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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF INORGANIC AND ORGANIC IONS USING LOW-CAPACITY ION-EXCHANGE COLUMNS WITH INDIRECT REFRACTIVE INDEX DETECTION

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SUMMARY

Non-UV absorbing inorganic and organic anions and cations may be detected by monitoring the decrease in refractive index of an eluent containing an aromatic counter ion. This approach is applied to the separation and quantitation of a variety of ionic species using commercially available low-capacity high-efficiency ion-exchange columns and conventional high-performance liquid chromatography instrumentation. The results obtained with indirect refractive index detection are compared with indirect UV absorbance and direct conductimetric detection. The analysis of wine for inorganic and organic ionic species is illustrated.

INTRODUCTION

In recent years, much effort has been applied to the use of high-performance liquid chromatography (HPLC) for the separation and quantitative determination of inorganic ions. The first reported method, called ion chromatography, was developed by Small *et al.*[1] and used a combination of an analytical column and a suppressor column (to decrease the conductivity of the eluent), together with conductimetric detection. Buytenhuys[2] has recently drawn attention to some inherent disadvantages of this approach; these include band broadening due to the suppressor column, the limited lifetime of the suppressor column and the requirement for special equipment.

A number of alternative methods have been reported in the literature, all of which use an analytical separator column (usually an ion-exchange column) without a suppressor column. These methods differ from each other chiefly in the mode of detection employed. Inorganic anions may be separated on a reversed-phase system either by prior formation of organic derivatives[3] or by using ion-pair formation with subsequent direct detection of UV-absorbing ions at low UV wavelengths (210–220 nm)[4,5]. This same approach has been applied to an amino column[6], however these methods are not widely applicable since only a limited number of inorganic ions show UV absorbance. A more general method is the use of reversed-phase ion-pair HPLC with UV-absorbing pairing ions[7], where samples gave positive or negative peaks,

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depending on their charge and retention relative to the UV absorbing pairing ion.

Conductivity detection is possible without the use of a suppressor column only if the background conductivity of the eluent is sufficiently low to enable it to be electrically offset on the conductivity detector. Low-capacity ion-exchange columns have been developed for this work, and their use with low conductivity eluents such as potassium hydrogen phthalate has led to some successful separations[8,9]. Furthermore, this approach is not restricted in its general applicability to anions.

Two indirect methods for detection of eluted anions have recently been reported. The first of these is based on the decrease in the refractive index of a phthalate buffer mobile phase occurring when an inorganic anion is eluted[2]. The second method has an identical basis, except that the UV absorbance (at 300 nm) of the phthalate buffer is monitored[10]. The refractive index detection mode was used with high-capacity anion-exchange columns which required the use of concentrated buffer eluents. The chromatographic efficiency achieved was relatively poor and broad, tailed peaks, with long retention times resulted[2].

We have investigated the use of refractive index detection of both cations and anions when high-efficiency, low-capacity ion-exchange columns are employed. The results obtained with refractive index detection are compared with the conductimetric and indirect UV absorbance detection methods, and the determination of inorganic and organic anions in wine is illustrated. All of the work reported in this paper was performed using conventional HPLC instrumentation.

EXPERIMENTAL

Instrumentation and reagents

The liquid chromatograph used consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model M-6000A pump, Model U6K injector, Model M-401 differential refractive index detector and Model M-730 data module. Alternative detectors used were a Waters Assoc. Model M-450 variable-wavelength detector and a Wescan Instruments (Santa Clara, CA, U.S.A.) Model 213A conductivity detector. Two columns were used; the first was a Vydac 3021C46 anion chromatography column, 250 × 4.6 mm I.D. (obtained from The Separations Group, Hesperia, CA, U.S.A.) and the second was a Wescan 269-004 cation column, 250 × 2.1 mm I.D. (obtained from Wescan Instruments).

All reagents used were the highest available purity and standard solutions of the ions were prepared by dissolving weighed amounts of the pure salts in water purified on a Millipore Milli-Q water purification system. Eluents were prepared by dissolving the desired amount of buffer salt in doubly distilled water and adjusting the pH to the required value with sodium hydroxide (for the phthalate buffer eluent) and nitric acid (for the anilinium eluent). The eluents were filtered through a 0.45-μm Millipore filter and degassed in an ultrasonic bath before use; a thermostatically controlled water bath was used to regulate the temperature of the eluents and also the column, through the use of a water jacket. In all cases, pure eluent was used to fill the reference cell of the refractive index detector.

Procedures

Stock solutions of individual ions (at 1000 ppm concentration) were prepared

as described above and appropriate mixtures (see Figs. 1 and 2) were obtained by mixing and dilution of these stock solutions. Aqueous solutions of the ions were injected directly onto the chromatograph using a microsyringe. All relevant experimental conditions are given in captions to the figures.

The wine sample was passed through a Waters Assoc. C_{18} Sep-Pak cartridge and the resultant solution degassed in an ultrasonic bath. An aliquot of the prepared wine was injected without further treatment.

RESULTS AND DISCUSSION

Separation of anions

A series of common inorganic and organic anions was chromatographed under a variety of mobile phase conditions and the retention times observed are given in Table I. The expected trends in retention times as the mobile phase is varied are clearly evident. That is, increasing the buffer concentration or the pH of the eluent causes a decrease in retention time due to the increased ionic strength of the eluent.

Fig. 1 illustrates the separation of six inorganic anions and compares the results obtained with conductivity, refractive index and UV absorbance detection. Fig. 2 provides the same comparison for the separation of a mixture of inorganic and organic anions.

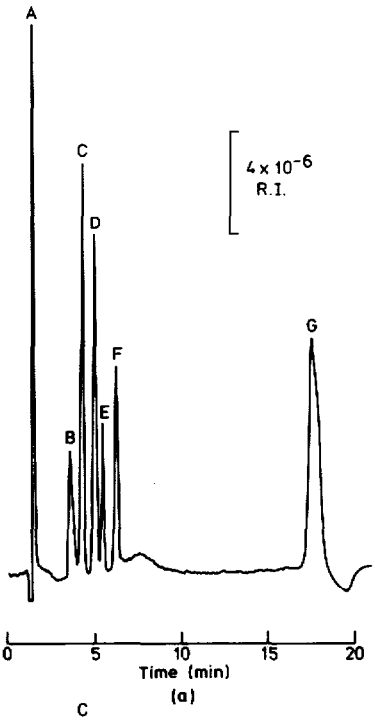
The level of baseline noise observed for all three detectors was somewhat higher than that generally encountered when the same sensitivity settings are used for routine HPLC work. This is attributable to the fact that a large amount of zero offset was required to compensate for the background signal of the eluent. This charac-

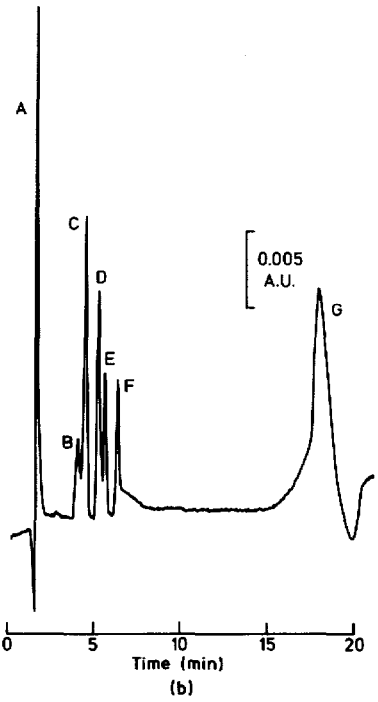
TABLE I

RETENTION TIMES (min) FOR INORGANIC AND ORGANIC ANIONS OBTAINED WITH A VYDAC ANION COLUMN WITH POTASSIUM HYDROGEN PHTHALATE (KHP) AND PHTHALIC ACID MOBILE PHASES AT A FLOW-RATE OF 2 ml/min

a = not determined; b = excessively long retention time.

Species	*Mobile phase*			
	4 mM KHP pH 4.0	*5 mM KHP pH 4.0*	*10 mM KHP pH 4.0*	*5 mM phthalic acid pH 5.9*
Acetate	2.42	2.27	a	a
Lactate	3.00	2.95	a	a
Dihydrogen phosphate	3.66	3.38	2.67	2.43
Chloride	4.48	3.98	2.95	2.86
Nitrite	5.19	4.58	a	a
Bromide	5.65	5.02	a	a
Nitrate	6.46	5.62	3.37	a
Malate	a	5.71	a	a
Maleate	9.56	8.40	a	a
Tartrate	13.81	10.73	5.02	5.61
Sulfate	18.30	14.80	6.80	a
Sulfite	18.62	14.86	6.84	a
Carbonate	19.07	16.00	a	a
Chromate	b	b	b	14.00





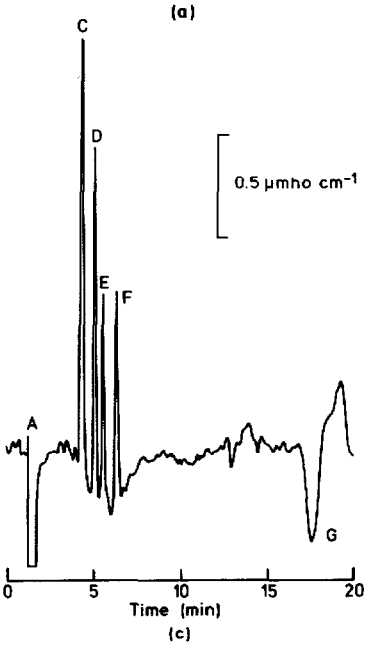
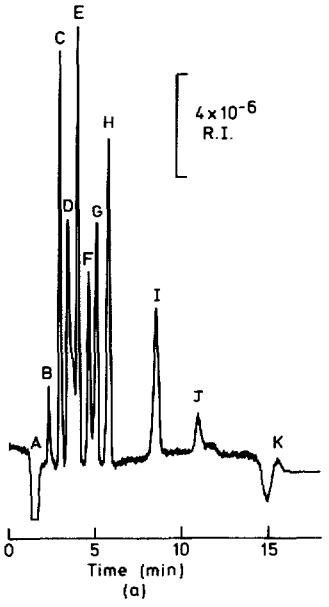
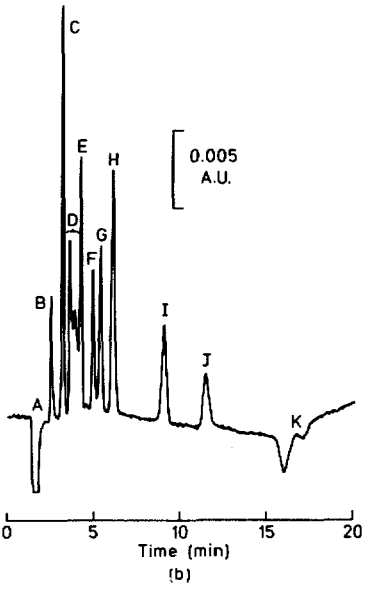


Fig. 1. Separation of inorganic anions with indirect refractive index (a), indirect UV absorbance (b) and direct conductimetric (c) detection. Conditions: column, Vydac anion column; flow-rate, 2 ml/min; mobile phase, 4 m*M* potassium hydrogen phthalate at pH 4.0; chart speed, 0.5 cm/min; injection volume, 100 μl; detector sensitivities: (a) R.I. × 1, (b) 0.04 a.u.f.s., (c) 3 μmho F.S.D. Peaks: A = solvent, B = $H_2PO_4^-$ (2 μg), C = Cl^- (2 μg), D = NO_2^- (2 μg), E = Br^- (2 μg), F = NO_3^- (2 μg), G = SO_4^{2-} (2 μg).





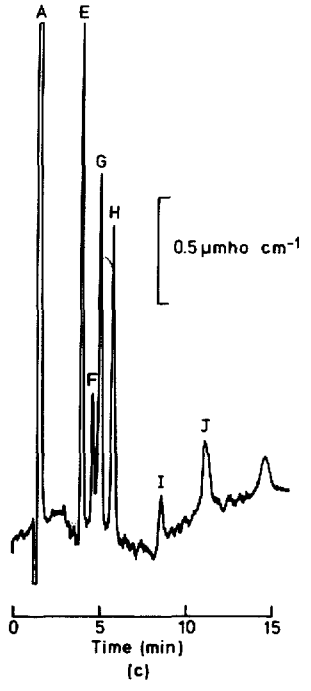


Fig. 2. Separation of a mixture of organic and inorganic anions with indirect refractive index (a), indirect UV absorbance (b) and direct conductimetric (c) detection. Conditions: mobile phase, 5 m*M* potassium hydrogen phthalate at pH 4.0; injection volume, 50 μl; other conditions as for Fig. 1. Peaks: A = solvent peak, B = acetate (7.6 μg), C = lactate (1.9 μg), D = $H_2PO_4^-$ (7.6 μg), E = Cl^- (1.9 μg), F = NO_2^- (1.0 μg); G = Br^- (3.8 μg), H = NO_3^- (1.9 μg) + malate (7.6 μg), I = maleate (7.6 μg), J = tartrate (7.6 μg), K = CO_3^{2-} (1.0 μg).

TABLE II

COMPARISON OF DETECTION LIMITS FOR INORGANIC AND ORGANIC IONS USING INDIRECT REFRACTIVE INDEX, INDIRECT UV ABSORBANCE AND DIRECT COMDUCTIMETRIC DETECTION METHODS

The detectors were operated at the following sensitivities. Indirect refractive index: R.I. × 1 (2.4×10^{-5} R.I. F.S.D.); indirect UV absorbance: 0.04 a.u.f.s.; direct conductometric: 3 μmho F.S.D., a = 2 μg of the anion was not detected; b = detection limit not determined.

Species	*Detection limit (μg)*		
	Indirect refractive index (a)	*Indirect UV absorbance (b)*	*Direct conductimetric (c)*
Acetate	1.4	0.80	a
Lactate	0.06	0.06	a
Dihydrogen phosphate	0.18	0.45	a
Chloride	0.06	0.09	0.15
Nitrite	0.08	0.26	0.77
Bromide	0.19	0.28	0.44
Nitrate	0.12	0.18	0.41
Malate	3.30	3.00	a
Maleate	0.70	0.92	7.00
Tartrate	3.00	1.78	5.10
Sulfate	0.11	0.12	0.78
Sulfite	0.10	0.12	b
Carbonate	0.95	0.19	b
Chromate	0.36	b	b

teristic is an inherent drawback to the use of these non-suppressed or indirect detection modes and is the chief limitation to their sensitivity. The refractive index and UV detection methods gave acceptable baseline stability, however the conductivity detector gave an erratic baseline at the sensitivity necessary to display the peaks, which rendered peak integration difficult. The detection limits obtained with the three detectors are given in Table II, which shows that the indirect refractive index and UV absorbance methods gave comparable detection limits, whereas direct conductivity detection gave much poorer detection limits, and in some cases, showed no response to ions at the concentration levels tested.

Some characteristics of the Vydac column deserve mention. The observed retention times were somewhat dependent on the amount of solute injected and the peak shape for a single species deteriorated noticeably if more than 2 μg of that species was injected. These characteristics result from the low capacity of the column. Peak shape was found to be independent of the injection volume used (up to a maximum injection volume of 500 μl), provided the column was not overloaded: for example, a 10 μl injection of 100 ppm Cl^- gave an identical peak to a 100 μl injection of 10 ppm Cl^-. The efficiency of the columns was relatively high (3800 theoretical plates for nitrate ion) when new, and satisfactory resolution of Br^- and NO_2^- (see Fig. 1) was obtainable after 200 injections.

Several of the anions tested (SO_4^{2-}, SO_3^{2-}, lactate and malate) gave a negative peak at a retention time of 15–20 min, depending on the ionic strength of the eluent (see Figs. 1 and 2). This peak was observed with both of the indirect detection

methods and appeared as a positive peak with conductivity detection. We were unable to find the cause of this peak, although a large number of ions were tested.

The refractive index mode of detection gave reliable, reproducible results, provided sufficient column equilibration time (20 min) was allowed and the column and eluent were temperature-controlled with a water jacket. Efficiency was somewhat dependent on column temperature, with a 5% increase in peak height observed for a temperature increase from 20°C to 30°C. The latter temperature was used throughout this work. Under optimum conditions, the peak area precision using refractive index detection was 2.2% R.S.D. for Cl^-, 3.2% R.S.D. for NO_2^- and 3.1% R.S.D. for NO_3^-, for a series of ten replicate injections.

Separation of cations

Monovalent and divalent inorganic cations have been separated using a low-capacity cation-exchange column and a dilute, low-conductivity eluent composed of nitric acid or an ethylenediammonium salt, together with a conductivity detector[11]. No suppressor column was used. Alternatively, indirect UV absorbance detection (at 252 nm) can be employed using a copper sulfate eluent and Dowex 50 cation-exchange resin[10]. Neither of these approaches is applicable to indirect refractive index detection since the eluents used do not provide a sufficiently high background refractive index relative to the solutes under study.

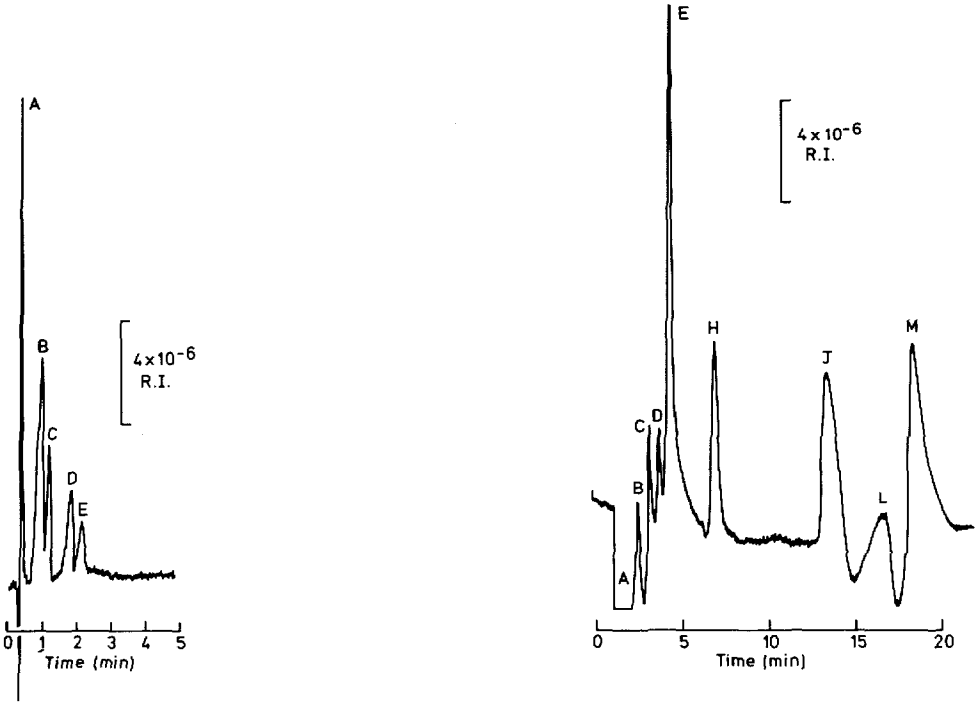


Fig. 3. Separation of some inorganic cations, with indirect refractive index detection. Conditions: column, Wescan cation column; flow rate, 2.0 ml/min; mobile phase, 2.74 m*M* anilinium ion at pH 4.65; chart speed, 1.0 cm/min; injection volume, 20 μl; detector sensitivity, R.I. × 1. Peaks: A = solvent peak, B = Li^+ (0.4 μg), C = Na^+ (0.4 μg). D = NH_4^+ (0.4 μg), E = K^+ (0.4 μg).

Fig. 4. Analysis of wine for anionic components using indirect refractive index detection. Conditions: column, Vydac anion column; injection volume, 20 μl; sample-treated white wine (Reisling); other conditions as for Fig. 1. Peaks: A–J as for Fig. 2; L and M are unidentified.

We have investigated a number of aromatic amines and diamines as eluents for inorganic cations, using a Wescan cation column. The diamines (such as isomers of phenylenediamine) proved to be very strong eluents, even when the pH was adjusted so that only one of the amino groups was protonated. Aniline (pK 4.63) at pH 4.65 was useful for the separation of Li^+, Na^+, NH_4^+ and K^+, with subsequent indirect refractive index detection (Fig. 3). The sensitivity of this method (as indicated by a detection limit for Li^+ of 12 ng at R.I. $\times$ 1) greatly surpasses that achieved with indirect UV absorbance detection[10], but is somewhat inferior to that achieved with direct conductivity detection[11].

Analytical applications

We have applied the anion separation with indirect refractive index detection to the analysis of wine. Injection of an undiluted sample of white wine, pretreated by passage through a C_{18} Sep-Pak cartridge, gave the chromatogram shown in Fig. 4. The inorganic components are clearly identifiable despite the presence of a large negative peak at the solvent front and the organic components are well resolved. We were unable to assign positively identities to the two latest eluting peaks in the chromatogram, because of interference due to the negative peak described above. However, citrate, carbonate, sulfate and sulfite all gave peaks with similar retention times to the unidentified peaks.

CONCLUSIONS

Inorganic and organic anions and cations may be determined by HPLC using conventional instrumentation with low-capacity ion-exchange columns and indirect refractive index detection. The sensitivity of this detection mode is comparable to indirect UV absorbance detection and superior to direct conductimetric detection. It is noteworthy that refractive index detectors are generally less expensive than variable-wavelength UV detectors, which are required for the indirect UV absorbance detection method.

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