The Determination of Anions and Cations, Transition Metals, Other Complex Ions and Organic Acids and Bases in Water by Chromatography 1990

Methods for the Examination of Waters and Associated Materials analysis

HMSO

The Determination of Anions and Cations, Transition Metals, Other Complex Ions and Organic Acids and Bases in Water by Chromatography 1990

Methods for the Examination of Waters and Associated Materials analysis

London: HMSO

First Published 1990

ISBN 0 11 752331 3

Within the Method for the Examination of Waters and Associated Materials series are four-ring binders suitable for use in storing reports. These are available from HMSO Price £4 (ISBN 0 11 7514373)

HMSO

Standing order service

Placing a standing order with HMSO BOOKS enables a customer to receive other titles in this series automatically as published.

This saves the time, trouble and expenses of placing individual orders and avoids the problem of knowing when to do so.

For details please write to HMSO BOOKS (PC 13A/1). Publications Centre, PO Box 276, London SW8 5DT quoting reference X22.04.22

The standing order service also enables customers to receive automatically as published all material of their choice which additionally saves extensive catalogue research. The scope and selectivity of the service has been extended by new techniques, and there are more than 3,500 classifications to choose from. A special leaflet describing the service in detail may be obtained on request.

The Determination of Anions and Cations, Transition Metals, Other Complex Ions and Organic Acids and Bases in Water by Chromatography 1990

Methods for the Examination of Waters and Associated Materials analysis

Chromatographic methods are very sensitive to minor physical and chemical variations in materials and apparatus. Hence this booklet mentions the actual materials and equipment used for the evaluation tests. This in no way endorses these as superior to other similar products. Equivalent materials or equipment are acceptable though it must be understood that the performance characteristics may be different, and can vary with batch or model. It is left to the senior supervising analyst to evaluate and choose from the appropriate brands available.

Users should remember that the accuracy of an analysis for specific ions is highly dependent on the other substances present including related complex ions (for example, cyanide and cyanometallates, and for fluoride – fluorometallates); therefore they should evaluate the suitability and performance of the methods for their own analytical problem. Consequently, all test data and examples herein can only be illustrative of what can be achieved.

Furthermore, as identification of ions is made by relative retention times, coupled in some instances with the use of various types of detector which respond selectively to particular ions, it is essential that analysts ascertain the provenance of their samples and the ions which they are likely to contain. This will enable them to check whether interferences are likely to occur, the approximate retention times of the sought ions and the best detectors, columns and eluents to use, thus minimising the risks of misidentification and false measurement.

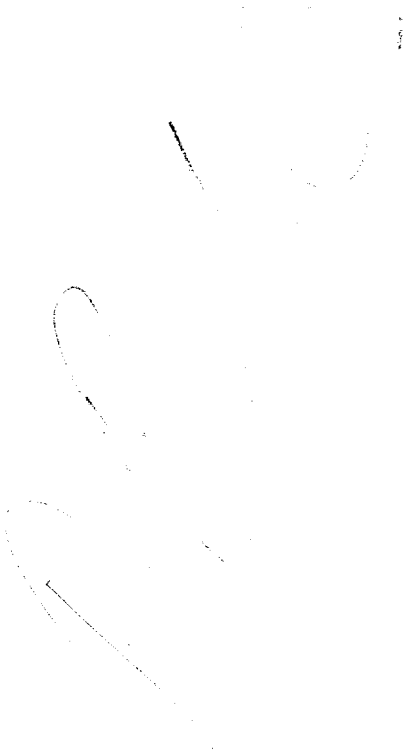
CONTENTS

Note: References are given by chapter at the end of each chapter in Part I and after methods in Parts II and III. Most, but not all, general references are listed after Chapter 1.
Figures, Chomatograms and Tables are numbered by Chapter. A list follows.

Part I

Part II

Part III



About This Series

This booklet is part of a series intended to provide recommended methods for determining the quality of water and associated materials. In addition short reviews of the more important analytical techniques of interest to the water and sewage industries are included.

In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare so that they were often partially out of date before they appeared in print. The present series is published as a series of booklets on single or related topics; thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method.

Although ideally, all methods published should be fully tested, this is not often possible without delay in publication. Furthermore, the limit of detection, range, precision and interference effects applying to instrumental methods can depend on the actual instrument used, as well as on sample type, reagent purity, operator skill, etc. Even methods tested in many laboratories have been known to acquire problems when a new domestic product appeared (introducing a new substance into effluents), changes in production methods, altered reagent quality, or the method was used to analyse a new type of sample (despite apparent similarity to samples already evaluated). As a guide, the following categories have been given to methods:
tested, usually in five or more laboratories
– no grade indicated;
tested in one to three or four laboratories
– Tentative;
evaluated, but not fully tested, but publication is urgently required
– Note;
tested and found to be satisfactory by several laboratories, but in the opinion of experts requires a high degree of skill or has some other difficulty such that the method would be replaced if a better method were discovered
– Provisional.

The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users, the senior technical staff, to decide which of these methods to use for the determination in hand. Whilst the attention of users is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water cycle). The Standing Committee of Analysts is a committee of the Department of the Environment set up in 1972. Currently it has nine Working Groups each responsible for one section or aspect of water cycle quality analysis. They are:

1.0 General Principles of Sampling and Accuracy of Results
2.0 Microbiological Methods
3.0 Empirical and Physical Methods
4.0 Metals and Metalloids
5.0 General Nonmetallic Substances
6.0 Organic Impurities
7.0 Biological Monitoring
8.0 Sewage Works Control Methods
9.0 Radiochemical Methods.

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in cooperation with the working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in that booklet.

L R PITTWELL
Chairman & Secretary

1 February 1990

Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary.

Local Safety Regulations must be observed.

Laboratory procedures should be carried out only in properly equipped laboratories.

Field Operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried.

Care should be taken against creating hazards for one's self, one's colleagues, those outside the laboratory or workplace, or subsequently for maintenance or waste disposal workers. Where the Committee have considered that a special ususual hazard exists, attention has been drawn to this in the text, so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. Reagents of adequate purity must be used along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards, if contamination is suspected, reagent purity should be checked before use.

The best safeguard is a through consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet) use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Safe practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory', issued by the Royal Society of Chemistry, London: 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection', Public Health Laboratory Services Monograph 6, HMSO, London.

It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected. If an amulance is called or a hospital notified of an incoming patient, give information on the nature of the injury especially if poisoning is suspected, as the patient may be taken directly to a specialized hospital.

Safety while Sampling

Prior consideration must be given, especially when sampling in confined spaces or where access is difficult, to guard against suffocation, drowning, falls, and poisoning or infection by ingestion, inhalation, or skin contact.

Good Laboratory Practice

The Department of Health issues a booklet entitled: Good Laboratory Practice; the United Kingdom Compliance Programme, 1989.
This can be obtained by writing to the Department in London. It deals chiefly with toxicity studies, but much can be applied to analytical chemistry.

Glossary of Acronyms Used

In order to avoid searching text for the first time an acronym is used, the more important ones are listed here with their meanings. Recognized chemical symbols and abbreviations for units of measurement are not included.

AMMS	Anion micromembrane suppressor
AU	Absorbance Unit
BS	British Standard number
CMMS	Cation micromembrane suppressor
DAP	Diaminopropane (1.3)
DPC	Diphenylcarbazide
EDTA	Ethylenediaminetetraacetate (or dinitrilo or tetraacetic acid)
fsd	full scale deflection
HDTMAH	Hexadecyl trimethyl ammonium hydroxide
HOA	Halogenated organic acids
HPLC	High performance liquid chromatography
IC	Ion(ic) chromatography
ICE	Ion exclusion chromatography
LOD	Limit of Detection (also Loss on Drying)
meq	Milliequivalent
MPDA	Meta-phenylene-diamine
NTA	Nitrilotriacetate (or triacetic acid)
PAR	4(2- pyridylazo) resorcinol
PDCA	2.6- pyridine dicarboxylic acid
PSDVB	Polystyrene divinyl benzene
PTFE	Polytetrafluoroethylene
RI	Refractive index
RSD	Relative standard deviation
TBA	Tetrabutylammonium
TBA H_2SO_4	Tetrabutylammonium sulphate
TBAOH	Tetrabutylammonium hydroxide
TDFHA	Tridecafluorohexanoic acid
TEAOH	Tetraethylammonium hydroxide
TMAOH	Tetramethylammonium hydroxide
UV	Ultra violet

Part 1 Review

Chapter 1 Introduction

Chromatography is a physical technique for the separation of substances by means of their equilibrium distribution between two phases – one of which is stationary and the other mobile.

Chromatography of ions is part of a family of liquid chromatographic techniques which are derived from high performance liquid chromatography (10). Compared with most other methods for the same determinands, it is convenient, precise, fast and able to isolate and quantify several species in one analysis. This is achieved by the use of closed, reusable columns containing microparticulate supports (3–25 μm) (usually with a bonded stationary phase) operated at high mobile phase flow rates and pressures. The technique is more capital intensive, but less labour intensive than the more 'classical' methods of ion determination.

Mobile phase (eluent) flow is provided by high-pressure pumps giving controlled, reproducible flow which gives rise to greater precision and speed of analysis. Precise sample introduction is easily achieved by a sample loop fitted to ports on an injection valve. Detection and quantification is achieved by use of continuous detectors which yield a final chromatogram without intervention by the operator. Computing integrators are available for the collection, reduction and manipulation of detector response data to provide a chromatogram. These are particularly useful where many sample measurements are made since they can allow real time display and data storage together with retrospective analysis (i.e. post-run) of stored data.

The number of published methods for the chromatography of ions is large and continues to grow rapidly, indicating the extent of the options which are available. An analyst may choose a method either on historical grounds or on the basis of particular features of available equipment. Two instruments, configured in different ways are capable of producing **equally** valid results; i.e. there is no single "correct" way of performing an analysis. Hence this booklet cannot provide multiple definitive methods, but aims to give **guidance** on the minimum performance criteria achievable. Examples of methods which achieve these criteria, and are available at the time of publishing this booklet, are presented.

The separation, detection and quantification of ions is achieved by using well established liquid chromatographic techniques. Both the equipment and principal modes of separation and detection apply equally to the chromatography of ions. Commonly, the term **Ion Chromatography** (IC) is used to cover the application of ion determination by chromatography. Ions or charged molecules exist only in predominantly aqueous solutions and need to be in the ionic (charged) form for separation to be achieved by equilibrium mechanisms based upon dynamic ion exchange. It is therefore more appropriate and descriptive to avoid this general term (ion chromatography) for simple ion exchange liquid chromatography, whilst not forgetting the inclusion of Ion Exclusion and Ion Pair techniques.

A chromatograph, when approximately set up, can separate and identify the individual components of a mixture, but to be useful, the amounts of these components must also be described. Since the procedure involves comparison of the sample to be analysed with external standards of known composition, it is clear that the accuracy with which the final result is known is strongly influenced by the accuracy with which these standards have been prepared. The possibility of other ions eluting after the same retention time needs to be considered.

It is the intention of this booklet to provide an overview of the application of the chromatography of ions to the field of water analysis.

Liquid chromatography now finds application in many branches of analytical chemistry. Difficulties can arise from the complexity of the sample matrix and a requirement for low limits of detection. The increasingly successful application of "ion chromatographic" determination of ionic moieties in water is a good indication of its scope and power. Other texts [1-10] and fields of application may also provide the basis for useful developments which can be applied to water analysis.

When compiling the methods to be included in this booklet it became apparent that certain aspects of experimental theory, practice or detail were common to all the different applications. To avoid repeating such information, these have been extracted and put into different sections at the front of the booklet.

Thus Chapter 2 deals with definitions that have particular applicability to chromatography of ions along with more general ones common to other fields of liquid chromatography.

Chapter 3 describes the principles and theoretical aspects of the application of chromatography of ions. The intention is to provide basic information of use in the practical application of chromatography, rather than a detailed account of all the theoretical concepts. An in depth understanding of the theory of chromatography is not an essential requirement for its successful application. However, a good basic knowledge of the theoretical aspects is essential for its efficient employment. Some attempt has been made in both Chapter 2 and this Chapter to use terminology common to all high performance liquid chromatography (HPLC). Trade names/terms used by different manufacturers of instruments for ion determination by chromatography have been equated where possible.

Chapter 4 describes the available components and instrumentation, and advocates the best practices which are common to most methods. There is now a very varied selection of both complete systems and also basic component units available on the commercial market. Only the essential requirements of the chromatography of ions are discussed in this series.

Applications of chromatography of ions to the analysis of water samples are described in Parts II and III grouped by ions commonly determined together:-

Anions (eg Cl^-, NO_2^-, $H_2PO_4^-$, Br^-, NO_3^-, SO_4^{2-})
Special ions (eg cyanide, sulphide, complex cyanides, fluoride)
Organic (eg formate, acetate, succinate, benzoate)
Cations (eg Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+})
Transition metals (eg Cd, Co, Cu, Fe, Pb, Mn, Ni, Zn)

Although the technique is widely used, there is a scarcity of published methods which give sufficient test data. However enough information is available to show that those separations and analyses which are accurately achievable are very dependent on the other ions and compounds present in the sample and on the equipment available.

There are two basic ways in which chromatography can be applied to ions, firstly as a general multi-determinand method and secondly the specific or target ion approach. It is rarely possible to achieve both objectives. For instance, if anions which elute early from the column are sought specifically, those which elute later on may take an unacceptably long time to elute. If, on the other hand, the latter are sought, the ions eluting early may appear as a confused inseparable jumble of peaks close to the start of the analysis. Some ions often coelute and require special separation procedures; several examples are cited later in this booklet. The methods here given are illustrative of what can be achieved. Furthermore, as the technique is developing rapidly, users are advised to consult both the scientific and trade literature for improvements. Table 1.1 lists the more important ions which can be determined at present using this technique. An index is appended listing ions for which chromatographic information is given in this booklet, and where this is located in the booklet.

There are many reasons why chromatography of ions is being increasingly applied to the analysis of water. These include high resolving power, great selectivity, fast analysis ease and simplicity of operation, continuous detection, precise identification based on accurate retention measurement, accurate quantitative measurement, repetitive analysis with the same column, and automation of the complete analysis and data-handling operations.

One disadvantage associated with all chromatographic techniques is the need to establish that the chosen chromatographic conditions lead to the unambiguous separation of the ions of interest from all the other components of the sample. If it is suspected that a particular peak is caused by more than one component, then a second more selective or specific detector should be used in series (e.g. suppressed conductivity, electrochemical, or UV absorption) in addition to the direct or indirect conductivity detector.

Recommended Texts for further reading:

1. *Introduction to Modern Liquid Chromatography, 2nd Edition.* L.R. Snyder and J.J. Kirkland. Wiley Interscience, 1979.
2. *Chromatographic Methods in Inorganic Analysis.* G. Schwedt, Huthig Verlag, 1981.
3. *Ion Chromatography, 2nd Edition.* D.T. Gjerde, and J. Fritz, Huthig Verlag, 1987.
4. *Ion Chromatographic Analysis for Environmental Pollutants.* E. Sawicki, J.D. Mulik and E. Wittgenstein, Ann Arbor Science, 1978.
5. Vol 2 of reference 4 above. J.D. Mulik and E. Sawicki, by Ann Arbor Science, 1979.
6. *Practice of Ion Chromatography.* F.C. Smith and C. Change, Wiley, 1983.
7. *Optimisation in HPLC.* R.E. Kaiser and E. Oelrich, Huthig Verlag, 1981.
8. *Handbook of Ion Chromatography.* J. Weiss, Dionex Corp USA, 1987.
9. *Ion Chromatography.* J.G. Tarter, Vol. 37 Chromatography Series, Dekker 1987.
10. *High performance Liquid Chromatography, Ion Chromatography, Thin Layer and Column Chromatography of Water Samples 1983.* HMSO, in this series.
11. *Ion Chromatography in Water Analysis.* O.A. Shipigun, and Yu A. Zololov, Horwood, Chichester 1988.
12. *Ion Chromatography Applicants.* R.E. Smith, CRC Press, Boca Raton (USA) 1988.
13. *Chemical Analysis of Ecological Materials.* Editor S.E. Allen. Blackwells 1989.
14. *Fundamentals of Analytical Chemistry, 5th Edition.* D.A. Skoog, D.M. West and F.J. Holler. Chapters 23, 25, 28 (some other chapters are also relevant). Saunders College Publishing. New York, London etc. 1988.

Table 1.1 *Ions Known To Have Been Determined By Chromatography*

Anions

Nitrogen
N_3^-, NO_2^-, NO_3^-

Sulphur
S^{2-}, HSO_3^-(+), SO_3^{2-}(+), SO_4^{2-}, $S_2O_3^{2-}$, $S_4O_6^{2-}$, SCN^-, $S_2O_6^{2-}$, $S_2O_8^{2-}$

*Aryl and Alkyl Sulphonates and Sulphates to C_{48}, Higher Polythionates

Phosphorous
HPO_2^{2-}, PO_3^{3-}, Ortho(+), Pyro, Tripoly, Trimeta and Tetrapoly Phosphates, *Polyphosphonates

Halogens
F^-, BF_4^-, PO_3F^{2-}
Cl^-, ClO^-, ClO_2^-, ClO_3^-, ClO_4^-
Br^-, BrO_3^-
I^-, IO_3^-
Chloride complex ions of Pt, Pd, Au, Fe, Pb

Carbon
CO_3^{2-}(+), CN^-, CNO^-

Alkyl Carboxylates
C_1 to *C_{18}, with both linear and branched carbon chains and with one, two, three or more carboxylate groups, halo-acetates, benzoate and ions of similar aromatic carboxylic acids, phthalates, EDTA, NTA.

Selenium
SeO_3^{2-}, SeO_4^{2-}

Arsenic
AsO_2^-, AsO_3^{3-}, AsO_4^{3-}(+)

Boron
Borate (+)

contd. (Notes on next page)

Silicon
Silicate (+)

***Cyanide Complexes**
Au(I and II), Fe(II and III), Co, Ag, Ni, Pd, Cu

Metal Oxyanions
CrO_4^{2-}(+), MoO_4^{2-}(+), WO_4^{2-}(+), ReO_4^-, MnO_4^-

Cations
Alkali and Alkaline Earth Metals
*Ethanolamines
Alkyl and *Aryl Amines
*Quaternary Ammonium ions

Heavy Metals (Post column colorimetry) (see also Anions)
Pb, Cu, Cd, Co, Zn, Ni, Fe, Mn, Cr, V, Ga, Sn, Tl, Hg, Ti, Y, Al, and Lanthanides, Fe(EDTA) complex

Comments
A number of the conducting and electrochemically active ions are also UV or Visible active to varying degrees.

Sensitivity for most of these species (except those marked *) is in the region of 5 to 100 ng mL^{-1} for a 100 µL injection. However this can often be improved by the use of larger sample loops, preconcentration techniques using a small concentrator column or by simply targetting the ion as a single species with a stronger eluent to produce a faster, more sensitive peak (at the expense of multiple ion determination).

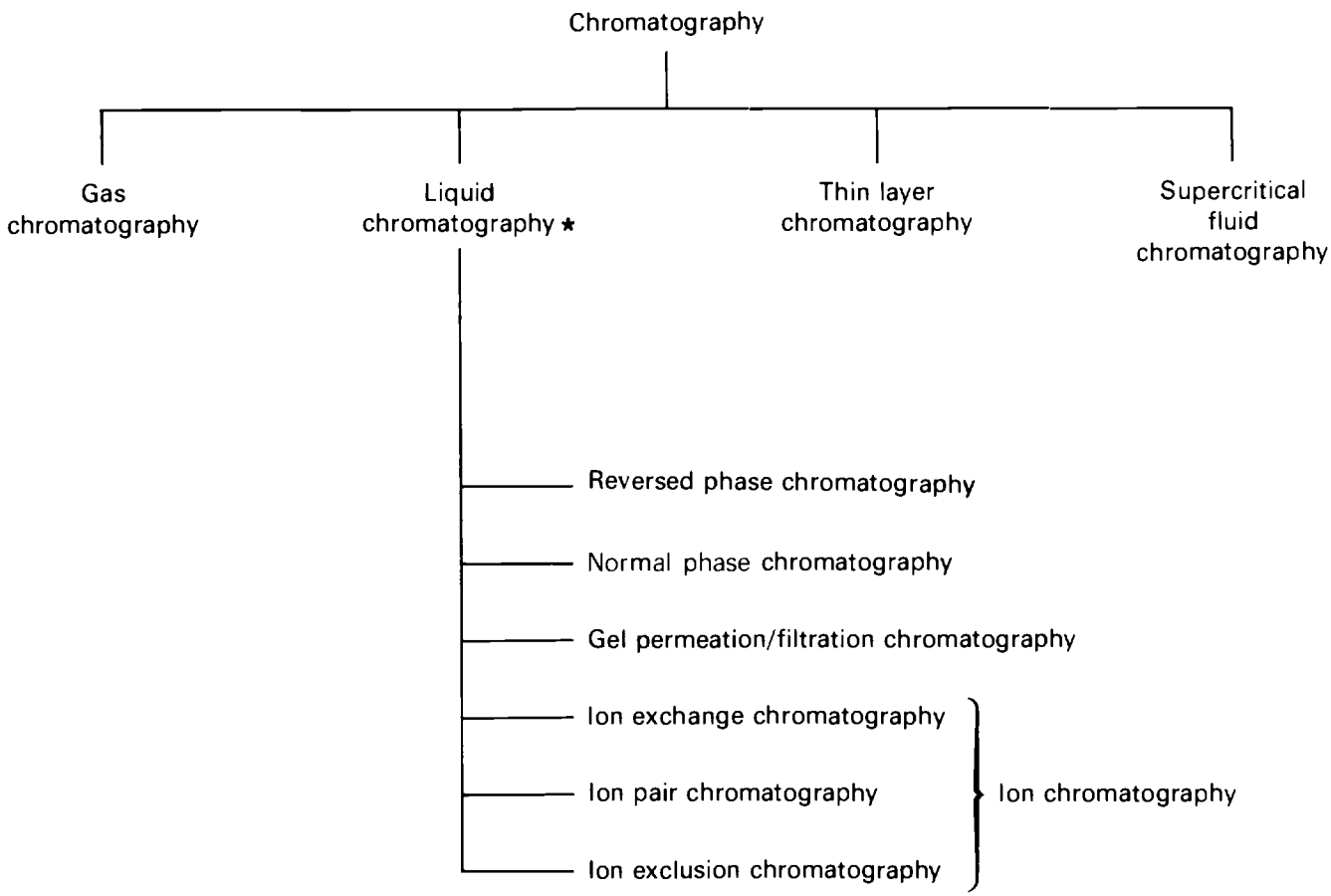
(+) The form in which these ions elute and hence the peak location is dependent on the elution pH and hence on the eluent buffer used (eg H_2CO_3, HCO_3^- or CO_3^{2-}).

Chapter 2 Definitions for the Chromatography of Ions

Chromatography involves the separation of components by differential migration. Samples require this separation prior to detection due to their complexity.

The chromatography of ionic species commonly called Ion Chromatography, is one branch of liquid chromatography, which is widely practiced as High Performance Liquid Chromatography (HPLC) (see Figure 2.1). Hence there is a well developed vocabulary available for its description. Consequently in the following listing of definitions, normal usage of chromatographic terms will be employed, and specialised terms will be explained relative to these and used only where necessary.

Figure 2.1 Interrelation of the various forms of chromatography



★ May be performed under gravity or hydraulic head, but usually carried out at elevated pressures

Glossary of Terms Relevant to Ion Chromatography

ANALYTICAL COLUMN
or separator column

The Liquid Chromatography (LC) column on which the separation of components is achieved.

BASELINE DISTURBANCE

There are several well-recognised sources of disturbances each of which generally prevent quantification of any over-lapping peaks.

Solvent Front
or water dip

The disturbance on the baseline due to any unretained components from the sample, ie $k'=0$, (See Fig. 2.2) corresponding to the void volume. All analyte peaks must be resolved from the solvent front which can be identified by injecting a sample of the "pure" water, used to prepare the mobile phase.

Systems Peaks

Peaks that are associated with the re-establishment of the dynamic equilibrium of the stationary and mobile phases. These artifacts derive from the analyte and can cause potentially interfering peaks. Mobile phases of greater complexity produce more system peaks.

CALIBRATION CURVE

Graph relating detector response, in peak area or height, to the weight of determinand introduced. It should be constructed for each component.

CAPACITY FACTOR (k')
or retention factor

A retention parameter, characteristic of the solute, dependent on the chromatographic conditions, but independent of the column dimensions, which can be used for identification purposes. It is defined as the ratio of the number of moles of solute in the stationary phase to that in the mobile phase. In practice, the most useful expression for k' is

$$k' = \frac{t_1 - t_0}{t_0}$$

where t_0 is the dead volume and t_1 is the retention time of peak 1 or in terms of net retention times, t', (see Fig. 2.2).

$$k' = \frac{t_1'}{t_0}$$

The most practical k' values lie in the range approximately 1 to 10, giving reasonably sharp peaks in acceptable analysis times.

CELL CONSTANT (K)

In conductivity detection, a parameter taking into account the electrode area (A), and distance (L), between the electrodes,

$$K=L/A$$

(units for practical purposes are cm^{-1}). This is generally determined experimentally, from a standard solution of known conductance.

CHEMICAL SUPPRESSION

A technique to remove the eluent ions that would otherwise cause a high background signal when using conductivity detectors. For example, with a sodium carbonate – bicarbonate eluent, suppression is achieved by substitution of the cations of the eluent and sample with H^+ ions, producing the weakly conducting carbonic acid.

COLUMN

An assembly comprising of a hollow tube with suitable end-fittings and support frits used to hold the packing material. This term is often used loosely to describe the packed column.

COLUMN CAPACITY
or loading capacity

The maximum amount of sample which can be loaded onto the analytical column without causing an overload. If this amount is exceeded, a loss in retention and a consequent non-linear response occurs, so that the results obtained are not normally acceptable. This amount is dependent on the ion-exchange capacity of the packing and the dimensions of the column.

COLUMN DISPERSION
(σ col)

The peak broadening that occurs in the column which, with the retention time (t_R), decides the efficiency (N).
The column efficiency is defined as

COLUMN EFFICIENCY (N)
or column plate count

$$N = \frac{t_R{}^2}{\sigma^2 \text{ col}}$$

where t_R = retention time, σ^2 col = column variance.

For Gaussian peaks then

$$N = 5.545 \frac{t_R{}^2}{w_{\frac{1}{2}}}$$

where $w_{\frac{1}{2}}$ = width at half height.

For a given packing, the column efficiency is proportional to the column length. For comparison of columns of different dimensions, the number of plates per metre determined using a common analyte should be used.

Column efficiencies are useful guides as they:

1. allow direct comparison of columns, in terms of separations achievable.
2. reveal the condition of a column (as N decreases with usage).
3. indicate the width of a peak with a given retention time.

COLUMN PACKING
support and, or stationary phase or packing material

A resin or silica based material of high surface area, which is packed at high pressures to give regular packing. The particles on which the chromatography is performed are of uniform size. Silica has a limited range of pH compatability (about 2–8), but resins have compatibility over a wider range (about pH 2–12), which is a great benefit. Generally, smaller particle diameters give higher efficiency columns, but the back pressures generated are correspondingly higher. New packing materials are constantly being evaluated.

COLUMN PLATE COUNT

see Column Efficiency.

CONCENTRATOR COLUMN

see Precolumn.

CONDUCTANCE (G)
(or specific conductivity)

This is equivalent to the inverse of the solution electrolytic resistance (measured in reciprocal ohms, or mhos, also called Siemens). For liquids the commonly used unit is the microSiemen (μS).

CONDUCTIVITY

see Specific Conductance.

DETECTION

This can be carried out in four main ways.

1. Direct Conductivity Detection

Ionic species are detected by conductivity at the end of the analytical column. Back-off of the high background signal (200–400 μS) from the conductivity of the eluent is achieved electronically (sometimes referred to as Electronic Suppression).

2. Suppressed Conductivity Detection — Detection of ions by conductivity in a modified eluent (see suppressor), giving a low background signal (0–50 μS) (sometimes called Chemical Suppression). (The most common form of detection).

3. Direct UV Detection — Detection of species in the eluent by monitoring of the UV absorbance. Some ions can be detected directly, but many ions are transparent to UV, thus requiring indirect UV detection.

4. Indirect UV Detection — Detection of non-UV absorbing species by use of an UV-absorbing buffer, e.g. phthalate, that reveals the separated UV transparent ions due to the one-for-one exchange (on a charge basis).

ELUENT
or mobile phase — The solution used in the analytical column to elute the analytes. Elution of ions from ion-exchange columns requires buffered eluents to provide solutions with carefully controlled pH and well-defined ionic strengths.

ELUTION — The removal of the solutes from the column, by the mobile phase.

EXTERNAL DISPERSION
or system dispersion, or extra column broadening — The broadening that occurs in the system excluding the column, which should be insignificant compared to the column dispersion. There are contributions from the injector tubing and connections, detector cell, and detector electronics, i.e. all parts between sample introduction and peak reporting. For columns of reduced volume, the external dispersion must be similarly reduced.

EXTRA COLUMN BROADENING — see External Dispersion

GRADIENT ELUTION — Elution in which the composition of the mobile phase is changed progressively.

GUARD COLUMN — see Precolumn.

ION EXCHANGE CAPACITY — Number of active ion-exchange sites per unit weight or volume of packing, measured in meq g^{-1} or meq mL^{-1}.

ION EXCHANGE PACKING — A silica or resin based packing with ion exchange sites that are chemically bonded to the surface.

ION EXCLUSION — Separation by exclusion of ions from the column packing. Ionic materials, of the same charge as the exchange sites, pass rapidly through the column. Non-ionic materials show retention, by partitioning between the flowing eluent and eluent trapped within the resin pores or matrix; thus making it well suited for salts of weak acids and weak bases. Careful pH control is vital. (See also Part 1 Chapter 3.3).

ION PAIR CHROMATOGRAPHY — Use of a charged species, which modifies the chromatography of ionic analytes (of opposite charge), either by adsorption of the ion-pairing agent onto the column packing material or formation of ion pairs.

LIMIT OF DETECTION — The limit of detection (LOD) is that concentration for which there is a desirably small probability, that the result obtained will be less than the criterion of detection. For 95% confidence, the LOD = $4.65s_w$, where s_w is the within batch standard deviation of the blank determinations. Strictly, the Limit of Detection is given by LOD = $2t_{0.1}\sqrt{2}s_w$, but when the estimation of the degrees of freedom is greater than 6, there will be very little difference between the values obtained from each expression, see Refs 2 and 3 ($t_{0.1}$ is the appropriate value of Student's t).

LOADING CAPACITY — see Column Capacity.

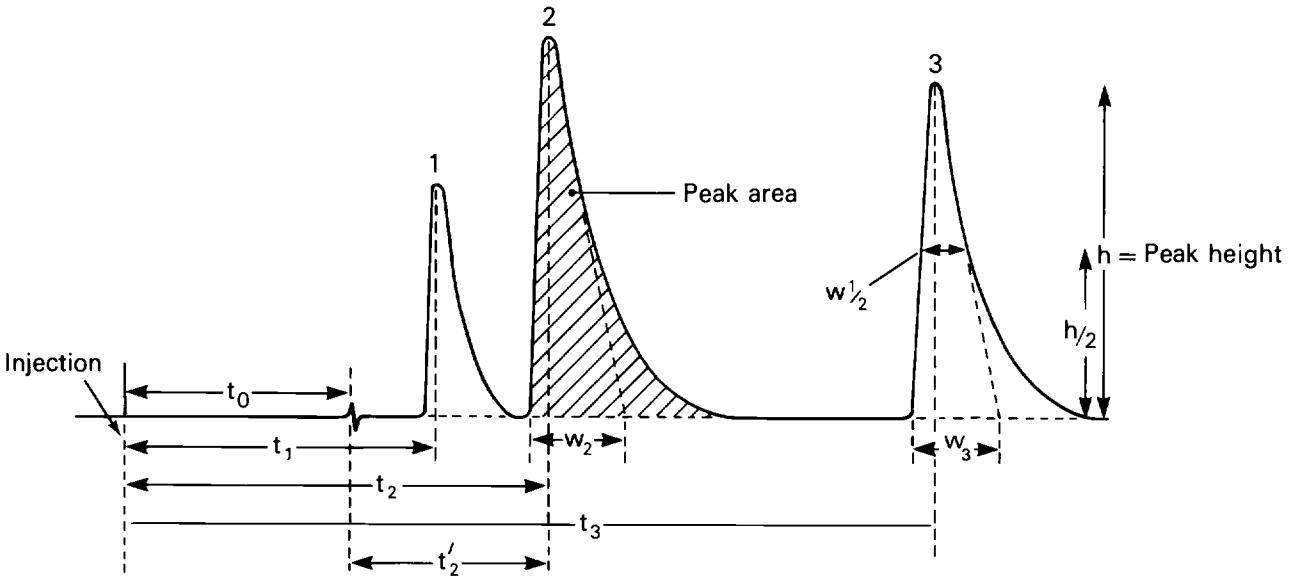
MOBILE PHASE — see Eluent.

PACKING MATERIAL — see Column Packing.

PEAK HEIGHT OR AREA

(see Fig 2.2). A measurable parameter, usually proportional to the concentration or weight of analytes. Calibration with standards is needed; – see Quantification.

Figure 2.2 Typical chromatographic peaks



Note: The trailing of peaks is exaggerated for clarity in the diagram. Actual peaks are almost always sharper.

PEAK IDENTIFICATION

The process of establishing analyte integrity. This can be achieved from:

1. retention times for very stable conditions;
2. relative retention times, to compensate for small variations;
3. capacity factors, to compare different dimensions of columns with the same packing under identical conditions.

PRECOLUMN
or guard column
or scavenger column

A short column used prior to the analytical column for various reasons. A Precolumn is designed to protect the analytical column from components which would deposit on the main column. It can act as a selective adsorber e.g. for removal of organics from inorganic ionic samples which are then separated on a resin-based column. If silica supports are used, a guard column may be inserted between the pump and the injector which presaturates the eluent with silica, thus prolonging the life of the analytical column.

Concentrator Column

A special form of precolumn, on which ions can be concentrated prior to the analysis. It is connected in-line between the point of sample introduction and the analytical column(s).

QUANTIFICATION The calculation of amounts or concentrations corresponding to each analyte. Quantification must be achieved relative to standard solutions, using either external or internal standardization to give calibration plots, according to standard practices (see Part 1 Chapter 4).

RESOLUTION A measure of the separation of two adjacent peaks, expressed as the ratio of the difference in retention times and an average of the two peak-widths.

$$R = \frac{2(t_2 - t_1)}{w_2 + w_1}$$

where t_1 and t_2 are retention times and w_1 and w_2, are baseline widths, of components 1 and 2 (see Fig. 2.2).

It can simply be shown that this can be expressed as a product of 3 terms; a separation factor $(\alpha-1)$, related to the selectivity (α), an efficiency term (N), and a capacity factor ratio, $(k'/1+k')$.

$$\text{Overall } R = \frac{1}{4}(\alpha - 1)\sqrt{N}\frac{(k')}{(1 + k')}$$

Thus in order to increase the resolution, the selectivity, the capacity factor ratio or the efficiency can each be increased. However, efficiency has a very limited effect on increasing the resolution.

RETENTION FACTOR see Capacity Factor.

RETENTION TIME (t') The time taken for an analyte to elute from a column. On the chromatogram it is proportional to the distance from the point of injection to the measured peak maximum (see Fig. 2.2). The net retention time is measured relative to the solvent front. This is the most practical means for peak identification.

Relative Retention Times These are calculated from analyte retention times relative to retention times of reference peaks. They are useful for identification purposes, allowing for the small chromatographic variations.

SCAVENGER COLUMN see Precolumn.

SELECTIVITY (α) Separation selectivity or the relative retention; the most useful form being

$$\alpha = \frac{k'_{(2)}}{k'_{(1)}}$$

where $k'_{(1)}$ and $k'_{(2)}$, are the capacity factors for peaks 1 and 2

or in terms of net retention times.

$$\alpha = \frac{t_2'}{t_1'}$$

Selectivity is the parameter most directly related to the selection of the particular eluent and column in use. Change in elution order and thus of selectivity can be affected by changes in eluent and/or column.

SENSITIVITY The slope of the calibration curve for a given analytical procedure; ie the signal output per amount of analyte introduced.

SEPARATOR COLUMN see Analytical Column.

SOLUTE A dissolved species. In the chromatography of ions, analyte solutes can be anionic or cationic species.

SOLVENT FRONT see Baseline Disturbances.

SPECIFIC CONDUCTANCE (k) or specific conductivity

The conductance taking into account the area of and the distance between the electrodes, with units of mhos cm^{-1} or Siemens(S) cm^{-1}. The practical units are μS cm^{-1}.

i.e. Specific Conductance = Conductance. Cell Constant
($S\ cm^{-1}$) (S) (cm^{-1})

SPECIFIC CONDUCTIVITY

see Conductance.

STATIONARY PHASE

see Column Packing

SUPPRESSION

see Chemical Suppression.

SUPPRESSOR

A device placed between the column and detector to minimise eluent conductivity and convert sample species to a common form, thus increasing detector sensitivity. This may be a packed-bed suppressor, hollow fibre suppressor, or, most recently, the micro-membrane suppressor.

SYSTEM DISPERSION

see External Dispersion.

SYSTEMS PEAKS

see Baseline Disturbance.

VOID VOLUME

The interstitial volume within a column accessible to the flowing eluent. Unretained components of the sample are eluted at the "solvent front" of the chromatogram.

WATER DIP

see Baseline Disturbance.

References

1. Snyder, L.R. and Kirkland, J.J., *Introduction to Modern Liquid Chromatography*, Wiley-Interscience 1979, 2nd edition.
2. Cheeseman, R.V. and Wilson, A.L. Revised M. Gardner. WRC Medmenham 1990. *A Manual on Analytical Quality Control for the Water Industry.*
3. Hunt, D.T.E. and Wilson, A.L., *The Chemical Analysis of Water*, 2nd Edition, 1986. The Royal Society of Chemistry.
4. Miller, J.C. and Miller, J.N., *Statistics for Analytical Chemistry*. 2nd Edn. Ellis Horwood Ltd 1988.

Chapter 3 Principles of Chromatography as Applied to Ionic Separations

3.1 Introduction

Unlike other forms of liquid chromatography, much of the driving force behind the success of the chromatography of ions, commonly referred to as ion chromatography has derived from one company patenting both column and detector technology and subsequently successfuly commercialising a method of analysis. This specialisation should not be allowed to confuse the understanding of the technique, because conventional HPLC equipment and column technology can be readily applied to the separation, characterisation, and quantification of solutions of ions. A comprehensive review of the determination of inorganic anions by HPLC appeared in 1984 (1). In broad terms, ion chromatographic methods, when applied to inorganic ions, chiefly exploit an ion-exchange separation stage followed by detection using one or more monitoring systems. The chromatogram generated provides the qualitative and quantitative information of interest. In the case of organic anions it is also possible to exploit the technique known as ion-exclusion chromatography.

3.2 Analytes

By definition, the analyte ions detected by chromatography need to be in an ionised form, and the most useful guide to the degree of ionisation of an acid is the pKa (i.e. the logarithm to the base ten of the dissociation constant of the compound). This value represents the pH at which the concentrations of the undissociated acid and the anion formed from it are equal. Lists of pKa values for various inorganic and organic acids are given in Tables 3.1 and 3.2). As a general rule, it is desirable to use eluents buffered to 1 or 3 pH units higher than the pKa of an analyte to ensure that it is separated and detected as an anionic species. Thus, common anions such as Cl^-, SO_4^{2-} and NO_3^- can be examined over a broad pH range (e.g. pH 1–11), whereas anions derived from very weak acids e.g. CN^-, S^{2-} are only substantially ionised over a narrow pH range (e.g. pH 11–12). Consideration of the pKa (or pKb for bases) is therefore an important first step in designing a separation procedure.

3.3 Separation Systems

The chromatographic methods used for ions involve either an ion-exchange, or ion-exclusion separation followed by detection using one or more monitoring systems. Although ion-exchange chromatography has been in use for many decades the design of a procedure specifically for the rapid chromatographic analysis of ions dates from 1975 (2). That system, and most of those subsequently developed, required careful co-ordination of the various stages in the analysis, so that the eluent system achieving the separation was also compatible with the final detection procedure.

3.3.1 Ion-exchange Procedures

The column packing materials used for separating inorganic ions fall into two classes:-

1. Silica-based materials
2. Organic polymers – typically based on polystyrene-divinylbenzene copolymers

Silica-based packing materials, although often providing more efficient separations than their organic polymer counterparts, have a slight but significant solubility in water. At pH values in excess of pH 7 such packings often deteriorate rapidly, but even at more acidic pH values some attack can occur. This can be minimised by using a silica pre-column to presaturate the eluent and/or by incorporating organic solvents such as methanol or acetonitrile in the eluent. The pH limitations of such packings restricts their application to the separation of anions with low pKa values.

Polymeric packings have a greater stability in aqueous eluents and can be used over a wide pH range (e.g. pH 2–12). The efficiency of these packings is generally lower than for silica-based materials of comparable size, but in recent years greater attention to controlling pore uniformity and particle size has led to improvements. In some circumstances, operation at an elevated temperature can also improve separation and column performance. Long-term stability and applicability to ions derived from weak and strong acids and bases, makes the polymer-based packings the more promising of the two types.

In common with developments in other areas of HPLC, the particle size of the packings used for the chromatography of ions has tended to get smaller. Pellicular materials based on 20 – 30 μm diameter sulphonated polystyrene-divinylbenzene (PSDVB)

beads coated with a thin layer of ion-exchanging latex beads of 0.1 – 0.5 μm diameter established this technique. The successful application of such materials prompted studies with the silica-based packings available at the time which had diameters of 5 – 10 μm. The improved efficiencies obtained, in turn promoted research into the improvement of latex PSDVB beads [4], so that alternative PSDVB and other polymer based packings with diameters similar to those of silica particles are now commercially available. Although they are still somewhat less efficient than their silica equivalent, it now seems that because of their wide pH stability PSDVB beads will dominate this field of chromatography.

The ion-exchange capacity of the packing materials used in IC is an important parameter. Columns packed with materials of high capacity permit a large mass of analyte to be injected, but they also necessitate the use of eluents of high ionic strength to displace the analytes from the column. In general, the constraints imposed by the detection method favour the use of low capacity packings and they are most frequently in the range 0.01 – 0.1 meq g^{-1}.

The chromatographic systems in use which exploit ion-exchange fall into two distinct classes:-

1. Systems using permanently modified packing materials. This type of separation uses a packing material containing ionisable functional groups (typically quaternary ammonium or sulphonic acid) covalently bound to the silica or PSDVB matrix. These are the most commonly used.

2. Systems based on dynamically modified packing materials (also called ion-interaction methods).

This type of separation operates by a process in which the ionisable functional group is introduced on to the surface of the packing materials by adsorption of lipophilic molecules containing ionisable groups (i.e. the so-called ion-pairing agents in HPLC). The lipophilic modifier is introduced with the eluent and ultimately a dynamic equilibrium is reached which permits reproducible ion-exchange chromatography to be carried out. Typical modifiers are quaternary ammonium salts and sulphonic acids both containing long alkyl chains.

Silica-based packings with surfaces covered with chemically bonded alkyl chains (e.g. C_{18}, C_8 etc.) and PSDVB packings can be dynamically modified as described above. An attraction of this type of approach is that a very broad range of ion-exchange capacities can be generated merely by compositional changes in the eluent. Typical aqueous eluents would contain 30% or more of an organic solvent (usually acetonitrile or alternatively methanol in the case of PSDVB packings, and usually methanol or otherwise acetonitrile for silica based packing), both with the ion-pairing agent at a concentration between about 0.1 and 10mM with accompanying counter ions.

A variant of the second type of separation procedure is one in which the packing material is first dynamically modified and is then used with a completely aqueous eluent containing no ion-pairing agent. The hydrophobic character of the reagent tends to leave it strongly bound to the packing material and it is possible to develop stable separation systems by this means. A complication with PSDVB packings is that low efficiency separations are obtained with completely aqueous eluents. This is attributed to the "non-wetting" of the PSDVB surface and can be overcome by incorporating 5 – 10% of acetonitrile into the eluent.

3.3.2 Ion Exclusion Procedures

The ion-exchange procedures described above do not always give adequate separations of anions derived from weakly ionised organic acids. For the analysis of mixtures of such compounds, it may be preferable to exploit the alternative technique of ion-exclusion. In this form of chromatography a high capacity PSDVB resin is used – the surface of this material acts as a semi-permeable membrane. Donnan exclusion prevents highly ionised inorganic species from entering the pore structure, whereas the weakly ionised molecules may enter and be retained.

3.4 Column Dimensions

With most analysts using commercially available columns, there has tended to be some standardisation of column dimensions. Columns of about 4.5 – 5 mm i. d. are in most common use with lengths ranging from 10 cm to 50 cm, (most are 25 cm or less). Enhanced sensitivity can be achieved by employing columns of 2 – 3 mm i.d., but, because column packing becomes more difficult with tubes of lower diameter, there can be some loss in chromatographic performance. PSDVB packings are more difficult to pack than silica, and pre-swelling of the material in the eluent is often an essential prerequisite for preparing columns of acceptable stability and efficiency.

3.5 Eluent Composition

The eluent composition is a critical factor in the design of a chromatographic method. Water is the major component of all eluents but organic solvents such as acetonitrile or methanol can be added to impart properties absent from the fully aqueous composition. The organic solvent rarely influences the elution sequence of inorganic anions, but it can improve column stability or column efficiency depending on the type of packing material being used. With dynamically modified systems, the presence of the organic solvent influences the equilibrium distribution of the ion-pairing agent, e.g. holding the ion-pairing reagent concentration constant whilst increasing the organic content of the eluent can result in more rapid elution. The ionic strength of the eluent and the nature of the counter-ion both influence the resolution achieved with a column of a specific ion-exchange capacity, for example, increasing ionic strength decreases resolution. Effects caused by variation of the pH are more complex since the degree of ionisation of both analyte ions and counter ions can be influenced. In most methods, the particular combination of variables usually represents a compromise which reconciles the demands of the detection system with the chromatographic separation required to perform the analysis. Conductivity detection is the most common method of monitoring. This demands a low ionic strength and eluent ions which have low conductivity (or eluent ions which can be converted to a low conductivity form after suppression – see later). Methods exploiting UV absorbance, electrochemical reactions, or refractive index monitoring have different requirements and these depend on whether direct or indirect detection is contemplated.

3.6 Detection Methods

3.6.1 Conductivity Detection

Because conductivity is a universal property of all ions, it is an obvious one to exploit for monitoring, although its application to HPLC was minimal before chromatographic techniques became widely used for ions. The design of these detectors is discussed later, but the way in which they are operated is as follows.

3.6.1.1 Direct monitoring without chemical suppression

This type of procedure results in a conductance signal being superimposed on a standing signal of moderate intensity. The ionic strength and conductivity of the ions used in the eluent determine the strength of the standing signal, and the lower this is the better. Chromatograms of ions of a mixture can display both positive and negative peaks.

3.6.1.2 Direct monitor with chemical suppression

This mode of detection is more sensitive than that of 3.6.1.1 in that the background signal from the eluent is reduced by installing a device which reduces the conductivity of the eluent. This device is installed between the chromatographic column and the detector and is termed a suppressor. In its original form it was a high capacity ion-exchange column which was able to convert a salt with high conductivity, being used as the source of eluent ions, into a low conductivity acid or base, whilst doing the reverse with the analyte ions. In the case of anion analysis, for example, the suppressor column contained a cation exchange resin in the hydrogen form. This column could convert eluents containing sodium carbonate and sodium bicarbonate (i.e. salts with high conductivity) into carbonic acid which is a very weak acid of low conductivity.

$$Resin^-H^+ + Na^+HCO_3^- \longrightarrow Resin^-Na^+ + H_2CO_3$$

Eluents containing sodium hydroxide could also be similarly suppressed

$$Resin^-H^+ + Na^+OH^- \longrightarrow Resin^-Na^+ + H_2O$$

Analyte ions, derived from strong acids, which pass through the chromatographic system in the form of their sodium salts, are converted by the suppressor into their free acids which have stronger conductivity.

$$Resin^{-}H^{+} + Na^{+}Cl^{-} \longrightarrow Resin^{-}Na^{+} + H^{+}Cl^{-}$$

The ion-exchange process taking place in the suppressor greatly enhances the sensitivity of the method and also simplifies the chromatogram, as all peaks are in one direction, whereas with unsuppressed systems peaks can be either positive or negative with respect to the background signal. The low background signal makes this mode of detection less sensitive to its thermal environment.

Columns used for suppression in the first commercially available instruments introduced significant band-spreading into the separation. In later developments hollow tubes or membranes of ion-exchanging polymer have been exploited. These achieve the same ionic reactions with significantly better resolution. The latest micromembrane suppressor can suppress eluents at 100–150 mM and be used with gradient elution.

3.6.1.3 Indirect detection

By using strongly conducting eluent ions it is possible to establish a relatively high background signal, such that when analyte ions are eluted, a fall in standing signal occurs, provided these ions are of lower conductivity than the eluent ion. This procedure requires good temperature control; room temperature control is advised to avoid the hunting which is liable if only the instrument is regulated.

3.6.2 UV Absorbance Detection

Conductivity is a property of all ions whereas they do not all display significant UV absorbance at wavelengths which can be conveniently monitored. However, by using different techniques it is possible to generate a signal to detect the presence of most ions. Two different approaches to exploiting UV absorbance have been used:

3.6.2.1 Direct detection

A number of inorganic anions display significant absorbance in the wavelength region 200 – 300 nm and, provided the background absorbance of the eluent is sufficiently low at the monitoring wavelength, such anions can be directly determined. Colourless anions containing no aromatic moiety generally display most absorbance in the region 200 – 220 nm. The 220 nm absorbance values provide a useful guide to detectability (see Table 3.3).

From this table it is possible to deduce whether a particular anion could be detected by its UV absorbance (for strict comparability equimolar rather than equal concentration solutions should be compared). In principle an anion will display absorbance provided it is eluted with an eluent ion of lower absorbance. Multivalent counter ions such as tartrate, citrate, phosphate, or sulphate provide useful low-absorbance materials for use as eluent ions.

3.6.2.2 Indirect detection

Provided a separation of ions is achieved by an ion-exchange process, it is possible to detect eluting analyte ions whenever there is a significant difference between their molecular extinction coefficient and that of the eluent ion at the monitoring wavelength (2). It has been common practice to employ eluent ions derived from an aromatic acid e.g. phthalic acid, to exploit the indirect photometric effect at wavelengths in the 300 nm region. At such wavelengths most inorganic and many non-aromatic ions display virtually no absorbance, and hence, they can be observed by a fall in the background signal as they elute. However, it is important to appreciate that the indirect photometric effect can be observed at any wavelength provided a suitable counter ion is used. For example, the use of citrate permits monitoring at 220 nm and at this wavelength both increases and decreases in the background signal may be observed – this phenomenon can provide useful qualitative information.

3.6.3 Electrochemical Detection

Detection of ions by amperometry, potentiometry, or coulometry is more selective than the detection techniques previously discussed. This selectivity can sometimes be used to good effect to provide acceptable data with analyte mixtures which are inadequately resolved, and which therefore give unsatisfactory results when monitored by other methods. In general, electrochemical detection provides a very sensitive method of analysis.

Amperometric techniques are chiefly applied to the detection of ions which readily oxidise in aqueous media under fairly low applied potentials. Reduction mode detection is rendered difficult by interference from atmospheric oxygen.

A summary of published work is given below:

Working Electrode	Counter Electrode	Reference Electrode	Anions detected	Ref
Pt	Pt	Ag/AgCl	OCl^-, Hydrazine, SO_3^{2-}, I^-	8
Ag	Pt	Ag/AgCl	CN^-, S^{2-}, Br^-,	3
Au	Pt	Ag/AgCl	CN^-, S^{2-}	6
Hg or Hg/Pt	Glassy C	Ag/AgCl	CN^-, S^{2-}	6
Hg	Pt	Ag/AgCl	CN^-, S^{2-}	6
Glassy C	Stainless steel	Ag/AgCl	NO_2^-, I^-, $S_2O_3^{2-}$, SCN^-, Oxalate	9
Glassy C	Stainless steel	Ag/AgCl	As above plus CN^-, S^{2-}, N_3^-	7

3.7 Equipment

As was mentioned in the introduction to this chapter, the chromatography of ions can be performed with conventional HPLC equipment. For a detailed description of such instrumentation refer to any of the standard texts on this subject (see Chapter 1).

3.7.1 Pumps

The pumps used have identical requirements to those used in other forms of HPLC, although some modern pumps are specifically designed so that there are no corrodable metal parts. This reduces the risk of eluent corroding the pump and also minimises the introduction of metal traces into the column and detector systems. Most separations can be performed under isocratic conditions but gradient systems have been developed for certain complex separations. Few separations are conducted at pressures in excess of 2000 psi.

3.7.2 Injectors

Introduction of the sample on to the column is usually performed with a high pressure injection valve fitted with a fixed volume loop. Such injectors can be filled manually or automatically

3.7.3 Detectors

The detector most widely used is the conductivity detector and various types have been specifically produced for this application. In addition, conventional HPLC detectors exploiting UV absorbance, electrochemical oxidation, and refractive index changes can also be used. With careful attention to eluent conditions, systems can range from highly selective to virtually universal in characteristics. For analytes which display radioactivity there are highly specific post-column monitoring systems available.

3.7.3.1 Conductivity Detectors

The cells used to monitor the conductivity of the eluates produced in the chromatography of ions are typically of low volume (e.g. less than 5 μL). This minimises band spreading of chromatographic peaks and provides the high sensitivity and rapid response required. Most conductivity cells contain two electrodes (usually platinum or some other inert material) across which a voltage is applied. An a.c. voltage is preferred to d.c. to minimise electrolysis. Improved linearity can be achieved by using a bipolar pulse technique in which two successive constant voltage pulses of opposite polarity to that of the cell are applied and the current flow is measured at the end of the second pulse. This procedure helps to overcome capacitance effects and permits the electronic nulling of the background conductance of the eluent. Some of these effects are also removed or minimise further by use of a four electrode system. Other developments with conductivity detectors include the introduction of a logarithmic response to increase the dynamic range, and cell designs in which the electrodes are not in galvanic contact with the solutions to be measured. The principal advantage of this latter innovation is that polarization, corrosion and other side effects that may change the measuring surface of the electrode and also the cell constant are eliminated.

Thermal stability is a most important parameter in the successful operation of conductivity detectors, particularly for high conductivity eluents, as the temperature coefficient of conductivity measurements is approximately 2% per °C. Thermostating of the cell reduces both noise and drift. Some systems also incorporate electronic means of providing temperature compensation by mathematical corrections based upon the laws of conductance.

3.7.3.2 UV Absorbance Detectors

The UV photometric detectors which are widely used for HPLC monitoring are specifically designed to provide high sensitivity, linearity of response and minimal band broadening and can be used for chromatography of ions without adaptation. In general, detectors operated to measure directly the absorbance of inorganic anions require wavelengths of 200 – 220 nm whereas indirect detection usually requires higher wavelengths in order that the background absorbance falls within the range 0.2 – 0.8 A.U. With counter-ions such as benzoate, phthalate, etc, wavelengths in the range 250 – 300 nm are commonly used, and for indirect monitoring of cations the monitoring wavelength may extend into the visible region of the spectrum. Some form of back-off is required to compensate for the high background absorbance of eluent in indirect monitoring. To meet the above requirements, a variable wavelength detector is usually a good choice for use in ion chromatography. The thermal stability of UV detectors is markedly superior to that of the conductivity and refractive index detectors. Post column reaction of inorganic species with spectrophotometric reagents requires UV-visible detectors capable of operating at wavelengths up to 600 nm.

3.7.3.3 Refractive Index Detectors

Since refractive index (RI) is a universal property, differential refractive index monitoring can be used for the indirect detection of ionic species, provided the counter ion and the analyte display significant differences in their RI. The most effective way to achieve this is to use an aromatic counter ion (e.g. phthalate, benzoate, salicylate, etc.) to detect non-aromatic ions. RI detectors are still in common use for the monitoring of carbohydrates separated by HPLC and can be applied without modification to the chromatography of ions. Major disadvantages are the relatively low sensitivity and poor thermal stability.

3.7.3.4 Electrochemical Detectors

Electrochemical detectors are used widely for selective and high sensitivity monitoring in HPLC and can be used without modification for the detection of certain analytes separated by ion chromatography. The design and operating conditions vary substantially from one procedure to another and reference to the publications (5–9) is recommended.

3.7.3.5 Radiochemical Detectors

Where radioactive analytes are to be detected it is possible to use detectors which are specifically designed to monitor phenomena associated with radioactive decay. The most well established radiochemical technique applied to the chromatography of ions uses the principles of scintillation counting to measure beta-emitting radionuclides. By post-column addition of a 'cocktail' of organic liquid scintillants to the eluate from the chromatographic column it is possible to detect emission of beta particles as photons of UV/visible light. The intensity of each light pulse gives an indication of the energy of the beta decay, whilst the rate of light pulse emissions provides a measure of the concentration. Since the energy discrimination of liquid scintillation counting is somewhat limited, and the energy of beta radiation itself is non-specific (i.e. merely ranges up to a maximum value for each radionuclide), chromatography provides a suitable method to carry out the necessary separation of individual nuclide ions. The use of two photomultipliers working in a coincidence mode can effectively reduce electronic and baseline noise, and improve the detection limits.

The major problems associated with radiochemical monitoring arise from signal quenching by:-

1. Chemical interferences in which compounds coeluting with the analyte interfere in the energy transfer to the scintillant.

2. Coloured compounds in the eluate which absorb emitted light from the scintillant.

Chemiluminescence can also introduce interferences caused by the interaction of eluting compounds with the scintillant to produce light.

Table 3.1a Dissociation Constants of some Organic Acids in Aqueous Solution

Compound	T°C	Step	K	pK	Compound	T°C	Step	K	pK
Acetic	25		1.76×10^{-3}	4.75	Maleic	25	1	1.42×10^{-2}	1.83
Ascorbic	24	1	7.94×10^{-5}	4.10	Maleic	25	2	8.57×10^{-3}	6.07
Ascorbic	16	2	1.62×10^{-12}	11.79	Malic	25	1	3.9×10^{-4}	3.40
					Malic	25	2	7.8×10^{-6}	6.11
Benzoic	25		6.46×10^{-4}	4.19	Malonic	25	1	1.49×10^{-2}	2.83
Benzosulphonic	25		2.00×10^{-1}	0.70	Malonic	25	2	2.03×10^{-3}	5.69
Bromoacetic	25		2.05×10^{-3}	2.09	Mandelic	25		1.4×10^{-4}	3.85
n-Butyric	20		1.54×10^{-3}	4.81					
iso-Butyric	18		1.44×10^{-3}	4.84	Naphalenesulphonic	25		2.7×10^{-1}	0.57
					o-Nitrobenzoic	18		6.95×10^{-3}	2.16
					o-Nitrophenol	25		6.8×10^{-2}	7.17
n-Caproic	18		1.43×10^{-5}	4.83	m-Nitrophenol	25		5.3×10^{-3}	8.28
iso-Caproic	18		1.40×10^{-3}	4.84	p-Nitrophenol	25		7.00×10^{-3}	7.15
Chloroacetic	25		1.40×10^{-3}	2.85					
o-Chlorobenzoic	25		1.20×10^{-3}	2.92	Oxalic	25	1	5.90×10^{-2}	1.23
m-Chlorobenzoic	25		1.51×10^{-4}	3.82	Oxalic	25	2	6.40×10^{-5}	4.19
p-Chlorobenzoic	25		1.04×10^{-4}	3.93					
Citric	18	1	7.10×10^{-4}	3.14	Phenol	20		1.28×10^{-16}	9.89
Citric	18	2	1.68×10^{-5}	4.77	Phenylacetic	18		5.2×10^{-3}	4.25
Citric	18	3	8.4×10^{-6}	6.39	o-Phthalic	25	1	1.3×10^{-3}	2.89
					o-Phthalic	25	2	3.9×10^{-6}	5.51
Dichloracetic	25		3.32×10^{-2}	1.48	m-Phthalic	25	1	2.9×10^{-2}	3.54
Dichlorophenol (2,3-)	25		3.6×10^{-3}	7.44	m-Phthalic	18	2	2.8×10^{-3}	4.69
					p-Phthalic	25	1	3.1×10^{-4}	3.51
					p-Phthalic	18	2	1.5×10^{-5}	4.82
Formic	20		1.77×10^{-4}	3.75	Picric	25		4.2×10^{-1}	0.38
Fumaric	18	1	9.30×10^{-4}	3.03	Propionic	25		1.34×10^{-1}	4.87
Fumaric	18	2	3.62×10^{-5}	4.44					
Fumaric	25		6.76×10^{-4}	3.17	Succinic	25	1	6.89×10^{-1}	4.16
					Succinic	25	2	2.47×10^{-4}	5.61
Gluturamic	25		3.98×10^{-5}	4.00	Sulphanilic	25		5.9×10^{-4}	3.23
Glycolic	25		1.48×10^{-4}	3.83					
					d or l-Tartaric	25	1	1.04×10^{-3}	2.98
o-Hydroxybenzoic	19	1	1.07×10^{-3}	2.97	d or l-Tartaric	25	2	4.55×10^{-5}	4.34
o-Hydroxybenzoic	18	2	4.00×10^{-14}	13.40	meso-Tartaric	25	1	6.00×10^{-4}	3.22
m-Hydroxybenzoic	19	1	8.7×10^{-3}	4.00	meso-Tartaric	25	2	1.53×10^{-5}	4.82
m-Hydroxybenzoic	19	2	1.2×10^{-10}	9.92	Terephthalic	25		3.1×10^{-4}	3.51
p-Hydroxybenzoic	19	1	3.3×10^{-3}	4.48	Thioacetic	25		4.7×10^{-4}	3.33
p-Hydroxybenzoic	19	2	4.8×10^{-10}	9.32	o-Toluic	25		5.32×10^{-1}	4.27
γ-Hydroxybutyric	25		2.00×10^{-5}	4.70	m-Toluic	25		4.33×10^{-1}	4.36
					Trichloroacetic	25		2.00×10^{-1}	0.70
					Trinitrophenol (2,4,6-)	25		2.1×10^{-2}	1.68
Iodoacetic	25		7.5×10^{-4}	3.12					
Itaconic	25	1	1.40×10^{-4}	3.85	Uric	12		1.3×10^{-4}	3.89
Itaconic	25	2	3.56×10^{-4}	5.45					
					n-Valeric	18		1.51×10^{-1}	4.82
Lactic	100		8.4×10^{-4}	3.08	iso-Valeric	25		1.7×10^{-5}	4.77

Table 3.1b Dissociation Constants in Inorganic Acids in Aqueous Solution (Approximately 0.1–0.01 M)

Compound	T°C	Step	K	pK	Compound	T°C	Step	K	pK
Arsenic	18	1	5.62×10^{-3}	2.25	o-Phosphoric	18	3	2.2×10^{-12}	12.07
Arsenic	18	2	1.70×10^{-7}	6.77	Phosphorous	18	1	1.0×10^{-2}	2.00
Arsenic	18	3	3.95×10^{-12}	11.60	Phosphorous	18	2	2.6×10^{-2}	0.89
Arsenious	25		6.00×10^{-20}	9.23	Pyrophosphoric	18	1	1.4×10^{-1}	0.89
o-Boric	20	1	7.3×10^{-10}	9.14	Pyrophosphoric	18	2	3.2×10^{-2}	1.49
o-Boric	20	2	1.8×10^{-11}	12.74	Pyrophosphoric	18	3	1.7×10^{-3}	5.77
o-Boric	20	3	1.6×10^{-14}	13.80	Pyrophosphoric	18	4	6.00×10^{-9}	8.22
Carbonic	25	1	4.30×10^{-7}	6.37	Selenic	25	2	1.2×10^{-2}	1.92
Carbonic	25	2	5.61×10^{-11}	10.25	Selenious	25	1	3.5×10^{-2}	2.46
Hydrofluoric	25		4.93×10^{-4}	9.31	Selenious	25	2	5.00×10^{-2}	7.31
Hydrogen sulphide	18	1	9.1×10^{-3}	7.04	m-Silicic	RT	1	2.00×10^{-10}	9.70
Hydrogen sulphide	18	2	1.1×10^{-12}	11.96	m-Silicic	RT	2	1.00×10^{-12}	12.00
Iodic	25		1.69×10^{-1}	0.77	o-Silicic	30	1	2.2×10^{-23}	9.66
Nitrous	12.5		4.6×10^{-4}	3.37	Sulphuric	25	2	1.20×10^{-2}	1.92
Nitrous	25			3.15	Sulphurous	18	1	1.54×10^{-7}	1.81
Periodic	25		2.3×10^{-2}	1.64	Telluric	18	1	2.09×10^{-8}	7.68
o-Phosphoric	25	1	7.52×10^{-2}	2.12	Tetraboric	25	1	$\sim 10^{-4}$	9.00
o-Phosphoric	25	2	6.23×10^{-3}	7.21					

Table 3.2a Dissociation Constants of some Organic Bases in Aqueous Solution

Compound	T°C	Step	pKa	Ka
n-Butylamine	20			
t-Butylamine	18		10.83	1.48×10^{-11}
Cyclohexylamine	24		10.66	2.19×10^{-11}
Diethylamine	40		10.489	3.24×10^{-11}
Dimethylamine	25		10.732	1.85×10^{-11}
Ethanol, 2-amino	25		9.50	3.16×10^{-10}
Ethylamine	20		10.807	1.56×10^{-11}
Ethylenediamine	0	1	10.712	1.94×10^{-11}
	0	2	7.564	2.73×10^{-8}
Hexamethylene-diamine	0	1	11.857	1.39×10^{-12}
	0	2	0.762	1.73×10^{-11}
Methylamine	25		10.657	2.70×10^{-11}
Nicotine	25		3.12	7.59×10^{-4}
Octylamine	25		10.65	2.24×10^{-11}
Piperazine	23.5		5.56	2.76×10^{-6}
Propane, 1,2-diamino	25	1	9.82	1.52×10^{-10}
	25	2	6.61	2.46×10^{-7}
Propane, 1,3-diamino	10	1	10.94	1.15×10^{-11}
	10	2	9.03	9.33×10^{-10}
Propane, 1,2,3-triamino	20	1	9.59	2.57×10^{-10}
	20	2	7.95	1.12×10^{-8}
Propylamine	20		10.708	1.96×10^{-11}
Triethylamine	18		11.01	9.77×10^{-12}
Trimethylamine	25		9.81	1.55×10^{-10}

Table 3.2b Dissociation Constants of Inorganic Bases in Aqueous Solution (Approximately 0.1–0.01 M)

Compound	T°C	Step	pKb	Kb
Ammonium hydroxide	25		4.75	1.79×10^{-5}
Calcium hydroxide	25	1	2.43	3.74×10^{-3}
Calcium hydroxide	30	2	1.40	4.0×10^{-2}
Hydrazine	20		5.77	1.7×10^{-6}
Hydroxylamine	20		7.97	1.07×10^{-8}

Table 3.3 UV Absorbance Values at 220 nm of Various Salts or Acids

Anion	Salt or acid	Absorbance value*
Iodide	KI	1.25
Nitrite	KNO_2	0.88
Nitrate	KNO_3	0.70
Thiocyanate	KSCN	0.62
Dichromate	$Na_2Cr_2O_72H_2O$	0.30
Thiosulphate	$Na_2S_2O_35H_2O$	0.29
Dithionite	$Na_2S_2O_4$	0.16
Sulphide	$Na_2S\ H_2O$	0.16
Iodate	KIO_3	0.15
Chromate	K_2CrO_4	0.14
Bromate	$KBrO_3$	0.08
Formate	HCOOH	0.05
Acetate	CH_3COOH	0.05
Bromide	KBr	0.03
Tartrate	$(CH(OH)COOH)_2$	0.03
Citrate	$C(OH)\ (COOH)\ (CH_2COOH)_2H_2O$	0.02
Oxalate	$(COOH)_2\ H_2O$	0.02
Phosphate	H_3PO_4	0.02
Chlorate	$KClO_3$	0.02
Metabisulphite	$Na_2S_2O_5$	0.02
Borate	$Na_2B_4O_7\ 10H_2O$	0.01
Chloride	KCl	0.01
Cyanide	KCN	0.01
Azide	NaN_3	0.01
Carbonate	Na_2CO_3	0.00
Bicarbonate	$NaHCO_3$	0.00
Fluoride	KF	0.00
Perchlorate	$NaClO_4$	0.00
Sulphite	Na_2SO_3	0.00
Sulphate	Na_2SO_4	0.00
Tetrafluoroborate	$NaBF_4$	0.00

* 20 μg ml^{-1} of salt or acid in distilled water measured against distilled water blank. Measurements made in a 1 cm cell.

3.8 References

1. Hadded, P.R. and Heckenberk, A.L., *J. Chromatogr. Sci., 300.* 357. (1984).
2. Small, H.S. and Bauman, W.C., *Anal. Chem. 47.* 1801. (1975).
3. Small, H.S. and Millar, T.E., *Anal. Chem. 54.* 462. (1982).
4. Dionex *IONPAC Columns Brochure* 1988.
5. Rocklin, R.D. and Johnson, E.L., *Anal. Chem. 55.* 4. (1983).
6. Bond, A.M. Heritage, I.D., Wallace, G.G. and McCormick, M.J., *Anal. Chem. 54.* 582. (1982).
7. Wheals, B.B., *J. Chromatogr. 402.* 115. (1987).
8. Dionex *Technical Note 19*
9. Wheals, B.B., *J. Chromatogr. 262.* 61. (1983).

Chapter 4 Chromatograph Practice Applied to Ionic Separation

4.1 Introduction

This chapter describes the best practical approach and procedures common to all methods which follow in Parts II and III.

4.2 Reagents and Equipment

Reagents of recognised analytical grade should be used.

Grade A calibrated glassware should be used to prepare standard solutions and to make sample dilutions.

Chemicals for the preparation of calibration standards may need to be dried to constant weight in a fan-assisted oven capable of being maintained at between 105° and 150°C. Commercial standards may be used if available and reliable. It is advisable to check the stability, purity and accuracy of new batches of stock standards.

The water referred to throughout the method shall be of HPLC grade free of organic and colloidal substances and have an electrical conductivity of < 0.1 μS cm^{-1}. Ideally the water should be purified using a reverse osmosis/deionisation unit. It should be free of particulate matter (> 0.2 μm) to prevent the introduction of bacteria, suspended material, or water purifier ion exchange resins into the reagents and subsequently onto the stationary phase. In general, no interference will be caused by an increase in conductivity due to uptake of carbon dioxide in the majority of applications, although the limit of detection may be affected in a sodium hydroxide mobile phase.

Ideally, the equipment should be located in a temperature controlled environment to minimise the drift due to temperature fluctuations. For conductivity detectors, especially, avoid direct sunlight or any rapid changes in temperature.

It is preferable to gravity feed the mobile phase to the pump. Degassing the mobile phase is not always necessary, but may be required to exclude carbon dioxide and gas bubbles. This is essential when gradient elution is used. Helium purging, or agitation of the liquid under vacuum are methods commonly used to degas the mobile phase.

Carry out regular maintenance of the equipment as outlined in the manufacturer's instructions; develop a routine cleaning schedule for the liquid lines and valves in the chromatographic system; and ensure that these procedures do not lead to contamination.

Once a routine analytical chromatographic procedure has been developed, establish a procedure to monitor the operating pressures and retention times for the sytem. Note the values in a log or on a control chart (see Section 4.7) and take corrective measures should changes occur. Replace bed supports when a significant increase in pressure occurs and clean up guard columns when a loss in separation is noted. Discard guard columns when separative performance has been lost or when they exert a high back pressure. The occurrence of split peaks may indicate the presence of a column void or channelling; discard such a column.

Before commencement of an analysis, confirm that the system has reached equilibrium by performing a checking routine to ensure that the sensitivity and resolution of the system are the same as they were last time the system was used. A system check standard or stable sample (e.g. tap water) should be used and the values obtained plotted on a control chart see (Section 4.7). Ignore the first injection of the run as this usually gives erroneous information.

4.3 Sample Collection, Preservation and Storage

Collect samples in high density polyethylene or PTFE containers that have been thoroughly rinsed with water. Avoid using strong mineral acids or alkaline detergents for cleaning vessels, if they are subsequently likely to affect results. Residual acids may remain in the polyethylene matrix and slowly leach back into the sample. Alkaline detergents may also leave residues which could affect the sample chemistry. Cap the collection bottle after cleaning to prevent contamination from airborne contaminants. If possible, pre-rinse the bottle with sample before taking the main sample.

Samples should be filtered through a membrane filter (< 0.45 μm) to avoid bacterial attack on the sample and the adsorption of ions on to particulate matter (see Ref. 5 for further details). If this is not done in the field and samples then transported under aseptic conditions, it must be done on receipt of the sample in the laboratory.

In general, samples should be stored in a refrigerator at 4°C and analysed with minimal delay after collection. For cations it may also be necessary to add a mineral acid to stabilize the metals in solution. It is most convenient to use the same acid as is used in the mobile phase (See ref. 5 for further details).

In order to avoid precipitation reactions on the column and also to eliminate baseline disturbance it may be beneficial to add concentrated mobile phase to each solution. Any dilution effects caused by such a process can be eliminated by giving the calibration solution the same treatment. Filter the sample again to remove any particulate matter before injection.

In addition, if the sample contains organic compounds such as humic acids, the use of a precolumn is recommended; this serves to protect the analytical column. This precolumn should contain either the same resin materials as the analytical columns or be filled with a suitable microporous polymer.

Treat a quality control reference solution in the same manner as the sample solutions to validate the filtration, sample preparation and clean-up techniques.

4.4 Method Validation

Following the commissioning or development of a chromatographic method it is important to validate the method using inter-method comparisons. For example, Cheam and Chau (3) compared various methodologies for sulphate and found that ion chromatography performed particularly well. Caution is necessary when comparing ion chromatography with less sensitive classical reference methods, e.g. the gravimetric sulphate method which is liable to small biases and interference effects.

4.5 Peak Identification

Record the retention time for each analyte in the standard. Measure the retention time from the initial starting point of the chromatogram and use this information to identify the components in the samples. Retention times can vary slightly due to minor variations in conditions, column deterioration and with changes in concentration of the ion. Some of these changes can be overcome by the use of relative retention times. For samples with many component ions, use of alternative columns, eluents and detectors is suggested. Standard additions should also be used; but care is necessary when coelution is possible, for example some organic acids coelute with sulphate, and so on.

4.6 Calibration

Use external standardisation for quantification providing that the errors from sample introduction are minimal.

Prepare all calibration standards by diluting the stock standard solutions using either glass or plastic pipettes.

Refer to the methods and reviews in Parts II and III for information on the stability of the various calibration solutions.

A minimum of five calibration solutions and one zero standard are needed to generate a reliable calibration curve. An indication of the appropriate analytical range for the various methods is given with each method in Parts II and III. The lowest calibration solution should contain analytes of interest at a concentration greater than or equal to the limit of detection. The highest standard solution should approach the expected upper limit of concentration of the analyte in the sample. The remaining solutions should be evenly distributed throughout the expected concentration range. If a second detector sensitivity scale setting is used in order to increase the analytical range, then both sensitivity levels should be calibrated using one standard solution common to both ranges. Initially, the highest standard should be injected and the chart recorder scale adjusted or the appropriate range selected on the integrator.

It may not always be possible to perform a full calibration, especially when run-times of the order of 20–30 minutes are involved. In this case, a normalisation procedure using a

check standard and quality control techniques (see Section 4.7) may be an appropriate alternative.

Inject the standards and record the peak height or area responses of the detector. From the data obtained, construct a calibration graph for each of the analytes of interest, in order to examine the shape of the analytical curve and reject outlying values. Use this graph to derive solution concentrations, or to cross check integrator values. Whenever a new mobile phase (or regenerant solution, if appropriate) is prepared, re-establish the calibration curve.

Obtain solution concentrations by interpolation, and not extrapolation. If necessary, dilute and re-run samples or prepare new standards to bracket the sample reading. Do not report data lower than the lowest calibration standard. Dependent on the background relative to the sample signal, and the closeness of the lowest standard to the limit of detection report 'not detected', 'less than . . . .', or 'probably present but not quantifiable'.

Alternatively use a computing integrator to calculate a linear least square fit of the standard concentrations as a function on the measured peak height or area. The correlation coefficient should be 0.9990 or greater.

Determine the concentration of analyte from the mathematical equation. If the relationship is non-linear, use a polynomial second or third order least squares equation to derive a curve with a correlation > 0.9990. An integrator may provide a direct read-out of concentration of the analyte, but it is essential to check regularly, by comparing the values so obtained with values from manual graphical techniques.

To check for drift, the calibration curve should be verified after every ten samples, and at the end of each day's analyses. To check calibration procedures, analyse separate independent standards at either end of the analytical range. Run control samples at regular intervals and plot the values on control charts.

4.7 Analytical Quality Control

Each laboratory should develop formalised quality control protocols to continually monitor the bias and precision of all measurements. Use of these protocols is required to ensure that the measurement system is in a state of statistical control. Procedures for the use of quality control charts for monitoring bias and precision should be developed to assess the data obtained from reagent blanks, duplicates, spiked samples, performance evaluation samples and check standards.

Values obtained from control samples should be plotted on control charts which give an indication of performance and contain lines indicating the mean, warning and action limits. Establish limits by performing at least ten determinations for each control sample on each of ten different days in order to provide a realistic estimate of variability. Calculate the mean and standard deviation(s) for each control and plot the warning and action limits, plus and minus two standard deviations and plus and minus three standard deviations respectively. These limits should be updated as additional data are acquired. Re-establish these limits whenever instrumental or operating conditions change.

Positive or negative deviation from the experimental mean is indicative of method or procedural bias. Two consecutive values outside the warning limit or a single value outside the action limit indicate that the system is out of control and corrective measures are required.

For further information on general statistical and quality control, a text by Miller and Miller (1) is recommended. In addition also refer to WRC Technical Report TR66 (2) and BS 5700–3 and 5750 (4) for details on inter-laboratory standardisation.

4.7.1 Control Samples

1. Analyse standards independent of the calibration solutions with concentrations at the low and high ends of the analytical range to check the calibration procedure.
2. Prepare a check sample, analyse it routinely with each batch of samples and use the data from it to determine if procedural bias is present.

3. Spiked sample solutions should be used to obtain a reliable estimate of accuracy. Values outside the control limit indicates matrix interference problems which should be resolved before routine analysis is continued. Coelution cannot be so detected.
4. Blank solutions (some including filtration and any sample preservation) should undergo identical processing to samples. Values exceeding the minimum detectable limit indicate a contamination problem.
5. Include random duplicates in the analytical batches and plot the differences on a control chart. For accurate work, duplicate analysis in separate batches, or on different days is recommended.

4.8 Reporting

The number of significant digits reported should be governed by the precision of the method and ideally be accompanied by confidence limits. Do not report more than three significant digits.

Records of the following information should be kept and passed onto the customer with each group of samples. The report shall refer to this method and state the following information:

a. exact identity of water sample

b. expression of results with error term

c. description of sample pretreatment if relevant

d. the absolute weight of analyte detected on the column

e. chromatographic conditions including type of instrument, mobile phase, flow rate, loop size, and detection mode and settings.

f. description of the method used for evaluation (Peak height or area)

g. Any deviation from this method and other information which might influence the result.

4.9 References

1. *Statistics for Analytical Chemistry, Miller, J.C. and Miller, J.N.* Ellis Horwood Ltd. 1984
2. *A Manual on Analytical Quality Control for the Water Industry.* (TR66) (2nd Edition). Water Research Centre, Medmenham.
3. Cheam, V. and Chau, A.S.Y., *Sulphate in Coloured Waters.* National Interlaboratory Quality Control Study No. 33, (1987), Environment Canada.
4. British Standards 5700–5703 and 5750.
5. *General principles of Sampling (2nd Edition with Supplements) 1990* in this series.

PART II Anions Methods

1. Common Anions, General Method

(Chloride, Nitrite, Orthophosphate, Bromide, Nitrate, and Sulphate etc in Water)

1.1 Performance Characteristics of the Method

1.1.1	Substances determined	Chloride, nitrate, orthophosphate, bromide, nitrite and sulphate, other conditions should be used for the determination of fluoride due to interference from short chain carboxylic acids.
1.1.2	Type of Sample	Slightly contaminated waters such as drinking water, rain water, ground water, and surface water.
1.1.3	Basis of the Method	Chromatography of and detection of ions.
1.1.4	Concentration	Chloride : 0.1 – 50 mg L^{-1} Nitrite : 0.05 – 20 mg L^{-1} Phosphate : 0.1 – 20 mg L^{-1} Bromide : 0.05 – 20 mg L^{-1} Nitrate : 0.1 – 50 mg L^{-1} Sulphate : 0.1 – 100 mg L^{-1} note: The range of application may be changed in particular cases by varying the working conditions (e.g. sample volume, dilution, detectors, separating columns etc).
1.1.5	Calibration Curve	May deviate slightly from linearity.
1.1.6	Limit of Detection	0.05 mg L^{-1} or better depending upon the conditions used.
1.1.7	Time required for analysis	Depends on the chromatographic analysis column. Sample run time typically 3–12 mins.

1.2 Principle of the method

The ions are separated in an ion chromatograph by means of an analytical column. Normally, an anion ion exchanger of low capacity serves as stationary phase. The mobile phase is usually an aqueous solution of the salt of a weak mono – or dibasic acid. Examples for each detection system are given in Chromatograms 1.1 to 1.3.

1.2.1 Direct conductivity detection (see Chromatogram 1.1 a-j).

1.2.2 Suppressed conductivity detection (see Chromatogram 1.2 a-c).

A suppressor column or membrane which continuously removes the cations from the eluent is placed after the separating column and before the conductivity detector. With the correct eluents a low conductivity background results.

1.2.3 Inverse UV Detection (see Chromatogram 1.3 a-d)

The eluent anion must be UV absorbing. The UV detector is used to measure the absorbance due to this anion. The separated anions are detected as a decrease in the background absorbance, hence low detection levels are difficult to achieve.

1.2.4 Direct UV measurement

This can be used to determine nitrite, bromide and nitrate with great sensitivity at 200–210 nm. An eluent with a low background absorbance at these wavelengths is required. References covering this analytical technique are summarised in section 1.12.

1.3 Interferences

In uncontaminated water samples there should be few interferences in this method. However, if contamination has occurred, a large number of anions which could interfere with the method may be introduced. These include some less common inorganic anions and low molecular weight organic anions such as aromatic and aliphatic carboxylates and dicarboxylates, hydroxy and halo substituted aliphatic

carboxylates, aromatic and aliphatic sulphonates and aliphatic sulphates. Some of these ions are listed in Table 1.1, where their approximate retention times are compared with those of the ions given in Figure 1.2. This is only a guide to possible interferences, as relative retention times may vary according to the nature of the column and the mobile phase. Use of samples spiked with determinand will not detect coelution.

If the appropriate equipment is available, it is possible to perform gradient elution (16) and sometimes obtain better separation from possible interferences. Examples are given in Chromatograms 1.4 and 1.5.

When an interference is suspected, the peak shape may reveal the presence of an unresolved peak. Similarly, the use of a second detector, especially a variable wavelength UV detector, may indicate the presence of an interference and enable unresolved components to be measured if their spectra are sufficiently different. Combined ion-exclusion chromatography and ion chromatography may also be used to reduce interferences. The anions of strong acids are largely unretained by the former and can then be separated by the latter. The carboxylates, in particular, are retained and separated by ion exclusion (see Part II Method 6).

Cross sensitivity (insufficient separation) may occur in cases of large differences in concentration. The concentration ratios in table 1.2 were checked; no interferences could be observed when 50 µL injection volumes were used.

In a buffered eluent (such as carbonate/hydrogen carbonate), the determination will not be influenced by the pH of the sample in the range of pH 2 to pH 9.

This information holds only as long as the quality requirements of the separating column are met (see section 1.7) and the electrical conductivity of the sample is less than 1000 μScm^{-1}. For natural samples, the peak resolution (R) should be at least 1.3 (see Part I figure 2.2).

Solid material and organic compounds (such as mineral oils, detergents, and humic acids) shorten the separation column's duration of life. They should therefore be removed prior to analysis (see section 1.9).

Table 1.1 Approximate Retention Times of Anions

Retention Time Min	Anion in Fig 1.2	Possible Interferences
	Fluoride	Acetate, Lactate
		C_3 to C_5 Carboxylates,
		Formate,
		Monochloroacetate, Pyruvate,
		Pentane sulphonate
2		Bromate, Phosphite
	Chloride	
		Methyl sulphate,
		2-bromoethane sulphonate,
		Chlorobenzoate
	Nitrite	
3		
		Dichloroacetate,
		Hexane sulphonate,
		o-Toluene sulphonate,
		Cyanate
4		Azide
	Phosphate	
5		Selenite
		Arsenate
		Hydroxyethylmethacrylate
		sulphate
6	Bromide	
7	Nitrate	Benzoate
		Chlorate
8		
9		Sulphite
		Malate,
		Benzene sulphonate,
		Octyl sulphonate
10	Sulphate	Maleate, Succinate, Tartrate
		p-Toluene sulphonate,
		Selenate

Table 1.2 Cross Sensitivity of Anions

Ions	Weight Ratio	Max tolerable relative concentration of interfering ions
Cl^-/NO_2^-	1 : 50	5 NO_2^-
Cl^-/NO_3^-	1 : 500	500 NO_3^-
Cl^-/SO_4^{2-}	1 : 500	500 SO_4^{2-}
NO_2^-/Cl^-	1 : 250	100 Cl^-
NO_2^-/PO_4^{3-}	1 : 50	20 PO_4^{3-}
NO_2^-/NO_3^-	1 : 500	500 NO_3^-
NO_2^-/SO_4^{2-}	1 : 500	500 SO_4^{2-}
PO_4^{3-}/Cl^-	1 : 500	500 Cl^-
PO_4^{3-}/NO_3^-	1 : 500	400 NO_3^-
PO_4^{3-}/Br^-	1 : 100	100 Br^-
PO_4^{3-}/NO_2^-	1 : 100	100 NO_2^-
PO_4^{3-}/SO_4^{2-}	1 : 500	500 SO_4^{3-}
Br^-/Cl^-	1 : 500	500 Cl^-
Br^-/PO_4^{3-}	1 : 100	100 PO_4^{3-}
Br^-/NO_3^-	1 : 50	100 NO_3^-
Br^-/SO_4^{2-}	1 : 500	500 SO_4^{2-}
NO_3^-/Cl^-	1 : 500	500 Cl^-
NO_3^-/Br^-	1 : 100	100 Br^-
NO_3^-/SO_4^{2-}	1 : 500	500 SO_4^{2-}
SO_4^{2-}/Cl^-	1 : 500	500 Cl^-
SO_4^{2-}/NO_3^-	1 : 500	400 NO_3^-

Conditions as for standard deviation (section 1.4).

1.4 Standard Deviation

Some typical precision results are given below.

1.4.1 Replicates of a Mixed Standard Solution.

Ion	Level mg L^{-1}	No of replicates mg L^{-1}	Standard deviation mg L^{-1}
Cl^-	3	10	0.020
NO_2^-	5	10	0.044
PO_4^{3-}	10	10	0.111
Br^-	10	10	0.094
NO_3^-	10	10	0.072
SO_4^{2-}	10	10	0.053

(Supplied by BP Research)

1.4.2 Replicate Analyses of Control mixtures over a year.

Ion	Given mg L^{-1}	Found mg L^{-1}	No of Replicates	Standard Deviation mg L^{-1}
Cl^-	0.18	0.19	132	0.02
	0.85	0.87	479	0.03
	1.78	1.88	255	0.05
PO_4^{3-}	0.05	0.05	10	<0.01
	0.05	0.05	10	0.01
NO_3^-	0.80	0.81	485	0.02
	3.54	3.64	415	0.12
SO_4^{2-}	0.72	0.72	340	0.03
	0.94	0.92	482	0.03
	3.60	3.69	122	0.11

(Supplied by British Gas)

Chromatographic conditions

Guard column	Dionex IONPAC AG3
Separator column	Dionex IONPAC AS3
Fibre suppressor	Dionex AFS
Flowrate	2 ml min^{-1}
Eluent	2.8 mM $NaHCO_3$/2.2 mM Na_2CO_3
Injection loop	1) 50 µL 2) 250µL
Conductivity Detector	10 µS full scale

1.5 Apparatus

1.5.1 Ion Chromatographic system

The system must be capable of detecting anions in the concentration ranges given in section 1.1.4 and separating them to the standard given in section 1.7. It will normally have the following components: (see figure 1.1)

Eluent reservoir

Eluent pump (low pulsing)

Sample injection loop, typically 50 µL

Separation column of the required performance

Detectors
either
Suppressor* and conductivity detector for section 1.2.2
or
Direct or indirect conductivity detector only for section 1.2.1

* is explained on the next page.

note: there are two types of suppressor, either of which may be used

(a) a column of hydrogen-form cation exchange resin. This must be regenerated when exhausted (after 2L of the eluent given in 1.6.1.2 for a 6 x 200 mm column).*
(b) Membrane of cation exchange material. A constant flow (3–4 $mLmin^{-1}$) of regenerant is required.

Variable range UV detector 195 to 350 nm only for section 1.2.3 (with inverse measurement).

Recording device and/or data processor.

1.5.2 Other Apparatus

Drying oven

Desiccator.

Membrane filtering apparatus with membrane filters, pore size 0.45 μm.

Common laboratory glassware.

1.6 Reagents

Reagents of recognised analytical grade must be used unless stated otherwise. The water must have an electrical conductivity of <0.1 μS cm^{-1} and must be free from particulate matter >0.2 μm. No interference in the determination will be caused by an increase in conductivity due to the uptake of carbon dioxide.

1.6.1 Eluents

Different eluents are used, their choice depending on the separating column and the detector. For some chromatography systems it may be necessary to degas the eluent. Renewed gas pick-up during operation must then be avoided (e.g. by a helium blanket). In order to avoid the growth of algae, the eluent must be stored in the dark and be renewed every 2 to 3 days.

Consult the column manufacturer's manual for the exact composition of the eluent.

1.6.1.1 Eluents for use with suppressor technique.

For the suppressor technique, sodium hydroxide and salt solutions of weakly dissociated acids are used, such as sodium carbonate/sodium hydrogen carbonate. Some typical column/eluent combinations are given with Chromatograms 1.2 a-c.

1.6.1.2 Eluents for use without the suppressor technique.

With the IC techniques having no suppressor facilities, salt solutions such as potassium hydrogen phthalate, p-hydroxybenzoic acid, sodium borate, and sodium benzoate are used. Normally, the concentrations of the salts are in the range of 0.0005 to 0.01 M. Some typical column/eluent combinations are given with Chromatograms 1.1 a-j.

1.6.1.3 Eluents for use with indirect (inverse) UV detection.

Dilute salt solutions with UV absorbing anions are used such as potassium hydrogen phthalate, potassium p-hydroxybenzoate and potassium benzoate. Normally the concentration is in the range 0.0005 to 0.01 M. Some typical column/eluent combinations are given with Chromatograms 1.3 a-d.

1.6.1.4 Eluent concentrate and eluent preparation.

Prepare an eluent concentrate 100 fold the concentration of the desired eluent. This will be stable for a long period if kept cool. Dilute the concentrate 100 times as required to make the eluent. When the pH is specified, adjust this after dilution.

1.6.2 Suppressor regenerant.

Sulphuric acid 0.125 M (column or packed bed).
Sulphuric acid 0.0125 M (membrane or fibre)
and see manufacturer's instructions.

* Little used today.

1.6.3 Anion Stock Solutions

1.6.3.1 Stock solutions 1000 mgL^{-1}, Cl^-, NO_2^-, Br^-, PO_4^{3-}, NO_3^-, SO_4^{2-}

For each stock standard dry the salt as stated in the table below. Allow to cool in desiccator for 45 min. Weigh out the quantity given in the table into a small beaker, dissolve in a little water. Transfer quantitatively to a litre calibrated capacity flask and make up to volume with water. These solutions are stable for several months if kept cool.

Anions	Substance	Amount Weighed out (gL^{-1})	Dry for (h)	At temperature °C
Chloride	NaCl	1.6484	2	150
Nitrite	$NaNO_2$	1.4998	1	150
Phosphate	KH_2PO_4	1.4330	1	105
Bromide	NaBr	1.2877	6	150
Nitrate	$NaNO_3$	1.3707	24	105
Sulphate	Na_2SO_4	1.4790	1	105

Commercially available stock solutions of the respective concentrations may also be used.

1.6.3.2 Standard Solutions.

According to requirements, standard solutions of different anion composition and concentration are prepared from stock solutions (section 1.6.3.1). The risk of changes of concentrations caused by interactions with the vessel material increases with decreasing anion concentrations. Polytetrafluoroethylene (PTFE) or polythylene vessels have proved suitable for the storage of chloride standard solutions. Experience has shown that nitrate standard solutions are more stable in borosilicate bottles. Polyethylene should not be used for samples containing phosphate.

Determine suitable bottles and solution storage life by practical tests, comparing freshly made and aged solutions.

To avoid cross contamination, the same vessels must be reserved for the same anions and concentrations.

1.6.3.3 Mixed standard solution I.

NO_2^-, PO_4^{3-} and Br^-	10 mgL^{-1}
Cl^-, NO_3 and SO_4^{2-}	100 mgL^{-1}

– Pipette the volumes given in the table below into a 100 mL calibrated flask and make up to volume with water. Stopper and mix well.

Volumes of stock solutions for the preparation of the mixed standard solution I.

Anions	Stock solution (mL)	Anion concentration (mgL^{-1})
Cl^-	10	100
NO_2^-	1	10
PO_4^{3-}	1	10
Br^-	1	10
NO_3^-	10	100
SO_4^{2-}	10	100

If kept cool, this solution is stable for about one week.

1.6.3.4 Mixed standard solution II:

NO_2^-, PO_4^{3-} and Br^-	1 mgL^{-1}
Cl^- NO_3^- and SO_4^{2-}	10 mgL^{-1}

– Pipette 10 mL of the anion mixed standard solution I into a 100 mL calibrated flask and make up to volume with water. Stopper and mix well.

This solution is only stable for 1 to 2 days even if kept cool.

1.6.3.5 Mixed standard solution III.

NO_2^-, PO_4^{3-} and Br^-	0.1 mgL^{-1}
Cl^-, NO_3^- and SO_4^{2-}	1.0 mgL^{-1}

– Pipette 1 mL of the anion mixed standard solution I into a 100 mL calibrated flask and make up to volume with water. Stopper and mix well.

Prepare the solution daily.

1.6.4 Anion Reference Solutions

Depending on the anion concentration expected in the samples, use the stock solution or the standard solutions I and II respectively to prepare at least 5 reference solutions which cover the working range to be expected as equi-distantly as possible. The working range must not be more than one order of magnitude, for example:

0.1 to 1.0 mgL^{-1}, NO_2^-, PO_4^{3-}, Br^- and
1 to 10 mgL^{-1} Cl^- NO_3^-, SO_4^{2-}.
Add 1 mL of the eluent concentrate (section 1.6.1) to each 100 mL of each reference solution. Mix well.

Prepare fresh reference solutions on day of use.

If samples differing widely in concentration are to be analysed, prepare and calibrate several working ranges of standards, as necessary.

note: The addition of 1 mL of eluent concentrate solution decreases the concentration of the reference solution. However, this dilution is compensated by the same treatment of the sample (section 1.9).

1.6.5 Blank solution

– A 100 mL calibrated flask is filled to the mark with water. 1 mL of an eluent concentrate (section 1.6.1.4) is added. Mix well.

1.7 Quality Requirements for the Separating Column

The separating column is an essential part of the ion chromatographic technique. Its separation performance is determined by several marginal conditions, such as material and eluents. Use only such columns which, after injecting a standard solution containing all six anions (Cl^-, NO_2^-, PO_4^{3-}, Br^-, NO_3^- and SO_4^{2-}) and also F^-, each having a concentration of 1 mgL^{-1}, will allow the base line resolved separation of all components (see figure 1.2). The peak solution should not be worse than R = 1.3 (see equation (1) and Part I Chapter 2 figure 2).

$$R = \frac{2(t_2 - t_1)}{W_2 + W_1} \qquad (1)$$

where:

R	peak resolution
t_1	retention time of the 1st peak
t_2	retention time of the 2nd peak
W_1	peak width of the 1st peak
W_2	peak width of the 2nd peak

1.8 Sampling and Sample Pretreatment

Vessels made of polytetrafluorethylene (PTFE) or polyethylene are suitable for sampling.

Immediately on arrival of the sample in the laboratory, filter it through a membrane filter (pore size 0.45 μm) in order to avoid bacterial action and the adsorption of the anions on to particulate matter.

If this can be done on site, immediately after sampling, and the sample transported aseptically, this is to be preferred (see Refs. 4 and 5).

The sample can be stablized by cooling or deep freezing until the analysis is performed.

In order to avoid precipitation reactions on the column and to eliminate the water dip, add one part of eluent concentrate (section 1.6.1.4) to one hundred parts of the sample. Dilution effects are eliminated by the same treatment of the calibrating solutions (section 1.6.4). It may be necessary to dilute the sample to be analysed with water and with eluent concentrate.

note: If necessary, filter the sample again through a membrane filter (pore size 0.45 μm) just before injection, to remove particulate matter.

If, in addition, the sample contains organic compounds such as humic acids, the use of a precolumn is recommended. It serves to protect the analytical separating column. Precolumns may contain the same resin material as the analytical separating column, or be filled with microporous polymer.

Reference solutions shall be treated in the same manner as the sample solutions.

1.9 Procedure

Step	Procedure	Notes
1.9.1	Start the ion chromatograph in accordance with the instrument manufacturer's instructions. (note a)	a. The instrument is ready for use when a stable baseline at the expected conductivity or UV absorbance reading is achieved. For a note on use of gradient elution see Section 1.12.
	Calibration.	
1.9.2	Prepare reference and blank solutions as described in section 1.6.4 and 1.6.5.	
1.9.3	Analyse the reference and the blank solutions.	
1.9.4	Plot a graph of peak height or area against concentration or calculate a polynomial regression. A smooth line should result which is either straight or slightly curved. (note b)	b. The anions are identified by comparing the retention times given by the samples with those of the reference solutions. The retention times may be slightly concentration and matrix dependent.
1.9.5	Measure concentration using the standard calibration procedure.	
1.9.6	Inject the pretreated sample (section 1.8) into the chromatograph (note c)	c. If the ion concentration to be determined exceeds the range of validity, the sample solution should be diluted (see section 1.8). It may become necessary to establish a new calibration curve for a lower concentration range.
1.9.7	After each series of samples, or at most after 10 to 20 measurements, reference solutions with concentrations in both the lower and upper parts of the working range should be measured, in order to check the validity of the calibration curve. If an unacceptable drift has occurred, recalibrate. If necessary, recheck the last results obtained.	
1.9.8	Estimate the mass concentration of the anion in the measuring solution using the peak areas or peak heights and the calibration graph. Multiply this by the dilution factor.	
1.9.9	The blank value should be obtained in like manner and, if significant, correction made by deducting it from the values found for the samples.	

1.10 Expression of Results and Reports of Analyses

See Part I Chapter 4 Section 8.

1.11 Effect of Temperature Variation

Although often insignificant, temperature variation affects many aspects of ion chromatography. For some precise measurements, variations of even a half a degree Centigrade have caused noticeable changes in the observed signal. If such variation is critical, the laboratory should be air-conditioned.

1.12 Use of Gradient Elution

Gradient elution can be used, just as in conventional HPLC analyses. It is sometimes useful if it is desired to analyse simultaneously for both rapidly and slowly eluting ions. Both direct and suppressed detection can be used. Examples are given in Chromatograms 1.4 and 2.4.

1.13 References

1. Angele, H.P., *Dictionary of Chromatography,* Huthig Verlag, Heidelberg, Basel, New York (1984).
2. Bennert, J. and Behrend, P., *Labor Praxis,* Nov (1984).
3. Bennert, J., Behrend, P. and Kipplinger, A., *Labor Praxis,* Jan. Feb. (1985).
4. Engelhard, H., *Hockdruck-Flussigkeitschromatographie, 2nd edition.* Springer Verlag, Heidelberg, Berlin, New York (1977).
5. Fritz, J.S., Gjerde, D.T. and Pohland, C. *Ion Chromatography,* Huthig Verlag. Heidelberg, Basel, New York (1982).
6. Haddad, P.R. and Heckenberg, A.L. *J. Chromatogr. Sci.,* **6,** 300, 357 (1984).
7. Jenke, D.R. and Pagenkopf, G.K., *J. Chromatogr. Sci.,* **6,** 231 (1984).
8. Jones, V.K. and Tarter, J.G., *Internat. Laboratory.* **9,** 36 (1985).
9. Meyer, V., *Praxis der Hochleistungs Flussigkeitschromatographie 2nd Edition.* Diesterweg Salle Sauerlander (1980).
10. Poole, C.F. and Schuette, S.A., *Contemporary Practice of Chromatography,* Elsevier, Amsterdam, Oxford, New York, Tokyo (1984).
11. Small, H.S. and Miller, T.E., *Anal. Chem.* **54,** 462 (1982).
12. Small, H.S., Stevens, T.S. and Baumann, W.C., *Anal. Chem.,* **47,** 1801 (1975).
13. Smith, F.C. and Chang, R.C., *The Practice of Ion Chromatography,* John Wiley & Sons (1984).
14. Schwedt, G., *Ionenchromatographie,* Vogel-Verlag, Wurzburg (1984).
15. Veis, J., *Handbuch der Ionenchromatographie 1st Edition,* VCH-Verlagsgesellschaft Weinheim (1985).
16. Dionex, *Technical Note* 19.

Figure 1.1 Schematic diagram of chromatographic system for ions

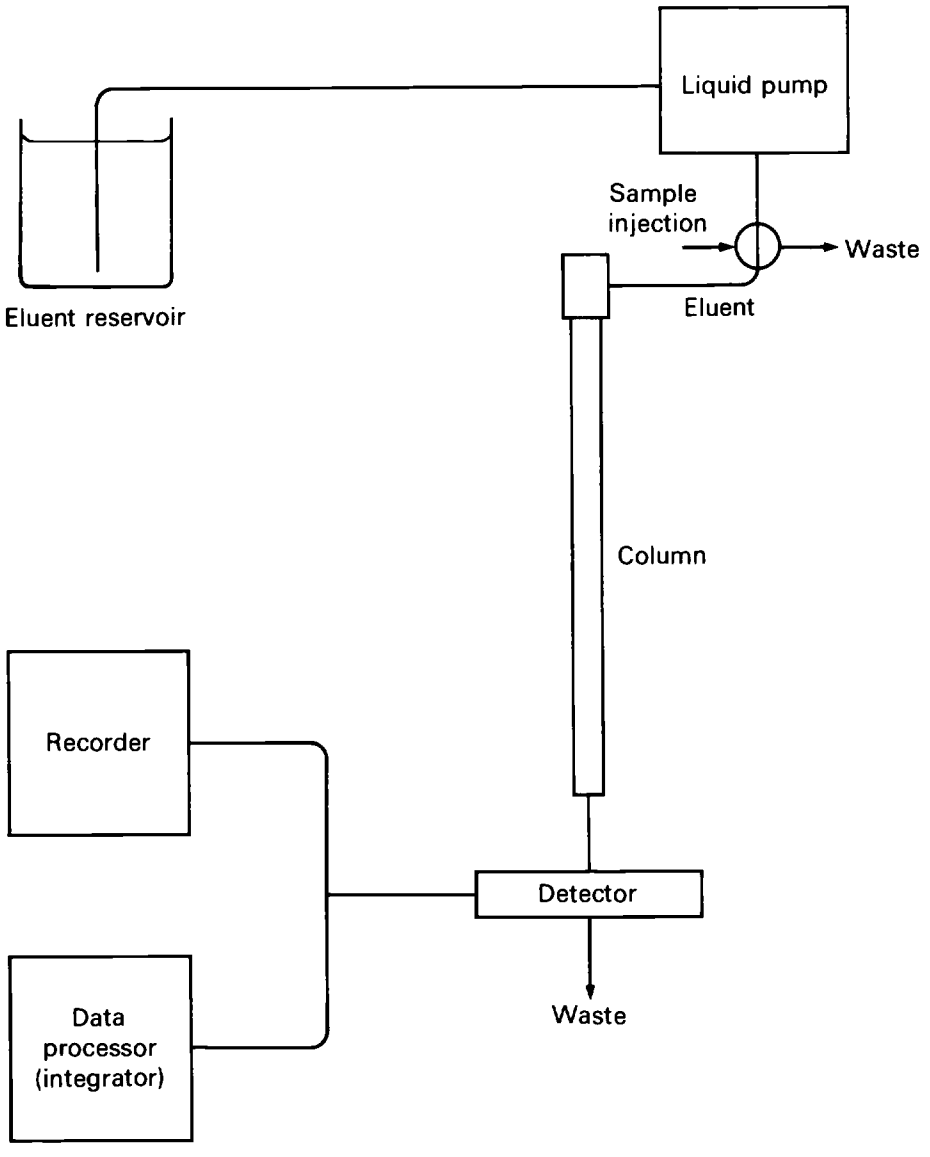
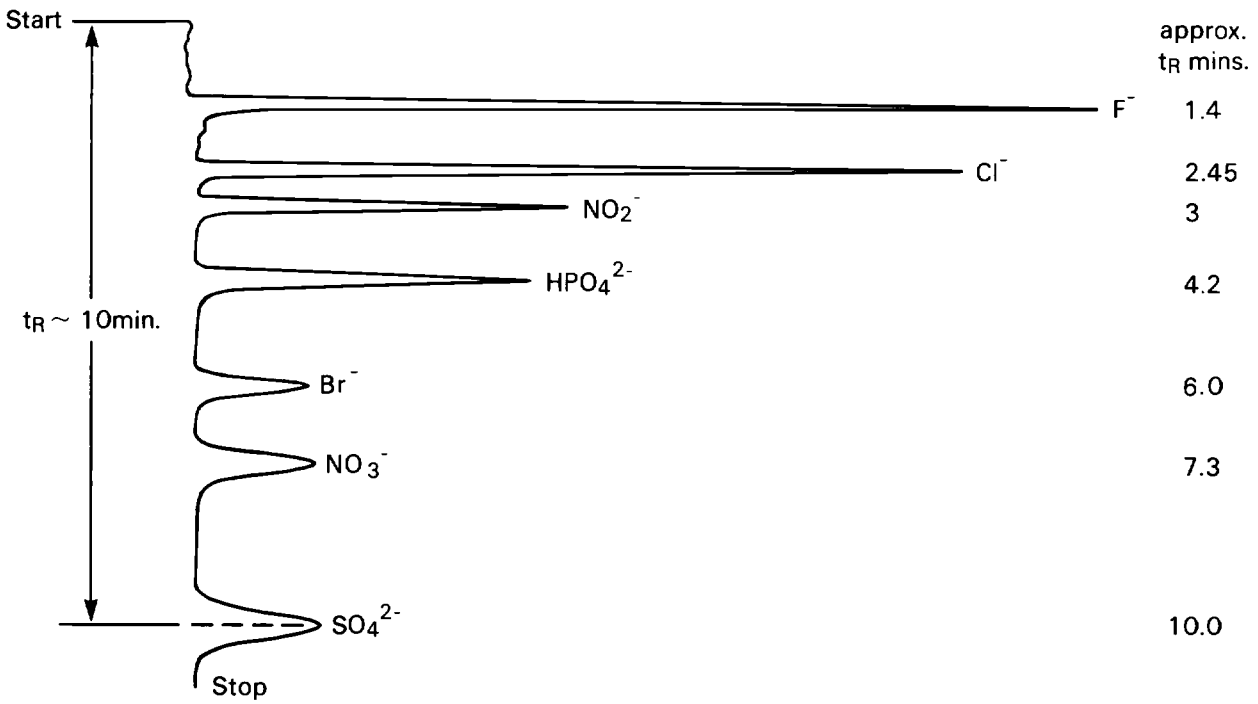


Figure 1.2 Illustration of retention times

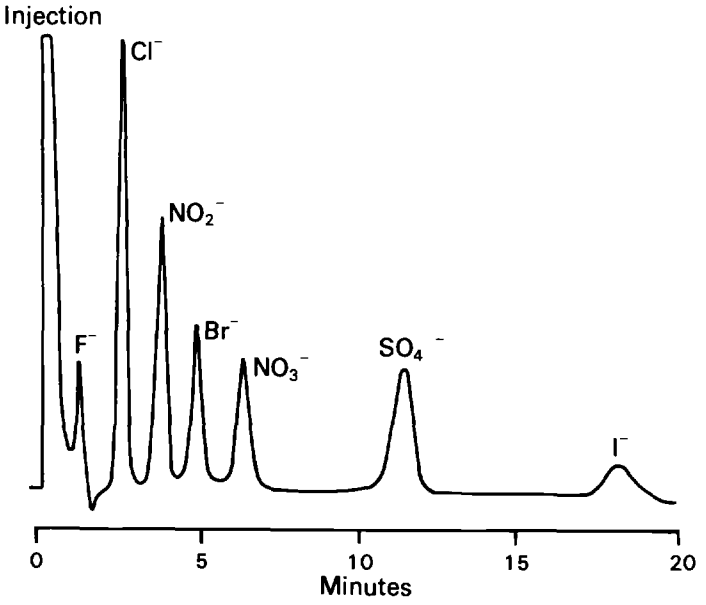
1mg L^{-1} standard; sample volume 50μL



Elution sequences and retention times may vary, depending on the type of column and the composition of the eluent.

Chromatogram 1.1

COMMERCIAL COLUMNS-DIRECT CONDUCTIVITY DETECTION



(a)

Bio-gel TSK IC-Anion-PW

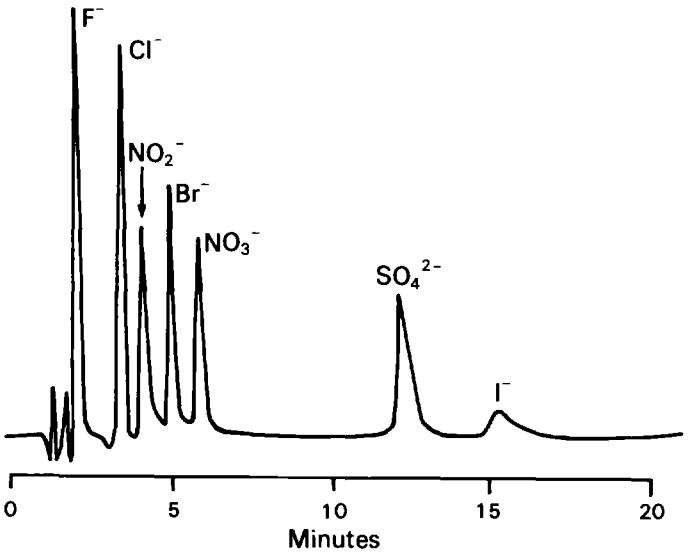
Eluent: 1mM potassium hydrogen phthalate pH5.3

Flow-rate: 2mL min^{-1}

Sample vol: 10μL

Concentrations mg L^{-1}:
Chloride 100, nitrite 100, bromide 100, nitrate 100, sulphate 100.

Source: Haddad *et al*, J. Chromat., 1985, 346, 139



(b)

Hamilton PRP-X100

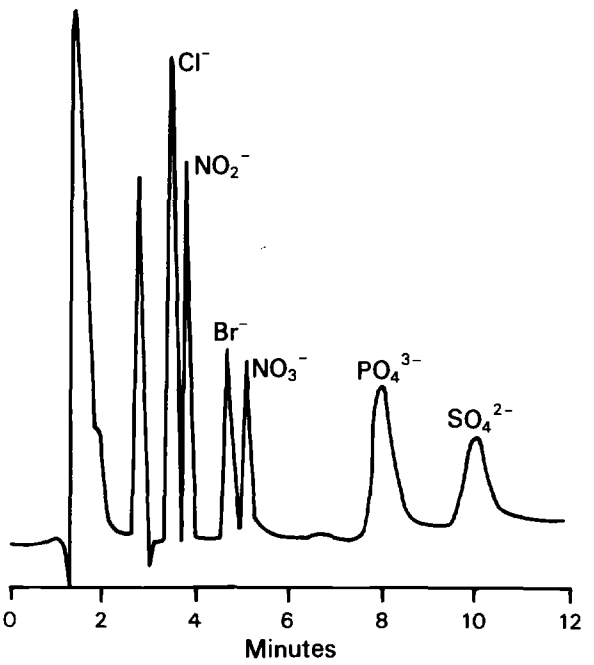
Eluent: 1mM potassium hydrogen phthalate pH5.3

Flow-rate: 2mL min^{-1}

Sample vol: 10μL

Concentrations mg L^{-1}:
Chloride 100, nitrite 100, bromide 100, nitrate 100, sulphate 100.

Source: Haddad *et al*, J. Chromat., 1985, 346, 139



(c)

Interaction ION-100

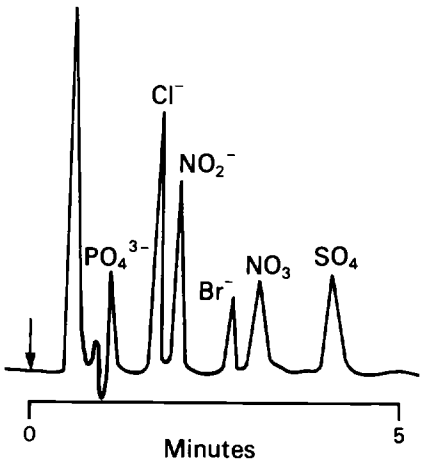
Eluent: 2.5 mM

Flow-rate: 0.8 mL min^{-1} sodium salicylate pH 8

Sample vol: 16 μL

Concentrations mg L^{-1}:
Chloride 10, nitrite 10, bromide10, nitrate 20, sulphate 20, phosphate 20.

Source: Interaction



(d)

IonoSphere A

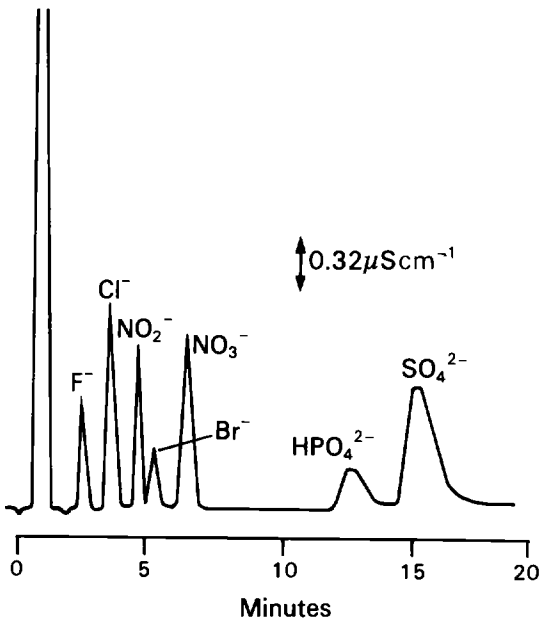
Eluent: 2.5 mM sodium salicylate pH4

Flow-rate: 1mL min^{-1}

Sample vol: 10μL

Concentrations mg L^{-}:
Chloride 10, nitrite 20, bromide 20, nitrate 20, sulphate 20.

Source: Chrompack



(e)

Shim-pack IC-A1

Eluent: 1mM p-hydroxybenzoic

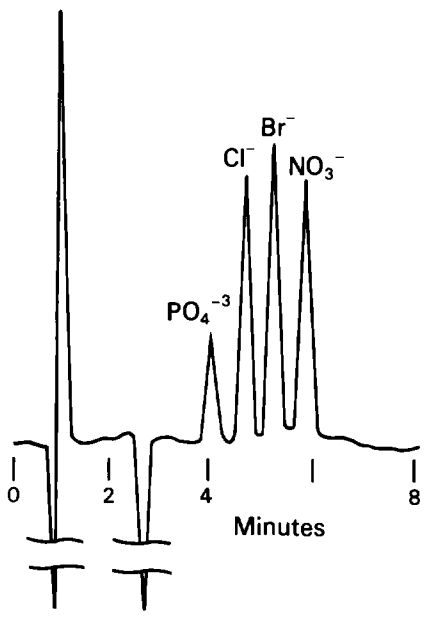
acid 1.1mM N,N- diethylethanolamine pH 7.9

Flow-rate: 1.5 mL min^{-1}

Sample vol: 20μL

Concentrations mg L^{-1}:
Chloride 10, nitrite 15, bromide 15, nitrate 30, sulphate 40, phosphate 30.

Source: Shimadzu



(f)

Supercosil LC-SAX

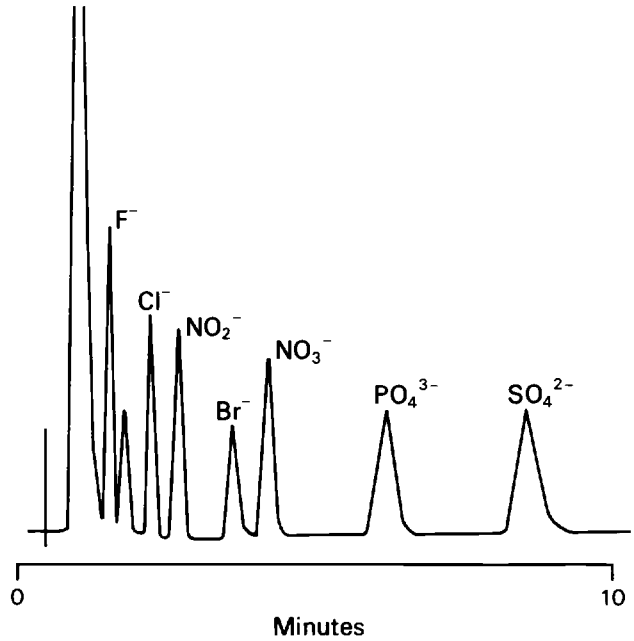
Eluent: 5mM potassium citrate pH3

Flow-rate: 3mL min^{-1}

Sample vol:10μL

Concentrations mg L^{-1}:
Chloride 10, bromide 30, nitrate 30, phosphate 30.

Source: Supelco



(g)

VYDAC 300 IC 405

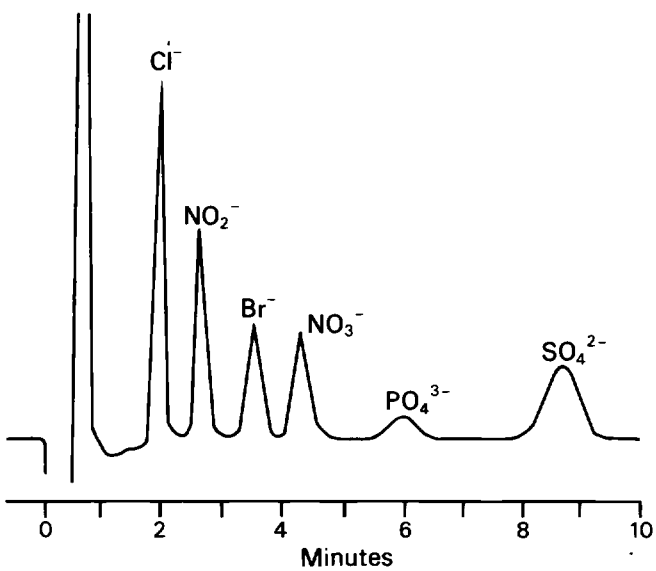
Eluent: 1.5mM phthalic acid pH8.9

Flow-rate: 1mL min^{-1}

Sample vol: 15μL

Concentrations mg L^{-1}:
Chloride 1, nitrite 1.5, bromide 3, nitrate 2.5, sulphate 3, phosphate 3.

Source: VYDAC



(h)

Waters IC Pac A

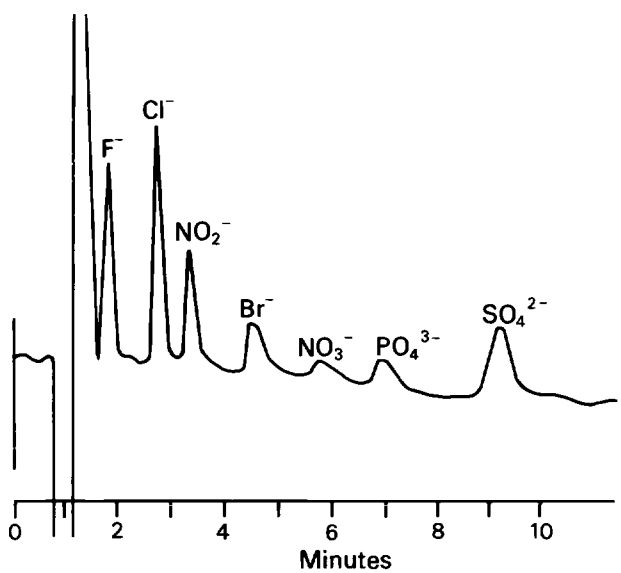
Eluent: mM phthalic acid pH7

Flow-rate: 1.2 mL min^{-1}

Sample vol: 100μL

Concentrations mg L^{-1}:
Chloride 5, nitrite 5, bromide 5, nitrate 5, sulphate 5, phosphate 5.

Source: BP Research Int.



(i)

Wescan Anion/R

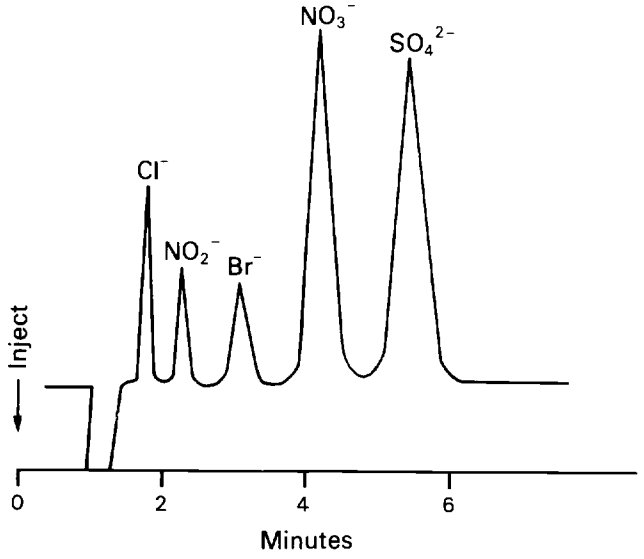
Eluent: 5mM p-hydroxybenzoic acid pH8.6

Flow-rate: 2mL min^{-1}

Sample vol: 100μL

Concentrations mg L^{-1}:
Chloride 2.8, nitrite 2.8, bromide 2.6, nitrate 2, sulphate 3, phosphate 3.2.

Source: Wescan



(j)

Zipax SAX

Eluent: 2mM disodium adipate pH7

Flow-rate: 2.5mL min^{-1}

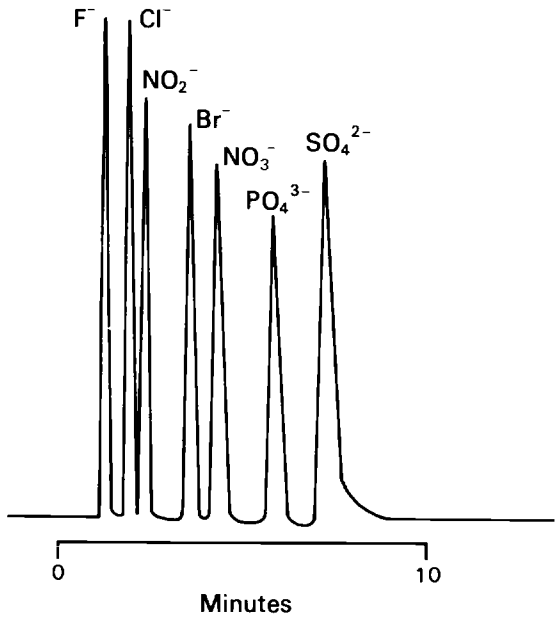
Sample vol: 50μL

Concentrations mg L^{-1}:
Chloride 2, nitrite 5, bromide 20, nitrate 20, sulphate 20.

Source: Van Os, M.J. *et al*,

Anal. Chim. Acta, 1982, 144, 73.

Chromatogram 1.2 Commercial columns – chemically suppressed conductivity



(a)

Dionex HPIC AS4A

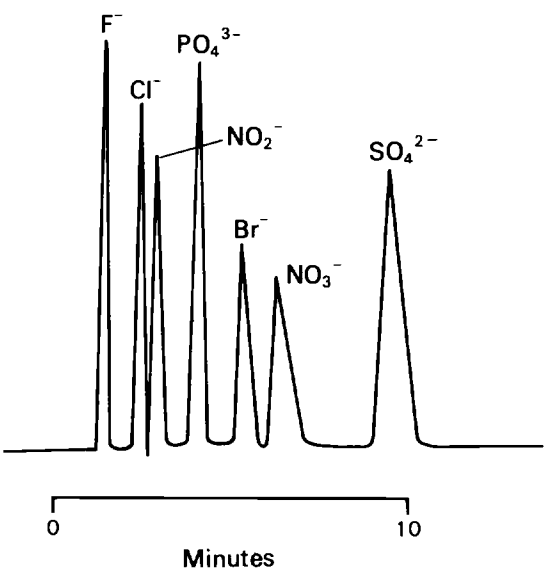
Eluent: 0.75mM sodium hydrogen carbonate, 2mM sodium carbonate.

Flow-rate: 2mL min^{-1}

Sample vol: 50μL

Concentrations mg L^{-1}:
Chloride 3, nitrite 5, bromide 10, nitrate 10, sulphate 15, phosphate 20.

Source: British Gas



(b)

Dionex HPIC AS4

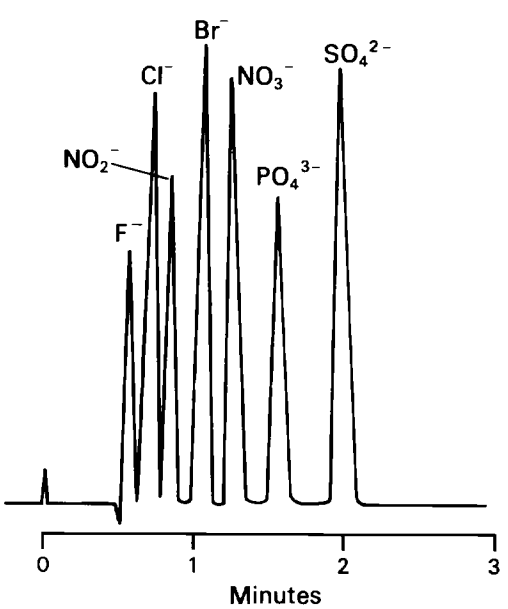
Eluent: 2.8mM sodium hydrogen carbonate, 2.4mM sodium carbonate.

Flow-rate: 2mL min^{-1}

Sample vol: 50μL

Concentrations mg L^{-1}:
Chloride 3, nitrite 5, bromide 10, nitrate 10, sulphate 15, phosphate 20.

Source: British Gas



(c)

Dionex Fast sep

Eluent: 0.15mM sodium hydrogen carbonate, 2mM sodium carbonate

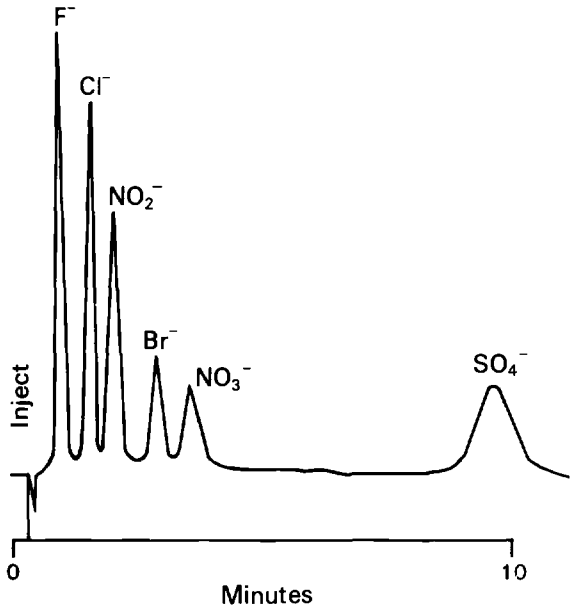
Flow-rate: 2mL min^{-1}

Sample vol: 20μL

Concentrations mg L^{-1}:
Chloride 1.5, nitrite 2.5, bromide 7.5, nitrate 10, sulphate 10, phosphate 15.

Source: Dionex Inc.

Chromatogram 1.3 Commercial columns – reverse UV detection



(a)

Interaction ION-100

Eluent: 1.5mM potassium phthalate pH4.3

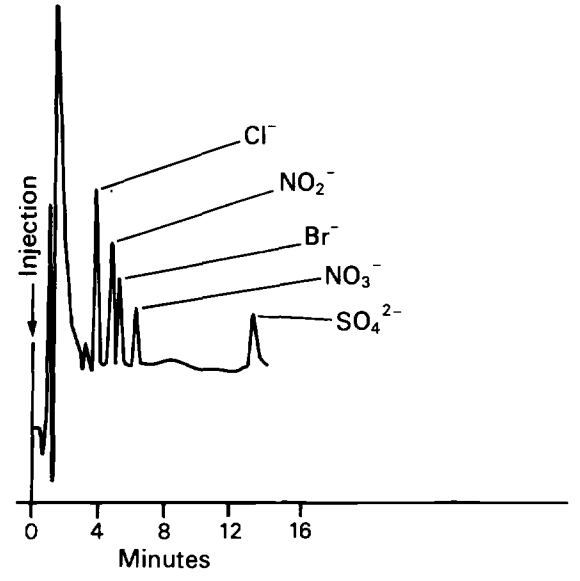
Flow-rate: 1.5mL min^{-1}

Sample vol: 20μL

Wave length: 264nm

Concentrations mg L^{-1}:
Chloride 17.1 nitrite 23.8, bromide 27.05, nitrate 20.45, sulphate 20.4.

Source: Philips Scientific & Analytical Equipment



(b)

VYDAC 302C

Eluent: 0.5mM potassium isophthalate

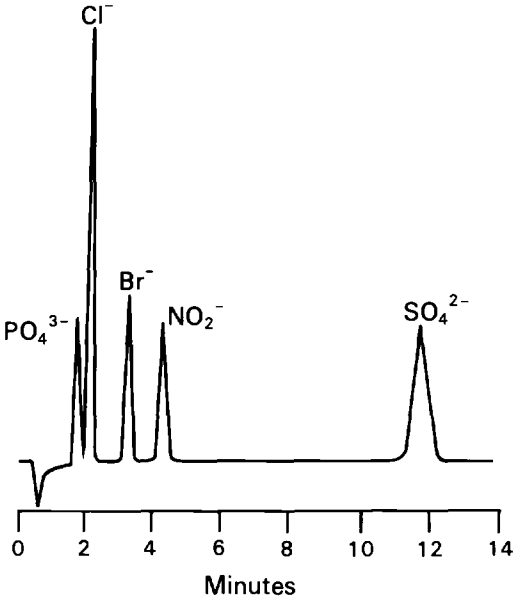
Flow-rate: 2mL min^{-1}

Sample vol: 50μL

Wave length: 254nm

Concentrations mg L^{-1}:
Chloride 1, nitrite 1.2, bromide 1.4, nitrate 1.4, sulphate 1.1.

Source: Naish, P.M., Analyst, 1984, 109, 809.



(c)

Supercosil LC-IC

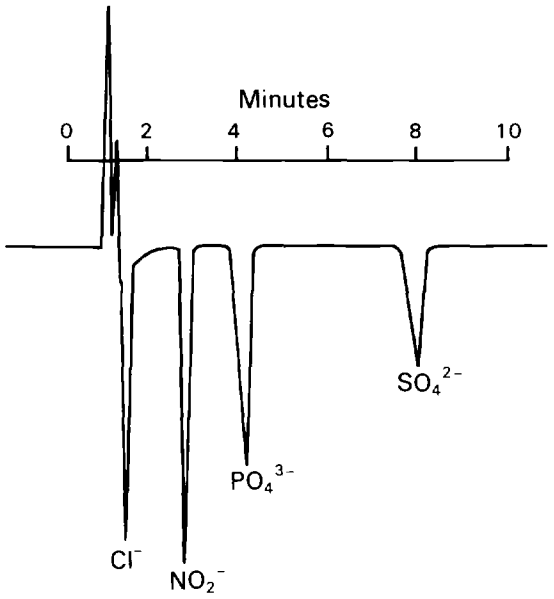
Eluent: 1mM potassium phthalate

Flow-rate: 0.7mL min^{-1}

Sample vol: 100μL

Concentrations mg L^{-1}:
Chloride 10, bromide 10, nitrate 10, sulphate 10, phosphate 10.

Source: Supelco.



*This column is no longer available. Use of an HPIC CS5 column is suggested by the makers

(d)

Dionex HPIC CAS1

Eluent: 0.5mM potassium phthalate, 0.61mM Sodium tetraborate.

Flow-rate: 1.5mL min^{-1}

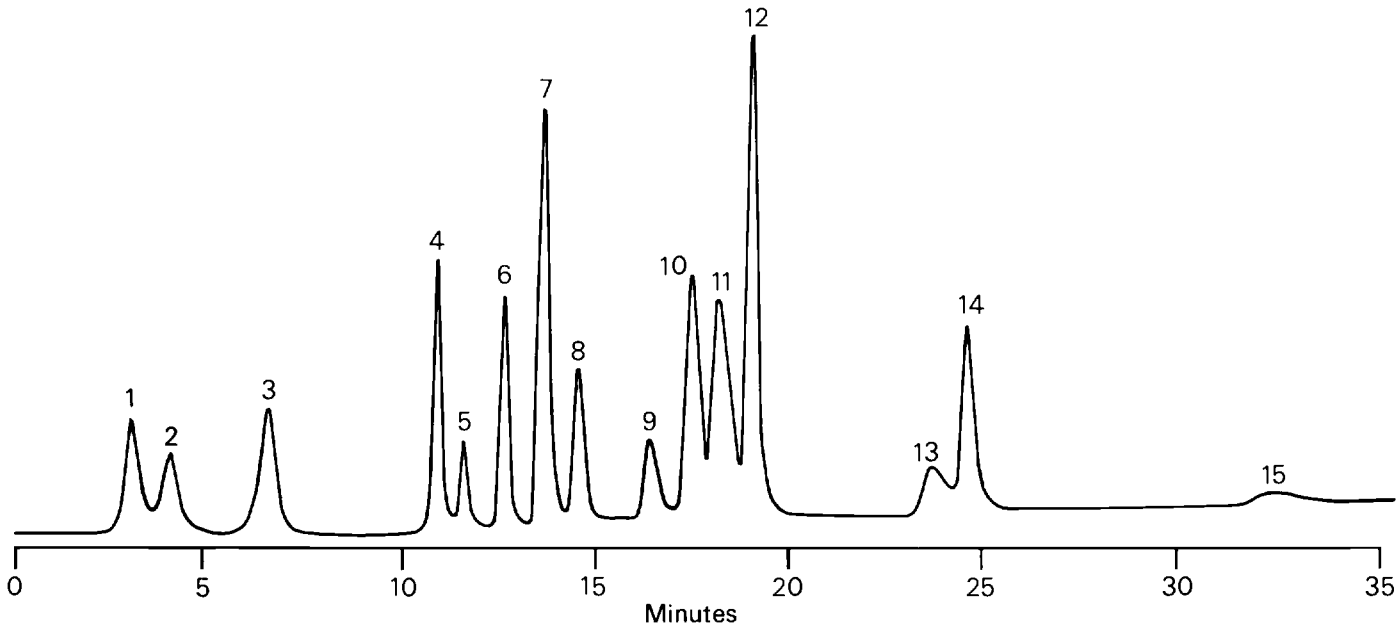
Sample vol: 50μL

Wave length: 254nm

Concentrations mg L^{-1}:
Chloride 3.5, nitrate 6.2, sulphate 9.6, phosphate 9.5.

Source: Dionex Inc.

Chromatogram 1.4
Gradient separation of halogened organic acids (HOA's) and common anions



Column: AS5A (5μ)

Eluent: NaOH

Gradient PGM: 0.75 – 70mM

Suppressor: AMMS

Regenerant: 12.5mM H_2SO_4 8mL min^{-1}

Detector: Conductivity 30μS FS

Flow rate: 1.0mL min^{-1}

Sample loop: 10μL

Peaks (10mg L^{-1} unless noted):

1. Fluoride (1.5mg L^{-1})
2. Acetate
3. Formate (5mg L^{-1})
4. Monochloracetate (MCA)
5. Monobromoacetate (MBA)
6. Chloride (3mg L^{-1})
7. Nitrite
8. Dichloroacetate (DCA)
9. Dibromoacetate (DBA)
10. Bromide
11. Nitrate
12. Sulphate
13. Trichloroacetate (TCA)
14. Phosphate
15. Tribromoacetate (TBA)

2. Fluoride

Fluorides are only weakly ionised in aqueous solution. They can be thought of as partially covalent. Hence, under most normal ion exchange chromatographic conditions this ion is hardly retarded in its passage through the column. As a result, fluoride appears as a sharp peak at the beginning of most chromatograms, often not uniquely identified or resolved from other analytes, especially formate, acetate and other organic ions (Chromatogram 2.1). Although this is now becoming less of a problem with the introduction of smaller particle size column packing material showing greater separation efficiencies (Chromatogram 2.2), there are a number of approaches that can be adopted to overcome this problem.

a. By using a much weaker mobile phase (eluent) the front end of the chromatogram can be stretched out to obtain baseline resolution for all the components of the sample in this area of the chromatogram (Chromatogram 2.3). By following this option a penalty has to be paid in that any ionic component held more strongly than chloride (particularly multivalent ions) will be retained on the column indefinitely. In consequence, if the column is not regularly cleaned with a much stronger or more competitive eluent, the column will finally be overloaded. This is indicated by loss of resolution.

b. By using more complex equipment, gradient elution can be carried out, starting with a weak, less competitive eluent, the benefit of the isocratic system above is achieved without the penalty. With this system the gradually increasing concentration of stronger more competitive eluent sweeps the more strongly held ions from the column (Chromatogram 2.4). Using this technique, baseline resolution is achieved for most ions, but only at the expense of a much longer chromatographic run time; extra time is also required to return the column to the weak starting eluent and re-establish equilibrium conditions before another sample can be introduced. Furthermore the complex equipment and control system required costs more than that needed for the simple isocratic approach.

c. Another approach is to use the power of modern chromatography; by changing the separation mechanism from ion exchange to ion exclusion partition, the order in which the analytes of interest are separated can be altered. For fluoride this means that all the strongly ionised analytes in the sample mixture pass through the system without being retarded whilst the fluoride, formate and acetate "unionised molecules" undergo partition with the stationary phase (column) and appear in the chromatogram separated from the other analytes with complete baseline resolution (Chromatogram 2.5).

The above approaches hold true for fluoride when present in the sample matrix as simple dissolved fluoride ion. However complex fluoride containing anions such as fluoroborate, monofluorophosphate and hexafluorophosphate will appear in different positions in the chromatogram (Chromatograms 2.6 and 2.7) under ion exchange conditions often coeluting with other ions eg phosphate/monofluorophosphate. So, in order to determine total fluoride in a sample, great care and caution must be taken to ensure that, before summing the parts to obtain a total fluoride value, the chromatographic conditions used do isolate the peaks of interest unambiguously. This is also mentioned in the booklet on Boron in Water in this series (1), as total boron in presence of fluoride must also be determined by summation of the various forms of borate and fluoroborate present.

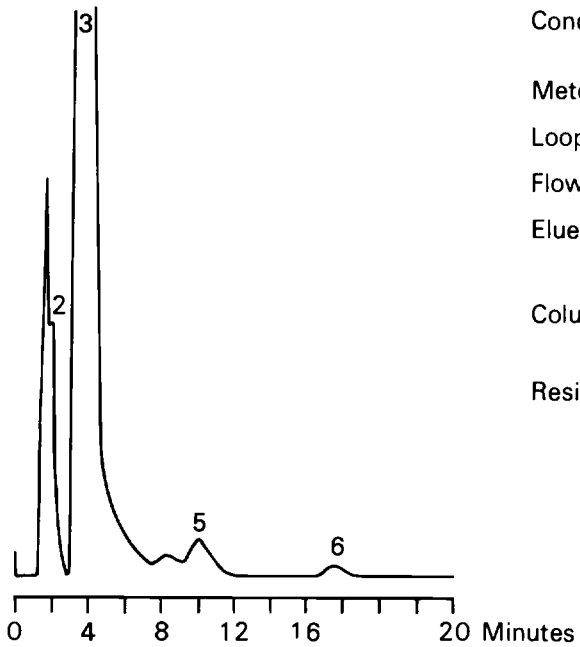
2.1 Reference

1. *Boron in Waters, Effluents, Sewages and Some Solids 1981.* HMSO in this series.

Chromatogram 2.1 Small anions

Species

1 Fluoride
2 Carboxylic acids
3 Chloride
4 $H_2PO_4^-$
5 Bromide
6 Sulphate



Conditions

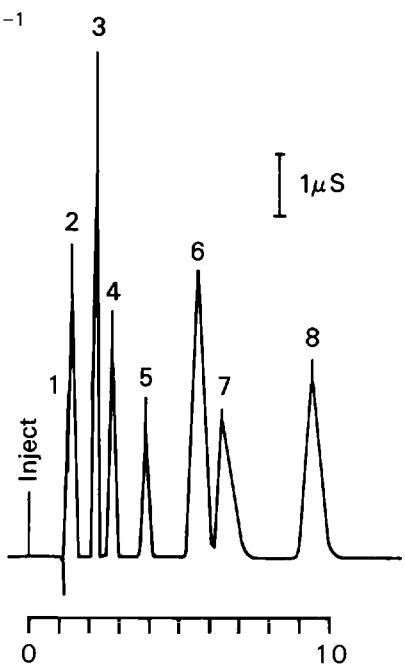
Meter: 100 μS FS
Loop size: 100 μL
Flow rate: 3mL min^{-1}
Eluent: 3mM $NaHCO_3$
2.4 mM Na_2CO_3
Columns: AG1 guard
AS1 separator
Resin suppressor

Chromatogram 2.2 Small anions

* 1	Fluoride	1mg L^{-1}
2	Formate	5
3	Chloride	5
4	Nitrite	5
5	Phosphate	10
6	Sulphite	20
7	Nitrate	10
8	Sulphate	10

Loop 50 μL

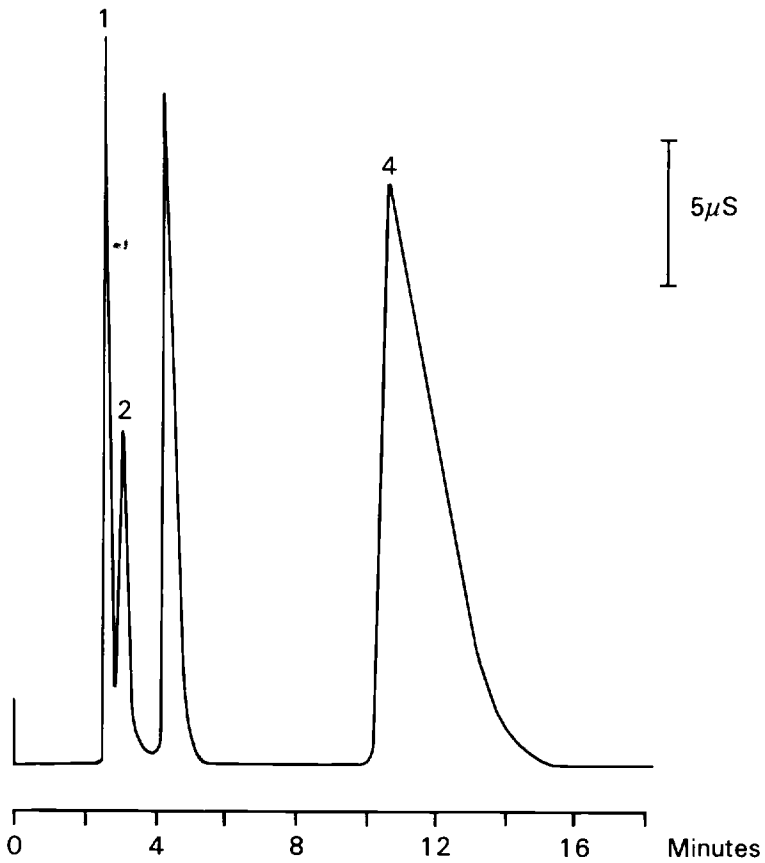
* Acetate coelutes with fluoride



Columns: Guard AG4
Separator AS4
Suppressor AFS1

Eluent: 2.8mM $NaHCO_3$
2.2mM Na_2CO_3
Flow rate: 2mL min^{-1}

Chromatogram 2.3 Carboxylic acids



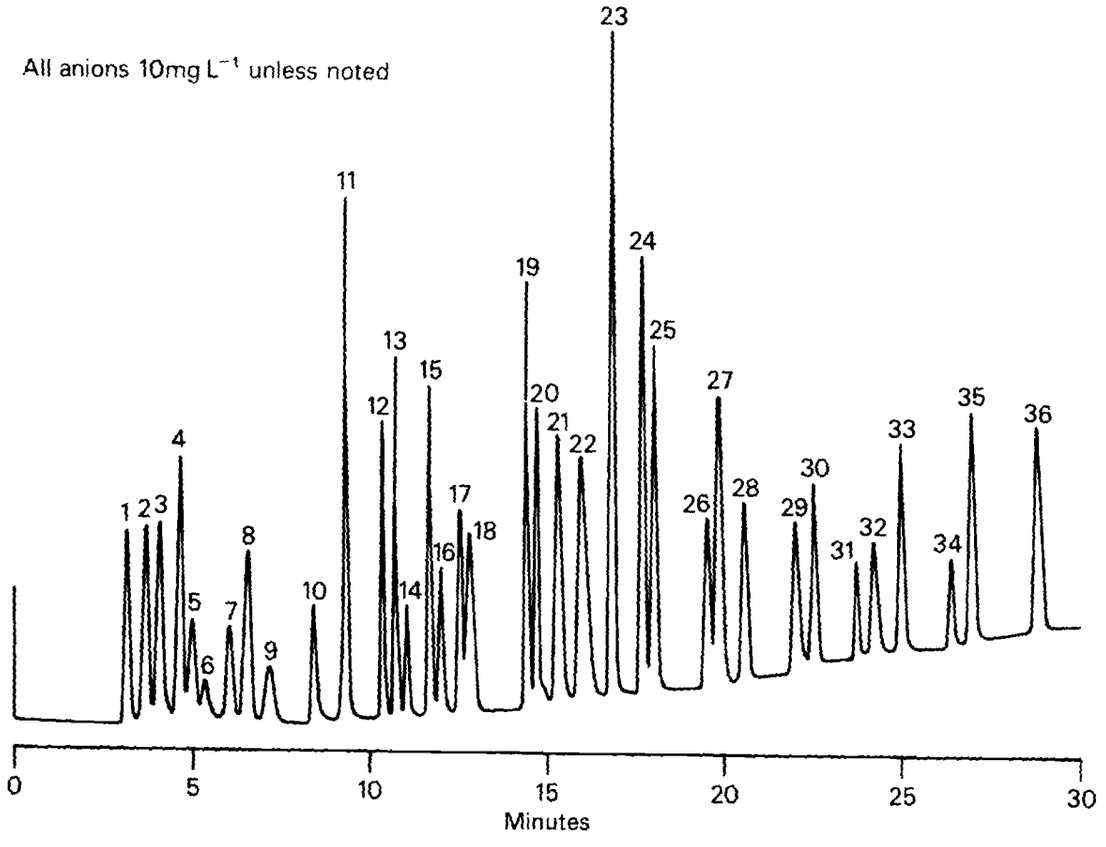
Conditions

Meter: 100μS FS
Loop size: 50μL
Flow rate: 2.2mL min^{-1}
Eluent: 5mM $Na_2B_4O_7$
Columns: AG3 guard
(Dionex) AS3 separator
AFS suppressor

Species ($mg\,L^{-}$)

1 Fluoride 10
2 Acetate 50
3 Formate 50
4 Chloride 50

Chromatogram 2.4 Gradient separation of anions



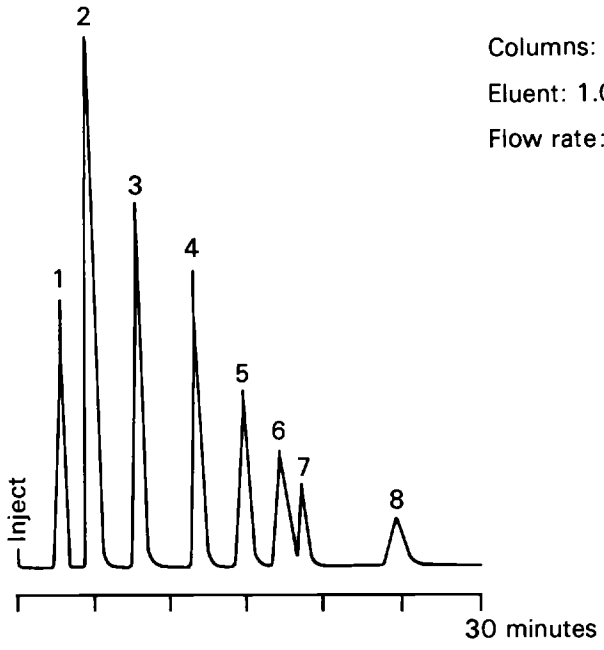
1 Fluoride (1.5mg L^{-1})
2 α-Hydroxybutyrate
3 Acetate
4 Glycolate
5 Butyrate
6 Gluconate
7 α-Hydroxyvalerate
8 Formate (5mg L^{-1})
9 Valerate
10 Pyruvate
11 Monochloroacetate
12 Bromate
13 Chloride (3mg L^{-1})
14 Galacturonate
15 Nitrite (5mg L^{-1})
16 Glucuronate
17 Dichloroacetate
18 Trifluoroacetate
19 Phosphite
20 Selenite
21 Bromide
22 Nitrate
23 Sulphate
24 Oxalate
25 Selenate
26 α-Ketoglutarate
27 Fumarate
28 Phthalate
29 Oxaloacetate
30 Phosphate
31 Arsenate
32 Chromate
33 Citrate
34 Isocitrate
35 Cis-aconitate
36 Trans-aconitate

Conditions as for Chromatogram 1.4

Chromatogram 2.5 Standard

1	Sulphate	2.5mg L^{-1}
2	Fluoride	2.5 "
3	Formate	5 "
4	Acetate	5 "
5	Propanoate	5 "
6	Carbonate	10 "
7	Butanoate	5 "
8	Pentanoate	10 "

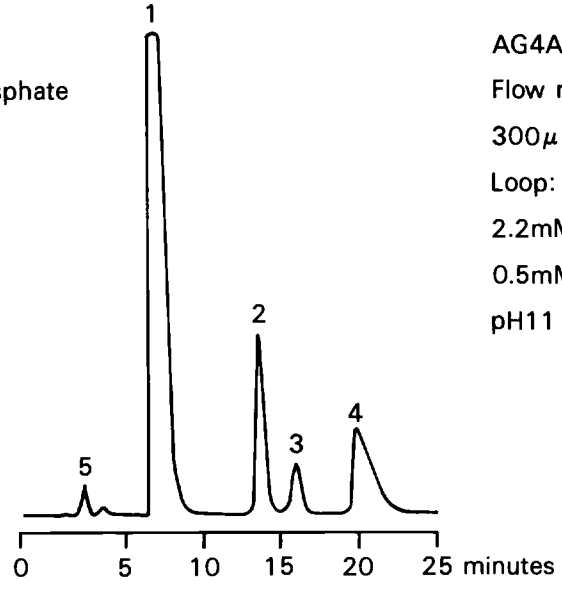
Loop: 50μL



Columns: ICE-ASI AFS 2
Eluent: 1.0mM TDFHA
Flow rate: 0.8mL min^{-1}

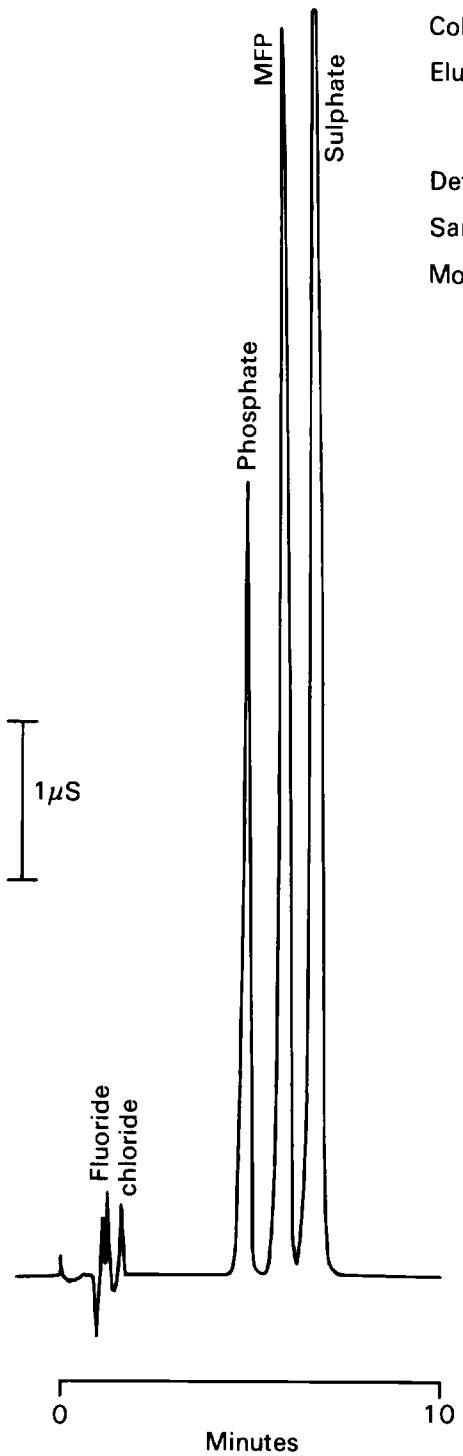
Chromatogram 2.6
Typical toothpaste chromatogram using modified anion mobile phase

1 Nitrate
2 Monofluorophosphate
3 Sulphate
4 Phosphate
5 Fluoride



AG4A AS4A
Flow rate: 1.2mL min^{-1}
300μS
Loop: 50μL
2.2mM Na_2CO_3
0.5mM NaOH
pH11

Chromatogram 2.7



Column: IONPAC AG4A, AS4A

Eluent: 1.8mM Na_2CO_3
1.7mM $NaHCO_3$
2mL min^{-1}

Detector: Suppressed conductivity

Sample: 2.5mL of toothpaste solution

Monofluorophosphate = 90mg mL^{-1}

3. Special Inorganic Anions

3.1 Introduction

In this section the chromatographic determination of various target anions and groups of anions is presented. In each case the conditions and detection systems are chosen specifically for the target anions rather than being a compromise to give a wide selection of anions as in Part II Method 1. Only limited information is available and so for each group only typical conditions for separation and detection are given with examples of chromatograms and a limited amount of statistical information.

It is clear that there are other anions not discussed here which could sensibly be determined by chromatography. If conditions are given in this booklet for anions similar to the one to be determined then those conditions could be suitable, or at least a good starting point, for the development of a working system. A list of inorganic anions for which peaks have been obtained using chromatographic systems similar to those given in this section is included in Part 1 Table 1.1. When a new species is determined chromatographically, precautions should always be taken to ensure that the peak produced is really the ion in question. These precautions would include the observation that the peak area or height is proportional to the concentration and that the sensitivity is in line with those of similar ions.

3.2 Cyanide and Sulphide in Water

3.2.1 Precautions

HCN and H_2S are both highly toxic. Materials containing cyanide and sulphide should be handled with care according to recognised procedures.

3.2.2 Principles

Cyanide and sulphide are both anions of acids too weak to be determined by suppressed conductivity detection but both can be determined with high sensitivity in a single run by amperometry at a silver electrode. The main difficulty in determining these ions by chromatographic procedures is that they are subject to rapid oxidation and the preservation procedures used with other techniques cannot be used for chromatography. Thus the method is most suitable for on site use.

The electrochemical reactions are:

$Ag + S^{2-} \rightarrow Ag_2S + 2e^-$, and
$Ag + 2CN^- \rightarrow Ag(CN)_2^- + e^-$

Both may also be determined by inverse UV or conductivity detection and, with less sensitivity, sulphide may be detected by direct UV photometry at 215 nm.

3.2.3 Characteristics of the method (amperometric detection)

3.2.3.1	Range of determination	Up to 1 mg L^{-1}
3.2.3.2	Detection limit	0.005 mg L^{-1}
3.2.3.3	Standard deviation	CN^- 1.0 mg L^{-1} 2.5% rel n = 6 S^{2-} 1.0 mg L^{-1} 2.2% rel n = 6

3.2.4 Interferences

Cyanide forms very strong complexes with certain metals. Ion chromatography does not recover any of the cyanide present as $Ag(CN)_2^-$, $Au(CN)_2^-$, $Au(CN)_4^-$, $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$, $Co(CN)_6^{3-}$ and $Co(CN)_5(OH_2)^{2-}$ because these complexes do not break down and are probably retained on the column (see Section 3.4). Distillation after acidification with sulphuric and phosphorous acid will release cyanide from all but the cobalt complexes. The distillate is collected in NaOH solution and cyanide is determined using the conditions in 3.2.6 below.

3.2.5 Preservation of the sample

If oxidising agents are present, arsenite should be added to prevent the oxidation of cyanide. Sulphide is very rapidly oxidised by dissolved oxygen. The sample should be adjusted to pH 10 with sodium hydroxide solution, deoxygenated and analysed without delay.

3.2.6 For typical chromatograms and conditions see Chromatogram 3.1.

3.2.7 Calibration

3.2.7.1 Sulphide

Dissolve 0.75 g of $Na_2S.10\ H_2O$ in distilled water and make up to 1 litre. Standardise by pipetting 50 mL into 25 mL of standard 0.1 M iodine solution containing 0.5 mL of concentrated HCl. Titrate the excess iodine with standard 0.1 M sodium thiosulphate solution using a soluble starch indicator. Just before use, dilute the standardised sulphide solution with deoxygenated water, adding sufficient deoxygenated 0.1 M NaOH solution to make the final solution 0.01 M.

3.2.7.2 Cyanide

Dissolve 2.5 g of KCN in distilled water, add 100 mL of 0.1 M NaOH and dilute to 1 litre. Titrate a 10 mL aliquot with a standard 0.1 M $AgNO_3$ solution using 3 drops of 0.03 g/100 mL dimethylaminobenzylidene rhodanine indicator solution. Dilute as necessary with 0.01 M NaOH solution.

3.3 Cyanate in Waters

3.3.1 Principle

Cyanate is determined by chromatography of ions using either direct UV absorption at 205 nm or suppressed conductivity detection. A column suppressor cannot be used because this hydrolyses cyanate to H_2CO_3 which is not detected with sufficient sensitivity by conductivity. The suppressor must be of the micromembrane or hollow fibre type.

3.3.2 Characteristics of the method

3.3.2.1 Range of determination

Up to 20 mg L^{-1} without dilution

3.3.2.2 Detection limit

0.03 mg L^{-1} by chemically suppressed conductivity detection
0.1 mg L^{-1} by direct UV detection

3.3.2.3 Standard deviation

1.2% relative for 10 replicate determinations at 10 mg L^{-1}

3.3.2.4 Interferences

Cyanate is only just separated from nitrite, so more than a two fold excess of the latter will interfere with conductivity detection, or an equal concentration if using UV photometry. Large amounts of chloride (>100 mg L^{-1}) interfere when using conductivity, but up to 1000 mg L^{-1} can be tolerated using UV.

3.3.3 For typical chromatogram and conditions see Chromatogram 3.2.

Any columns and conditions capable of separating nitrite from chloride will probably be suitable for this analysis.

3.3.4 Calibration

Cyanate hydrolyses quite rapidly. A 1000 mg L^{-1} stock solution should be prepared or standardised each week.

Standards in the range 1 to 20 mg L^{-1} should be prepared daily.

3.4 Metal Cyanide Complexes in Water

3.4.1 Principle

The stable metal cyanide anions* are separated by ion pair reverse phase chromatography. All the complexes are detected by either direct UV absorbance or chemically suppressed conductivity measurements. Good sensitivity is obtained for all the complexes by using UV detection at 210 nm, but other wavelengths can be chosen to increase selectivity for particular complexes such as $Co(CN)_6^{3-}$ which has an absorption maximum at 310 nm, whereas $[Co(CN)_5OH_2]^{2-}$ has a maximum at 380 nm.

*Note: A number of stable metal cyanide complexes exist. For a list of metals forming such cyanides see Cyanide in Waters etc. 1988. HMSO in this series.

3.4.2 Characteristics of the method

3.4.2.1 Range of determination

Up to 20 mg L^{-1} of $Cu(CN)_2^{2-}$, $Ag(CN)_2^-$, $Fe(CN)_6^{4-}$.
$Fe(CN)_6^{3-}$, $Co(CN)_6^{3-}$, $[Co(CN)_5OH_2]^{2-}$, $Au(CN)_2^-$ and $AuCN_4^-$.

3.4.2.2 Detection limits

About 0.1 mg L^{-1}

3.3.2.3 Standard deviations

1 – 3% relative at 10 mg L^{-1} of
$Ag(CN)^-_2$, $Fe(CN)_6^{4-}$ and $Fe(CN)_6^{3-}$

3.4.2.4 For typical chromatograms and conditions see Chromatograms 3.3 and 3.4.

3.5 Iodide, Thiocyanate and Thiosulphate in Waters

3.5.1 Principle

These three anions are grouped together because they are usually determined under similar conditions. They may be determined by ion exchange chromatography or reverse phase ion pair chromatography. Direct or suppressed conductivity may be used. All three ions absorb strongly in the UV, 225 nm being a convenient wavelength for detecting them. Electrochemical detection using a silver electrode is particularly sensitive for the detection of iodide. Iodide can often be determined as a late peak in the chromatograms given in Part II Method 1 but thiosulphate and thiocyanate are too well retained to give well shaped peaks.

3.5.2 Characteristics of the method

3.5.2.1 Range of determination

Conductivity and UV detection: Up to 20 mg L^{-1} without dilution

Electrochemical detection of I^-: Up to 1 mg L^{-1} without dilution

3.5.2.2 Detection limits (40 μL injections)

Conductivity detection I^- 0.05 mg L^{-1}
$S_2O_3^{2-}$ 0.02 mg L^{-1}
SCN^- 0.04 mg L^{-1}

UV detection I^- 0.01 mg L^{-1}
$S_2O_3^{2-}$ 0.02 mg L^{-1}
SCN^- 0.02 mg L^{-1}

Electrochemical detection I^- 5 μg L^{-1}

3.5.2.3 Standard deviation

UV detection I^- 5 mg L^{-1} 1.5% RSD
$S_2O_3^{2-}$ 5.6 mg L^{-1} 1.4% RSD
SCN^- 5 mg L^{-1} 1.5% RSD
(6 replicates)

Conditions as in Chromatogram 3.5a

Electrochemical detection I^- 0.1 mg L^{-1} 2.1% (RSD)
(5 replicates)

Conditions as in Chromatogram 3.5b

Interferences: There are relatively few interferences, but some organic anions may coelute.

Chromatograms from the same run in which two different detectors were used are given in Chromatograms 3.5a and b. Sample, column and conditions used are given with Chromatogram 3.5a, detector data accompanying each chromatogram.

3.5.3 Alternative Separations

Details of an alternative separation procedure are given in Chromatogram 3.6. Chromatogram 3.7 gives a simple procedure for iodide.

3.6 Large Oxysulphur Anions

3.6.1 Principles

The sulphur oxy anions larger than thiosulphate are determined by reverse phase ion pair chromatography. Direct UV detection is possible for all except peroxydisulphate. All of them can be detected by suppressed conductivity.

3.6.2 Characteristics of the method

3.6.2.1 Range of determination up to 20 mg L^{-1}

3.6.2.2 Detection limits in the range 0.02 – 0.05 mg L^{-1}

3.6.2.3 Interferences not known

3.6.3 Typical conditions and chromatograms see Chromatograms 3.8 and 3.9.

3.7 Sulphite in Water

3.7.1 Principle

Sulphite is determined using conditions similar to those for the common anions. The pH of the mobile phase is important since retention varies considerably if it is present as SO_3^{2-} (at high pH) or HSO_3^- (at lower pH). This may be used to achieve a separation from any interfering species as singly charged ions are eluted early whereas doubly charged ones are more strongly retained.

Conductivity detection is usually employed. UV absorbance is less sensitive. The spectrum has a maximum at 192 nm. The absorbance is greater at high pH.

3.7.2 Characteristics of the method

3.7.2.1 Range of determination up to 20 mg L^{-1}

3.7.2.2 Detection limit (40 μL injection)

0.1 mg L^{-1} with suppressed conductivity detection

0.3 mg L^{-1} UV at 210 nm

3.7.2.3 Interferences

Oxidising agents interfere, the effects of other anions are as discussed in Method 1 of this part, for common anions.

3.7.3 For typical conditions and chromatograms see Chromatogram 3.10.

3.7.4 Preservation of the sample

Sulphite is readily oxidised to sulphate, but the complexes formed with aldehydes are more stable and sulphite is released again on the analytical column. The addition of 20 μl of 40% formaldehyde solution to a 20 mg L^{-1} sample will prevent oxidation for about one week.

3.7.5 Calibration

Determine the purity of the anhydrous sodium sulphite by adding an excess of standard iodine solution to about 0.1 g (accurately weighed) and back titrating with standard sodium thiosulphate solution. Weigh out the calculated quantity for 100 mL of 1 mg mL^{-1} of SO_3^{2-}. Dissolve in distilled water, add 1 mL of 40% formaldehyde solution and make up to 100 mL. Dilute as necessary to make working standards which are stable for at least 5 days. Renew the stock solution each month.

3.8 Carbonate and Borate in Water

3.8.1 Principle

Carbonate and borate are weak acids and can thus be separated by ion exclusion chromatography. Both, however, are so weak that they cannot be detected by direct conductivity measurement using the usual dilute acid eluent. Post column reaction

using an ion exchange membrane to give the salts facilitates sensitive conductimetric detection. Ammonium hydroxide is used as the regenerant for the membrane as this gives reproducible results. The use of NaOH, KOH, or tetrabutylammonium hydroxide gives poor reproducibility for carbonate due to a broad negative peak which follows the carbonate peak.

Deionised water alone as the eluent phase also gives reproducible results. Measurement of low levels is possible because the background conductivity is then very small.

3.8.2 Characteristics of the method

3.8.2.1 Range of determination

Up to 30 mg carbonate L^{-1}, up to 10 mg boron L^{-1} as borate

3.8.2.2 Detection limits (40 μL injection)

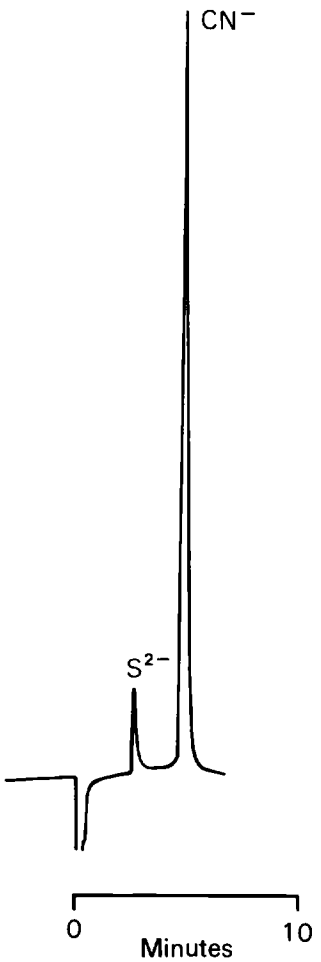
About 0.2 mg L^{-1} carbonate
0.05 mg L^{-1} boron as borate

3.8.2.3 Interferences

Propionate is only partially separated from carbonate and if more than half the concentration of carbonate will affect the peak height. Borate elutes just before formate which may interfere.

3.8.3 For typical conditions and chromatogram see Chromatogram 3.11.

Typical chromatograms and conditions



Chromatogram 3.1

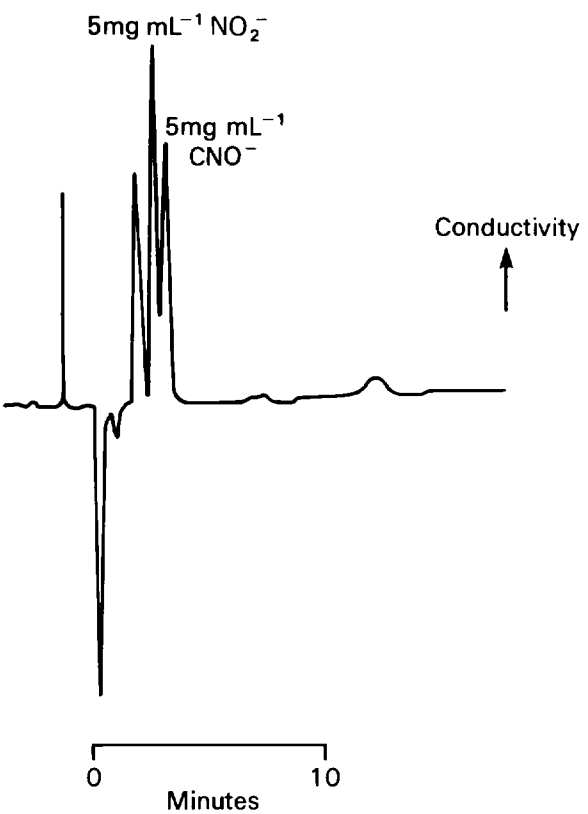
Analytical column: Dionex AS6

Mobile phase: 0.1M NaOH
0.5M Na acetate
0.5g/100mL ethylenediamine

Flow rate: 1.5mL $min.^{-1}$

Detection: Electrochemical silver electrode
0.0V vs Ag/AgCl

Injection: 40 μL
S^{2-} 0.1mg L^{-1}
CN^- 1mg L^{-1}



Chromatogram 3.2

Analytical column: Dionex AS3

Mobile phase: 2.8mM Na_2CO_3 2.4mM NaHCO

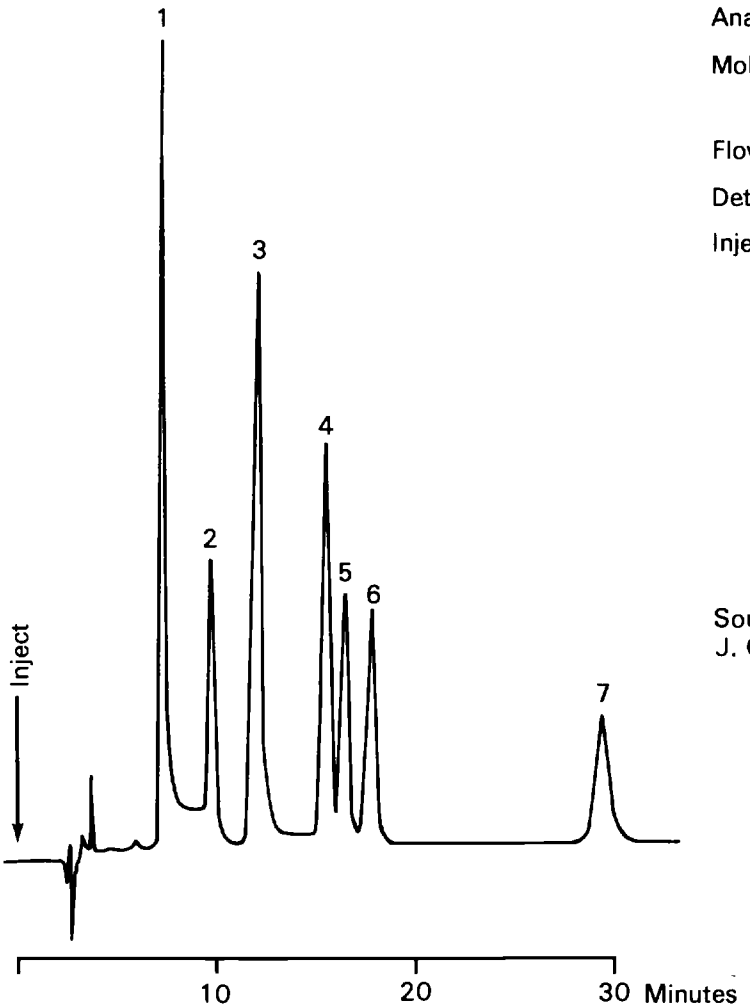
Micromembrane suppressor

Regenerant: 25mM H_2SO_4

Injection: 40μL

Source: BP Research

Chromatogram 3.3



Analytical column: C18 Novapak 4 μm

Mobile phase: 2.5mM TBA H_2SO_4 70
Methanol 30

Flow rate: 1mL min^{-1}

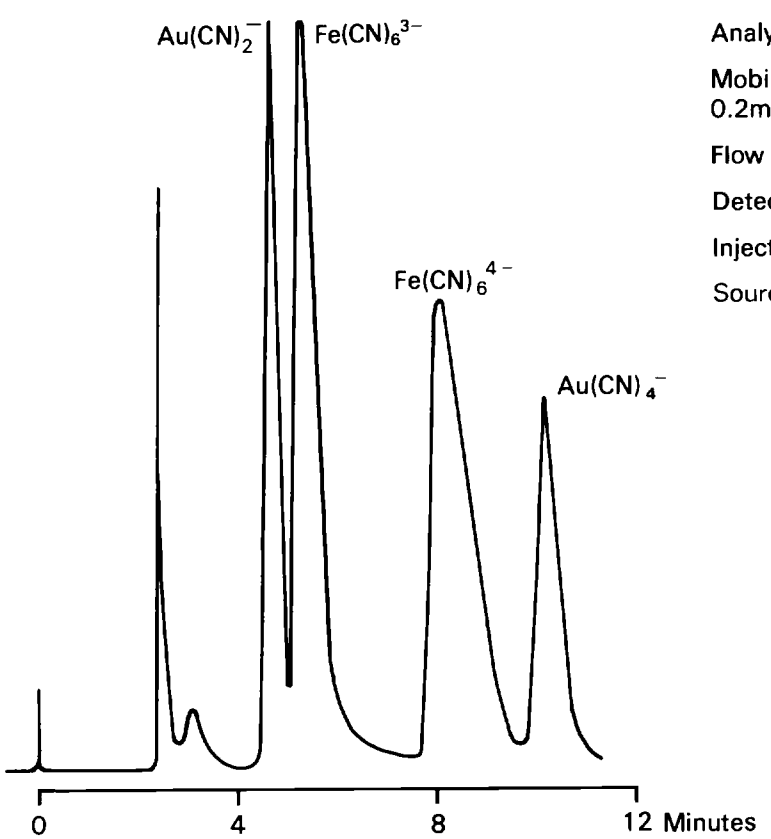
Detection: UV 210nm

Injection: 20μL 10mg L^{-1}each of:

1 $Cu(CN)_2^-$
2 $Ag(CN)_2^-$
3 $Fe(CN)_6^{4-}$
4 $Co(CN)_6^{3-}$
5 $Ni(CN)_4^{2-}$
6 $Fe(CN)_6^{3-}$
7 $Au(CN)_2^-$

Source: B Grigorora S A Wright M Josephson - J. Chromatography 1987

Chromatogram 3.4



Analytical column: Dionex MPIC NSI

Mobile phase: 2mM TBAOH 40% CH_3CN
0.2mM Na_2CO_3

Flow rate: 1mL min^{-1}

Detection: Suppressed conductivity

Injection: 50μL

Source: Dionex Inc.

Chromatogram 3.5a
Conductivity detection

Analytical column: Dionex AS 5

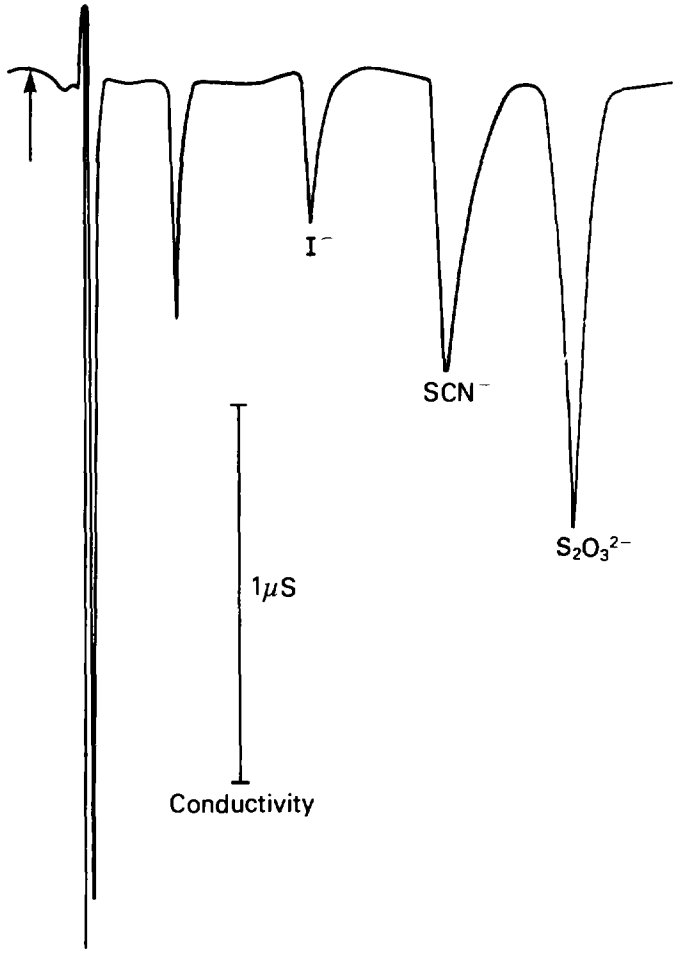
Flow rate 2mL.mm^{-1}

Suppressed conductivity: micromembrane suppressor regenerant 25mM H_2SO_4

Injection: 40μL I^- 5mg L^{-1}
SCN^- 5mg L^{-1}
$S_2O_3^{2-}$ 5.6mg L^{-1}

Mobile phase: $NaHCO_3$ 4.3mM
Na_2CO_3 3,7mM

Source: BP Research

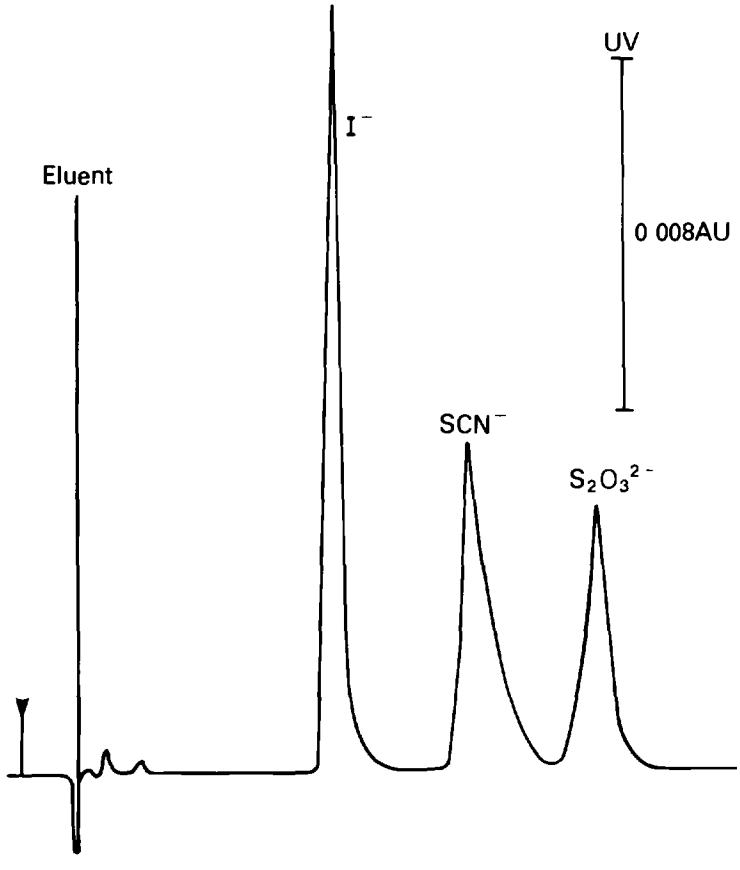


Chromatogram 3.5b
UV detection

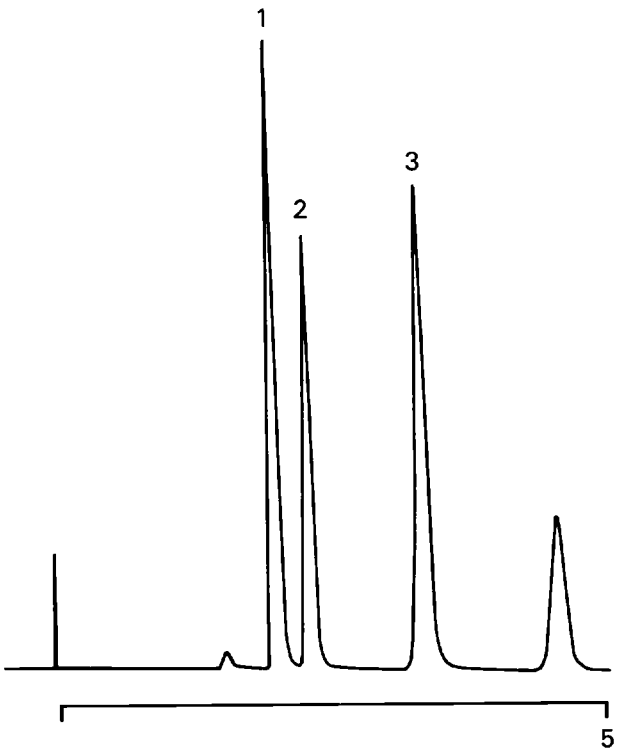
Detection: UV 225nm

Same run as for Chromatogram 3.5a

Source: BP Research



Chromatogram 3.6



Analytical column: PLRP-S 5μ 150×5mm

Mobile phase: Acetonitrite/water 3/7 v/v with 0.7g L^{-1} citric acid and 0.05g L^{-1} HDTMAH adjusted to pH 11.0 with KOH

Flow rate: 1.05mL min^{-1}

Detection: UV 220nm

Injection: 20μL 1 NO_2^- 6.3ng

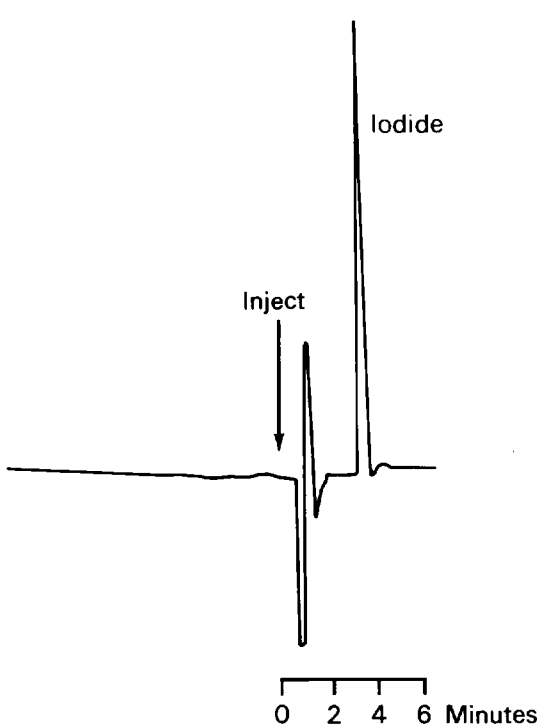
2 NO_3^- 5.8ng

3 I^- 6.1ng

4 SCN^- 4.8ng

Source: Metropolitan Police Forsenic Laboratory

Chromatogram 3.7



Analytical column: Dionex AS 5

Mobile phase: 50mM HNO_3

Flow rate: 1mL min^{-1}

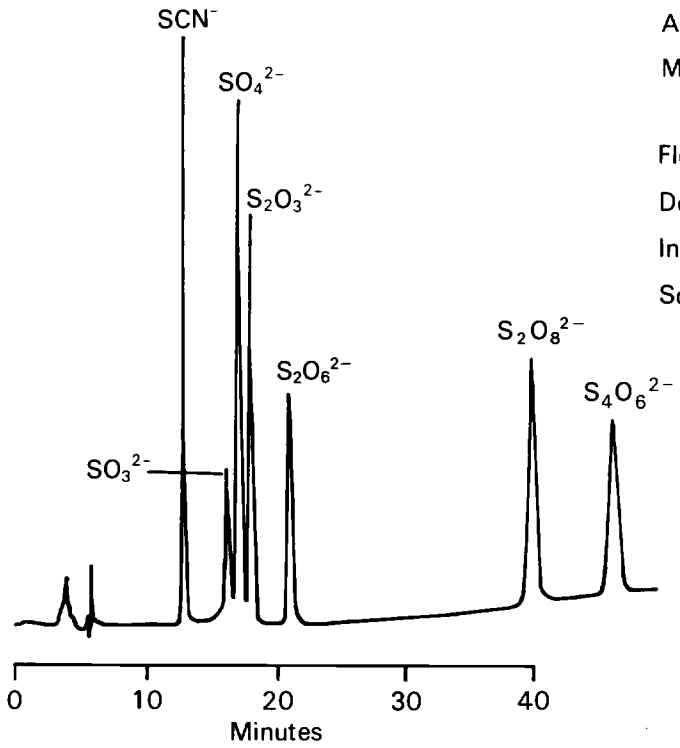
Detection: Electrochemical

Silver electrode 200mV vs Ag/AgCl

Injection: 40μL I^-0.25μ mL^-

Source: BP Research

Chromatogram 3.8



Analytical column: Lichrosphere - 100CH

Mobile phase: 10^{-3}M TBAOH 14% acetonitrile
7.5×10^{-3}M H_3BO_3

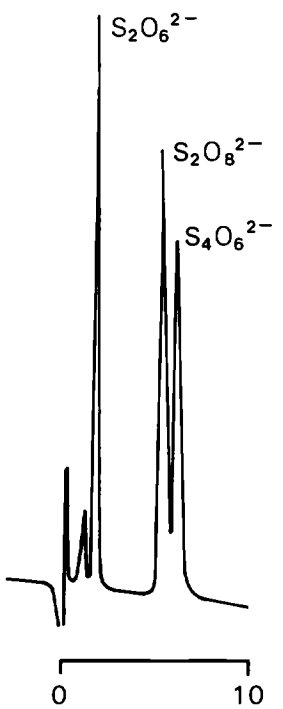
Flow rate: 0.8mL min^{-1}

Detection: Suppressed conductivity

Injection: 50μL 1mg L^{-1} except $S_2O_8^{2-}$ and $S_4O_6^{2-}$ 2mg L^{-1}

Source: M Werdenauer P Hoffmann K H Lieser Z Anal. Chem. 1988 331 372

Chromatogram 3.9



Analytical column: Dionex HPIC NSI

Mobile phase: 1mM Na_2CO_3 2mM TBAOH
30% acetonitrile

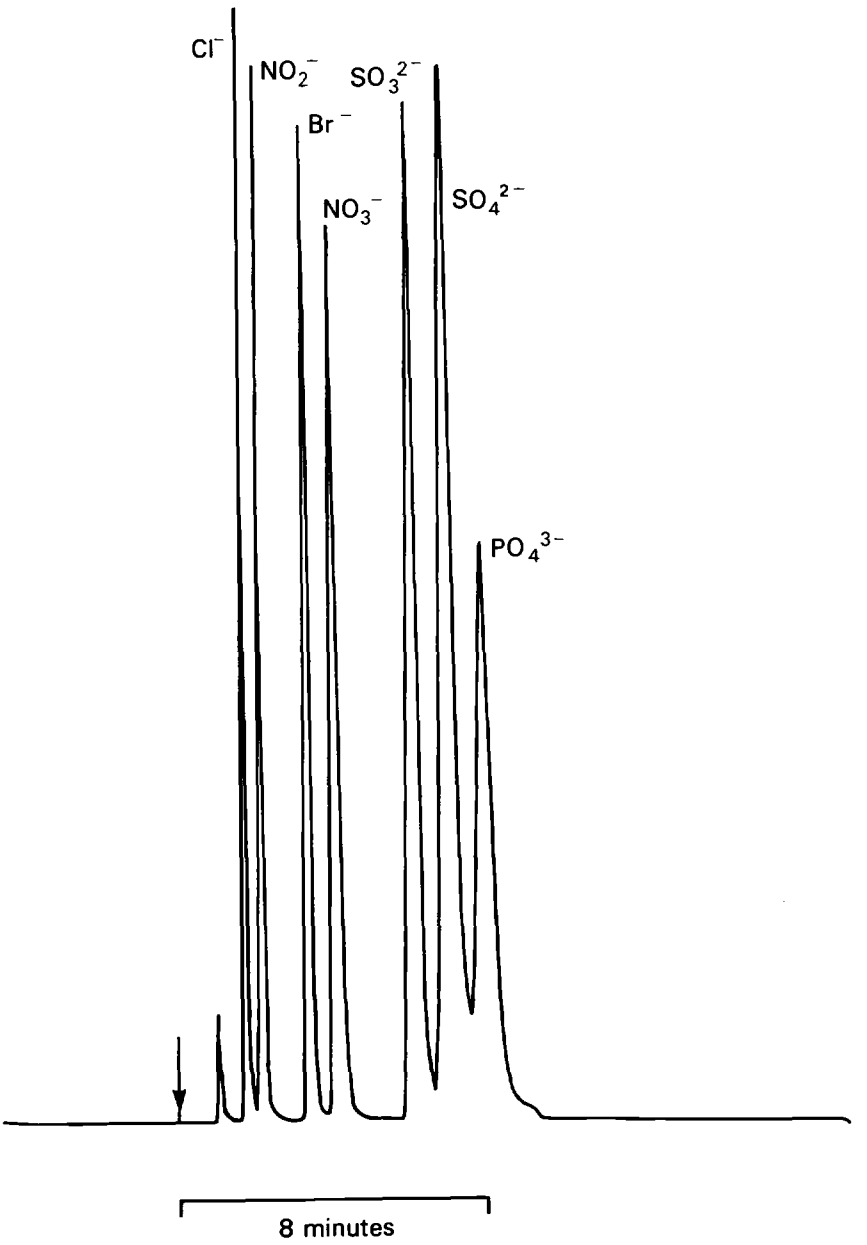
Flow rate: 1mL min^{-1}

Detection: Suppressed conductivity

Injection: 40μL 20mg L^{-1} of each ion

Source: BP Research

Chromatogram 3.10



Analytical column: Dionex AS4–A

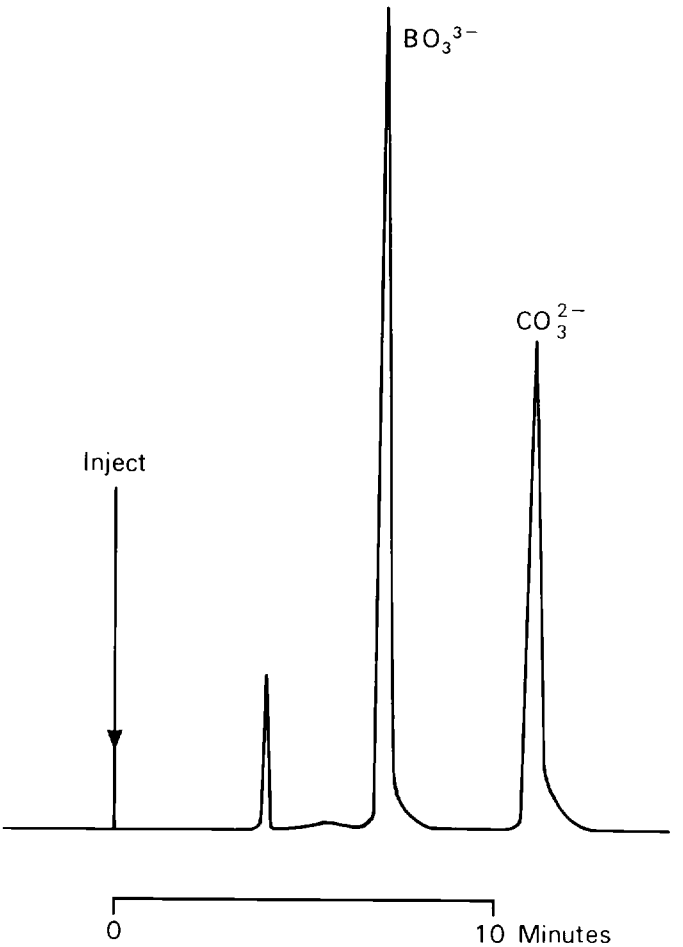
Eluent: 2mM Na_2CO_3 0.5mM NaOH 2mL min^{-1}

Detection: Suppressed conductivity

Injection: 40μL SO_3^{2-} 20μg mL^{-1} other ions Cl^- 3, NO_2^- 5, Br^- 10, SO_4^{2-} 15, PO_4^{3-} 20μg mL^{-1}

Source: British Gas plc

Chromatogram 3.11



Analytical column: Dionex ICE AS1

Mobile phase: 20mg L^{-1} HCl

Suppressor: Dionex ICE micromembrane

Regenerant: 40mM NH_4OH

Detector: Conductivity

Injection: 40μL

Borate: 4mg L^{-1}

Carbonate: 10mg L^{-1}

Source: BP Research

4. Sequestering Agents (including Ethylenediamine tetraacetate, Nitrilotriacetate and Polyphosphonates) in Water

4.1 Scope and Field of Application

4.1.1 Type of Sample

This method is applicable to the determination of NTA, EDTA, orthophosphate, pyrosphosphate, tripolyphosphate, higher polyphosphonates in slightly contaminated waters such as drinking water, rain water, ground water and surface water. (Typical polyphosphonates are illustrated in Fig 4.1).

4.1.2 Concentration Ranges

Using an eluent concentration of 70 mM and conditions as defined in section 5, the following expresses the usable working range from the limit of detection to the linear maximum.

Orthophosphate	: 1.0 to 100 mgL^{-1}	
NTA	: 0.1 to 105 mgL^{-1}	RT 3.8 min
Pyrophosphate	: 0.1 to 125 mgL^{-1}	RT 3.8 min
EDTA	: 0.2 to 125 mgL^{-1}	RT 6.5 min
Tripolyphosphate	: 1.0 to 500 mgL^{-1}	RT 13.5 min
Polyphosphonates	: 0.1 to 100 mgL^{-1}	

4.2 Principle of the Method

The ions are separated in an ion exchange chromatograph by means of an anion separating column of high capacity. The mobile phase is an aqueous solution of nitric acid. Post column reaction with iron(III) allows detection by visible region absorption at 330 nm.

4.3 Interferences

The very few known interferences are mostly other organic chelating agents such as organic acids (e.g. citrate), but their detector response is often much lower. Sulphate co-elutes with EDTA when an eluent concentration of 70 mM is used. If both iron and EDTA are present, an iron EDTA complex elutes close to free EDTA. As other similar unreported metal sequesterant complexes may exist, users are advised to be alert.

4.4 Standard Deviation

Some typical precision results are given below. The conditions used were as in section 4.5, using an eluent of 70 mM nitric acid.

Replicates of a mixed standard (9 degrees of freedom)

Ion	Level mgL^{-1}	%RSD
NTA	50	0.54
Pyrophosphate	50	0.52
EDTA	50	0.57
Tripolyphosphate	100	2.25

4.5 Apparatus

The system shall consist of the following items in series:

Eluent reservoir
Eluent pump set at 0.5 mL min^{-1}
Sample injector with a 50μL loop
Dionex Ionpac–AG7 anion guard column
Dionex Ionpac–AS7 anion separator column
Post column reactor set at 0.5 mL min^{-1} reagent flow
UV-Visible range absorptiometric detector set at 330 nm

Recording device and/or data processing device

4.6 Reagents

Reagents of recognised analytical grade must be used unless otherwise stated. The water must have a negligible visible absorbance at 330 ± 10 nm and must be free from particulate matter (>0.2 μm).

4.6.1 Eluents

The eluent consists of a solution of nitric acid in the range 30 to 70 mM depending on the separation required. It may be necessary to degas the eluent.

4.6.2 Post Column Reagent

1 g of $Fe(NO_3)_3.9H_2O$ in 1 litre of 2%(v/v) Perchloric acid

4.6.3 Calibration Solution

Depending on the concentrations expected in the samples, use stock solutions of each individual ion or mixed standard solutions to prepare at least 5 reference solutions which should cover the working range to be expected.

Prepare these solutions afresh each day.

4.7 Separation Quality

Using an eluent concentration of 50 mM HNO_3, a mixed solution of the following ions should be resolved as in Chromatogram 4.1.

A = Phosphate (150 mgL^{-1})
B = NTA(25 mgL^{-1})
C = Pyrophosphate (40 mgL^{-1})
D = EDTA (5 mgL^{-1})
E = Sulphate (30 mgL^{-1})

An eluent concentration of 30 mM is used to separate polyphosphonates (Chromatogram 4.2). A concentration of 70 mM can be used to reduce run times provided both sulphate and EDTA are not present in the same sample.

4.8 Sample Pretreatment

Sample pretreatment involves only filtration through a 0.45 μm membrane filter. It may be necessary to dilute the sample with water or eluent concentrate. A neutral guard column is used to remove highly retained organics and prevent poisoning of the analytical column. The column is cleaned regularly for 15 minutes with 90% acetonitrite 10% water mixture followed by a 15 minute water wash.

4.9 Procedure

Start the ion chromatograph in accordance with the manufacturer's instructions. The instrument is ready for operation as soon as the base line is stable. Perform the calibration as described in section 4.9.1 using solutions prepared as in Section 4.6.3. Inject the pretreated sample into the chromatograph.

4.9.1 Calibration

The ions are identified by comparing the retention times of the sample with those of the reference solutions. The retention times may be concentration and matrix dependent.

After establishing the calibration curve, the pretreated sample can be measured.

A calibration check should be performed every 10 to 20 measurements.

4.10 Evaluation

Estimate the mass concentration of the anion in the measuring solution using the peak areas or peak heights and the calibration graph. Multiply this by the dilution factor.

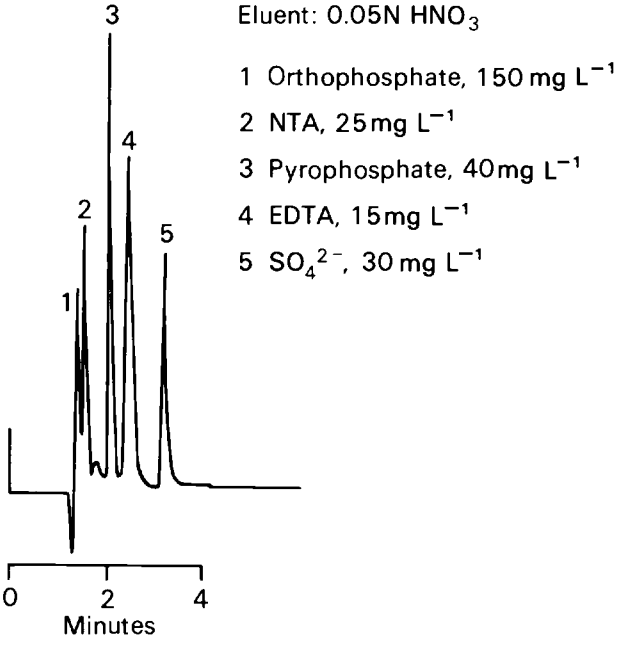
4.11 Expression of Results

Report the result to three significant digits.

4.12 References

1. Dionex *application note* 44.
2. Fitchett, A.W. and Woodruff, A., 'Determination of polyvalent ions by Ion Chromatography', *Liquid Chromatography Magazine*,**1** (1), 48–49, 1983.

Chromatogram 4.1
Separation of EDTA and SO_4^{2-}



Chromatogram 4.2
Polyphosphonates

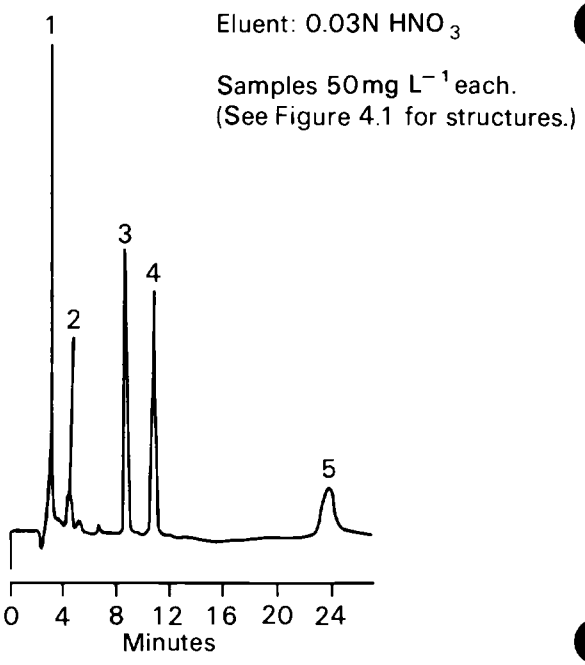
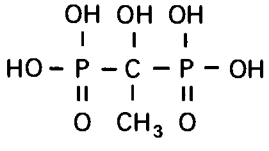
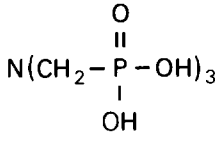


Figure 4.1 Polyphosphonate structures

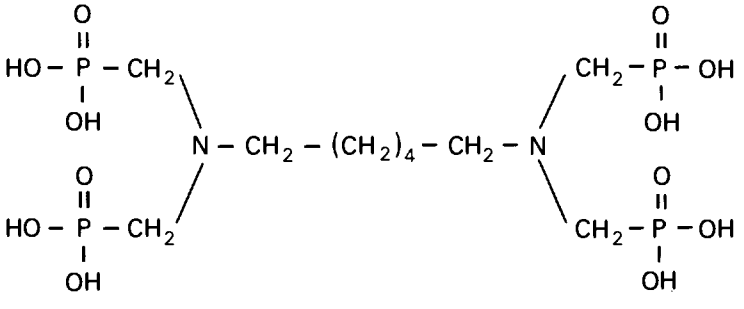
1.



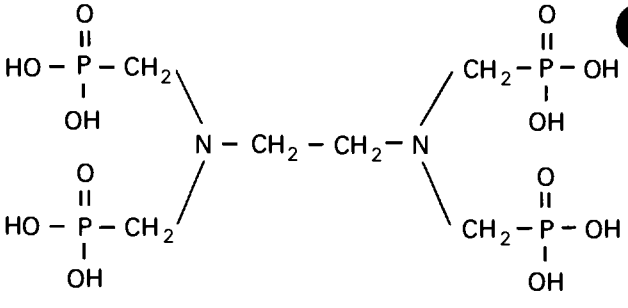
3.



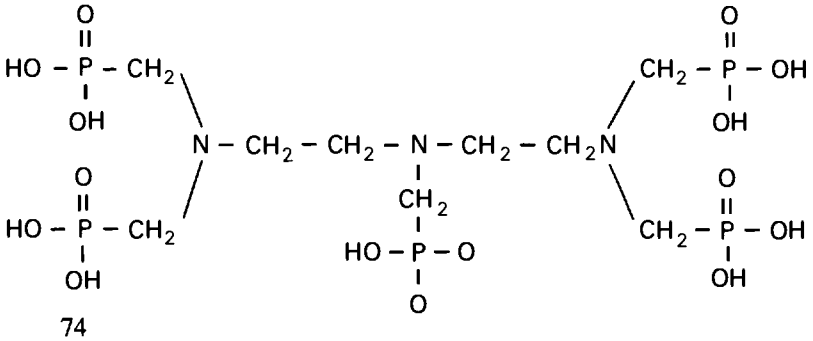
2.



4.



5.



5. Chlorite, Chlorate and Other Anions

5.1 Scope and Field of Application

5.1.1 Type of Sample

This method is applicable to the determination of chlorite and chlorate as well as common anions such as chloride, nitrite, bromide, nitrate, phosphate and sulphate in slightly contaminated waters, such as drinking water, rain water, ground water and surface water.

This method is ideal for all breakdown products of a chlorination process (1) in the presence of large amounts of other anions.

5.1.2 Concentration Ranges

Using an eluent concentration of 2.0 mM sodium carbonate and 0.75 mM sodium bicarbonate and conditions as defined in section 4 the usable working ranges for chlorite, chloride and chlorate were:

Chlorite : 0.05 to 20 mg L^{-1} RT = 1.35 mins
Chloride : 0.1 to 50 mgL^{-1} RT = 1.85 mins
Chlorate : 0.1 to 50 mgL^{-1} RT = 4.0 mins

5.2 Principle of the Method

The ions are separated in an ion exchange chromatograph by means of an anion separating column designed specifically for the analysis of chlorite, chlorate and nitrate and other inorganic anions.

The column has an ion exchange capacity of approximately 30 μeq/column and the resin is composed of 15 μm particles. The nominal efficiency for sulphate is 14,000 plates/meter.

The mobile phase is an aqueous solution of sodium carbonate/sodium bicarbonate and detection is by suppressed conductivity.

It is also possible to determine both **hypochlorite** and **perchlorate** with this procedure. Hypochlorite is an unstable species and a weakly conducting ion, however it is easily reduced and is therefore preferentially detected using an electrochemical detector under the following conditions:

Electrode = Platinium
Potential = −0.2 volts

The electrochemical cell is placed between the injector and guard column and detected after a delay loop of 500 μL. This allows detection of hypochlorite before possible reaction with the column, which is known to occur. The working range is approximately 0.1 to 20 mg L^{-1}.

5.3 Interferences

No direct interferences are known, however problems may arise when high levels of one ion affect lower concentrations of adjacent peaks. For example chlorate levels may be affected by high levels of either bromide or nitrate. This may result in a change in retention time and/or peak shape which can affect quantification. This becomes more apparent as the column degrades.

5.4 Apparatus

The system shall consist of the following items in series:

Eluent reservoir
Eluent pump set at 2.0 ml min^{-1}
Sample injector with a 50μL loop
Dionex Ionpac–AG9 anion guard column
Dionex Ionpac–AS9 anion separator column
Anion micromembrane suppressor
Conductivity detector
Recording or data device

5.5 Reagents

Reagents of recognised Analytical grade must be used unless otherwise stated. The water must be dionised and have a conductivity of 18 megohm cm^{-1} or less.

5.5.1 Eluent

The eluent is a solution of 2.0 mM sodium carbonate and 0.75 mM sodium bicarbonate. If necessary degas the eluent.

5.5.2 Reference Solution

Depending on the concentrations expected in the samples, use stock solutions of each individual ion or mixed standard solutions to prepare at least 5 reference solutions which should cover the working range to be expected. Prepare the solutions freshly for each day of measurement.

5.6 Separation Quality

Using an eluent concentration in 5.5.1 a mixed solution of the following ions should be resolved as in Chromatogram 5.1.

1 = Fluoride (1 mg L^{-1})
2 = Chlorite (5 mg L^{-1})
3 = Chloride (1.5 mg L^{-1})
4 = Nitrite (6 mg L^{-1})
5 = Bromide (10 mg L^{-1})
6 = Chlorate (15 mg L^{-1})
7 = Nitrate (20 mg L^{-1})
8 = Phosphate (20 mg L^{-1}
9 = Sulphate (25 mg L^{-1})

5.7 Sample Pretreatment

Sample pretreatment involves only filtration through a 0.45 μm membrane filter. It may be necessary to dilute the samples with water or eluent concentrate.

5.8 Procedure

Start the chromatograph in accordance with the manufacturer's instructions. The instrument is ready for operation as soon as the baseline is stable. Inject the standards, pretreated samples and blanks.

5.8.1 Calibration

The ions are identified by comparing the retention times of the sample with those of the reference solution. The retention times may be concentration and matrix dependent.

After establishing the calibration curve, the pretreated sample can be measured.

A calibration check should be performed every 10 to 20 measurements.

5.9 Evaluation

Estimate the mass concentration of the anion in the measuring solution using the peak areas or heights and the calibration graph. Multiply this by the dilution factor, see Section 5.1.2 for suitable concentration ranges.

5.10 Expression of Results

Report the result to three significant digits.

5.11 Perchlorate Analysis

Due to the hydrophobic nature of the perchlorate molecule this ion has a high affinity for polymeric columns. The retention using normal eluent conditions is therefore long and impracticable and in some cases the ion does not elute at all. However, the above column will elute perchlorate provided the eluent strength is sufficient, the use of reagents such as p-cyanophenate may be necessary to desorb the column and act as a more efficient pusher. This can be done as a step gradient addition to the above method so that perchlorate is eluted along with the other ions mentioned or analysed for as a single ion determination using the stronger eluent. The alternative is to target perchlorate as a single ion on a lower capacity column which would require a lower strength but more practicable eluent concentration.

The following conditions have been found to be satisfactory

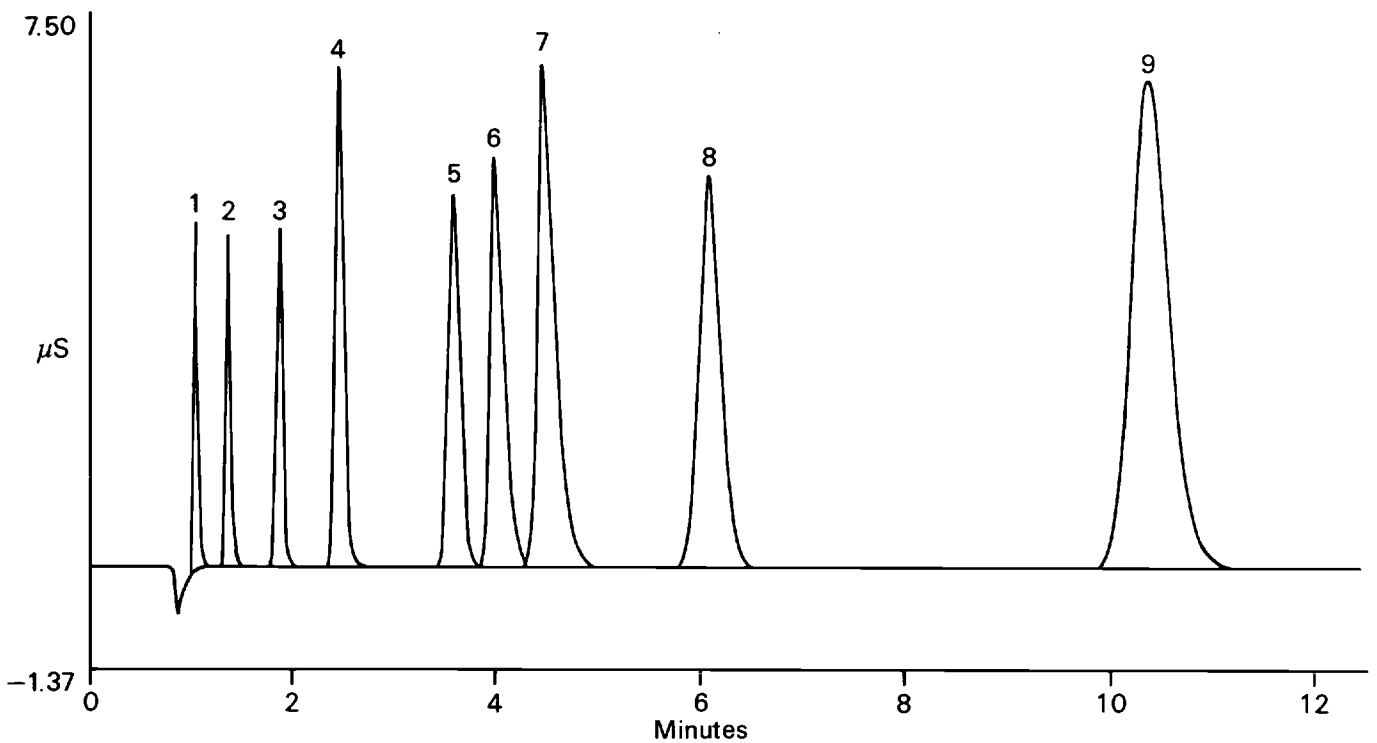
Eluent	= 120 mM Sodium Hydroxide
Flow	= 1.0 mL min^{-1}
Column	= Dionex IONPAC AS5
Suppressor	= Anion Micromembrane
Regenerant	= 25 mM Sulphuric Acid, 10 mL min^{-1} Flow
Detector	= Conductivity
Retention Time	= 9.6 Mins

This can be speeded up by the addition of p-cyanophenate as a replacement for some of the hydroxide.

5.12 References

1. DIONEX DOCUMENT NO 034104, *"Instructions and trouble shooting guide for the IONPAC AS9 column"*.

Chromatogram 5.1



Concentrations of injected anions

1. 1.0 mg L^{-1} Fluoride
2. 5.0 mg L^{-1} Chlorite
3. 1.5 mg L^{-1} Chloride
4. 6.0 mg L^{-1} Nitrite
5. 10.0 mg L^{-1} Bromide
6. 15.0 mg L^{-1} Chlorate
7. 15.0 mg L^{-1} Nitrate
8. 20.0 mg L^{-1} Phosphate
9. 25.0 mg L^{-1} Sulphate

25μL injection value

Eluent flow rate: 2.0mL $min.^{-1}$

6. Weak Inorganic and Organic Acids

6.1 Introduction (1)

Method 1 can separate a large variety of anions by conventional ion exchange chromatography. This includes a number of organic acids such as oxalate, tartrate and citrate which are relatively strong acids. For them Method 1 becomes the method of choice. Other separations are given in Part II Methods 2 and 3 (see Chromatograms 1.7, 2.4 and 2.5), and also Method 3 section 6. Method 1 may sometimes be improved by use of gradient elution. However, there are much weaker acids such as acetate and formate, which show little retention on these columns. This can be overcome by using much weaker eluents, but without frequent column washes with a stronger eluent or the ability to perform gradient elution, this is not a practical answer. The alternative is to change separation mechanism and ION EXCLUSION is a natural choice (2, 3, 4).

A column containing a strong cation exchange resin, which is a negatively charged column, will repel all strong anions such as chloride, nitrate and sulphate. However in an acidic medium, weak organic acids will have their ionisation suppressed according to the following mechanism:

$$A^- + H^+ \rightleftharpoons HA.$$

The unionised form has the ability to partition into the pores of the resin and be retained. The weaker the acid (i.e. the higher the pK of the acid) the longer it will be retained.

This type of column will separate a range of weak aliphatic carboxylic acids from C_1 to C_7, with both straight and branched chains, as well as a variety of weak inorganic anions such as fluoride, borate and carbonate. Due to the weak nature of the mechanism, column efficiencies are poor; but most of the common organic acids of interest are separated (Chromatogram 6.1). Elevated temperatures of up to 80°C are sometimes used to improve separation. All the strong anions come out in the void volume.

A variant of the conventional ion exclusion column has two functional groups bound to the resin. In addition to sulphonate groups, there are also carboxylic groups which retain hydroxy acids longer by a hydrogen bonding mechanism. Thus citrate, which is a strong tribasic anion and unretained on a convential exclusion column, shows good retention on this column. (Chromatogram 6.2).

Higher fatty acids (C_4 to C_{18}) can be separated on a reverse phase type column in an ion suppression mode, using an acetonitrile gradient (Chromatogram 6.3).

In all cases conductivity is the detector of choice since most organic acids have very little UV absorbance, and refractive index detectors are not sufficiently sensitive at low concentrations.

6.2 General Principles and Methodology

The separating column is usually an HPLC grade sulphonated cation exchange cross linked resin. The negatively charged resin repels anions such as chloride, nitrate and sulphate which are not retained and elute together. Weak acids are only partially ionised. The undissociated acid will enter the resin, interact with it and be retained on the column. Separations can be effected with water as eluent, but the stronger the acidity of the eluent the less dissociated is the weak acid and the more it is retained. Hence the eluent is usually a dilute solution (0.5 – 10 mM) of an acid such as hydrochloric acid, sulphuric acid, octane or hexanesulphonic acid, or tridecafluoroheptanoic acid. The latter acid helps keep the conductivity background low when using direct conductivity detection (see Tables 6.2 and 6.3).

When direct conductivity detection is used, sensitivity and linearity are dependent on the pKa of the analyte. Sensitivity drops off markedly from formic acid, which is a relatively strong acid, through the series of less dissociated acids. The more concentrated the eluent, the less dissociated and less sensitive the analytes become and the higher the background conductivity will be.

Two types of suppression are used in ion-exclusion chromatography. In the first which is only applicable to hydrochloric acid eluents, a cation exchange resin in the silver form removes the chloride:

$$\text{resin} - SO_3Ag + HCl \rightarrow \text{resin} - SO_3H + AgCl$$

resulting in a very low conductivity background and the prevention of loss of sensitivity due to the suppression of ionisation of the analyte. Sensitivity still drops off with analyte pKa and the linearity is poor at higher concentrations. The resultant silver chloride partially blocks the front of the suppressor column increasing the pressure drop. Eventually the blocked part of the column must be removed.

The second type of suppression involves the use of a cation exchange membrane continuously regenerated with sodium, potassium, tetra-alkylammonium or ammonium hydroxides, which convert both the eluent and analyte to salts. The salts of weak acids are dissociated in solution and thus have a greater conductivity and hence greater sensitivity than the free acids. The molar sensitivity of the carboxylate ions is also almost independent of pKa, whilst the background conductivity is reduced because hydrogen ions have a much higher molar conductivity than the cations which replace them.

6.3 Performance

Table 6.1 and Chromatogram 6.4 give typical relative standard deviations for suppressed conductivity detection for a range of acids. The detection limits under the conditions given in table 6.1 are usually in the range 0.02–0.2 mg L^{-1} for a 50 μL sample loop. Typical chromatograms are given in Chromatograms 6.1, 6.2, 6.3 and 6.4.

A number of co-elutions can occur. Some systems (eg Chromatogram 6.1) will not separate propionate and carbonate. This is particularly troublesome, as many aqueous samples contain carbonate. However, up to 50 mg L^{-1} of carbonate is hardly detected by direct or silver suppressed conductivity detection, while acidification and vacuum degassing can be used to remove carbonate if micromembrane suppression is used. Some systems (e.g. that for Chromatogram 6.1) allow propionate to be separated from small quantities of carbonate, but lactate and succinate are often poorly resolved. Separation can be improved by adding up to 5% of propan-1-ol to the eluent and/or raising the temperature to 50°C.

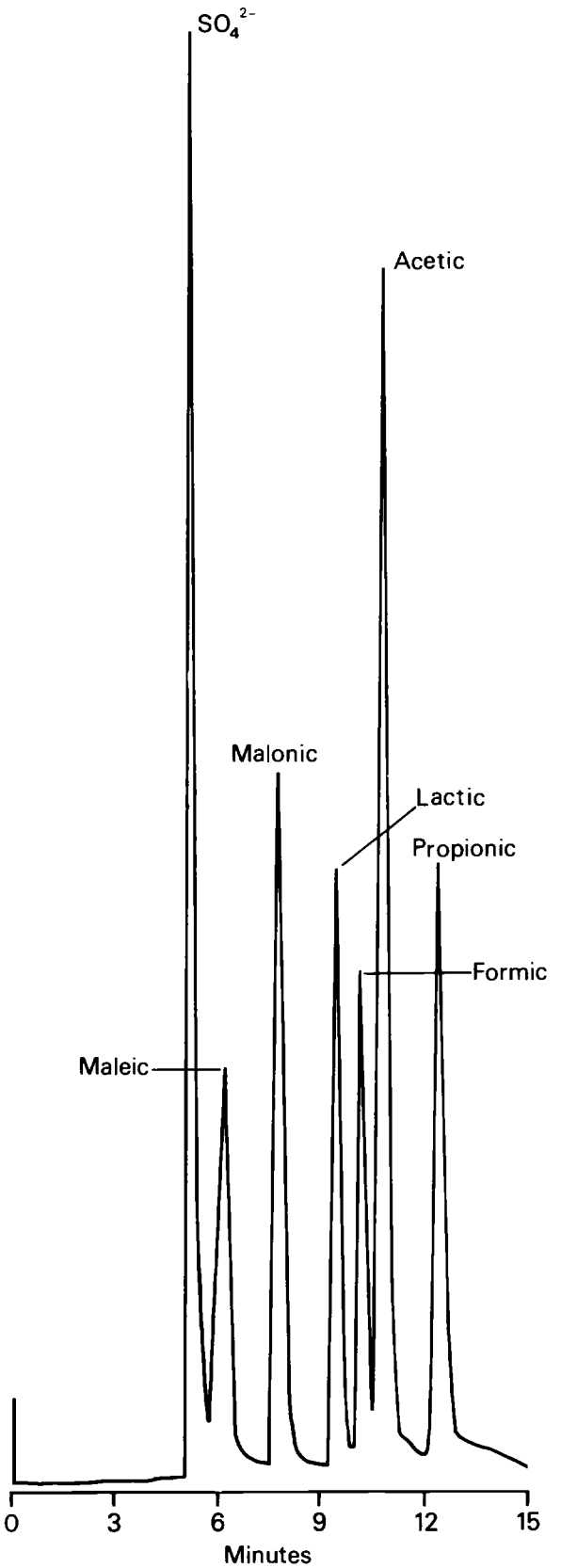
Tests have also shown that double and triple bonded organic acids have relatively short retention times, compared with the corresponding saturated acids. As they elute close together, their separation is more difficult. Phosphonic acids and nitrites elute in the same region. Some peaks such as those of carbonic and butanoic acids, tend to be broad. This too can cause problems.

Substituted acids, such as hydroxy acids, can sometimes be separated by the use of resins containing more than one type of active site. Thus resins having both sulphonic and carboxylic acid groups have been used for separating hydroxy-carboxylic acids (see section 6.1 above).

6.4 References

1. Rocklin, R.D. et al., 'The Separation and Detection of Carboxylic Acids by Ion Chromatography' *J. Liquid Chrom.*, **9** (4), 757–75. (1986).
2. Wheaton, R.M. and Bauman, W.C., *Ind. Eng. Chem. (Anal. Edn.)*, **45,** 228. (1953).
3. Harlow, G.A. and Morman, D.H., *Anal. Chem.*,**36,** 2438. (1964).
4. Goodman, G.W., Lewis, B.C. and Taylor, A.F., *Talanta,* **16,** 807. (1969).

Chromatogram 6.1

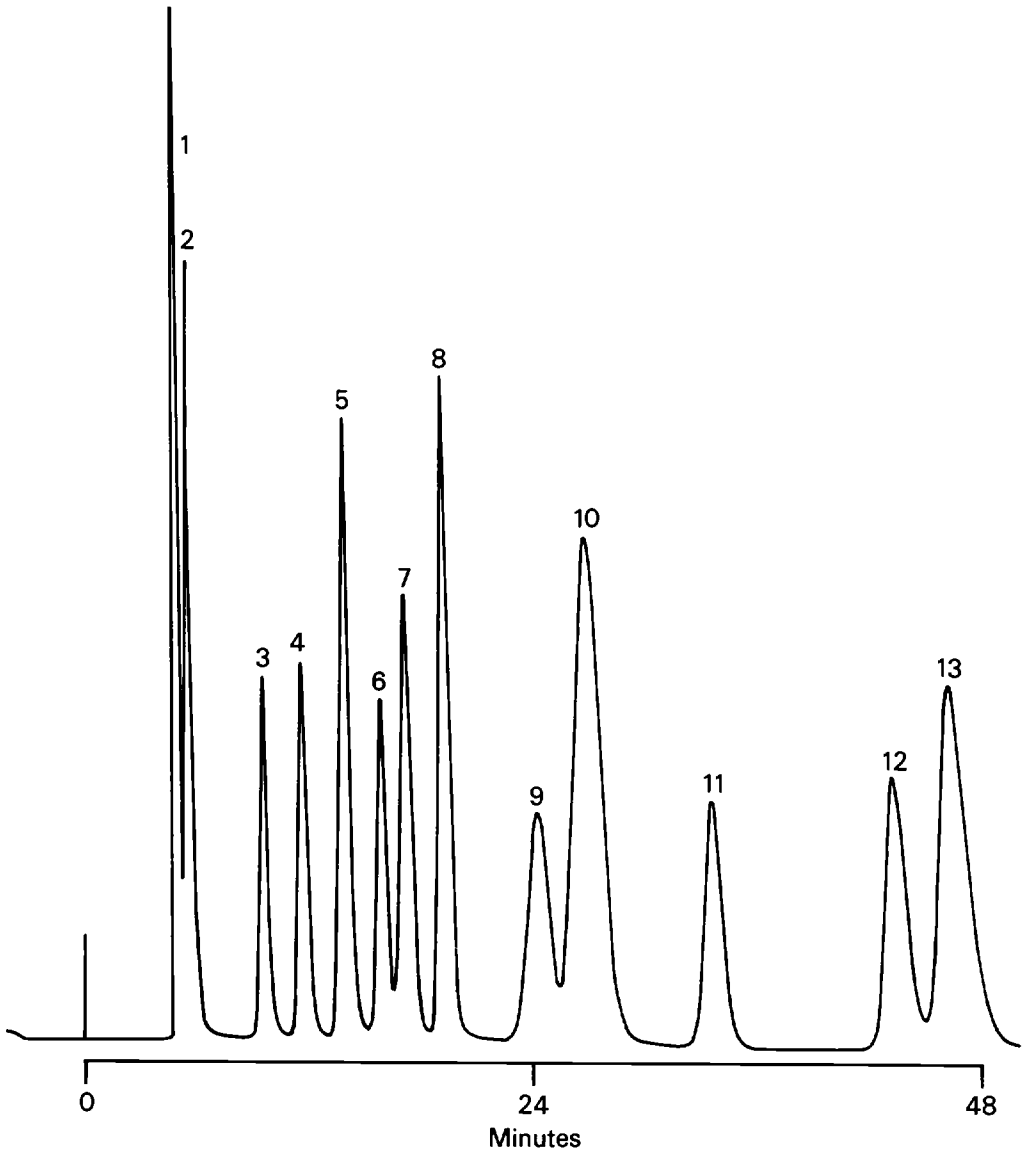


500ng SO_4^{2-}
500ng maleic
500ng malonic
1000ng lactic
1000ng formic
1000ng acetic
1000ng propionic

Column: Ion exclusion–AS1
Eluent: Octane sulphonic acid
Flow rate: 1mL min^{-1}
Detector: Conductivity

1μS

Chromatogram 6.2



Column: AS5

Eluent: 1.6mM perflurobutyric acid
pH = 2.8
0.3mL $min.^{-1}$

Suppressor: AMMS–ICE

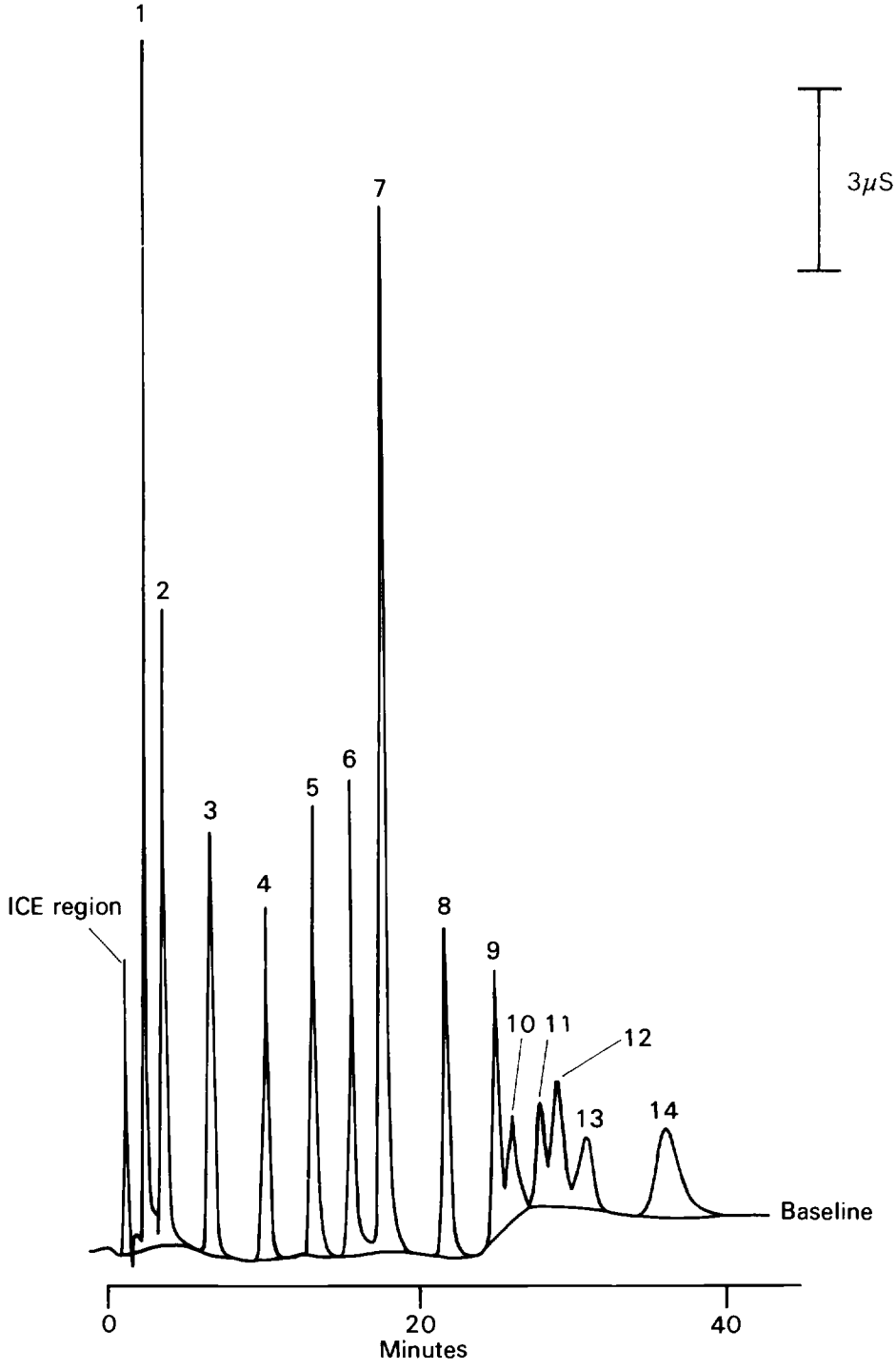
Regenerant: 5mM TBAOH 2mL min^{-1}

Detector: Conductivity 30mS

1. Chloride
2. Oxalate
3. Pyruvate
4. Tartrate
5. Malonate
6. Lactate
7. Malate
8. Acetate
9. Isocitrate
10. Citrate
11. β–Hydroxy–n–butyrate
12. Succinate
13. Propionate

Chromatogram 6.3

Ion suppressed gradient separation of the homologous series from butyric to stearic acids, including some unsaturated fatty acids

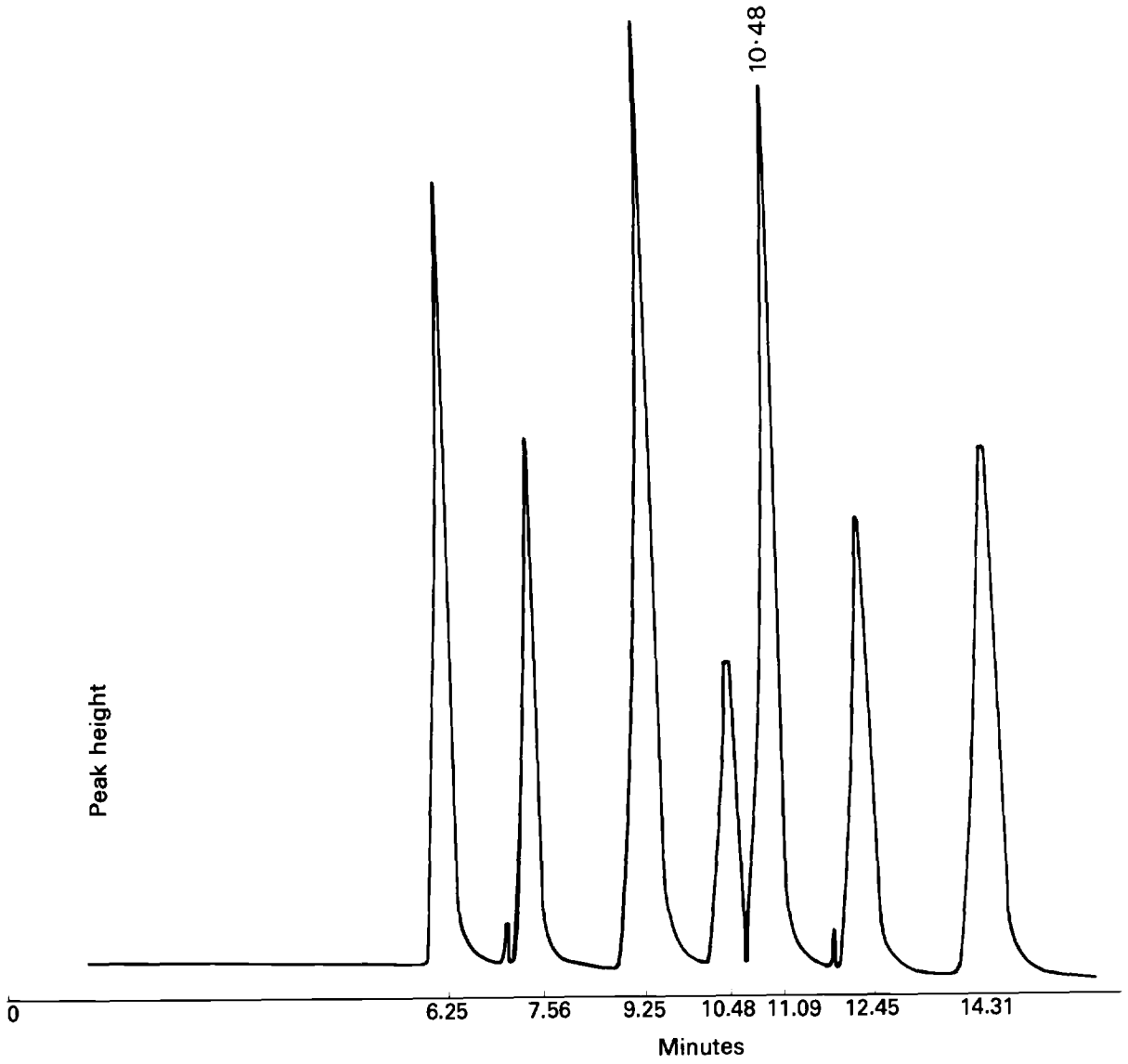


Peaks: "ICE Region" acids more hydrophilic than butyric acid;

1. 100mg L^{-1} butyric
2. 100mg L^{-1} valeric
3. 100mg L^{-1} caproic
4. 100mg L^{-1} oenanthic
5. 100mg L^{-1} caprylic
6. 175mg L^{-1} pelargonic
7. 200mg L^{-1} capric
8. 250mg L^{-1} lauric
9. 250mg L^{-1} myristic
10. 250mg L^{-1} linolenic
11. 250mg L^{-1} linoleic
12. 375mg L^{-1} palmitic
13. 375mg L^{-1} oleic
14. 500mg L^{-1} stearic

Chromatographic conditions: Column, MPIC–NSI, 20cm x 0.4cm I.D.
Eluent A: 24% acetonitrile 6% methanol in 0.03mM HCl.
Eluent B: 60% acetonitrile 24% methanol in 0.05mM HCl
Flow-rate: 1.0ml min^{-1} Detection: Electrical conductivity, 30 μS fsd
Chart speed, 0.25cm min^{-1} Injection volume, 50μL
Temperature, 42°C; gradient profile as indicated below chromatogram

Chromatogram 6·4



Acid	mg L^{-1}	RT	AVERAGE FOUND	REL SD%
Oxalic	50.646	6.25	49.878	0.646
Tartaric	49.731	7.56	49.786	0.327
Fluoride	25.438	9.25	25.335	0.653
Lactic	50.388	10.48	49.723	0.387
Formic	49.759	11.09	49.767	0.332
Acetic	51.131	12.45	49.846	0.796
Propionic	99.117	14.31	99.587	0.465

Table 6.1 Typical Conditions for the Analysis of Organic Acids and Other Weak Acids

Conditions:

Column	= Dionex IONPAC – ICE – ASI
Eluent	= 1 mM Octanesulphonic Acid
Flow	= 1 ml min^{-1}
Sample Size	= 50 μL
Detector	= Suppressed conductivity
Regenerant	= 10 mM Tetrabutyl Ammonium Hydroxide 2 ml min^{-1}
Temperature	= Ambient

NAME	RT min	K T_0=4.5	Mean Limit of Detection mgL^{-1}	Upper Limit of Calibration mgL^{-1}	RSD% (n=20)
Oxalate	4.5	0.00	0.04	500	0.65
Maleic	5.0	0.11	0.02	100	
Pyruvate	5.25	0.17	0.15	100	
Citrate	5.7	0.27	0.025	100	
Tartrate	5.9	0.31	0.15	250	0.33
Malonate	6.0	0.33	0.10	100	
Malate	6.9	0.53	0.10	250	
Fluoride	7.7	0.71	0.02	100	0.65
Borate	8.6	0.91	0.20	250	
Lactate	8.8	0.96	0.10	500	0.39
Succinate	9.1	1.02	0.10	500	
Formate	9.3	1.07	0.04	100	0.33
Nitrilotriacetate	9.5	1.11	0.10	50	
Acetate	10.7	1.38	0.10	250	0.80
Propionate	12.5	1.78	0.10	500	0.47
Carbonate	12.5	1.78	0.25	250	

Table 6.2 A comparison of elution data obtained from a range of acids on both the Dionex AS1 and AS3 columns using the recommended eluents of TDFHA and HCl both at 0.001M.

		Retention Times Relative to Formic Acid	
Acid	**pKa**	**Retention volume ASI Eluent TDFHA**	**Retention volume AS3 HCl**
Sulphuric	1.92	0.58	0.58
Hydrochloric		0.58	*
Nitric		0.58	0.58
Oxalic	1.23	0.59	–
Thiosulphuric	1.70	–	0.60
Phosphoric	2.12	0.60	0.61
Pyruvic	2.49	0.65	0.63
Tartaric	2.98	0.67	0.72
Citric	3.08	0.69	0.71
Nitrous	3.37	0.75	0.76
Malic	3.40	0.75	0.83
Hydrofluoric	3.45	0.82	0.83
Lactic	3.86	0.91	0.97
Formic	3.75	1.00	1.00
Acetic	4.75	1.12	1.13
Propanoic	4.87	1.29	1.28
Butanoic	4.81	1.56	1.48
Carbonic	6.37	1.37	1.66
Pentanoic	4.82	2.26	2.00

– not determined
* chloride absorbed on silver form resin suppressor

1. Strong mineral acids like sulphuric, hydrochloric and nitric elute early and cannot be separated from each other on the basis of retention time by the ICE technique.
2. The weaker mineral acids like nitrous and hydrofluoric acids are well separated on both columns and ICE can be used for their determination.
3. Carbonate, fluoride and the short chain monocarboxylic acids are well separated from each other. This should enable such mixture to be determined by ICE thus solving the problem set out in the introduction when ion exchange chromatography is used.
4. In homologous series like formic through to pentanoic acid, the elution order depends on the size of the component anions.

Table 6.3 Effect of Hydrochloric Acid Strength and Elution Rate

Eluent	**Retention Time Min**						
	Flow mL min^{-1}	SO_4^{2-}	F^-	Formate	Acetate	Propan-oate	Pentan-oate
0.5mM HCl	1.0	8.42	11.49	14.95	18.41	21.09	34.28
1.0mM HCl	1.0	9.55	14.26	17.75	20.47	23.28	38.40
1.0mM HCl	1.1	8.62	12.84	15.98	18.34	20.90	33.90
1.0mM HCl	1.5	6.12	9.00	11.24	12.97	14.70	23.85
1.0mM HCl	2.0	4.48	6.63	8.23	9.51	10.83	17.50

PART III Cation and Transition Metal Methods

1. Common Cations – General Methods

(Sodium, Ammonium, Potassium, Calcium and Magnesium, etc in Water)

1.1 Performance Characteristics of the Method

1.1.1	Substances determined	Sodium, ammonium (as NH_4^+), potassium, calcium and magnesium. The methods described can be extended to incorporate lithium, other alkali and alkaline earth metals and some amines.*.
1.1.2	Type of Sample	Slightly contaminated waters such as drinking water, rain water, ground water, and surface water.
1.1.3	Basis of the Method	Chromatography of and detection of ions.
1.1.4	Concentration Ranges	Sodium : 0.01 – 20 mgL^{-1} Ammonium 0.03 – 10 mgL^{-1} Potassium : 0.03 – 10 mgL^{-1} Magnesium 0.02 – 10 mgL^{-1} Calcium : 0.2 – 30 mgL^{-1} note: The range of application may be changed in particular cases by varying the working conditions (e.g. sample volume, dilution, detectors, separating columns etc).
1.1.5	Calibration Curve	May deviate slightly from linearity.
1.1.6	Limit of Detection	0.01 mgL^{-1} or better depending upon the cation and conditions used.
1.1.7	Time required for analysis	Depends on the chromatographic analysis column. Sample run time typically 3–12 mins.

*See 1.12

1.2 Principle of the method

The ions are separated on an analytical column. A low capacity cation exchange material normally serves as the stationary phase. The eluent is usually an aqueous solution of a strong acid; for some applications this solution is modified by the addition of other substances, usually organic compounds.

The method described in this chapter for the analysis of divalent cations involves the use of an eluent which is strongly retained on the guard and separator columns. This makes the subsequent analysis of monovalent cations after an analysis for divalent ions impractical on the same column. Two sets of columns are therefore necessary, one dedicated to each analysis. Methods for the analysis of monovalent and divalent cations simultaneously, using column switching or gradient elution have been developed; details of some of these are given at the end of the chapter.

The ions are detected and quantified after separation. Examples of chromatograms for detection systems used in cation analysis are given in Chromatograms 1.1 to 1.3.

These detection systems are:

Direct conductivity detection (Chromatograms 1.1 (a-e))

Suppressed conductivity detection (Chromatograms 1.2 (a-f))

A suppressor column or membrane, placed after the separator column, continuously converts the eluent to a solution of lower conductivity by an ion exchange process.

Inverse UV and Novel detection systems (Chromatograms 1.3 (a-e))

1.3 Interferences

Interferences may be caused by ions with retention times similar to those of the analyte, thus resulting in peak overlap. This type of interference is rare for the cations covered in this chapter. Unresolved peaks will also result if the concentration of one sample component is 10 to 20 times higher than that of the component of the adjacent peak. Decreasing the eluent concentration or flow rate may help to ameliorate these problems.

When analysing for monovalent cations with a dilute solution of hydrochloric acid as the eluent any divalent cations will accumulate on the separator column. This will cause an increasing loss of retention and resolution. When this occurs the column must be cleaned with stronger eluent, for example 1 M HCl at approximately 1 mL min^{-1} for 15 minutes.* The system must then be allowed to equilibriate with eluent until a stable baseline is obtained.

Contaminants, such as solid material, organic compounds (e.g. detergents, mineral oils and humic acids) and transition metals will also cause a deterioration of column performance. They should therefore be removed prior to analysis.

Further information on column care can be obtained from the manufacturers.

1.4 Standard Deviation

The precision of the method is generally good, even at comparatively low concentrations. Precision data has been collected using rainwater samples and synthetic rainwaters. Some results, obtained using different columns, different eluents and at various concentration levels, are shown in Tables 1.1 to 1.3 below. More modern columns may achieve even better statistics.

Table 1.1 Single-operator precision and bias for cations in a synthetic rainwater

Cation	Given Concentration mg L^{-1}	Measured Concentration mg L^{-1}	Number of Replicates n	Bias		Precision	
				mg L^{-1}	%	s_w mg L^{-1}	RSD %
Sodium	0.082	0.090	7	0.008	9.8	0.009	10.0
	0.465	0.454	7	−0.011	−2.4	0.019	4.2
Ammonium	0.063	0.067	7	0.004	6.4	0.011	16.4
	0.400	0.400	7	0.000	0.0	0.032	8.0
Potassium	0.021	0.024	7	0.003	14.3	0.004	16.7
	0.098	0.098	7	0.000	0.0	0.005	5.1
Magnesium	0.018	0.026	7	0.008	44.4	0.008	30.8
	0.084	0.085	7	0.001	1.2	0.018	21.2
Calcium	0.053	0.058	7	0.005	9.4	0.006	10.3
	0.406	0.405	7	−0.001	−0.2	0.045	11.1

Table 1.2 Single-operator precision and bias for cations in a spiked rainwater sample

Analyte	Amount added mg L^{-1}	Number of replicates	Mean % recovery	Mean bias mg L^{-1}	Standard deviation mg L^{-1}	Statistically significant bias (95%)
Sodium	0.108	10	95.3	−0.001	0.010	No
	0.273	9	94.4	−0.015	0.010	No
Ammonium	0.188	10	113.8	0.026	0.030	No
	0.473	9	107.5	0.025	0.025	No
Potassium	0.014	8	157.1	0.008	0.009	No
	0.034	8	132.4	0.011	0.016	No
Magnesium	0.018	9	89.5	−0.002	0.004	No
	0.044	9	92.3	−0.003	0.002	No
Calcium	0.079	10	93.9	−0.005	0.008	No
	0.199	10	97.1	−0.008	0.014	No

Concentrations are significant to two decimal places.

* For stainless steel columns use of nitric acid is recommended.

Chromatographic Conditions for Tables 1.1 and 1.2
(supplied by British Gas plc)

Monovalent cations	Divalent cations
Eluent 5mM HCl	Eluent 1.5 mM HCl/1.5 mM mPDA
Flow rate 2.3 mL.min^{-1}	Flow rate 2.3 mL.min^{-1}
Guard Column	Dionex IONPAC CG1
Separator Column	Dionex IONPAC CS1
Fiber Suppressor	Dionex CFS
Regenerant	40mM TMAOH
Flow Rate	3mL.min^{-1}
Detector	6 μl conductivity cell
Detector sensitivity	meter 0–1000 μS.cm^{-1}
Sample loop	100 μL

Table 1.3 Precision data for monovalent cations in synthetic rainwater

(data collected at the rate of one analysis on each day the instrument was used, over a 3 month period)

Cation	Theoretical Concentration mgL^{-1}	Measured Concentration mgL^{-1}	Number of Replicates	Precision σ(n−1) mgL^{-1}	Precision RSD %
Sodium	2.06	2.06	25	0.046	2.3
Ammonium*	0.65	0.70	25	0.063	9.0
Potassium	0.66	0.64	25	0.035	5.5

* Reported as N

Analysis conditions

Eluent	8.5 mM HCl
Flow Rate	2 mL min^{-1}
Guard Column	Dionex IONPAC CG2
Separator Column	Dionex IONPAC CS2
Micromembrane Suppressor	Dionex CMMS
Regenerant	40 mM TBAOH
Flow Rate	4 mL min^{-1}
Sample loop	200 μL

(data supplied by DTI, Warren Spring Laboratory)

1.5 Apparatus

1.5.1 Chromatographic System

The system must be capable of detecting cations in the concentration ranges given in section 1.1.4 and of separating them to the standard given in section 1.7. To determine mono- and divalent cations simultaneously a dual channel chromatograph may be necessary. This will be equipped with 2 separating columns and 2 detectors.

Eluent Reservoir
Containers should, ideally, be designed to minimize introduction of air or be capable of being degassed.

Eluent Pump
Must be capable of delivering a low pulsing reproducible flow

Sample Injection System
Typically a loop of 50 to 200 μL.

Pre-column (metal free)
A column, usually containing a chelating ion exchange resin, which is placed between the pump and the injection system. This removes low levels of cations from the eluent thus preventing the exchange sites being permanently occupied by transition metals.

Pre-column (or guard column)

A short column placed before the separator column, and usually containing the same resin. Its purpose is to protect the separator column from being fouled by particulates and firmly binding organic constituents from the sample matrix.

Separator Column of the required performance.

Detector

either
Conductivity Detector
In the case of suppressed ion chromatography this is preceded by a membrane type suppressor (see Chromatograms 1.2 (a-f)).

or
Variable wavelength UV detector (see Chromatograms 1.3 (a-e)).

Recording Device and/or Data Processor

1.5.2 Other Apparatus

Drying oven

Desiccator

Membrane filtration apparatus with membrane filters, pore size 0.45 μm

Normal Laboratory glassware

1.6 Reagents

Reagents of recognised analytical grade must be used. The water must have an electrical conductivity of <0.1 μS cm^{-1} and must be free of particulate matter >0.2 μm.

1.6.1 Eluents

Different eluents are used, their choice depending on the separator column, analytes and detection mode. For some systems it may be necessary to degas the eluent. In order to prevent algal growth eluents should be stored in the dark and may need to be renewed every 2 to 3 days.

Consult the column manufacturer's manual for the exact composition and treatment of eluents. See Chromatograms 1.1 and 1.3 for examples of eluents.

Eluent concentrate and eluent preparation

Eluents can be prepared and stored, for convenience, in concentrated form. The concentrate will remain stable for several months if kept cool. It can be diluted as required to make the eluent.

1.6.2 Suppressor regenerant

Usually a solution of an hydroxide with a large cation. Examples are tetramethyl- or tetrabutyl-ammonium hydroxide and concentrations are normally higher than those of the eluent and depend on columns used – consult manufacturers guidelines for details.

1.6.3 Cation Stock solutions

Stock solutions 1000 mg L^{-1} NH_4^+, Ca^{2+}, Mg^{2+}, K^+, Na^+:

For each stock standard dry the salts in the table below for 1 hour at 105°C. Allow to cool for 45 minutes in a desiccator. Weigh the quantity given in the table into a small beaker and dissolve in a little deionised water. Transfer quantitatively to a 1L standard flask and make up to volume with water. These solutions are stable for several months if kept cool.

Cation	Substance	Weighed (g)
Ammonium	NH_4Cl	2.9654±0.0002
Potassium	KCl	1.9067±0.0002
Sodium	NaCl	2.5420±0.0002

Magnesium Standard – Dissolve 1.000±0.0002g magnesium ribbon in a minimal volume of 6M HCl and dilute to 1 L.

Calcium standard – Dry 2.5 to 3 g of calcium carbonate at 180°C for one hour, and cool for 45 mins. Weigh out 2.4973±0.0002 g into a beaker. Add 600±50 ml of water, then slowly add concentrated hydrochloric acid dropwise until all the solid has dissolved. Transfer quantitatively to a 1 litre calibrated flask and make up to the mark with water.

Alternatively, commercial stock standards may be used if of adequate quality. (See Part I Section 4.2).

Standard Solution Storage.

High density polyethylene bottles have generally proved suitable for storage of standard solutions. Storage life and bottle suitability can be determined by practical tests.

To avoid cross contamination, the same vessels must be reserved for the same cations and concentrations.

Standard Solutions

According to requirements, mixed standard solutions of different cation concentrations and composition are made from the stock solutions. There will normally be separate mono- and divalent solutions prepared.

Mixed standard stock solutions

Mixed stock solutions must be prepared with reference to the levels of analytes in the samples. Examples of the concentrations which may be used are:

Monovalent cations

Sodium	50 mgL^{-1}
Ammonium	50 mgL^{-1}
Potassium	100 mgL^{-1}

Divalent cations

Calcium	100 mgL^{-1}
Magnesium	100 mgL^{-1}

If kept cool these solutions will be stable for several weeks.

Cation Standard Solutions

Using volumetric glassware (Grade A) and the mixed standard stock solution, prepare at least 5 reference solutions of appropriate concentrations. Prepare these solutions daily, unless experience proves them to have greater stability.

1.6.4 Blank solution

Any sample pretreatment, e.g. acidification, must be duplicated with the blank solution.

1.7 Quality Requirements of the Separating Column

Ideally, the performance of the column will allow baseline resolved separation of all components. The peak resolution (R), as defined in Part I Chapter 2 will not be worse than 1.3 for concentrations in the region of 1 mg L^{-1} of each component. However, the separation of ammonium from potassium, particularly if one component is present in significant excess, is often less satisfactory. Analysts will have to decide, with reference to their particular samples, whether this is acceptable.

The calibration curve obtained when analysing a sample for ammonium ion is often non-linear. This is due to the change in degree of dissociation at higher concentrations. When using suppressed conductivity, a carbonate regenerant may be used in place of the hydroxide in order to increase the response to ammonium ion concentration. This does, however, lead to a decrease in the response to sodium and potassium.

1.8 Sampling and Sample Treatment

Sampling and sample pretreatment is described in Part I Chapter 4. However, when water samples are to be analysed for metals it is advisable to stabilize the sample by acidifying it, usually with the same acid as the eluent. It may prove necessary to acid wash sample bottles before use. If samples are to be analysed for both anions and cations, acidification can only be done if the samples are split.

It is particularly important that samples for analysis for ammonium ion be filtered as soon as possible after collection. The samples should then be stored at 4°C in the dark, in order to inhibit any microbial activity, and be analysed within seven days.

1.9 Procedure

Step	Procedure	Notes
1.9.1	Start the ion chromatograph in accordance with the instrument manufacturer's instructions. (note a).	a. The instrument is ready for operation as soon as a stable baseline at the expected conductivity or UV absorbance is achieved.
	Calibration	
1.9.2	Prepare reference and blank solutions as described in section 1.6.	
1.9.3	Inject the reference and the blank solutions.	
1.9.4	Plot a graph of peak height or area against concentration or calculate a polynomial regression. A smooth line should result which is either straight or slightly curved. (note b).	b. The cations are identified by comparing the retention times of the sample with those of the reference solutions. The retention times may be slightly concentration and matrix dependent.
1.9.5	Measure using the standard calibration procedure.	
1.9.6	Inject the pretreated sample (section 1.8) into the chromatograph. (note c).	c. If the ion concentration of the sample to be analysed exceeds the range of validity, the sample solution should be diluted. It may become necessary to establish a new calibration curve for a lower concentration range.
1.9.7	After each sample series, or at most after 10 to 20 measurements, two reference solutions of different concentrations in the lower and upper part of the working range should be measured in order to check the validity of the calibration curve. If need be perform a new calibration.	

1.10 Recent Developments

Since the methods described in this section were investigated, there have been several developments in the chromatography of ions. Some of these have enabled mono- and divalent cations to be determined in one chromatographic run. Brief details and sample chromatograms are given, but no performance data were available at the time of writing this booklet.

1.10.1 Gradient elution

The advent of the more efficient membrane suppressors has allowed the use of stronger eluents. These have made it possible to elute the monovalent cations with a relatively weak eluent and then to increase the eluent strength to elute the divalent cations. (The apparatus is similar to that used for gradient elution HPLC).

1.10.2 Column switching

A novel technique has been developed in which the divalent cations are separated on the precolumn only. The monvalent cations which pass through the precolumn first, are then switched onto a separator column and then returned to the precolumn. These ions then emerge at the end of the precolumn ahead of the divalent cations which are still coming through. This procedure requires an automated instrument and fine timing of the switching step.

1.11 Reporting of Results

Report results to no more than three significant digits. See Part I section 1.4 for more information on the expression of results and the information which must be given with the report of analysis.

1.12 The Determination of Alkyl Amines

Using an eluent concentration of 5 mM HCl on a Dionex IONPAC CS1 column and a flow rate of 2.3 mL min^{-1} the following amines have been determined using suppressed conductivity detection (1).

Cation	Retention time in min
Ammonium	15.0
Monomethylamine	20.3 (Potassium 19.0)
Monoethylamine	24.3
Dimethylamine	26.3
Trimethylamine	31.0
Diethylamine	34.3
Hydrazine	35.0
Tetramethylammonium	38.3
Triethylamine	43.0

Interferences are few using the above conditions because no other inorganic cations either elute or are compatible with suppressed conductivity detection, for example transition metals. The only known interferences are all organic amines, some of which elute close together, for instance the mono, di and tri ethanolamines all elute close to ammonia.

The detection limit is dependent on the overall retention, but is generally higher than for the alkali metals, but down to at least 0.1 mgL^{-1} should be achievable.

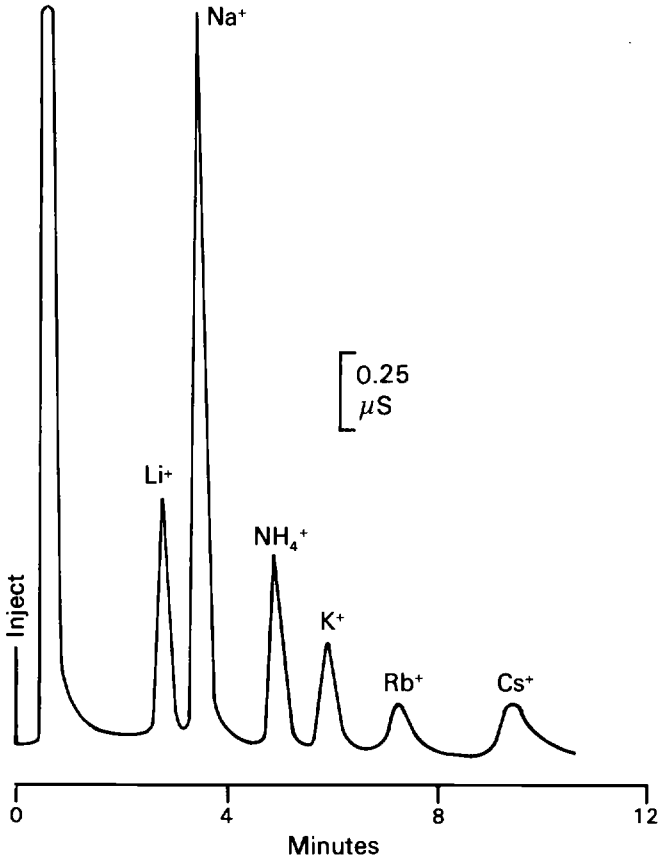
Over the years developments in column and suppression technology have allowed the use of stronger eluents and even the ability to use gradient elution. This in general has reduced analysis times, increased sensitivity and allowed the determination of a wider range of cations in a single run. Some examples are shown in Chromatograms 1.4 and 1.5.

Hydroxylamine is also of interest and can be determined under the above conditons, but using electrochemical detection with a platinum electrode set at 0.8 volts versus the standard calomed electrode. This discriminates it from ammonium ion as its retention time is similar to that of ammonia.

1.13 Reference

1. The Evaluation of a DIONEX ION CHROMATOGRAPH by R.A. Cochrane & D.E. Hillman. *Technical Paper No 890.* DGDQA/TS(MOD) Royal Arsenal, East Woolwich, London.

Chromatogram 1.1 Commercial columns – direct conductivity detection



(a)

Column type: Waters IC Pak C

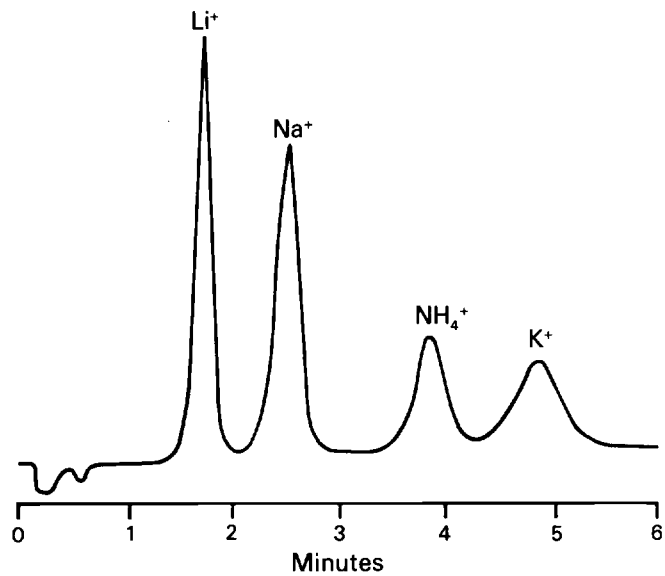
Eluent: 1mM picolinic acid pH 3.0

Flow rate: 1mL min^{-1}

Sample volume: 100μL

Concentrations mg L^{-1}: Lithium 0.14, sodium 0.92, ammonium 0.36, potassium 0.78, rubidium 1.7, caesium 2.7

Source: Millipore Waters



(b)

Column type: Wescan cation

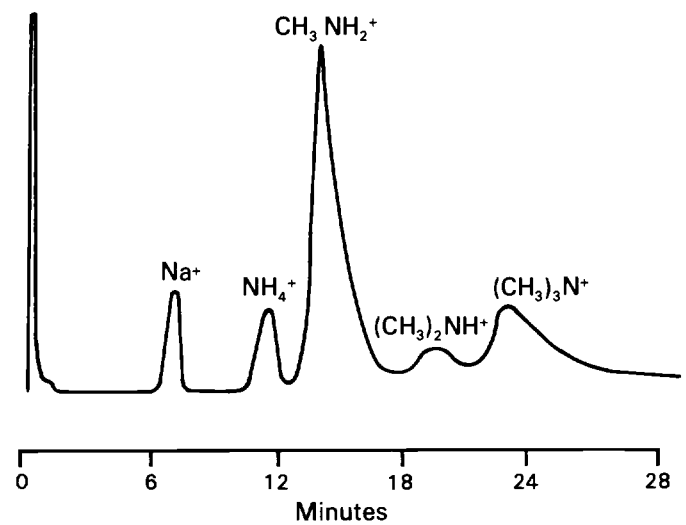
Eluent: 5mM HNO_3 pH 2.3

Flow rate: 2mL min^{-1}

Sample volume: 20μL

Concentrations mg L^{-1}: Lithium 5, sodium 10, ammonium 20, potassium 20

Source: Wescan



(c)

Column type: Wescan cation

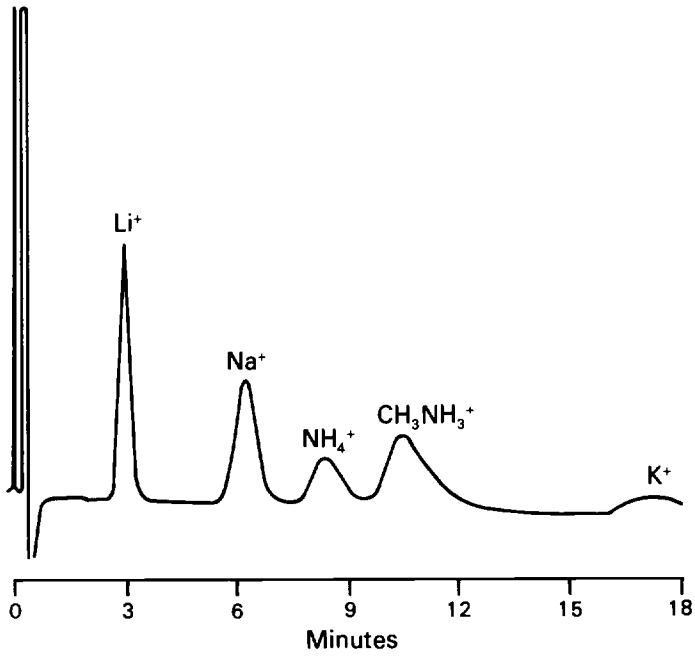
Eluent: 3.2mM HNO_3

Flow rate: 1.5mL min^{-1}

Sample volume: 20 μL

Concentrations mg L^{-1}: Sodium 1.6, potassium 6, methylamine, dimethylamine, trimethylamine

Source: Wescan



(d)

Column type: Wescan cation

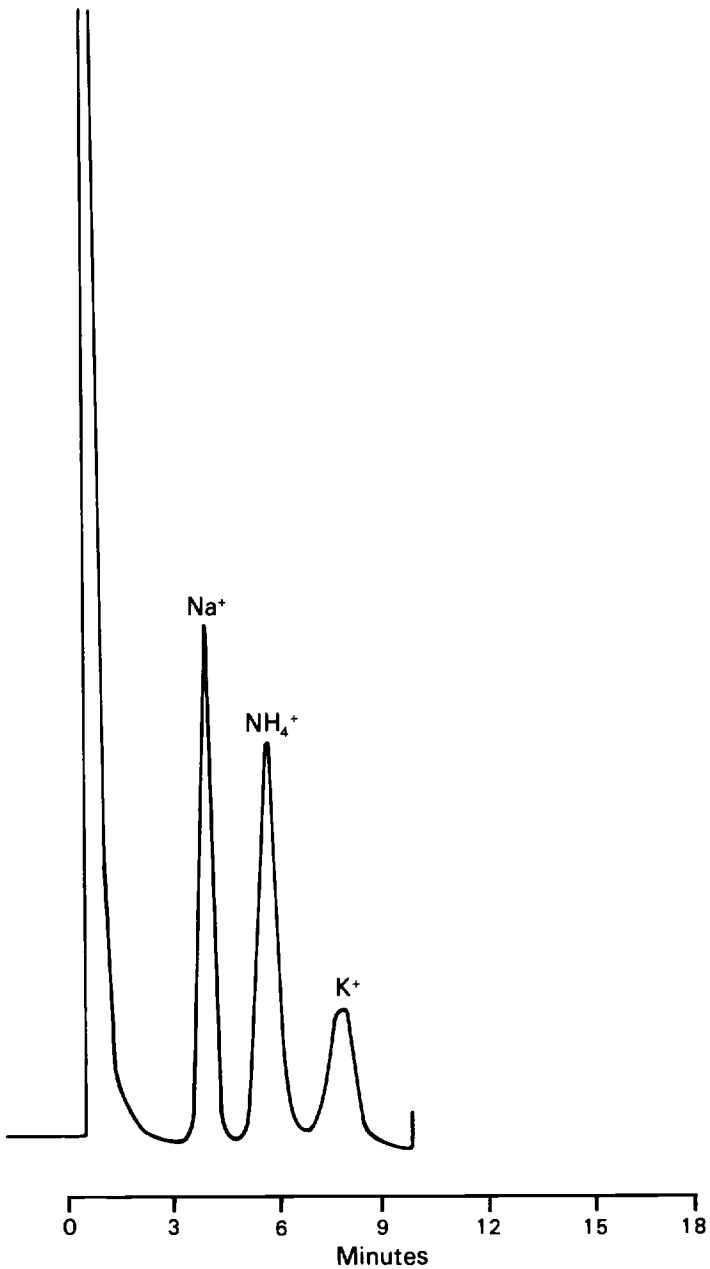
Eluent: 3mM HNO_3 in 40% MeOH

Flow rate: 2mL min^{-1}

Sample volume: 100μL

Concentrations mg L^{-1} Lithium 1.6, sodium 6, ammonium 3, methylamine 16, potassium 6

Source: Wescan



(e)

Column type: Benson A104

Eluent: 1.5mM HNO_3

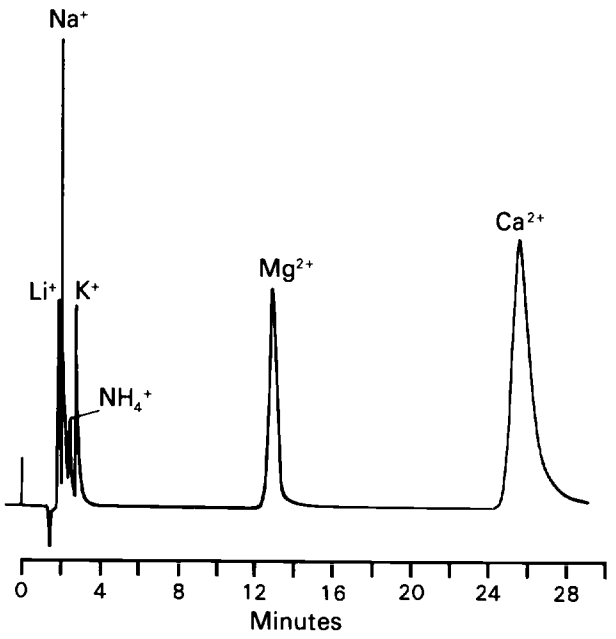
Flow rate: 1mL min^{-1}

Sample volume: 50μL

Concentrations mg L^{-1}: Potassium 60, ammonium 20, sodium 100

Source: Unknown

Chromatogram 1.2 Commercial columns – suppressed conductivity detection



(a)

Column type: Dionex HPIC CS3 (Isocratic)

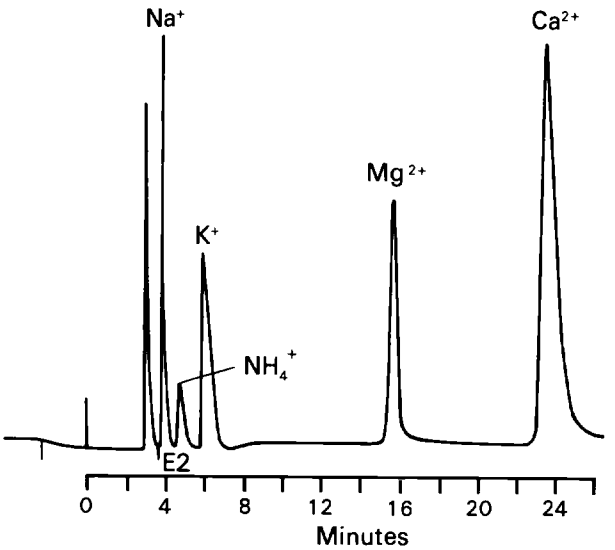
Eluent: 30mM HCl 3mM DAP 1.5mM $ZnCl_2$

Flow rate: 1mL min^{-1}

Sample volume 50μL

Concentrations mg L^{-1}: Lithium 0.4, ammonium 3, magnesium 10, sodium 3, potassium 3, calcium 15

Source: Dionex



(b)

Column type: Dionex HPIC CS3 (Step gradient)

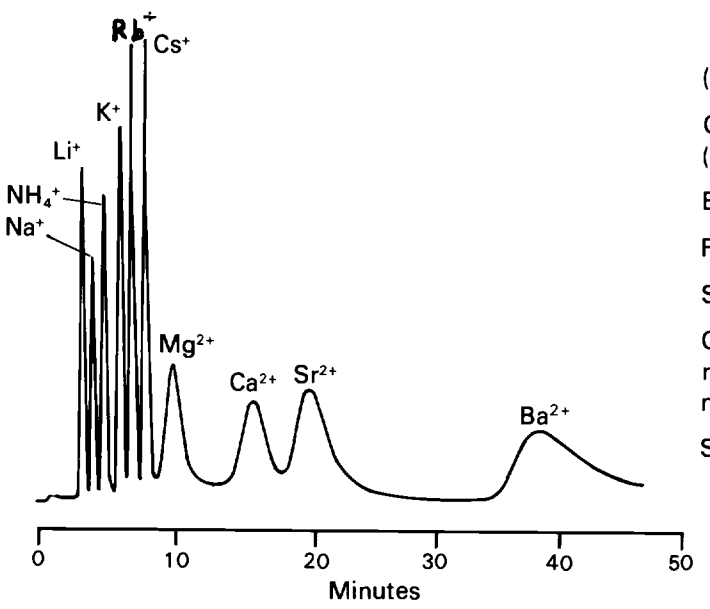
Eluent: E_1 10mM HCl 0.19mM DAP 0.09mM $ZnCl_2$
E_2 40mM HCl 3mM DAP 1.5mM $ZnCl_2$

Flow rate: E_1 1mL min^{-1}
E_2 1mL min^{-1}

Sample volume: 50μL.

Concentrations mg L^{-1}: Lithium 10, ammonium 100, magnesium 100, sodium 40, potassium 100, calcium 150

Source: Dionex



(c)

Column type: Dionex Fast Sep™ Cation I & II (Switched column)

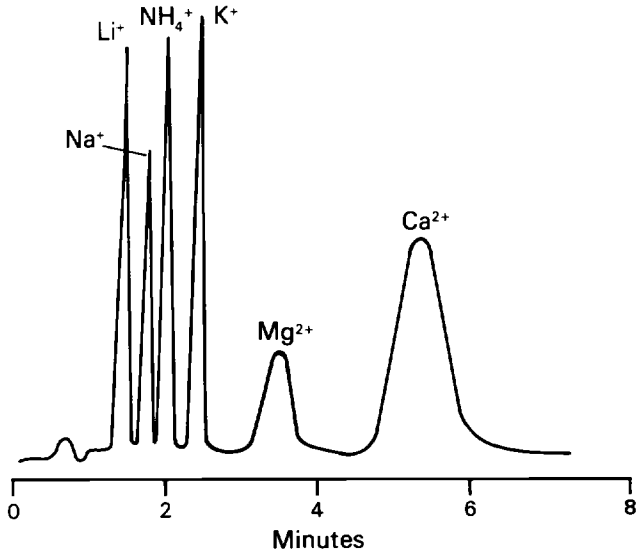
Eluent: 1.7mM HCl 0.255mM DAP HCl

Flow rate: 1mL min^{-1}

Sample volume 20μL

Contentrations mg L^{-1}: Lithium 0.8, ammonium 3, rubidium 20, sodium 2, potassium 6, caesium 30, magnesium 3, calcium 5, strontium 20, barium 50

Source: Dionex



(d)

Column type: Dionex Fast Sep™ Cation I (Isocratically)

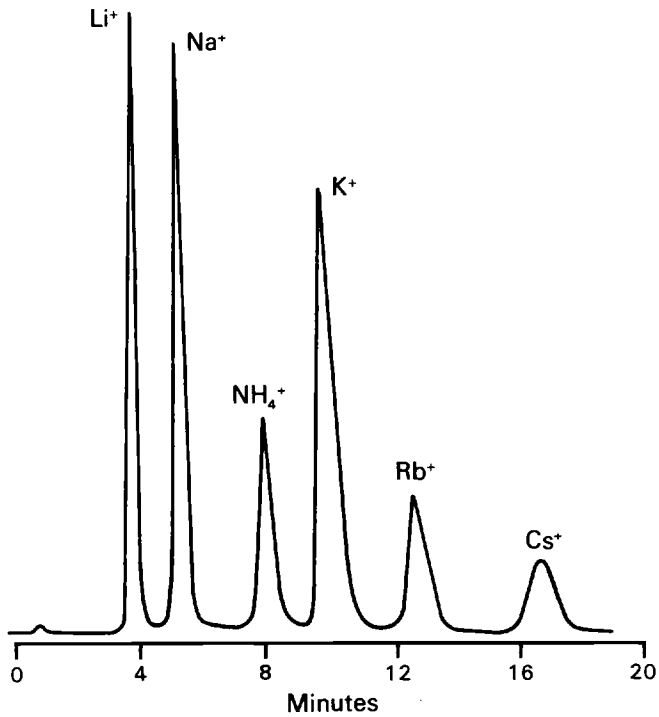
Eluent: 20mM HCl + 0.3mM DAP HCl

Flow rate: 1mL min^{-1}

Sample volume: 20μL

Concentrations mg L^{-1}: Lithium 0.8, sodium 2, ammonium 3, magnesium 3, potassium 6, calcium 5

Source: Dionex



(e)

Column type: Dionex HPIC CSI

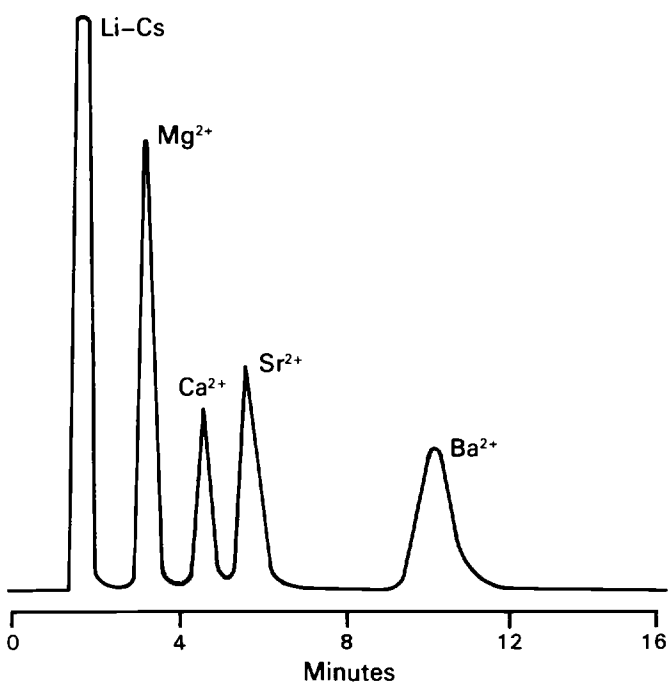
Eluent: 5mM HCl

Flow rate: 2.3 mL min^{-1}

Sample volume: 50μL

Concentrations mg L^{-1}: Lithium 5, sodium 5 ammonium 10, potassium 10, rubidium 20, caesium 30,

Source: Dionex



(f)

Column type: Dionex HPIC CSI

Eluent: 2mM m–phenylenediamine dihydrochloride
2mM HCl

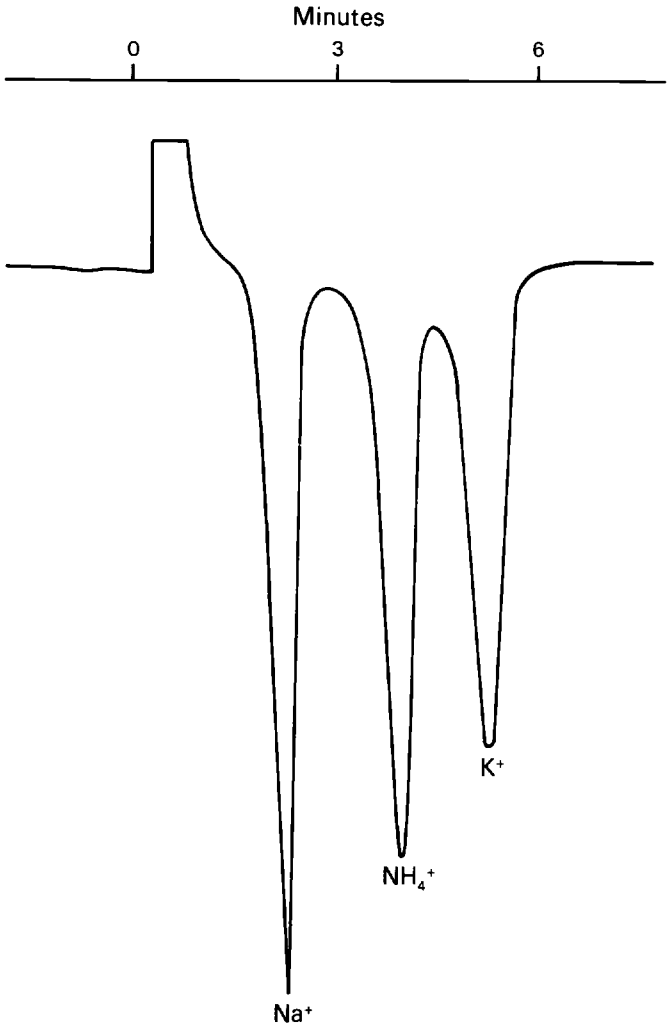
Flow rate: 2.3 mL min^{-1}

Sample volume: 50μL

Concentrations mg L^{-1}: Magnesium 3, strontium 10, calcium 3, barium 25

Source: Dionex

Chromatograms 1.3 Inverse UV and Novel detection systems



(a)

Column type: Dionex 50 Resin

Eluent: 0.2mM copper sulphate

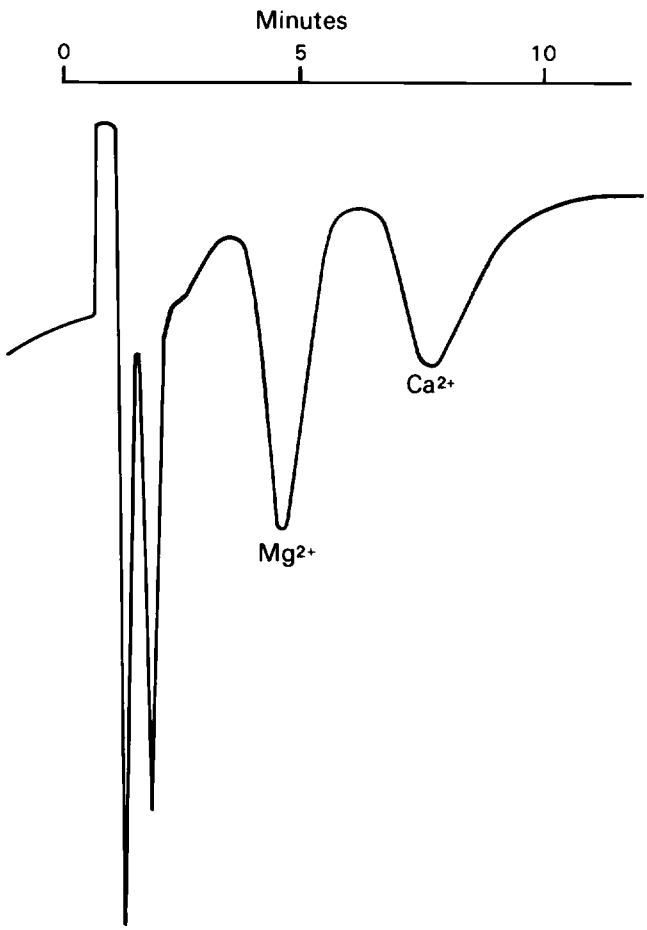
Flow rate: 0.7mL min^{-1}

Sample Volume: 200μL

Concentrations mg L^{-1}: 10mM sodium, ammonium, potassium

Detector: UV 252nm

Source: Small *et al* Anal. Chem. (1982) 54 462



(b)

Column type: Surface sulphonated styrene DVB

Eluent: 1.25mM copper sulphate

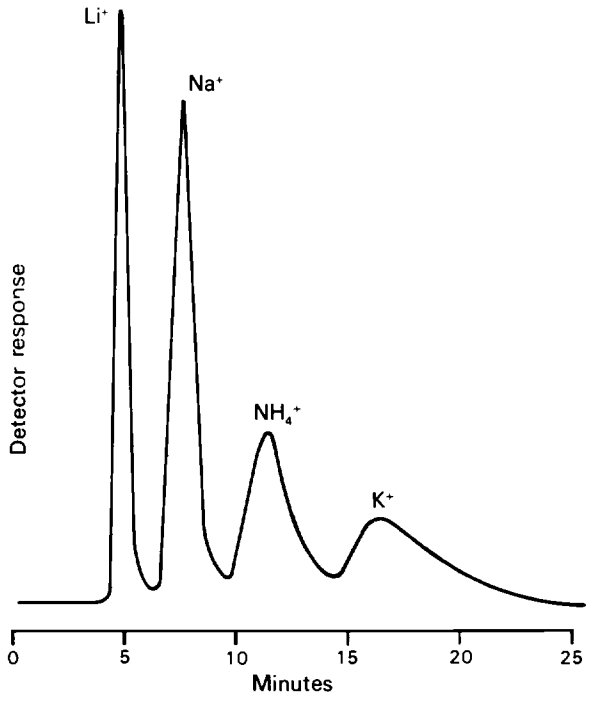
Flow rate: 1mL min^{-1}

Sample volume: 100μL

Concentrations: Sodium, potassium, magnesium, calcium (all equal)

Detector: UV 216nm

Source: Small et al Anal. Chem.(1982) 54 462



(c)

Column type: Wescan cation

Eluent: 5mM HNO_3 pH 2.3

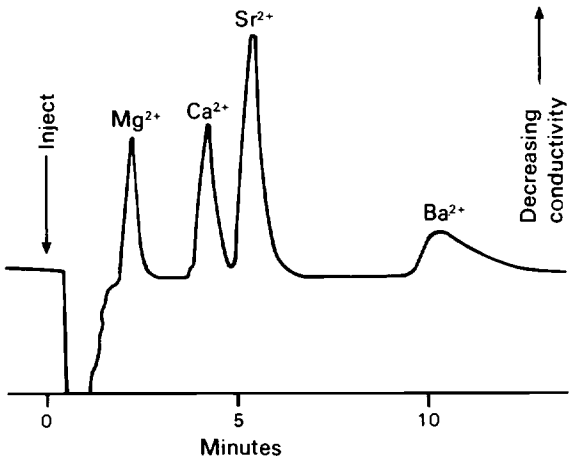
Flow rate: 2mL min^{-1}

Sample volume: 20μL

Concentrations mg L^{-1}: Lithium 7, sodium 23, ammomium 18, potassium 39

Detection: Emission at 670nm

Source: Wescan



(d)

Column type: Resin BN–X4

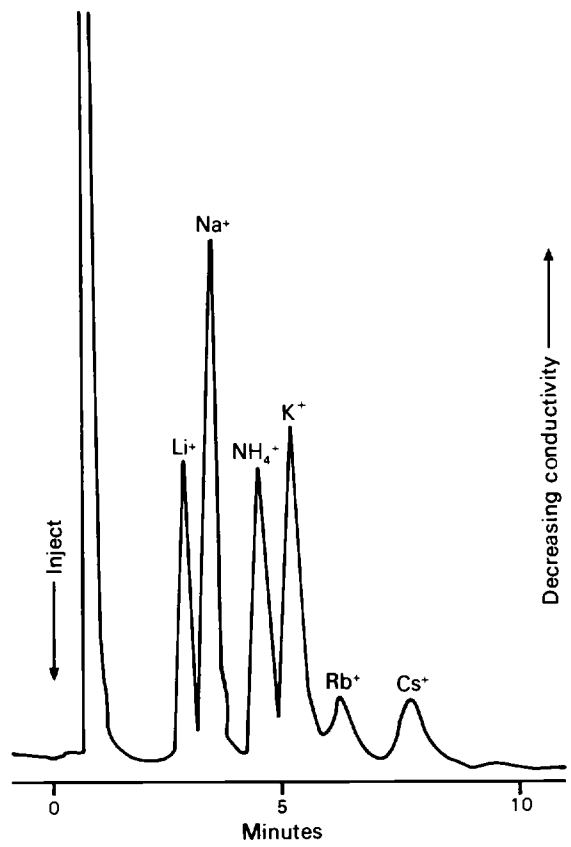
Eluent: 1mM ethylenediammonium nitrate pH = 6.1

Flow rate: 1mL min^{-1}

Sample volume 100μL

Concentrations mg L^{-1}: Magnesium 17.1, strontium 40.9, calcium 50.8, barium 40.9

Source: Fritz *et al* Anal. chem. (1980) 52 1519



(e)

Column type: BN–X4 blend 3.2 ratio neutral

Eluent: 1.25mM HNO_3

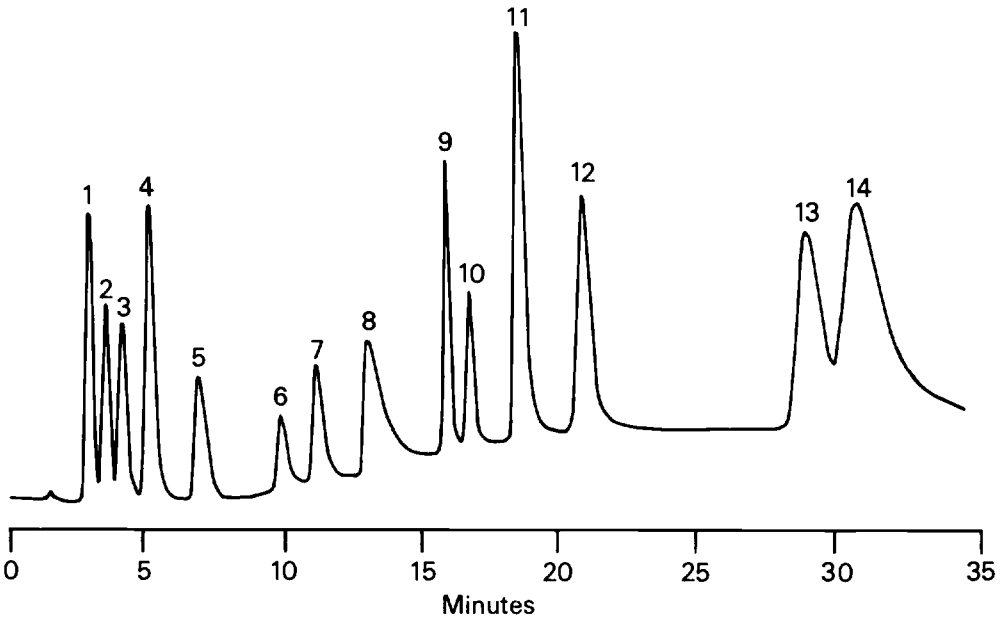
Flow rate: 1mL

Sample volume: 100μL

Concentrations mg/L: Lithium 0.09, ammonium 0.22 rubidium 0.47, sodium 0.26, potassium 0.31, caesium 0.86

Source: Fritz *et al* Anal. chem. (1980) 52 1519

Chromatogram 1.4 Gradient separation of inorganic and organic cations



Column: Fast sep cation 1

Eluent:
Flow rate: 1mL min^{-1}
Gradient: HCl 9mM, DAP 0.5mM from 0 to 5 minutes then a linear gradient to HCl 56mM, DAP 8mM after 20 minutes.

Suppressor: CMMS

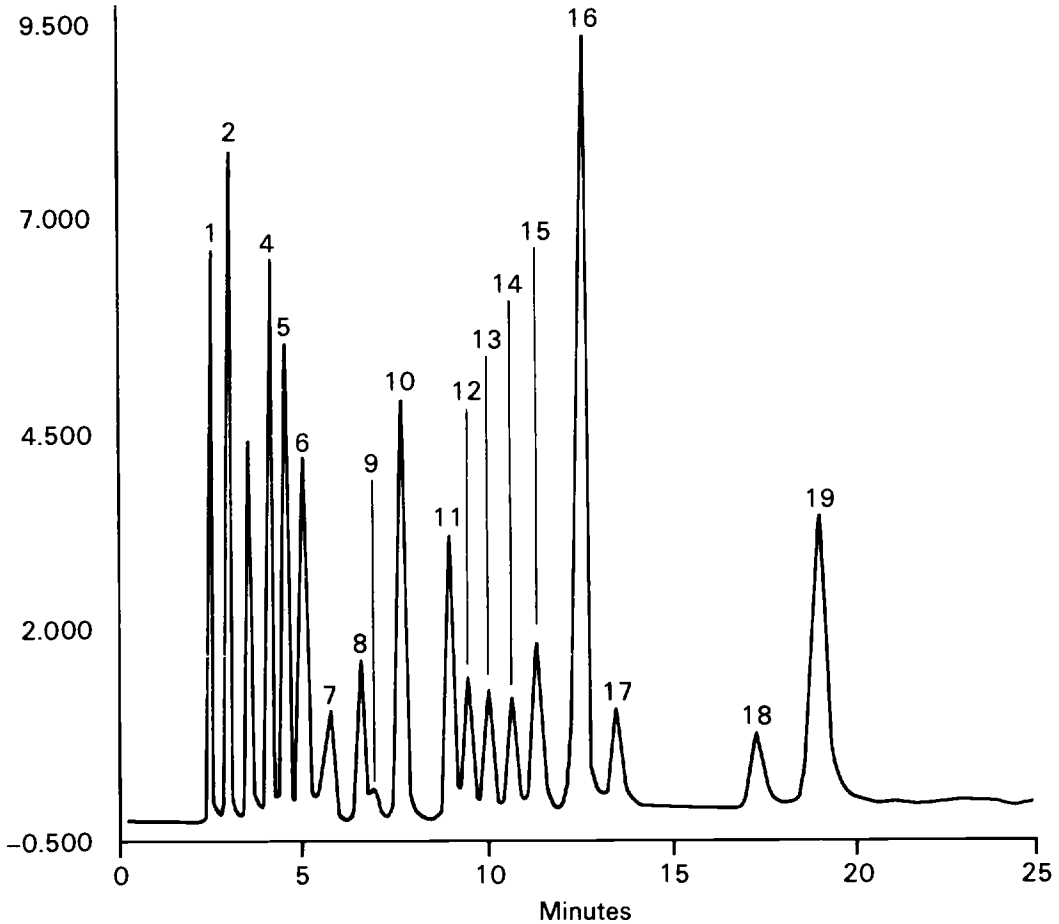
Regenerant: 100mM TBAOH 14mL min^{-1}

Detector: Conductivity 30μS FS

Peaks:

1. Lithium
2. Sodium
3. Ammonium
4. Potassium
5. Triethylamine
6. Tripropylamine
7. Cyclohexylamine
8. Tetrabutylammonium
9. Magnesium
10. Manganese
11. Calcium
12. Strontium
13. Ethylenediamine
14. Barium

Chromatogram 1.5



Column: Omnipac PCX-500

Gradient mixture of HCl 15mM in DAP 100-0/0-100 acetonitrile

Flow rate: 1mL min^{-1}

Source: Dionex Inc.

Peaks:

1. Lithium
2. Sodium
3. Ammonia
4. Methylamine
5. Potassium
6. Ethylamine
7. Trimethylamine
8. Iso -Propylamine
9. Morpholine
10. Diethylamine
11. Pyrrolidine
12. Sec-Butylamine
13. Iso -Butylamine
14. Triethylamine
15. Piperidine
16. Magnesium
17. Tetraethylammonium
18. Cyclohexylamine
19. Calcium

2. Selected Soluble Transition Metals, and Aluminium in Waters

The first method covers the determination of cadmium, copper, iron, lead, manganese, nickel and zinc in water. Two techniques are described here; one based upon the use of special anion exchange resins in a non-metallic system and a second technique based upon separation using a dynamically coated reverse phase HPLC column in a 'conditioned' stainless steel system. Detailed information on the application and performance characteristics of the first technique in a routine working environment was available from a critical independent study for DOE carried out by the Analytical Chemistry Section, AEE Winfrith of the United Kingdom Atomic Energy Authority (1).

A separate method for the determination of aluminium is also appended (Section 2.3).

Information on the second technique was available only from the Applications Laboratory of the instrument manufacturer, and this did not include any detailed peformance data. It is hoped that such data, independently obtained under the same stringent assessment criteria, would be available for any revision of this booklet. Thus the detailed procedure outlined below is based upon the first technique, but the principal differences of the reverse phase HPLC method and other applications are given in Section 2.2. Other alternatives are also mentioned.

2.1 Chromatography Using Low-Capacity Anion Exchange Resin

This method has been developed and evaluated mainly for the analysis of trace levels in natural non-saline waters by direct injection of samples, but the range of application can be varied by either sample dilution or preconcentration. Thus, elemental levels are expressed in the absolute amount analysed rather than the concentration in the original solution.

2.1.1 Peformance Characteristics

2.1.1.1 Substances Determined

Cadmium, cobalt, copper, iron (as iron II), lead, manganese, nickel and zinc ions in solution.

2.1.1.2 Types of Sample

Distilled water, tap water, river waters and other non-saline waters. It may also be applied to trade and sewage effluents provided any known specific interferences are checked or assessed.

2.1.1.3 Basis of Method

200 μL of sample volume is injected into the flowing mobile phase of an inert polymeric ion chromatograph and the time-resolved elemental species detected and quantified by reaction with a spectro-photometric post-column reagent.

2.1.1.4 Range of Application

Performance determined on the basis of absolute amounts of each element being determined. The range of application can be varied by suitable pre-concentration or dilution of the sample. Approximate levels of analyte tested were:

Cadmium	: 40	and 230	ng,
Cobalt	: 0.5	and 5	ng,
Copper	: 10	and 70	ng,
Iron	: 15	and 120	ng,
Lead	: 15	and 70	ng,
Manganese	: 2	and 20	ng,
Nickel	: 2	and 20	ng,
Zinc	: 10	and 110	ng,

2.1.1.5 Calibration

Standard solutions are prepared to give a response above and below that of the test solution (the sample is bracketed by standards). Calibration should be carried out before and after the analysis of a set of samples.

2.1.1.6 Range of Linearity

Linearity of response depends upon the detector used, together with the concentration and flow rate of the spectrophotometric post-column reagent. All elements give a linear response up to at least 500 ng under the conditions specified in Section 2.1.3. Linearity up to higher levels has been found for some elements, e.g.

Iron 1500 ng, Nickel 2500 ng,
Lead 2000 ng, Cadmium 2000 ng

2.1.1.7 Standard Deviation	Within-batch (sw), between-batch (sb) and total (st) standard deviations for the two concentration levels are reported in Tables 2.1 and 2.2. These were obtained in a routine working laboratory environment.
2.1.1.8 Limits of Detection	Based on 4.652 SD of the baseline noise of the blank, the limits of detection have been found to be: Cd : 13 ng, Pb : 13 ng, Co : 0.5 ng, Mn : 1 ng, Cu : 0.5 ng, Ni : 1 ng, Fe : 2 ng, Zn : 1 ng
2.1.1.9 Interferences	Carbonate, chloride and fluoride ions cause some interference at concentrations of over 100, 1000 and 100 mgL^{-1} respectively. Cyanide ions, when present at concentrations above 5 $\mu g\ mL^{-1}$, cause gross interference. These and other sources of interference are described more fully in Section 2.1.5.
2.1.1.10 Time for Analysis	Time taken to record each chromatogram containing the eight elements is approximately 16 minutes.

2.1.2 Principles of the Technique

The soluble transition metal cations together with cadmium and lead are injected onto an anionic exchange resin, and eluted as anionic complexes with an eluent containing pyridine-2, 6-dicarboxylic acid (PDCA) having a fixed pH. Total soluble iron is determined after pre-reduction to iron II by the addition of ascorbic acid to this eluent (see Section 2.1.5.3). The time-resolved species from the chromatographic column are measured in a spectrophotometer following the addition of 4-(2 pyridylazo) resorcinol (PAR) as a colorimetric post-column reagent.

2.1.3 Chromatographic Conditions

The following conditions are recommended:

Sample loading	Direct injection of 200 µL of calibration standards or test solutions. Alternatively, much larger volumes of sample can be pre-concentrated onto a cationic exchange resin and subsequently injected.
Guard Column	Dionex IONPAC CG5 anion column
Separator Column	Dionex IONPAC CS5 anion column
Eluent	Modified reagent based upon pyridine −2, 6-dicarboxylic acid (PDCA – see 2.1.4.2).
Eluent Flowrate	1.0 mL min^{-1}
Post-column Reactor	Pressurised semi-permeable hollow fibre membrane
Post-column Reagent (PCR)	4-(2-pyridylazo) resorcinol (PAR) in an ammonium acetate/ammonium hydroxide solution having a pH of 10.
PCR Flowrate	0.75 mL min^{-1}
Post-column Detector	UV-visible spectrophotometer set to a wavelength of 520 nm and a sensitivity of 0.5 absorbance units full scale (AUFS) for the higher concentrations and 0.2 AUFS for the lower concentrations.

2.1.4 Preparation of Reagents and Standards

'Aristar' grade reagents or reagents of similar purity should be used wherever possible for preparation of the mobile phase and post-column reagent.

2.1.4.1 Preparation of Standards

Multi-element standards for calibration should be prepared in a chemical matrix which is similar to the samples to be analysed. Such calibration standards can be prepared by serial dilution of suitable primary standards (see Part I Chapter 4 Section 6).

2.1.4.2 Water

High quality de-ionised water (i.e. >18 M.ohm.cm) should be used for the preparation of reagents and standards.

2.1.4.3 Eluent

The eluent is based upon pyridine 2.6-dicarboxylic acid (PDCA) with lithium hydroxide for pH control. The addition of other reagents is required to modify the chromatography. It can be prepared by dissolving the following in high purity de-ionised water:

0.500 ± 0.005 g sodium sulphite
0.570 ± 0.005 g sodium sulphate
5.840 ± 0.005 g sodium chloride
0.720 ± 0.005 g lithium hydroxide monohydrate
2.000 ± 0.005 g pyridine-2, 6-dicarboxylic acid

followed by the addition of:

0.70 ± 0.01 g ascorbic acid
40.0 ± 0.1 mL methanol

The solution is then made up to 4 litres with de-ionised water. The reagent should be filtered through a 0.45 μm membrane filter and stored in a collapsible eluent container.

2.1.4.4 Post-column Reagent (PCR)

The PCR is prepared by dissolving 0.100 ± 0.005 g of 4-(2-pyridylazo) resorcinol (PAR)* in approximately 700 mL de-ionised water followed by the addition of 180 ± 2 mL ammonium hydroxide (d_{20} 0.88) and 67 ± 1 mL glacial acetic acid. This solution is made up to 1 litre with deionised water. This reagent, which has a pH of about 10, should be filtered through a 0.45 μm filter membrane before use and stored in a suitable PCR container of the type supplied with the ion chromatograph.

2.1.5 Interferences

Interference effects considered in this section are those due to mono- and di valent cations, certain common anions, and inter-element effects between selected transition metals.

2.1.5.1 Effect of Mono-valent and Di-valent Cations

The technique has been applied to both the analysis of natural lake water and certain biological fluids (2). No problems were identified from the presence of alkali or alkaline earth elements, but their concentrations were not reported. In the same presentation, a chromatogram from the analysis of the lead content of beer showed calcium eluting immediately before iron II. However, the detection system used was based upon a modified PAR reagent which affected the sensitivity for calcium. If large amounts of any major cations such as sodium, calcium, or magnesium may be present in the samples submitted, the analyst is recommended to assess each application using synthetic samples with and without the possible interferences.

2.1.5.2 Effect of Common Anions

Effects due to the presence of carbonate, chloride, cyanide, fluoride and sulphate ions have been specifically addressed at concentrations up to 10 mg mL^{-1} (1). Carbonate ions modify the behaviour of the post-column reagent by changing the reaction kinetics between certain metal ions and the PAR. The presence of carbonate ions at concentrations between 100 and 1000 μg mL^{-1} causes enhanced responses for Cd, Cu, Mn, and Ni. Cobalt and lead responses are enhanced at the lower carbonate concentration, but these elements show reduced sensitivity at the higher carbonate concentration. Typical enhancement factors are between 1.5 and 3. Conversely, iron responses can be reduced, and carbonate causes unreliable chromatography for all elements, when present at 10 mg mL^{-1}. It is recommended that samples known to contain carbonate are pretreated (e.g. acidified to 0.001M mineral acid), or that standards are 'matrix matched' for carbonate concentrations < 1000 μg mL^{-1}.

* 0.118 ± 0.005g of the monosodium PAR salt monohydrated or 0.137 ± 0.005g of the disodium PAR salt dihydrated may be used instead.

Chloride and sulphate ions are already present in the eluent, and no interference was reported from the presence of either these anions or fluoride at concentrations of up to 100 $\mu g\ mL^{-1}$. However, considerable disruption of the chromatography occurs at anion concentrations of 10 $mg\ mL^{-1}$.

The presence of cyanide ions has a marked effect on the chromatography at concentrations above 5 $\mu g\ mL^{-1}$. Nickel and cobalt responses disappear at cyanide concentrations of 10 $\mu g\ mL^{-1}$, whilst at a concentration of 50 $\mu g\ mL^{-1}$ the chromatography becomes very poor and the results are meaningless.

It is important that standards are matrix matched to samples, and thus compensate for some of the minor interference effects from common anions. the chromatographic method can tolerate up to 1000 $\mu g\ mL^{-1}$ of common anions, but only up to a maximum cyanide concentration of 5 $\mu g\ mL^{-1}$. Chelating agents such as EDTA can also affect metal responses, particularly those of iron and nickel, for instance 60 mgL^{-1} EDTA completely suppresses 10 mgL^{-1} Iron III.

2.1.5.3 Determination of Iron

Whilst is is technically possible to separate and quantify both iron II and iron III ions, in practice, problems can be experienced with erratic results due to changes in oxidation state of the iron during analysis. These changes are probably due to traces of oxidising impurities in the reagents used, or, less likely, to the ingress of oxygen. Moses et al. (3) were able to make such measurements, but found that photochemical reduction of iron III is a potential cause of interference when measuring FeII/FeIII ratios. Thus, they had to pay considerable attention to sample preparation and storage (i.e. to acidify with HCl, store at <5°C (without freezing) in the dark and analyse as soon as possible, preferably within minutes). Such preparation and storage was also recommended in a much earlier report (4). It should be noted that a few anions such as nitrate can oxidise iron II to III, others such as sulphide may do the reverse. A few iron II complexes can reduce water to hydrogen. These effects are very pH dependent.

Reliable determination of the total soluble iron content of samples can be achieved by reducing all the iron species to the two valent state. This can be done by the addition of ascorbic acid and sodium sulphite to the PDCA eluent. This has been used in the continuous analysis of reactor coolant waters for total soluble iron content.

2.1.5.4 Inter-element effects

Problems sometimes occur if the concentrations of sequentially eluting analytes differ widely. The later eluting species may appear as a poorly resolved peak on the tail of the earlier eluting peak. It is possible to modify the chromatography by making minor changes in the composition of the mobile phase (e.g. by changes in pH), but such modifications may be only partially successful. Alternative mobile phases can be used to change the elution order and overcome a specific problem, but this may result in certain elements not being determined. One example is the use of an oxalate-based eluent (the elution order is Pb, Cu, Cd, Mn, Co, Zn, Ni), but in this case iron cannot be determined due to the stability of the iron II-iron III oxalato complexes which prevent formation of a coloured deriviative with the PAR reagent. Thus, as is often the case, certain compromises have to be made to allow a capability for multi-element analysis. Problems have been experienced in resolving nickel and cobalt peaks where the concentration ratio (Ni:Co) was greater than 500:1. Similar problems might be expected if the ratio between any two sequentially eluting peaks was of a similar order.

The determination of zinc at the lower concentrations can be influenced by adventitious contamination from sample bottles and plastic pipettes. Zinc is a common filler and 'lubricant' used in the manufacture of plastics and care is needed to avoid contamination from this source. Such materials should be rigorously cleaned by soaking in high purity 50% nitric acid followed by demineralised water.

2.1.5.5 Nickel Sensitivity

Kinetics of the nickel/PAR reaction are temperature dependent and attempts should be made to avoid wide temperature variations during analysis (i.e. <5°C). Alternatively, for maximum sensitivity the post column reagent and column effluent can be heated to 50°C.

2.1.6 Analytical Procedure

Step	Procedure	Notes
2.1.6.1	Switch on the instrument and analytical pump for the mobile phase (eluent) as per the manufactuer's instructions. Set the detector to a suitable range. (Note a).	a. It is important when using a computing integrator to match the detector output and the integrator input. It is necessary to record the range setting and integrator attenuation when using different range settings on modern detectors with digital output. Generally, detector ranges of 0.2 and 0.5 AUFS are appropriate depending on analyte concentrations.
2.1.6.2	Turn the injection valve to the 'inject', position (Figure lb) and pump eluent through the chromatography system. (Note b).	b. The configuration of the recommended double 4-way valve is shown in Figure 2.1. A double 3-way valve may be used, but this is less flexible in operation when both direct injection or concentration are options.
2.1.6.3	Pressurise the post column reagent delivery reservoir and establish an adequate flow of reagent through the hollow-fibre membrane (Note c).	c. It is essential that the eluent is allowed to flow through the separator column before the post-column reagent delivery is actuated. Failure to observe this rule can result in damage to the column resin due to backflushing of the PAR reagent.
	Calibration	
2.1.6.4	When a stable baseline signal is attained, switch the injection valve to the 'load' position (Figure 2.1a) and using a syringe, fill the injection loop with the first standard. (Note d).	d. Any tubing and the injection loop itself should be thoroughly flushed with the standard or sample under test.
2.1.6.5	Inject the standard by switching the injection valve to the 'inject' position (Figure 2.1b) and simultaneously start data collection by the integrator.	
2.1.6.6	After completion of the first standard chromatogram, repeat steps 2.1.6.4 and 2.1.6.5 for the second standard used. (Note e).	e. Generally, it has been found that results from the first chromatogram of each day are unreliable, probably due to non-equilibrium conditions existing in the system. Thus, the first chromatogram of each day should be repeated. Typical responses from an ion chromatograph under the conditions listed in Section 2.1.3 are shown in Chromatograms 2.1, 2.2 and 2.3.
2.1.6.7	Repeat steps 2.1.6.4 and 2.1.6.5 using only high quality deionised water to check any blank contribution. (Note f).	f. Responses can be corrected for any blank contributions if any significant amounts are found.
2.1.6.8	Repeat the calibration (i.e. steps 2.1.6.4 to 2.1.6.6) after completing the analysis of the mean response of each standard (Note g).	g. If there is a significant difference between the two calibrations, check and correct for instrumental drift. If the difference is still unacceptable the analysis will need to be repeated.

2.1.6.9	Plot total weight (in ng) against peak height and obtain the regression lines for the individual transistion metal ions (note i).	i. Certain integrators and/or calculators have the ability to calculate 'best fit' regression lines from either stored data or input data and these should be used in preference to manual plotting.
	Sample Analysis	
2.1.6.10	Load the sample (test solution) and then inject into the chromatograph using the method outlined in steps 2.1.6.4 to 2.1.6.5.	
2.1.6.11	On completion of the first sample chromatogram (typically < 20 minutes) repeat step 2.1.6.10 to obtain the duplicate results.	
2.1.6.12	Peak heights for each element are measured and compared with the linear regression of the calibration standards to obtain the concentration for each injection of the sample (Note j).	j. Baseline fitting is required for those peaks which do not have background resolution with an adjacent peak. This can be achieved either by manual or automatic methods depending upon the integrator used and/or the chromatographic resolution.
2.1.6.13	Repeat steps 2.1.6.10 to 2.1.6.12 for further samples (test solutions) and finally re-calibrate as indicated in step 2.1.6.8 (note k).	k. Calibration must be carried out every day on which samples are analysed.

2.2 Alternative Procedures

2.2.1 Reverse Phase Chromatography of Transition Metal Ions

This method is based upon work undertaken by the Waters Chromatography Division of Millipore at their Milford Applications Laboratory (USA) as part of a study for the US Environmental Protection Agency. Neither independent assessment or independent performance data were available for the method at the time of publication of this booklet. Many of the characteristics of the method are similar to those identified in the sections above. Discussion in this section will be limited to the main differences between this technique and that using a low-capacity anion exchange resin. The instrument manufacturer claims that it is possible to make iron II-iron III ratio measurements, but comments concerning this determination in section 2.1.5.3 should again be noted. Typical analysis time is 25 minutes.

2.2.1.1 Principles of the Technique

Soluble transition metals are injected onto a standard reverse phase HPLC column which has been dynamically coated with sodium octanesulphonate to give it a cation-exchange character. The method is an extension of one proposed by Cassidy (5). Cationic transition metal species have an affinity for the anionic sites and will be retained for varying times depending upon their complexation with a mobile phase based on tartaric acid of pH 3.4. The retention times of different transition metal ions can be altered by adjusting the ionic strength and pH of the mobile phase. Detection is again based upon derivatisation of separated metal ions with PAR, and pulse free addition of this reagent can be provided by use of a pressurised reagent vessel. If a stainless steel system is used, it is essential that it is passivated and conditioned with nitric acid (see 2.2.1.4).

2.2.1.2 Chromatographic Conditions

Aliquots (100μL) of standards or samples are injected onto a dynamically coated Waters Bondapek C18 column (e.g. 3.9 mm id by 30 cm), and separation is achieved using a mobile phase of 2mM sodium octanesulphonate/50 mM tartaric acid (having a pH 3.4) flowing at 1 mL min^{-1}. A pressurised vessel can be used to deliver the post-column reagent at a flow rate of 0.5 ml min^{-1} (0.2 mM PAR in 3M ammonium hydroxide/1M acetic acid). Detection is again based upon a UV-visible spectrometer set at 520 nm and 0.01 to 0.5 absorbance units full scale.

2.2.1.3 Reagent Preparation

The mobile phase is prepared by dissolving 0.443 ± 0.001 g sodium octanesulphonate and 7.510 ± 0.005 g tartaric acid in 1 litre of high purity water. The pH of this reagent is adjusted to pH 3.4 using sodium hydroxide solution. It should be filtered through a 0.2 μm membrane and degassed, and although stable for 2 to 3 days, it is susceptible to bacterial growth. The post-column reagent is prepared by dissolving 0.026 ± 0.001 g PAR in a mixture of 167.0 ± 0.02 mL of concentrated ammonium hydroxide and 57.0 ± 0.2 mL glacial acetic acid. It should then be diluted to 1 litre, filtered through a 0.2 μm membrane, degassed, and stored under a nitrogen atmosphere. This reagent is stable for 2 weeks.

2.2.1.4 Analytical Procedure

The analytical procedure is similar to that described in section 2.1.6, although in this case special attention is required to the conditioning of the chromatographic system and column. Since the system is stainless steel, it is essential to passivate the internal surfaces by forming an oxide film. This is achieved by flushing the entire system, without a column present, using 6M nitric acid at a flow rate of 1 mL min^{-1} for 30 minutes. The system should then be flushed with high purity water for a further 30 minutes. Such passivation must be done before commencing analysis for transition metals, and it is recommended that the process is repeated at periodic intervals. Users are advised to check that other ions which may be present in the sample (e.g. borate, citrate, chloride) do not lead to reductive dissolution of the 'protective' oxide film and hence cause interference with the transition metal measurements.

Once the system has been passivated, the column should be installed and flushed with high purity water for 5 minutes at 1 mL min^{-1} to remove the methanol/water storage medium. The eluent, based on sodium octane-sulphonate, should then be pumped through the column for 15 hours (i.e. overnight) to condition it for separating transition metal ions. The basic operating steps are similar to those outlined in section 2.1.6.

Typical chromatograms, provided by the Winfrith AEA Applications Laboratory, are shown in Chromatograms 2.4 and 2.5.

2.2.2 Other Applications

Two other approaches, which have been applied to the analysis of transition metals, have been based upon the use of complexation and elution using low-capacity cation exchange columns. Complexing anion reagents can react with metal cations to form uncharged or reduced charge metal-ligand complexes. This effect can be used to control the amount of metal cation available for exchange with a cation-exchange column. Reducing the fraction of metal cation (or charge) causes shorter retention times in the column.

Sevenich and Fritz (6) used a 'home-packed' column of Benson low-capacity cation exchange resin (0.06 meq g^{-1}) and an eluent with a high background conductance to separate certain transition metal and alkaline earth cations. The eluent was 1.5 mM ethylenediammonium cation and 2.0 mM tartaric acid at pH 4.00. Very small amounts of complexing reagent were used and a major proportion of the separation relied upon the competition for exchange sites between the analyte ions and the ethylenediammonium ion. Post-column detection was by conductivity and a chromatogram of a solution containing cadmium, calcium, cobalt, lead, manganese, strontium and zinc at concentrations between 10 and 20 μg mL^{-1} is reproduced in Chromatogram 2.6.

The other complexation approach is to use higher concentrations of the complexing reagent, but much weaker concentrations of the competing anion. In order for a chelating (acid) reagent to be used, it must be adjusted to a more basic pH. Lithium hydroxide is often used for pH adjustment since it contributes very little to the chromatographic behaviour. Gjerde (7) reported the determination of cobalt, copper, ferrous, lead, nickel and zinc using a Wescan cation column (catalogue number 269004) and an eluent composition of 20 mM tartaric acid and 4 mM oxalic acid adjusted to pH 4 with lithium hydroxide (Chromatogram 2.7). It was also reported that it is possible to determine weakly bound cations (e.g. Fe) by using an eluent adjusted to pH 2.4. Elchuk and Cassidy (8) have also applied this complexation approach to the determination of the lanthanides using a gradient of 0.17M to 1M 2-hydroxyisobutyric acid at pH 4.6.

Jones et al (9) used Bio-Rad Aminex A9 ion exchange resin and an eluent based upon 0.2 M tartaric acid (pH adjusted to 4.3) to separate cadmium, cobalt, copper, ferrous, ferric, lead, manganese, magnesium, nickel and zinc. Analyte concentrations were 5 μg mL^{-1} (except Cd and Pb at 50 μg mL^{-1}), the injection volume was 100 μL, and post-column detection was based upon an inverse photometric technique using eriochrome black-T. A chromatogram produced from this high capacity ion exchange material is shown in Chromatogram 2.8. The chromatography of a similar range of ions at 0.5 μg mL^{-} is shown in Chromatogram 2.9, produced using a low capacity silica-based material (Partisil 10 SCX) with 0.1 M lactic acid adjusted to pH 3.6 as the eluent.

Table 2.1 Precision Data for Elements at the Higher Concentrations

Element	Mean Weight(ng)	S_w ng	S_b ng	S_t ng	RSD %
Cadmium	230	+3.0	+11	+11	4.8
Cobalt	4.67	+0.04	+ 0.13	+ 0.14	3.0
Copper	68.9	+0.4	N/S	+ 0.6	0.9
Iron	115	+2.0	+ 5.0	+ 6.0	5.1
Lead	69.2	+2.0	N/S	+ 2.7	4.0
Manganese	23.2	+0.3	+ 0.6	+ 0.7	3.0
Nickel	22.2	+0.3	+ 0.8	+ 0.8	3.8
Zinc	114	+1.0	+ 4.0	+ 5.0	4.0

N/S indicates that the between batch standard deviation is not significant.

Table 2.2 Precision Data for Elements at the Lower Concentrations

Element	Mean Weight(ng)	S_w ng	S_b ng	S_t ng	RSD %
Cadmium	41.1	+3.0	N/S	+7.0	17
Cobalt	0.553	+0.041	N/S	+0.04	7.0
Copper	0.96	+0.25	N/S	+0.27	2.7
Iron	15.5	+1.6	N/S	+2.4	15
Lead	16.0	+1.3	N/S	+1.7	11
Manganese	1.82	+0.12	+0.20	+0.23	13
Nickel	1.58	+0.04	+0.12	+0.13	8.2
Zinc	9.71	+0.21	N/S	+0.23	2.4

N/S indicates that the between batch standard deviation is not significant.

2.3 Determination of Aluminium

2.3.1 Introduction

The routine determination of low levels of aluminium in water and foodstuffs requires simple, sensitive and specific methods. Unfortunately, most atomic spectroscopy methods are not very sensitive. Ion chromatography can be applied successfully using a cation exchange separation, post column reaction and UV or visible range detection of the coloured aluminium complex.

Depending on the wavelength of detection desired, two colour reactions are used. Tiron is preferred for UV detection at 310 nm whereas pyrocatecechol violet is preferred for visible detection at 570 nm. Both of these reagents are very selective for aluminium, and they are useful for minimising matrix interferences and determining aluminium in the presence of other metals.

2.32 Instrumental Conditions

Eluent:	0.01 M Sulphuric acid 0.2 M Ammonium sulphate
Column:	Dionex IONPAC-CG2 Cation Guard Column Dionex IONPAC-CS2 Cation Separator Column
Post Column Reagent for UV Range Detector:	0.0003 M Tiron in 3 M Ammonium acetate, adjusted to pH = 6.2 with Sodium hydroxide. 310 nm.
Post Column Reagent for Visible Range Detector:	0.0002 M Pyrocatechol Violet in 0.25 M Acetic Acid and 0.25 M Sodium acetate. 570 nm.
Eluent Flow:	0.7 to 1.0 mL min^{-1}
Post Column Flow:	0.6 mL min^{-1}
Retention Time:	4.0 min

2.33 Discussion

Most performance data relates to the use of Tiron as post column reagent (10). Using a 100 μL sample loop, good linearity was obtained over two orders of magnitude, from 10 to 5000 μg L^{-1} of Al. The limit of detection using the definition of three times the standard deviation (3σ) was 7 μg L^{-1} of Al. Repeatability was better than 4% RSD for a 100 μg L^{-1} standard. No metal interferences were observed, although iron (II) and iron (III) do respond to tiron and elute at 3 mins. Detection in the UV however will be more prone to direct organic interferences. Problems may result if the aluminium is complexed with other organic materials. If so, it may be necessary to acidify the sample to pH 1 or perform an acid digest on the sample.

The use of pyrocatechol violet is more recent (11) and results in a more specific form of detection. The limit of detection is very similar (see Acid Soluble Aluminium in Marine, Raw and Potable Waters (2nd Ed) 1987. HMSO in this series).

Examples using both reagents are shown in Chromatograms 2.10 and 2.11.

2.4 References

1. Amey, M.D.H., Symons, W.J., Mills, S.P., *UKAEA REPORT AEEW-R-2340* (1988).
2. Herbling, S.S. and Rivello, J.M., Presentation at 27th Rockey Mountain Conference, Denver, Colorado (July 1985).
3. Moses, C.A., Herlihy, A.T., Herman, J.S. and Mills, A., *Talanta,* **35,** 1, 15–22. (1988).
4. McMahon, J.W., *Limnol, Oceanog,* **12,** 437, (1967).
5. Cassidy, R.M., Elchuk, S. and McHugh, J.O., *Anal. Chem.,* **54,** 727. (1982).
6. Sevenich, G.J. and Fritz, J.S., *Anal. Chem.,* **55,** 12. (1983).
7. Gjerde, D.T., *J. Chromatography,* **439,** 49–61. (1988)
8. Elchuk, S. and Cassidy, R.M., *Anal. Chem.,* **56,** 474. (1984).
9. Jones, P., Barron, K. and Ebdon, L., *Anal Proc.,* **22,** 373–375. (1985).
10. Dean, J.R., *Analyst,* **114,** 165–168, 1989.
11. Dionex, *Application Note,* 42, 1986.

Figure 2.1a Sample loading

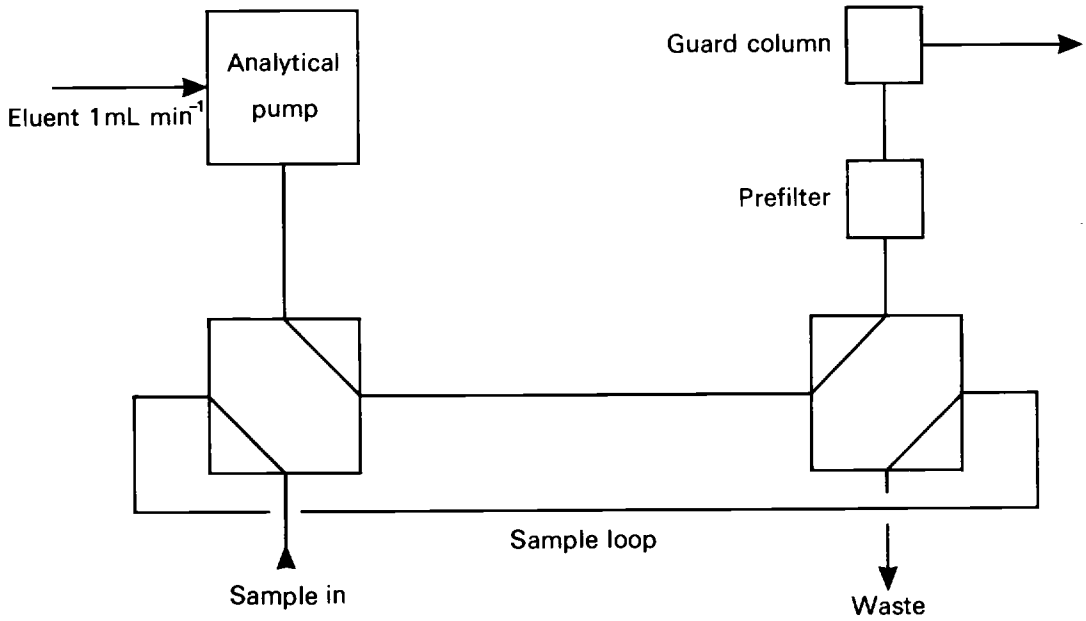
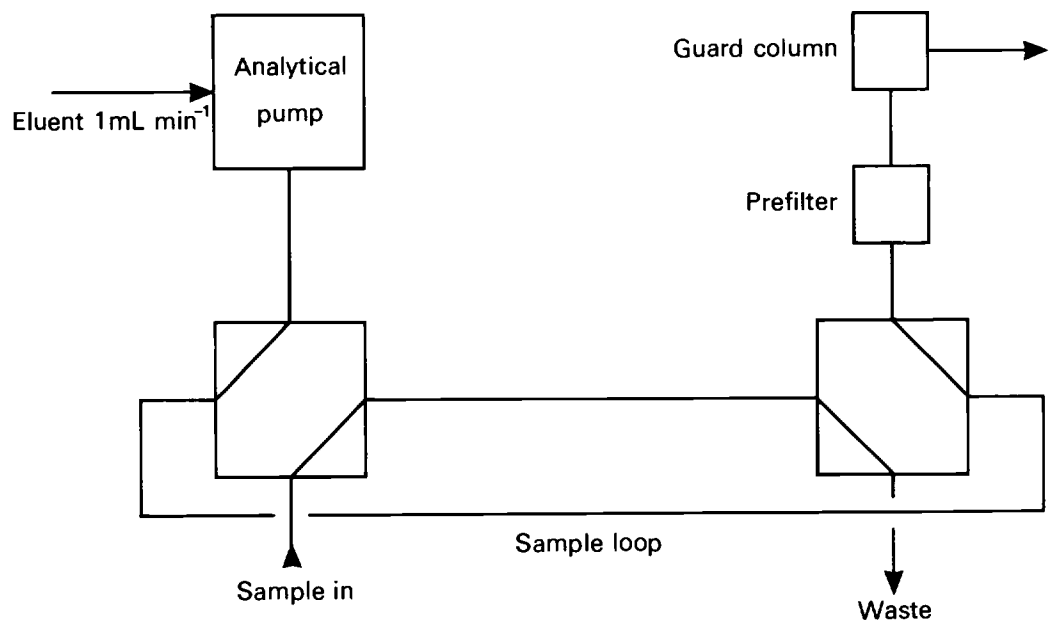
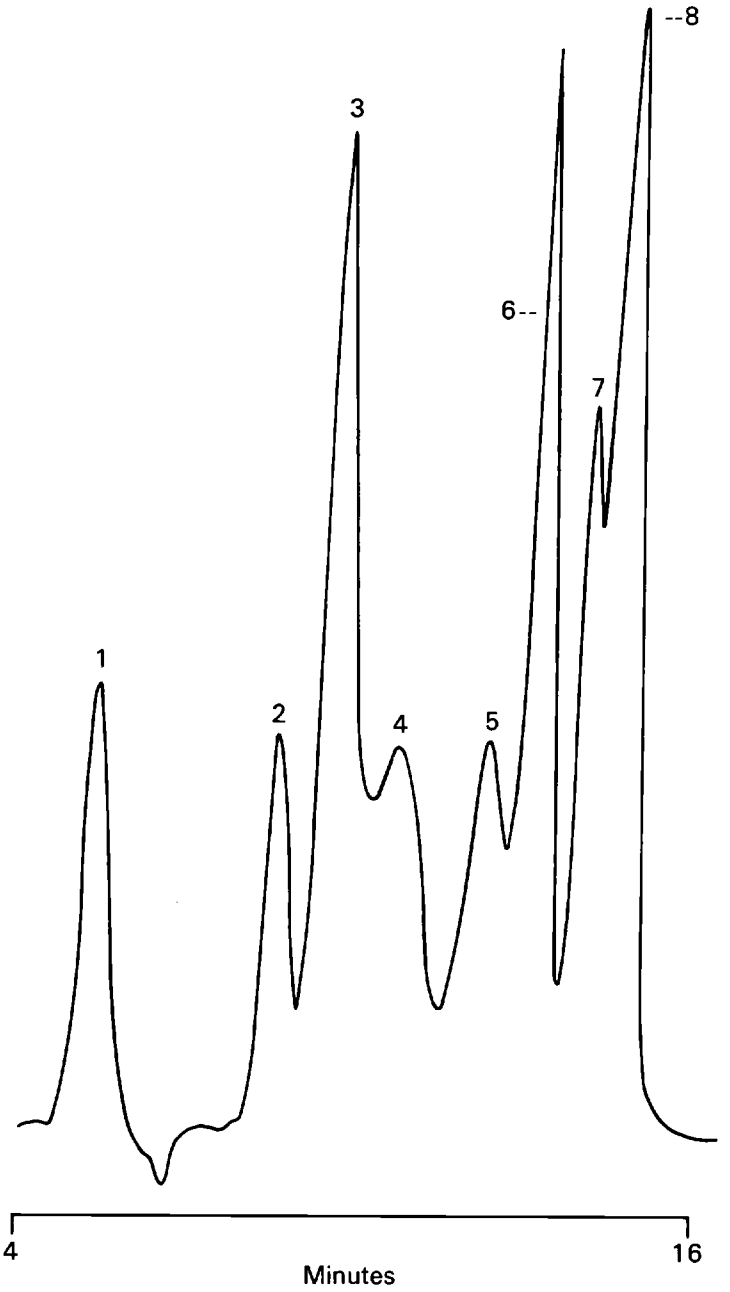


Figure 2.1b Sample injection

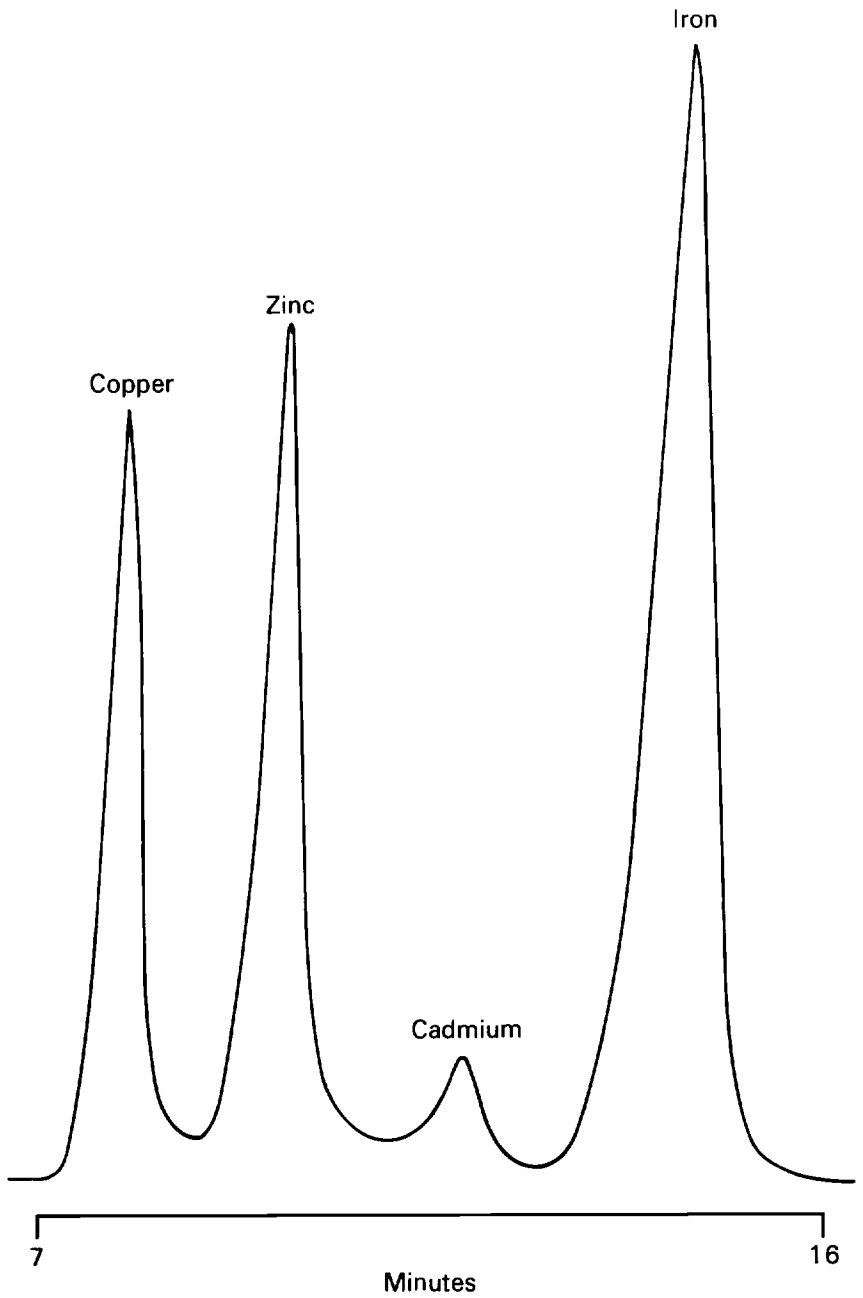


Chromatogram 2.1 Solution containing all eight elements

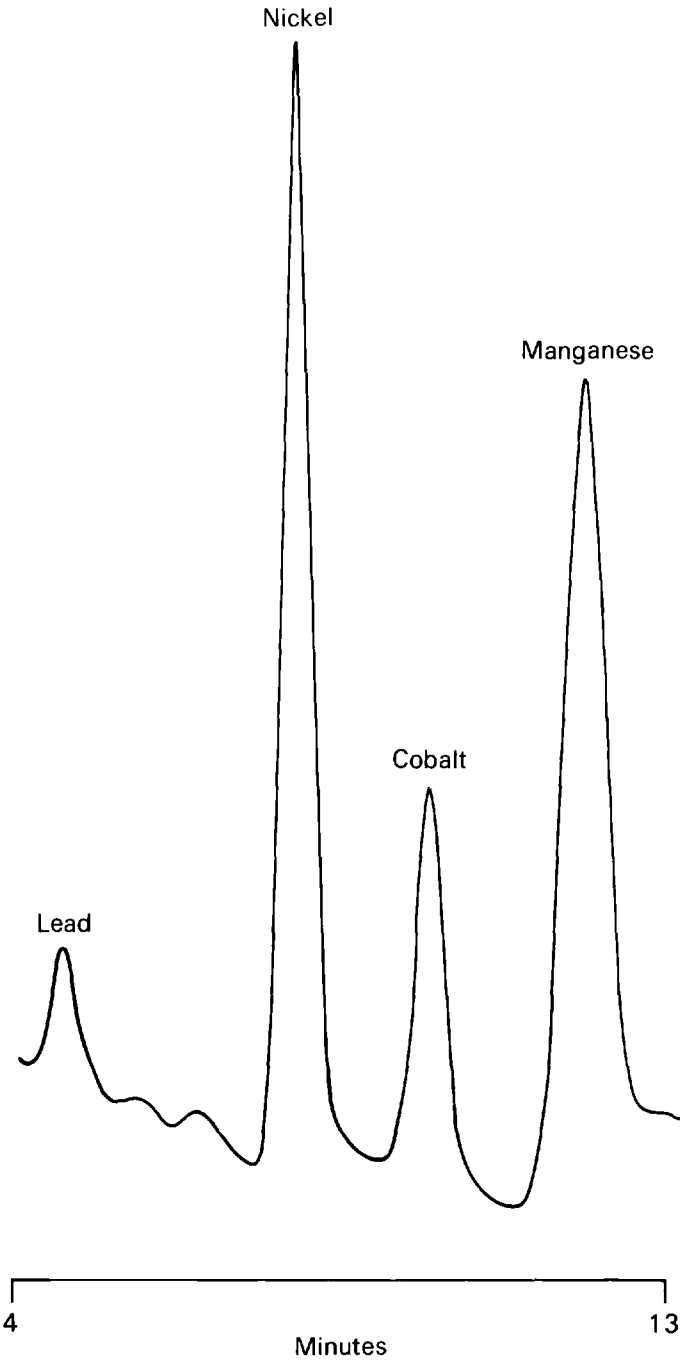


Peak	Element	Retention time min	Concentration ng
1	Lead	4.97	400
2	Copper	7.40	6
3	Nickel	8.20	20
4	Zinc	9.03	10
5	Cobalt	10.23	5
6	Cadmium	11.00	400
7	Manganese	12.20	15
8	Iron	13.20	20

Chromatogram 2.2 Typical chromatogram of group B elements (cadmium, copper, iron and zinc)



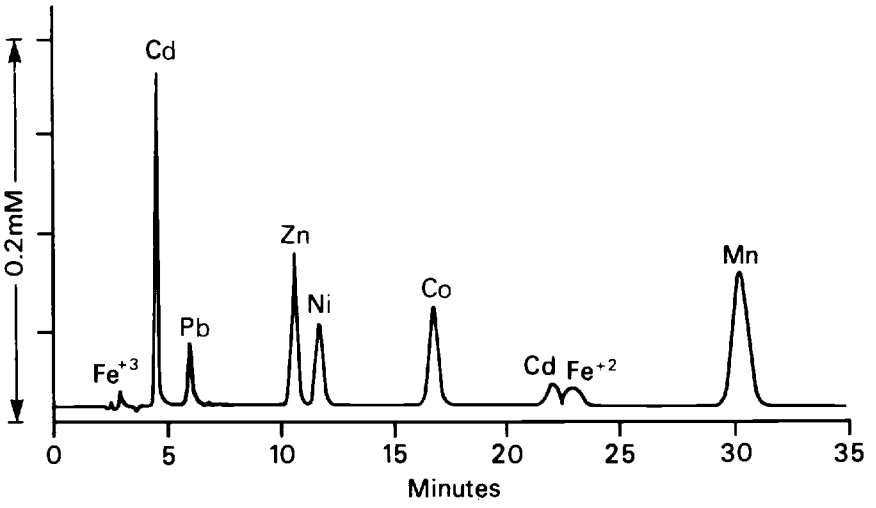
Chromatogram 2.3 Typical chromatogram of group A elements (cobalt, lead, manganese and nickel)



Chromatogram 2.4

Reverse phase chromatogram for some divalent transition metal

using 2mM sodium octane sulphonate/50mM tartaric acid pH 3.4 with NaOH as eluent

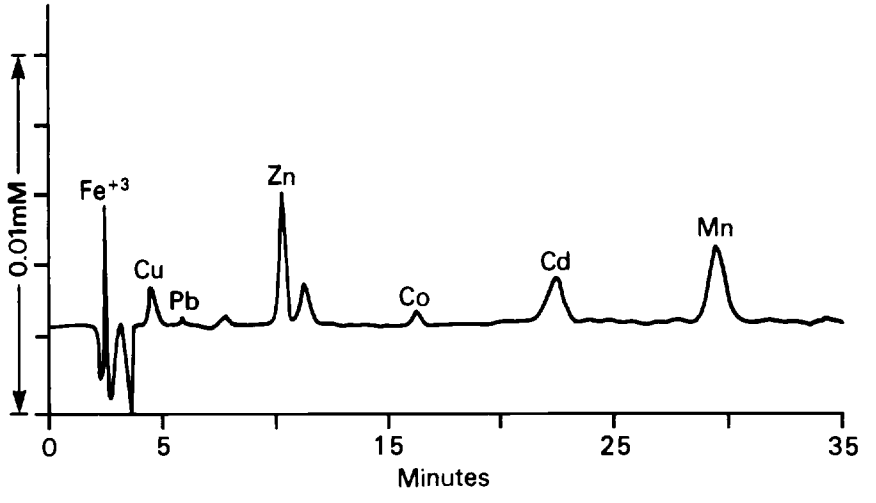


	Amount ng
Copper	50
Lead	100
Zinc	50
Nickel	100
Cobalt	50
Cadmium	100
Ferrous	100
Manganese	100

Chromatogram 2.5

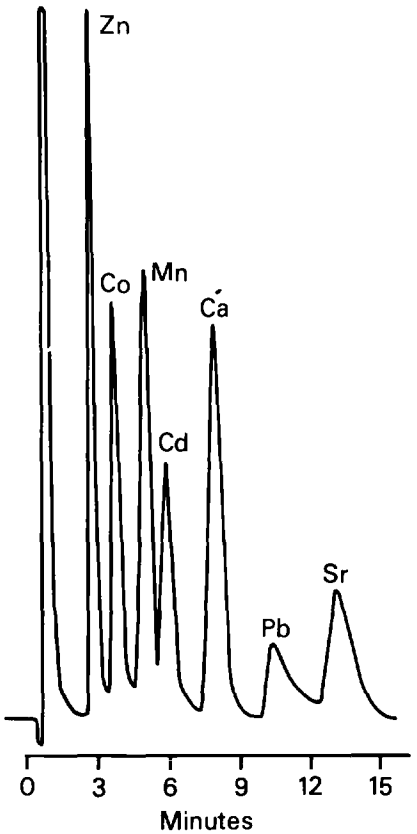
Reverse phase chromatogram of a waste water sample

shows the divalent transition metal profile of a wastewater digested in HNO_3 /H_2O_2 and resuspended in 0.5% HNO_3 using the same eluent as for Chromatogram 2.4



	Amount ng
Copper	0.8
Lead	0.8
Zinc	2.4
Nickel	3.0
Cobalt	0.3
Cadmium	15
Manganese	3.4

Chromatogram 2.6
Low-capacity ion exchange with low concentration of complexing ion



Detection of transition metal and alkaline earth cations, using an eluent with high background conductance.

Column: home-packed with Benson Company low-capacity cation exchange resin.

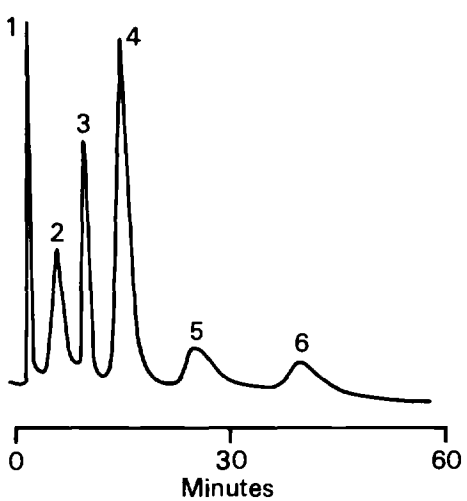
Eluent: 1.5 mM ethylenediamine and 2.0 mM tartrate at pH 4.0.

Solute concentrations: 10-20 mg L^{-}

The peaks are in the direction of decreasing conductance.

(From Ref. 6)

Chromatogram 2.7
Transition metals Wescan cation column and high concentration of complexing reagent



Separation of transition metal cations with a 20 mM tartaric acid and 4mM oxalic acid, pH 4 with lithium hydroxide eluent. Weakly bound cations (Fe^{3+}, Cu^{2+} etc.) are separated using a pH 2.4 eluent with the same acid concentrations.

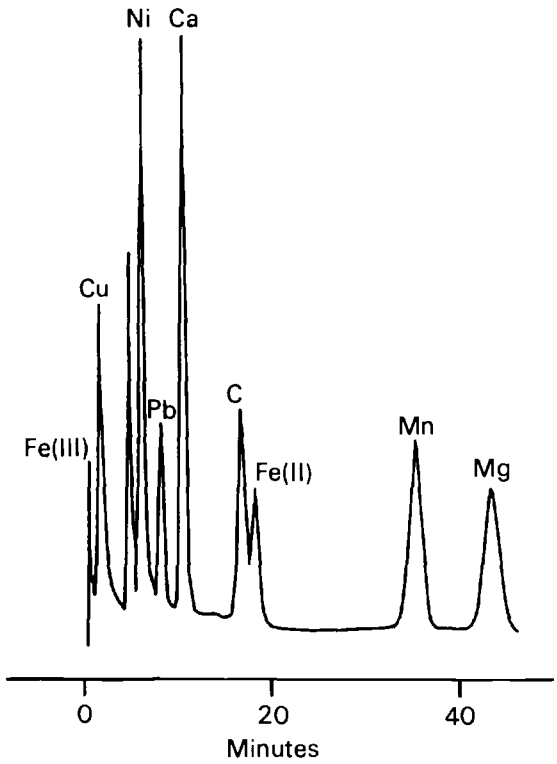
Column: Wescan Cation Cat. No. 269004

Detector: Wescan Model 273 PCR, Cat. No. 273 100.

Peaks: 1 = Cu^{2+}, 2 = Ni^{2+}, 3 = Zn^{2+}, 4 = Co^{2+}, 5 = Pb^{2+}, 6 = Fe

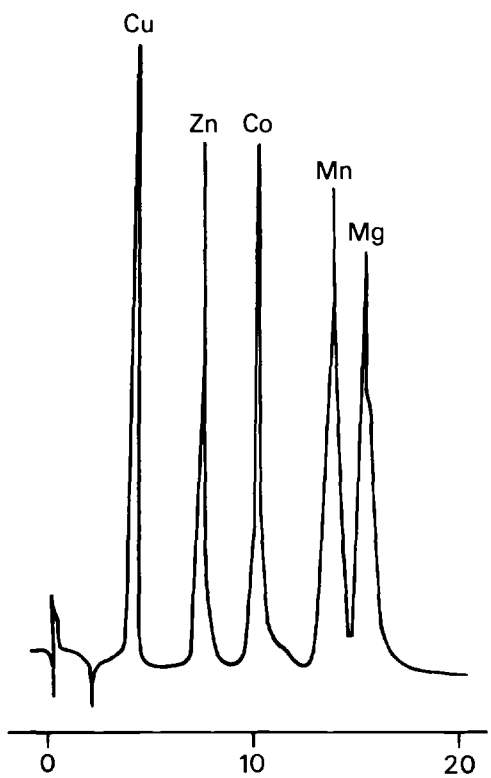
(From Ref. 7)

Chromatogram 2.8 Aminex AG - tartrate system



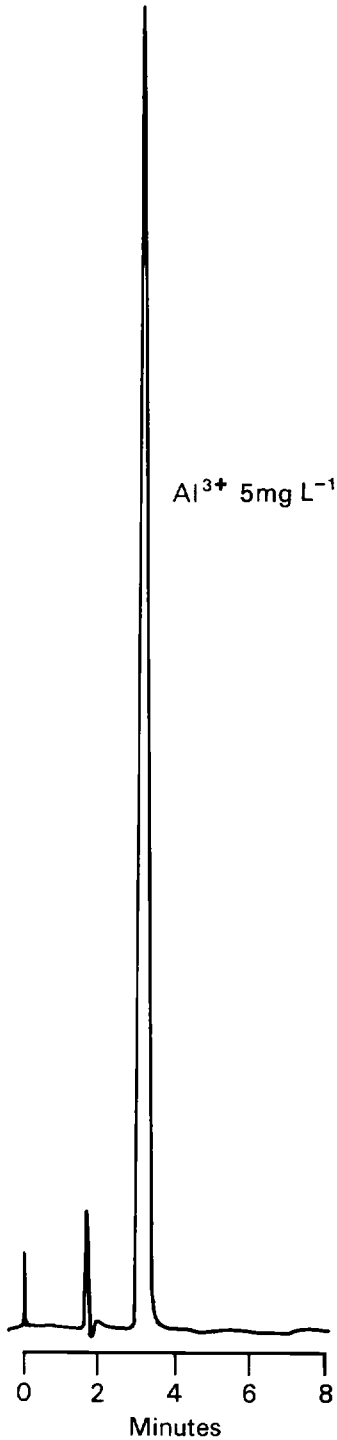
Chromatogram of a solution containing 5mg L^{-1} of Fe(III), Cu, Zn, Ni, Co, Fe(II), Mn, Mg and 50mg L^{-1} of Pb and Cd, (100-μL injection). Mobile phase 0.2 M. tartaric acid at pH 4.3. Detector as Fig.2.9

Chromatogram 2.9 Partisil (SX10) - lactate system



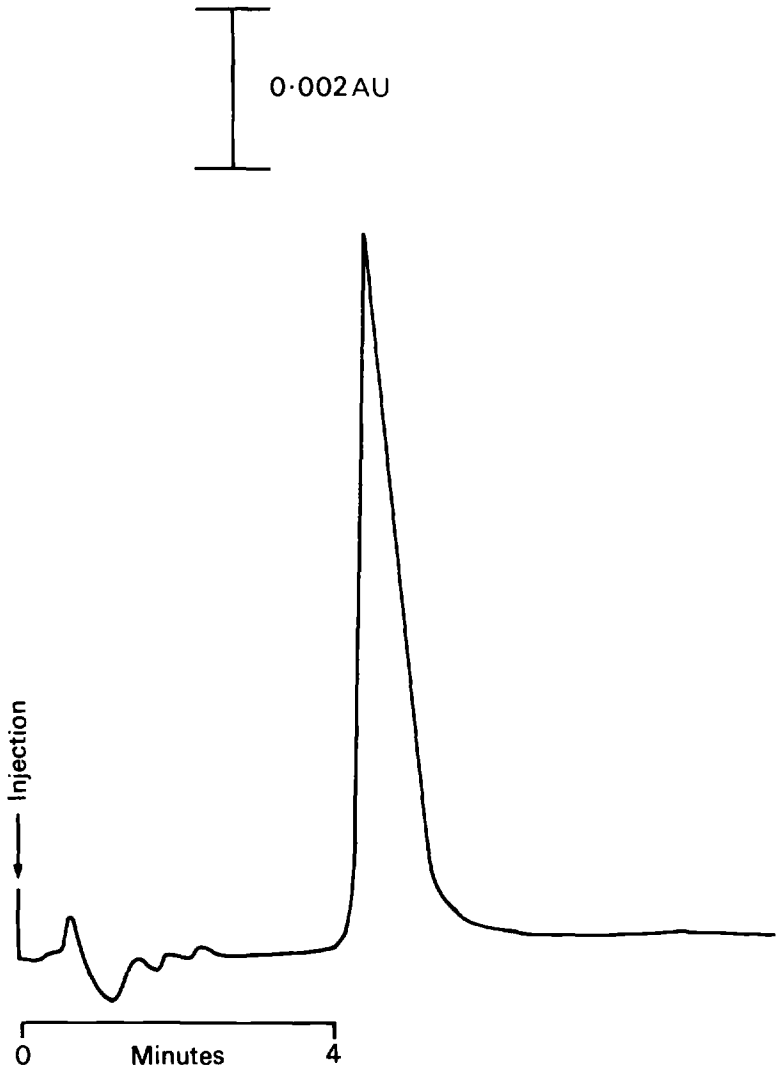
Chromatogram of a solution containing 0.5 p.p.m. of Cu, Zn Zn, Co, Mn, and Mg (100-μL injection). Mobile phase, 0.1 M. lactic acid at pH 3.6. Detection is based on eriochrome black-T as post-column reagent, monitored at 610 nm

Chromatogram 2.10 Determination of aluminium by cation exchange



HPIC-CS2, PCR (Tiron), 313nm

Chromatogram 2.11



Sample at RT mins	AL $\mu g\,L^{-1}$
4.13	1.001

Injection: 1μg mL^{-1} of aluminium, 150μL

Detection: Pyrocatechol violet

3. Chromium

3.1 Introduction

The variations between the toxicity of chromium III and chromium VI and the oxidative properties of the latter have led to the need for a sensitive method of analysis of both in environmental and biological systems. Ion chromatography utilising anion/cation exchange followed by post column reaction and visible detection allows sensitive and specific simultaneous determination of both forms of chromium.

3.3 Instrumental Conditions

Sample Size;	250 μL
Column:	Dionex IONPAC-CG5 Guard Column Dionex IONPAC-CS5 Separator Column
Eluent:	2 mM Pyridine dicarboxylic acid (PDCA) 2 mM Disodium hydrogen phosphate 10 mM Sodium iodide 50 mM Ammonium acetate 2.8 mM Lithium hydroxide
Flow Rate:	1.0 mL min^{-1}
Post Column Reagent:	2 mM Diphenyl carbazide (DPC) 10% Methanol 0.45 M Sulphuric acid
Post Column Flow:	0.5 mL min^{-1}
Detector Wavelength:	520 nm

3.3 Discussion

The visible absorbances of the chromium (III)–PDCA complex and the chromium (VI)–DPC complex at 520 nm allow photometric detection of chromium III and VI. As shown in Chromatogram 3.1, elution times are 3 and 5 minutes for trivalent and hexavalent chromium, respectively. The eluent system is PDCA based. The trivalent chromium is separated as the $Cr(PDCA)^{2-}$ complex whereas the hexavalent chromium is separated as the chromate ion. Hexavalent chromium does not form a complex with PDCA. Because of the slow kinetics of ligand exchange of chromium III, a precolumn derivatisation with PDCA is used to form the Cr(III)–PDCA complex in the samples. This is achieved by taking 10 mL of a 10× eluent concentrate and 10 mL of sample in a 100 mL volumetric flask and boiling for one minute. After cooling the sample is diluted to 100 mL with deionised water. The Cr(III)–PDCA complex is a stable anion.

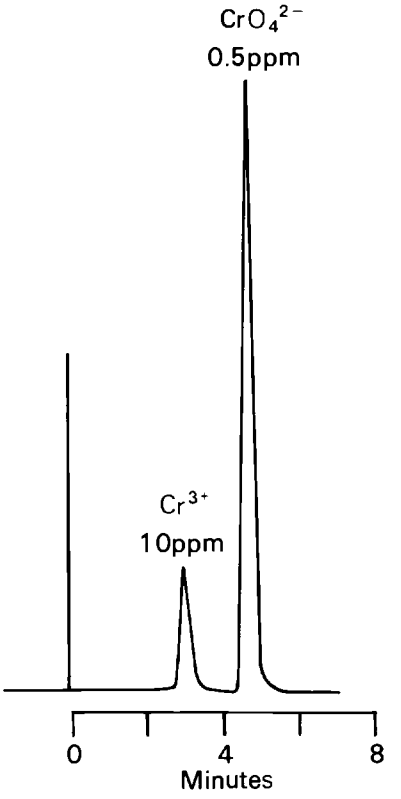
The pH of the sample and eluent systems are critical to the efficiency of the separation. For typical wastewater samples adjust the pH of samples to 6.8 with sodium hydroxide or hydrochloric acid. After separation the Cr(VI)–DPC complex is formed using post column derivatisation. Using an injection volume of 250 μL, detection limits for Cr(III) and Cr(VI) is 30 μg L^{-1} and 0.3 μg L^{-1} respectively. No interference data are available, however, this type of detection system (post column reaction) is very specific.

In a variation of this method both Cr(III) and Cr(VI) complexes are detected directly at 365 nm. No post column hardware is required, however the absorbance of chromate at 365 nm is not nearly as great as that of the Cr(VI)–DPC complex at 520 nm. See Chromatogram 3.1.

3.4 Reference

1. Dionex, *Technical Note*,24, May 1987

Chromatogram 3.1 Determination and speciation of trivalent and hexavalent chromium by ion chromatography



Column: HPIC–CS5

Eluent: 20mM PDCA
2.0mM disodium phosphate
10.0mM sodium iodide
50.0mM ammonium acetate
2.8mM lithium hydroxide

Post column reagent:
2.0mM diphenylcarbazide
10 %/o methanol
0.5M sulphuric acid

Detector: Visible range (365nm) 0.05 AU

Index of Ions Mentioned

	Part I Chapter	Part II Method	Part III Method
Acetate	3	1, 2 & 6	
Aconitate		2	
Alkylamines	1 & 3		1
Alkyl sulphonates	1		
Alkyl sulphates	1		
Aluminium	1		2
Ammonium	1		1
Argenticyanide		3	
Arsenate	1 & 3	1 & 2	
Arsenite	1 & 3		
Arylamines	1		
Aryl sulphonates	1		
Aryl sulphates	1		
Auricyanide		3	
Aurocyanide		3	
Azide	1 & 3	1	
Barium	1		1
Benzene Sulphonate		1	
Benzoate		1	1
Borate	1 & 3	3 & 6	
Bromate	1 & 3	1 & 2	
Bromide	1 & 3	1, 2, 3 & 5	
2-Bromoethane Sulphonate		1	
Butanoate		2	
Butylamines			1
Butyrate (Butanoate)		2 & 6	
Caesium	1		1
Cadmium	1		2
Calcium	1		1 & 2
Caprate		6	
Caproate		6	
Caprylate		6	
Carbonates	1 & 3	2, 3 & 6	
Carboxylates (linear, branched chain and unsaturated	1	1 & 2	
Chlorate	1 & 3	1 & 5	
Chloride	1 & 3	1, 2, 3, 5 & 6	
Chlorite	1	5	
Chloroaurates	1		
Chlorobenzoate		1	
Chloroferrates	1		
Chloropalladates	1		
Chloroplatinates	1		
Chloroplumbates	1		
Chromates	1 & 3	2	3
Chromium	1		3
Citrate	3	2 & 6	
Cobalt	1		2
Cobalticyanides	1	3	
Copper	1		2
Cuprocyanide	1	3	
Cyanate	1	1 & 3	
Cyanide	1 & 3	3	
Cyanoargentate	1	3	
Cyanoaurates	1	3	

	Part I Chapter	Part II Method	Part III Method
Cyanocobaltates	1	3	
Cyanocuprate	1	3	
Cyanoferrates	1	3	
Cyanonickelate	1	3	
Cyanopalladate	1		
Cyanoplatinate	1		
Cyclohexylamine			1
Dibromoacetate	1	1	
Dichloroacetate	1	1 & 2	
Diethylamine			1
Dimethylamine			1
Dithionate	1	3	
Dithionite	3		
Ethylenediamine tetraacetate	1	4	
Enanthate		6	
Ethanolamines	1 & 3		
Ethylamines (mono, di and tri)			1
Ethylene diamine (Diaminoethane)			1
Ferric iron			2
Ferricyanide	1	3	
Ferrous iron			2
Ferrocyanide	1	3	
Fluoride	1 & 3	1, 2, 5, & 6	
Fluoroborate	1 & 3	1	
Fluorophosphate (mono and hexa)	1	1 & 2	
Formate	3	1, 2 & 6	
Fumarate		2	
Galacturonate		2	
Gallium	1		
Gluconate		2	
Glucuronate		2	
Glycolate		2	
Gold	1	3	
Hexanesulphonate		1	
Hydrazine	3		1
Hydroxybutyrate (alpha and beta)		6	
Hydroxycarboxylates		6	
Hydroxyethylmethacrylate		1	
Hydroxylamine	1 & 3		1
Hydroxyvalorate (alpha)		2	
Hypochlorite	1 & 3	5	
Hypophosphite	1		
Iodate	1 & 3		
Iodide	1 & 3	3	
Iron (ferrous, ferric, chloro, cyano II and III and EDTA complexes)	1	3	2
Isobutylamine			1
Isocitrate		2 & 6	
Isopropylamine			1
Ketoglutarate (alpha)		2	

	Part I Chapter	Part II Method	Part III Method
Lactate		1 & 6	
Lanthanides	1		2
Laurate		6	
Lead	1		2
Linoleate		6	
Linolenate		6	
Lithium	1		1
Magnesium	1		1 & 2
Malate		1 & 6	
Maleate		1 & 6	
Malonate		6	
Manganese	1		2
Mercury	1		
Metaarsenite	1		
Metaphosphate (tri)	1	4	
Methylamines (mono, di and tri)			1
Methylsulphate		1	
Molybdates	1		
Monobromoacetate		1 & 2	
Monochloroacetate	1	1	
Monoethylamine			1
Monomethylamine			1
Morpholine			1
Myristate		6	
Nickel	1	3	2
Nitrate	1 & 3	1, 2, 3, 5 & 6	
Nitrilotriacetate	1	4 & 6	
Nitrite	1 & 3	1, 2, 3, 5 & 6	
Octylsulphonate		1	
Oenanthate (Enanthate)		6	
Oleate	3	6	
Organic Acids	3	1, 2 & 6	
Organosulphates	1	1	
Organosulphonates	1	1	
Orthophosphates	1 & 3	1	
Oxalacetate		2	
Oxalate	3	2 & 6	
Palladium	1		
Palmitate		6	
Pelargonate		6	
Pentanoate		2 & 6	
Pentanesulphonate		1	
Perchlorate	1 & 3	5	
Perdisulphate	1	3	
Periodate	3		
Permanganate	1		
Perrhenate	1		
Phosphate ortho	1 & 3	1, 2, 3, 4, 5 & 6	
pyro	1 & 3	4	
tetrapoly	1	4	
trimeta	1	4	
tripoly	1	4	
Phosphite	1 & 3	1 & 2	
Phosphonates poly	1	4 & 6	
Phthalates	1	2	

	Part I Chapter	Part II Method	Part III Method
Piperidine			1
Platinum	1		
Polyphosphonates		3	
Potassium	1		1
Propanoate (Propionate)		2 & 6	
Propylamines			1
Pyrophosphate	1 & 3	4	
Pyrrolidine			1
Pyrurate			1, 2 & 6
Quaternary Ammonium	1		1
Rhenium	1		
Rubidium	1		1
Selenate	3	1 & 2	
Selenite	3	1 & 2	
Silicate	1 & 3		
Silver	1		
Sodium	1		1
Stearate		6	
Strontium	1		1 & 2
Succinate		1 & 6	
Sulphate	1 & 3	1, 2, 3, 4, 5 & 6	
Sulphide	3	3	
Sulphite	1 & 3	1, 2 & 3	
Sulphonates	1	1	
Tartrate	3	1 & 6	
Tellurate	3		
Tellurite	3		
Tetrabutylammonium			1
Tetraethylammonium			1
Tetramethylammonium			1
Tetrapolyphosphate	1		
Tetrathionate	1	3	
Thallium	1		
Thiocyanate	1 & 3	3	
Thiosulphate	1 & 3	3 & 6	
Tin	1		
Titanium	1		
Toluenesulphonates		1	
Tribromoacetate	1	1	
Tributylammonium			1
Trichloroacetate	1	1	
Triethylamine			1
Trifluoroacetate	1	2	
Trimetaphosphate	1		
Trimethylamine			1
Tripolyphosphate	1		
Tripropylamine			1
Tungstate	1		
Valerate		6	
Vanadium	1		
Yttrium	1		
Zinc	1		2

Address for Correspondence

However thoroughly a method may be tested, there is always the possibility of a user discovering a hitherto unknown problem. Users with information on this method are requested to write to:

The Secretary
The Standing Committee of Analysts
The Department of the Environment (Drinking Water Inspectorate)
Romney House
43 Marsham Street
London SW1P 3PY

Department of the Environment

Standing Committee of Analysts

Membership responsible for these methods.

A. Adams	1.2 (3 occasional)	D.S. McIntyre	2
M.D.H. Amey	3	D. Mealor	3
H.T. Barnhoorn	1	D. Midgley	2
F.B. Basketter	1	J.J. Monaghan	1
G.A. Best	1	R.P. Mounce	2.3
B. Buckley	1	C.J. Nash	1
P. Chamberlain	3	H.A. Painter	1
R.A. Cochrane	3	M. Parker	3
J.L. Cocksedge	3	K.W. Petts	2
M. Cooke	3	L.R. Pittwell	1.2.3
B.T. Croll	1	L.D. Purdie	1
E. Crowther	3 (occasional)	G.F. Phillips	1
J.V. Dadswell	1	J.P. Riley	1
J. Dalziel	3 (occasional)	N. Ramsey	3 (occasional)
E. Dryburgh	1	A.P. Rowland	3
J.A. Dunning	3	D. Taylor	1
M.C. Finniear	1	K.C. Thompson	1
I. Fox	2	R.J. Vincent	1.2
M. Gardner	1	P.J. Walker	1
J.R. Garlick	1	A. Ware	1
D. Gelsthorpe	2	B.B. Wheals	3
G.I. Goodfellow	2	K. Wheatsone	1
K.J. Greedy	3	P.A. White	3 (occasional)
P. Green	2	R. White	3
A.S. Holmes	2	D. Woods	2.3
M.R. Hurcombe	1	R. Wood	1
J.G. Jones	1		
R. Law	1		

Main committee Member 1
Working Group Member 2
Panel Member 3

Printed in the United Kingdom for HMSO
Dd.0292731 9/90 C10 488/2 12521

HMSO

HMSO publications are available from:

HMSO Publications Centre
(Mail and telephone orders only)
PO Box 276, London, SW8 5DT
Telephone orders 071-873 9090
General enquiries 071-873 0011
(queueing system in operation for both numbers)

HMSO Bookshops
49 High Holborn, London, WC1V 6HB 071-873 0011 (counter service only)
258 Broad Street, Birmingham, B1 2HE 021-643 3740
Southey House, 33 Wine Street, Bristol, BS1 2BQ (0272) 264306
9-21 Princess Street, Manchester, M60 8AS 061-834 7201
80 Chichester Street, Belfast, BT1 4JY (0232) 238451
71 Lothian Road, Edinburgh, EH3 9AZ 031-228 4181

HMSO's Accredited Agents
(see Yellow Pages)

and through good booksellers

£25.00 net

ISBN 0-11-752331-5