Monograph

Advanced Detection Techniques in Ion Chromatography

Metrohm

Advanced detection techniques in ion chromatography

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Preface

Ion chromatography is a determination method used in ion analysis that has undergone great commercial expansion in the last thirty years and has also undergone much further development. Numerous reports have been made, particularly about innovations in separation columns, suppressors as well as instruments and application techniques. In addition, the most important routine methods have been incorporated in standards: anion determinations in the ISO 10304-series and ISO 15061, cations in ISO 14911 and complexing agents in DIN 38413-8.

In a similar way to the development in the classical (reversed phase) HPLC, coupling methods for a more selective and more sensitive detection are playing an ever greater role. Above all, LC-MS and IC-MS coupling have already reached a high level of development. In the research sector both IC-ICP and IC-ICP-MS have already been used successfully for the speciation of heavy metals and their complexes. The classical ion chromatography detection techniques (conductivity, UV/VIS, amperometry) have also been further developed, which has resulted in a further increase in the performance of IC as an analytical method for ion analysis.

On 14 and 15 June 2005, the Metrohm seminar «Advanced detection techniques in ion chromatography» was held in Filderstadt.

After the introduction, the presentation of the speakers and participants, as well as the presentation of the Metrohm group of companies (Jochen Schäfer), an overview of the detection techniques available for ion chromatography was given first (Seubert). After this the advantages and disadvantages of IC-MS coupling, which is currently of particular importance, were described (Wille).

New developments in the field of IC-ICP-MS (Seubert) and IC with post-column derivatization (Frenzel) were presented in a clear and up-to-date manner.

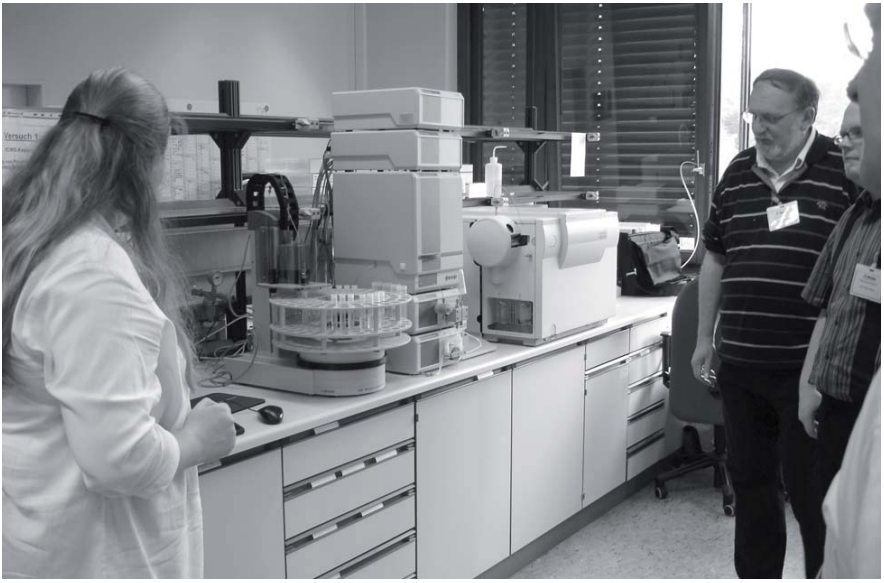
The fact that conductivity detection using CO_2 suppression downstream from the chemical suppression has achieved new dimensions in performance, was presented in an impressive manner (Helwig Schäfer).

Dr. A. Wille, Metrohm AG Herisau; Prof. Dr. A. Seubert, University of Marburg;
Dr. H. Schäfer, Metrohm AG Herisau; Priv.-Doz. Dr. W. Frenzel, TU Berlin (from left to right).

The (pulsed) amperometric detection method should also not be forgotten, not only for the detection of sugars and carbohydrates, but also for amino acids and alcohols.

On both afternoons very lively discussions took place in the IC application lab of Deutsche Metrohm in Filderstadt. All the detection techniques presented (with the exception of ICP) were demonstrated in practice using real problems. The expert round was also very well received, in which each participant was able to discuss particular problems with the speakers.

The fact that the dialog between the participants and the speakers and between one another is an important component of such seminars was again demonstrated in a pleasing manner.



Practical work in the IC application lab (IC-MS coupling).

Discussion with the speakers.

The questionnaire filled in by all the participants confirmed the high level of the seminar with regard to its content and organization. This has persuaded us to publish a revised version of this seminar as a monograph.

Dear Readers, we hope that you will enjoy reading this monograph and find many stimulating ideas for your practical work.

Filderstadt, July 2005

A – Theory, principles and applications of advanced detection techniques in ion chromatography

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1 Introduction

Analytical techniques such as chromatography that are based on separations are – without a further technique used for detection – not capable of use as complete, quantifying analytical methods. The choice of the detection principle is determined by the requirements of the selected separation principle and the properties of the analytes or the matrix. In ion chromatography the answer to the detector question is usually «conductivity detection».

Detection techniques that differ from conductivity detection will be briefly described under the term «advanced detection techniques» and compared with one another. The spectrum ranges from UV/VIS detection through electrochemical detection principles right up to modern atomic spectrometry and mass spectrometry detectors.

1.1 Resolution and information content

For ion chromatography there is no detector that can fulfill any imaginable requirement profile. Each detection principle has its own specific strengths and weaknesses. It can be characterized by the sensitivity as a measure for the lower working range limit, the linearity range as a measure of the dynamic range of the method as well as by the terms resolution and information content. What applies for our eyes when, for example, we look at pictures with different resolutions and information depths (black/white, gray levels, color) is also found in chromatography detectors. Typical «gray level detectors» are conductivity or photometric detectors, which provide the alteration in the measured quantity as information (uni-dimensional detector). «Color detectors» in the sense of ion chromatography are multi-dimensional detectors such as mass spectrometers, diode-array detectors or simultaneously working atomic spectrometry detectors. According to the requirement profile the most suitable detector for the particular problem will be chosen.

1.2 Necessary conditions for ion chromatography detection

For a particular detector to provide a universal solution for a particular type of chromatography depends either on the working principle of the type of chromatography, or on the properties of the analytes, or on both to a similar extent. Taking gas chromatography (GC) as an example, it becomes clear that flame ionization detector (FID) has been able to establish itself because of its outstanding sensitivity to the typical analytes encountered in GC (small, volatile, nonpolar). The eluent used in GC (N_2, H_2 or He) does not really represent a problem for any of the detectors used in GC. When considering liquid chromatography the necessary conditions must be discussed in greater detail. On the one hand the range of analytes and eluent compositions is much greater. On the other hand a great number of different retention modes can be activated (adsorption, distribution, reversed-phase, ion pair formation, ion exchange, etc.). This explains why the photometric detector is far less capable of universal use than FID in GC.

Ion exchange as a stoichiometrically occurring chemical reaction (Figure A1, right) is again a truly special case, because really only an alteration of the exchange equilibrium by changing the eluent anion concentration promises a successful elution of the analytes. This is made clear in the following section by using a simple retention model for ion chromatography. In the two other variants the interaction of the analytes with the stationary phase that is necessary for retention, adsorption (Figure A1, middle) and distribution (Figure A1, left), the particular adsorption and distribution isotherms can be varied by altering the solvent mixture or the temperature. This is made clear in the following section by using a simple retention model for ion chromatography.

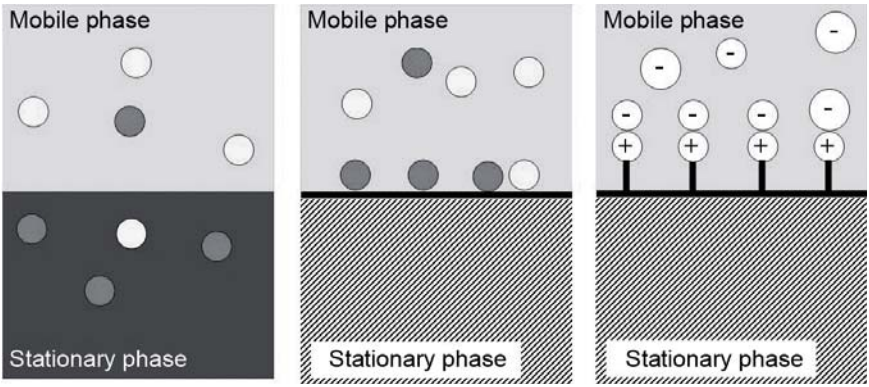

Figure A1: *Distribution, adsorption and ion exchange chromatography (from left to right) – differences in the interaction of the analytes with the mobile and stationary phase.*

1.2.1 A simple retention model for anion chromatography

Under the assumption of electroneutrality the simplest approach for a retention model is isoionic displacement, in which only a single eluent anion E^{y-} competes with an analyte anion A^{x-} for the functional groups of the stationary phase [A1]. The concentration of the eluent anion E^{y-} should be constant with time (isocratic elution). The exchange places on the separation column with capacity Q are occupied by the eluent anions E^{y-} at the start of the chromatographic process. If a sample with the analyte anion A^{x-} is added then the following equilibrium becomes established between the stationary phase (index S) and mobile phase (index M):

$$y\ A_M^{x-} + x\ E_S^{y-} \rightleftharpoons y\ A_S^{x-} + x\ E_M^{y-} \qquad (1)$$

According to the law of mass action the equilibrium can be described by a thermodynamic equilibrium constant. If the concentration of the analyte anion A^{x-} in the stationary and mobile phases is now replaced by the distribution coefficient D^A and retention factor k'^A:

$$D_A = \frac{[A]_S}{[A]_M} \qquad \text{with } k'_A = D_A \cdot \frac{V_S}{V_M} \qquad (2)$$

then the following Equation 3 can be derived from the law of mass action:

$$K_{A,E} = \left(k'_A \cdot \frac{V_M}{V_S} \right)^y \left(\frac{[E_M^{y-}]}{[E_S^{y-}]} \right)^x \qquad (3)$$

As the concentration of the eluent ion E is normally greater than that of the analyte anion A^{x-} by several powers of ten, then in a good approximation it can be assumed 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 parameter exchange capacity Q (i.e. the number of functional groups per column or per gram of anion exchanger) and charge of the eluent anion y:

$$[E_S^{y-}] = \frac{Q}{y} \qquad (4)$$

After inserting Equation 4 in Equation 3 and solving for k', the straight line Equation 5 is obtained in logarithmic form.

$$\log k'_A = \frac{1}{y}\log K_{A,E} + \frac{x}{y}\log\frac{Q}{y} + \log\Phi - \frac{x}{y}\log[E_M^{y-}] \quad \text{with} \quad \Phi = \frac{V_S}{V_M} \qquad (5)$$

The following consequences result from Equation 5:

- The retention of an analyte can only be reduced by increasing the eluent concentration [E^{y-}], reducing the exchange capacity Q or by a smaller selectivity coefficient $K_{A,E}$.
- Multivalent analytes A^{nx-} are more strongly retained 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 greater elution force than monovalent eluents E^{y-}
- The elution of multivalent analytes A^{nx-} is more strongly influenced by increased concentrations of monovalent eluent ions E^{y-} than monovalent analytes A^{x-}.

These consequences are synonymous with the crucial development problem in ion chromatography:

- Ions can only be eluted again from a permanent ionic, stationary phase by the use of ions as the eluent.

This means that the non-selective «conductivity» detector has the problem of recognizing the few analyte anions in a high concentration of eluent anions. One solution to this dilemma is the use of derivatization (section 2.1).

1.2.2 Concentration and mass-flow-dependent detectors

Chromatography detectors can be roughly divided into two groups. In one of these groups the signal is directly proportional to the concentration of the analyte, in the other group it is proportional to the mass flow. The difference is simple. If the detector signal falls to zero when the eluent flow is stopped then it is a mass-flow-dependent detector. The most common detectors used in ion chromatography (conductivity detector, photometric detector) are concentration-dependent detectors. On the other hand, some of the «advanced» detectors are mass-flow-dependent detectors. Whenever the detector includes a nebulizer system it is a mass-flow-dependent detector. This difference in the characteristics of the detectors is of importance whenever the optimization of the sensitivity of the application is concerned. The mass flow and the concentration of the analytes can be optimized in different ways. The constancy of the flow rate also has a varying influence on the correctness of the results. In this case a mass-flow-dependent detector tolerates any flow rate irregularities better than a concentration-dependent detector.

1.3 «Less» advanced detection possibilities in ion chromatography – «what's the problem?»

For 95 to 99% of all ion chromatography applications conductivity detection is certainly the most suitable type of detection. However, it reaches its limits when faced with extreme demands such as the determination of element species or extreme trace analysis in difficult matrices. The same applies when no suitable eluent is available for conductivity detection with and without chemical suppression, i.e. when working at low pH levels or similar. Figure A2 makes clear the close link-up between eluent, separation column and detector. Only when the intercept between all three sections is sufficiently large will the application be workable and sensible. An unsuitable detector can ruin an ion chromatography separation just as easily as an unsuitable column or an unsuitable eluent.

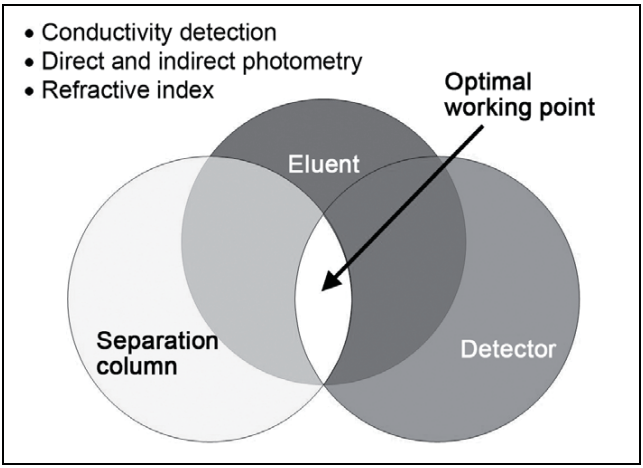

Figure A2: *Visualization of the close relationship between separation column, eluent and detector. All components must be exactly matched to one another in order to achieve the best possible results.*

The selectivity of the conductivity detection usually is the actual limitation in classical ion chromatography. As the conductivity represents a cumulative parameter of the ions contained in the solution, it is hardly possible to make any discrimination between the types of ion. The differences in the equivalent conductivities of the sample components (including matrix) are usually much too small to be exploited analytically. In this case other differing chemical-physical properties of the analyte and interferent are utilized. These could, for example, differ in their chemical reactivity, which is made use of in derivatization. The utilization of electrochemical peculiarities or differences in molecular or atomic weight are also exploited.

2 Advanced detection techniques

The choice of a suitable advanced detection technique must depend on the particular conditions regarding the analysis and the laboratory. There are different ways of reaching the target for practically every problem. The complexity of the solution, the necessary investments and the benefits of advanced detection must stand in a reasonable relationship to one another.

2.1 Derivatization

A common extension to an ion chromatograph is the use of a derivatization module. The diagram of an ion chromatograph shown in Figure A3 practically always includes a derivatization module. The most frequently used type is the suppressor for the chemical reduction of the eluent conductivity. With similar equipment it is also possible to implement derivatization for photometric or electrochemical detection. The demands placed on the derivatization module can be adapted from the flow injection analysis sector. Crucial quantities are the degree of mixing, the resulting signal dispersion and the residence time in the reactor (see Chapter E).

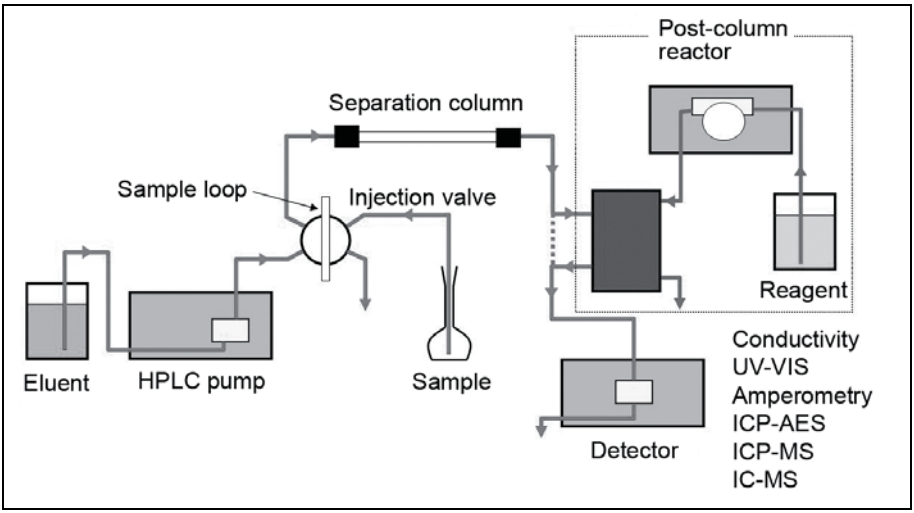

Figure A3: *Schematic diagram showing an ion chromatograph with built-in post-column reactor. Derivatization is not limited to the suppression of the eluent background conductivity but can also include complex conversions.*

2.1.1 Suppression

The successful use of ion chromatography for the determination of anions is closely linked with the solution of the background conductivity problem (eluent background conductivity). In chemical suppression of the background conductivity, it is possible to choose between column-based and membrane-based suppressors. The latter work continuously and, with regard to their running time, have a higher suppression capacity. Although with the former the suppression is more complete, they have only a limited suppression capacity and therefore need to be regenerated at regular intervals.

As well as chemical suppression, eluents with a lower equivalent conductivity can also be used. In this case a different design of column and eluent is required. The sensitivity of the method is also reduced by an approximate factor of ten.

For the majority of applications, suppression or a particular type of suppression is not even absolutely necessary, as it is rather the robustness and running time of the analysis that are the decisive criteria [A2].

2.1.2 Post-column derivatization for the determination of cations and anions

The photometric reaction systems commonly used today for the continuous detection of multivalent ions have their origins in the early work carried out on the separation of mixtures of substances using ion exchange. The individual fractions of these still non-continuously detected separations were treated with a color reagent solution which, in addition to the color reagent itself, also contained a suitable buffer system. The concentration of the analyte was then determined photometrically.

First studies into the application of this detection principle to continuous systems were carried out in 1971. In addition to apparatus parameters such as mixing chamber, reaction conditions and dwell time of the analytes in the detection system, the suitability of different color reagents was also investigated. Among others, PAR (4-(2-pyridylazo)-resorcinol) proved to be a universal reagent with a total of 40 detectable cations; it is shown in Figure A4 (below). Unfortunately these 40 cations cannot be

determined under identical reaction conditions. An interesting extension is the addition of ZnEDTA for complex conversion of several metal ions, which can then also be determined with PAR. As the product of this complex conversion is always free Zn^{2+}, the reaction conditions are now practically identical for numerous metal cations. Figure A5 shows the chromatogram of the separation of 12 cations using a sulfonic acid exchanger together with a complexing eluent and photometric detection after post-column derivatization with ZnEDTA-PAR [A3]. A further important color reagent is arsenazo III, which is used for the determination of rare earth elements and some actinides.

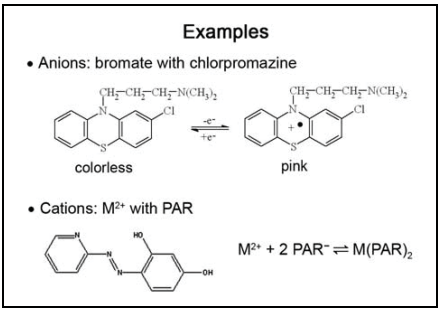

Figure A4: *Conversion of chlorpromazine with bromate (top) and 4-(2-pyridylazo-)resorcinol (PAR) with metal cations (below).*

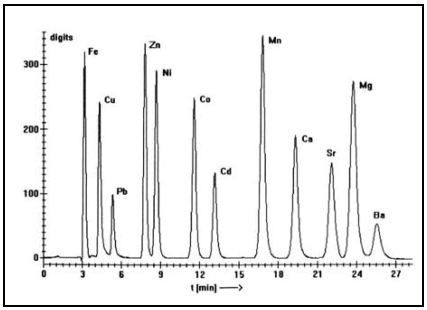

Figure A5: *Separation of 12 cations on a sulfoacylated exchanger using a tartaric acid eluent and photometric detection after post-column derivatization with ZnEDTA-PAR.*

In the field of anion analysis isolated solutions with reaction systems for a single anion or a few anions are usually encountered. An approach with the ability for multi-anion determination is represented by the derivatization of the eluate with $Fe(NO_3)_3$ under acidic conditions. With many anions this produces a shift of the absorption maximum to longer wavelengths.

Special conversions such as the color reagents for bromate analysis require specific features of the analyte. For bromate its oxidizing power at low pH values is used as a discriminating property. The following reaction, which is indicated photometrically, can again be very different. An example is shown in Figure A4 (top) for the conversion of bromate with chlorpromazine, in which a pink-colored radical cation is formed as the oxidation product of the chlorpromazine. This can be detected photometrically with great sensitivity. The practical application is shown Figures A6 to A8, in which both the apparatus used (Figure A6) and examples of chromatograms for the corresponding conductivity (Figure A7) and UV/VIS detection (Figure A8) are shown. The conductivity and UV/VIS detection can simply be carried out in series. In this way both high and low concentrations of the anions can be determined in a single run [A4, A5].

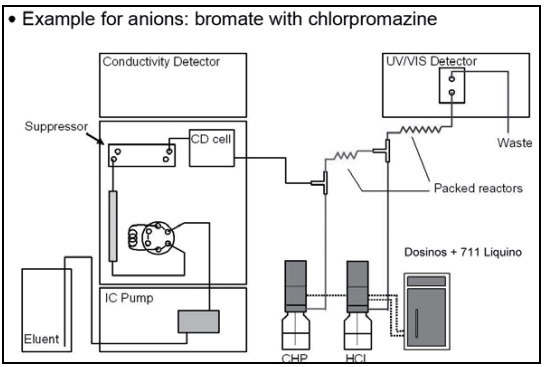

Figure A6: *Ion chromatograph for the simultaneous determination of the main components by conductivity detection and traces of bromate by photometry and post-column derivatization with chlorpromazine (CHP). Derivatization must take place in two steps, which means that intelligent dosing systems (Dosino/Liquino) are ideally suitable for reagent addition.*

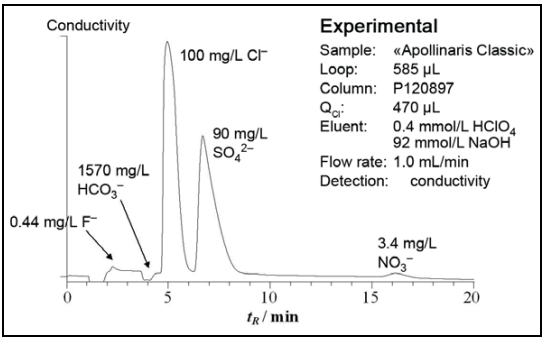

Figure A7: *Conductivity chromatogram of a mineral water, recorded with the apparatus shown in Figure A6.*

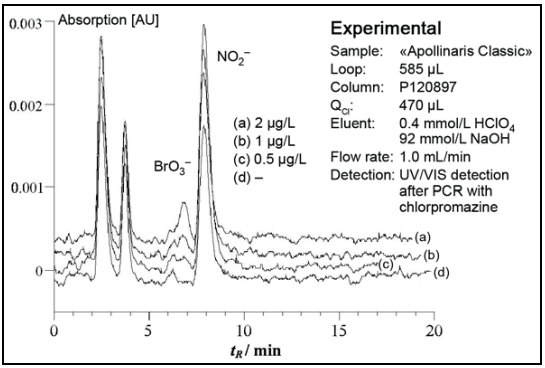

Figure A8: *UV/VIS chromatogram of the sample of mineral water shown in Figure A7 after derivatization with chlorpromazine. Curve (d) shows the original sample, curves (a) to (c) were obtained by adding amounts of bromate corresponding to 2 µg/L, 1 µg/L and 0.5 µg/L to the original sample.*

2.2 Electrochemistry

Advanced detection techniques based on electrochemical conversions normally face problems in their practical use. The classical amperometric detector is unbeatably selective and sensitive for a number of analytes. However, in daily use it only has a sufficiently long working life for certain classes of samples.

Detectors based on potentiometric sensors have not established themselves under rough practical conditions. On the other hand, the pulsed amperometric detector is extremely robust and practically without competition. Within a short time it has become established for the determination of oxidizable organic substances.

2.2.1 Pulsed amperometric detection

In the classical amperometric detector a potential that is constant with time is applied between the working electrode and a reference electrode. If an electrochemically active analyte (whose half-wave potential is such that either oxidation or reduction takes place under the applied potential) now reaches the electrode surface, then a current flows which represents the measuring signal.

In the field of ion analysis it is possible to determine, as listed in Table A1, not only some cations (Fe^{3+}, Co^{2+}), but above all anions such as nitrite, nitrate, thiosulfate as well as halides and pseudohalides.

Table A1: Uses of amperometric detection in ion chromatography

Anions	Cations
$H_2AsO_3^-$	Transition metals
N_3^- / CN^- / SCN^-	Metal chelates
NO_2^- / NO_3^-	
Br^- / BrO_3^-	
Cl^- / ClO^- / ClO_2^- / ClO_3^-	
I^- / IO_3^-	
$C_2O_4^{2-}$	
S^{2-} / SO_3^{2-} / $S_2O_x^{2-}$	
Sugars	
O_2 / OH^- / O^{2-}	

However, the most important applications are in the analyses of sugars using anion chromatography and in clinical analyses. A disadvantage is that it is rarely possible to determine more than one or two classes of analytes under the same conditions (electrode, eluent, potential). In the case of the oxidation of sugars the eluent is even so crucial that alterations in the eluent pH value toward lower pH values make post-column derivatization with NaOH necessary. Except in its main fields of use, the amperometric detector is often used as an additional detector that can detect individual analytes selectively and sensitively.

Although the turnover in amperometry is only about 10%, the method is very sensitive. At the same time the turnover is the basis of the main problem with amperometry; this is the poisoning of the electrode surface. To avoid this, the potential applied to the electrodes is not constant, but varies with time. A typical potential curve used in the analysis of sugars is shown in Figure A9 [A6].

Pulsed amperometric detection (PAD) normally makes use of three different potentials (Figure A9). The first potential step is selected so that the analyte can be oxidized. This deposits it on the surface

(A1 and A2). The second potential is selected to be more positive than the first so that the electrode surface is completely oxidized, which causes desorption of the analyte (B). A negative potential is then applied in order to return the electrode surface to its original condition (C). After this, the potential is again set to the start potential. The measuring time begins shortly after the application of the start potential (A2).

Amperometric detection is very suitable for the analysis of analytes with high pK_A values as because of their low dissociation these are difficult to determine by conductivity measurements. As an example, Figure A10 shows the separation of sugars on a high-capacity anion exchange column.

The amperometric measuring cell is usually constructed as a three-electrode measuring cell, consisting of a working electrode, a reference electrode and an auxiliary electrode. Noble metals such as gold, nickel, copper or platinum are usually used for the working electrode. Alloys or chemically modified electrodes, in which the metals are oxidized in an organic/inorganic matrix, are also used. The electroactive species are oxidized or reduced on such electrodes.

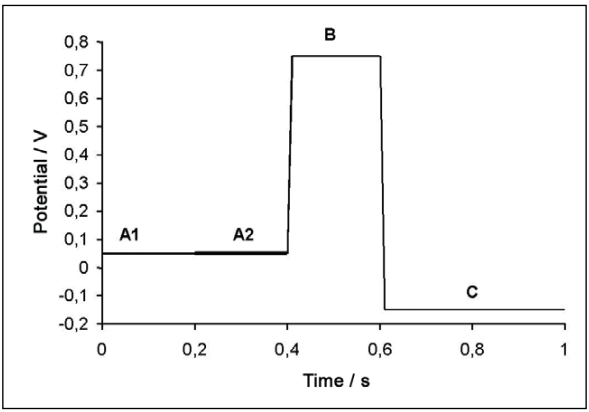

Figure A9: *Typical potential curve at the working electrode for pulsed amperometric detection.*

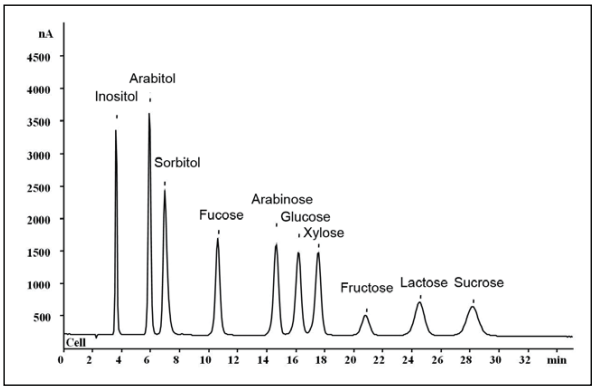

Figure A10: *Example of a chromatogram for the separation of sugars and sugar alcohols on a strongly basic anion exchanger with pulsed amperometric detection.*

2.3 Multi-dimensional detectors

The combination of ion chromatography with multi-dimensional detectors belongs to the category of the so-called hyphenated techniques, which represent the link-up of a separating system (chromatography or electrophoresis) with an independent analytical method, usually a spectrometric one. These methods have greatly increased in importance in recent years. Whereas in the field of gas chromatography couplings such as with mass spectrometry (GC-MS) are well established, coupling HPLC with spectrometric methods presents greater technical problems. For classical HPLC, i.e. the analysis of organic compounds, couplings with mass spectrometry (LC-MS), IR spectroscopy (LC-FTIR) and nuclear magnetic resonance spectroscopy (LC-NMR) are available. Particularly powerful atomic spectrometric detectors are used for ion chromatography (IC). Examples are atomic emission and mass spectrometry with inductively coupled plasma (IC-ICP-AES, IC-ICP-MS) which, because of their element specificity and sensitivity, provide excellent performance data. This is why, despite their comparatively high acquisition costs, such types of coupling are used in the fields of species analysis and the ultratrace analysis of elements.

A feature of these detectors is that they can provide several pieces of information per time segment (retention time). This can be the intensity as a function of the wavelength, as in the diode-array detector, atomic spectrometric detector (ICP-AES, MIP-AES, etc.; MIP = microwave-induced plasma) or an infrared detector.

If element mass spectrometry (ICP-MS) or molecular mass spectrometry (MS) is used for detection, then the intensity can be recorded as a function of the mass/charge ratio per time segment. It is also possible to imagine recording the intensity as a function of the electrochemical potential with a voltammetric detector or the intensity as a function of the chemical shift with an NMR spectrometer.

A further special feature of multi-dimensional detectors is the evaluation of chromatograms which also consist of several dimensions. The form of data evaluation resulting from this varies in its degree of establishment. For GC-MS or HPLC with diode-array detector the software has achieved a high degree of maturity, for chromatography-atomic spectrometry couplings the development is still in its starting phase.

2.3.1 Atomic spectrometry

Atomic spectrometric detectors are based either on atomic emission spectrometry with an inductively coupled plasma as energy source (ICP-AES), or on a mass spectrometer, which extracts and analyzes the ions of the elements present in the plasma of the ICP (ICP-MS).

ICP-AES was already discovered in the early eighties for online couplings [A7]. The variety of the applications is organized on the same pattern as the applications of the LC-ICP-MS coupling. The interest in the LC-ICP-AES coupling has strongly decreased as ICP-MS has become more widespread [A8]. This is due to the low sensitivity of the ICP-AES, which is unsatisfactory for problems such as speciation in environmental samples. The cause is the simple replacement of the HPLC detectors by ICP-AES. As a result of the nebulizer system, an ICP-AES loses sensitivity by approx. 2 orders of magnitude when compared with a photometer. The standard separation columns developed for HPLC are only designed for low sample injection volumes, which together with the unavoidable signal broadening during the chromatographic separation results in a detection which, all in all, is too insensitive.

What remains as a general advantage for ion chromatography is the element-specific detection of ICP-AES, which in the analysis of complex mixtures still allows deductions to be made about the presence and distribution of individual elements. An important precondition for the extended use of the online LC-ICP-AES coupling is the further spread of simultaneous ICP-AES instruments. Modern ICP-AES instruments based on echelle grating spectrometers are suitable detectors for online coupling. In comparison to sequential spectrometers the flood of information from such spectrometers is extremely large. Software support for the utilization of the information for transient measured value recording is available from many manufacturers.

Figure A 11 shows the chromatogram of a sample of drinking water using ICP-MS as the detector. The signals can be assigned to individual elements by using the m/z ratios. Species information (BrO_3^- or Br^-) is hidden in the retention time.

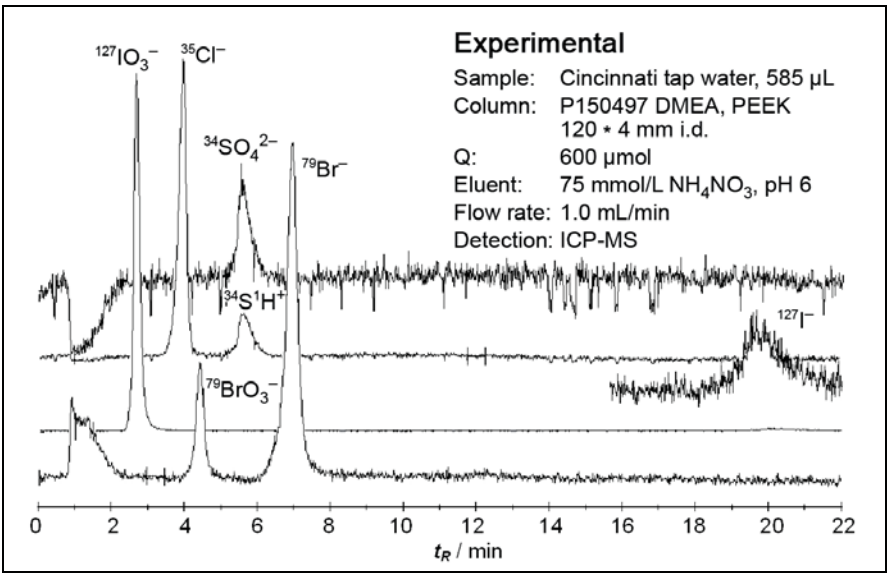

Figure A11: *IC-ICP-MS chromatogram of a water sample. Mass traces for sulfur (m/z 34), chlorine (m/z 35), bromine (m/z 79) and iodine (m/z 127) have been recorded.*

An important difference between ICP-AES and ICP-MS is the clearly higher loading capacity of ICP-AES with respect to the type and concentration of the sample matrix and eluents. A decisive difference is the direct mass transport of the analytes to the mass spectrometer of the ICP-MS, which therefore reacts much more sensitively to salt loading and high concentrations of loaded particles. In addition, a further plus point for ICP-AES in comparison with ICP-MS is the greater detection strength for elements that are subject to massive interference in ICP-MS or only provide a low ionization yield. For ICP-AES only the atomization and excitation are decisive. Examples are elements such as Be, K, Ca and Fe.

2.3.2 Molecular mass spectrometry

Molecular mass spectrometers, whose general construction is shown in Figure A12, were not compatible with liquid chromatography for a long time. The main reason for this were problems in transferring a liquid into the high vacuum of a mass spectrometer. When the liquid vaporizes spontaneously, large volumes of gas are produced which are too much for the vacuum system to handle. In addition, in a coupling with a chromatograph, only a very small fraction of the liquid to be transferred, namely the analyte, is of any interest. In gas chromatography, the differentiation between analyte and eluent takes place relatively simply by the use of momentum separators; in liquid chromatography, techniques that selectively ionize the analytes without ionizing the eluent are used. Again, for reversed phase chromatography this is considerably easier than for ion chromatography. The breakthrough for linking molecular mass spectrometers to ion chromatographs was provided by the development of electrospray ionization (ESI). By using ESI and its derivatives such as APCI (Atmos-

pheric Pressure Chemical Ionization) or ionspray, it is easily possible today to transfer ionic analytes to the mass spectrometer.

From the recorded mass spectra, information about the molecular mass and molecule substructures (fragmentation patterns) are obtained. High-resolution mass spectrometers (FT-ICR-MS) allow the determination of the empirical formula from the molecule ion peak. The frequency or intensity of individual fragments can be used for quantitative analysis.

Whereas in the meantime LC-MS coupling is well established, IC-MS is still in the starting blocks. Problems are a strictly limited choice of eluent (usually depending on chemical suppression), interference with the ion source, and extreme variations in sensitivity depending on the analyte and operating mode. On the plus side a remarkable high selectivity as a result of recording the M^+ or M^- signal must be mentioned and, under favorable conditions, a high sensitivity.

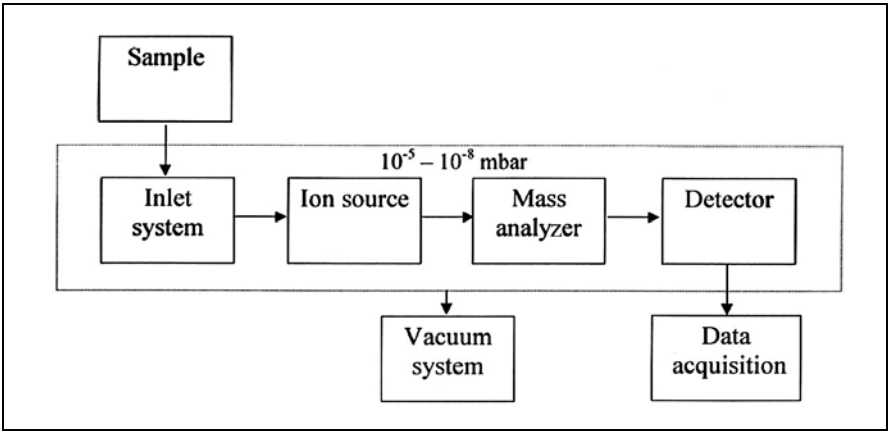

Figure A12: *Schematic of a mass spectrometer. The crucial developments for using a mass spectrometric detector in ion chromatography have concerned the sample introduction system.*

2.3.3 UV/VIS diode-array detectors

A multi-dimensional detector with a relatively limited use in ion chromatography is the diode-array detector. It permits the simultaneous recording of the absorption curve for numerous wavelengths. This information can be used, for example, for checking the peak purity. Table A2 lists the absorption maxima of some analytes. In addition, the diode-array detector is also useful in post-column derivatization with photometric detection. If the absorption maxima of the educt (photometric reagent) and the product (conversion product of the analyte) are both visible in the observation window then multi-wave detection can be used to reduce the reactor-based noise fraction. This strategy has been used with particular success for PAR-based systems.

Table A2: Possible applications for photometric detection in ion chromatography

Analyte	λ_{max}
F^-	<< 190 nm
CH_3COO^-	≤ 190 nm
Cl^-	≤ 190 nm
NO_2^-	210 nm
BrO_3^-	195 nm
Br^-	214 nm
NO_3^-	214 nm
SO_4^{2-}	≤ 190 nm
HPO_4^{2-}	≤ 190 nm
Anions after PCR	> 300 nm
Cations after PCR	> 300 nm

2.3.4 NMR, IR …

Up to now the link-up of ion chromatography with structure-resolving spectroscopic techniques such as infrared or NMR spectroscopy has not yet been realized. A reason for this is the lack of suitable applications and the lack in sensitivity of the combinations. Interesting applications for using the link-up between NMR and IC are to be found in the field of species analysis for the identification of previously unknown species. It is similar in the field of environmental chemistry, where there is still a great requirement for structure-resolving analytical methods for complex samples.

3 Conclusions

Ion exchange as a chemical reaction considerably limits the choice of eluent for non-selective detectors, as elution is only successful by isoionic displacement or, in favorable conditions (such as for cations), by complexing. Each of the different detectors limits the choice of eluent to a greater or lesser degree and the range of compromise is very narrow. The fields of application of the individual detectors in ion chromatography are shown in Table A3.

Although it is no longer apparent to most users, in ion chromatography with conductivity detection it is the detector that determines to a large extent the choice of eluent. This means that with alternative detection techniques, the whole ion chromatography application must be adapted. This includes the separation column and its dimensions, the eluent and, of course, the detector, with the choice of eluent being dominated by the limits imposed by the detector.

The «advanced detection techniques» extend ion chromatography with regard to sensitivity, selectivity and information content and are welcome alternatives for particular problems. They can be used to address fully new problems such as element speciation analysis by ion chromatography.

Table A3: Use of various standard and advanced detectors in ion chromatography

Version	Direct	Indirect	After derivatization
Conductivity	A / C	A / C	CO_2 suppressor
Photometry (UV/VIS)	A	A / C	A / C
Fluorimetry	-	C	C
Amperometry	A / (C)	–	C
Atomic spectrometry	A / C	–	–
Mass spectrometry	A / C	–	conceivable

A = anion chromatography
C = cation chromatography

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B – IC-MS coupling – theory, concepts and applications

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1 Introduction

IC-MS coupling is a modern determination method for qualitative and quantitative analysis. The ion chromatograph (IC) is responsible for the separation of similar chemical analytes. Detection then takes place using a mass spectrometer (MS). Both ionic substances (anions and cations) and polar substances (e.g. organic acids or sugars) can be determined with this very sensitive detection system. In each case, MS detection of ions or molecular ions is based on their mass/charge ratio (m/z).

IC-MS coupling is used when the selectivity or sensitivity must be increased. Analysis using an MS detector (MSD) is also characterized by a very low matrix influence and is thus ideally suitable for cases involving coelution, eluent interference or if the sample matrix influences the analysis. This means that MSD represents a real alternative to conventional IC detectors such as conductivity, electrochemical or UV/VIS detectors. By the use of IC-MS coupling, direct qualitative analysis of the species is possible. The mass/charge ratio is used for peak identification and resolving the molecular structure.

Metrohm cooperates closely with Agilent in the field of coupling ion chromatography with MS and ICP-MS detection. In the case of IC-MS coupling, a fully automated Advanced Modular IC System (Metrohm) is connected with a high-resolution MS detector (Agilent) via an electrospray ionization module (Agilent). The separation of the substances by ion chromatography before MS detection significantly improves the sensitivity and at the same time minimizes matrix influences. This means that interpretation of the results is simplified. Dimer and adduct patterns are easier to recognize and are used for identification of the substances.

2 The ion chromatography system

In the IC-MS system a high-performance ion chromatograph from Metrohm is used; this consists as standard of a high pressure pump, an injection valve, a separation column and a conductivity detector. In addition, in anion chromatography a chemical suppressor is used to suppress the background signal in the conductivity detector and to reduce the salt load of the eluent. This suppressor can, for example, be a «microcapillary packed bed» suppressor such as the Metrohm Suppressor Module II «MSM II» (see Section C). In addition to its resistance to pressure, it is characterized in particular by being completely resistant to solvents. For IC-MS coupling the detector is directly connected to the inlet filter of the MS detector. Optionally a T-piece with a mixing capillary can be incorporated in the connection; this can be used for mixing in a further reagent (addition option). In this way, for example, the pH or the amount of readily volatile organic solvents can be altered after the analytes have been separated.

3 MS detection

In principle a mass spectrometer consists of an ion source, ion optics, mass filter and detector. The ion source is used to transfer the IC eluate into the mass spectrometer. This is done by converting the liquid eluent containing the analytes into the gas phase under normal pressure and generating ions, should this be necessary. The analytes must be thermally stable and the eluent must evaporate without depositing salts. Depending on the application, the salt load of the eluent can be reduced by chemical suppression or by using thermally unstable eluent components. The evaporability of aqueous eluents can be improved by the addition of organic modifiers such as acetonitrile or methanol. In liquid chromatography with MS detection (LC-MS), various ion sources can be used:

- Electrospray ionization (ESI)
- Chemical ionization (APCI)
- Photochemical ionization (APPI)

The field of use depends on the polarity of the analytes, the chromatography flow rate and the molecular mass to be determined. In IC-MS coupling only electrospray ionization is used. Figure B1 is a

schematic diagram showing how electrospray ionization works. Eluent and sample are nebulized with an auxiliary gasflow of nitrogen. The eluent evaporates from the droplets formed and the analyte is concentrated. When a charge density of 10^8 V/cm^3 (Raleigh limit) is exceeded in the droplets, then even smaller droplets are formed as a result of the Coulomb explosion. In this way, under the influence of the electrostatic conditions inside the ESI chamber, the sample ions are desolvated and then transferred to the gas phase. Because of the potential and vacuum gradients, they finally enter the capillary which is the starting point of the ion optics.

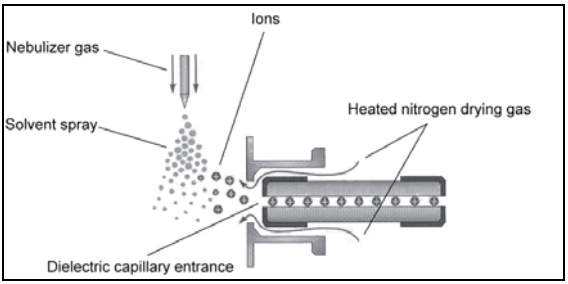

Figure B1: *Schematic diagram showing how electrospray ionization works [B1].*

Electrospray ionization is a very gentle ionization method. It shows the best sensitivity in LC-MS for polar and ionic analytes. In contrast to other ionization methods, with ESI, multivalent ions can also be converted to the gas phase. Further advantages of electrospray ionization are its robustness, simple handling and maintenance as well as its wide range of use for polar and ionic substances in any molecular mass range.

As in ESI no charge transfer occurs, the analyte must be present in the form of an ion before it enters the ion source. As a result, this means that the chemistry of the eluate has a decisive influence on the ionization process. The pH of the eluate determines the degree of dissociation of the analyte. Varying the pH of the eluate can result in increased ion formation, which allows to achieve a considerable increase in sensitivity. In contrast to the conductivity detector, the MS detector is a mass-flow-dependent detector. This means that the flow rate influences the sensitivity of the signal. Flow rates below 1.0 mL/min should be used with ESI. To favor the sensitivity of the detector the flow rate should be as low as possible.

In the ion optics the beam of ions is focused and uncharged particles are separated off. As a result of the potential and vacuum gradients, the ions move through the capillary, pass through the fragmentation zone (CID) and skimmer and finally through the octopole to the entrance lens of the mass filter. The degree of fragmentation of the analyte can be actively influenced by the fragmentation potential. Molecular cleavage of the ions also occurs by collision with nitrogen molecules.

The mass filter has the task of separating the analytes according to their mass/charge ratios. Various types of mass filters are used in LC-MS. The quadrupole is the most widespread one. As it completely satisfies the requirements of the IC-MS analysis with its robustness and accuracy, it is used for the IC-MS coupling. Alternative mass filters are used in time-of-flight (TOF) or ion trap MS detectors. The quadrupole is made from four parallel metal rods that are arranged in a square and point in the longitudinal direction. In each case, opposite poles are coupled by a potential, made up of a direct voltage fraction and a high-frequency alternating voltage fraction. This causes the ions to describe an oscillating path in the space between the rods. Whether this path is stable or whether the ions are accelerated against the walls of the quadrupole depends on their m/z-ratio and the selected settings of the direct and alternating currents. The m/z-ratio of an ion is proportional to the amplitude of the alternating voltage.

The downstream detector has the job of counting the number of ions passing the mass filter. An electron multiplier is used to amplify the resulting current, which is proportional to the ion concentration.

4 IC-MS coupling

IC-MS coupling is used in many sectors. It is frequently used if detection must be extremely sensitive and very selective. These advantages of IC-MS coupling are noticeable in the analysis of drinking water (e.g. for the determination of perchlorate) or the analysis of haloacetic acids. Other fields of application are in clinical and biochemical research (determination of organic acids, amines or sugars), the pharmaceutical industry (peak identification and purity tests), the petrochemical industry (determination of indicator substances) as well as in the food industry, electroplating industry, the analysis of natural substances or environmental analysis. For IC-MS coupling the typical detection limits for ionic analytes are in the ng/L range. This means that in comparison to conventional IC detectors, the sensitivity is much higher.

For the applications described below, a fully automated Advanced Modular IC System (Metrohm) was used in combination with a single quadrupole MS detector (Agilent). The MSD can work in an m/z range from 2 to 3000. With its multiple signal recording function, both specific masses and mass ranges can be detected for anions and cations. The MSD is complemented by an electrospray ionization module (Agilent). Because of the orthogonal design of the electrospray ionization module the ionization is particularly robust and the influence on the background signal by matrix effects is minimized.

A common software solution is available for the IC-MS coupling, in which simple operation and user-friendliness in particular are in the foreground. The user works only with the ChemStation software (Agilent); the IC Net chromatography software (Metrohm) runs in the background. In ChemStation the MS method and the sample table with all information about the injections are set up. ChemStation can control the IC system, start data acquisition (conductivity and MS), record the IC and MS signals and carry out the complete data evaluation with the generation of a report.

IC-MS coupling provides a wealth of information which can be evaluated in various ways. With respect to IC both the retention time and the quantitative response of the conductivity detection are available. MS detection can be carried out in different modes. In the «selected ion monitoring» mode (SIM) information about specific masses is obtained (retention time, quantitative response). This mode is characterized by its very high sensitivity and selectivity and is used for quantitative analysis. In the scan mode a range of masses (m/z) is detected. Information about retention times, mass spectra at a point in time or over a period of time as well as the distribution of selected masses in the chromatogram is available. The scan mode is used for qualitative analysis. However, due to the bandwidth of the mass range to be detected a loss of sensitivity must be accepted.

The interpretation of the mass spectra is easy for small molecules. As the molecular size increases the number of possible compounds with a particular mass/charge ratio also increases. This is why various pieces of information from the IC-MS chromatograms obtained are used for peak identification. In addition to the mass/charge ratio of the main peak the results obtained from conductivity detection, mass distribution in the MS chromatogram, isotope distribution, patterns of molecule fragments, adduct and oligomeric formation and mass defects can all be used for peak identification.

5 Applications of IC-MS coupling

5.1 Oxyhalide analysis in drinking water

In addition to the standard anions, the oxyhalides present in drinking water also need to be analyzed. It is primarily the determination of bromate in drinking water and mineral water that has increased considerably in recent years because of the potential carcinogenic effects of bromate. During the disinfection of water (e.g. by ozonization) bromate is produced by the oxidation of traces of bromide. The legal limits differ from country to country. The German drinking water regulations stipulated a temporary limit of 25 µg/L, which has been reduced to 10 µg/L in 2008 [B2]. The treatment of mineral water is also subject to a similar problem. Since July 01, 2004 a limit of 3 µg/L applies according to the mineral and table water regulations [B3].

The MS chromatogram shown in Figure B2 can be obtained by IC-MS coupling; in addition to the other anions bromate is also shown with the mass 127. A comparison of methods in the trace range (Table B1) shows that the IC-MS coupling leads to a considerable increase in sensitivity when compared with other IC detection systems. It also offers the possibility of determining the main and secondary components of the water in addition to the oxyhalides in a single chromatographic run.

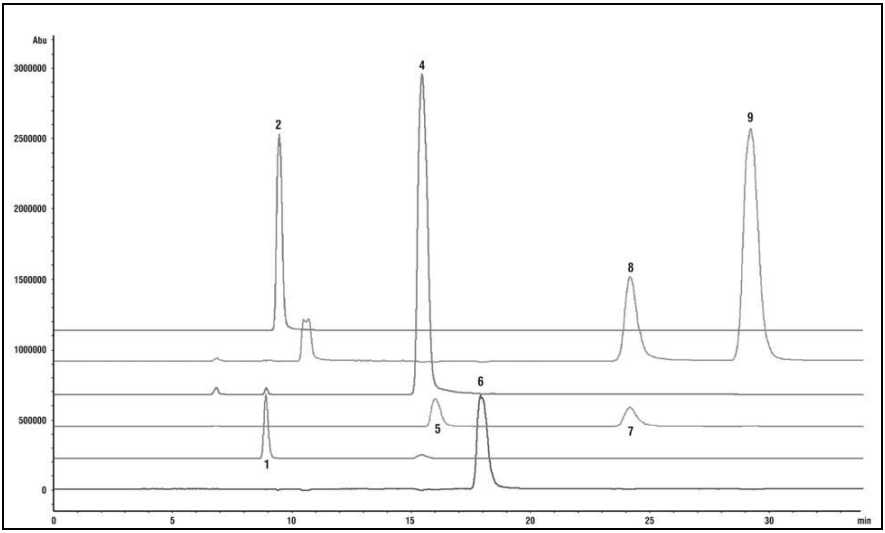

Figure B2: *MS chromatogram of a 10 mg/L standard for the standard anions and oxyhalides*

Column: Metrosep A Supp 5 – 250; eluent: 3.2 mmol/L Na_2CO_3 + 1.0 mmol/L $NaHCO_3$; flow rate: 0.7 mL/min;

ESI: 13 L/min N_2, 350 °C; MSD: SIM neg., Fragmentor: 70 V

Peak assignment: 1: chlorite (67 m/z), 2: bromate (127 m/z), 4: chlorate (83 m/z), 5: bromide (79 m/z), 6: nitrate (62 m/z), 7: phosphate (79 m/z), 8: phosphate (97 m/z), 9: sulfate (97 m/z)

Table B1: Detection limits as per DIN 32 645 of various bromate determination methods [B4]

	Injected volume µL	Detection limit in ultrapure water µg/L	Detection limit in drinking-water matrix* µg/L
Direct conductivity detection	100	0.13	0.39
IC-MS coupling with MS detection	100	0.0064	0.0067
Post-column derivatization with VIS detection (o-dianisidine)	100	0.21	0.64
Post-column derivatization with UV detection (triiodide)	1000	0.032	0.066

* Drinking water matrix: 100 mg/L chloride, carbonate, sulfate

5.2 Perchlorate determination in drinking water

Perchlorate is used as an oxidant in rocket fuel, in explosives and in the electroplating industry. Studies have shown that perchlorate, among other things, is enriched in water. For example, as a result of numerous rocket test flights it has been possible to demonstrate perchlorate contamination in surface waters in the US States of California, Nevada and Arizona [B5]. The effects of perchlorate on human beings are still insufficiently known. However, one thing is certain: perchlorate inhibits iodine uptake in the thyroid gland. This means that the determination of perchlorate is very important.

The analysis is defined in various international methods. EPA Method 332.0 describes the determination of perchlorate using IC-MS coupling [B6]. The detection limit has been published as 0.02 µg/L. Matrix influences can be decisively reduced by the use of IC-MS coupling. In contrast, EPA Method 314.0 documents the determination of perchlorate with a IC system alone [B7]. Perchlorate analysis by conductivity detection becomes increasingly difficult as the amounts of chloride, carbonate and sulfate increase in the sample matrix. However, with this method it is still possible to reliably determine down to 0.5 µg/L perchlorate in 1 g/L chloride [B8].

Perchlorate supplies two main signals in the mass spectrum. Because of the isotope abundance ratio of chlorine the perchlorate is present as m/z 99 (74.8%) and m/z 101 (25.2%). Figure B3 shows the analysis of a ground water using SIM trace m/z 99. The chromatogram of the sample spiked with 1 µg/L perchlorate is also shown. The recovery of the perchlorate in the spiked solution corresponds to the ideal value of 100%. Typical recovery rates are in the 90 – 105% range when a concentration range below 1 µg/L is considered [B5]. As shown in Figure B4, the calibration is linear throughout a wide range.

Plants can accumulate perchlorate from contaminated surface waters and fertilizers containing perchlorate. This is why perchlorate is also determined in vegetables and other agricultural products by using IC-MS coupling as the analysis method [B9].

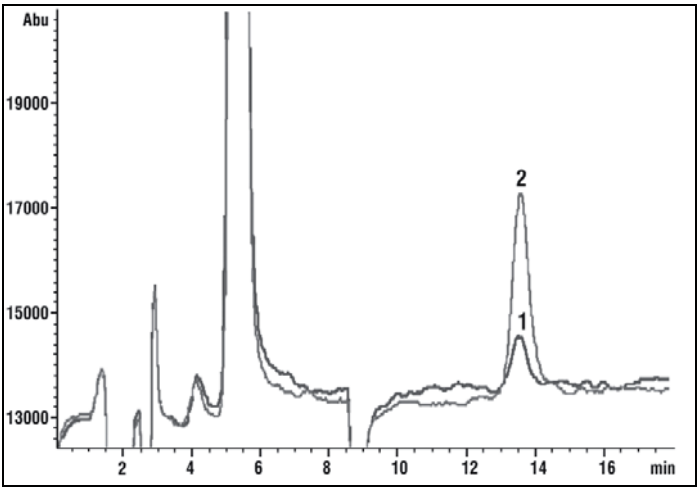

Figure B3: *MS chromatogram of a ground water*

Column: Metrosep A Supp 5 – 100; eluent: 30 mmol/L NaOH + 30% methanol; flow rate: 0.8 mL/min;

ESI: 10 L/min N_2, 320 °C; MSD: SIM neg., Fragmentor: 140 V

Peak assignment: 1: ground water (unchanged): 0.35 µg/L perchlorate (99 m/z), 2: ground water spiked with 1 µg/L perchlorate: 1.35 µg/L perchlorate (99 m/z)

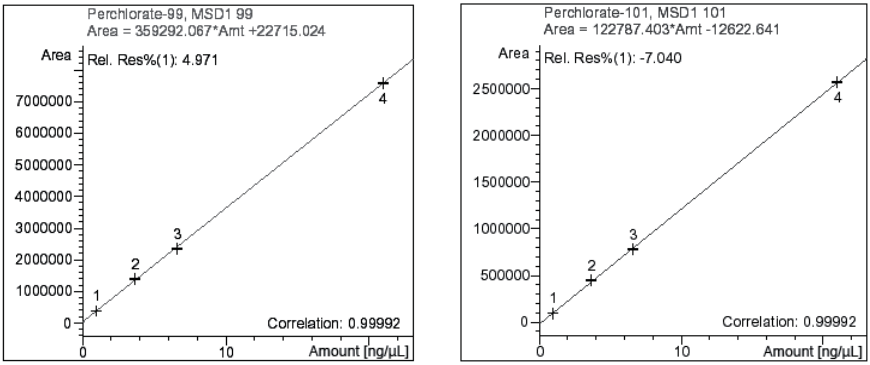

Figure B4: *Perchlorate calibration using the mass/charge ratios m/z 99 and 101*

5.3 Nitrate determination as explosives screening

The danger from terrorist attacks has increased in recent years. In particular, the use of explosives in public transport systems can result in catastrophes. This is why an attempt is made, e.g. at airports, to minimize the risk by checking baggage and travelers. IC-MS coupling is a possible analysis method for detecting explosives in various materials by nitrate screening. Studies have been carried out on hand wipes, corrugated board and other packaging materials [B10] (Figure B5); these showed a good agreement between the results obtained by conductivity and MS detection (Table B2). IC-MS coupling offers the advantage of unambiguous peak identification.

Internal standards can also be used in the analysis using IC-MS coupling. This is done by using the same analytes, but spiked with isotopes that rarely occur naturally, for example ^{18}O. A nitrate enriched with ^{18}O is then used as an internal standard for the analysis of the nitrate.

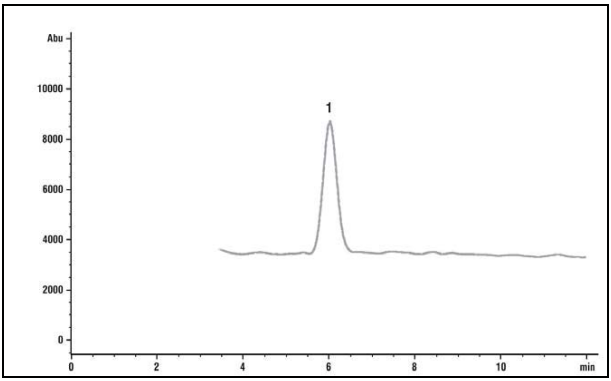

Figure B5: *MS chromatogram of a hand wipe*

Column: Metrosep A Supp 5 – 100; eluent: 3.2 mmol/L Na_2CO_3 + 1.0 mmol/L $NaHCO_3$ + 5% acetonitrile; flow rate: 0.7 mL/min; ESI: 10 L/min N_2, 300 °C; MSD: SIM neg., Fragmentor: 100 V

Peak 1: 11.6 mg/L nitrate (62 m/z)

Table B2: Comparison of nitrate concentrations in contaminated and non-contaminated materials using conductivity and MS detection [B10]

	Conductivity detection, nitrate in mg/L	MS detection, nitrate in mg/L
Contaminated hand tissues	11.39	11.57
Non-contaminated hand tissues	0.41	0.44
Contaminated corrugated cardboard	69.80	69.90
Non-contaminated corrugated cardboard	0.20	0.21

5.4 Determination of phosphate and organic acids in samples of the petrochemical industry

In the oil industry test boreholes are drilled to discover profitable crude oil fields. The process waters of the drillings are tested for indicator substances such as phosphates and aliphatic short-chain carboxylic acids. These indicate the existence of specific bacteria, among other things.

In addition to up to 100 g/L chloride the samples also contain crude oil in the percentage range. This means that they cannot be injected directly. Inline dialysis, as one of the «Metrohm Inline Sample Preparation» (MISP) techniques, allows the oil fraction to be kept away from the injected sample in a fully automated IC system. This is then coupled with an MSD. In order to keep the salt load for column and detector low the samples are diluted accordingly. Figure B6 shows the determination of phosphate using m/z 79. Measurement has been deliberately made at a secondary mass of the phosphate ($H_2PO_4^-$ – H_2O: 79 m/z) in order to exclude sulfate interference (97 m/z) and to be able to measure precisely and sensitively [B11].

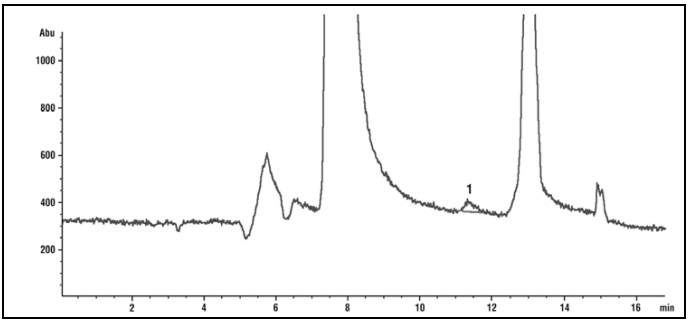

Figure B6: *MS chromatogram of a process water with phosphate and 100 g/L chloride*
Column: Metrosep A Supp 5 – 150; eluent: 3.2 mmol/L Na_2CO_3 + 1.0 mmol/L $NaHCO_3$; flow rate: 0.7 mL/min; sample preparation: dilution 1:500, dialysis; ESI: 13 L/min N_2, 350 °C; MSD: SIM neg., Fragmentor: 70 V
Peak 1: 2.0 µg/L phosphate (79 m/z)

In contrast to the previously described separations on anion exchanger columns, the separation of the aliphatic monocarboxylic acids takes place on an ion exclusion column. This separation mechanism is based on the formation of a Donnan membrane by using an acid as the eluent, which permits the retention of undissociated acids depending on their dissociation constants. For this separation mechanism mineral acids such as sulfuric acid or perchloric acid are typically used as the eluent. However, these cannot be used in an IC-MS coupling, as they would be concentrated in the ESI and their aggressiveness would cause corrosion. For this reason organic acids such as oxalic acid or citric acid are used as the eluent [B12].

The separation of the analytes as undissociated acids is in conflict with the fact that only ions can be analyzed in the MS detector using the electrospray ionization module. This means that in an intermediate step the pH of the eluate must be altered so that the organic acids are then present in a dissociated condition and their anions can be detected. The addition possibility is used for this (T-piece with mixing capillary); this is incorporated between the conductivity detector of the IC system and the inlet filter of the MSD. For example, it is used to continuously add dilute ammonia solution and in this way increase the pH from 2 to 10.5. This robust method for determining the organic acids using IC-MS coupling is very selective (Figure B7).

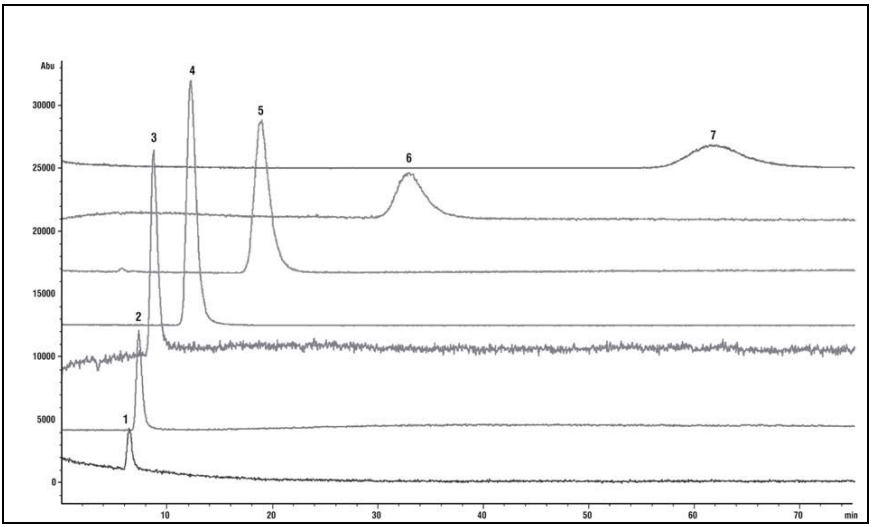

Figure B7: *MS chromatogram of a 10 mg/L standard for aliphatic monocarboxylic acids*

Column: Metrosep Organic Acids – 250; eluent: 0.25 mmol/L oxalic acid; flow rate: 0.4 mL/min; sample preparation: dialysis; addition possibility: 300 mmol/L ammonia solution (0.25 mL/min); ESI: 13 L/min N_2, 350 °C; MSD: SIM neg., Fragmentor: 70 V

Peak assignment:1: acetic acid (59 m/z), 2: propionic acid (73 m/z), 3: butyric acid (87 m/z), 4: valeric acid (101 m/z), 5: caproic acid (115 m/z); 6: heptanoic acid (129 m/z), 7: caprylic acid (143 m/z)

5.5 Analysis of amines

Short-chain aliphatic amines such as methylamines or ethanolamines are used as organic neutralization agents or buffer substances. They are also used as corrosion inhibitors. The analysis takes place after the separation of the various substituted amines on a cation exchanger column. Also in this case organic acids are used instead of mineral acids as the eluent in the IC-MS coupling (Figure B8).

Higher amines such as the biogenic amines putrescine, cadaverine and histamine play a decisive role in food analysis. They may be contained in foodstuffs or formed subsequently as a result of microbial decarboxylization of the corresponding amino acids, primarily during fermentation processes. Increased concentrations of biogenic amines are an indicator for poor hygienic conditions during the fermentation process. This means that they are used as «freshness indicators» for sea fishes and for the quality control of wines. Higher concentrations of biogenic amines can result in intolerance reactions such as, for example, headaches. This is why limits have been set for various foods, in particular regarding their histamine content [B13].

IC-MS coupling can be used as a reference method for amine determination by ion chromatography, as in the analysis of biogenic amines (Figure B9).

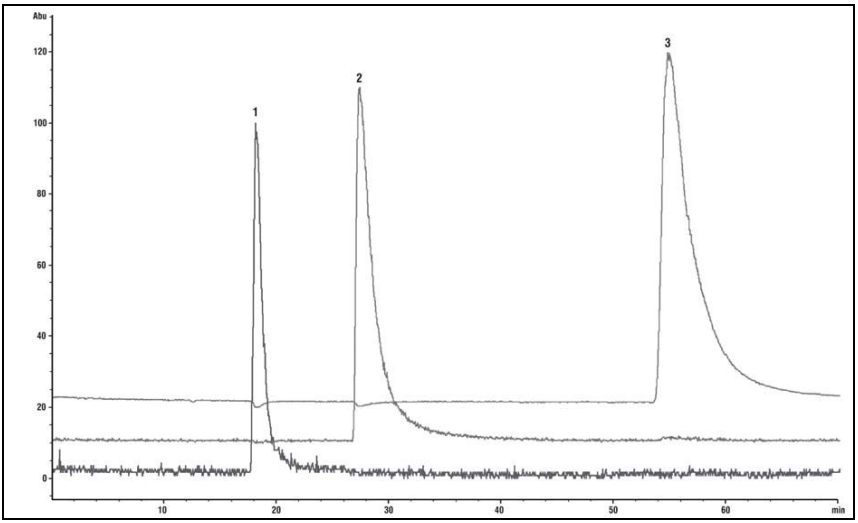

Figure B8: *MS chromatogram of a 10 mg/L standard for various methylamines*
Column: Metrosep C2 – 150; eluent: 4.0 mmol/L tartaric acid; flow rate: 0.5 mL/min; ESI: 13 L/min N_2, 350 °C; MSD: SIM pos., Fragmentor: 70 V
Peak assignment: 1: monomethylamine (32 m/z), 2: dimethylamine (46 m/z), 3: trimethylamine (60 m/z)

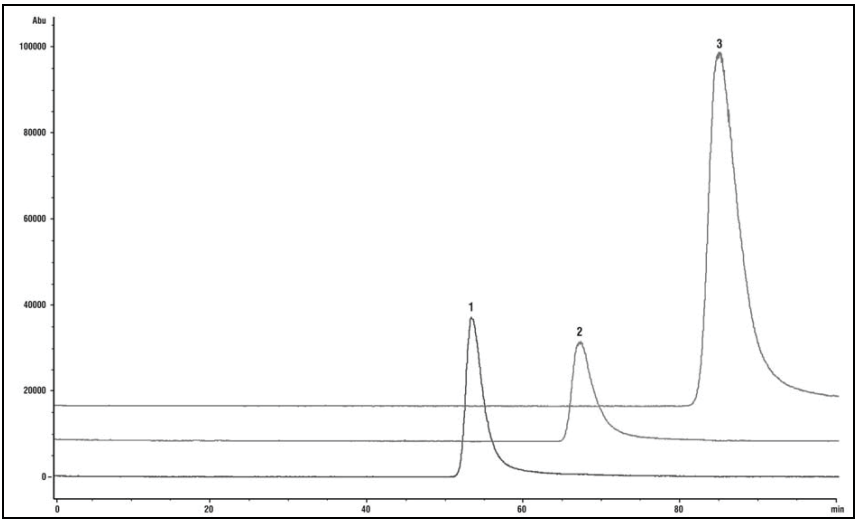

Figure B9: *MS chromatogram of a 10 mg/L standard for biogenic amines*
Column: Metrosep C2 – 150; eluent: 4.0 mmol/L tartaric acid; flow rate: 0.5 mL/min; ESI: 13 L/min N_2, 350 °C; MSD: SIM pos., Fragmentor: 70 V
Peak assignment: 1: putrescine (89 m/z), 2: cadaverine (103 m/z), 3: histamine (112 m/z)

5.6 Sugar analysis by IC-MS coupling

Carbohydrates are a further class of molecules that can be determined by IC-MS coupling. Because of their polarity they can be separated as anions or as undissociated substances. In the latter case an ion exclusion column is used. As well as sugars it is also possible to determine sugar alcohols, alcohols and organic acids in a single chromatographic run. After the column the separated undissociated carbohydrates are converted to their lithium or sodium salt by inverse suppression. This process takes place in the «Metrohm Suppressor Module II» (MSM II), whose cation exchanger material is continuously occupied by lithium or sodium ions. The lithium and sodium adducts of the carbohydrates are very stable and can be detected very sensitively as cations (Figure B10).

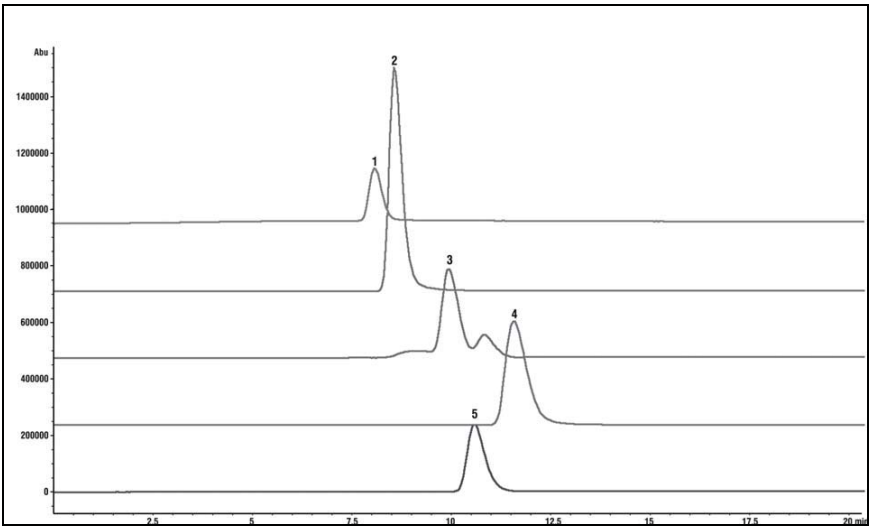

Figure B10: *MS chromatogram of a 10 mg/L standard for various sugars*

Column: Metrosep Organic Acids – 250; eluent: 2.0 mmol/L oxalic acid; flow rate: 0.5 mL/min; ESI: 13 L/min N_2, 350 °C; MSD: SIM pos., Fragmentor: 70 V

Peak assignment: 1: maltotriose (511 m/z), 2: sucrose (349 m/z), 3: glucose (187 m/z), 4: fucose (157 m/z), 5: xylose (171 m/z)

6 Conclusions

The combination of a highly efficient ion chromatograph with a high-resolution mass spectrometer represents a modern detection technique whose strengths lie in its extreme sensitivity and excellent selectivity. IC-MS coupling is also used when matrix effects have to be suppressed or analytes have to be identified. Because of its flexibility such a coupling method opens up numerous possible applications in ion analysis. Previously IC-MS coupling was mainly used in the environmental and health sectors. The Metrohm/Agilent combination of IC-MS coupling is characterized by its very good robustness and simple handling. This makes it ideally suitable for routine analyses. Detection limits in the ng/L range underline the very good sensitivity of this detection method.

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C – High-performance CO_2 suppression

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1 Introduction

Suppression has been an important component of IC ever since the inclusion of ion chromatography (IC) into the group of analytical techniques. As the suppression technique is regarded in part as being IC dogma, the further development of IC was subjected to very one-sided influences for some time.

Due to their susceptibility to pressure and poisoning, membrane suppressors could make the analytical system trouble-prone and expensive to maintain. Only the introduction of the one-column technique (ion chromatography without suppression) – primarily for cation chromatography – demonstrated that considerable advantages in these fields could be achieved.

A compact micro-suppressor filled with ion exchanger, for example the MSM, is to be preferred to a membrane suppressor for anion chromatography.

In contrast to anion analysis, the use of suppression for cation analysis does not result in any increase in sensitivity. However, without the use of a suppressor the background conductivity is considerably higher. This is why it is the performance of the detector that decides whether a suppressor has to be used for the determination of cations.

2 The CO_2 suppressor

The use of a chemical suppressor in anion chromatography reduces the background conductivity dramatically which considerably lowers the detection limit. Nevertheless the acid produced from the eluent during suppression basically remains in the eluate and has the effect of slightly increasing the background conductivity.

Table C1 summarizes the most important eluents used for anion chromatography and gives the resulting values for the background conductivity for the same eluent concentration of 3 mmol/L. In each case the background conductivities are given without suppression and with suppression using the Metrohm Suppressor Module (MSM).

On the one hand the elution power of the individual eluents is roughly classified by a rating from very weak to very high and on the other hand by giving the retention time for the chloride ion under identical chromatographic conditions.

In addition, the Table contains those background conductivities that are obtained by combining the usual chemical suppressor (MSM) with the new Metrohm CO_2 suppressor (MCS).

Table C1: Comparison of background conductivities and chloride retention times of eluents used in anion chromatography

Eluent (3 mmol/L)	Elution power (A Supp 5)	R_T* Cl^- (min)	Suppression effect (baseline in µS/cm)		
			without MSM	with MSM	with MSM+MCS
Sodium carbonate	very high	1.8	564	9.6	0.6
Sodium hydrogen carbonate	very weak	10.8	216	9.6	0.6
Sodium borate	weak	5.3	370	0.4	–
Sodium hydroxide	weak	6.8	552	0.5	–
p-Cyanophenol**	very high	1.5	119	24	–

*A Supp 5 – 100 column **pH above 7.5

As can be clearly seen from the values given in the table, the optimization of the suppression, i.e. the introduction of the Metrohm CO_2 suppressor, has considerably reduced the background conductivity.

The setup of an IC system with chemical suppression and CO_2 suppression is shown as a flow diagram in Figure C1. The CO_2 suppressor is built in downstream from the MSM, as the carbonic acid and therefore the CO_2 is formed in the MSM.

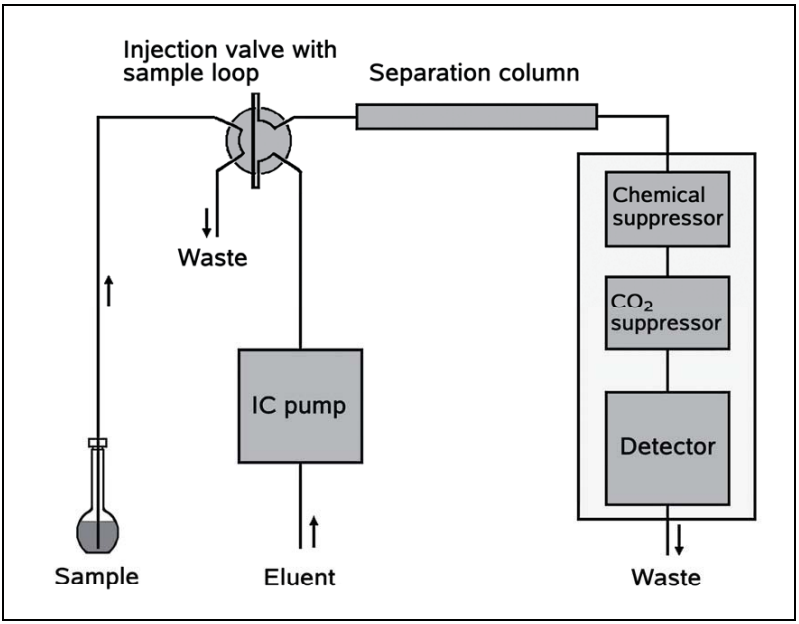

Figure C1: *Flow diagram of an IC system with chemical and CO_2 suppression*

3 Construction and operation of a CO_2 suppressor

It is known that in the chemical suppressor (MSM) the sodium carbonate and sodium hydrogen carbonate are converted to carbonic acid by ion exchange. The protons of the ion exchanger are exchanged for the sodium ions of the eluent.

As the carbonic acid is in equilibrium with the CO_2 dissolved in the water and the water itself, the conductivity of the carbonic acid is considerably lower than can be derived from the dissociation constants (see equations 1 and 2).

Equation 1: Exchange reaction at ion exchanger

$$R — SO_3^- H^+ + NaHCO_3 \rightleftharpoons R — SO_3^- Na^+ + H_2CO_3$$

Equation 2: Equilibrium reaction for carbonic acid

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$$

The CO_2 released in the chemical suppressor (MSM) is removed from the eluent by the use of membrane technology, so that only the analyte ions dissolved in water reach the detector as free acids (flow diagram - see Figure C1).

Because of the slow equilibration of carbonic acid there is always a slight fraction of CO_2 remaining in the eluent; depending on the eluent concentration and flow rate this produces a background conductivity value of approx. 0.2 to 2 µS/cm. Table C1 gives some typical data for this. Please note that ionic contaminants in the water and chemicals used will result in a somewhat increased background conductivity even after total CO_2 suppression, i.e. after complete removal of the CO_2 from the eluent.

Figure C2 shows the construction of the CO_2 suppressor, Figure C3 shows the 853 CO_2 Suppressor including the two necessary absorption cartridges.

In the CO_2 degasser cartridge the CO_2 is removed from the eluent under vacuum. The CO_2 diffuses through the Teflon wall (membrane) of a gas-permeable capillary through which the eluent flows.

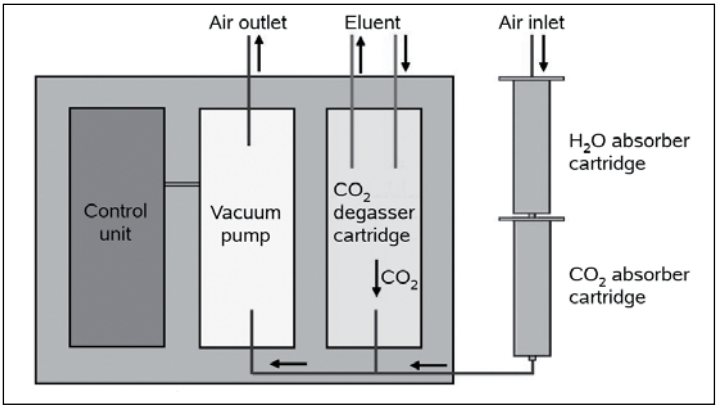

Figure C2: *Schematic of CO_2 suppressor.*

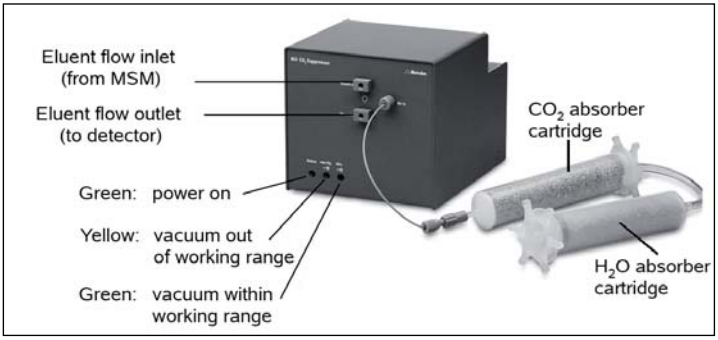

Figure C3: *853 CO_2 Suppressor.*

An additional small airflow enhances transport of the diffused CO_2 to the vacuum pump. As this airflow should be CO_2-free, the atmospheric CO_2 is removed from the required air by a CO_2 absorber cartridge. An upstream water absorber cartridge which can be regenerated additionally prevents atmospheric humidity from quickly blocking the CO_2 cartridge.

Control diodes show the state of the vacuum and the operating mode of the 853 CO_2 Suppressor: as long as the instrument is switched on the left-hand green diode remains lit up. If the vacuum is still inadequate then the central yellow diode will remain lit up continuously. A change to the right-hand green diode is made when the working vacuum has been achieved. When the cartridges start to become blocked and this reduces the flow of air to the CO_2 suppressor the central yellow diode will start to blink.

Figures C4 and C5 show typical chromatograms recorded with and without the CO_2 suppressor. The differences with respect to peak heights, the absence of the system peak and the extreme reduction of the water peak can be clearly recognized. The considerable reduction in the background conductivity is not visible as the chromatograms have been superimposed. In determinations with CO_2 suppression the shift of the retention times towards larger values is caused by the volume of the capillary in the vacuum cell.

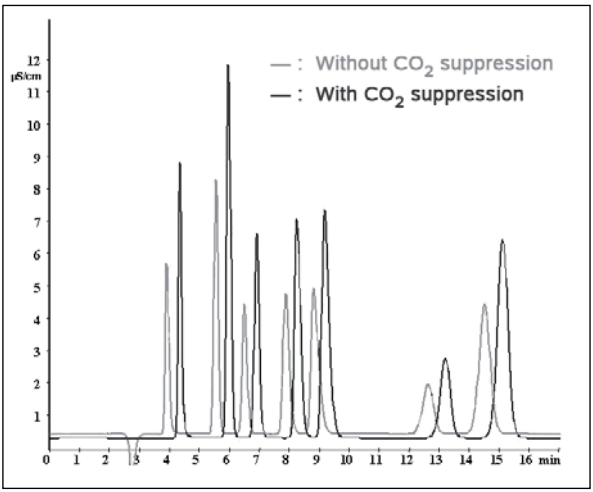

Figure C4: *Comparison of ion chromatograms in the mg/L range, recorded with and without CO_2 suppression.*

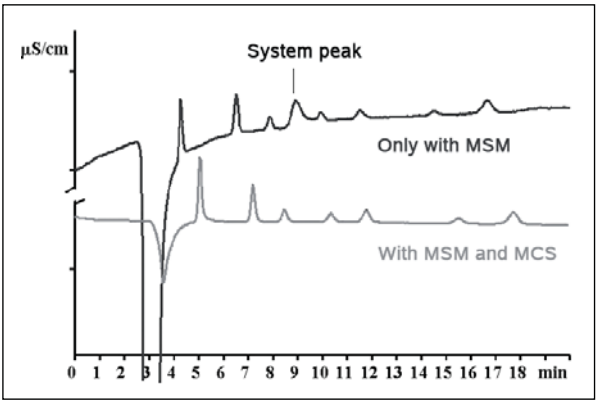

Figure C5: *Comparison of ion chromatograms in the µg/L range, recorded with and without CO_2 suppression.*

4 Increasing the sensitivity by using a CO_2 suppressor in IC

If a CO_2 suppressor is used then an increase in sensitivity can be observed only in the mg/L range, but not in the µg/L range (see Figures C4 and C5). The reason for the increase in sensitivity when the CO_2 suppressor is used becomes obvious when the chemical equilibrium between the eluent components and the analyte ions is considered.

The analyte ion, for example Cl^- with the counter-ion Na^+, is converted in the MSM to the free acid of the analyte ion, i.e. to HCl (see Equation 3). The protons of the HCl influence the carbonic acid equilibrium by displacing it toward CO_2 and water. This becomes clear by considering the law of mass action corresponding to Equation 4a: if the proton concentration is increased by the protons of the analyte acid then, because K is constant, the hydrogen carbonate fraction must be reduced automatically. The hydrogen carbonate concentration influences the background conductivity. The higher the analyte concentration, the lower the hydrogen carbonate concentration and therefore the background conductivity contribution made by carbonic acid in the peak. The peak consists of the share of the background conductivity and the signal of the analyte. The dependency of the background conductivity on the analyte concentration and the resulting peak is shown schematically in Figure C 6.

Equation 3: Reaction of the analyte with the ion exchanger of the MSM

$$R\text{—}SO_3^-\ H^+ \ + \ NaCl \longrightarrow R\text{—}SO_3^-\ Na^+ \ + \ H^+\ Cl^-$$

Equation 4a: Dissociation equilibrium of carbonic acid and CO_2 dissolved in water

$$H^+ + HCO_3^- \rightleftharpoons H_2O + CO_2$$

Equation 4b: Law of mass action for Equation 4a

$$K = \frac{[H^+] \cdot [HCO_3^-]}{[H_2O] \cdot [CO_2]}$$

As the background conductivity drops strongly during the simultaneous detection of analyte acids, approximately one-third of the possible analyte ions are not included in the peak, but appear in the background conductivity. In this way about one third of the detection sensitivity is lost.

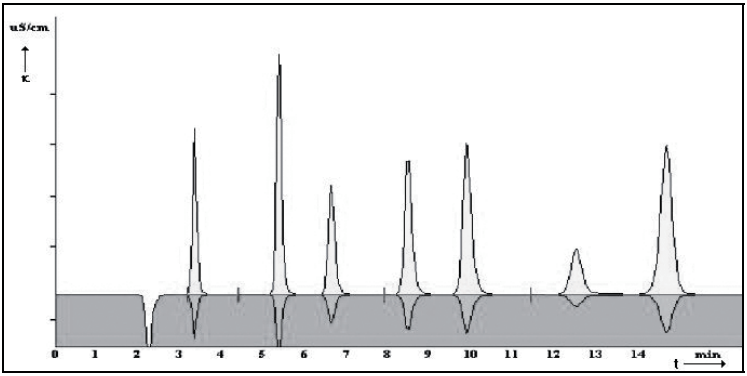

Figure C6: *Schematic presentation of the effects of the influence of the protons of the analyte ions on the carbonic acid equilibrium (dark gray: background conductivity; light gray: signals of the analyte ions).*

As the CO_2 suppressor removes the CO_2 from the eluent before the detector, the background conductivity decreases no further during the elution of analyte ions and the peak heights and areas become correspondingly larger, as can be clearly recognized in Figure C 4. In contrast, no increase in sensitivity in the µg/L range is observed (see Figure C5). The reason for this is the low CO_2 residual fraction in the eluent. In this case the same effect again occurs as is seen in the mg/L range when no CO_2 suppressor is used.

In order to achieve an optimal effect of the CO_2 suppression, this residual CO_2 content must also be removed from the eluent. An even longer gas-permeable capillary would be necessary which, however, cannot be justified.

If, however, a low concentration of the sodium salt of a strong acid is added to the eluent then additional acid is formed in the MSM; this influences the carbonate equilibrium in accordance with Equations 4a and 4b. The increased acid concentration displaces the carbonate equilibrium almost fully to the CO_2 and water side. As a result the efficiency of the CO_2 suppressor is considerably increased and the CO_2 is completely removed from the eluent. As an unwanted side effect the additional acid fraction results in a somewhat increased background conductivity (see Figure C7).

These relationships are summarized in Figure C8.

In order to be able to use this technique (so that no system peak is obtained) a separation column must be used that binds the corresponding anion extremely strongly. These conditions are met by the Metrosep A Supp 10: perchlorate is bound very strongly by this column material so that no system peak is observed at low concentrations.

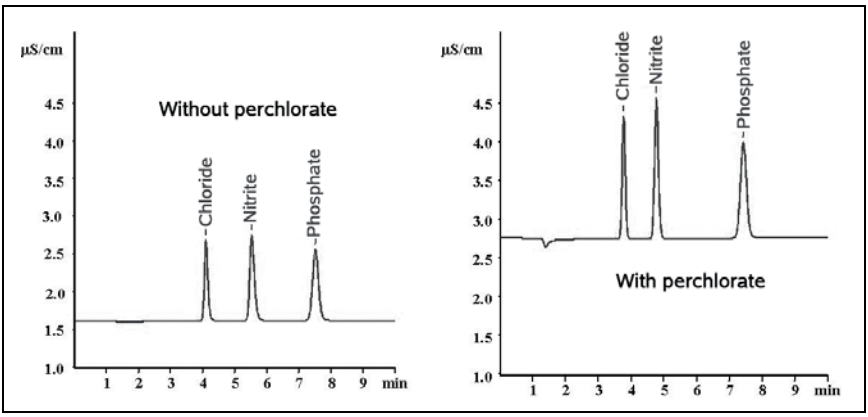

Figure C7: *Comparison of ion chromatograms with and without addition of perchlorate to the eluent. Suppression with MSM and CO_2 suppressor.*

Sample: 1 ppm chloride; 2 ppm nitrite and 5 ppm phosphate

Column: A Supp 10 – 100; loop: 20 µL

Figure C8 clearly shows that the addition of perchlorate results in considerably larger peaks. This is made even clearer by Figures C9 and C10, in which the corresponding bar diagrams are shown for the determination of chloride, nitrite and phosphate in the mg/L and µg/L ranges. It can be clearly seen that larger values are obtained for both the area and height of the peaks.

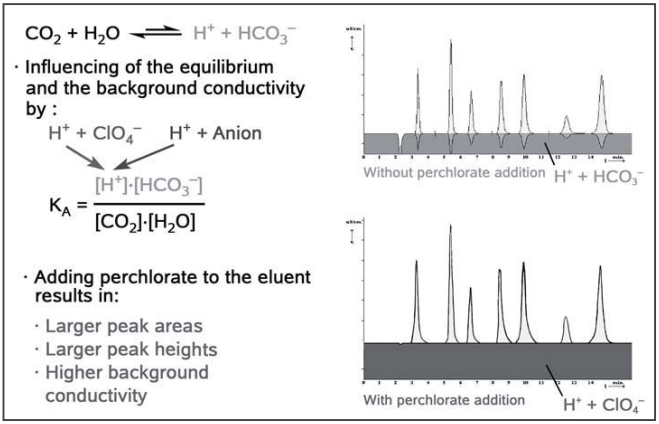

Figure C8: *Perchlorate added to the eluent and its effects on the background conductivity, carbonic acid equilibrium and the effective peak areas and heights*

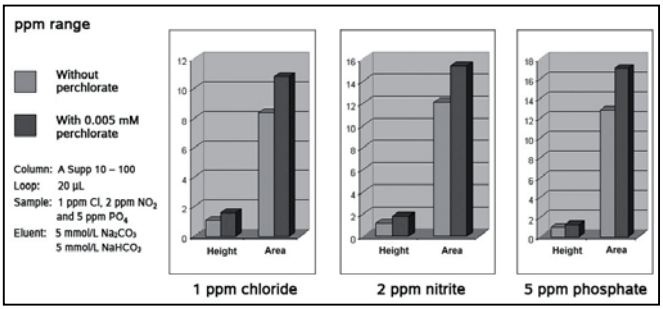

Figure C9: *Differing peak heights and areas as a function of the added perchlorate when using a CO_2 suppressor in the mg/L range*

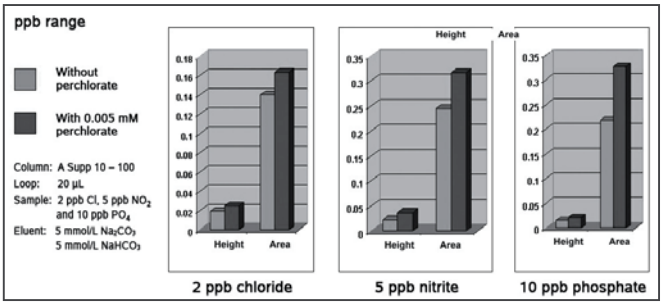

Figure C10: *Differing peak heights and areas as a function of the added perchlorate when using a CO_2 suppressor in the µg/L range*

Removal of all the carbon dioxide from the system leads not only to an improvement of the detection limits, but also results in linear calibration curves over several orders of magnitude; these are not obtained by using commercially available suppressors. Figure C11 shows the calibration curves for the range from 10 µg/L to 3 mg/L obtained with conventional chemical suppression (shown in light gray) and with the combination of chemical and CO_2 suppression (shown in dark gray). In the first case the

measured values in the mg/L range lie below the linear calibration curve and in the enlargement (in the µg/L range) the axis intercept caused by the CO_2 can be seen.

When the combination of chemical and CO_2 suppression is used (dark gray curve) then the measuring points lie on the calibration curve throughout the whole range; no axis intercept is obtained.

In this case the marked increase in sensitivity is again clearly recognizable: the slope of the dark gray curve is considerably greater than that of the light gray curve.

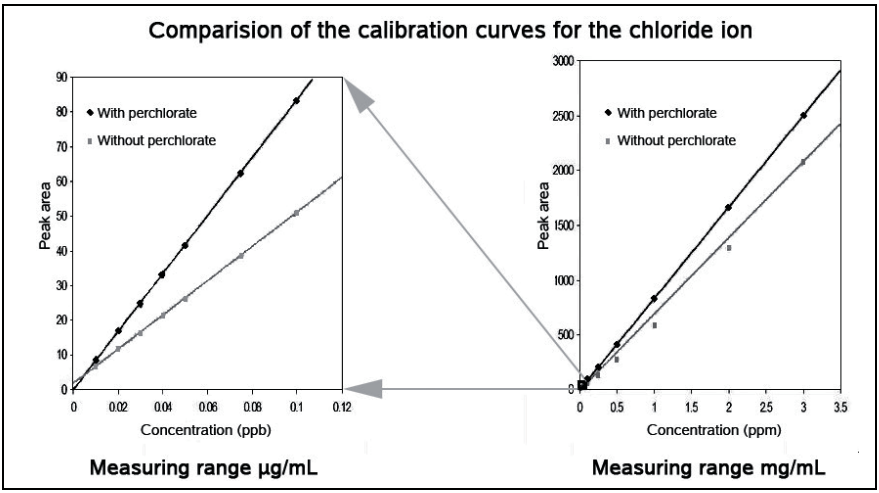

Figure C11: *Calibration curves for chloride with (upper straight line) and without (lower straight line) addition of perchlorate. In the right-hand figure the whole range is shown; in the left-hand one only the section from 0.01 to 0.1 µg/L.*

5 The effect of the CO_2 absorber cartridge

In Figure C3 it can be seen that a small flow of air is led through the CO_2 suppressor in order to optimize the suppression. A CO_2 absorber cartridge ensures that additional CO_2 from the atmosphere does not enter the CO_2 suppressor from this flow of air. There is also a further upstream cartridge that absorbs atmospheric humidity and gives the CO_2 cartridge a considerably longer working life. The drying agent of this second cartridge can be regenerated to save costs.

The use of both cartridges results in a baseline with extremely low noise.

However, when the baseline noise is not important, i.e. for work in the mg/L range, it may be possible to do without the cartridges.

The baselines of chromatograms of a standard solution containing a few ppm of anions that have been recorded with and without the cartridges are shown in Figure C12. This clearly shows the slight baseline offset of less than 0.1 µS/cm which is caused by atmospheric CO_2. In this case the baseline noise is almost identical.

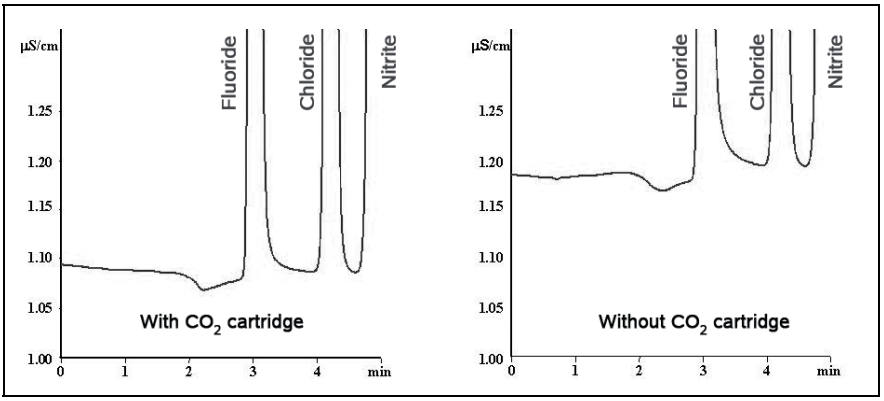

Figure C12: *The effect of the absorber cartridges; chromatograms of a standard solution recorded with (left) and without (right) cartridges.*

Sample: 2 ppm fluoride; 2 ppm chloride; 5 ppm nitrite; 10 ppm each of bromide, nitrate, phosphate and sulfate

Column: Metrosep A Supp 5 – 100; eluent: 3.2 mM Na_2CO_3/1.0 mM $NaHCO_3$; flow rate: 0.7 mL/min; loop: 20 µL

Figure C13 shows the chromatogram of a standard solution containing a few µg/L of anions that has been recorded without the two cartridges. It shows a very good baseline with slight noise and peaks that can be evaluated very easily. In this case again no real difference can be recognized in comparison to chromatograms recorded using both the cartridges.

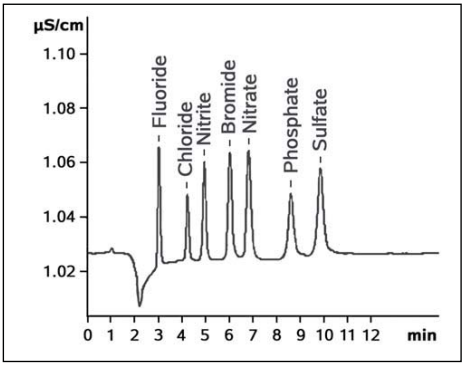

Figure C13: *Chromatogram of a µg/L standard solution recorded without absorber cartridges*

Sample: 2 µg/L fluoride, 2 µg/L chloride, 5 µg/L nitrite and 10 µg/L each of bromide, nitrate, phosphate and sulfate

Column: Metrosep A Supp 5 – 100; eluent: 3.2 mM Na_2CO_3/1.0 mM $NaHCO_3$; loop: 20 µL; flow rate: 0.7 mL/min

If the CO_2 suppressor is located in surroundings with a constant CO_2 content then a very good baseline can be obtained without the use of the two absorber cartridges. However, if the level of the atmospheric CO_2 fluctuates then the effects on the baseline are obvious (see Figure C14). Opening the door of the IC instrument, breathing into the open instrument as well as persons moving around in front of the IC instrument are examples of activities which slightly alter the CO_2 content of the air that is drawn into the CO_2 suppressor. This causes a fluctuation of the background conductivity and

results in the baseline interference shown in Figure C14. In this case the use of the two absorber cartridges would have prevented such interference.

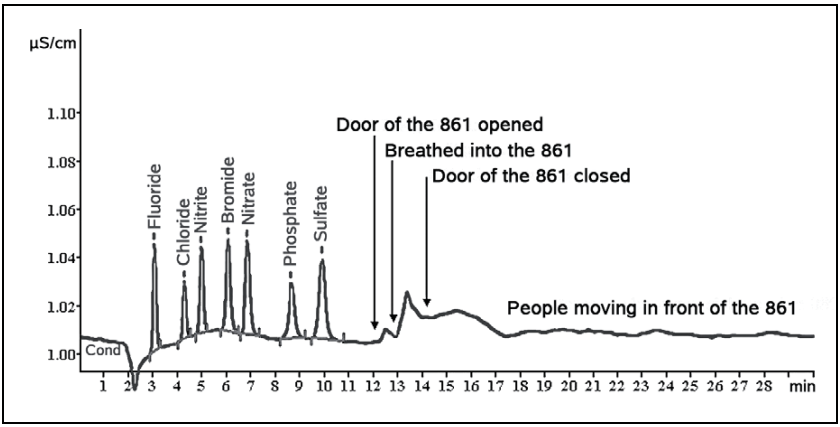

Figure C14: *Alteration in the background conductivity as a function of the variation in the CO_2 content of the surrounding air when using a CO_2 suppressor without absorption cartridges*

Sample: 2 µg/L fluoride; 2 µg/L chloride; 5 µg/L nitrite; 10 µg/L each of bromide, nitrate, phosphate and sulfate

Column: Metrosep A Supp 5 – 100; eluent: 3.2 mM Na_2CO_3/1.0 mM $NaHCO_3$; flow rate: 0.7 mL/min; loop: 20 µL

6 Flow-dependency of the CO_2 suppression

The performance of the CO_2 suppressors depends not only on the carbonate concentration of the eluent, but also on its flow rate. The removal of CO_2 from the eluent depends not only on the equilibration and the applied vacuum, but the properties of the gas-permeable capillary in the CO_2 suppressor also have an influence (e.g. chemical composition, length and wall thickness). However, as these parameters are constant, it is understandable that the carbonate concentration and the flow rate determine the background conductivity.

Table C2 lists the values for flows from 0.3 to 0.9 mL/min for an eluent containing 3.2 mM Na_2CO_3/ 1.0 mM $NaHCO_3$. The value for water measured in the same system (flow: 0.5 mL/min) is given for comparison. The value of 0.46 µS/cm is higher than expected by theory and indicates that the detector has not been accurately calibrated. However, this is not astonishing as absolute measurements are not made in IC (quantification is done using standards). This is the reason why the detector is rarely or never calibrated in normal laboratory use.

Table C2: Dependency of the background conductivity on the flow rate of the eluent

Flow rate (mL/min)	Baseline (µS/cm)
0.9	1.12
0.7	1.09
0.5	0.95
0.3	0.88
Water as eluent	0.46

Column: Metrosep A Supp 5 – 100
Eluent: 3.2 mM Na_2CO_3/1.0 mM $NaHCO_3$
Metrohm CO_2 Suppressor with water and CO_2 cartridges

7 Influence of the carbonate content of the sample solutions

Carbonate or dissolved CO_2 present in the sample can have a further influence on the performance of the Metrohm CO_2 Suppressor. While dissolved CO_2 is best removed by a sample degasser, which also reliably removes other dissolved gases in addition to CO_2 as well as air bubbles in the sample aspiration tubing, only slight amounts of carbonate or hydrogen carbonate can be removed from the sample. In this case the carbonate enters the suppressor via the column and alters the system peak. In order to clarify the effect caused by samples containing carbonate, Figures C15 to C17 show chromatograms of solutions containing carbonate and hydrogen carbonate.

In all cases the chromatograms with (dark gray curve) and without (light gray curve) CO_2 suppression are shown. The experimental conditions were always identical, except for the chromatograms without CO_2 suppression, in which the CO_2 suppressor was bypassed. As the chromatograms have been superimposed, the absolute value of the background conductivity is only valid for the chromatogram without CO_2 suppression.

All three examples (Figures C15, C16 and C17) show that, despite the carbonate content, all the ions present can be determined and the very marked interference caused by the water peak and the system peak in the chromatograms without CO_2 suppression is eliminated by CO_2 suppression.

Please note that during the separation of the ions in the column the large amount of carbonate moves through the column as an ionic load (and is not continuously added to the column, as is this case with the carbonate eluent). If the sample contains a further ion with a retention time very similar to that of the carbonate then an interference must be expected. In most cases this appears as a deformation of the smaller peak, so that the peak height and peak shape no longer correspond to those of the standard solution, but the peak area remains comparable. Examples of co-eluting ions and the resulting effects on the affected peaks are shown in Figures C15 and C17. In Figure C15 the peak of an unknown ion with a retention time of approx. 10.9 minutes lies exactly in the middle of the system peak. In Figure C17 the unknown ion co-elutes with bromide at a retention time of approx. 12.5 minutes.

8 Gradient techniques in combination with CO_2 suppression

In principle gradients cannot be used together with an eluent containing carbonate, because as the eluent concentration increases, the background conductivity increases strongly. However, as eluents containing carbonate are the best and most versatile eluents due to the strong elution power of the carbonate ion and the very weak elution power of the hydrogen carbonate ion (see Table C1), it is very desirable to be able to work with gradients using this eluent combination. Approaches have already been made by first running a blank sample (ultrapure water). Subtracting the blank chromatogram from the sample signal then resulted in the actual chromatogram.

A far more elegant way of implementing the gradient technique today is to use the CO_2 suppressor, as the CO_2 is removed from the eluent before the detector. This means that the background conductivity remains virtually constant even with high carbonate concentrations in the eluent (see Figure C18).

In the example shown in Figure C18 a binary gradient consisting of the eluents water and sodium carbonate was used for the separation of 20 anions. The chronological changes in eluent composition as the chromatogram was recorded are shown in Table C3.

From the chromatogram it is obvious that even those ions that cannot be separated by isocratic elution can easily be separated within 35 minutes by using a binary carbonate gradient. The slight baseline increase is caused by residual CO_2 and ionic contaminants in the eluent.

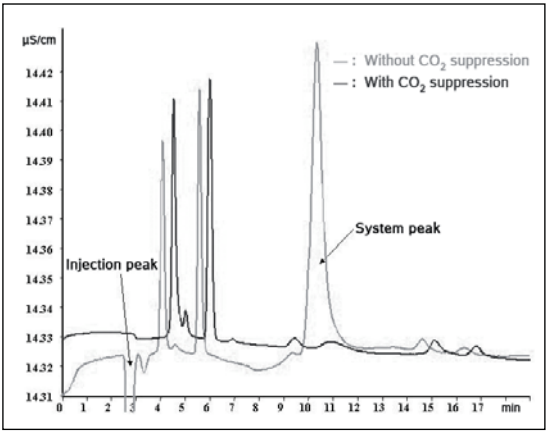

Figure C15: *Ion chromatograms of a carbonate solution with a carbonate concentration of 250 mg/L*

Column: Metrosep A Supp 4; eluent: 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$; flow rate: 1 mL/min; loop: 20 µL

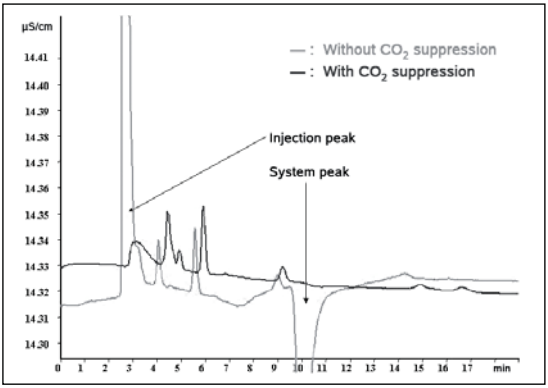

Figure C16: *Ion chromatograms of a hydrogen carbonate solution with a hydrogen carbonate concentration of 250 mg/L*

Column: Metrosep A Supp 4; eluent: 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$; flow rate: 1 mL/min; loop: 20 µL

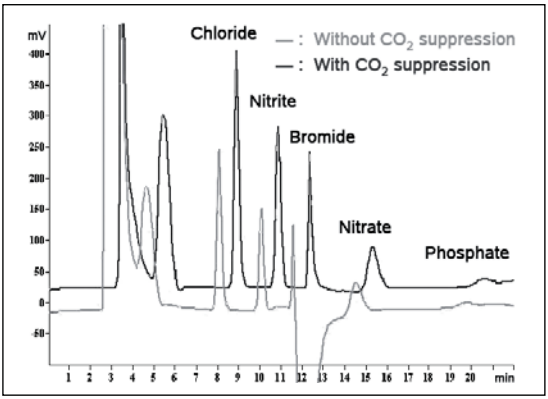

Figure C17: *Ion chromatograms of a carbonate solution with 10'000 mg/L carbonate to which 1 mg/L each of chloride, nitrite, bromide and nitrate have been added*

Column: Metrosep A Supp 5 – 250; eluent: 3.2 mM Na_2CO_3/1 mM $NaHCO_3$; flow rate: 0.7 mL/min; loop: 20 µL

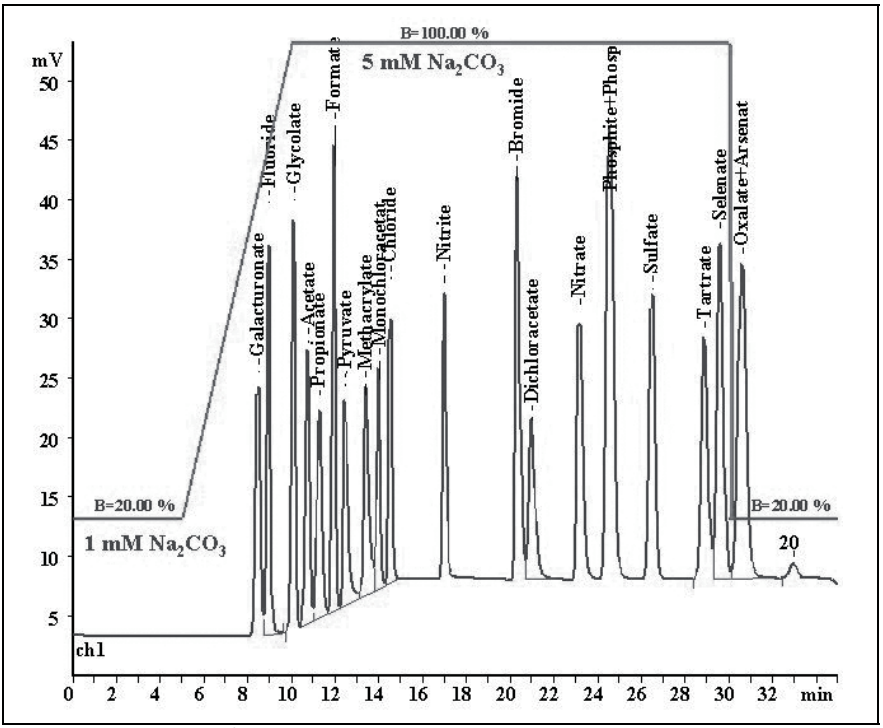

Figure C18: *Separation of 20 anions using a binary gradient*

Sample: 20 ppm galacturonate, 2 ppm fluoride, 10 ppm glycolate, 10 ppm acetate, 10 ppm propionate, 5 ppm formate, 10 ppm pyruvate, 10 ppm methacrylate, 5 ppm monochloroacetate, 2 ppm chloride, 5 ppm nitrite, 10 ppm bromide, 10 ppm dichloroacetate, 5 ppm nitrate, 10 ppm phosphite, 10 ppm phosphate, 5 ppm sulfate, 10 ppm tartrate, 10 ppm selenate, 5 ppm oxalate and 10 ppm arsenate

Column: Metrosep A Supp 7; eluent 1: ultrapure water; eluent 2: 5 mM/L Na_2CO_3

Flow rate: 0.8 mL/min; loop: 20 µL

Table C3: Gradient composition for the separation of the 20 anions

Time (min)	Eluent 1: H_2O	Eluent 2: 5 mM Na_2CO_3
0.0	80%	20%
5.0	80%	20%
10.0	0%	100%
30.0	0%	100%
30.1	80%	20%
35.0	80%	20%

9 Application examples

9.1 Determination of oxyhalides in the presence of standard ions

Even with other difficult analytical samples the use of the Metrohm CO_2 Suppressor produces ion chromatograms that are considerable easier to evaluate, as can be seen in Figure C19. The determination of oxyhalides in the presence of standard anions in the lower µg/L range presents a challenge to ion chromatography. Figure C19 shows the ion chromatograms obtained with a water sample spiked with 10 µg/L oxyhalides. The positive effect of the CO_2 suppressor can easily be recognized. The water peak and the system peak have disappeared and the baseline is much more stable.

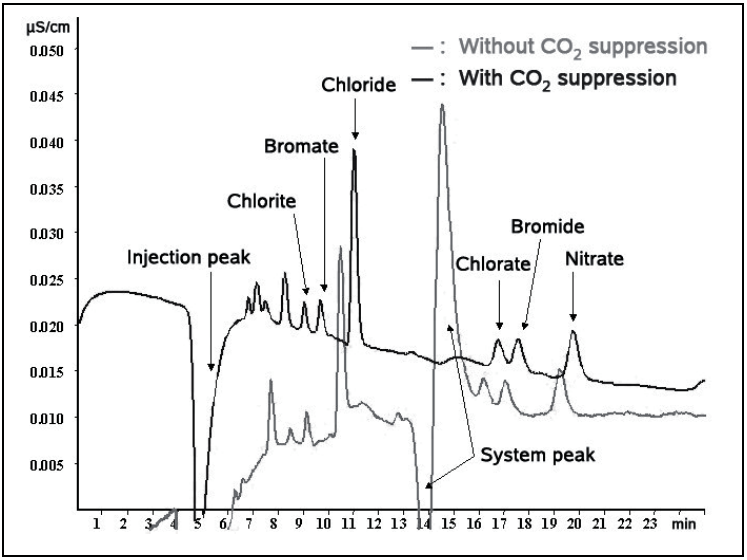

Figure C19: *Ion chromatograms of a water sample spiked with 10 µg/L of each oxyhalide*
Column: Metrosep A Supp 5 – 250 with A Supp1 Guard; eluent: 3.2 mM Na_2CO_3 /1.0 mM $NaHCO_3$; flow rate: 0.7 mL/min; loop: 20 µL

9.2 Drinking water from Herisau, Switzerland

In general, the analysis of drinking water by ion chromatography places no great demands on the analytical chemist. However, if small amounts of bromide and phosphate are to be determined in samples of very hard water then a system that has been optimized in all respects must be used. In this case the CO_2 suppressor should not be neglected.

Figure C20 shows the chromatograms of a sample of drinking water from the community of Herisau, Switzerland. It contains 0.04 ppm fluoride, 7.8 ppm chloride, 7.8 ppm nitrate und 5.0 ppm sulfate. The difference between the chromatograms with and without CO_2 suppressor is marginal and in both cases there is no interference with the evaluation. If, however, the chromatogram is interpreted more exactly, i.e. the conductivity scale is spread from approx. 30 µS/cm to 0.07 µS/cm then further peaks become visible, namely bromide and phosphate with a content of 0.004 and 0.001 mg/L, respectively.

This case also clearly shows the benefits of CO_2 suppression (compare with Figure C21).

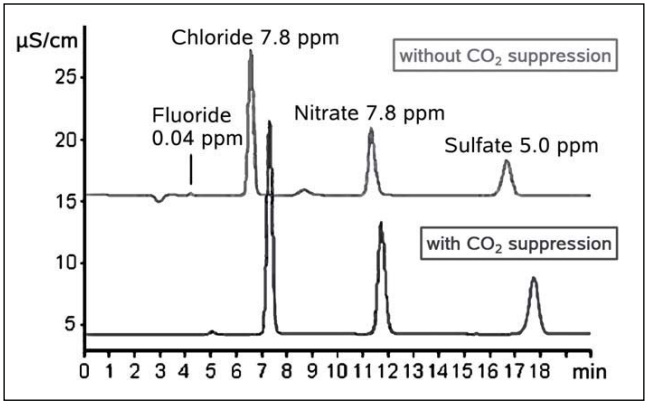

Figure C20: *Ion chromatogram of a drinking water sample from Herisau*

Column: Metrosep A Supp 5 – 150 with A Supp1 Guard; eluent: 3.2 mM Na_2CO_3 /1.0 mM $NaHCO_3$; flow rate: 0.7 mL/min; loop: 20 µL

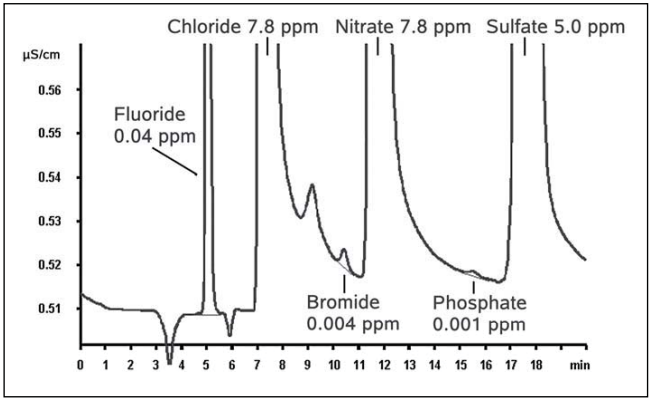

Figure C21: *Detailed view of the ion chromatogram (with CO_2 suppression) of a drinking water sample from Herisau*

Column: Metrosep A Supp 5 – 150 with A Supp1 Guard; eluent: 3.2 mM Na_2CO_3 / 1.0 mM $NaHCO_3$; flow rate: 0.7 mL/min; loop: 20 µL

9.3 Determination of the anion content in ultrapure water

The water and system peaks also interfere strongly with the evaluation of the chromatogram in the analysis of ultrapure water. Figure C22 shows only the chromatogram that was recorded using the CO_2 suppressor. Of particular interest is the fact that even a sample volume of only 20 µL still produces chromatograms in the µg/L range that are easy to evaluate.

The fact that not even a very small water peak can be observed is due to the CO_2 dissolved in the sample of ultrapure water.

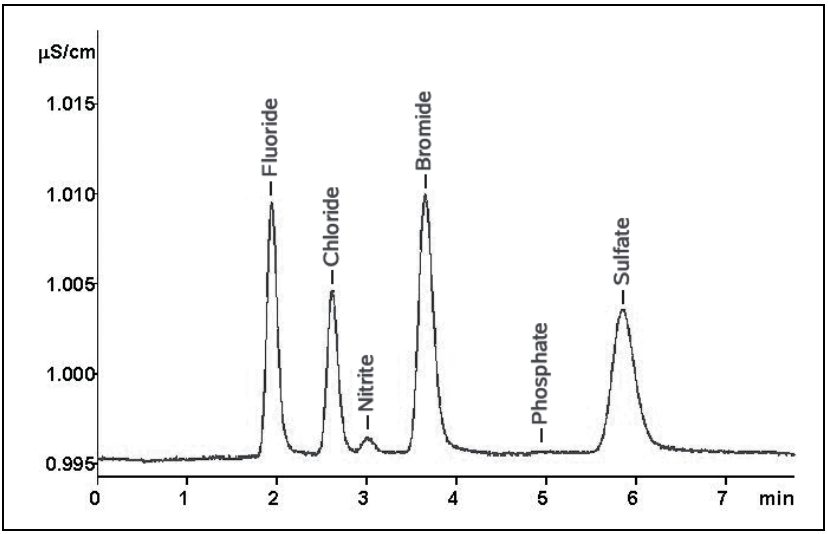

Figure C22: *Concentrations: 15 µg/L fluoride; 18 µg/L chloride; 3 µg/L nitrite; 86 µg/L bromide; approx. 2 µg/L phosphate and 42 µg/L sulfate*

Column: Metrosep A Supp 5 – 50; eluent: 3.2 mM Na_2CO_3/1.0 mM $NaHCO_3$; flow rate: 0.7 mL/min; loop: 20 µL

10 Conclusions

By the combination of chemical suppression and CO_2 elimination considerably lower background conductivities can be realized in anion chromatography than without CO_2 suppression. The benefits for the user are:

- linear calibration curves from the µg/L to the mg/L range
- better detection limits due to an extremely smooth baseline and the absence of the system peak
- problem-free peak integration
- can be used with gradients

All in all the user receives a much improved conductivity detection that opens up new possibilities.

D – Theory, principles and applications of IC-ICP-AES and IC-ICP-MS coupling

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1 Introduction

In the course of the further development of chromatographic methods, limits imposed by the system are encountered at many places. In order to overcome such limits, drastic alterations are required in the chromatography system (column, separation principle and eluent) or the detection system (selectivity, sensitivity).

If the IC-ICP-AES and IC-ICP-MS online couplings are considered then it becomes immediately clear that the detection represents the most drastic alteration. Plasma-based atomic spectrometry analytical methods such as ICP-AES or ICP-MS convince by their extremely high element selectivity paired with very low detection limits for most elements. However, an atomic spectrometer places different demands on the chromatography system than a conductivity detector would. If a look is taken at the history of ion chromatography then it quickly becomes clear that the combination of ion chromatography and conductivity detection has only been possible by making numerous adaptations to the chromatography system. Special low-capacity stationary phases had to be developed; the eluent had to be prepared on the basis of the anions of weak acids and, last but not least, this only worked in combination with a type of suppression. If an ion chromatography application that has been developed for use with a conductivity detector is now transferred directly to an IC-ICP-AES or IC-ICP-MS coupling then the fact that unsatisfactory or at least not optimal results are obtained should not be surprising.

2 The coupling partners

2.1 Ion chromatography as a coupling partner

Today the term ion chromatography is used both as an abbreviation for ion exchange chromatography and also as a collective term for the three methods associated with the separation of ions or potentially ionic compounds: ion exchange, ion exclusion and ion pair chromatography.

2.1.1 Ion exchange chromatography (IEX)

Ion exchange is a well-understood retention mechanism that is based on a chemical reaction. The resulting consequences are described in detail in Chapter A, Section 1.2, «Theory, principles and applications of advanced detection techniques in ion chromatography».

Small anions and cations are separated from one another on an oppositely charged ion exchanger by the driving force of an equally charged ion (see Figure D1). The ion exchange function for the separation of anions is based on quaternary ammonium groups using hydroxide or carbonate as the eluent ions [D1 – D3].

Complex-forming ligands are frequently added to the eluent for the separation of cations; these reduce the effective charge on the cations and promote differences in retention by different degrees of complexing.

A typical elution system for cations is based on a driving cation such as H^+ or Na^+ and a strong complexing agent such as dipicolinic acid or tartaric acid [D3]. If necessary, the driving cation is used at the same time for pH adjustment.

The basic ion exchange process on a strongly acidic or strongly basic ion exchanger can normally be easily controlled. It becomes more difficult when secondary retention mechanisms such as the adsorption of ions interfere. Furthermore, the packing materials used have a low exchange capacity as a consequence of using conductivity detection. This limits the maximum sample concentration and amount, and thus directly influences the achievable detection limit.

Depending on the type of basic material and the construction principle, the following effects can be observed in addition to adsorption (polystyrene resins): ion exchange of opposite polarities (agglomerated ion exchanger), oxophilic interactions (silica gel) or complex formation (methacrylate resins).

A considerably more selective version of ion exchange is based on complex formation on the exchanger. A prominent representative is immobilized iminodiacetic acid as quasi half an EDTA molecule. In these chelating ion exchangers kinetic problems are frequently encountered in the form of an unsatisfactory equilibration rate. Its occurrence depends on the complex formation kinetics of the cation and the number of branches of the complexing agent [D4].

All types of ion exchange are outstandingly suitable for the preconcentration and separation of chemically similar cations and anions.

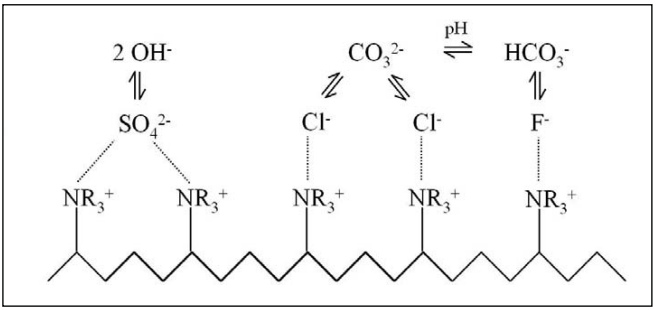

Figure D1: *Exchange equilibria on an anion exchanger. The elution of the analytes can only be realized by isoionic displacement by the eluent anions.*

2.1.2 Ion pair chromatography (IPC)

The fields of application of ion pair and ion exchange chromatography clearly overlap. IPC fills the gap up to reversed phase chromatography (RP) and is particularly interesting for less polar and for only partly stable ions such as complex species [D3]. By the ion pair formation mechanism the sometimes critical ionic interactions between the analyte and the stationary phase can be reduced.

At its limits IPC can either be characterized as being ion exchange chromatography with a dynamically generated ion exchanger, or as the reversed phase separation of less-dissociated ion pairs. In the case of a dynamically generated ion exchanger the ion pair reagent is simply adsorbed on the surface of the reversed phase packing material.

An important advantage of IPC is the possibility of simultaneously separating anionic, cationic and nonionic compounds in a single system. The excellent possibility of regenerating the basic reversed phase column is also of interest. By using a strong eluent based on organic solvents the ion pair reagents and any adsorbed substances can be completely removed again. This type of column poisoning presents great problems, particularly in ion exchange chromatography. It is just these interesting properties that have made IPC together with atomic spectrometry into a very suitable method for element species analysis. However, a disadvantage is the amount of organic modifiers which makes it necessary to modify the plasma with oxygen at higher concentrations.

Further disadvantages are the long equilibration times and the limited resistance to concentrated or large-volume samples. The long equilibration times result from the complex dynamic adsorption equilibriums on the column. Their interference by concentrated samples is at the same time the reason for the sensitivity of IPC to concentrated and large-volume samples. A further consequence is a reduced retention time stability of IPC compared with IEX and RP chromatography.

2.1.3 Ion exclusion chromatography (IEC)

IEC separates the analytes according to their pK_a values [D3]. The anions of strong acids are excluded from the pore volume of a low cross-linked strongly acidic cation exchanger by its negative surface potential. This surface potential, which is known as the Donnan membrane, occurs with highly functionalized ion exchangers with permanently dissociated functionalities.

The retention volume of the analytes increases as the pK_a values of the analyte increase. Up to now this type of ion separation is the only mechanism that has not yet been used in online coupling with ICP. This is because of the analytes that are typically analyzed by IEC. Although the detection of carbon by ICP-AES and ICP-MS is basically possible and also sensitive, the argon used for ICP generation contains too much CO_2. This results in a high background which depends largely on the plasma conditions. In this case other detection principles such as photometry or conductivity are more suitable.

2.2 ICP as a source for atomic emission and mass spectrometry (ICP-AES and ICP-MS)

Atomic spectrometry methods based on ICP permit the sensitive determination of almost all elements in a virtually unlimited bandwidth of gaseous, liquid and solid matrices. Important limitations result from the plasma gas and its contaminants (Ar, other inert gases, N, O and some halogens) as well as the elements of typical solvents such as water (H, O) and by physical restrictions such as, for example, inadequate ionization (F) or emission lines in the vacuum UV range (< 190nm) (F, Cl, Br) [D5].

2.2.1 Inductively coupled plasma

ICP with argon as the plasma gas is today the most important excitation source for atomic spectrometry [D5]. Argon plasmas are compatible with aqueous aerosols, have a sufficiently high thermal capacity for drying aerosols, for the dissociation of compounds and for the excitation of the participating atoms. As a result of the ionization energy of argon it is also ideally suitable for ionizing elements. It is convenient that this produces practically only single ionized element ions. The temperatures (kinetic temperature, electron temperature, atomization temperature, ionization temperature and others) in the argon plasma vary from 4500 to 10000 K, depending on the particular temperature definition and the local position in the plasma. This means that ICP is a so-called thermal non-equilibrium plasma. The temperature in the analytically usable inner sample channel of an ICP of 5500 to 6500 K is high enough to destroy all the chemical bonds of a molecule.

Typical components and the configuration of an ICP-AES and ICP-MS spectrometer are shown in Figure D2. The ICP part is practically identical for ICP-AES and ICP-MS.

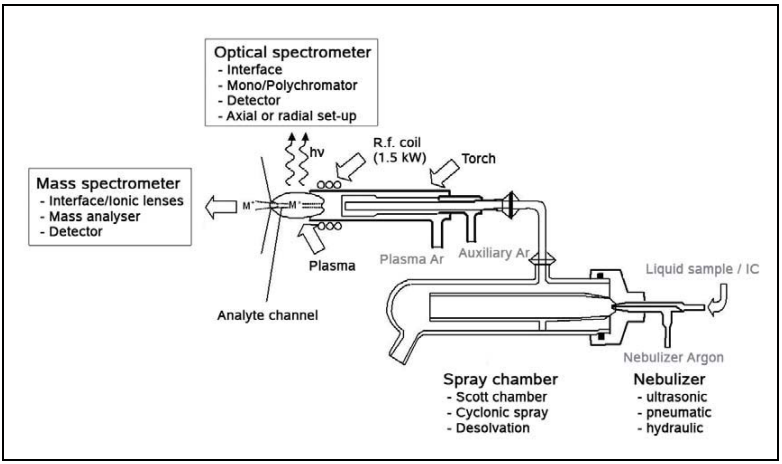

Figure D2: *Setup of an ICP-MS and ICP-AES spectrometer with typical components. The ICP part is practically identical for both versions.*

The torch (burner) of the ICP consists of three concentric quartz tubes, through the outer one of which plasma argon flows at a typical rate of 14 L/min. The gas stream is used to maintain the plasma and for cooling.

The middle channel transports the auxiliary gas flow, which is responsible for the shape and axial position of the plasma. The inner channel transports nebulizer gas flow coming from the nebulizer/ nebulizer chamber combination. This gas stream transports the analytes to the plasma. Both the auxiliary gas flow rate and that of the nebulizer gas flow are typically 1 L/min.

The energy for maintaining the plasma is transferred by a water-cooled copper coil with one, two or three windings. A high-frequency generator with a rating of 1 to 1.5 kW and a frequency of 27.12 or 40.68 MHz is used as the energy source. The plasma is formed by using an ignition spark which produces an adequate amount of electrons and argon cations.

The sample feed system is typically set up for liquid samples, but suitable solutions also exist for gases and solids. The standard configuration of an ICP consists of a pneumatic nebulizer for producing very fine aerosol droplets and a nebulizer chamber, which uses kinetic discrimination to allow only the finest fraction of the aerosol droplets to enter the plasma. Otherwise the plasma would either become unstable or be extinguished completely.

Table D1 compares the two detection methods ICP-AES and ICP-MS using the estimated detection limits for 49 elements.

Table D1: Summary of the roughly estimated detection limits (DL) for 49 elements using the most sensitive emission line in ICP-AES and the most sensitive isotope in ICP-MS (quadrupole ICP-MS). The data has been collected from information provided by the manufacturers and adapted by overlapping elements.

Element	$DL_{ICP-AES}$ µg/L	DL_{ICP-MS} µg/L	Element	$DL_{ICP-AES}$ µg/L	DL_{ICP-MS} µg/L
Al	5	0.05	Mn	0.4	0.005
Sb	40	0.005	Hg	20	0.001
As	30	0.01	Mo	1	0.005
Ba	0.5	0.001	Ni	7	0.005
Be	0.1	0.001	Pd	70	0.005
Bi	10	0.001	Pt	30	0.005
B	3	0.07	K	80	0.5
Br	800 / 250	0.05	P	20	6
Cd	2	0.005	Se	50	0.05
Ca	0.1	0.5	Si	5	8
Ce	100	0.001	Ag	5	0.005
Cr	5	0.005	Na	6	0.05
Cl	250 / 80	160	Sr	0.2	0.001
Co	3	0.001	S	50	50
Cu	0.3	0.005	Te	20	0.005
Ge	100	0.05	Tl	50	0.001
Au	25	0.005	Sn	7	0.005
I	100 / 10	0.005	Ti	3	0.05
In	100	0.001	W	20	0.005
Fe	1	0.1	U	400	0.001
La	6	0.005	V	2	0.005
Pb	7	0.001	Zn	1	0.005
Li	3	0.005	Zr	5	0.005
Mg	0.5	0.05			

2.2.2 ICP-AES as coupling partner

Atomic emission spectrometry using ICP as a source of photons is a well-established and easily understood routine method [D5]. The photons can be observed either radially as the standard method or axially as a more sensitive, but at the same time more interference-susceptible alternative method. With radial observation different temperature zones of the ICP can be used by varying the observation height. Modern instruments are usually equipped for both observation methods.

ICP produces a wavelength-dependent background, which is primarily produced by the energy released by the recombination of argon cations and electrons. This continuum is also overlayered by the emission lines of argon and a number of molecule emissions. The basic problem with ICP-AES is the correct detection of the analyte line against this background, which naturally also varies with all the operating parameters of the plasma.

Today practically only polychromators are used as the spectral apparatus; these permit the simultaneous observation of many emission lines and their backgrounds. The task of the monochromator or polychromator is to differentiate the selected analyte emission line from interfering lines of other elements, ions or molecules. The necessary resolution varies from less than 3 pm (hopeless due to the Doppler broadening of the emission lines) up to a typical distance of at least 20 to 30 pm between the lines. The achievable resolution of the spectral apparatus usually lies between 4 and 20 pm.

Polychromators are based either on the Paschen-Runge setup (concave grating with detection of the emission lines on the Rowland circle of the grating) or on echelle optics, which utilizes a grating with low ruling densities in high orders and together with an order separator (prism) ensures high resolutions. [D5]. Photomultipliers, photodiodes, diode arrays or surface detectors such as CCD or CID-chips are used as detectors.

Further important characteristics of the optics used are the usable wavelength range, the number of lines that can be observed simultaneously, the degree of noise and last but not least the transmission. Today's top models have a usable spectral range starting at 120 nm [D6] up to the near infrared range for the determination of alkali metals and offer the choice of observing the plasma axially and radially.

2.2.3 ICP-MS as coupling partner

In ICP most elements are present practically exclusively in the form of singly charged ions [D7]. The ions are extracted via a multi-stage, differentially pumped interface consisting of aperture plates. The first stage consists of a water-cooled «sampler» made of nickel or platinum which is immersed directly in the plasma and has an opening of approx. 1 mm. The expansion chamber is located behind this sampler. Here a powerful rotary slide-valve pump lowers the pressure from atmospheric pressure down to approx. 2 mbar. A further aperture, known as the «skimmer», separates the expansion or interface section from the high-vacuum section. In the latter there is a residual pressure of 10^{-4} to 10^{-7} mbar. The large pressure difference between the atmospheric pressure plasma and the mass spectrometer generates an ultrasonic expansion of the plasma in the mass spectrometer.

Positively charged ions are accelerated into the mass spectrometer using an ion optics system, while negatively charged and neutral particles are preferentially discharged or pumped off. The beam of ions produced still has to be separated from photons and refocused by a photon stop («on-axis» design) or by an «off-axis» mass filter [D8].

The latest generation of quadrupole-based ICP-MS instruments offers a collision cell (also known as reaction cell, hexapole or octopole), which is located between the classical ion optics and the quadrupole. It is used for focusing the energy of the ions and the destruction of molecular ions [D9]. The collision cell influences the transmission of the ions (both positive and negative) and destroys molecular ions more or less effectively, with molecular ions containing H-atoms being particularly unmanageable whereas, for instance, argon dimers are easily dissociated.

The standard mass filter used in ICP-MS is still the quadrupole. It allows the resolution of nominal mass differences of 0.2 to 0.5 amu and is therefore usually adequate for problems involving ele-

ments. Crucial factors for the performance of all ICP-MS instruments are the transmission of the interface and the mass analyzer, the background count (triggered by residual gas and photons) as well as interference caused by molecular ions and doubly charged ions.

The instrumental background count of typical quadrupole instruments is 10 cps (counts per second, measured at m/z 220), modern instruments with an off-axis quadrupole achieve 0.2 to 1 cps.

A medium resolution mass filter is the time-of-flight mass spectrometer (TOF) [D10]. When connected to ICP resolutions in the range m/Δm 1000 are obtained. The main advantage of the TOF-ICP-MS for applications in the coupling sector is the rapid recording of complete mass spectra, which is particularly advantageous for the short-term signals produced by chromatography. With quadrupole instruments a quick decision must be taken to limit oneself to only a few masses depending on the half-width values of the signals. This is due to the sequential working method of the quadrupole.

The extraction time (starting time of the acceleration) which is identical for all masses in TOF-ICP-MS leads to accurate isotope ratio measurements, which again can be used for calibrating online applications.

High-resolution ICP-MS instruments are all based on double focusing mass spectrometers in the reversed Nier-Johnson design with one electrostatic and one magnetic mass filter [D8]. This allows resolutions from 3000 to 10000 m/Δm to be achieved routinely.

This resolution is sufficient to avoid numerous interferences by molecular ions. In this way ArO^+ can be differentiated from ^{56}Fe and $ArCl^+$ from ^{75}As. Nevertheless some insoluble interferences remain.

2.3 The interface between IC and ICP

The behavior of an ICP spectrometer as detector in ion chromatography is dominated by the eluent composition and the flow rate; the flow rate can be influenced throughout a wide range by the column geometry. If the column diameter is varied from 8 mm (preparative columns) through 4 mm (standard analytical columns) down to 1 or 2 mm (microbore columns) then the flow rate will vary from 10 to 0.1 mL/min for the same linear flow rate of the mobile phase.

Larger column diameters permit larger sample amounts and are easier to handle. Smaller packing materials are normally used with smaller column diameters. The reduced flow places different demands on the HPLC pump. The sensitivity of chromatography to external volumes increases; this is critical, particularly with respect to tubing connections and the nebulizer chamber of the ICP [D23].

Figure D3 shows the practical setup for coupling an ion chromatograph with the ICP-MS instrument. All sample manipulation takes place in the ion chromatography unit on the left. In addition to the essential components (pump, injection valve and column) a further detector (conductivity) and an autosampler can be seen. On the ion chromatography side the outlet capillary of the column is the only interface component.

On the ICP side the nebulizer and the nebulizer chamber are the crucial interface components (see Figure D4). The most common types and their characteristics are listed in Table D2.

The general demand placed on the nebulizer is that it is compatible with the flow rate and eluent composition. Water-based eluents often contain high salt concentrations, which could block the nebulizer during the nebulization process. If organic solvents are used then the plasma will be disturbed by the increased vapor pressure and the resulting higher carbon content in the gas phase.

For both water and organic solvents this can be counteracted by cooling the nebulization chamber. For higher water loading desolvatization must be used. With higher solvent fractions oxygen must be added to the plasma so that the carbon can be burned off to form CO_2. In comparison to ICP-AES, ICP-MS tolerates lower salt and solvents contents.

Pneumatic nebulization transforms the ICP into a mass-flow-dependent detector. A typical pneumatic nebulizer possesses a relatively large range throughout which the efficiency of the aerosol formation (and therefore the observed signal intensity) remains constant. Below this range the signal decreases clearly, above it relatively little.

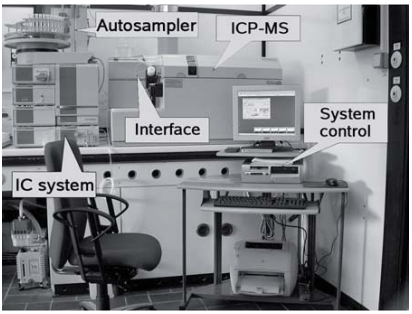

Figure D3: *Setup of an IC-ICP-MS system. On the left-hand side an ion chromatograph can be seen with detector and autosampler, on the right is an ICP-MS system.*

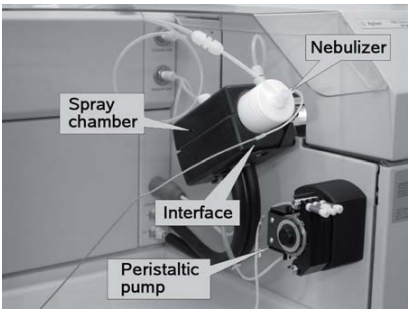

Figure D4: *Detailed view of the physical interface between chromatography and atomic spectrometry. The only connection that needs to be made is between the outlet capillary of the column and the nebulizer system of the ICP-MS.*

Table D2: Nebulizer types and spray chambers used in IC-ICP-AES and IC-ICP-MS applications

Nebulizer type	Mass-flow-dependent	Sensitivity	Desolvatization required	Used for
Pneumatic nebulizer (Meinhard, cross-flow, V-groove)	yes	low	no	Aqueous eluents, with limitations also for organic solvents
High-efficiency nebulizer (HEN)	yes	high	yes	Aqueous eluents, with limitations also for organic solvents
Ultrasonic nebulizer (USN)	yes	high	yes	Functions best or clean water samples; efficiency depends strongly on viscosity and salt content
High pressure nebulizer (HHPN)	yes	high	yes	Best choice for viscous samples, high efficiency for many sample types
Direct injection nebulizer (DIN)	no	low/ high (relative/ absolute)	no	For all types of eluents, useful for Hg, B, I and other elements with high carryover using conventional nebulization
Scott spray chamber	–	–	no	Standard equipment of many ICP-AES and ICP-MS
Cyclone chamber	–	–	no	Small inner volume, rapid stripping times
Desolvatization	–	–	–	Large volume, large inner surface; causes physical and chemical interferences

This means that at low flow rates the relationship between mass flow of the analyte and signal intensity is linear and at higher flow rates the signal intensity is constant. This behavior can be utilized to increase the sensitivity of the coupling.

If an external calibration is to be used for the online application then the flow rate used for the chromatography must agree exactly with that used for the calibration. The flow rate stability is also extremely important for low scattering of the results [D11].

Due to their large surface areas, some easily adsorbable analytes cause considerable problems in pneumatic nebulization systems. These include compounds of the elements mercury, iodine, thallium and silver, which interact with polymer components of the sample injection system. Boron compounds cause other problems, which lead to complications on glass surfaces. This means that the nebulization system cannot be neglected as a source of adsorption and carryover.

A further quality feature is the signal broadening triggered by the nebulization system. The typical signal width is determined by the column geometry. In microbore applications signals with half-width values of less than 1 s are obtained, whereas standard applications using columns with an inner diameter of 4 mm produce typical signal widths of 5 to 30 s. Signal broadening can be influenced by the choice of nebulizer chamber; the inner volume of the nebulizer chamber should be kept as low as possible. However, this has an adverse effect on the signal stability in ICP.

A general advantage of the IC-ICP online coupling is the constant eluent composition. This means that ICP-AES or ICP-MS can be operated with a constant matrix for the whole time, which has a positive effect on the long-term stability of the spectrometer. Performance, gas flows and other instrument-specific settings can be optimized for exactly defined operating conditions with ICP-AES and ICP-MS.

2.4 Data acquisition and software

The detection of individual elements by using an emission line or a mass-to-charge ratio (m/z) presents no problems to either ICP-AES or ICP-MS spectrometers. If the number of lines or masses to be observed simultaneously increases, then special requirement profiles are the result. With ICP-AES a polychromator is essential, because monochromators cannot change wavelengths quickly enough. In the use of echelle spectrometers with surface detectors the reading speed of the array is the limiting factor. Considerable differences exist depending on the construction principle.

In the case of ICP-MS with a quadrupole the transition is smoother. As a result of the high rate of change of the m/z-ratio (up to 3000 mass units per second) the quadrupole can observe several masses quasi-simultaneously. Only above this level does a simultaneously operating mass spectrometer such as a TOF or a multi-collector system become necessary.

In modern instruments software support for the rapid, time-resolved readout of several channels is provided by the manufacturer; as a rule with older spectrometers an individual solution must be found.

The only quasi-simultaneous recording of the quadrupole ICP-MS influences the accuracy of the peak height determinations and the determination of possible isotope ratios in IC-ICP applications. The relative error in the peak height determination becomes important when the time for a scan and the signal half-width value are of the same order of magnitude. If the scan time is 1 to 2 s then this source of error is normally negligible.

3 Applications of IC-ICP-AES and IC-ICP-MS online coupling

Examination of previously published applications shows that they are divided into three fields [D12], whose strategy is explained in Figure D5. The most successful field of application to date is element species analysis, for which in many cases there is no alternative to online coupling. In the variety of possible combinations and applications of IC-ICP-AES and IC-ICP-MS online couplings other highly interesting fields of work are still slumbering. While for element species analysis normally only an HPLC detector is replaced by an efficient atomic spectrometer, the second main focus – multi-element ultratrace analysis – requires a higher degree of adaptation on the chromatography side. With consequent utilization of the strengths of atomic spectrometry, which are characterized by the terms element specificity, sensitivity and multi-element capability, the coupling can be upgraded to form an ultratrace method by adapting the chromatography system to meet the requirements of atomic spectrometry, at least for suitable chemical matrices.

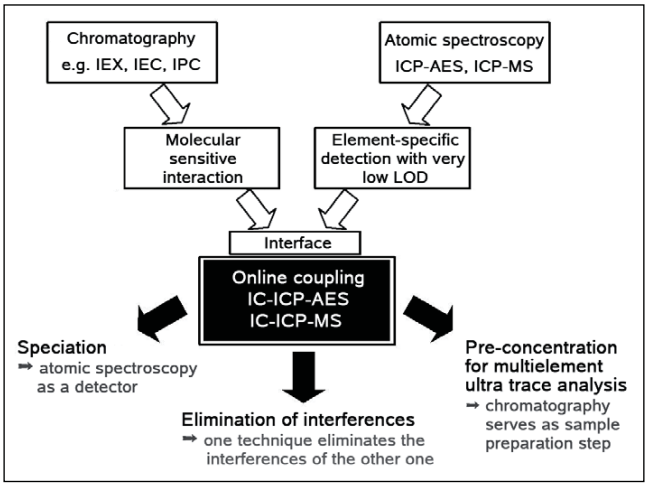

Figure D5: *Schematic of IC-ICP-AES and IC-ICP-MS online couplings.*

A somewhat different field is the selective elimination of interferences to atomic spectrometry by using a liquid chromatography separation. The elimination of interferences places an approximately equal weight on the problem-solving abilities of chromatography and atomic spectrometry. Applications working with such a distribution come from the field of trace and ultratrace analysis of complex systems. A special mention must be made here of isotope dilution analysis as an analytical technique that on the one hand works with a high degree of accuracy and on the other hand places high demands on freedom from interference.

3.1 Element species analysis

The field of element species analysis has already been the subject of many reviews [D13-D17]. The philosophy on which it is based can be found in several reviews and monographs [D13, D14]. Before 1990 the type of detection used was usually AAS and ICP-AES, since 1990 ICP-MS has been used almost exclusively. Table D3 provides an overview of the elements that have been subjected to a species analysis by ion chromatography. The separation system was designed according to the species to be separated. Permanent ionic species are typically separated by ion exchange chromatography, with arsenic being the most versatile element in this case. Arsenic has numerous stable anionic, nonionic

and cationic species. Other elements such as lead and mercury form nonionic species and are therefore preferably separated by reverse phase or ion pair chromatography.

In addition to the removal of interferences (Section 3.3), element species analysis is the most important field of application for IC-ICP-AES and IC-ICP-MS, because good utilization of the benefits of IC is possible here. Often there is no alternative to speciation by ion chromatography. An important disadvantage of IC is the limited chromatographic efficiency, which normally does not permit the separation of more than 10 components in a single system.

Table D3: Examples of IC-ICP-AES and IC-ICP-MS applications in the element species analysis sector

Element	Separation mode	Separated species	Comments	Literature references
Al	Anion/cation	Al(III) and Al-complex species	Chromatographic separation of unstable complex species	[D25]
As	Anion/cation	As(III), As(V), anionic or cationic, organic As compounds	Most frequent species application	[D26]
Cr	Anion/cation/ ion pair	Cr(III), Cr(VI)	Slow kinetics of Cr(III) complex formation causes problems	[D27]
Hg	Ion pair	Cationic Hg compounds	Critical element in ICP applications	[D28]
Pt	Anion	Anionic Pt complexes	Strongly adsorbed anions, catalyst abrasion	[D29]
S	Anion	S^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, SCN^-	Detection as $^{32}S^{16}O^+$	[D30]
Sb	Anion	Sb(III), Sb(V), organic Sb anions	Non-assigned signals	[D31]
Se	Anion	Se(IV), Se(VI), organic Se anions	Hydride formation possible	[D27]
Sn	Cation	Tin organyls	Usually sediments	[D32]

3.2 Preconcentration for multi-element ultratrace analysis

Preconcentration is an established method in analytical chemistry which can be used when the required detection limits cannot be achieved.

The literature concerning solutions to such problems for older instrumental techniques such as photometry, X-ray fluorescence, AAS and also including ICP-AES supplies many applications based on ion exchange [D18, D19]. Most of these techniques can also be transferred to IC-ICP-AES and IC-ICP-MS systems. Table D4 summarizes the activities of IC-ICP-AES and IC-ICP-MS in the preconcentration and ultratrace analysis fields. The most important fields of application lie in the development of ultratrace methods for environmental problems and for high-tech chemicals in the semiconductor sector.

Preconcentration as an extreme example of chromatographic separation immediately offers two ways of increasing the sensitivity. Each chromatographic separation step results in dilution of the analytes, as the peak volume is normally far larger than the injected sample volume. In comparison to offline fraction collection, online coupling at least allows the time-resolved determination of the analyte and in this way permits the utilization of the higher analyte concentration in the maximum of the elution signal. The simultaneous matrix separation also reduces its negative influence in the form of interferences and signal suppressions.

Implementation of a preconcentration step in a chromatography system offers the following advantages:

- the closed chromatography system reduces the risk of contamination by environmental influences
- the completely metal-free IC systems are ideally suitable for metal trace analysis in particular
- modern IC systems permit the use of high-efficiency packing materials with rapid mass transfer and therefore quicker equilibration; the elution process is also simplified.
- the highly reproducible flow rates of modern IC systems ideally match the requirements of a mass-flow-dependent detector

Table D4: Examples of IC-ICP-AES and IC-ICP-MS applications in the preconcentration sector.

Sample / matrix	Separation mode	Separated analytes	Preconcentration column	Detection limits	Literature references
As, Mo, W, P, Re	Cation Anion	Li, Be, Na, Mg, Al, Ca, Sc, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Sr, Y, Ag, Cd, Ba, rare earths, Tl, Pb, Bi, Th, U	SCX (AG 50W) SAX (AG1X-8)	0.1...80 ng/g referred to the solid	[D11, D20]
MoSix, WSix	Cation	Analog to As application	After volatilization of SiF_4 SCX (AG 50W)	0.2...200 ng/g in solid	[D33]
Seawater, wastewater	Complex formation	Cd, Co, Cu, Fe, Mn, Ni, Pb, Ti, V	Iminodiacetic acid	Not given	[D34]
Alkali and alkaline earth metals, anions, seawater	Complex formation	V, Cr, Ni, Co, Cu, Mo, Pt, Hg, Bi	Bis(carboxymethyl)-dithiocarbamate adsorbed on XAD-4	9...80 ng/L (0.5 mL sample loop, no dilution)	[D35]
Seawater, river water	Complex formation	Mn, Co, Cu, Cd, Pb, U Fe, Mn, Co, Ni, Cu, Zn, Cd, Pb	Iminodiacetic acid, 8-hydroxyquinoline on silica gel	6...200 ng/L 0.8 ng/L (with IDA*)	[D21, D36, D37]

* IDA = isotope dilution analysis

An interesting group of applications comes from the analysis of semiconductor chemicals. The simplest application is the determination of traces in ultrapure water or hydrogen peroxide as working and etching media. In hydrogen peroxide analysis the lack of resistance of the ion exchangers normally used causes problems. By switching to highly cross-linked ion exchangers it is possible to considerably extend their working lives. The gas evolution of solutions containing H_2O_2 continues to cause problems which can be avoided by chemical decomposition of the H_2O_2 or by an appropriate overpressure in the system [D30].

The determination of traces in pure elements represents the next higher degree of difficulty. The actual extent of this difficulty is determined by differences between the chemistry of the matrix element and that of the interesting trace contaminants. The purity of the chemicals necessary for the digestion and the separation step is also an important aspect [D20]. The frequently used matrices Mo and W are characterized by an enormous variety of interferences in all atomic spectrometry working techniques. From the point of view of ion chromatography Mo and W as anion formers clearly have a different chemical behavior from that of the most interesting trace contaminants. With a suitable choice of digestion and separation conditions more than 30 elements can be preconcentrated as

cationic species from peroxomolybdenic acid and peroxotungstic acid. Further matrices that have proved to be ideal for the online coupling are the material Re and the classical n-doping arsenic and phosphorus.

The basis of the outstanding detection limits of the IC-ICP online coupling is already provided by the separation step. The combination of excellent separation with residual amounts of a few µg of the matrix element and the preconcentration volume of the analyte, which can correspond to more than 2 g of the matrix element, are decisive. A corresponding experimental setup is shown in Figure D6. The use of three valves in the flow path allows automatic sample injection and rinsing, alternating preconcentration on two preconcentration columns and also inline calibration using a sample loop. Because of the element selectivity of the ICP techniques no problems are caused by overlapping chromatographic peaks of species of different elements. The combination of the element selectivity of the detection and time-resolved data recording permits maximum sensitivity of the online coupling. Numerous optimizations such as splitting the separation column in the «dual-column» technique or the consequent utilization of the control possibilities of modern IC instruments also contribute to the low detection limits. These are approx. 10 to 100 pg/g for IC-ICP-MS and 10 to 100 ng/g for IC-ICP-AES for the 30 to 40 elements that can be determined by each technique. The time required allows the analysis of 2 samples per hour, which is far superior to all other alternatives. As an example, Figure D7 shows the preconcentration and elution of uranium from a Mo matrix, with increasing preconcentration volumes being used. The large signals at the start and after 23 min come from inline calibration using an uranium standard. Figure D8 shows the excellent accuracy of the coupled IC-ICP-AES and IC-ICP-MS methods in comparison to isotope dilution analysis with isotope dilution mass spectrometry (IDMS).

For trace analysis in complex matrices such as seawater the selectivity of simple ion exchangers is inadequate [D21], which is why chelating ion exchangers are used. The high selectivity of the complex formation permits the analysis of selected groups of analytes, with the multielement capability being clearly reduced in comparison to simple ion exchange.

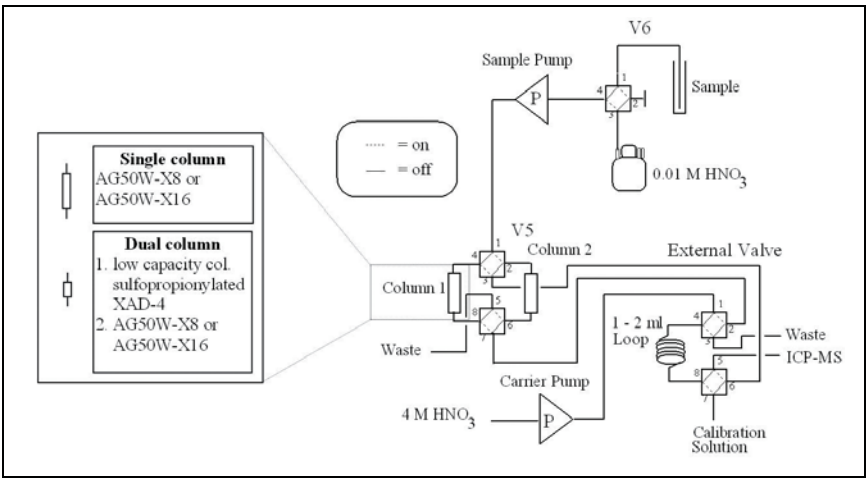

Figure D6: *Setup and flow path for an application for the preconcentration of cationic traces from peroxotungstenate and peroxomolybdate solutions.*

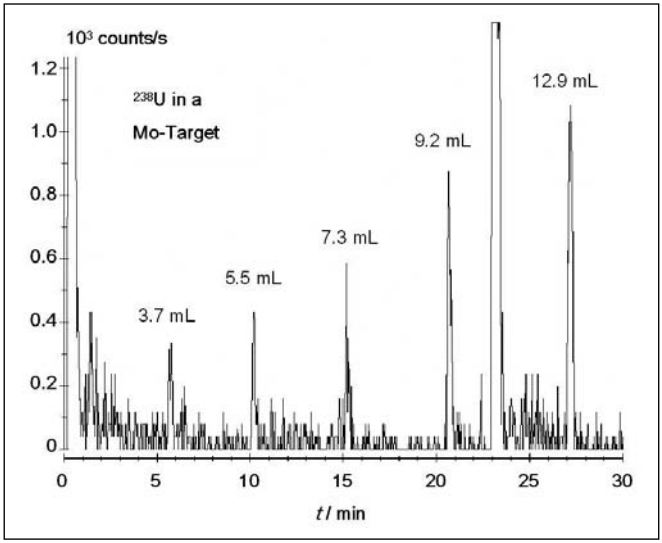

Figure D7: *Chromatogram of a preconcentration – five times repeated – of uranium traces in the digestion solution of a high-purity Mo target. In each case the preconcentrated volumes appear above the signals.*

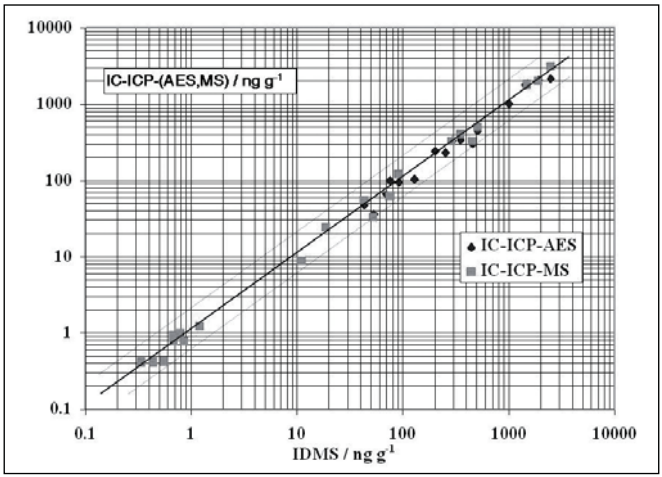

Figure D8: *Comparison of the results of IC-ICP-AES and IC-ICP-MS for the trace preconcentration of molybdenum and tungsten samples with the data of the isotope dilution analysis with thermionic mass spectrometry as the final determination (IDMS).*

3.3 Elimination of interferences

The elimination of interferences in IC or in ICP-AES and ICP-MS can be carried out by using the other complementary technique. Spectroscopic interferences are a well-known problem in ICP-AES and ICP-MS, for whose elimination there are, of course, other solutions available apart from the use of IC.

The first approaches in ICP-MS utilized the natural abundance of the isotope of an element to eliminate inter-element interferences by molecular ions or doubly charged ions by mathematical means.

More efficient, but also more complex ways utilize special instrumental properties such as «cool plasma», collision cells, high resolution or specific sample application such as the hydride system [D5, D8].

If such aids are not available or do not work then IC-ICP coupling becomes increasingly more interesting. A prominent example is the resolution of interferences in the rare earth metals (Table D5). This relatively small group of analytes with very similar chemical behavior can – by making use of the small differences in complex formation – be separated very well by ion chromatography using several reagents. The separation with an α-hydroxyisobutyric acid gradient (HIBA) on a sulfonic acid exchanger permits elimination of the interferences by isobaric ions, by isobaric oxide ions and even by isobaric hydrides, which cannot be separated or decomposed by any of the other techniques.

Table D5: Examples of IC-ICP-AES and IC-ICP-MS applications involving the removal of interferences on the atomic spectrometry or IC side.

Sample / Matrix	Separation mode	Interference ICP	Interference IC	Comments	Literature references
As species in urine	Anion	$^{40}Ar^{35}Cl$ on ^{75}As	–	Prototype of this application type	[D39, D40]
Mo	Anion	MoO_2 on Te	Other anions	Ultratrace analysis	[D11]
Rare earths and their oxides	Cation	Oxide and hydride cations of light rare earths have the same masses as the heavy ones	Signal overlap due to inadequate selectivity	HIBA and butyric acid gradients	[D38]
Transition metals in S and Cl matrix	Anion	S and Cl-based interferences on V, Cr, Cu, Zn, As and Se	No IC application	Offline application with possibility of online coupling	[D41]
Se in human urine and serum	Anion	ArCl on ^{75}As and ^{77}Se	Other anions	Both online and offline	[D42]
BrO_3^- in water	Anion	Ar_2H on ^{81}Br	Cl^-, NO_2^-	Important in drinking water analysis	[D24]
IO_3^- in water	Anion	–	Early eluting anions	Also element species analysis	[D43]
Fission products	Anion/ Cation	$^{90}Sr/^{90}Zr$ $^{137}Cs/^{137}Ba$ Rare earths	Other anions / cations	Resolution of unusual isobaric interferences	[D44]

Newer applications are concerned with the determination of oxohalides in water samples and make use of the outstanding sensitivity of ICP-MS as well as its element selectivity for solving problems in ion chromatography. The most important application is the determination of bromate, which can serve as a textbook example for the development steps of an online coupling application. Figure D9 shows the typical concentration ranges and retention factors $k' = (t_R - t_M)/t_M$ (previously known as capacity factor k'; t_R = retention time of a compound, t_M = dead time) for anions in water samples. It can be easily seen that bromate elutes at practically the same time as chloride, but is present at much lower concentrations in the sample.

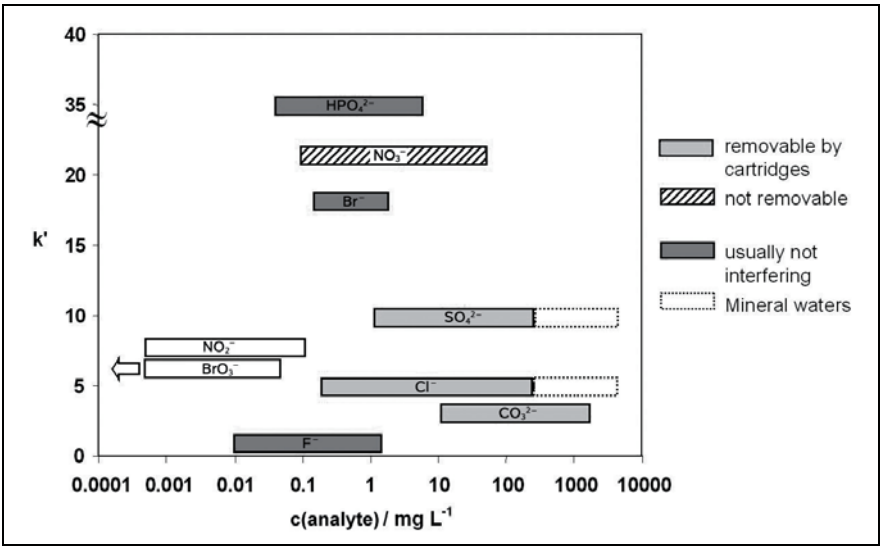

Figure D9: *Comparison of the typical concentration ranges of some anions in drinking water and their retention factors k' in anion chromatography.*

The first IC-ICP-MS applications for the determination of bromate used a pure IC application and replaced only the conductivity detector by ICP-MS. Even the suppressor was retained [D22]. Improvements in IC and ICP-MS increased both the speed and the accuracy while lowering the detection limits. On the IC side the low-capacity ion exchangers for use with conductivity detection were replaced by high-capacity types with a comparable performance [D23, D24]. This permitted considerably larger sample volumes and lowered the detection limits. The next step was the replacement of the usual eluents NaOH and Na_2CO_3, which were used in concentrations of up to 180 mmol/L and therefore made suppression essential, by NH_4NO_3 eluents. NH_4NO_3 is a virtually perfect eluent for ICP-MS, as all its decomposition products are already present in the plasma and therefore no additional interference is produced. From the IC viewpoint nitrate is also a suitable eluent; although it is sufficiently strong it does not lead to dynamic blocking of the exchange functions. The sensitivity of the coupling technique achieved by these optimizations can be seen in Figure D10, in which the analysis of a mineral water spiked with 0.1 µg/L bromate is shown.

Optimization on the ICP-MS side started with a special setup for the determination of bromine. The detection limits could then be improved by the use of high-efficiency nebulizers as well as the possibility of isotope dilution analysis by the use of the collision cell technique. For isotope dilution it is necessary to eliminate interfering molecular ions (see Figure D11).

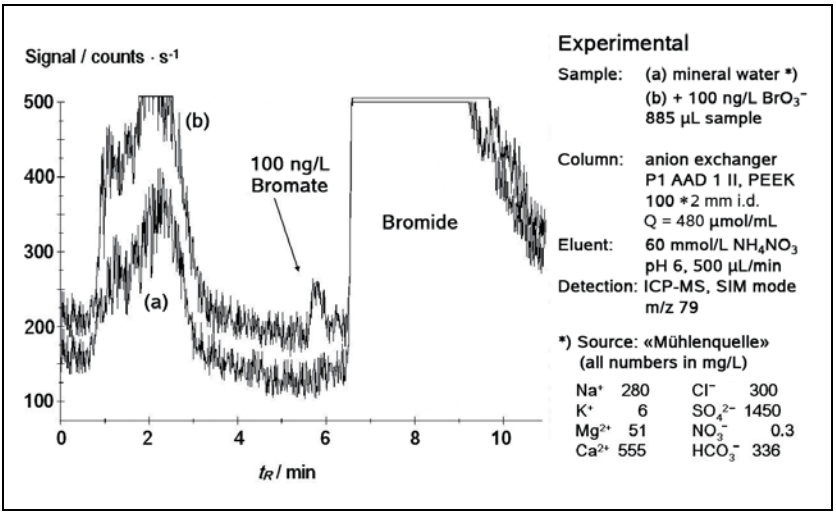

Figure D10: *Sensitivity of the IC-ICP-MS method for the determination of bromate in mineral waters with a high salt content. The lower curve shows the pure mineral water, the upper one the same water spiked with 0.1 µg/L bromate.*

By using isotope dilution analysis for calibration [D24], an IC-ICP-MS application results that allows bromate analyses to be carried out in the sub-µg/L range in less than 10 min with a high degree of accuracy and precision. As an example of the accuracy, Figure D12 shows the results obtained by IC-ICP-MS within the framework of a ring test in comparison with the theoretical values.

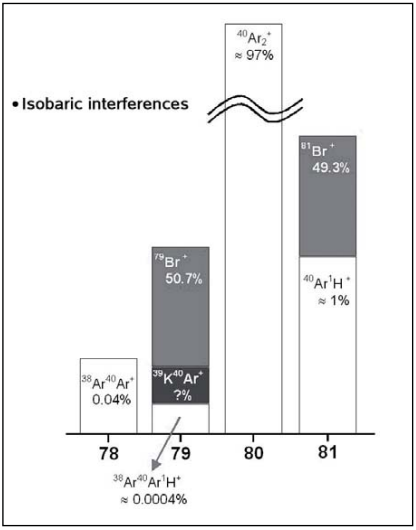

Figure D11: *Isobaric interferences in the mass range 78 to 81. In order to be able to use isotope dilution analysis, the molecular ions interfering at mass 81 must be eliminated.*

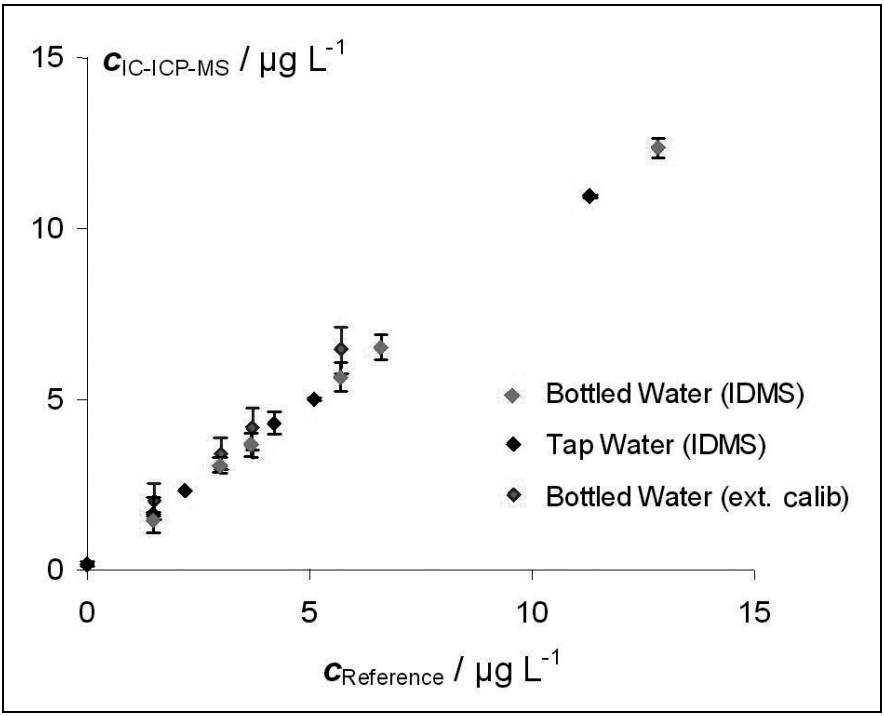

Figure D12: *Comparison of the results of IC-ICP-MS for bromate with the reference values of a round-robin test.*

4 Conclusions

The IC-ICP-AES and IC-ICP-MS online couplings are always attractive when the advantages of one partner can be used for solving the problems of the other partner. In this way molecular or charge information can be transferred to atomic spectrometry by using ion chromatography. The opposite case is the upgrading of ion chromatography to an element-specific separation of individual species.

For a successful application both partners must be adapted to suit the particular demands of the coupling (column, choice of eluent, nebulizer system, operating modes). The sensitivity of ICP-MS for many analytes exceeds by far that of conductivity detection, whereas ICP-AES only offers advantages with respect to the sensitivity for some elements. However, ICP-AES also offers element-specific detection, which can be very important with complex samples. While the realization of the coupling is relatively simple on the hardware side of the ICP, separation columns adapted to the coupling are often lacking on the ion chromatography side. The software integration of the IC-ICP coupling is usually adapted from LC-MS or from diode-array detectors.

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E – Post-column derivatization in ion chromatography

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1 Introduction

In the context of analytical chemistry methods derivatization means the conversion of an analyte into a different compound which, because of its better/more favorable properties, is easier to determine than the original substance. For example, in spectrophotometry this idea is used in a very versatile manner by converting the substance that is to be determined, which is often colorless, selectively (and with as high a degree of selectivity as possible) into a colored compound. Derivatization reactions also play a role in chromatography. A basic differentiation is made between precolumn and post-column derivatization. In this case the aim of a derivatization can be better separability (in precolumn derivatization) or the better detection of the already separated components (in post-column derivatization) [E1].

Precolumn derivatization plays almost no part in combination with ion chromatography (IC). The manageable number of ions (in comparison to the determination of organic substances using HPLC methods) that can be separated by IC and the relatively large differences in their physical-chemical properties usually do not cause any great difficulties during the separation. Solutions for complex separation problems are achieved by improved column materials, optimization of the mobile phase and/or the combination with various (sometimes highly selective) detectors.

Post-column derivatization is a technique that is relatively rarely used in IC, and virtually no summarizing literature exists on this subject [E2-E4]. Among other things, this is due to the fact that with conductivity detection an almost universal detection method is available that is also characterized by a high degree of sensitivity for the determination of most ions. But all the same there are, as already mentioned in other contributions to this monograph, special problems or particular reasons that require the use of a different detector or its use in addition to conductivity detection.

Because of the fact that post-column derivatization offers a number of interesting and attractive possibilities for detection it will be covered in detail in this contribution. The basic concepts, instrumental aspects as well as various applications are presented below.

2 Post-column derivatization

2.1 The ideas behind post-column derivatization

Post-column derivatization can be regarded as being a special form of IC detection. This is why the term «reaction detector» is frequently used for this technique. This already implies the combination of the derivatization step with the subsequent detection of the reaction products formed. Both steps take place independently of one another, but it makes sense to always regard them as being a combination. In this way the choice of the derivatization step is always linked with a particular detection method. Generally speaking, each selective alteration made to the mobile phase or the analytes contained in it that takes place on the way between elution from the column and transfer to the detector can be regarded as being a post-column derivatization. This means that one can (and one does) also regard the chemical suppression of the conductivity with column or membrane suppressors as a special form of post-column derivatization. If this is further extended intellectually then the nebulization of the mobile phase and the subsequent atomization and formation of ions in the gas phase, which occurs when IC is coupled with atomic spectrometric and mass spectrometric detection techniques, can also be assigned to post-column derivatization (see Sections B and D).

As far as the possible combinations of derivatization step and detection method go, numerous different versions can be imagined and some of them have been realized [E2, E3]. Apart from chemical reactions, electrochemical conversions at electrodes and light-induced (photolytic) processes have been described for converting the analyte into a species that can be detected more easily. In addition to spectrophotometry, detectors are also based on other molecular spectrometric methods (e.g. luminescence), various electrochemical detection methods (potentiometry, amperometry) as well as mass spectrometry.

2.2 The aims of post-column derivatization

As already mentioned in the introduction, the ultimate aim of post-column derivatization is the improvement of the detection properties of the analytes separated on the column. This basically has two aspects: an improvement of the sensitivity as well as an improvement or alteration in the selectivity.

An increase in sensitivity is frequently of interest as numerous IC applications concern trace analysis problems. A particular necessity for increasing the sensitivity is required for those ions that cannot be detected at all (or not sensitively enough) with the predominantly used conductivity detector. For direct conductivity detection (without suppression) this applies to analyte ions whose equivalent conductivity is of a similar magnitude to that of the corresponding ions of the mobile phase. With the suppressor technique weakly dissociated ions (e.g. sulfide, cyanide) only cause a slight alteration in the conductivity and therefore can only be detected very insensitively.

In general, the aspect of the selectivity of the detection is to be considered ambivalently for chromatographic methods. On the one hand the idea behind chromatography is to separate all the components contained in the sample, so that the real function of the detector can be regarded as being only the localization of the eluting species (via the retention times) and their quantification. The latter is done by calibration and measuring the peak heights or areas. Conductivity measurement as a universal detection method for ions has therefore achieved its own particular importance in IC. There are also reasons for using detectors with a higher selectivity (that do not «see» everything). In this way during simultaneous elution, or not completely separated analytes eluting in succession, a selective detector that only recognizes one of the two components provides a solution to the problem of unsatisfactory separation. The combination of universal and selective detectors (e.g. in serial arrangement) can be of particular interest in such cases. A further problem with universal/non-specific detectors is the fact that they indicate the elution of an analyte, but cannot provide any further information about its identity. The assignment of the signals is carried out solely by using the retention times that have previously been determined for the various analytes under the given experimental conditions in a calibration step. This means that components eluting at the same retention time cannot be differentiated, which leads to an incorrect interpretation and/or falsification of the quantitative determination. In some cases it may be possible to remedy this shortcoming by selective detection. However, the price for this is the loss of information about all the other components that the selective detector does not record. In this case the combination of universal and selective detectors is an interesting possibility that has also been realized in some cases. A detector that combines both properties, i.e. is universal in the sense that it detects all analytes while at the same time ensuring their identity, would be the optimal version. With mass spectrometry (see Sections A and B) as the chromatographic detector this ideal has been realized to a certain extent.

The possibilities of improving the sensitivity and increasing the selectivity, which can be achieved by post-column derivatization, depend to a crucial extent on the selection of the derivatization reaction, the layout of the reactor (in which the derivatization takes place) as well as the detection method used. These aspects are discussed in detail in the following sections.

2.3 Basic configuration

Possible basic configurations of an ion chromatography system with post-column derivatization are show in Figure E1. Three different versions can be differentiated. If detection takes place only by post-column derivatization then after leaving the column the analyte ions are derivatized in a reactor (post-column reactor, PCR) and transported to the detector. There is also the possibility of combining post-column derivatization with the usual conductivity detection. For this the PCR can either be switched downstream in series or a parallel arrangement can be used. Which of these basic configurations is selected in a particular case depends on the problem to be solved. The use of a PCR alone is advisable when either a single ion is to be determined selectively or if a reaction is to be used that is so unselective that all the ions of interest can be determined in this way.

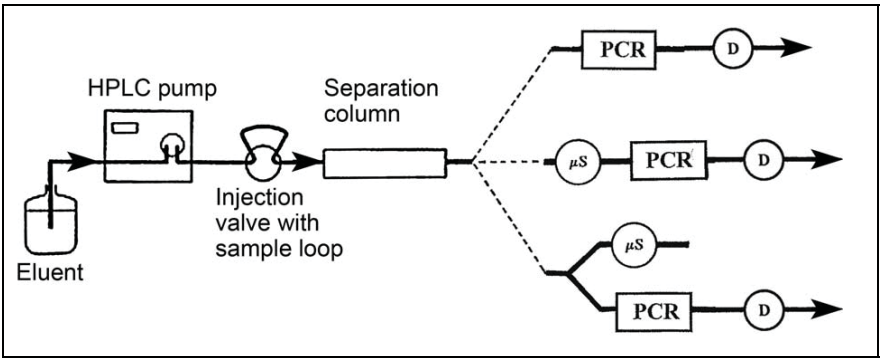


Figure E1: *Possible IC configurations with post-column derivatization. The diagram shows the use of a PCR (post-column reactor) alone as well as serial and parallel operation together with a conductivity detector.*

The combination of conductivity detection and PCR is used if the determination of numerous ions can be carried out with adequate sensitivity by conductivity detection, but a further ion of interest (or even a few others) can only be detected by PCR. In this way the range of ions that can be determined simultaneously is extended, but compromises frequently have to be made concerning the choice of the mobile phase and the PCR conditions.

In order to carry out a post-column derivatization the mobile phase leaving the separation column must be mixed with the derivatization reagent in a suitable manner so that conversion occurs. This takes place in a reactor, which can have various shapes. In principle a differentiation can be made between solid phase reactors and those based on a reaction in a homogeneous phase.

For the reaction in a homogeneous solution in the simplest (and most frequently used) case the derivatization reagent is combined with the mobile phase using a T- or Y-piece. The mixing and reaction then take place in a tube of varying length that is connected to the flow-through detector. If necessary, several reagents can be added in succession and mixed.

This form of post-column derivatization is very flexible in use as the experimental conditions can be varied very easily and adapted to meet the specific demands for optimal reaction conditions. In the following section the theoretical aspects of the mixing process and zone broadening in tube reactors as well as the resulting aspects concerning the optimal arrangement of the instrumentation are treated separately because of their particular importance.

A different type of reagent mixing can be realized with membranes [E3, page 393]. In this case the reagent is kept under a slight overpressure in a reservoir behind a membrane; this achieves a uniform rate of permeation through the membrane. The mobile phase is led through a specially constructed module on the permeate side of the membrane and the reaction takes place in a downstream mixing loop. A pump for transporting the reagent is not required. Dilution by the addition of reagent is slight and virtually no pulsation occurs. This type of mixing requires special membrane modules with suitable membrane materials and is therefore less flexible than mixing via a T-piece.

An alternative to mixing loops and membrane addition is offered by fixed-bed reactors in the form of packed columns or cartridges located downstream from the separation column. In this case the reagent is immobilized on a solid (if necessary the solid itself can be the reagent). When the mobile phase passes through, the conversion reaction takes place in the form of a surface reaction. However, this type of post-column derivatization is seldom used, as the immobilization of reagents is not always easy to realize and is limited to only a few substances. In addition, the working life of fixed-bed reactors is limited by their nature. There is an advantage in that no additional pump is required

and that dilution by the mixing and reaction process is minimal. Examples of fixed-bed reactors for post-column derivatization are redox reactors, enzyme cartridges and the so-called dual electrodes. However, such systems are almost exclusively described in original publications and until now have found virtually no use in practical IC analysis.

2.4 Instrumentation

In the previous sections some of the general aspects of post-column derivatization have been presented. The original literature contains virtually all the possibilities that can be imagined concerning the combination of reactor configuration and detector. However, by far the most important combination in almost all applications is chemical derivatization in tube reactors followed by spectrophotometric detection. Therefore only this version will be covered below. The general arrangement of such a system is shown in Figure E2.

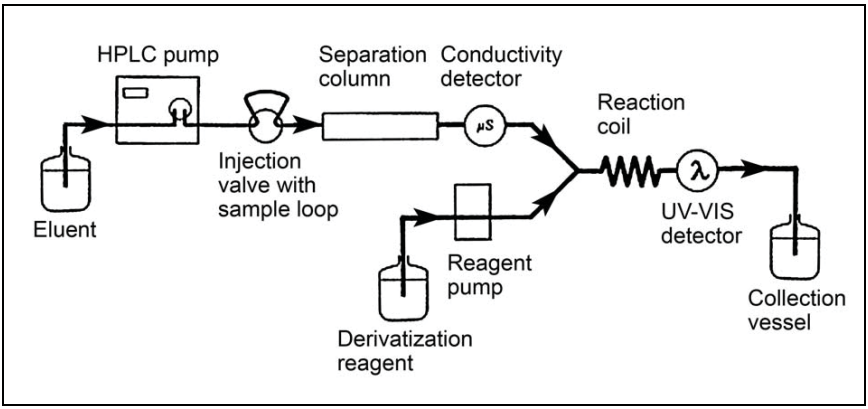


Figure E2: *Setup of an IC system with post-column derivatization in homogeneous solution and photometric detection.*

The selection of the correct components and the optimization of the operational parameters are of crucial importance for the successful use of post-column derivatization. This applies to the transport of the derivatization reagent, the arrangement of the tube reactor and the configuration of the photometric flow-through detector. These are covered in detail below.

2.4.1 Transporting the reagent solution

For optimal operation it is desirable that the transport of the reagent solution can take place at a constant flow rate, pulsation-free and continuously (possibly throughout a long time span). It should also be possible to vary the flow rate for different applications. Further requirements are the compatibility with different reagents (acids, alkalis, organic solvents) as well as operation that requires as little maintenance as possible. Piston pumps, syringe pumps, peristaltic pumps as well as compressed gas systems can be used for liquid transport. None of these possibilities meets all the criteria equally well. Pulsation-free transport is possible with compressed gas systems and syringe pumps. However, these require interrupting the operation to refill the reagents. The use of peristaltic and piston pumps permits interruption-free operation with flow rates that have a very good long-term stability and are also easy to regulate. However, their working principle results in a pulsation of the flow which can have a negative effect on the stability of the baseline as well as on the reproducibility of the measuring signals. The use of pulsation dampeners may help in such cases. In the simplest case this could be

a very long piece of moderately flexible tubing. There are also other versions with membranes or with small air-filled columns (equivalent to electronic damping using an R/C element).

2.4.2 Arrangement of mixing and reaction paths

Various aspects must be taken into consideration when designing a reactor for post-column derivatization (see Figure E3). Good mixing of the mobile phase and the reagent solution must be ensured, the reaction time must be long enough to generate a detectable product and finally the resolution of the ions eluting from the column must not alter to any great extent. This final aspect is linked with signal-broadening processes, which are unavoidable when transporting the analyte zones from the column to the detector (see Box 1).

- Constant mixing ratio between mobile phase and reagent solution
- Reproducible mixing conditions
- Intensive mixing
- Minimal dilution of the eluent
- Sufficiently large reaction time
- Small contribution to peak broadening of the chomatographically separated components

Figure E3: *Design criteria for mixing and reaction paths.*

The choice of instrumental configuration and the definition of the operating parameters result in consequences that are outlined below.

A constant mixing ratio is ensured by the constant flow rates of the IC and reagent pumps. To keep the dilution of the mobile phase as low as possible mixing-in the reagent should take place at as low a flow rate as possible. However, there must be a sufficiently high reagent concentration present in the mixing loop, which may make the use of highly concentrated reagent solutions necessary. The reproducibility of the mixing process is usually guaranteed by the defined shape of the tube reactor (length and inner diameter of the tube and type of arrangement) and the given flow rates. Intensive mixing can be realized in various ways. Mixing already takes place when the liquids are brought together in the T- or Y-piece; this continues during transport through the tube reactor. The geometrical arrangement of the tube is to a high degree responsible for the mixing intensity (see Box 1). Special mixing chambers (sometimes with built-in magnetic stirrer) or so-called jet-mixers are also proposed; these produce spontaneous mixing.

With good mixing the available reaction time for post-column derivatization is the same as the dwell time of the reactants in the reactor. This is determined by the total flow rate and the volume of the tube reactor. An increase in the dwell and reaction time is accompanied by a broadening of the analyte zone. Band broadening can be kept sufficiently small even with longer dwell times by suitably designing the shape of the tube reactor (see Box 1). It can be mentioned here that with exactly defined experimental conditions a complete reaction (in the sense of achieving equilibration) is not necessary, so that an acceptable compromise must be found with respect to band broadening, reaction time and conversion rate.

BOX 1

Peak broadening in chromatography – reasons for reduced resolution and sensitivity

The aim of optimizing chromatography is to obtain a good separation (resolution) of the eluting components with as narrow peaks as possible – and this in as short a time as possible. Band broadening unavoidably occurs in all chromatographic methods as a result of diffusion and phase-transfer processes within the separation column. The basic theoretical relationships are mostly described on the basis of the model observations of the chromatography process introduced by van Deemter and later refined.

For the optimal arrangement of a chromatography system it is not just the processes that take place on the separation column that need to be taken into consideration, but also band broadening processes that occur on the way between injection and the separation column and, in particular, those that can occur after the elution of the analytes. These are often summarized under the term «extra column band broadening».

For example, in the configuration of a flow-through detector the dead volume is extraordinarily important. If it is too large then an overlapping detection of analytes that have actually been separated could occur. In post-column derivatization band broadening processes occur in the transport and mixing processes in the reaction loop and in the flow-through detector. These must be given sufficient attention when designing the whole system.

In the past attempts have not failed to describe band broadening using models (based on general fluid dynamics) and then using these as a basis for optimizing the configuration of flow-through detectors [E8]. In the arrangement of tube reactors the most important parameters to be considered are the length and inner diameter of the tube, the way in which the tube is arranged and the flow rate. In principle band broadening decreases as the flow rate decreases and the inner diameter becomes smaller. However, the minimum flow rate in post-column derivatization is dictated by the chromatographic conditions and is therefore more or less fixed.

With regard to the arrangement of tube reactors it is beneficial to either coil them or, even better, to knot them in an orderly manner. In this way the primary axial flow is overlayered by a secondary radial flow; this results in good mixing with the simultaneous reduction in band broadening [E8, 9].

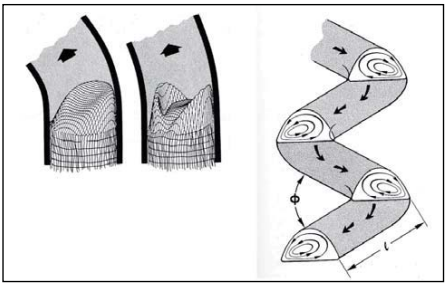


Figure E15: *Schematic diagram of the flow lines when deflecting the flow direction in a thin tube [E10].*

Figure E16: *Knotted (a) and knitted (b) tube reactors for minimizing dispersion with simultaneous good mixing of the reaction partners. The enlargement shows the regularity of the knitting pattern.*

An alternative configuration to the wound tube reactors are microcolumns packed with inert material. In this case good mixing is also achieved by the branching flow in the interstitial spaces. A particularly low dispersion is shown by the so-called single bead string reactors (SBSR) [E11].

2.4.3 Detector configuration

Only the requirements placed on detectors for spectrophotometric determination are formulated here and their different versions and options are presented below. Important aspects that have to be taken into account are the geometry/shape of the flow-through cell, light path length, dead volume, chemical resistance, necessary wavelength range and finally practical considerations such as the possibility of cleaning and the risk of air bubble adherence. In practice virtually only U- or Z-shaped flow-through cells with glass or quartz windows are used. The light path length is typically 1...10 mm and the cross-section of the liquid-transporting channel 0.3...1 mm. Although in principle longer light paths permit higher sensitivities (Lambert-Beer law) this often results in an increased dead volume. In addition, the signal-to-noise ratio is also not improved. If the cell has a small diameter then higher demands have to be placed on the focusing of the light beam.

With regard to the optical system of a spectrophotometer a differentiation must be made between those that measure at fixed wavelengths and those that can cover a wide spectral range simultaneously. In conventional spectrophotometers with filters or grating monochromators the measuring wavelengths are preset and usually cannot be altered during the chromatography run. This is adequate when only a single component is to be determined, or also when the reaction products of the derivatization reaction have the same or approximately the same absorption maxima for different ions. Diode-array spectrometers can simultaneously record the absorption maxima throughout a wide spectral range in a few milliseconds and also repeat this after only a few more milliseconds. This results in a sort of 3-dimensional chromatogram with time, wavelength and absorption axes. This has two advantages; on the one hand the maximum sensitivity for each component can be achieved independently of the absorption maximum and on the other hand the UV/VIS spectrum gives a good indication of the identity of the analyte (by comparison with a reference). Signal overlapping can be detected, if necessary, by assessing the peak purity.

2.4.4 Selection of derivatization reagent

A summary of the most important properties of derivatization reagents and their associated selection criteria is given in Figure E4.

- Selectivity for the analytes concerned
  - Determination of a single analyte
  - Multi-component determination
- Sensitivity of derivative determination (absorption coefficient)
- Wavelength of maximal absorption (UV or VIS range)
- Contribution of reagent to the detector signal (blank)
- Required reaction time
- Miscibility with the mobile phase
- Solubility of reagent and reaction product in the mobile phase
- Stability of reagent solution
- Availability, purity and cost of reagents

Figure E4: *Selection criteria for the derivatization reagent.*

The most important property of the derivatization reagent is its selectivity with respect to the analyte ions to be determined. It may be necessary for it to have a high selectivity (which also naturally means that it may only be possible to determine a single ion) or to have a low selectivity, so that as a result several ions can be simultaneously determined. Both ideas are used in practice and are covered in the following section on examples of applications.

The detection sensitivity is determined by the absorption coefficients of the derivatives. However, the cuvet geometry and the dilution resulting from mixing and dispersion in the reaction loop also influence the sensitivity of the method. A further criterion is the position of the absorption maximum. Photometric measurement in the visible range is instrumentally simpler and can be realized at a more favorable price than in the UV range. In the simultaneous determination of several ions the relative positions of the absorption maxima to one another are also important. If the absorption of the derivatives takes place at the same or approximately the same wavelength, then measurement can be made at a defined wavelength. If this is not the case then losses in sensitivity of the detection for individual analytes must be put up with, or a diode-array spectrometer must be used.

The self-absorption of the derivatization reagent at the measuring wavelength should be as low as possible, as otherwise considerable baseline noise could result due to the incomplete (and not quite uniform) mixing of mobile phase and reagent solution. From purely photometric/technical considerations it is also favorable when the blank value is as low as possible so that the maximum radiated power of the light source reaches the detector.

The necessary reaction time for the derivatization step is of exceptional importance. If it is short then the reaction path between the column outlet and the detector can be kept short, which leads to a lower dispersion of the analyte zone that is linked to a retention of the chromatographically achieved resolution and a higher sensitivity. On the other hand, in reactions with slower kinetics long reaction paths must be selected (which leads to unwanted band broadening; see Box 1), or detection takes place at a time at which the reaction has not yet been completed. Because of the reproducible conditions in a flow-through system this is possible without any loss in reproducibility, but the sensitivity of the measurement is of course reduced. The reaction time can be shortened by increasing the temperature in the flow-through reactor. In this case very good thermostatting must be guaranteed, as otherwise the reproducibility can deteriorate considerably. A further problem could also be caused by the reaction solution degassing at increased temperatures with the result that small air bubbles interfere with the photometric detection.

Further criteria for the selection of the derivatization reagent are its miscibility with the mobile phase as well as the solubility of the reagent and the reaction product in the mobile phase. Finally the stability of the reagent solution as well as the availability, purity and costs of the reagents also play a role.

3 Application examples

As mentioned at the beginning, post-column derivatization has not achieved very much importance as a detection possibility in IC and is mostly used as an extension to the usual conductivity detection. It is used whenever the sensitivity of conductivity detection is inadequate for special problems (or for particular ions) or when the higher selectivity of post-column derivatization can remedy problems caused by inadequate separation.

However, there are also applications in which the combination of IC with post-column derivatization is used very selectively for the determination of a single ion or a few ions. This may then have a great effect on the optimization of the IC, which then no longer needs to demonstrate a high separating capacity, but is possibly only used for the analyte-matrix separation. Another field of application is metal speciation, in which the different chemical bonding forms or oxidation states of a metal are to be determined.

The most important examples of post-column derivatization in IC and their associated analytical specifications are presented below.

3.1 Determination of chromate

Because of its high toxic potential the determination of chromate (CrO_4^{2-}) has received increased attention in recent years. Among other things, this has led to the fact that limits have been set for chromate in drinking water and wastewater as well as for other environmental samples; these make quantification in the lower µg/L range (and partially even below this) necessary. A suitable method for this task is IC with post-column derivatization. This is why this method is also planned in several American standard procedures and standards, and in some cases even stipulated as the only method (see Figure E5). However, this method has not yet been incorporated in German or European standards (DIN/ISO, VDI).

- Drinking water, ground water and industrial effluents
  - US EPA 218.6, issued 1991 (revised 1996, EPA 7199)
  - ASTM D 5257-97
- Characterization of waste and soils (after alkaline digestion)
  - CEN TC 292 (WI 292037 2004-06)
  - US EPA 3060A and 7199
- Workplace surveillance (NIOSH air-filter analysis; OSHA 215)

Figure E5: *Official IC methods for chromate determination with DPC as the derivatization reagent.*

The reaction used for post-column derivatization is that which is also described for the direct photometric determination of chromate (e.g. DIN/ISO 53 314). The reaction is based on the oxidation of diphenylcarbazide (DPC) to form carbazone with Cr(VI) as the oxidizing agent which is reduced to Cr(III). The latter forms an orange-red complex with carbazone with an absorption maximum in the range 520-540 nm. The reaction requires a medium containing sulfuric acid; the reagent concentration is relatively non-critical. The reaction kinetics are rapid (approx. 15-30 s for complete color development), so that a short reaction loop is sufficient.

Chromate elutes relatively late under the typical conditions for anion separation with carbonate/ hydrogen carbonate, which in addition to a long analysis time also results in a lower sensitivity. This is why the simultaneous determination of chromate with other anions by derivatization following conductivity detection is normally not practiced.

If chromate alone is to be determined than the chromatographic conditions can be optimized so that a short retention time results. Figure E6a shows a typical chromatogram. By using a strong eluent, a short separation column and a relatively high flow rate for the mobile phase the retention time can be shortened even more. The literature contains examples in which the total analysis time is less than 1 min.

An interesting aspect of IC is the possibility of significantly increasing the sensitivity of the method by «large-volume injection» [E3, page 442]. If the ionic strength of the sample solution is sufficiently low then the chromate will be concentrated at the head of the column (and, of course, all other later eluting ions); this means that the concentration in the peak maximum is many times higher than in the initial sample. In this way a sensitivity can be achieved that is much higher than that in a direct photometric determination. A typical chromatogram for the determination of chromate in the trace range is shown in Figure E6b. Under the given experimental conditions the determination limit was about 20 ng/L Cr(VI).

The determination of chromate in leather extracts is a special but environmentally critical and analytically challenging task. The sample solutions concerned are strongly colored and can only be analyzed by direct photometry after a tedious sample preparation procedure. Atomic spectrometry methods (if

they are not combined with a preliminary separation step) cannot differentiate between Cr(III) and chromate.

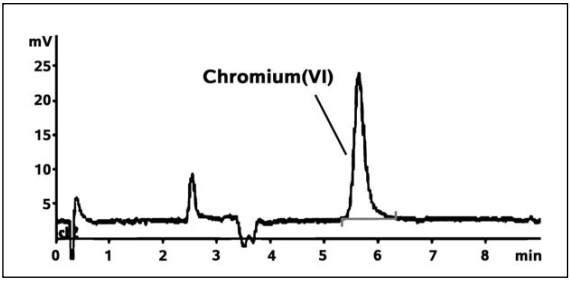


Figure E6a: *1 µg/L chromate; injection volume 1000 µL*

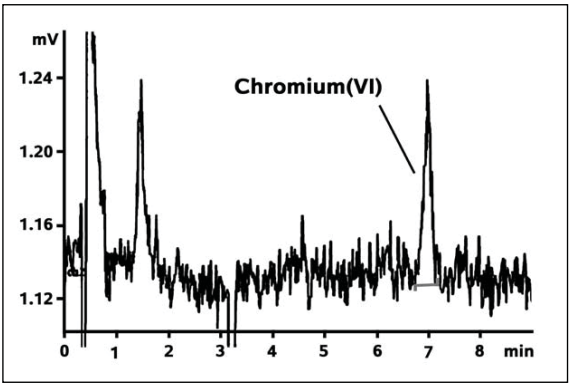


Figure E6b: *0.05 µg/L chromate; injection volume 1000 µL*

Figure E6: *Chromate determination with diphenylcarbazide as the derivatization reagent*

Separation:	*Metrosep A Supp 1*
	Eluent: 16 mM $(NH_4)_2SO_4$ / 6.5 mM NH_3
	Flow rate: 0.7 mL/min
Derivatization/detection:	*0.5 g/L DPC, 0.5 M H_2SO_4 / 10% methanol*
	Flow rate: 0.5 mL/min; λ = 530 nm

An elegant IC method for solving this problem has recently been worked out and presented [E5]. It uses an inline sample preparation procedure with dialysis and a post-column derivatization with DPC. The flow chart is shown in Figure E7. With this fully automated IC method interferences by colored and turbid solutions can be completely eliminated and determination limits obtained that are better then the previously used manual photometric method. It has proved to be very reliable in practical use.

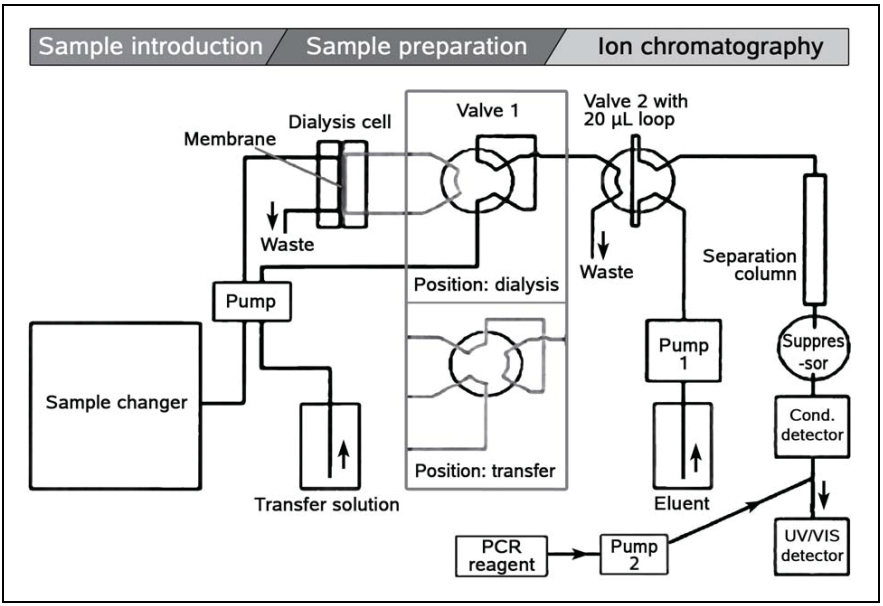


Figure E7: *System configuration for the determination of chromate in leather extracts: inline dialysis for sample preparation coupled with DPC post-column derivatization.*

3.2 Determination of bromate

In recent years bromate has made ignominious headlines as an unwanted ozonization byproduct in the purification of drinking water. The proven toxicity and suspected mutagenicity of bromate have very quickly led to the establishment of limits in the relevant regulations (EPA guidelines, EU directives, drinking water regulations). The current limit for bromate in the drinking water regulations is 10 µg/L. Determination limits of less than 1 µg/L are considered to be desirable.

There are hardly any other alternative methods to IC for the sensitive and selective determination of bromate. However, the typical determination limit for conductivity detection (about 5-20 µg/L) is not sufficient to ensure that the limits are observed. Coupling IC with mass spectroscopy detection (IC-ESI-MS and IC-ICP-MS, see Sections B and D) offers outstanding analytical specifications – with very good detection limits for bromate – but is currently still extremely expensive and therefore not widely available.

For this reason various post-column derivatization methods have been developed for the determination of bromate, some of which have already been stipulated in standards and standard specifications (see Figure E8). One method is based on the conversion of bromate with o-dianisidine (e.g. EPA 317.0). The reaction conditions can be adapted relatively easily and determination limits below 1 µg/L bromate can be achieved. However, o-dianisidine is suspected of being carcinogenic, is not readily commercially available and does not always have a uniform and adequate degree of purity.

- Reaction with o-dianisidine (EPA 317.0)
  (suspected carcinogen; purity problems)
- Reaction with chlorpromazine
  (in 12 mol/L HCl; two-step reaction)
- Reaction with bromide and formation of bromine
- Reaction with iodide and formation of triiodide (EPA 326.0)

Figure E8: *Overview of different versions for determining bromate by post-column derivatization.*

Another reagent with a very high sensitivity for bromate is chlorpromazine. The reaction requires extremely acidic conditions (12 mol/L HCl) and takes place in two steps. Both have no favorable features for practical realization by post-column derivatization.

Probably the best method for the determination of bromate is the triiodide method. It is an adaptation of the classical iodometric titration and is based on the following reactions, which take place in a medium containing sulfuric acid:

$$BrO_3^- + 6\ I^- + 6\ H^+ \rightleftharpoons 3\ I_2 + 3\ H_2O + Br^-$$

$$3\ I_2 + 3\ I^- \rightleftharpoons 3\ I_3^-$$

The addition of catalytic amounts of molybdate ions is required to achieve sufficiently rapid kinetics. Triiodide, which is easily soluble in water, is detected at 352 nm. The reagent solution should be thoroughly degassed with nitrogen to prevent the formation of iodine by oxygen oxidation.

The choice of chromatographic conditions depends on whether bromate alone is to be determined, or if other ions are also to be determined simultaneously. For example, for the determination of bromate alone a sulfuric acid eluent is proposed in which the molybdate is already contained. Only a solution of potassium iodide needs to be added as the derivatization reagent. A serial arrangement of conductivity detection with suppression and post-column derivatization is also possible. In this case the usual carbonate/hydrogen carbonate eluent is used with an acidic solution containing ammonium molybdate and potassium iodide being used as the derivatization reagent. Typical chromatograms for the determination of bromate at very low concentrations are shown in Figures E9a and b.

An extension of the use of the triiodide method includes the simultaneous determination of several strongly oxidizing anions. A corresponding chromatogram is shown in Figure E10. It must be pointed out that despite their strongly oxidizing properties nitrate, chlorate and also perchlorate are not determined, as these reactions are apparently kinetically strongly inhibited.

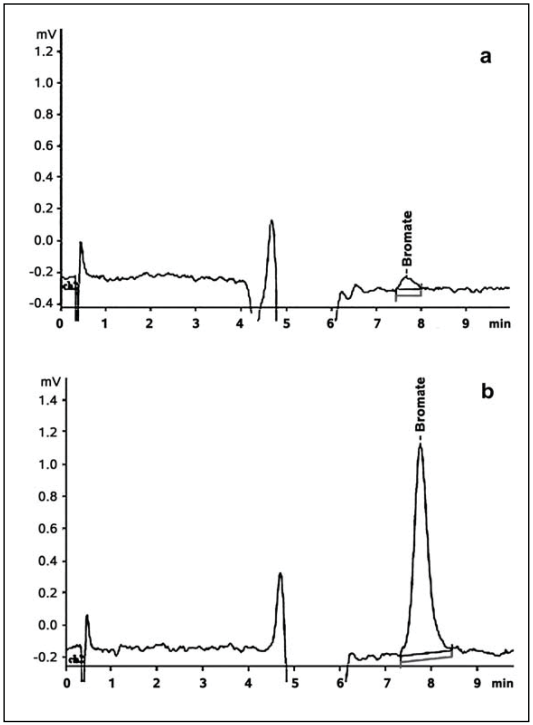


Figure E9a: *0.05 µg/L bromate; injection volume 1000 µL*

Figure E9b: *1 µg/L bromate; injection volume 1000 µL*

Figure E9: *Examples of chromatograms for the determination of bromate by the triiodide method*
Separation: Phenomenex Star Ion A300 HC
Eluent: 100 mM H_2SO_4 / 45 µM ammonium molybdate
Flow rate: 1 mL/min
Derivatization/detection: 260 mM KI
Flow rate: 0.5 mL/min; λ = 352 nm

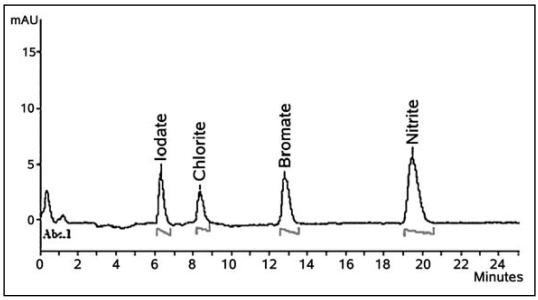


Figure E10: *Simultaneous determination of strongly oxidizing anions by the triiodide method*
Separation: *Metrosep A Supp 10*
Eluent: 20 mM sodium hydrogen carbonate
Flow rate: 1 mL/min
Derivatization/detection: *0.2 mM ammonium molybdate / 260 mM KI*
Flow rate: 0.5 mL/min; λ = 352 nm
Analyte concentrations: *10 µg/L each*

3.3 Determination of aluminum

The determination of aluminum and in particular the speciation of the reactive aluminum ions is an important problem in the medical field and in limnology (study of inland fresh waters) for the evaluation of its bioavailability and possible mobilization from soils and sediments. In addition to various photometric methods, IC has proven itself to be one of the possible alternatives with attractive properties [E6]. Because of limitations in direct conductivity detection (with respect to sensitivity and clear assignment of the elution peaks in sometimes complex cation chromatograms) a post-column derivatization is sometimes used. A sensitive version (also used in manual methods for photometry) is the conversion of aluminum with pyrocatechol violet (PCV). This reaction requires that the reaction conditions are very strictly observed; in particular the pH must be kept constant within a very narrow window (6.1±0.1 pH). This is why standard solutions are preferred for use in the mobile phase and it may be necessary to adapt the acid content of the samples. An example of a chromatogram is shown in Figure E11.

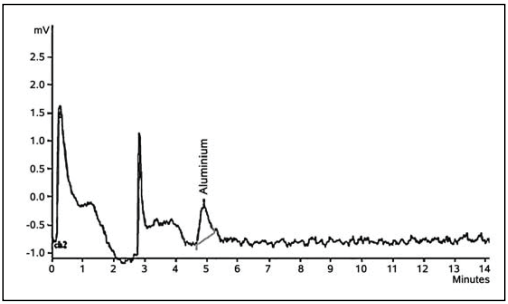


Figure E11: *Determination of aluminum with pyrocatechol violet (PCV) as the derivatization reagent*
Separation: *Metrosep C2 – 250*
Eluent: 5.6 mM H_2SO_4/30 mM $(NH_4)_2SO_4$
Injection volume: 900 µL
Flow rate: 1 mL/min
Derivatization/detection: *0.2 mM PCV; 500 mM NaAc/HAc*
pH 6.1 (exactly!) for the eluent/reagent mixture
Flow rate: 1 mL/min, λ = 570 nm
Analyte concentration: *10 µg/L Al*

The detection limits that can be achieved with this method are no better than with the photometric method, but problems from interferences with other cations (iron in particular) do not exist because of the chromatographic separation. An alternative method for the determination of aluminum using post-column derivatization is the conversion with morin, which can also be detected photometrically (but particularly sensitively by fluorimetry) [E6].

3.4 Simultaneous determination of some transition metals

An enormous number of reagents exist for the photometric determination of the transition metals; the reactions are mostly based on the formation of chelate complexes. Some of these reagents exhibit a high selectivity (e.g. o-phenanthroline for Fe(II) or dimethylglyoxime for Ni(II)), on the other hand some are less selective and form colored complex compounds with many metal cations. Representatives of this latter category are 8-hydroxyquinoline, 1-(2-pyridylazo)-2-naphthol (PAN) and 4-(2-pyridylazo)-resorcinol (PAR). In principle such reagents are suitable for the simultaneous determination of several metal cations in combination with a chromatographic separation. However, it must be taken into consideration that if metal ion complexing eluents (e.g. tartaric or oxalic acids) are used then the stability of the complex formed by the color complexing agent must be sufficiently high to release the metal ions from their compounds (see Section A). For this reason the correct choice of the pH value (which determines the stability of the complex) and the reagent concentration are very important.

The conversion with PAR (as a representative of numerous other possibilities) is described in more detail. Metal-PAR complexes are formed in a slightly alkaline medium with approximately the same yield. The maximum absorption wavelength is similar for most PAR complexes and also the absorption coefficients are of the same order of magnitude. This is why all complex-forming metal cations

can be detected at a fixed wavelength (500-520 nm) with virtually the same sensitivity. The determination limits are in the lower µg/L range (and with «large-volume injection» or inline preconcentration even below this). A representative chromatogram is shown in Figure E12.

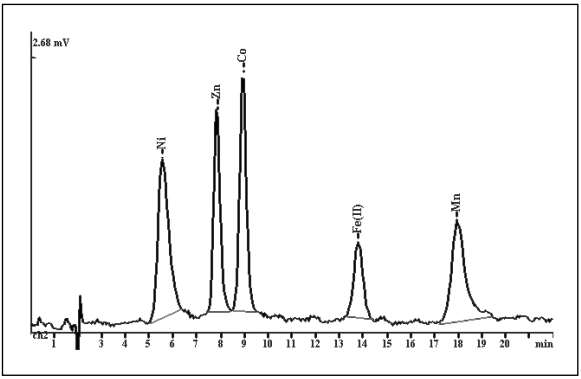


Figure E12: *Simultaneous determination of various transition metals using PAR as the derivatization reagent*
Separation: *Metrosep C2 – 150 + Metrosep C2 Guard*
Eluent: 1.75 mM oxalic acid / 2 mM ascorbic acid
Derivatization/detection: *PAR (4-(2-pyridylazo)-resorcinol), λ = 520 nm*
Analyte concentrations: *50 µg/L each of Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}*

3.5 Determination of ions that form complexes with Fe(III)

Iron(III) ions are able to form complexes with numerous anions. Nearly all of these have a yellow color and absorb in the wavelength range λ = 300-330 nm. This fact can be utilized for the simultaneous determination of various anions by post-column derivatization. The reagent solution usually used is Fe(III) perchlorate/perchloric acid. The sensitivity of the detection is not very high because of the absorption coefficients of the iron complexes also being not all too high. This can, but must not necessarily, be a limitation in the use of the method. An interesting use of this reaction is the simultaneous determination of anions containing phosphorus (besides ortho-phosphate) as well as organic complexing agents and detergents which, among other things, are components of washing and cleaning agents. An example of a chromatogram is shown in Figure E13.

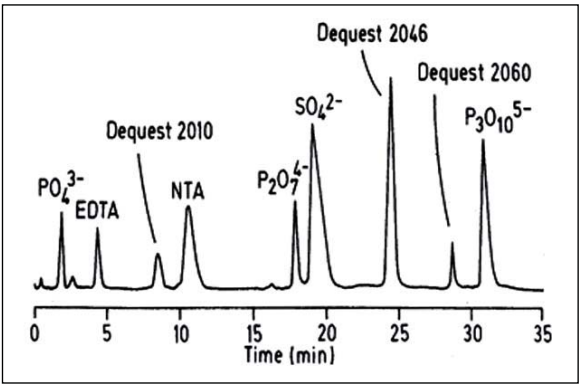


Figure E13: *Iron(III) as a reagent for post-column derivatization. Determination of anions containing phosphorus, complexing agents and surfactants*
Separation: *Waters IC Pak A*
Eluent: nitric acid (step gradient)
Derivatization/detection: *0.05 M Fe^{3+} perchlorate / 0.8 M perchloric acid, λ = 570 nm*
Analyte concentrations: *all components in the lower mg/L range*

3.6 Post-column derivatization for the determination of amino acids

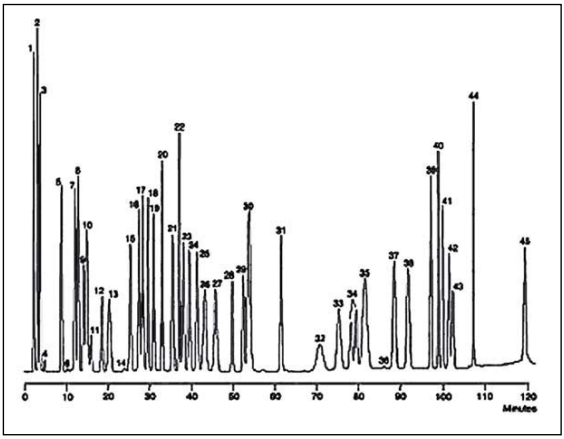
Post-column derivatization for the determination of amino acids is probably the most widespread application with the greatest importance [E1-E3, E7]. Although amino acids can easily be separated by ion exchange chromatography (as anions or cations), they either cannot be detected at all or only poorly with the usual detection methods. Most amino acids have virtually no UV absorption, cannot be oxidized amperometrically and the sensitivity is very low even with conductivity detection.

Photometric methods for the determination of amino acids show a good to outstanding sensitivity, but are not very selective. However, it is just this low selectivity which in combination with a chromatographic separation proves to be very advantageous, as consequently many (if not all) amino acids in a sample can be determined simultaneously. The two most important reactions for post-column derivatization of amino acids are the conversion with ninhydrine or o-phthalic aldehyde/ mercaptoethanol.

The ninhydrine method can be used for the determination of primary and secondary amines. However, the optimal detection wavelengths for the corresponding reaction products are very different. With primary amines products with an intense blue coloration are produced with an absorption maximum at 570 nm. Secondary amines produce yellow derivatives that are preferably detected at 440 nm, so that good discrimination in comparison to the self-absorption of the reagent is achieved. A disadvantage of the ninhydrine reaction is the high reaction temperature of approx. 60 °C that is necessary.

Primary amines can preferably be converted using o-phthalic aldehyde (usually abbreviated as OPA) in the presence of thiols (e.g. 2-mercaptoethanol or the more stable 2-mercaptoethanol derivative Thiofluor™). The reaction takes place rapidly at room temperature and the blue derivatives can be detected photometrically, but preferably by fluorimetry. The sensitivity of fluorimetric detection is higher than that of photometric detection by a factor of about 100. Secondary amines can also be determined by inline oxidation (e.g. with hypochlorite).

The example of a chromatogram shown in Figure E14 illustrates the outstanding separating performance of ion exchange chromatography for amines as well as the high sensitivity of photometric detection after derivatization.



Separation: *Pickering dedicated amino acid column (totally sulfonated cation exchanger)*
Eluent: Li^+ gradient, 0.35 mL/min
Derivatization: *300 mg/L OPA / 2 g/L Thiofluor™, reactor temperature 130 °C; flow rate: 0.3 mL/min*
Detection: *Primary amino acids λ = 570 nm;*
Secondary amino acids λ = 440 nm
Analyte concentration: *0.25 µmol/mL (10 µL injection)*

Figure E14: *Post-column derivatization for the determination of amino acids – cation exchange chromatography in combination with o-phthalic aldehyde (OPA)/Thiofluor™ as the derivatization reagent*

4 Summary

Post-column derivatization in combination with spectrophotometric detection is an interesting addition to the existing detection techniques for IC. It can be used alone or in combination with other detectors and is used for the determination of individual ions or to extend the range of ions that can be determined simultaneously by IC.

It is particularly attractive for individual analytes (e.g. chromate, bromate) as in this case an extraordinarily high sensitivity can be achieved that is not (or hardly) achieved with other detectors. The detection can be selectively influenced by the choice of the derivatization reagent. The high selectivity of some derivatization reactions can be an advantage when, for example, matrix influences cause the appearance of so-called system peaks in conductivity detection or the complete separation of neighboring signals cannot be achieved by the chromatographic separation. However, information about other separated ions is lost.

A disadvantage of post-column derivatization is the increased complexity of the instrumentation and the necessity of optimizing the separation and reaction part of the whole system. This means that it may be necessary to make compromises in the choice of flow rates and the composition of the mobile phase or the reagent solutions. The increased complexity is not only a cost factor, but also increases the susceptibility of the method to interferences. The preparation of reagent solutions for the particular derivatization reaction is time-consuming, and the successful execution of a post-column derivatization requires some degree of chemical understanding.

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F – Practical work

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1 Determination of perchlorate in drinking water by IC-MS coupling

Branches: quality control in water analysis, environment

by Andrea Wille

Keywords

IC / MIC / MS / ASUPP5 / perchlorate / bromate / complexing agents / drinking water

Further applications

Bromate determination by IC-MS coupling in drinking water

Complexing agents (EDTA, DTPA) in surface water by IC-MS

Method description

An outstanding selectivity and a remarkably low detection limit can be achieved by coupling the IC system with a mass-specific detector. This extends the range of IC applications, which has a positive effect on the analysis of water and in other branches.

Perchlorate can be determined in the µg/L range by utilizing the advantages of the IC-MS coupling. The chromatographic separation is carried out using a short polyvinyl alcohol column and a concentrated hydroxide eluent. The water sample is injected directly.

Two naturally occurring perchlorate ions exist with the compositions $^{35}Cl^{16}O_4^-$ (m/z 99) and $^{37}Cl^{16}O_4^-$ (m/z 101); these are present in a ratio of 3.086:1. Both mass traces are used for quantification. The isotope-enriched perchlorate ion with the composition $^{35}Cl^{18}O_4^-$ (m/z 107) is used as the internal standard. It is used for quantification and also helps to improve the accuracy of the method and its robustness to interferences. This commercially available internal standard already contains perchlorate ions that contain less than four ^{18}O-Isotope (e.g. m/z 103, 105). In addition such compounds can also be formed by isotope exchange in the measuring solution. This is why the ratio of the traces m/z 107 and 105 is used to check the quality and stability of the internal standard.

The capabilities of the IC-MS coupling can be impressively demonstrated by a comparison of the chromatograms obtained by suppressed conductivity detection and mass-specific detection. Although with conductivity detection a signal can be obtained for the perchlorate standard solution in ultrapure water, this is no longer possible in the presence of a matrix (1 g/L each of chloride, carbonate and sulfate). In comparison, with the MS detector very small amounts of perchlorate, both in the standard solution (with or without matrix) and also in the sample solution, can be detected and quantified without any problems.

Sample
Drinking water

Eluent
30 mmol/L NaOH
30% methanol v/v

Standards

The standard solutions are prepared with ultrapure water:

Standard	Perchlorate conc. [µg/L]
1	1
2	5
3	10
4	20

Instruments and accessories

2.830.0020	830 Advanced IC Interface
2.819.0110	819 Advanced IC Detector
2.820.0230	820 Advanced IC Separation Center
2.818.0110	818 Advanced IC Pump
2.833.0010	833 Advanced IC Liquid Handling Pump Unit
6.2620.150	Pulsation dampener
6.1006.510	Metrosep A Supp 5 – 100
	Agilent mass spectrometer 1100 MSD

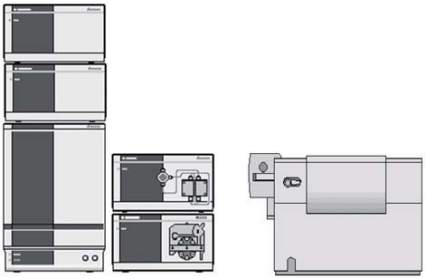
Chromatographic parameters

Flow rate	0.8 mL/min	**Pressure**	7.5 MPa
Sample loop	100 µL	**Technique**	chem. suppression
Background conductivity	5 µS/cm	**Full scale range**	10 µS/cm
Measuring range	100 µS/cm		

Agilent 1100 MSD mass spectrometer

Tune mode	negative mode auto-tune
V cap.	1400 V
Drying gas flow and temperature	9 L/min, 320 °C
Nebulizer pressure	20 psi (0.138 MPa)
Fragmentor	140 V

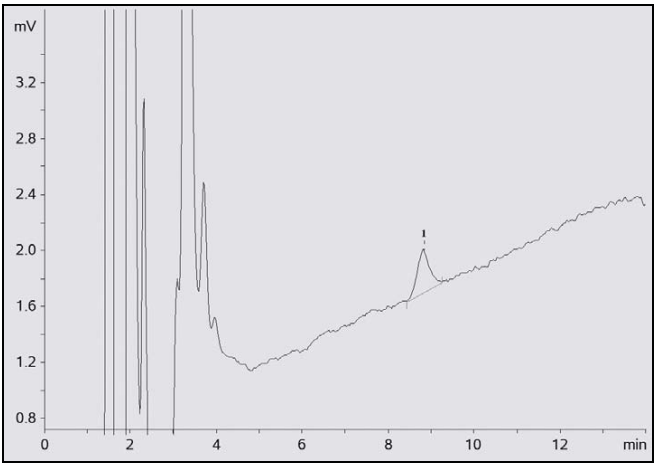
Chromatography system



Procedure

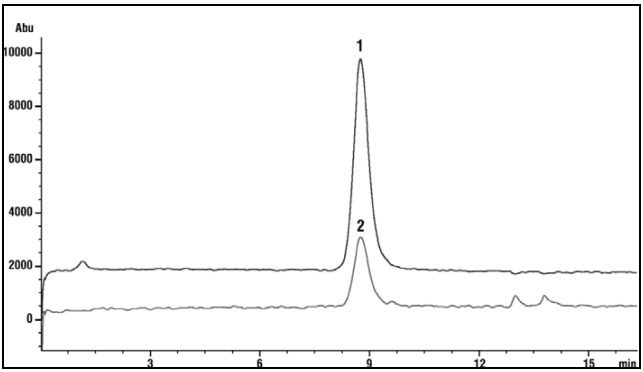
The samples are injected directly.

Chromatograms



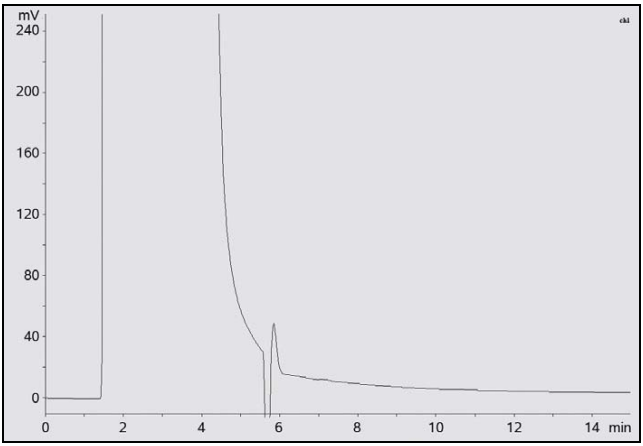
Conductivity detection with suppression: 5 µg/L perchlorate standard in ultrapure water

No.	Retention time	Height	Area	Conc.	Name
	min	mV	mV*sec	µg /L	
1	8.82	0.31	6.049	5.027	Perchlorate



MS detection: 5 µg/L perchlorate standard in ultrapure water

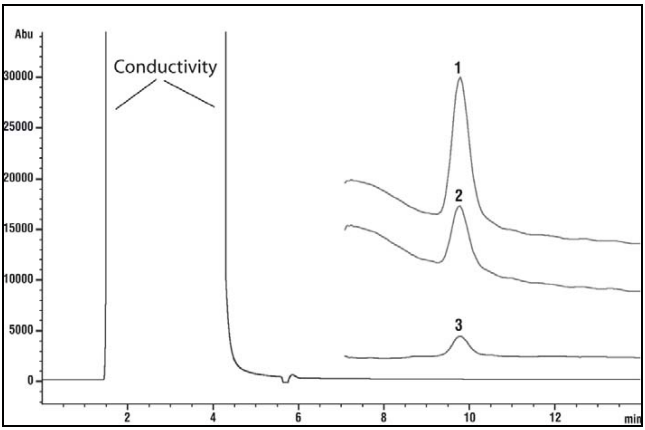
No.	Retention time	Height	Area	Conc.	Name
	min			µg/L	
1	8.89	7909	164994	5.02	Perchlorate m/z 99
2	8.89	2598	52213	5.01	Perchlorate m/z 101



*Conductivity detection with suppression: 5 µg/L perchlorate standard in matrix**

➔ no perchlorate signal visible

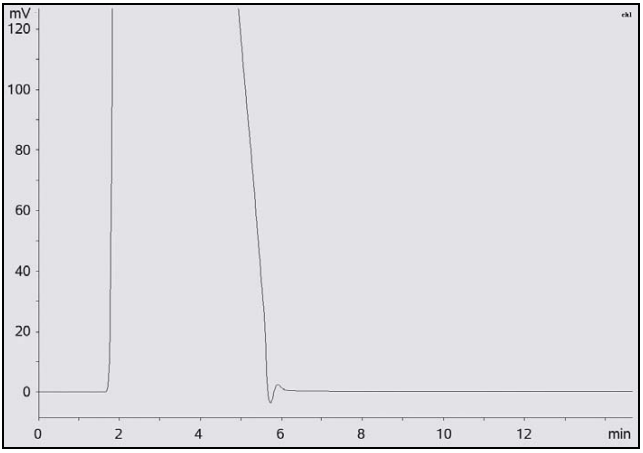
* Matrix: 1 g/L each of chloride, carbonate, sulfate



MS detection: 5 µg/L perchlorate standard in matrix (1 g/L each of chloride, carbonate, sulfate); also shown: conductivity signal (not sensitive enough for the detection of perchlorate in the matrix)

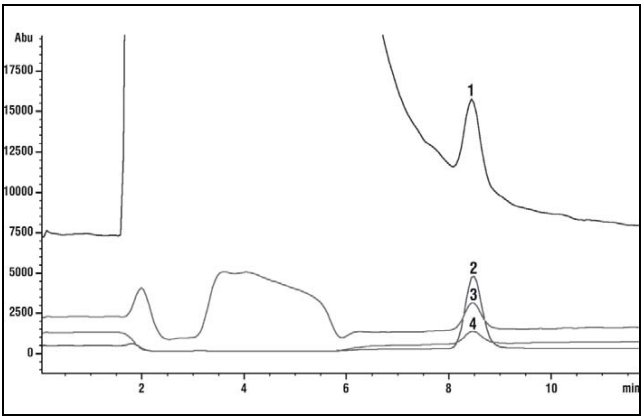
No.	Retention time	Height	Area	Conc.	Name
	min			µg/L	
1	9.77	13960	406414	5.01	TIC for perchlorate m/z 99 + 101
2	9.75	6079	179228	5.01	Perchlorate m/z 99
3	9.77	1968	57232	5.01	Perchlorate m/z 101

TIC = total ion current chromatogram



Conductivity detection with suppression: sample solution with 5 µg/L internal standard

➔ no perchlorate signal visible



MS detection: sample solution with 5 µg/L internal standard

No.	Retention time	Height	Area	Conc.	Name
	min			µg/L	
1	8.44	4877	104118	5.01	Perchlorate m/z 99
2	8.47	4511	106728	5.00	Perchlorate m/z 107
3	8.46	1673	39207	5.01	Perchlorate m/z 101
4	8.46	793	20444	5.00	Perchlorate m/z 105

2 Determination of bromate in water samples

2.1 Determination of bromate in various waters by conductivity detection after sequential suppression

Branches: quality control in water analysis, environment

by Michael Wahl

Keywords

IC / MIC / Metrosep A Supp 5 – 250 / bromate / environment / drinking water

Further applications

Carbonate gradient applications
Elimination of system peak
Removing CO_2 interference by the sample

Method description

Bromate is determined in various waters after direct injection. A high-capacity polyvinyl alcohol column is used for the chromatographic separation. A buffer mixture of $NaHCO_3$/Na_2CO_3 is used as the eluent. In order to reduce the background conductivity and thus increase the sensitivity for bromate the chemical suppressor (Metrohm Suppressor Module II «MSM II») is combined with the Metrohm CO_2 Suppressor «MCS» (sequential suppression). The linear measuring range is increased by using CO_2 suppression.

Samples

Water samples

Eluent

3.2 mmol/L Na_2CO_3 / 1.0 mmol/L $NaHCO_3$

Standard

The standard solutions are prepared with ultrapure water.

Standard	BrO_3^- conc. [µg/L]
1	2
2	4
3	6
4	8
5	10

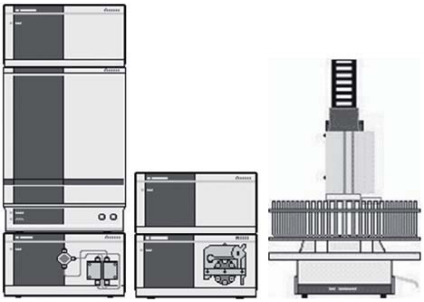
Instruments and accessories

2.830.0020	830 Advanced IC Interface
2.819.0110	819 Advanced IC Detector
2.820.0230	820 Advanced IC Separation Center
2.818.0110	818 Advanced IC Pump
2.853.0010	853 Metrohm CO_2 Suppressor «MCS»
2.833.0010	833 Advanced IC Liquid Handling Pump Unit
2.838.0020	838 Advanced IC Injection Sample Processor
2.800.0010	800 Dosino
6.3032.120	Dosing Unit 2 mL
6.2620.150	Pulsation dampener
6.1006.530	Metrosep A Supp 5 – 250

Chromatographic parameters

Flow rate	0.7 mL/min	**Pressure**	10.9 MPa
Sample loop	100 µL	**Technique**	chem. suppression
Background conductivity	0.80 µS/cm	**Full scale range**	2.0 µS/cm

Chromatography system



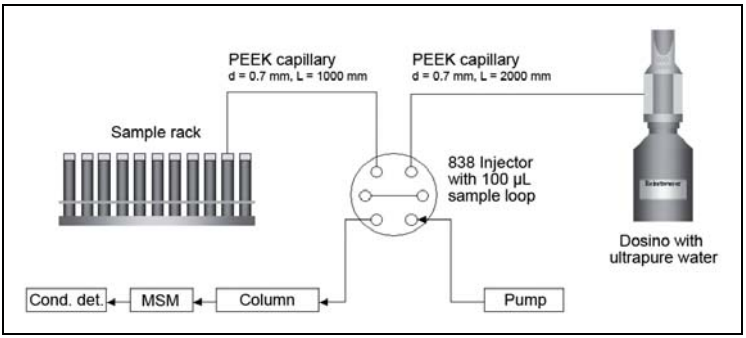
Procedure

All the samples were injected directly using the 838 Advanced IC Injection Sample Processor.

Special features

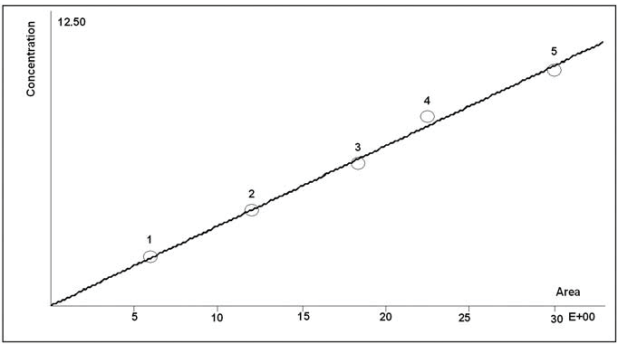
The principle of the partial loop injection is used in this application. Depending on the species to be determined, an 800 Dosino is used to inject various sample volumes. This means that a volume of 50 µL is injected for bromate determination and a volume of 20 µL for determining the other anions. In this way a single system can be used to determine other anions in addition to bromate, without needing to convert the system or carry out a further manual dilution step.

The Dosino is used to fill the 100 µL sample loop with the required sample volume which is taken from a sample vial in the sample rack of the 838 Advanced IC Injection Sample Processor. The remaining volume in the sample loop is filled with ultrapure water. The ultrapure water is also used to rinse the PEEK capillaries; the waste vessel can be located in the sample rack itself or at one of the external positions of the Sample Processor.



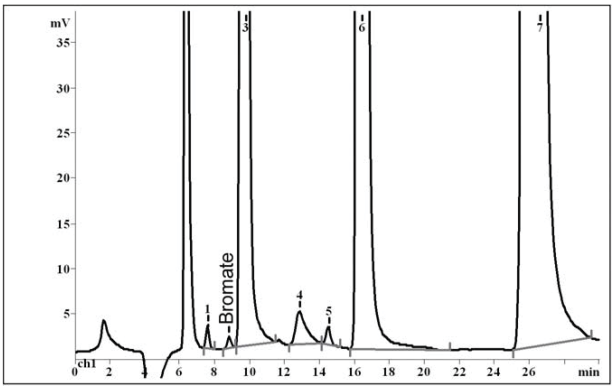
Sketch of partial loop injection.

Calibration curve

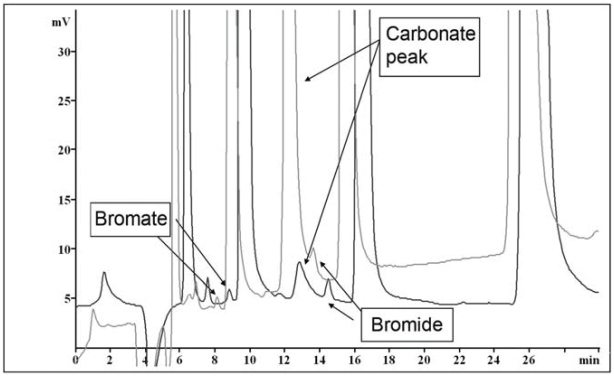


The calibration curve is obtained by plotting the concentrations of the five standards against the measured peak areas.

Chromatograms



Drinking water spiked with 5 µg/L BrO_3^-, recorded with sequential suppression.



Sample chromatograms of drinking water spiked with 5 µg/L BrO_3^-, recorded without (light gray) and with CO_2 suppressor (dark gray).

2.2 Determination of bromate in mineral water and drinking water by post-column derivatization and UV/VIS detection (triiodide method)

Branches: beverages, environment

by Michael Wahl

Keywords

IC / MIC / UV/VIS Compact IC / 6.1005.110 / Phenomenex Star Ion A300 HC / PCR / bromate / triiodide / mineral water / drinking water / environment

Further applications

Chromate determination

NTA, EDTA, DTPA determination

Heavy metal determination

Method description

This method is suitable for the selective determination of bromate. The potassium iodide added for post-column derivatization reduces any bromate present to bromide under the catalytic influence of ammonium heptamolybdate. The triiodide formed in this reaction is detected by UV/VIS spectroscopy at 354 nm. 100 mmol/L sulfuric acid with 45 µmol/L ammonium heptamolybdate is used as the eluent. The concentration of the KI solution, which is added as a reagent via a mixing spiral, is 0.75 mol/L.

The high-capacity polystyrene/divinylbenzene column «Phenomenex Star Ion A300 HC» is used as the separation column.

Samples

Mineral water, drinking water

Eluent

100 mmol/L sulfuric acid + 45 µmol/L ammonium heptamolybdate

Reagent

0.75 mol/L KI solution

Standard solutions

The standard solutions are prepared with ultrapure water.

Standard	BrO_3^- conc. [µg/L]	Standard	BrO_3^- conc. [µg/L]
1	0.5	5	6.0
2	1.0	6	8.0
3	2.0	7	10.0
4	4.0		

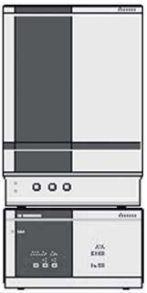
Instruments and accessories

2.844.0020	844 UV/VIS Compact IC with PCR
2.837.0020	837 Advanced IC Sample Degasser
6.2620.150	Pulsation dampener
6.1005.110	Phenomenex Star Ion A300 HC

Parameters

Eluent flow rate	1.0 mL/min	**Pressure**	1.0 MPa
PCR reagent flow rate	0.2 mL/min	**Technique**	PCR with UV/VIS detection
Sample loop	1000 µL	**Wavelength**	354 nm

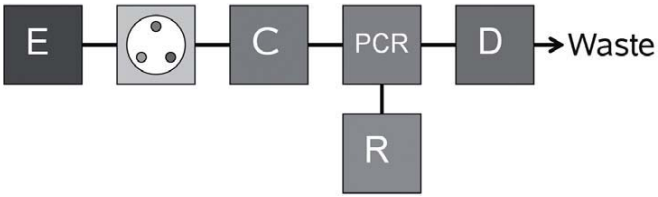
Chromatography system



Procedure

The samples are degassed inline in the 837 Advanced IC Sample Degasser and then injected directly.

Flow chart



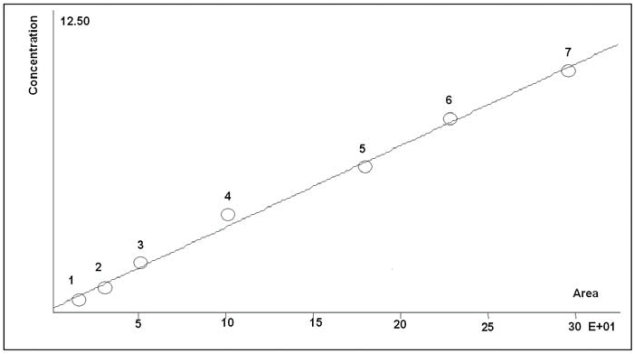
E Eluent; 0.1 mol/L H_2SO_4 + 45 µmol/L ammonium heptamolybdate
C Separation column
PCR IC post-column reactor
D UV detector
R Reagent; 0.75 mol/L KI

Reaction equations

$$BrO_3^- + 6\ I^- + 6\ H^+ \rightleftharpoons 3\ I_2 + 3\ H_2O + Br^-$$

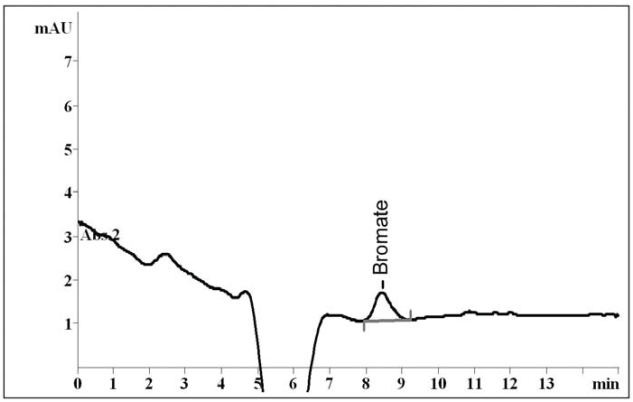
$$3\ I_2 + 3\ I^- \rightleftharpoons 3\ I_3^-$$ (detected at 354 nm)

Calibration curve

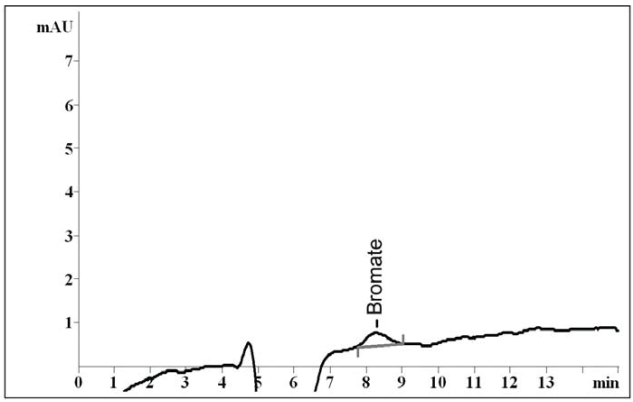


The calibration curve is obtained by plotting the concentrations of the seven standards against the measured peak areas.

Chromatograms



Standard 1: 0.5 µg/L BrO_3^-



Sample: mineral water + 0.5 µg/L BrO_3^-

3 Simultaneous determination of borate, chloride and sulfate in a nickel plating bath

Branch: electroplating industry

by Andreas Walter

Keywords

IC / MIC / 828 / Phenomenex Star Ion A 300 / borate / chloride / sulfate / step gradient / electroplating

Further applications

Simultaneous determination of the anions of weak and strong acids, e.g. borate, silicate, cyanide, sulfide, selenite, selenate, arsenite, arsenate, etc.

Method description

Ion chromatography in combination with a detection system consisting of two conductivity detectors represents an uncomplicated and problem-free analytical method for the determination of borate, chloride and sulfate in a nickel electroplating bath.

The combination of two conductivity detectors for direct conductivity measurement and for conductivity measurement after chemical suppression is also known as «high/low» detection. The weakly dissociated acids, which after suppression cannot be measured with the second conductivity detector, are determined beforehand with the first conductivity detector by direct conductivity measurement. Subsequently the suppressor is connected and the anions of the strongly dissociated acids are detected by the second conductivity detector.

Elution is realized by using a step gradient. A polystyrene/divinylbenzene column is used for the separation.

Sample

Chemical nickel bath

Eluents

Eluent 1 (for direct conductivity)	5.0 mmol/L KOH
Eluent 2 (for chemical suppressor technique)	2.0 mmol/L Na_2CO_3 / 5.0 mmol/L KOH

Eluent change (step) after 4 minutes

Standards

The standard solutions are prepared with ultrapure water and injected directly.

Standard	Concentration [mg/L]		
	Boric acid	Chloride	Sulfate
1	25	10	75
2	40	20	100

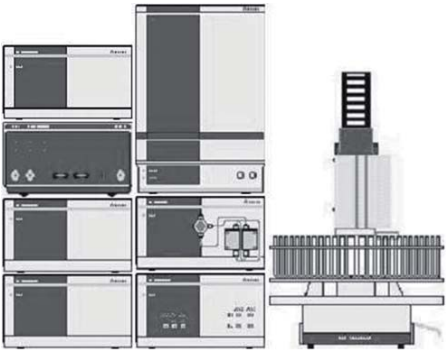
Instruments and accessories

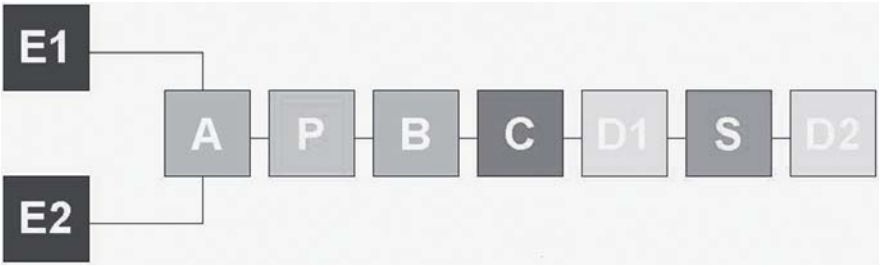
2.830.0020	830 Advanced IC Interface
2.819.0110	819 Advanced IC Detector (2 pieces)
2.820.0220	820 Advanced IC Separation Center
2.818.0110	818 Advanced IC Pump
2.828.0010	828 IC Dual Suppressor
2.838.0010	838 Advanced IC Sample Processor
2.837.0010	837 Advanced IC Eluent Degasser
6.2620.150	Pulsation dampener
6.1005.100	Phenomenex Star Ion A 300
6.1014.000	Metrosep A Trap 1
6.1012.010	IC sample preparation cartridge IC-H

Chromatographic parameters

Direct conductivity detection		**Conductivity detection after chemical suppression**	
Background conductivity	968 µS/cm	Background conductivity	0.52 µS/cm
Full scale range	20.0 µS/cm	Full scale range	10.0 µS/cm
Flow rate	1.5 mL/min	Pressure	4.1 MPa
Sample loop	20 µL		

Chromatography system





Sketch illustrating step gradient method

E1 Eluent 1
E2 Eluent 2

A valve for selecting the eluent
P high-pressure pump for transporting the particular eluent
B injection valve for injecting 20 µL sample
C column

D1 Detector 1 for direct conductivity detection (high background conductivity)
S Suppressor
D2 Detector 2 for conductivity detection after chemical suppression (low background conductivity)

Procedure

The samples are diluted with ultrapure water and injected via an IC-H cartridge. The IC sample preparation cartridge IC-H contains an acid-form cation exchanger, which removes interfering cations (in this case nickel cations) from the sample. This prevents interferences to the separation by unwanted interactions between the cations and the stationary phase in the column.

The simultaneous determination of borate, chloride and sulfate requires the use of a step gradient; this can be realized with two different eluents in the manner described below.

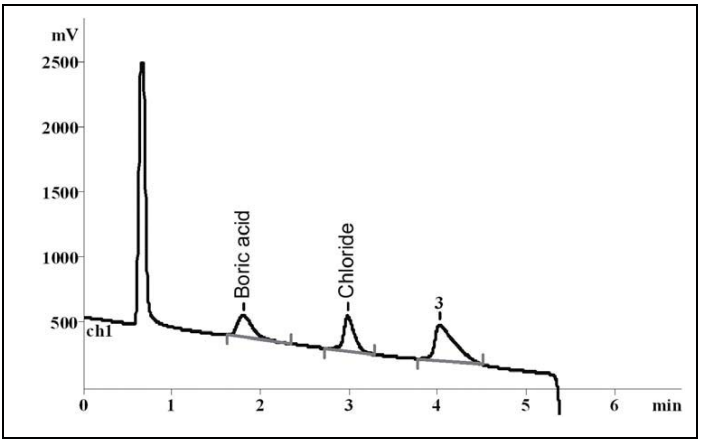
The eluents can be selected automatically by switching Valve A. A high-pressure pump is used for transporting them. Eluent 1, with low elution strength, is used first. The sample is injected via the sample loop in Valve B. After 4 minutes Valve A is switched to the other position to transport Eluent 2. With the aid of the stronger Eluent 2 the later eluting ions are eluted more rapidly from the column; this reduces the chromatography time.

Detection takes place with two conductivity detectors. The weakly dissociated acids, e.g. boric acid, are determined directly – without suppression – and measured at a high background conductivity by Detector 1. After Detector 1 the suppressor and Detector 2 are connected in series. In Detector 2 the anions of the strong acids are detected at a low background conductivity.

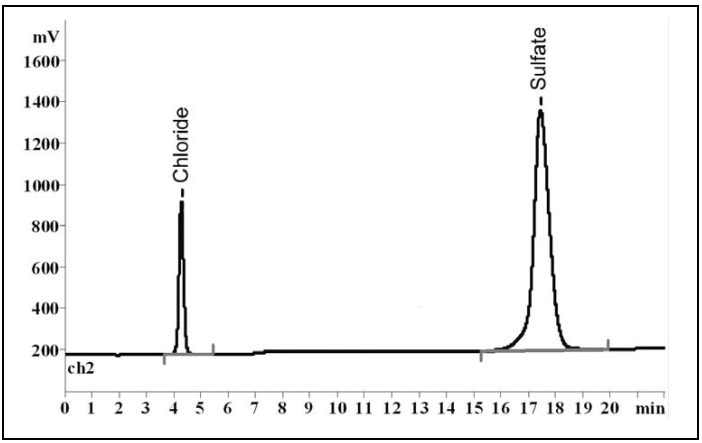
Results

Type of detection	Concentration [g/L]		
	Boric acid	Chloride	Sulfate
Direct conductivity	44.4	19.0	–
Conductivity after chemical suppression	–	19.2	108

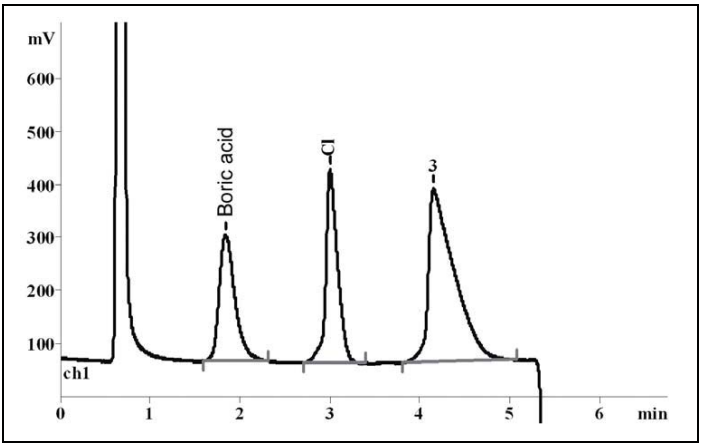
Chromatograms



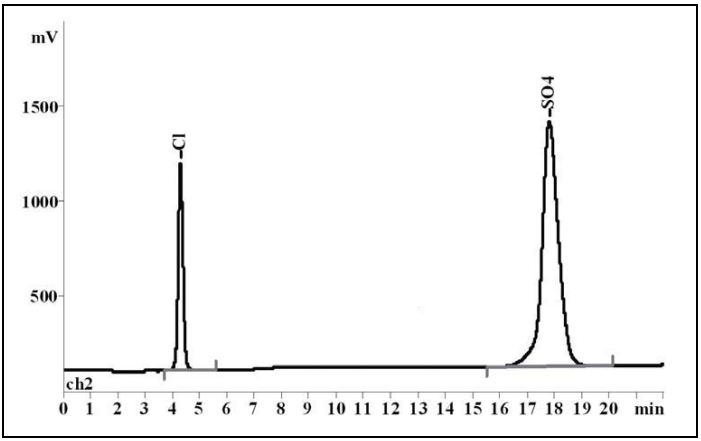
Standard 1 (direct conductivity detection, detector 1)



Standard 1 (conductivity detection after chemical suppression, detector 2)



Nickel bath (diluted 1:1250, direct conductivity detection, detector 1)



Nickel bath (diluted 1:1250, conductivity detection after chemical suppression, detector 2)

4 Determination of saccharose, glucose and sucralose in soft drinks by ion exclusion chromatography and pulsed amperometric detection (PAD)

Branch: food analysis

by Thomas Kolb

Keywords

IC / CIC / Bioscan / 817 / PAD / PCR / organic acids / saccharose / glucose / sucralose / soft drinks

Further applications

Detection of hydroxylamine after cation exchange chromatography

Method description

Saccharose, glucose and sucralose are separated by ion exclusion chromatography. Pulsed amperometric detection (PAD) is used after post-column derivatization (PCR).

After the separation column the acidic sulfuric acid eluent is made strongly alkaline by the addition of sodium hydroxide solution. At this high pH value the carbohydrates are deprotonated and can be oxidized at a gold electrode at a potential of 50 mV. In order to achieve a reproducible sensitivity, positive and negative cleaning pulses are applied which recondition and activate the electrode surface before each measurement. The potentials applied to the gold electrode (E1, E2 and E3) and their associated time spans (t1, t2, t3 and ts) are listed in the «Chromatography parameters» table.

A cation exchanger column (with sulfonic acid groups) on a styrene-divinylbenzene basis is used as the separation column.

Sample

Soft drinks

Eluent

20 mmol/L H_2SO_4

PCR reagent

300 mmol/L NaOH

Standards

The standard solutions are prepared with ultrapure water:

Standard	Concentration [mg/L]		
	Saccharose	Glucose	Sucralose
1	0.5	5	0.5
2	1	10	1
3	2	20	2
4	5	30	5

Instruments and accessories

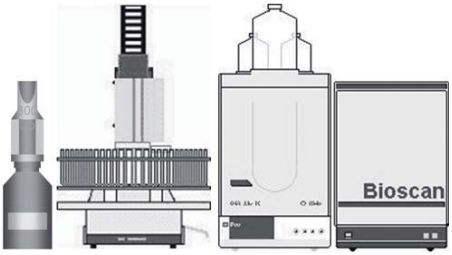
2.861.0010	861 Advanced Compact IC
2.817.0010	817 Bioscan (predecessor of the current 871 Advanced Bioscan)
2.838.0120	838 Advanced IC Dilution Sample Processor
2.800.0010	800 Dosino
2.801.0010	801 Magnetic Stirrer
6.2836.000	IC post-column reactor
6.3032.210	Dosing unit 10 mL
6.1005.200	Metrosep Organic Acids

Parameters

Pulsed amperometric detection (PAD)

Applied potential		Time span	
E_1	+50 mV	t_1	400 ms
E_2	+750 mV	t_2	200 ms
E_3	−150 mV	t_3	400 ms
Current	195 nA	Measuring time t_s	100 ms
Working electrode	Au	Sample loop	20 µL
Pressure	3.4 MPa	Temperature	34 °C
Eluent flow rate	0.6 mL/min	PCR reagent flow rate	0.5 mL/min

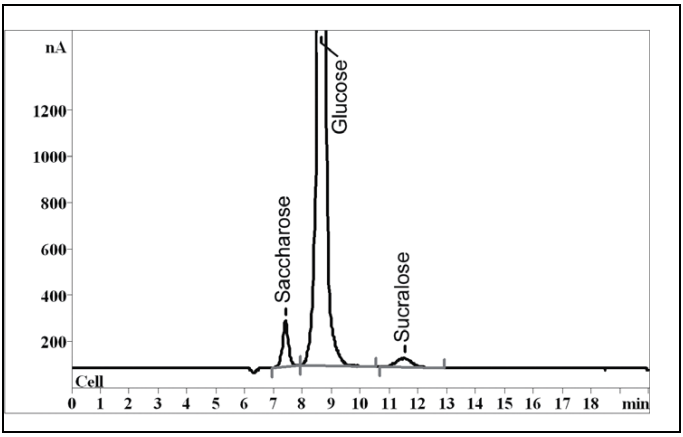
Chromatography system



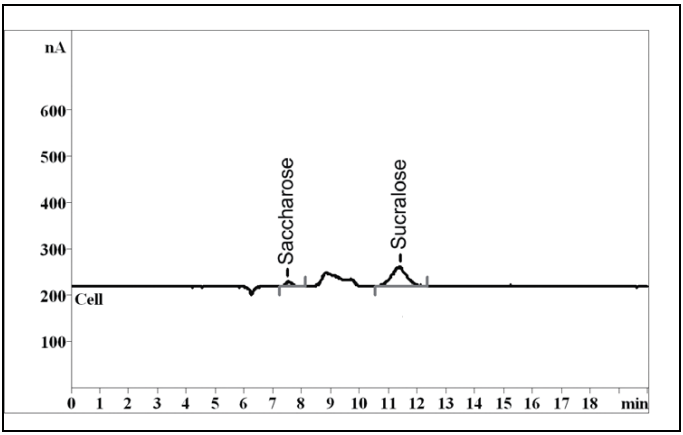
Procedure

The samples are automatically diluted 1:50 with ultrapure water and injected by the 838 Advanced Dilution Sample Processor.

Chromatograms



Standard 20 mg/L glucose, 2 mg/L saccharose, 2 mg/L sucralose



Sample: lemon diet lemonade, spiked with 100 mg/L sucralose, diluted 1:50

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