Journal of Chromatography, 449 (1988) 103–113
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 679

ROLE OF THE ALKYL CHAIN LENGTH OF THE ION INTERACTION REAGENT, FLOW-RATE, COLUMN PACKING AND DETECTION IN ION INTERACTION REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN SEPARATIONS OF ANIONS USING AMINE SALICYLATES

M. C. GENNARO
Department of Analytical Chemistry, University of Torino, Via P. Giuria, 5-10125 Torino (Italy)
(First received April 21st, 1988; revised manuscript received May 24th, 1988)

SUMMARY

The effect of different factors on retention in ion interaction reversed-phase high-performance liquid chromatography was investigated. In particular, the factors considered were the alkyl chain length of the lipophilic cation of the ion interaction reagent, the flow-rate, the size of the stationary phase packing and the choice of detector. The reagents used and compared were hexylamine, octylamine and decylamine salicylates, and the stationary phases were C_{18} reversed phase with packing sizes of 5 (spherical) and 10 μm (irregular). A comparison was made between UV spectrophotometric (direct and indirect) and conductometric detections. Mixtures of inorganic and organic anions were separated and also some real samples. The system studied was shown to be suitable for analysis of nitrates in drinking waters and for the evaluation of the contents of some organic acids (acetic, succinic, malic and tartaric) in vinegars.

INTRODUCTION

Previous work performed in this laboratory[1,2] dealt with separations of anions: the use of different ion interaction reagents was compared and the mechanism which governs retention discussed. The results obtained correspond well with proposed mechanisms[3–5], which postulate for lipophilic ions the formation of an electrical double layer on the surface. In this way the adsorption process is associated with a step involving electrostatic forces. This hypothesis is also able to explain[2] the formation of the so-called system peaks, namely "injection" and "system" peaks, whose presence characterizes this chromatographic technique.

Literature reports concerning ion interaction chromatography[6–10] all agree as to its versatility, due to the possibility of changing different parameters, such as the alkyl chain length of the lipophilic cation of the ion interaction reagent, the flow-rate, the stationary phase packing, and the method of detection.

In the present paper the use of octylamine salicylate is considered, due to its

0021-9673/88/$03.50

ability to retain both organic and inorganic anions and its good general propertie. As salicylate ions are characterized by an high molar absorptivity ($\varepsilon = 308 \pm 2$ mol^{-1} cm^{-1} at $\lambda = 254$ nm) and a relatively low ionic conductivity, the use of octylamine salicylate allows spectrophotometric (both direct and indirect) and conductometric detections.

For comparison, some experiments were also performed by using hexylamine salicylate and decylamine salicylate as ion interaction reagents, at different flow-rates.

Mixtures of typical anions and examples of the analysis of anions in real samples were considered.

EXPERIMENTAL

Apparatus

Analyses were carried out using a Varian LC 5000 chromatograph, equipped with a Vista 401 data system and an UV-100 spectrophotometric detector. Alternatively, a Wescan 213 A conductometric detector was employed; a 1 V exit was used in order to interface it to the Vista 401 data system. Merck Lichrospher RP-18, 5 μm, and LiChrosorb RP-18, 10 μm, columns were used. For pH measurement, an Orion 811 pH-meter equipped with a combined glass–calomel electrode was employed.

Chemicals

Ultra-pure water from a Millipore Milli-Q was used for preparation of solutions. Hexylamine, octylamine and decylamine were Fluka analytical grade reagents. Salicylic acid and all other reagents were Carlo Erba analytical grade chemicals. The solutions to be used as eluents were prepared by dissolving weighed amount of the corresponding amines in ultra-pure water and bringing the solutions to a pH 6.2 $\pm$ 0.4 by additions of salicylic acid. Taking into account the acidic formation constants of the amines, the eluent compositions so prepared are not exactly stoichiometric. Nevertheless, for simplicity, they will be mentioned henceforth as amine salicylates.

In order to condition the chromatographic system, eluent was allowed to flow through the column until a stable baseline was obtained. Generally, times not less than 1 h were necessary. Eluent solutions were prepared each second day.

The reproducibility of retention times was very good for sequential measurements, but it was a little poorer for different eluent preparations and column conditionings. This effect has been observed by other authors and can be ascribed to a very low residual irreversible functionalization (*i.e.*, the alteration induced on the stationary phase by the interactions with the alkyl chain of the amines of the eluent) of the stationary phase. Retention data listed in the tables refer to the reproducibility obtained for different preparations.

Between uses, the column was regenerated by passage of water–methanol (1:1). No particular deterioration of the column was observed with respect to its use in other chromatographic techniques.

The samples of vinegars were prepared only for filtering up to 0.45 μm and diluting 1:10 (v/v) in ultra-pure water.

RESULTS AND DISCUSSION

Table I shows retention times for some typical inorganic anions, obtained by using octylamine salicylate as the eluent (flow-rate 2.0 ml/min) and Lichrospher

TABLE I

RETENTION TIMES, t_R, FOR SOME INORGANIC ANIONS

Ion interaction reagent: 0.0050 *M* octylamine salicylate; flow-rate 2.0 ml/min. Column: Merck Lichrospher RP-18, 5 μm.

Anion	t_R *(min)*
Carbonate, chloride, sulphide, bromide	2.0 ± 0.3
Nitrite, fluoride	2.3 ± 0.3
Iodide, nitrate, chlorate, bromate, iodate, perchlorate	2.5 ± 0.3
Thiocyanate	3.8 ± 0.3
Arsenate	5.4 ± 0.4
Orthophosphate	5.6 ± 0.5
Pyrophosphate	9.7 ± 0.4
Chromate	11.8 ± 0.5
Sulphide, sulphate	20.0 ± 0.8

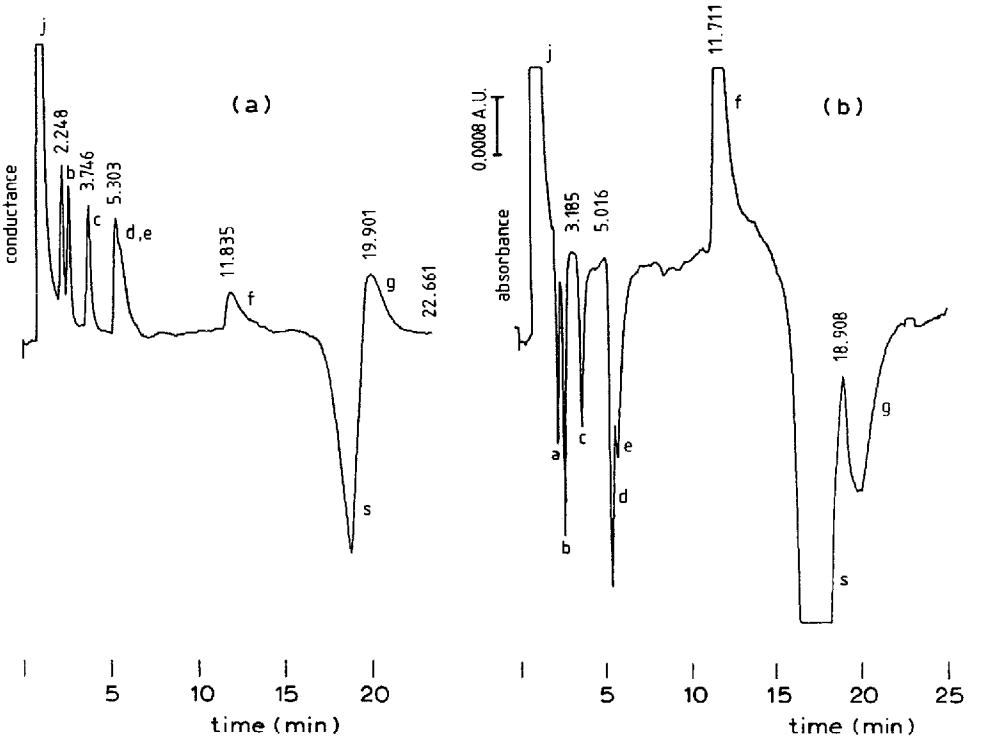

Fig. 1. Comparison between conductometric (a) and spectrophotometric (λ = 254 nm) (b) detection in the separation of a typical mixture. Ion interaction reagent: 0.0050 *M* octylamine salicylate. Flow-rate: 2.0 ml/min. Column: Merck Lichrospher RP-18, 5 μm. Peaks: j = injection peak; a = nitrites (30.0 ppm); b = nitrates (30.0 ppm); c = thiocyanates (50.0 ppm); d = arsenates (100.0 ppm); e = orthophosphates (60.0 ppm); f = chromates (100.0 ppm); g = sulphates (50.0 ppm); s = system peak.

RP-18, 5 μm, as the stationary phase. The chromatograms of Fig. 1, recorded under these conditions for a mixture of nitrites, nitrates, thiocyanates, arsenates, orthophosphates, chromates and sulphates, compare the use of two different detectors: conductometric and spectrophotometric. It is seen that spectrophotometric detection (Fig. 1b) permits a general enhanced sensitivity. This applies both to anions which being transparent at $\lambda = 254$ nm are detected indirectly as peaks of negative sign and to chromates which, due to their molar absorptivity at this wavelength, appear as positive peaks. Spectrophotometric detection offers as well a general enhanced resolution and, in particular, allows separation between arsenates and orthophosphates.

Fig. 2 shows, for a mixture of nitrites, nitrates, thiocyanates, arsenates and orthosphosphates, the effect on the retention of a variation of the flow-rate from 2.0 (a) to 1.0 ml/min (b), all other conditions being constant. The retention times increase as the flow-rate decreases, consequently, even if the baseline noise increases, a good separation between arsenates and orthophosphates is obtained.

The effect of flow-rate was the same for all the cases investigated, as evidenced by the retention data in Table II recorded at flow-rate ranging between 0.8 and 3.5 ml/min for both octylamine salicylate and decylamine salicylate eluents. The increase in retention times with decreasing flow-rate also applies to the system peaks.

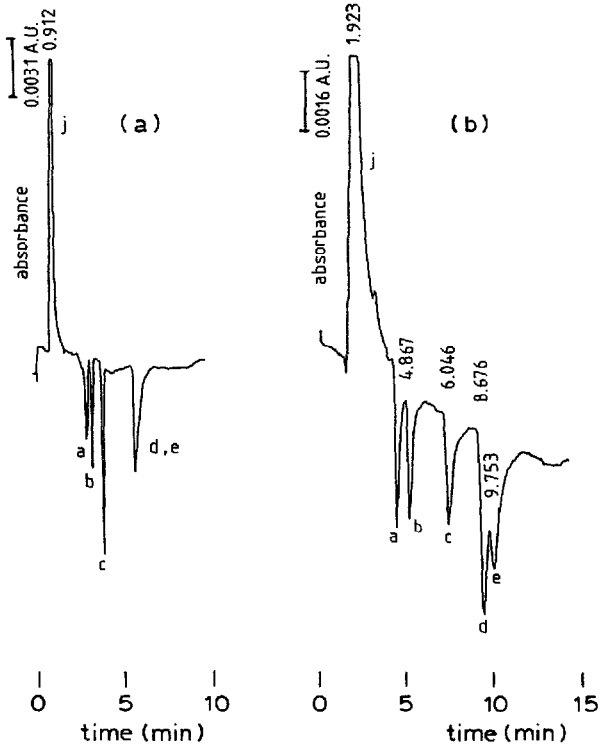

Fig. 2. The effect of the flow-rate on the separation of a typical mixture. Injection: 100 μl. UV detection ($\lambda = 254$ nm). Ion interaction reagent: 0.0050 *M* octylamine salicylate. Column: Merck Lichrospher RP-18, 5 μm. Peaks: j = injection peak; a = nitrites (15.0 ppm); b = nitrates (15.0 ppm); c = thiocyanates (25.0 ppm); d = arsenates (60.0 ppm); e = orthophosphates (60.0 ppm). Flow-rates: 2.0 (a); 1.0 ml/min (b).

TABLE II

EFFECT OF FLOW-RATE ON THE RETENTION TIMES OF SOME TYPICAL ANIONS

Average times are reported. Column: Merck Lichrospher RP-18, 5 μm.

	Flow-rate (ml/min)							
	3.5	*3.0*	*2.5*	*2.0*	*1.5*	*1.2*	*1.0*	*0.8*
Ion interaction reagent: 0.0050 M octylamine salicylate								
System peak	14.0	16.0	18.5	23.0	31.0	38.0	56.0	
Carbonate				2.0				4.9
Nitrite				2.2			4.9	5.5
Iodide				2.5				6.5
Nitrate	1.7	1.7	2.4	2.5	3.6	4.5	5.5	6.6
Perchlorate				2.6				8.5
Thiocyanate	2.2	2.4	3.2	3.8	4.8	6.0	6.7	9.2
Arsenate				5.8			9.2	11.9
Phosphate				6.0			9.8	12.6
Sulphate		12.9	15.4	20.0	25.4			
	Flow-rate (ml/min)							
	3.3	*3.0*	*2.5*	*2.0*	*1.5*			
Ion interaction reagent: 0.0050 M decylamine salicylate								
System peak	17.2	19.0	21.5	26.5	35.0			
Chloride	4.3	4.4	5.4	6.5	8.7			
Bromide			5.9	7.0				
Fluoride			6.2	7.3				
Iodide			6.5	8.0				

Fig. 3 shows the chromatogram recorded, under the same experimental conditions as in Fig. 1b, for a mixture of carbonates (50.0 ppm), nitrites (5.0 ppm), iodides (10.0 ppm), nitrates (5.0 ppm), perchlorates (15.0 ppm), thiocyanates (15.0 ppm), arsenates (50.0 ppm) and orthophosphates (50.0 ppm). A good separation for the eight anions is obtained in as little as 8 min. Thus interference from the system peak is avoided as its retention time is about 23 min under these conditions.

For comparison, similar mixtures were analyzed after equilibration of the flow-rate at 0.8 ml/min (Fig. 4). As expected, increased retention times are obtained, but no particular advantage is achieved on this occasion, nor in the separation of arsenates and orthophosphates. As above, spectrophotometric detection generally allows a better resolution than conductometric detection. In particular, the separation between iodides and nitrates, whose retention times are very similar to each other, is of special interest. The molar absorptivity of iodides at this wavelength results in the formation of a positive peak which can easily be identified and separated from the near negative one due to the transparent nitrates.

The effect on retention of the alkyl chain length of the lipophilic cation of the interaction is shown in Table III, in which retention times for some anions and hexylamine, octylamine and decylamine salicylate as interaction reagents (flow-rate

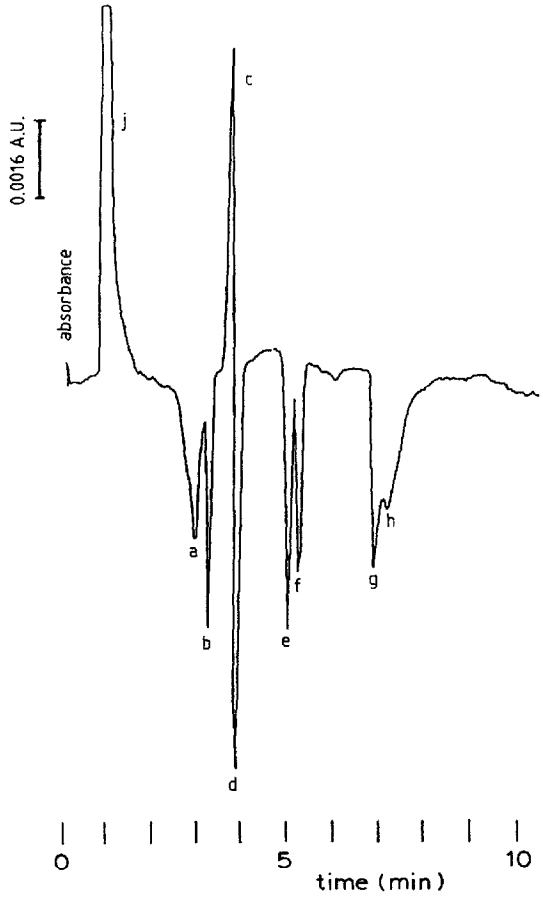

Fig. 3. Separation of a typical mixture. Volume injected: 100 μl. λ = 254 nm. Ion interaction reagent: 0.0050 *M* octylamine salicylate. Flow-rate: 2.0 ml/min. Column: Merck Lichrospher RP-18, 5 μm. Peaks: j = injection peak; a = carbonates (50.0 ppm); b = nitrites (5.0 ppm); c = iodides (10.0 ppm); d = nitrates (5.0 ppm); e = perchlorates (15.0 ppm); f = thiocyanates (15.0 ppm); g = arsenates (50.0 ppm); h = orthophosphates (50.0 ppm).

2.0 ml/min) are listed. The longer the alkyl chain, the longer are the retention times, *cf.*, the chromatograms in Fig. 5. The same mixture of eight anions, previously separated with octylamine salicylate (Fig. 3), shows practically null retention for all its components when the interaction reagent is hexylamine salicylate (Fig. 5a). On the other hand, the use of decylamine salicylate permits (Fig. 5b) an acceptable separation between chlorides, bromides, fluorides and iodides.

Table IV lists retention times obtained for some organic acids for different stationary phase packings and octylamine salicylate concentrations (0.0050 and 0.100 *M*).

For all the anions investigated, increased concentration leads to increased retention. This can be explained through a greater functionalization of the stationary phase induced by a more concentrated eluent.

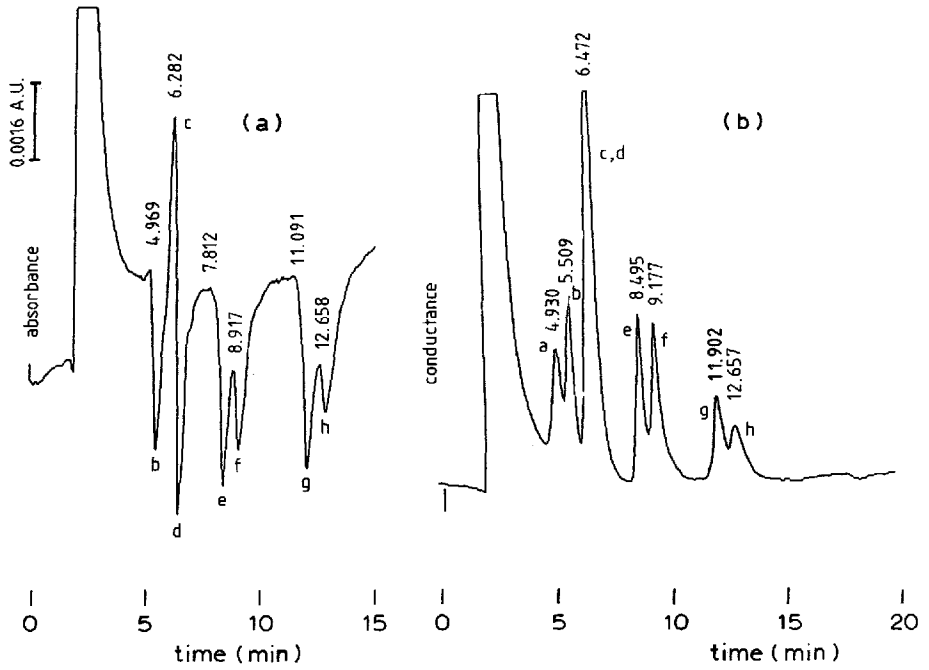

Fig. 4. Effects of the flow-rate (*cf.*, fig. 3) and detection. Ion interaction reagent: 0.0050 *M* octylamine salicylate. Flow-rate: 0.8 ml/min. Column: Merck Lichrospher RP-18, 5 μm. (a) UV detection (λ = 254 nm): separation of the mixture as in Fig. 3, without carbonates. (b) Conductometric detection: separation of the mixture as in Fig. 3.

As far as the column packings are concerned, retentions on the RP-18, 10 μm, packing are less than those obtained on the RP-18, spherical 5 μm. This difference can reasonably be ascribed to the greater total surface area available for functionalization by the 5-μm particles, even when the extent of endcapping and the percentage binding in the two columns are taken into account.

TABLE III

EFFECT OF THE ALKYL CHAIN LENGTH OF THE ION INTERACTION REAGENT ON THE RETENTION TIMES OF SOME TYPICAL ANIONS

Column: Merck Lichrospher RP-18, 5 μm. Flow-rate: 2.0 ml/min. n.r. = Not retained.

	t_R (min)		
	Hexylamine salicylate	*Octylamine salicylate*	*Decylamine salicylate*
System peak	16.0 ± 0.8	23.0 ± 0.8	27.0 ± 0.8
Chloride	n.r.	2.0 ± 0.3	6.5 ± 0.3
Bromide	n.r.	2.0 ± 0.3	7.0 ± 0.3
Fluoride	n.r.	2.3 ± 0.3	7.3 ± 0.4
Iodide	n.r.	2.5 ± 0.3	8.0 ± 0.4
Nitrate	n.r.	2.5 ± 0.3	8.3 ± 0.4

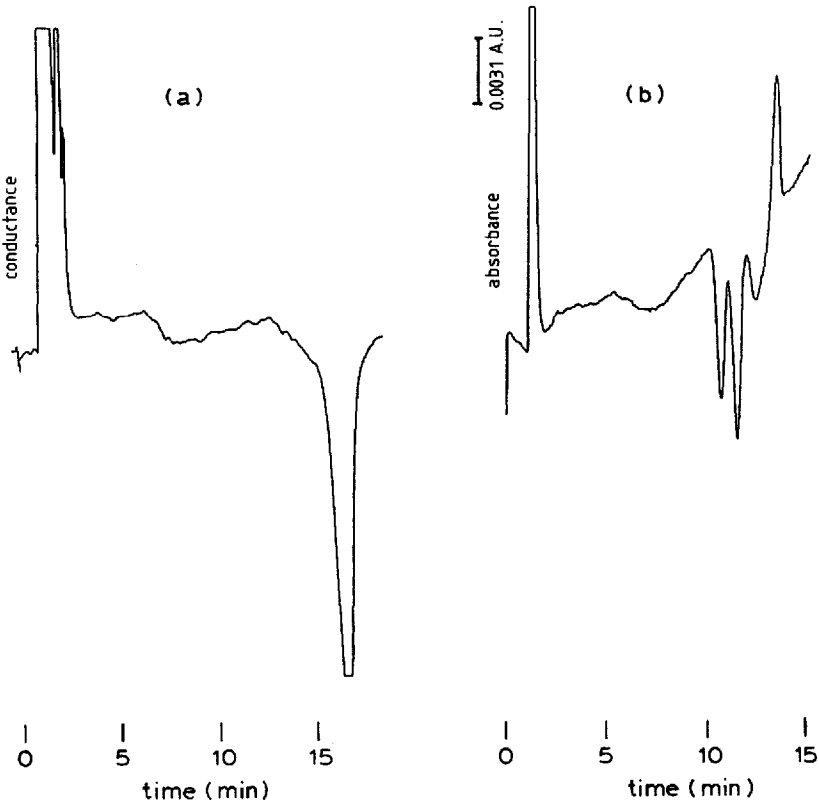

Fig. 5. (a) Ion interaction reagent: 0.0050 *M* hexylamine salicylate. All other conditions as in Fig. 3. (b) Ion interaction reagent: 0.0050 *M* decylamine salicylate. Flow-rate: 1.5 ml/min. UV detection (λ = 254 nm). Separation of a mixture containing chlorides, bromides, fluorides, iodides (each 30.0 ppm). Volume injected 100 μl.

TABLE IV

EFFECT OF THE ION INTERACTION REAGENT CONCENTRATION, C_{IIR}, AND COLUMN PACKING SIZE ON THE RETENTION TIMES OF SOME ORGANIC ANIONS

Ion interaction reagent: octylamine salicylate.

Acid	C_{IIR} = 0.0050 *M* *LiChrosorb RP-18* 10 μm	C_{IIR} = 0.0100 *M* *LiChrosorb RP-18* 10 μm	C_{IIR} = 0.0050 *M* *Lichrospher RP-18* 5 μm
Acetic	2.4 ± 0.2	2.5 ± 0.2	2.7 ± 0.2
Lactic	2.7 ± 0.2	2.7 ± 0.2	3.1 ± 0.3
Butyric	4.4 ± 0.4	4.5 ± 0.2	4.6 ± 0.3
Succinic	6.0 ± 0.4	7.2 ± 0.2	10.6 ± 0.2
DL-Malic	7.5 ± 0.5	8.8 ± 0.3	14.8 ± 0.2
Tartaric	8.5 ± 0.5	10.5 ± 0.3	20.3 ± 0.5

Applications to real samples

Water supply. Fig. 6 shows an example of the quantitation of nitrates and chlorides in a water from a rural pipeline in the Italian Piedmont region, suspected of an anomalous content of nitrates. The chromatographic conditions are as follows: 0.0050 *M* octylamine salicylate as ion interaction reagent; flow-rate 1.5 ml/min; RP-18, spherical 5 μm column; UV (254 nm) detection. The figure shows some typical chromatograms recorded after injection of 100 μl pipeline water (Fig. 6a), standard solution of nitrates (50.0 ppm) (Fig. 6b), standard solution of chlorides (10.0 ppm) (Fig. 6c), pipeline water after standard addition of 40.0 ppm nitrates (Fig. 6d) and pipeline water after standard addition of 15.00 ppm of chlorides (Fig. 6e).

The quantitation was performed by use of a standard calibration graph of peak area and confirmed by the method of internal standard additions. The good agreement of the two series of measurements yielded the content of chlorides as 4.7 $\pm$ 0.5 ppm. The content of nitrates is 58 $\pm$ 1 ppm and exceeds the amount permitted for drinking water.

Vinegars. Data in Table IV permitted the best conditions to be chosen for the analysis of some vinegars for the content of organic acids. By using 0.0050

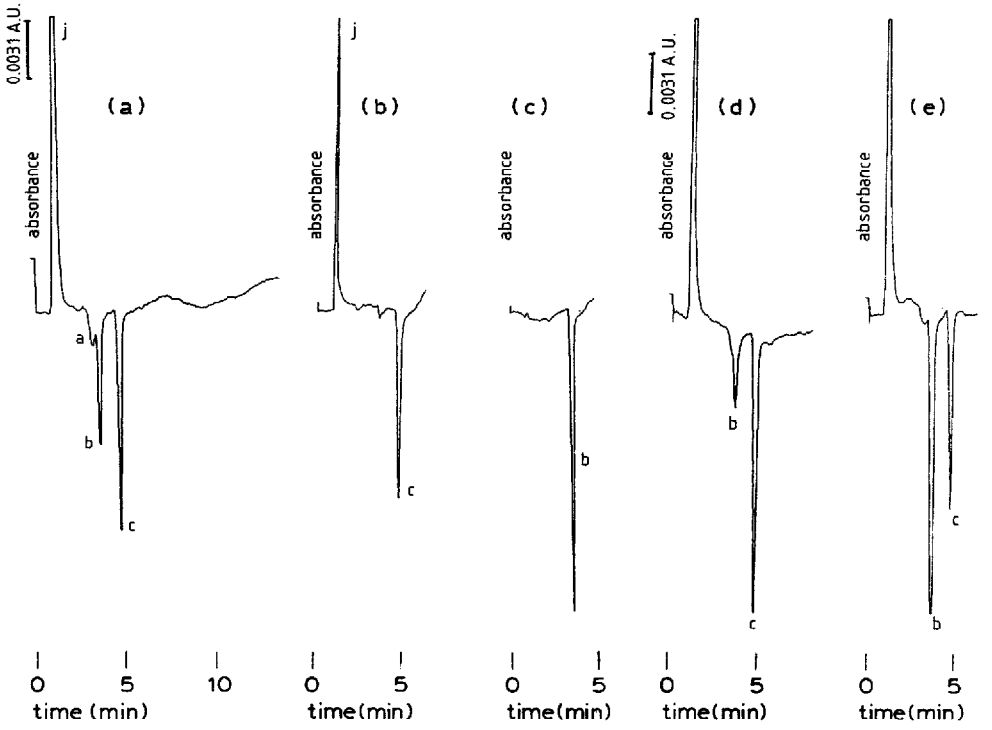

Fig. 6. Chromatograms of water from a rural pipeline. Ion interaction reagent: 0.0050 *M* octylamine salicylate. UV detection (λ = 254 nm). Column: Merck Lichrospher RP-18, 5 μm. Peaks: j = injection peak; a = carbonates; b = chlorides; c = nitrates. Volume injected: 100 μl. (a) Pipeline water; (b) standard nitrates (50.0 ppm); (c) standard chlorides (10.0 ppm); (d) pipeline water after standard addition of 40.00 ppm of nitrates; (e) pipeline water after standard addition of 15.0 ppm of chlorides.

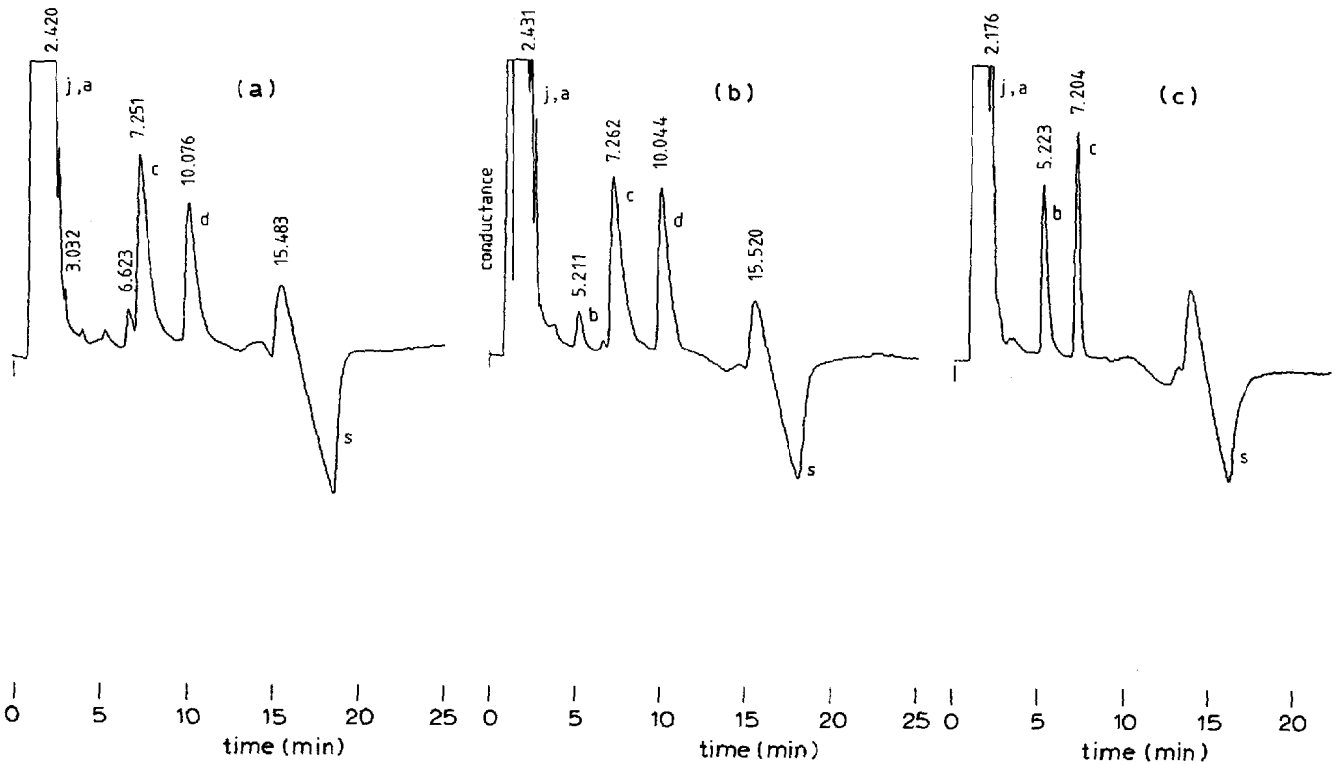

Fig. 7. Chromatograms of samples of vinegars diluted 1:100 (v/v). Volume injected: 100 μl. Ion interaction reagent: 0.0050 *M* octylamine salicylate. Flow-rate: 2.0 ml/min. Column: Merck LiChrosorb RP-18, 10 μm. Conductometric detector. Peaks: j = injection peak; a = acetic; b = succinic; c = malic; d = tartaric acid; s = system peak. (a) Laboratory vinegar; (b) and (c) commercial vinegars.

M octylamine salicylate, conductometric detection and an LiChrosorb RP-18, 10 μm, column, a laboratory-made vinegar and two commercial products were compared. From the chromatograms in Fig. 7 it is seen that succinic acid is practically absent from the laboratory-made vinegar, whilst the two commercial vinegars show substantial differences as regards the contents of succinic and tartaric acids.

In conclusion, this chromatographic technique can be applied to different real samples. Liquid samples require no pretreatment other than filtration. The biggest advantage offered by the technique is its versatility because it is possible to change so many different parameters that are able to affect retention. Moreover the sensitivity is satisfactory, taking into account that no derivatization reaction is employed. Nevertheless, the sensitivity for the particular species of interest can be enhanced or optimized through suitable choice of the conditions.

REFERENCES

1 M. C. Gennaro, M. Sbuttoni, E. Mentasti, C. Sarzanini and V. Porta, *Ann. Chim. (Rome)*, 78 (1988) 137.
2 M. C. Gennaro, *J. Liq. Chromatogr.*, 10 (1987) 3347.
3 B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, 18 (1980) 525.
4 W. E. Hammers, C. N. M. Aussems and M. Janssen, *J. Chromatogr.*, 360 (1986) 1.
5 J. Ståhlberg, *J. Chromatogr.*, 356 (1986) 231.
6 B. B. Wheals, *J. Chromatogr.*, 262 (1983) 61.
7 W. E. Barber and P. W. Carr, *J. Chromatogr.*, 316 (1984) 211.
8 M. Dreux, M. Lafosse, P. Agbo-Hazoumé, B. Chaabane-Doumandji, M. Gibert and Y. Léui, *J. Chromatogr.*, 354 (1986) 119.
9 B. A. Bidlingmeyer, C. T. Santasania and F. V. Warren, Jr., *Anal. Chem.*, 59 (1987) 1843.
10 M. Lookabaugh, I. S. Krull and W. R. LaCourse, *J. Chromatogr.*, 387 (1987) 301.