Journal of Chromatography, 262 (1983) 61–76
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,737

MULTIPLE COLUMN AND DETECTOR APPROACH TO ANION SCREENING BY ISOCRATIC HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received January 26th, 1983)

SUMMARY

A high-performance liquid chromatographic method for detecting and characterising a wide variety of inorganic and organic anions in a range of substrates is described. Separations are achieved on columns packed with micro-particulate silica modified to display strong anion-exchanging properties, or a conventional C_{18} packing material. All columns are eluted under isocratic conditions with a single solvent system which incorporates an ion-pairing agent and has been formulated to permit monitoring with ultra-violet, electrochemical, refractive index and conductivity detectors; in the case of the ultra-violet detector the eluent composition allows both absorbing and non-absorbing anions to be sensed. A preparative method for making strong anion exchangers is also described.

INTRODUCTION

Characterising samples of unknown composition is a facet of forensic science that can pose problems and the qualitative analysis of anions is an area which can prove particularly difficult. Compared with other types of analysis this field continues to rely extensively on the use of spot tests based on specific chemical reactions and quantitation by colorimetry. Such methods often produce ambiguous results when mixtures are encountered and the innovative work of Small *et al.*[1] has illustrated how a chromatographic approach to anion analysis can provide many benefits. The commercialisation of Small's method as "Ion Chromatography"[2] has been highly successful, but the equipment is expensive and there are some disadvantages in its operation. This has prompted research into alternative methods of anion analysis using conventional high-performance liquid chromatographic (HPLC) equipment and publications have appeared in which UV absorbance[3,4], refractive index[5] and conductimetric[6] detectors have been used to monitor eluates for the presence of anions.

The study reported in this paper was initiated to provide an HPLC method for screening solutions for anions and places particular emphasis on characterising inorganic and organic anions by providing a range of retention time and response

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ratio data. One eluent has been formulated that can be used with any combination of several different columns and detectors; this is a particularly convenient way of enhancing qualitative information in HPLC and has been described before in a different context[7].

EXPERIMENTAL

The packing materials

Five packing materials were studied all of which were based on chemically modified microparticulate silica; one was a conventional "end-capped" C_{18} modification and the others were strong anion exchangers (SAXs) synthesised by a new procedure. The base silica used throughout had the following characteristics: particle size, 3–7 μm; pore diameter, 130 Å (mean value); pore volume, 1.25 ml/g; surface area (BET), 320 m^2/g.

The C_{18} modification was carried out as follows. The silica was dried overnight at 150°C and 50 g were refluxed for 1 h with 250 ml of xylene (sodium dried) and 25 ml of octadecyltrichlorosilane. 25 ml of trimethylchlorosilane were then added and refluxing was continued for a further hour. The product was filtered through a sintered glass filter funnel and washed several times with xylene, then acetone and finally methanol. The packing was dried in a vacuum oven at 70°C.

The strong anion exchangers were prepared by mixing 100 g of dried silica with 500 ml of xylene and 100 ml of 3-glycidoxypropyltrimethoxysilane. The suspension was heated at 45°C for 24 h in a stoppered conical flask mounted in a water-bath. The flask was swirled intermittently, and at the end of the reaction the product was filtered and dried as described above. 20-g quantities of the product were mixed with 0.1 *M* of a tertiary amine (triethylamine, tributylamine, trihexylamine and trioctylamine were studied) and 200 ml of xylene, and were heated as before for 24 h at 45°C. The resulting anion exchangers were filtered off, washed and dried as before. An estimate of the organic content of all packing materials was made by ashing at 600°C.

The packing materials were slurry packed into 5 mm I.D. stainless-steel columns using methanol or chloroform–methanol (1:1) as the slurrying solvent. The lengths of column packed were 12.5 cm (for the SAX materials) and 5 cm (for the C_{18}). The columns were terminated with 1/4–1/16 in. zero dead volume end fittings and the packing materials were retained by using stainless-steel mesh discs. In addition to the above columns several commercially available SAX materials were assessed under the same eluent conditions.

The eluent

This was prepared by dissolving 1.75 g of citric acid and 0.125 g of Cetrimide (cetyltrimethylammonium bromide) in 2.5 l of water–methanol (70:30). The pH of the solution was then adjusted to 5.5 by the dropwise addition of ammonium hydroxide. To rapidly equilibrate the C_{18} column it was found advisable to pump an eluent with an eight-fold higher concentration of Cetrimide for about 2 h. Eluent was pumped at 1 ml/min using an LDC Consta-Metric I pump, and before entering the analytical column was passed through a 10-cm column packed with a coarse grade of silica, which acted as a way of enriching the silica content of the eluent.

Detection

Four different monitoring principles were investigated and details of the detectors used are summarised below:

UV absorbance	Knauer filter photometer operated at 220 nm or an LDC SpectroMonitor III variable wavelength UV detector operated between 210 and 230 nm
Electrochemical	Metrohm Model 656 electrochemical detector operated in the oxidation mode at an applied potential of 1 V. The amplifier used was a variant on one previously described[8]
Refractive index	Knauer differential refractometer Model 98
Conductivity	Applied Chromatography Systems conductivity detector Model No. 750/15

The detectors were either used singly or more frequently with two mounted in series. The sensitivity conditions, etc. were adjusted to suit the type of samples being examined.

Sample preparation

The eluent described above has relatively little buffering capacity and whenever possible it was found advisable to adjust the pH of the sample to within 0.5 pH unit of that of the eluent. For this purpose ammonium hydroxide or citric acid was used. Samples were diluted with either water or the eluent and injections were made via a valve injector with a 20-μl loop (Negretti and Zambra or Rheodyne).

RESULTS AND DISCUSSION

The packing materials

The organic content of the various packing materials used in this study is indicated in Table I. Those materials studied were of two distinct types. The C_{18} material was converted to an *in-situ* anion exchanger by extracting the lipophilic

TABLE I

THE WEIGHT LOSS ON ASHING THE PACKING MATERIALS USED IN THIS STUDY

Nature of the chemical modification	*Code*	*% Weight loss**
Octadecyl	C_{18}	18.4
Glycidoxypropyl	–	9.0
Triethylamine/glycidoxypropyl	SAX 1	10.0
Tributylamine/glycidoxypropyl	SAX 2	10.4
Trihexylamine/glycidoxypropyl	SAX 3	11.0
Trioctylamine/glycidoxypropyl	SAX 4	13.0

* The value shown was calculated using the following relationship:

$$\% \text{ Weight loss} = \frac{\text{weight loss at } 600°C \times 100}{\text{residue weight at } 600°C}$$

TABLE II

ANION RETENTION VOLUMES ILLUSTRATING THE EFFECT OF ADDING AN ION-PAIRING AGENT TO THE ELUENT

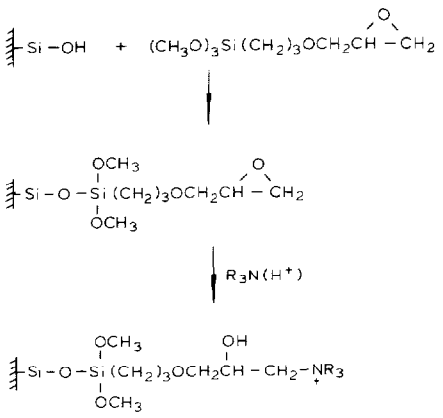
The columns used in providing the above data were 12.5 cm × 5 mm. The packing material code is as in Table I. + Indicates an eluent with Cetrimide, − indicates an eluent without Cetrimide. The eluent composition was as follows: 1.75 g of citric acid (with or without 0.125 g of Cetrimide) were dissolved in 2.5 l of water–methanol (70:30) and the pH was adjusted to 5.5 with ammonium hydroxide. The molar concentrations were 0.004 *M* in citric acid and 0.0001 *M* in cetrimide. N.E. = Not eluted in 60 ml.

Anion	*SAX 1*		*SAX 2*		*SAX 3*		*SAX 4*	
	+	−	+	−	+	−	+	−
Non-retained	1.8	1.8	1.8	1.8	1.8	1.8	1.9	1.9
Iodate	3.1	3.0	3.0	2.9	2.8	2.8	3.1	3.1
Bromate	3.5	3.4	3.3	3.2	3.1	3.1	3.5	3.3
Nitrite	3.2	3.1	3.6	3.3	3.4	3.3	4.6	4.5
Nitrate	3.6	3.5	3.9	3.7	3.8	3.7	6.4	5.6
Maleate	3.8	3.6	4.3	4.1	4.9	4.5	10.0	9.0
Phenylacetate	4.0	3.8	4.2	4.0	4.4	4.2	10.8	9.4
Benzoate	4.4	4.2	4.7	4.4	5.0	4.9	12.0	11.2
Cinnamate	6.2	5.9	7.0	6.4	8.4	8.1	29.5	26.0
Salicylate	6.2	6.0	8.2	6.9	11.2	10.4	N.E.	39.6

quaternary ammonium cation from the eluent until a dynamic equilibrium was established. By way of contrast the packings prepared via the glycidoxypropyl/trialkylamine route have inherent anion-exchanging properties. Even so the retention of anions on these SAX materials was modified by the presence of Cetrimide in the eluent. This is shown in Table II, and it is apparent that organic anions in particular are retained to a greater extent when the eluent contains the ion-pairing agent: the effect being enhanced as the alkyl chain length of the tertiary amine increases. It was assumed that the cluster of alkyl groups around the quaternary nitrogen atom in these anion exchangers forms a lipophilic site capable of binding with the ion-pairs formed by the interaction of organic analyte ions and the pairing agent.

Commercial anion exchangers for use in HPLC are based either on chemically modified silicas or are polymeric beads of the styrene/divinylbenzene class which have tetraalkylammonium groups introduced[9]. The methods used in preparing such packings are usually proprietary secrets, but several methods for making silica-based anion exchangers have been described in the literature. Weak anion exchangers can be made by reacting silylating agents such as 3-aminopropyltrimethoxysilane or 3-(2-aminoethylamino)propyltrimethoxysilane[10]. Strong anion exchangers are invariably made by a two-part reaction. The first stage introduces a reactive surface coating bonded to the silica support via a siloxane bond, and the second stage of the process involves reaction with a compound containing an amino group[11,12]. 3-Glycidoxypropyltrimethoxysilane has been shown to be a valuable reactive modifier and weak anion exchangers have been prepared in both aqueous[13] and non-aqueous[14] media. During experiments with reactions of the latter type it was found that glycidoxypropyl modifications yielded products with tertiary amines that displayed useful anion-exchanging properties. The structure of the products has not been established but

their chromatographic properties are explicable by a reaction sequence of the type shown below:



The catalysis of epoxy resins by tertiary amines is believed to proceed via an analogous route to that described above[15].

The chromatographic properties of the various packings displayed the expected dependence on eluent pH, ionic strength and methanol content[16] and the effects of altering these parameters are mentioned in the discussion of eluent composition. Tables II and III summarise the chromatographic data obtained with the various packing materials. It is apparent that the retention characteristics of the SAX materials were influenced by the nature of the tertiary amine used in their preparation and increasing the alkyl chain length had the following effects:

(I) The elution sequence of most inorganic anions remained essentially unchanged.

(II) The retention of most inorganic anions increased slightly.

(III) The retention of organic anions increased substantially.

In the case of the C_{18} packing the retention of aromatic anions was so strong that they were not eluted, whereas aliphatic and inorganic anions eluted in an acceptable time. To use the described columns for screening purposes necessitates a preliminary run on a SAX material (*e.g.*, SAX 1) to permit the elution of both inorganic and organic anions followed by a confirmatory separation performed on a C_{18} or more lipophilic SAX packing (*e.g.*, SAX 4) which gives greater retention and discrimination. Fig. 1 shows the separation of a test mixture of anions on the five column systems investigated and Fig. 2 shows the separation of anions of aromatic acids on the four SAX columns (note these acids did not elute from the C_{18} column). The chromatograms in Fig. 2 provide a graphic illustration of the data summarised in Table II.

The ion-exchange capacities of these materials were not measured directly, but when compared with commercially available silica-based anion exchangers they were found to be less retentive than LiChrosorb AN (Merck), slightly more retentive than Zorbax SAX (DuPont) and substantially more retentive than Whatman SAX (Whatman). With the described eluent none of these proprietary anion exchangers gave

TABLE III

RETENTION VOLUME, DETECTOR RESPONSE AND SENSITIVITY DATA ON ANIONS

The chromatographic conditions were as described in the text, but note that the C_{18} column was 5 cm in length, whereas the two SAX columns were 12.5 cm long. The symbols for detector response have the following significance: UV detector, + = increased absorbance, − = decreased absorbance; conductivity detector, + = increased conductivity, − = decreased conductivity; electrochemical detector, + = those anions giving a response at an applied potential of 1 V. The sensitivity data shown gives an indication of the minimum weight of a particular anion which must be injected on to the system of minimum retention to give a detectable chromatographic peak. The code used was as follows: a = less than 1 ng; b = 1–10 ng; c = 10 100 ng; d = 100–1000 ng. Anions not detected included: fluoride, cyanide, carbonate, bicarbonate, bromide and citrate.

Anion	*Retention volume (ml)*			*Detector response*			*Sensitivity*	
	C_{18}	*SAX 1*	*SAX 4*	*UV*	*Elec.*	*Cond.*	*UV*	*Elec.*
Non-retained	0.6	1.8	1.9					
Iodate	1.4	2.9	3.1	+		−	b	
Phosphate	1.7	3.0	3.0	−		−	c	
Lactate	1.8	3.0	3.3	−		−	d	
Acetate	2.2	3.0	3.4	−		−	d	
Formate	2.5	3.4	3.8	−		+	d	
Chloride	2.6	3.1	3.8	−		+	c	
Bromate	3.0	3.1	3.5	+		+	b	
Nitrite	3.8	3.2	4.6	+	+	+	b	a
Succinate	4.8	4.8	5.8	−		−	d	
Nitrate	5.6	3.6	6.4	+		+	b	
Malonate	5.6	4.5	5.9	−		−	d	
Chlorate	8.8	3.4	6.7	−		+	c	
Tartrate	9.0	6.8	8.6	−		+	d	
Maleate	9.2	3.8	10.0	+		−	b	
Sulphate	11.0	7.6	9.2	−		+	c	
Fumarate	11.2	8.6	13.0	+		+	c	
Oxalate	13.6	8.2	11.0	+	+	+	c	b
Iodide	16.8	4.0	13.0	+	+	+	b	a
Thiosulphate	31.0	8.6	12.8	+	+	+	b	a
Thiocyanate	47.6	4.8	32.8	+	+	+	b	a
Phenylacetate	N.E.	4.0	9.5	+		−	b	
Benzoate	N.E.	4.4	12.0	+		−	b	
Cinnamate	N.E.	6.2	29.5	+		−	b	
Salicylate	N.E.	6.2	N.E.	+		−	b	
o-Phthalate	N.E.	7.3	N.E.	+		−	b	

separations as good as those achieved with the laboratory-made materials. However, it is fair to say that no attempt was made to adjust the eluent conditions to optimise separations on these packings.

A problem encountered throughout this work was a slow but measurable decrease in the retention times of anions. No complete solution to this was found but a series of precautions reduced the deterioration substantially. These were based on the assumption that the major cause of difficulty was attributable to silica dissolution in the highly aqueous media used in anion-exchange chromatography, and evidence to support this was generated by the atomic absorption analysis of eluates for silicon. With completely aqueous eluents it was found that the first column volume of eluate

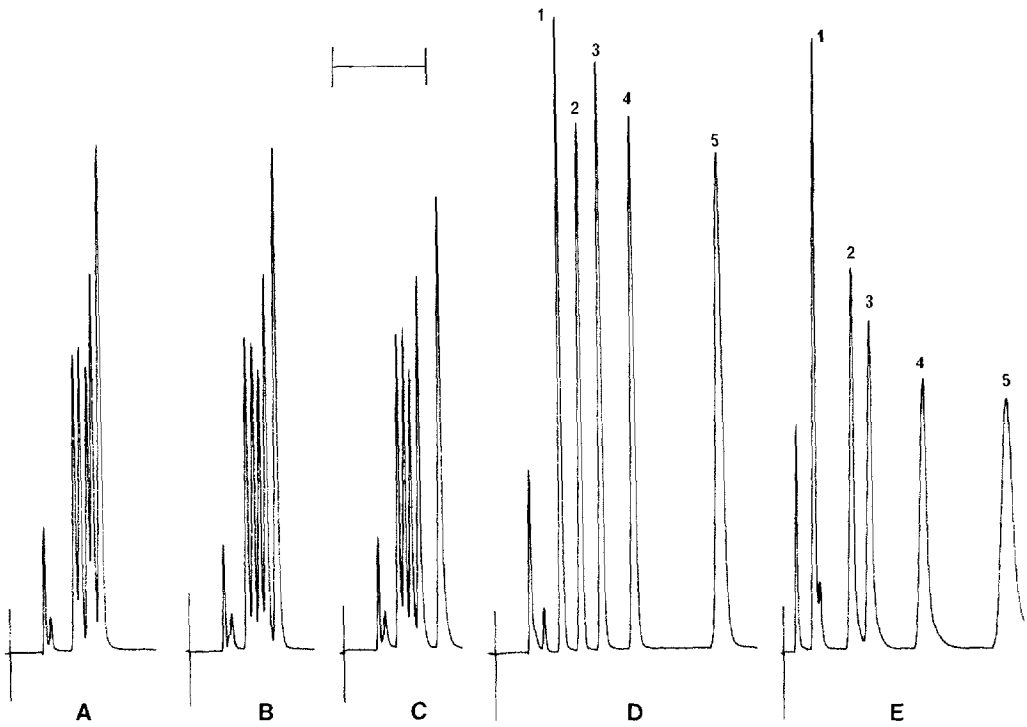


Fig. 1. The separation of UV-absorbing anions on various columns eluted with a single solvent. Columns: A–D, 12.5 cm in length; E, 5 cm in length; A = SAX 1; B = SAX 2; C = SAX 3; D = SAX 4; E = C_{18}. Test anions: 1 = iodate; 2 = bromate; 3 = nitrite; 4 = nitrate; 5 = maleate; 1.6, 4.0, 0.4, 0.4 and 0.3 μg of potassium salts injected. Sensitivity: A, B, C, 0.2 a.u.f.s.; D, E, 0.1 a.u.f.s. The scale on this and all subsequent chromatograms is such that the elapsed time marked corresponds to 5 min.

after overnight standing contained silicon levels of *ca.* 15–20 μg/ml, falling to less than 1 μg/ml on subsequent elution at 1 ml/min. By adding 30% of methanol to the eluent the silicon concentration in the first column volume was reduced to *ca.* 10–15 μg/ml. Introducing a pre-column containing silica[17] between the pump and injector reduced the rate of deterioration, presumably by acting as a means of increasing the silica content of the eluent before it contacted the analytical column. It was also found that the more hydrophobic packings (*i.e.*, the C_{18} and SAX 4, see Table I) were more stable than those SAX materials prepared from amines of shorter chain length. Columns could usually be used for periods of 2–3 months, but during that time overall retention times tended to fall, sometimes as much as 10–30%. Nevertheless virtually no change in retention was noticeable during a working day.

The eluent composition

The eluent used in this study was arrived at after considerable experimentation. It had to:

(1) Act as an ion-pairing medium in order to give separations on the C_{18} column.

(2) Contain a multivalent counter-ion to permit the rapid elution of mono- and divalent anions under isocratic conditions.

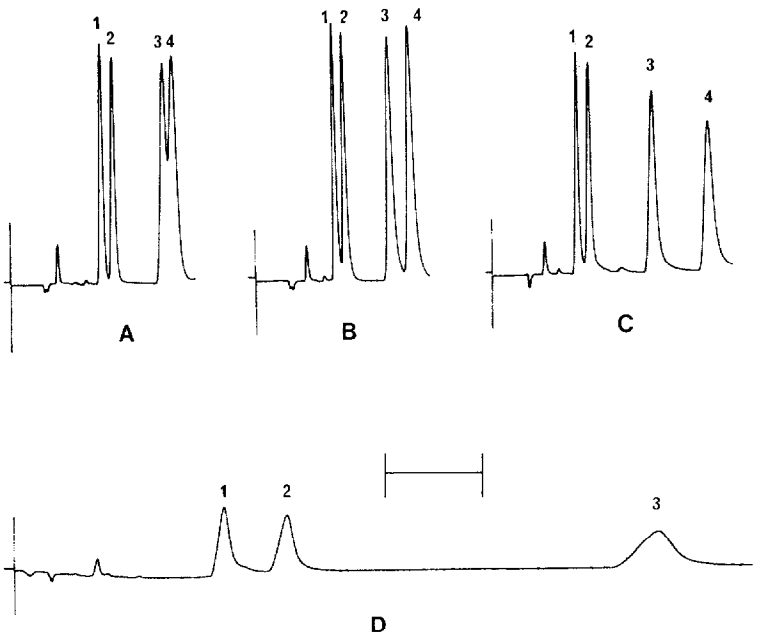


Fig. 2. The separation of anions of aromatic acids on various columns eluted with a common solvent. Columns: length and coding as indicated in Fig. 1. Test anions: 1 = phenylacetate; 2 = benzoate; 3 = cinnamate; 4 = salicylate. Sensitivity: 0.2 a.u.f.s. All other conditions as indicated in text.

(3) Have some UV absorbance in the 220-nm region to allow both UV absorbing and non-absorbing anions to be detected.

(4) Have a low ionic strength to facilitate conductivity monitoring.

(5) Have some buffering capacity to allow reproducible chromatography.

(6) Have as high a methanol content as possible to reduce silica dissolution.

(7) Contain counter-ions that were unlikely to be of major importance in the samples being screened. With the described eluent, for example, citrate and bromide anions are precluded from being detected in samples.

(8) Permit both organic and inorganic anions to be eluted.

The ion-pairing agent incorporated in the eluent was Cetrimide (cetyltrimethylammonium bromide) which has been used before to separate inorganic anions[3]. The reagent is obtainable in the hydroxide form, and although this was not tested it should have the advantage of permitting bromide ions to be detected. Citric acid with pK_a values of 3.1, 4.7 and 6.4 proved to be an effective counter-ion, and by having some trivalent character at pH 5.5 was able to displace most mono- and divalent anions from columns in an acceptable time. In addition citric acid has some UV absorbance in the desired region, and the importance of this property will be described later. Lowering the pH, ionic strength or the methanol content of the eluent gave rise to increased retention of anions, and hence control of these parameters is important.

Detection

During the early stages of developing the described method eluents were used

which had negligible UV absorbance in the 210–230 nm region. At these wavelengths several inorganic anions absorb strongly, *e.g.*, nitrite, nitrate, bromide, iodide, etc., and provided they can be made to elute, UV monitoring is an excellent method of detection. Unfortunately several important inorganic anions, *e.g.*, chloride, phosphate and sulphate remain undetected and their elution was initially monitored by having a conductivity detector mounted in series with the UV detector. Subsequently in attempting to study the effects of different counter-ions in the eluent it was necessary to work with a background absorbance between 1.0 and 0.1 in the 210–230 nm region, and it was then noticed that negative peaks occurred when a non-absorbing anion eluted. This detection phenomenon was obviously very useful as it permitted the screening of absorbing and non-absorbing anions with a UV monitor. Small and Miller[18] coined the expression "indirect photometric chromatography" for separations of ions detected by this method and others[19,20] have also observed the effect. In two of the papers cited the eluent composition and the UV monitoring region are such that all anions give a negative response whereas in the work described in this publication some anions absorb and some do not. The additional sensitivity and extra qualitative information accorded by the latter approach (*i.e.*, the response may be positive or negative) are points in its favour.

Despite the difference in the detection strategy both approaches exploit a phenomenon which arises from the fact that when an ion-exchange process governs a separation the passage of analyte ions down the column is accompanied by a fall in the counter-ion concentration in order to maintain electro-neutrality. Provided there is a difference between the molar extinction coefficient of the analyte and the counter-anion at the monitoring wavelength then a baseline change will occur as the former passes through the detector. The eluent described has a background absorbance resulting from the presence of citrate and bromide counter-ions, and has a value of about 0.5 units at 220 nm. The slope of the absorption curve of the eluent is quite steep in the 210–230 nm region, but the "indirect photometric effect" is observable throughout this wavelength region provided the detector and recorder have adequate backing-off facilities. With a variable wavelength detector it is possible to "tune" to an optimum wavelength depending on the type of sample mixture being examined.

In using an electrochemical detector it is usually necessary to operate at an applied voltage which gives an acceptable response from materials of interest without generating a high standing current from the eluent, and it was found that an applied voltage of 1 V was about optimum in the work described. Under these conditions the electrochemical detector was found to be highly selective and only nitrite, iodide, oxalate, thiocyanate and thiosulphate were detectable amongst the various inorganic anions tested. This high selectivity is illustrated in Fig. 3 which shows the chromatogram produced by the sequential monitoring of an anion mixture with UV and electrochemical detectors. The electrochemical detector is also highly sensitive to the anions mentioned and this is brought out in Table III. The refractive index and conductivity detectors lack the sensitivity displayed by the previously mentioned detectors, but this can be advantageous in some instances. For example, it is useful to have a means of distinguishing major from minor components and mounting a UV and RI detector in series can aid in this situation. The conductivity detector, which we found even more sensitive to temperature fluctuations than the RI detector has

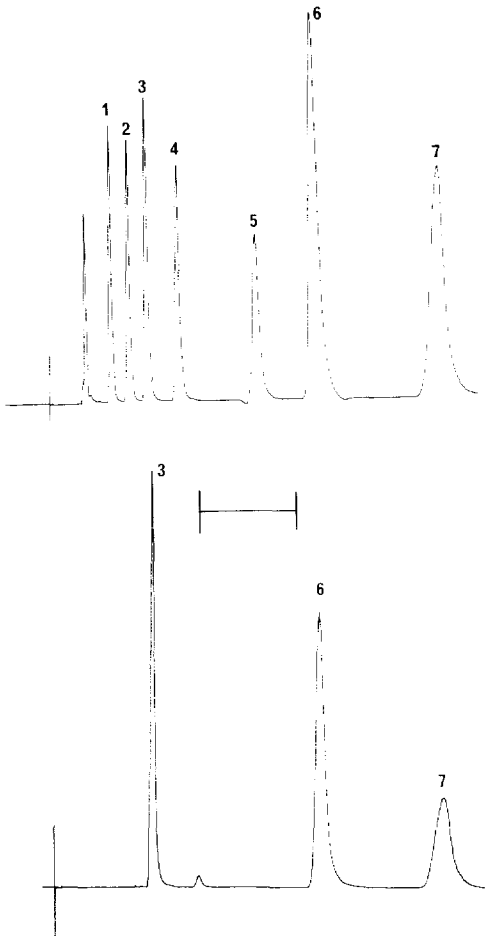


Fig. 3. A comparison of UV absorbance and electrochemical detection for the detection of anions. Column: 12.5 cm in length packed with SAX 4. Test anions: 1 = iodate; 2 = bromate; 3 = nitrite; 4 = nitrate; 5 = maleate; 6 = iodide; 7 = thiosulphate; 2, 4, 0.4, 0.4, 0.2, 0.4 and 8 μg injected as potassium salts. Top = UV detection at 0.2 a.u.f.s. Bottom = Electrochemical detection. All other conditions as indicated in text.

the merit of providing additional qualitative information in that it can respond in a positive or negative sense in a manner analogous to the UV monitor; the information is summarised in Table III. Quantitative analysis is possible with all four modes of detection, and requires the usual construction of calibration curves using known standards. When exploiting the "indirect photometric effect" for quantitation it is necessary to work at sample dilutions below about 0.004 M. This corresponds to the molarity of the citric acid in the eluent and if analyte concentrations greatly in excess of this are used the curve will flatten off into a plateau form. Clearly once the citrate counter-ion has been exceeded by the analyte the absorbance of the solution will not greatly change.

Applications

The method described in this paper has been in routine use in this Laboratory for about a year and has been applied to a wide range of forensic samples. The major precaution that needs to be taken is to adjust the pH of samples injected on to the chromatographic columns to *ca.* 5–6. Failure to do this gives rise to a long retained peak which can confuse the interpretation of chromatograms. Some of the applications of the methods are described below.

Strong mineral acids. Characterising and quantitating strong mineral acids in admixture has been a common problem and is amenable to an HPLC approach. Fig. 4 shows the chromatograms of a mixture of the four common acids when monitored by three different detectors. Only nitric acid displays UV absorbance at 220 nm and hence UV detection offers a sensitive way of detecting nitrate anions. The other anions exhibit the "indirect photometric effect" with the UV detector, and the phosphate anion gives a different response on the conductivity detector to the other three anions.

Waters. The high sensitivity of the procedure for nitrate makes it useful for comparing water samples, because tap and river waters vary appreciably in their nitrate level. Fig. 5 shows the chromatograms of three different tap waters and chloride, nitrate and sulphate anions present in each 20-μl water sample can be directly

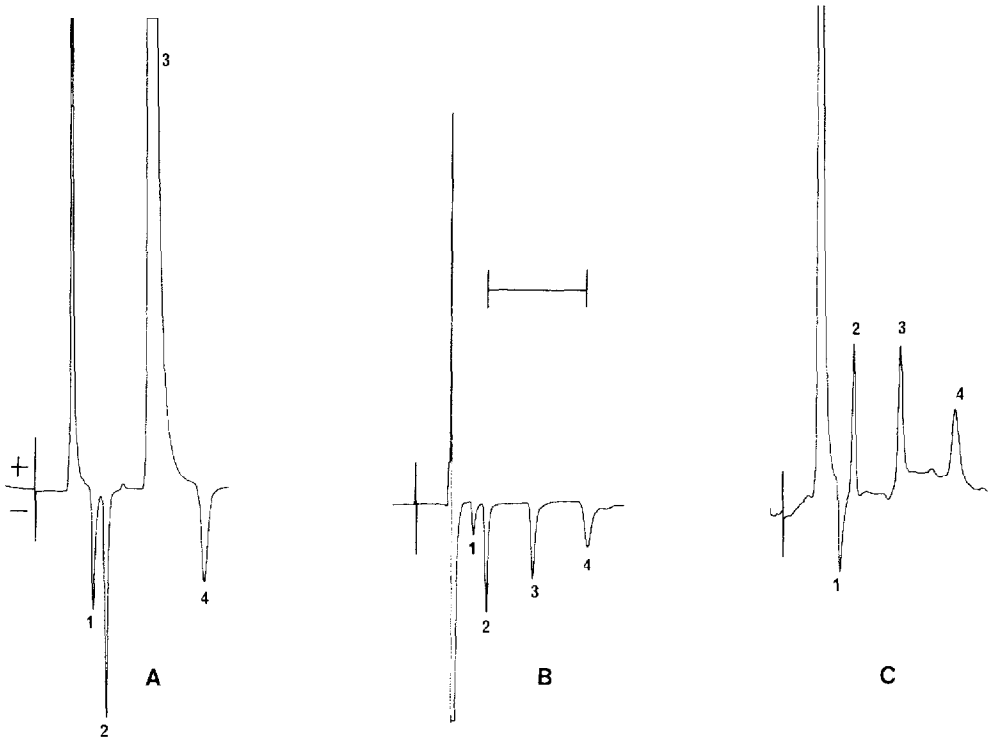


Fig. 4. The separation of the potassium salts of strong mineral acids. Column: 12.5 cm packed with SAX 4. Detection: A, UV absorbance, 0.1 a.u.f.s.; B, RI; C, conductivity. Test anions: 1 = orthophosphate; 2 = chloride; 3 = nitrate; 4 = sulphate; 4 μg of each (as potassium salt) injected. All other conditions as indicated in text.

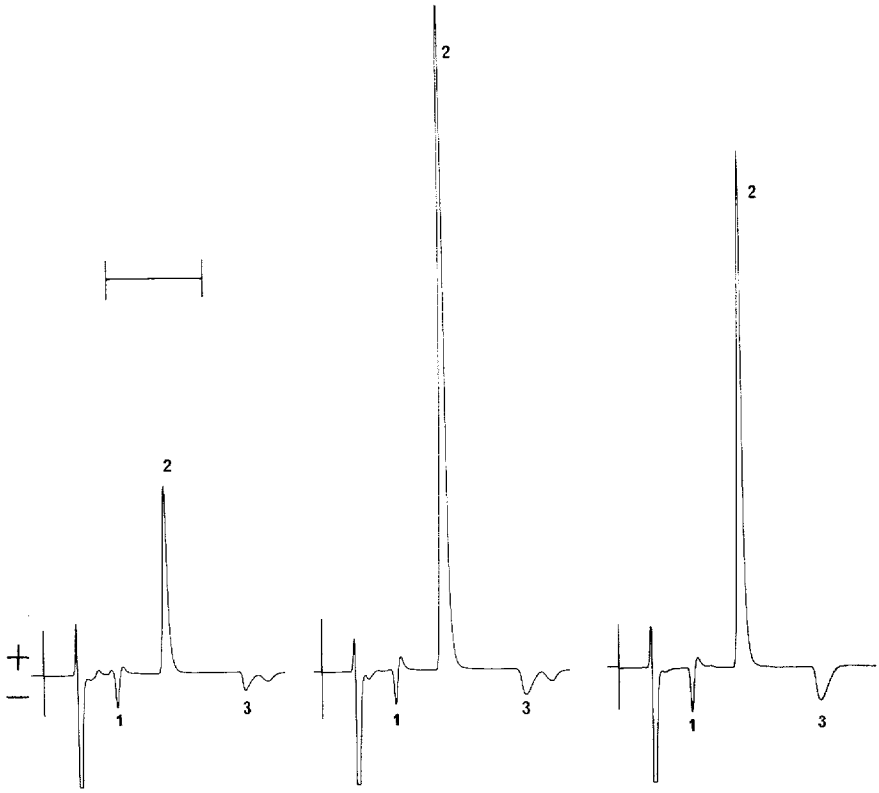


Fig. 5. The detection of anions in tap waters. Column: 12.5 cm packed with SAX 4. Detection: UV, 0.2 a.u.f.s. Ions detected: 1 = chloride; 2 = nitrate; 3 = sulphate. Sample size: 20 μl. All other conditions as described in text.

monitored. The wide variation of nitrate levels (it ranged from 2.5 to 10 mg/l in the tap waters tested) makes discrimination possible. None of the tap waters tested exceeded the World Health Organisation recommended limit of 11.3 mg/l of nitrate nitrogen.

Soils. Soil samples can be readily compared for extractable anions by adding 1 ml of eluent to 10 mg of soil, and after adequate shaking and centrifuging directly injecting a 20-μl aliquot of the supernatant on to the columns. Fig. 6 illustrates typical chromatograms monitored by UV and electrochemical detector. The UV traces show that the proportions of several UV-absorbing species in these soils displayed variation and the electrochemical detector confirms the presence of nitrite in each sample in addition to another unidentified oxidisable component.

Body fluids. A particularly interesting illustration of the value of the electrochemical detector in providing selectivity and sensitivity is when it is applied to the examination of body fluids. Fig. 7 shows chromatograms of saliva, urine and blood serum following sub-dilution in eluent. The UV trace of the saliva sample is not easy to interpret as it contains several unresolved UV-absorbing species and a negative peak due to the presence of chloride. The electrochemical detector simplifies the chromatogram and reveals the presence of uric acid, nitrite and thiocyanate anions

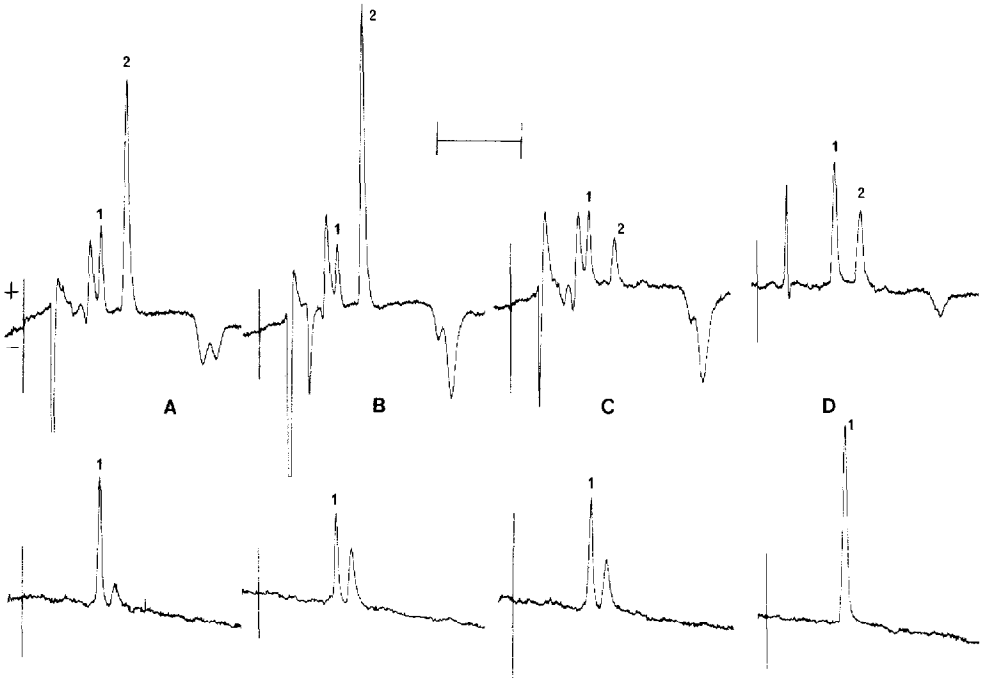


Fig. 6. The detection of anions in soil extracts. Column: 12.5 cm packed with SAX 4. Detection: top, UV detection at 0.01 a.u.f.s.; bottom, electrochemical detection. Samples: A, B, C are soil extracts (10 mg soil per ml); D = a mixture of nitrite and nitrate, 0.5 and 0.9 ng (as potassium salts) respectively. 20 μl injected in each case. Ions detected: 1 = nitrite; 2 = nitrate. All other conditions as indicated in text.

(all characterised by retention volume, and response ratio comparison); all have previously been reported as present in saliva. The column used for the separation SAX 1 did not adequately resolve nitrite and nitrate anions but by repeating the analysis on more retentive columns (*e.g.*, C_{18} or SAX 4) these two anions are readily distinguished, although thiocyanate becomes long retained. The saliva sample illustrated contained nitrite, nitrate and thiocyanate anions at concentrations of *ca.* 7, 30 and 85 μg/ml (as potassium salts) respectively. The formation of nitrite from nitrate in the saliva could be easily followed by the method and has been attributed to the presence of reducing bacteria in the mouth. The blood serum and urine samples whilst both containing uric acid did not contain the other anions noted in the saliva sample, although there is a small peak which might be attributable to nitrite in the serum sample. The high sensitivity of the electrochemical detector for nitrite anions has shown a previously unnoticed problem in that all the low-cost, disposable, screw-capped, glass vials used in sub-diluting samples contain from 0.1 to 0.6 μg of nitrite (vial capacity 2–10 ml). Careful washing with de-ionised water was an essential precaution.

Paint resin analysis. An example of an application of the method in organic anion analysis is illustrated in Fig. 8 in which the *ortho* and *meta* isomers of benzenedicarboxylic acid (*i.e.*, phthalic and isophthalic acids) are separated. Alkyd resins, which are the organic binder used in most decorative gloss paints, are polyesters in

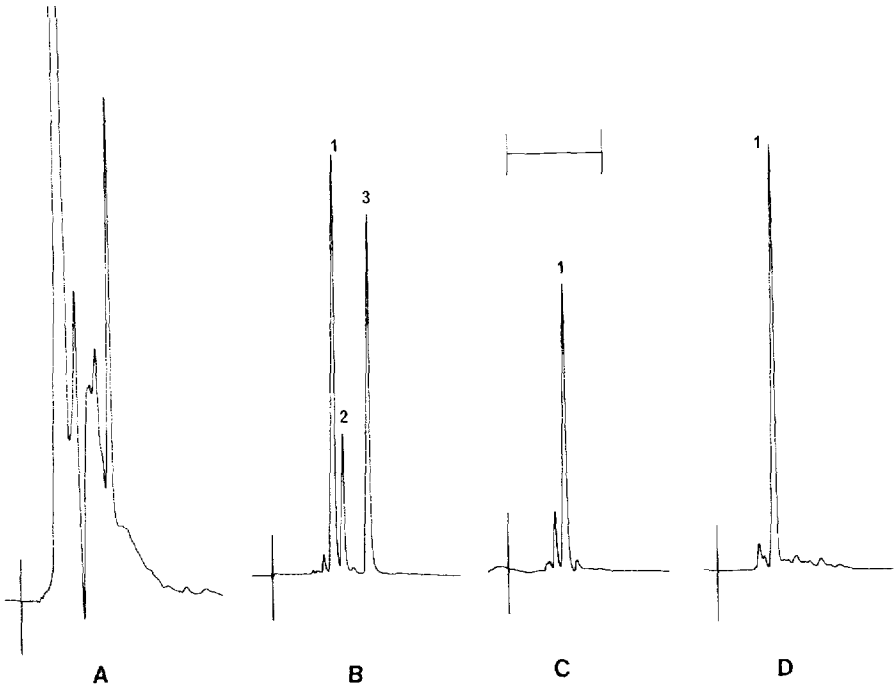


Fig. 7. The detection of anions in body fluids. Column: 12.5 cm packed with SAX 1. Detection: A, UV at 0.2 a.u.f.s.; B, C, D, electrochemical detector. Samples: A, B = 1 part saliva to 2 parts eluent; C = 200 μl of blood serum in 1 ml of eluent; D = 50 μl of urine in 1 ml of eluent; 20 μl of each solution was injected. Ions detected: 1 = uric acid; 2 = nitrite; 3 = thiocyanate. All other conditions as indicated in text.

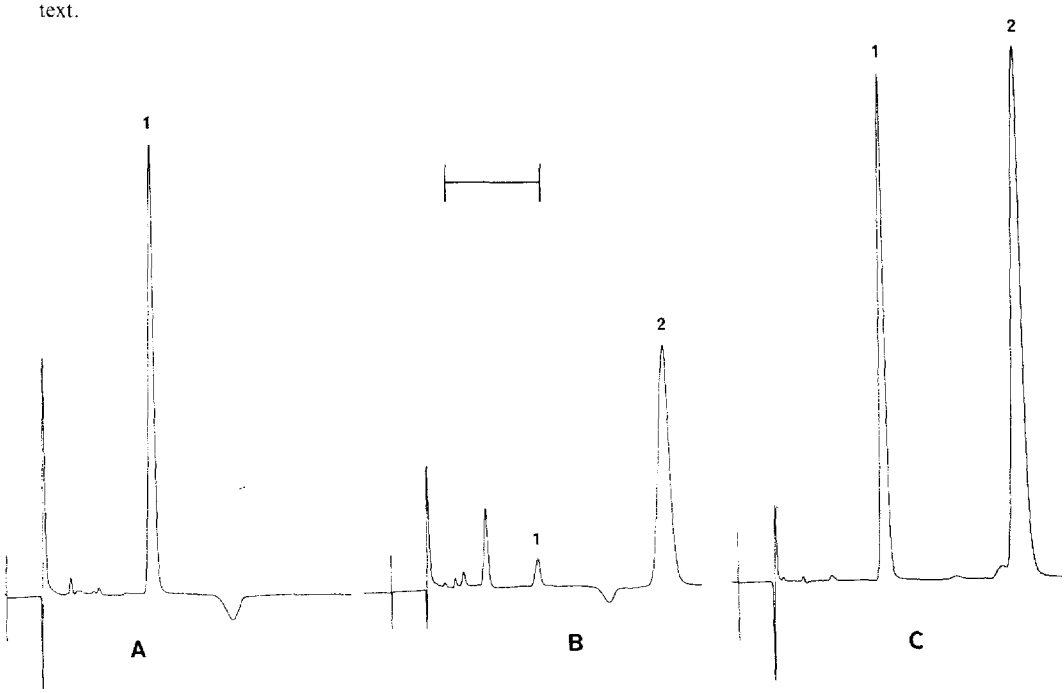


Fig. 8. The detection of phthalic and isophthalic acid in paint hydrolysates. Column: 12.5 cm packed with SAX 1. Detection: UV at 0.2 a.u.f.s. Samples: A and B = paint hydrolysates (the equivalent of 0.4 and 0.5 μg of paint was injected); C = mixture of phthalic acid (1) and isophthalic acid (2). All other conditions as described in text.

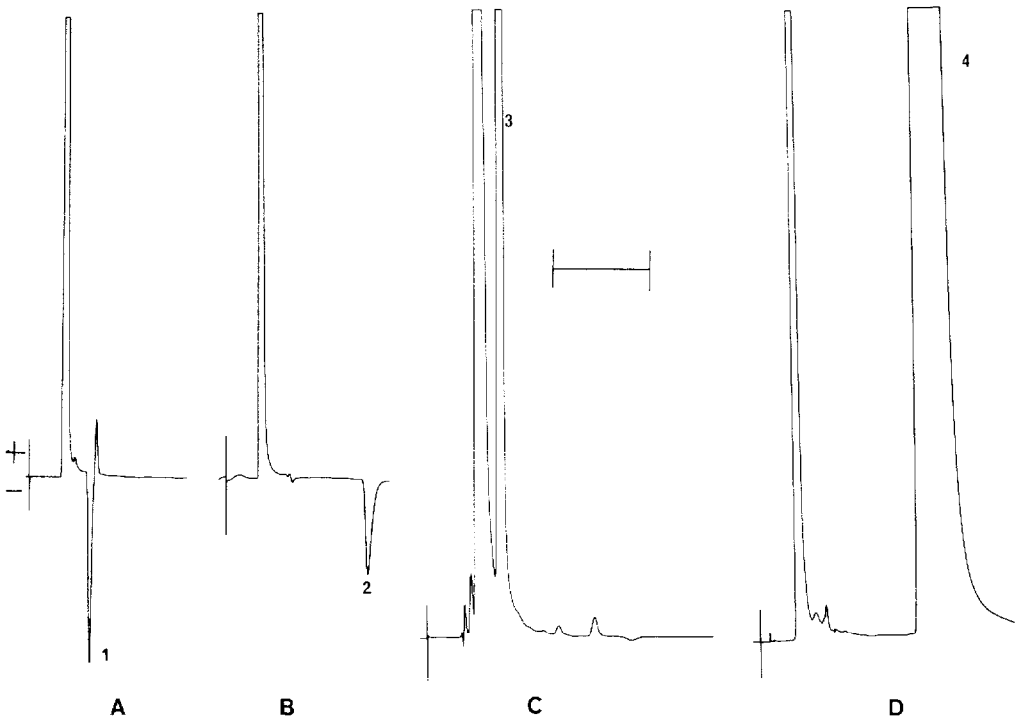


Fig. 9. The detection of anions in solutions of basic drugs. Column: 12.5 cm packed with SAX 1. Detection: UV at 0.2 a.u.f.s. Samples: A = amphetamine chloride; B = amphetamine sulphate; C = dexbromphen-iramine maleate; D = dextropropoxyphene napsylate. About 20 μg of each sample injected. Ions detected: 1 = chloride; 2 = sulphate; 3 = maleate; 4 = naphthyl sulphonate. All other conditions as described in text.

which the two acids may occur as major components. The free acids can be readily liberated from a dried paint flake by hydrolysis with methanolic potassium hydroxide, and the hydrolysate analysed by the HPLC method. The resins in the two paint film hydrolysates illustrated are quite different, one containing only phthalic acid and the other mainly isophthalic acid. Although the small size of most forensic paint flakes precludes using this type of method for comparative analysis, it is useful in correlating other methods (*e.g.*, pyrolytic procedures) with the chemical composition of paints.

Characterising the salt forms of basic drugs. Basic drugs are invariably formulated as their salts and for some purposes, particularly in a forensic context, it is required to know the nature of the anion present. The HPLC method being applicable to the separation of both inorganic and organic anions can be readily applied to this type of problem. Fig. 9, for example shows chromatograms of amphetamine chloride and sulphate and two other basic drugs in which the counter-ion present is organic in character. In most cases the basic drug, being cationic when dissolved in the HPLC eluent, is not retained on the columns, but in some instances retention due to lipophilic interactions can occur, and hence retention alone cannot be taken as evidence of the presence of an anion.

Further developments

The method described in this paper was specifically developed as a screening method and inevitably represents a compromise. For the separation and detection of known anions the eluent could be adjusted to provide greater resolution and sensitivity. If SAX materials alone were used the ion-pairing agent could be eliminated, and if only UV-absorbing anions were of interest the citrate counter-ion could be replaced by a non-absorbing anion such as phosphate. Similarly in the choice of detector there is a wide scope for optimising the method to suit a specific application; clearly the HPLC approach to anion analysis is full of potential.

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