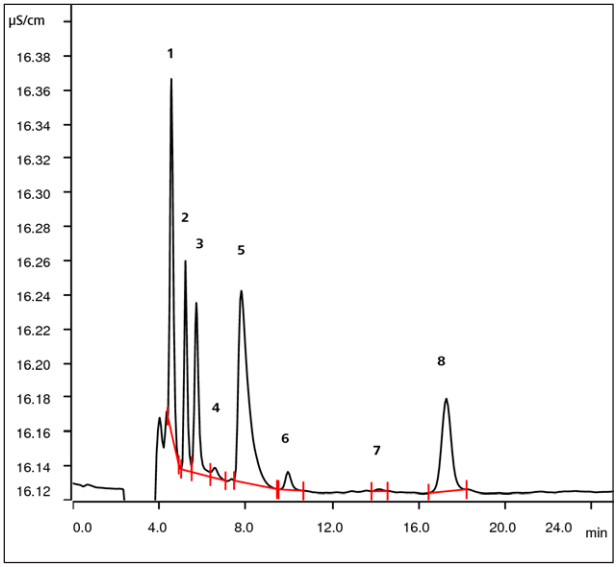
Table 51 Parameters – Experiment 14a and 14b

Column	6.01032.420 Metrosep A Supp 17 - 150/4.0
Eluent	5.0 mmol/L Na_2CO_3 / 0.2 mmol/L $NaHCO_3$ Conductivity after chemical suppression approx. 16 µS/cm
Sample	a) White sugar b) Brown sugar
Flow	0.6 mL/min
Pressure	7.6 MPa
Analysis time	25 min
Loop	20 µL
Suppressor	Regenerant: 100 mmol/L H_2SO_4, STREAM
Polarity	+

Sample preparation
Filtering of the samples is absolutely essential. Use one-way filters with a pore size of 0.2 µm or less. Even seemingly clear solutions can contain very fine particles that damage the column.

Sample preparation
Dissolve the sugar in ultrapure water (dilution 1:10) and filter through a 0.2 µm filter

Experiment 14a – White sugar

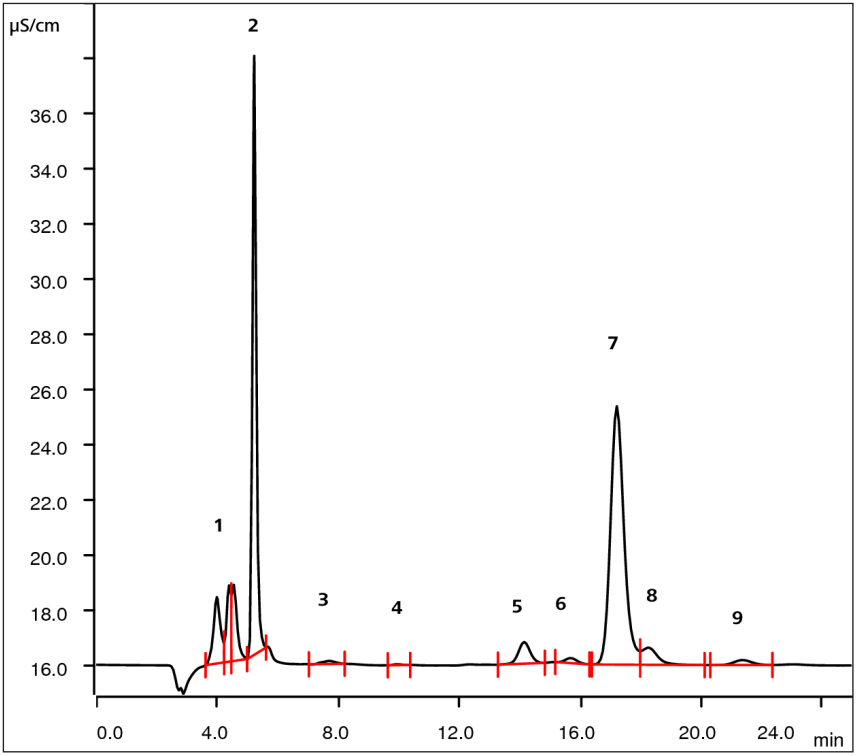


Chromatogram 35 Analysis of white sugar (dilution 1:10)

Table 52 Components – Experiment 14a – White sugar

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	unknown	4.5			
2	Chloride	5.2	7.8	7039	0.020
3	unknown	5.7			
4	Nitrite	6.6	0.7	2773	0.002
5	unknown	7.8			
6	Nitrate	9.9	1.4	6818	0.003
7	unknown	14.1			
8	Sulfate	17.3	2.2	7159	0.028

Experiment 14b – Brown sugar



Chromatogram 36 Analysis of brown sugar (dilution 1:10)

Table 53 Components – Experiment 14b – Brown sugar

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	unknown	4.0-4.6			
2	Chloride	5.2	95.8	8420	3.222
3	System peak	7.7			
4	Nitrate	9.9	1.9	6581	0.011
5	unknown	14.1			
6	unknown	15.7			
7	Sulfate	17.2	212.2	7404	4.873
8	unknown	18.3			
9	Phosphate	21.4	18.0	5958	0.124

Remarks
With brown sugar, contaminants may elute at later retention times; this could interfere with the following chromatograms if the run time is too short. This is why the white sugar should be measured first, followed by the brown sugar.

4.4 Experiments for determining cations

4.4.1 Experiment 18 – Alkali and alkaline earth metals in drinking water

Apart from the dissolved gases O_2, N_2 und CO_2, natural water also contains salts that are mainly leached out of the soil and rocks and enter the ground water. Further ingredients of natural water are polar organic compounds originating, for example, from the humus layers of the soil. Contamination by wastewater is also a possible source of various salts and organic compounds. The most important salts are the chlorides, sulfates and hydrogen carbonates of sodium, calcium and magnesium.

An important parameter of drinking water, both for its use as a foodstuff and its use in washing processes or industrial processes, is the so-called water hardness.

The total hardness of the water is understood as being the sum of the molar concentrations (mmol/L) of the calcium and magnesium ions. That part of the hardness that can be removed by boiling is known as the carbonate hardness (previously temporary hardness). The residual hardness is caused by sulfate and chloride ions, whose Ca and Mg salts cannot be precipitated by boiling.

With the classical soaps – sodium salts of fatty acids – calcium and magnesium ions form insoluble calcium and magnesium compounds. These are both ineffective in washing and are also deposited on textiles (grayness). Modern washing agents contain alkyl sulfates which do not form insoluble calcium and magnesium compounds. The zeolites that are also added to these washing agents function as ion exchangers and bind Ca^{2+} and Mg^{2+} ions. This effectively prevents the precipitation of insoluble $CaCO_3$ and basic magnesium carbonate on heating.

The drinking water regulations, which are the implementation of an EC drinking water directive (80/778/EEC), contain the following values for cations:

NH_4^+	0.5	mg/L
Na^+	150	mg/L
K^+	12	mg/L
Mg^{2+}	50	mg/L
Ca^{2+}	400	mg/L

An increased intake of sodium ions by human beings causes high blood pressure, among other things. The daily Na^+ requirement of 1 g is greatly exceeded today (3-7 g).

In all analyses carried out the samples must be acidified by the addition of nitric acid (pH 2.5 -3.5), as otherwise no reproducible results will be obtained for divalent cations.

If the sodium content of the sample is substantially higher than that of the described standard solution, the latter's sodium concentration should be adapted to that of the sample.

Learning content

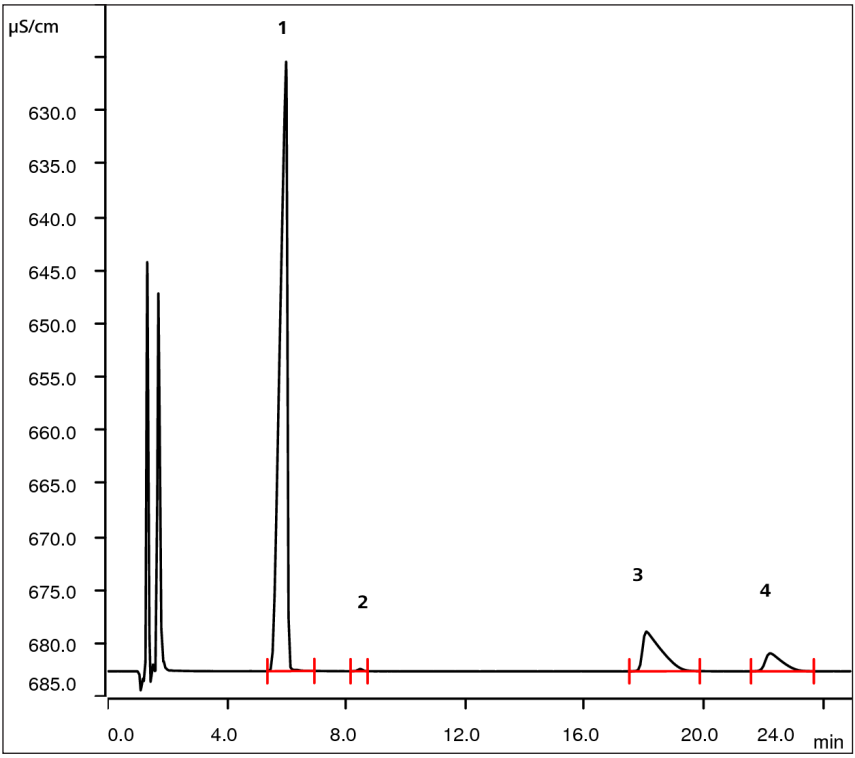
- Checking the «Number 1» foodstuff
- Checking the information given on bottles of mineral water
- Why is it necessary to acidify the samples?

Table 54 Parameters – Experiment 15

Column	6.1050.420 Metrosep C 4 - 150/4.0
Eluent	1.7 mmol/L nitric acid / 0.7 mmol/L dipicolinic acid
Sample	a) Tap water, acidified to pH = 2.5-3.5 (approx. 100 µL 2 mol/L HNO_3 for 100 mL) b) Mineral water, acidified to pH = 2.5-3.5 (approx. 100 µL 2 mol/L HNO_3 for 100 mL)
Flow	0.9 mL/min
Pressure	6.2 MPa
Analysis time	25 min
Loop	10 µL
Polarity	-

Eluent preparation

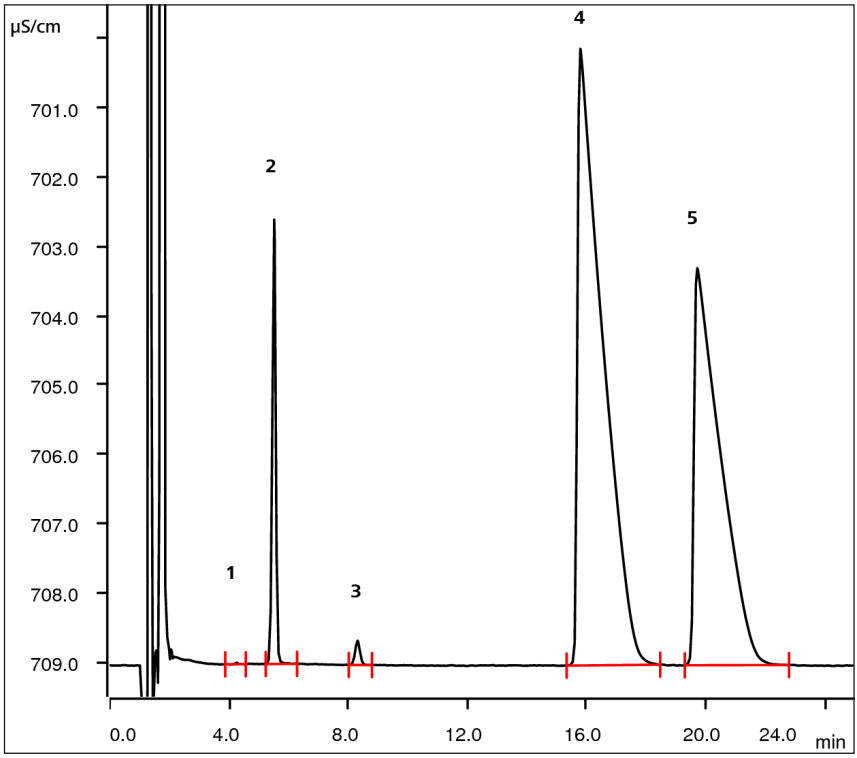
Dissolve 1.7 mL nitric acid (c = 1 mol/L) and 117 mg dipicolinic acid in 100 mL ultrapure water under heating and then make up to 1 L with ultrapure water.



Chromatogram 37 Tap water from Herisau, Switzerland

Table 55 Components – Experiment 15a – Tap water

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Sodium	6.0	133.7	2289	15.96
2	Potassium	8.5	0.7	12470	0.041
3	Calcium	18.1	25.1	3492	2.709
4	Magnesium	22.2	5.1	6915	1.106



Chromatogram 38 Mineral water (dilution 1:10)

Table 56 Components – Experiment 15b – Mineral water

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Lithium	4.3	n.q.		
2	Sodium	5.5	7.1	10836	0.856
3	Potassium	8.3	1.1	11916	0.068
4	Calcium	15.8	80.0	1504	8.608
5	Magnesium	19.7	26.8	2076	5.847

n.q.: not quantified

Remarks

All solutions must be stored in plastic vessels. All contact with glass must be avoided for a correct sodium determination. The pH value of the standard and sample solutions must be between 2.5 and 3.5.

4.4.2 Experiment 16 – Determination of transition metals

Complexing agents are added to the mobile phase in order to separate the transition metal ions. They reduce the charge density and improve the selectivity of the separation, which is relatively low for transition metal ions having the same charge. Apart from the equilibrium between exchanger resin and analyte ion, if complexing agents are added there is a further equilibrium, namely between metal ion and complexing agent. This equilibrium is also influenced by the pH of the solution if various dissociation steps of the complexing agent are possible or if the metal ion forms hydroxy complexes.

Weak organic acids are often used as complexing agents, e.g. tartaric acid, citric acid, oxalic acid, 2,6-pyridinedicarboxylic acid (dipicolinic acid – see chapter 4.2.6 – Experiment 6 – Altering the selectivity by using complexing agents). The number of ligands L surrounding the central metal ion Me – the coordination number – can vary, e.g. MeL, MeL_2, MeL_3. The resulting charge can also differ. Depending on the charge of the metal ion, the ligands and their number, either cationic, neutral or anionic complexes can be present. This means that, depending on their charge, the complexes can be separated on cation exchangers or anion exchangers. Theoretically the use of exchangers containing both cation and anion exchanger groups is possible.

The separation can be influenced and optimized by varying the pH value and the complexing agents – two or more different complexing agents can be added to the mobile phase. In part the influences are contrary and difficult to predict. Some general information is given below:

- Different stabilities (complex formation constants) influence the separation.
- The addition of an organic solvent, e.g. acetone, to the eluent also influences the separation. Lipophilic ions elute more quickly after the addition.
- In cation exchange chromatography, ethylenediamine has proven itself as an eluting agent. Under normal conditions it is present as a cation carrying two protons.
- An increase of the pH results in a reduction of the retention time by reducing the total charge.
- An increase in the ligand concentration in the mobile phase, apart from displacing the complexing equilibrium,also increases the concentration of the counterion H^+ or Na^+ and accelerates the displacement of the complex from the negatively charged exchange sites, i.e. the separation performance is reduced.
- An increase in the ligand concentration causes an increase in the retention time as the coordination number of the complex increases, but the free anionic ligands also promote the elution of the anionic metal complex, so that two opposing effects exist with regard to the retention time.

Detection of the complexes can be carried out either by conductivity or photometrically (post-column derivatization).

For **conductivity detection** only detection without chemical suppression can be used as insoluble hydroxides would be formed by the suppression reaction and the only eluent anions available are carbonate or hydroxide.

Photometric detection requires post-column derivatization in which an additional, chromophoric complexing agent displaces the one originally present (e.g. tartaric acid, oxalic acid, etc.). The newly formed and UV- or VIS-absorbing complex is detected, whereby the absorption maximum of the free ligand must not be in the region of the maximum of the complex. As, for most complexing dyestuffs, the absorption maxima of the individual transition metal complexes lie in different wavelength regions, the detection limits of the individual metals differ greatly.

Learning content

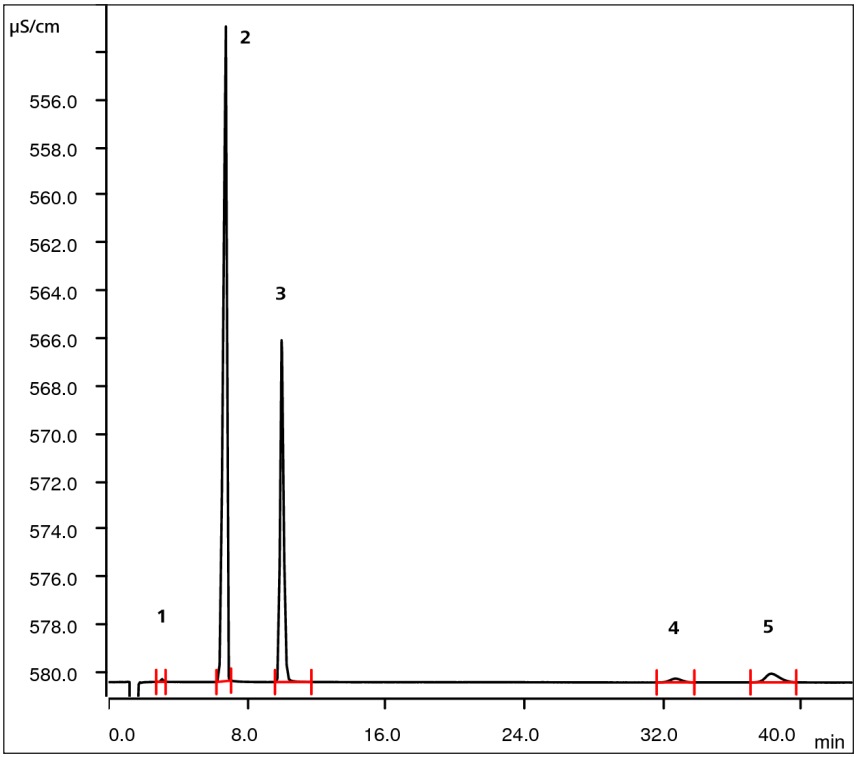
- Influence of the eluent on the selectivity
- Complexing of transition metal ions
- Complexing agents for photometric detection

Table 57 Parameters – Experiment 16a

Column	6.1050.420 Metrosep C 4 - 150/4.0
Eluent	1.7 mmol/L nitric acid / 0.1 mmol/L dipicolinic acid Conductivity approx. 550 µS/cm
Sample	Standard
Flow	0.9 mL/min
Pressure	6.2 MPa
Analysis time	25 min
Loop	10 µL
Polarity	-

Eluent preparation

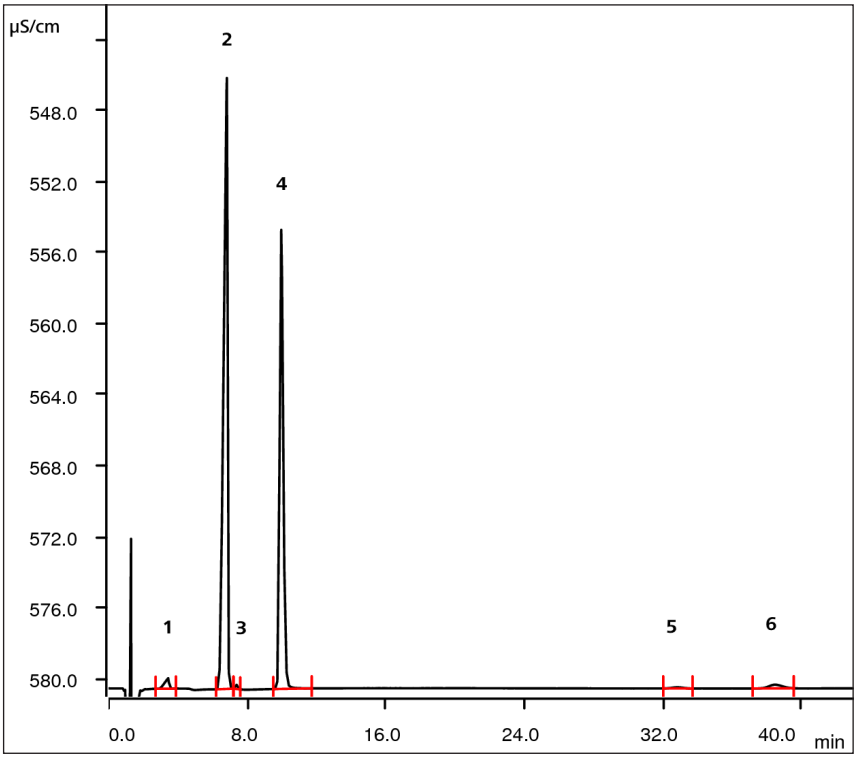
Dissolve 1.7 mL nitric acid (c = 1 mol/L) and 16.8 mg dipicolinic acid in 100 mL ultrapure water under heating and then make up to 1 L with ultrapure water.



Chromatogram 39 Standard cations including zinc

Table 58 Components – Experiment 16a – Standard

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Zinc	3.1	0.5	3644	0.015
2	Sodium	6.8	50	4789	6.189
3	Potassium	10.0	50	11676	3.377
4	Magnesium	32.7	0.5	12217	0.123
5	Calcium	38.2	2.5	10403	0.346



Chromatogram 40 Toothpaste (dilution 1:200)

Table 59 Components – Experiment 16a – Toothpaste (dilution 1:200)

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Zinc	3.1	5.2	717	0.165
2	Sodium	6.8	72.1	3494	8.968
3	Ammonium	7.4	n.q.		
4	Potassium	10.0	90.6	11898	6.101
5	Magnesium	32.7	0.2	13640	0.050
6	Calcium	38.2	1.3	12130	0.187

n.q.: not quantified

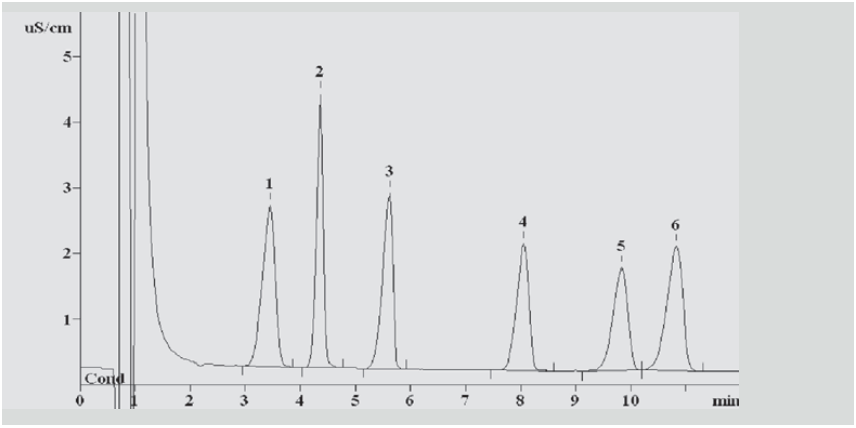
Table 60 Parameters – Experiment 16b and 16c

Column	6.1007.000 Nucleosil 5SA - 125/4.0
Eluent	a) 4 mmol/L tartaric acid, 0.5 mmol/L citric acid, 3 mmol/L etylenediamine, 5% Acetone Conductivity approx. 500 µS/cmb) b) 3.5 mmol/L oxalic acid, 5% acetone, pH = 4 (approx. 120 µL ethylenediamine), Conductivity approx. 350 µS/cm
Sample	Tap water
Flow	1.5 mL/min
Pressure	13 MPa
Analysis time	a)12 min b)13 min
Loop	100 µL
Polarity	–

Experiment 16b – Determining transition metals with an eluent containing tartaric acid, citric acid, ethylenediamine and acetone (eluent a)

Eluent preparation

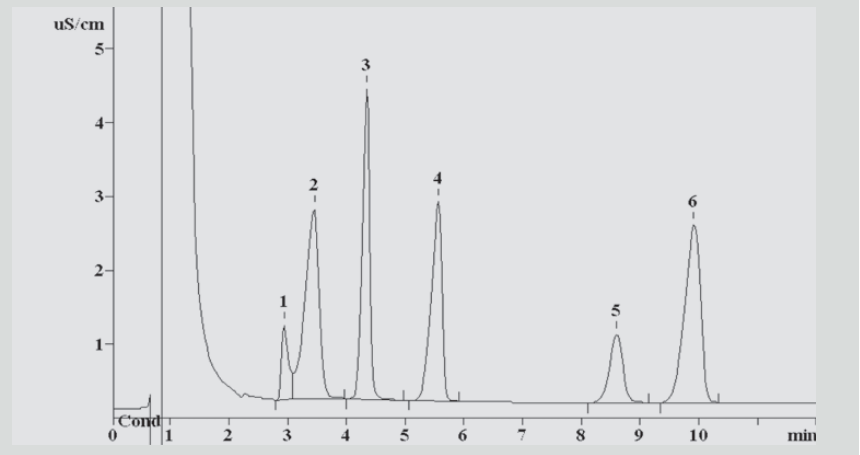
Dissolve 600 mg tartaric acid and 105 mg citric acid in ultrapure water. Add 200 µL ethylenediamine solution (3 mmol/L) and 50 mL acetone and make up to 1 L with ultrapure water.



Chromatogram 41 Standard solution 1 for determining the transition metals

Table 61 Components – Experiment 16b – Standard 1

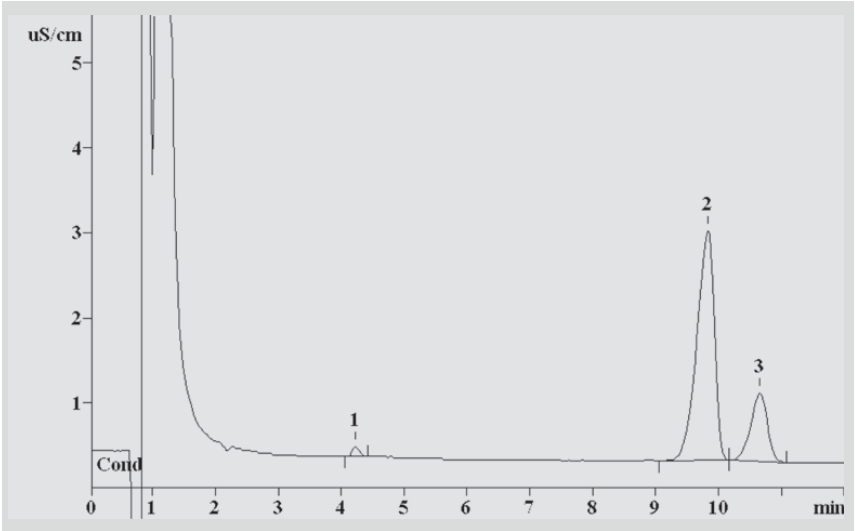
Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
1	Nickel	3.4	5	1020	40
2	Zinc	4.4	5	5640	36
3	Cobalt	5.6	5	4370	34
4	Iron(II)	8.1	10	6150	30
5	Calcium	9.8	5	6340	29
6	Magnesium	10.8	5	6390	39



Chromatogram 42 Standard solution 2 for determining the transition metals

Table 62 Components – Experiment 16b – Standard

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
1	Lead	2.9	5	2680	8.6
2	Nickel	3.4	5	930	43
3	Zinc	4.3	5	5510	37
4	Cobalt	5.6	5	4310	34
5	Cadmium	8.6	5	6600	15
6	Manganese	9.9	10	6270	45



Chromatogram 43 Analysis of tap water (dilution 1:10)

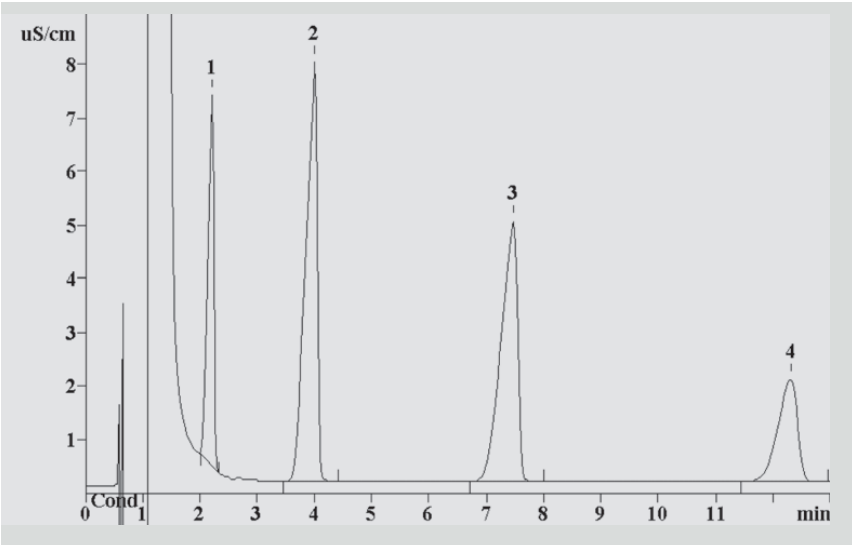
Table 63 Components – Experiment 16b – Tap water

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
1	Zinc	4.2	1.2	6360	0.9
2	Calcium	9.8	89	5750	53
3	Magnesium	10.6	19	7460	15

Experiment 16c – Determining transition metals with an eluent containing oxalic acid, ethylenediamine and acetone (eluent c)

Eluent preparation

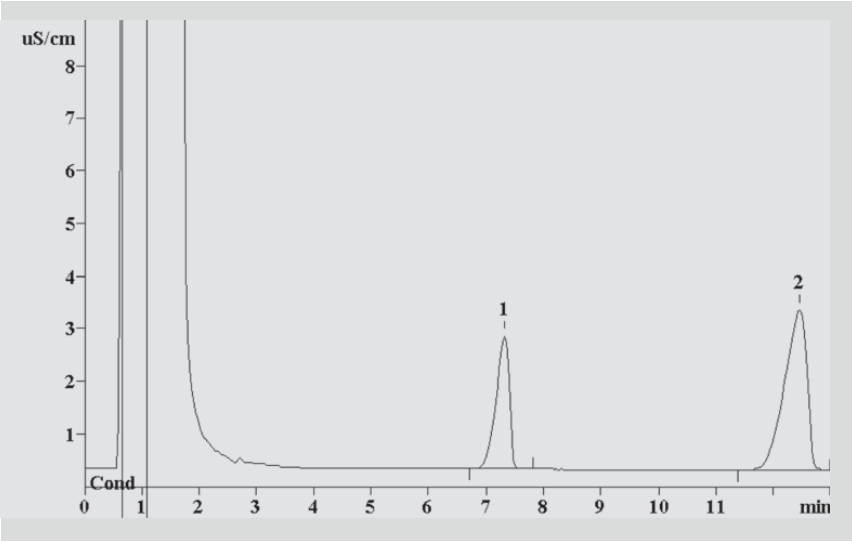
Dissolve 315 mg oxalic acid in ultrapure water, add 50 mL acetone and make up to 1 L with ultrapure water. Adjust to pH = 4 with ethylenediamine solution (3 mmol/L, approx. 120 µL).



Chromatogram 44 Standard solution for determining the transition metals

Table 64 Components – Experiment 16c – Standard

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
1	Iron	2.2	10	2110	50
2	Manganese	4.0	10	1830	110
3	Magnesium	7.5	5	3110	98
4	Calcium	12.3	5	6000	45



Chromatogram 45 Analysis of tap water (dilution 1:10)

Table 65 Components – Experiment 16c – Tap water

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
1	Magnesium	7.3	21	4690	41
2	Calcium	12.4	91	4780	83

Remarks
Calcium and manganese coelute if eluent a is used. With eluent b calcium and manganese are separated; nickel, zinc and cobalt are strongly complexed and elute in the front peak.

4.4.3 Experiment 17 – Contaminants in silica gel – determination of calcium and magnesium ions

Silica gel is produced, for example, by the hydrolysis of sodium silicate and the almost complete removal of water; it has the following structure:

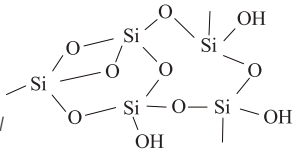


Figure 34 Structure of silica gel

Apart from its use as a technical adsorption agent, silica gel is used in gas chromatography and high-pressure liquid chromatography.

In **gas chromatography** originally only packed columns were used – they still are used to a small extent today. A liquid phase, e.g. silicone oil, is applied to a particulate carrier such as silica gel. The number of theoretical plates of such columns is low compared with those of the capillary columns that are mainly used today.

In **high-pressure liquid chromatography (HPLC)** silica gel is used as a stationary phase or carrier material for adsorption, reversed phase (RP), normal phase and ion chromatography. For some of these applications the silica gel surfaces have to be modified with functional groups (e.g. octadecyl or ion exchanger groups).

In **adsorption chromatography** silica gel is also used as a stationary phase or in addition to Al_2O_3. The chromatographic separation is based on dipole-induced dipole linkage, dipole-dipole linkage, hydrogen bridge linkage and π-complex linkage. Adsorption chromatography is used for the separation of nonpolar substances which are difficult to dissolve in water.

In **RP chromatography** the free silanol groups of the silica gel are hydrophobicized by the chemical bonding of longer alkyl chains (e.g. octyl or octadecyl groups)

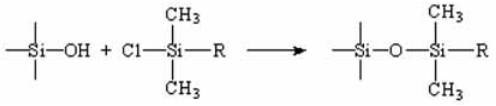


Figure 35 Reaction of silica gel with chlorosilane (R = C_8H_{17} to $C_{18}H_{37}$)

In this conversion free residual silanol groups remain; these can adsorb polar compounds and cause peak tailing. These residual silanol groups can be deactivated by trimethylchlorosilane – «end capping».

In **normal phase chromatography** the polar residues are chemically bonded to the silanol groups, e.g.:

$$\text{—}(CH_2)_n\text{—}C{\equiv}N \quad \text{or} \quad \text{—}(CH_2)_n\text{—}NH_2$$

A less polar or nonpolar mobile phase is used with this polar stationary phase for chromatographic separation. In RP chromatography, which is used in approx. 75% of all HPLC applications, the polarities are reversed and a nonpolar stationary phase is used with a polar mobile phase. This means that the name reversed phase has a purely historical basis as this method was only developed later.

In **ion chromatography** silica gel to which sulfonic acid groups have been bonded is used as a cation exchanger for the analysis of alkali ions.

Contamination of silica gel by metal ions is important in chromatography as these can cause non-specific interactions or blank values. A strongly acidic cation exchanger is used for the determination of these contaminants; monovalent cations elute in the dead volume and only the divalent cations are separated. Fe(III) must be reduced to Fe(II) with ascorbic acid prior to the analysis.

Learning content

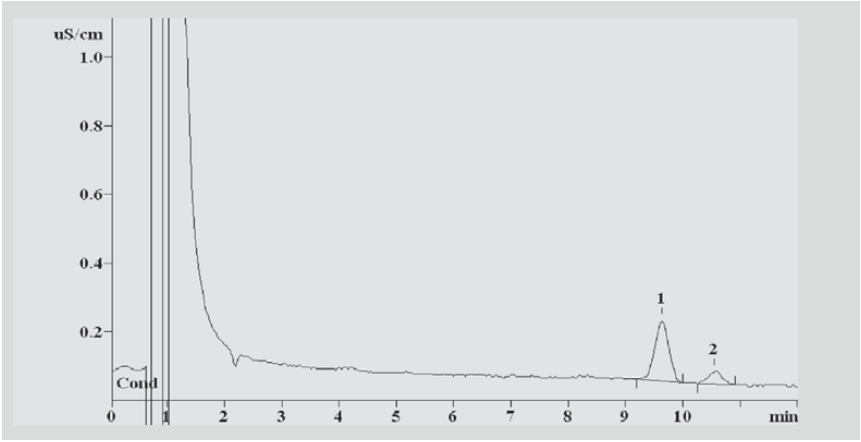
- Quality control
- Additional experiment: spiking the extract with Fe(III); determination with and without the addition of ascorbic acid (vitamin C)
- HPLC separating columns based on silica gel

Table 66 Parameters – Experiment 17

Column	6.1007.000 Nucleosil 5SA - 125/4.0
Eluent	4 mmol/L tartaric acid, 0.5 mmol/L citric acid, 3 mmol/L ethylenediamine, 5% acetone Conductivity approx. 500 µS/cm
Sample	Silica gel (5% solution)
Pressure	13 MPa
Analysis time	12 min
Loop	100 µL
Polarity	–

Eluent preparation

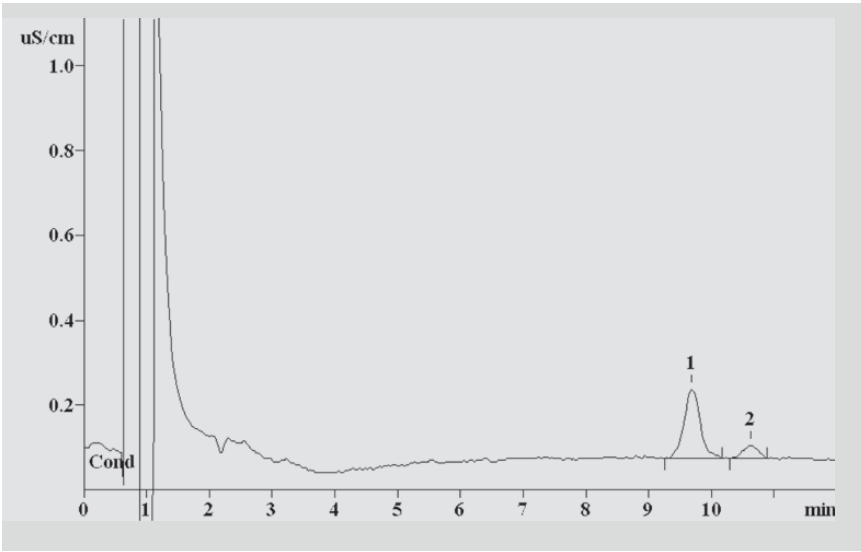
Dissolve 600 mg tartaric acid and 105 mg citric acid in ultrapure water. Add 200 µL ethylenediamine solution (3 mmol/L) and 50 mL acetone and make up to 1 L with ultrapure water.



Chromatogram 46 Standard solution for the determination of cations in silica gel

Table 67 Components – Experiment 17 – Standard

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
1	Calcium	9.6	0.5	6990	3.1
2	Magnesium	10.5	0.1	7260	0.7



Chromatogram 47 Determination of cations in silica gel

Table 68 Components – Experiment 17 – Silica gel

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*s]
	Iron	8.1	n.d.	–	–
1	Calcium	9.7	9.4	6850	2.9
2	Magnesium	10.6	1.4	10100	0.4

4.4.4 Experiment 18 – Cosmetics and corrosion protection: determination of ethanolamines and alkali metals

The following three ethanolamines, whose systematic names are aminoethanol, iminodiethanol and nitrilotriethanol, are made by reacting ammonia and ethylene oxide.

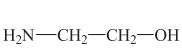


Figure 36
Monoethanolamine

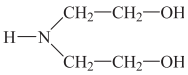


Figure 37
Diethanolamie

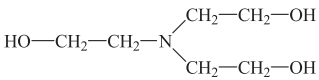


Figure 38
Triethanolamine

All three substances are used for removing CO_2 and H_2S from mixtures of gases, e.g. for removing carbon dioxide from synthesizer gas and in the synthesis of ammonia as well as for the removal of hydrogen sulfide from refinery gases.

$$NR_3 + H_2O + CO_2 \underset{T}{\rightleftharpoons} HNR_3^+ + HCO_3^-$$

At a higher temperature CO_2 is again released and the ethanolamine NR_3 is returned to the absorption process.

$$2NR_3 + H_2S \underset{T}{\rightleftharpoons} (HNR_3)_2S$$

The H_2S released again at the higher temperature is oxidized to liquid sulfur in «Claus units»; the sulfur is then converted to sulfuric acid via SO_2 and SO_3.

Monoethanolamine (MAK = maximum allowable workplace concentration = 3 ppm) is used in surfactants as a fatty acid ester. Diethanolamine is used in furniture and floor care products, shoe polish and lubricants.

Triethanolamine forms triethanolamine soaps with fatty acids, e.g. with stearic acid $C_{17}H_{35}COOH$. These are easily soluble in water and mineral oils and are therefore used as emulsifiers. They may also be contained in cosmetic preparations (e.g. shaving foam).

Monoethanolamine is used as corrosion inhibitor in power stations as it maintains a weakly alkaline pH and scavenges excess H^+ ions.

For their determination by ion chromatography the ethanolamines are protonated by the addition of acid and can then be determined as their ammonium derivatives.

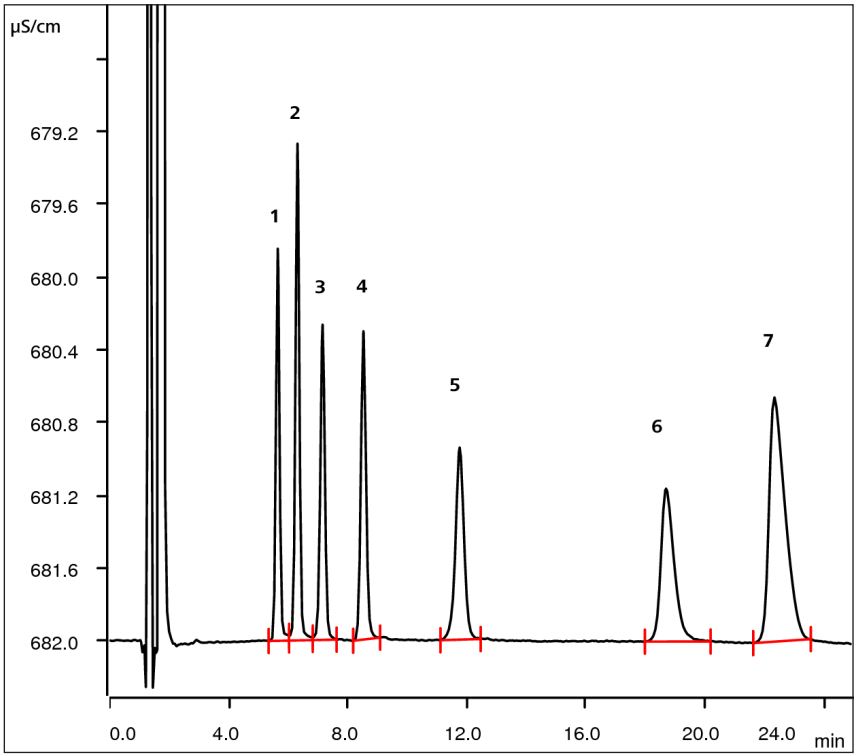
- Determining inorganic and organic cations
- Changing the selectivity by adding complexing agents
- Determination of amines by ion chromatography

Table 69 Parameters – Experiment 18

Column	6.1050.420 Metrosep C 4 - 150/4.0
Eluent	1.7 mmol/L nitric acid / 0.7 mmol/L dipicolinic acid
Sample	Standard (monoethanolamine, triethanolamine + standard cations)
Flow	0.9 mL/min
Pressure	6.2 MPa
Analysis time	25 min
Loop	10 µL
Polarity	-

Eluent preparation

Dissolve 1.7 mL nitric acid (c = 1 mol/L) and 117 mg dipicolinic acid in 100 mL ultrapure water under heating and then make up to 1 L with ultrapure water.



Chromatogram 48 Standard solution – Standard cations with monoethanolamine and triethanolamine

Table 70 Components – Experiment 18 – Standard cations with monoethanolamine and triethanolamine

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	Sodium	5.7	2	10309	0.309
2	Ammonium	6.3	2	11296	0.420
3	Monoethanolamine	7.2	7	10434	0.313
4	Potassium	8.5	5	11577	0.344
5	Triethanolamine	11.8	20	8408	0.355
6	Calcium	18.7	5	8949	0.437
7	Magnesium	22.3	5	7644	0.843

4.4.5 Experiment 19 – Alkali and alkaline earth metals in wine

The mineral substances in the grape juice (grape must) are chiefly phosphates of calcium, magnesium and potassium. The concentrations of these compounds are 3 to 5 g/L. The mineral content, also known as «ash» of the wine, is lower than that of the juice as a part of the minerals is taken up by the yeasts in their metabolism. The mineral content is also reduced by the separation of tartrates. This means that wine contains only about 1.8 to 2.5 g/L «ash». Important cationic constituents are given as oxides; these are K_2O (approx. 40%), MgO (approx. 6%), CaO (approx. 4%) and Na_2O (approx. 2%).

See chapter 4.3.5 – Experiment 11 – Organic acids in wine.

Learning content
- Analysis of foodstuffs
- Process analysis – checking tartrate precipitation

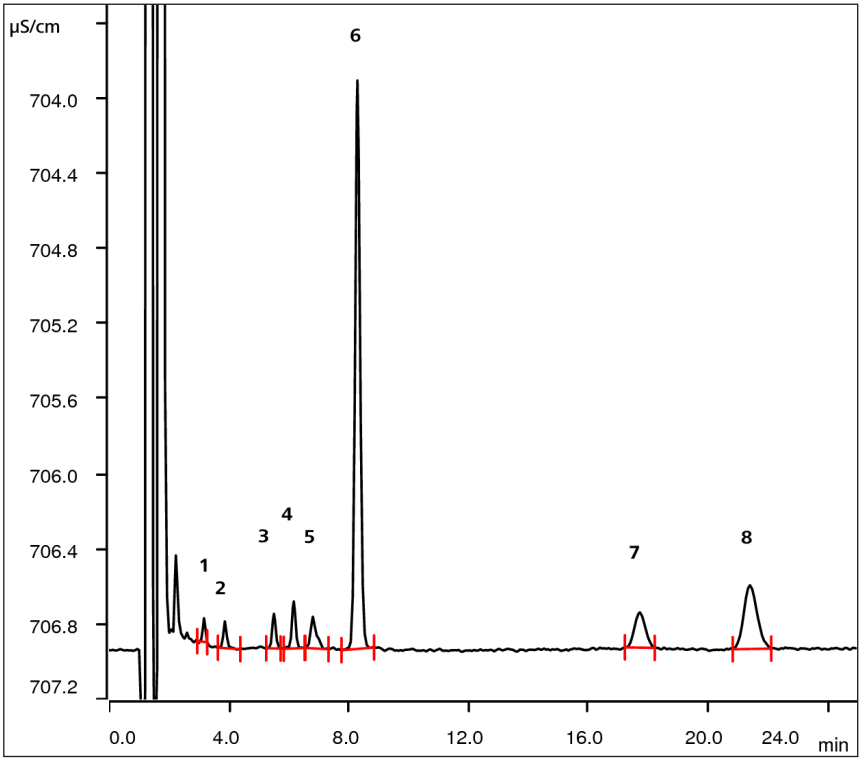
Table 71 Parameters – Experiment 19

Column	6.1050.420 Metrosep C 4 - 150/4.0
Eluent	1.7 mmol/L nitric acid / 0.7 mmol/L dipicolinic acid
Sample	Standard (monoethanolamine, triethanolamine + standard cations)
Flow	0.9 mL/min
Pressure	6.2 MPa
Analysis time	25 min
Loop	10 µL
Polarity	-

Eluent preparation

Dissolve 1.7 mL nitric acid (c = 1 mol/L) and 117 mg dipicolinic acid in 100 mL ultrapure water under heating and then make up to 1 L with ultrapure water.

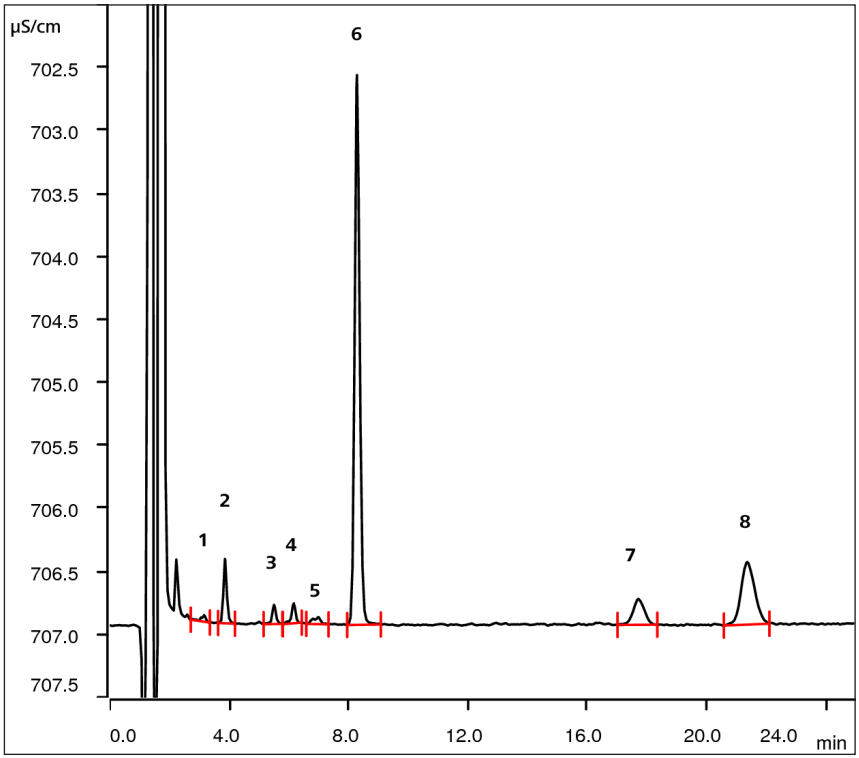
Sample preparation
Filter and dilute the wine sample (1:10).



Chromatogram 49 Determination of cations in white wine (dilution 1:10)

Table 72 Components – Experiment 19 – White wine

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	unknown	3.2			
2	unknown	3.9			
3	Sodium	5.5	15.2	10407	0.025
4	unknown	6.2			
5	unknown	6.8			
6	Potassium	8.3	930.7	11801	0.586
7	Calcium	17.7	64.3	11114	0.078
8	Magnesium	21.4	80.4	11381	0.177



Chromatogram 50 Determination of cations in red wine (dilution 1:10)

Table 73 Components – Experiment 19 – Red wine

Peak	Component	t_R [min]	Conc. [mg/L]	No. of plates	Area [µS/cm*min]
1	unknown	3.2			
2	unknown	3.9			
3	Sodium	5.5	13.4	10088	0.23
4	unknown	6.2			
5	unknown	7.0			
6	Potassium	8.3	1351	11590	0.853
7	Calcium	17.7	75.9	10363	0.091
8	Magnesium	21.4	121.2	10418	0.266

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