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Simultaneous determination of nitrites, nitrates and amines by ion-interaction reversed-phase high-performance liquid chromatography

Application to lagoon water

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ABSTRACT

The conditions for the simultaneous separation of aliphatic and aromatic amines, nitrites and nitrates by ion-interaction reversed-phase high-performance liquid chromatography (HPLC) were optimized. The mechanism that governs retention in this HPLC technique is discussed. Sensitivity of the order of 0.10 ppm for nitrites and nitrates can be obtained with octylamine orthophosphate as eluent at 210 nm. Amounts of 0.20 ppm of nitrates and 0.50 ppm of 1,4-phenylenediamine can be easily identified and determined in matrices as complex as sea water.

INTRODUCTION

Previous studies have shown the suitability of ion-interaction reversed-phase high-performance liquid chromatography (RP-HPLC) in the analysis of both organic and inorganic anionic species[1–3] and aliphatic and aromatic amines[4]. Reversed-phase C_8 and C_{18} columns with different stationary phases and salicylates of aliphatic amines with different chain lengths as the ion-interaction reagents were used.

The aim of this work was to develop improved methods for the simultaneous separation of anions and amines. Mixtures of this type are frequently present in environmental and food samples.

EXPERIMENTAL

Apparatus

Analyses were carried out with a Varian LC 5000 chromatograph (equipped with a Vista 401 data system and a UV-100 spectrophotometric detector) and a Merck–Hitachi Lichrograph Model L-6200 chromatograph (equipped with a Model D-2500 Chromato-Integrator and a Model L-4200 UV–VIS detector).

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Alternatively, a Wescan 213A conductometric detector was employed; 1 V exit was used in order to interface it to the Vista 401 data system or to the Chromato-Integrator. The latter is provided with two channels and allows a direct comparison between chromatograms obtained for the same injection by the use of the two detectors.

A Merck C_{18} Hibar LiChrospher RP-18 (5 μm) column (250 $\times$ 4.0 mm I.D.) was used.

For pH measurements, a Metrohm 654 pH meter equipped with a combined glass–calomel electrode was employed.

Chemicals

Chemicals of analytical-reagent grade and ultra-pure water from a Millipore Milli-Q system were used.

Pentylamine, heptylamine and octylamine were obtained from Fluka and salicylic acid and all other reagents from Carlo Erba.

Solutions to be used as eluents, namely pentylamine salicylate, heptylamine salicylate, octylamine salicylate and octylamine orthophosphate, were prepared by dissolving a weighed amount of the amine in water and adjusting the pH to 6.3 $\pm$ 0.4 with salicylic or orthophosphoric acid[1–4].

In order to condition the chromatographic system, eluent was passed through the column until a stable baseline was obtained (not less than 1 h). Eluent solutions were prepared freshly each third day.

The reproducibility of measurements was very good for sequential measurements and slightly poorer for different eluent preparations and column conditionings. Average data and reproducibilities of the retention data listed in Tables I–IV were calculated for different preparations.

Between uses, the column was regenerated by passing water–methanol (1:1, v/v). No particular decrease in the column lifetime was observed with respect to its use in other chromatographic techniques.

RESULTS AND DISCUSSION

Optimum conditions for the simultaneous determination of anionic species and amines were established. Parameters that were previously shown[2] to affect retention, such as the alkyl chain length of the eluent, eluent flow-rate and the stationary phase packing, were varied systematically and the results compared (Tables I–IV). UV and conductometric detectors were employed. Reasonable retention times were obtained for both amines and anions by the use of a LiChrospher RP-18 column, which was subsequently adopted throughout.

It can be concluded that the alkyl chain length of the ion-interaction reagent has the greatest influence on retention. This effect differs when the analytes are anions or amines, an increase of the alkyl chain length leading to a greater retention of anions and a lower retention of amines. The effect, which has been observed previously[4], holds for all the investigated systems.

It follows that, when the resolution between the components of a mixture needs to be improved, an eluent must be chosen that is characterized by a longer alkyl chain when dealing with anions and by a shorter chain in the analysis of amines. Thus, as an

TABLE I

RETENTION TIMES OF SOME NITROGEN-CONTAINING SPECIES

Chromatographic conditions: stationary phase, LiChrospher RP-18 (5 μm); ion-interaction reagent, pentylamine salicylate (0.0050 M) at different flow-rates.

Species	*Retention time (min) ± S.D. (n ⩾ 4)*				
	Flow-rate (ml min^{-1})				
	0.8	*1.0*	*1.2*	*1.5*	*2.0*
Ammonium	3.1 ± 0.3				
Hydrazine	3.4 ± 0.3				
Hydroxylamine	3.4 ± 0.3				
Methylamine	3.7 ± 0.3	3.3 ± 0.3	2.2 ± 0.3	2.1 ± 0.3	
Ethylamine	4.6 ± 0.3	3.8 ± 0.3	2.7 ± 0.3	2.5 ± 0.3	
Propylamine	7.2 ± 0.3	6.0 ± 0.3	4.5 ± 0.3		
tert.-Butylamine	12.7 ± 0.4	10.4 ± 0.3	7.2 ± 0.3	6.0 ± 0.3	4.7 ± 0.3
sec.-Butylamine	14.2 ± 0.4	11.6 ± 0.2	8.5 ± 0.3	8.0 ± 0.3	5.5 ± 0.3
n-Butylamine	16.8 ± 0.5	13.6 ± 0.4	9.7 ± 0.3		6.4 ± 0.3
neo-Pentylamine	34.9 ± 0.6				

TABLE II

RETENTION TIMES OF SOME NITROGEN-CONTAINING SPECIES

Chromatographic conditions: stationary phase, LiChrospher RP-18 (5 μm); ion-interaction reagent, octylamine salicylate (0.0050 *M*) at different flow-rates.

Species	*Retention time (min) ± S.D. (n ⩾ 4)*			
	Flow-rate (ml min^{-1})			
	0.8	*1.2*	*1.5*	*2.0*
Ammonium	2.0 ± 0.2			
Hydrazine	2.4 ± 0.2			
Hydroxylamine	2.4 ± 0.3			
Ethylamine	2.2 ± 0.3	1.5 ± 0.3		
Thiourea	2.3 ± 0.3			
Propylamine	3.3 ± 0.3	1.8 ± 0.3		
Butylamines	3.5 ± 0.6	3.0 ± 0.5		
p-Phenylendiamine	4.3 ± 0.3			
Benzylamine	5.5 ± 0.3			
Pentylamines	6.5 ± 0.4	4.4 ± 0.3	3.4 ± 0.3	2.2 ± 0.3
Nitrites	7.7 ± 0.4			
Nitrates	9.0 ± 0.3			
Hexylamine	12.7 ± 0.4			

TABLE III

RETENTION TIMES OF PENTYLAMINE ISOMERS

Chromatographic conditions: stationary phase, LiChrospher RP-18 (5 μm); ion-interaction reagent, heptylamine salicylate (0.0050 *M*) at different flow-rates.

Isomer	*Retention time (min) ± S.D. (n ⩾ 4)*	
	Flow-rate (ml min^{-1})	
	0.8	*1.0*
2-Methyl-2-butylamine	7.0 ± 0.4	5.4 ± 0.3
2,2-Dimethylpropylamine	7.7 ± 0.4	6.0 ± 0.3
1,2-Dimethylpropylamine	7.9 ± 0.4	6.2 ± 0.3
3-Pentylamine	8.1 ± 0.4	6.5 ± 0.3
2-Pentylamine	9.0 ± 0.4	6.8 ± 0.4
2-Methylbutylamine	9.3 ± 0.4	7.2 ± 0.4
n-Pentylamine	11.5 ± 0.4	8.9 ± 0.4

TABLE IV

RETENTION TIMES (min) OF SOME NITROGEN-CONTAINING SPECIES

Chromatographic conditions: stationary phase, LiChrosopher RP-18 (5 μm); ion-interaction reagent, octylamine orthophosphate (0.0050 *M*) at different flow-rates.

Species	*Retention time (min) ± S.D. (n ⩾ 4)*		
	Flow-rate (ml min^{-1})		
	0.8	*1.0*	*2.0*
Propylamine	2.7 ± 0.2	2.2 ± 0.2	
n-Butylamine	3.0 ± 0.3	2.6 ± 0.3	
Thiourea	3.5 ± 0.3	2.7 ± 0.3	1.4 ± 0.2
n-Pentylamine	5.5 ± 0.4	3.7 ± 0.3	2.2 ± 0.3
Hexylamine	10.2 ± 0.4	8.2 ± 0.4	4.3 ± 0.2
Nitrites	12.5 ± 0.4	10.3 ± 0.3	5.5 ± 0.3
Nitrates	16.9 ± 0.5	14.4 ± 0.5	7.6 ± 0.3

example, when octylamine salicylate is used as the ion-interaction reagent, *tert.*-, *sec.*- and *n*-butylamines show the same retention time and cannot be separated. However, separation can be achieved by the use of pentylamine salicylate, as shown in Fig. 1, recorded for a mixture of ethylamine, propylamine and the three butylamine isomers.

A similar trend was observed in the separation of pentylamine isomers. This separation is impossible when using octylamine salicylate as the ion-interaction whereas with heptylamine salicylate the separation of six of the seven isomers is possible (Table III).

If the retention times obtained for the butylamine and pentylamine isomers are compared, it is seen that the greatest retentions correspond to the normal isomer. It can be argued that the alkyl chain length, more than the molecular structure, determines retention. This different behaviour of anions and amines can be interpreted

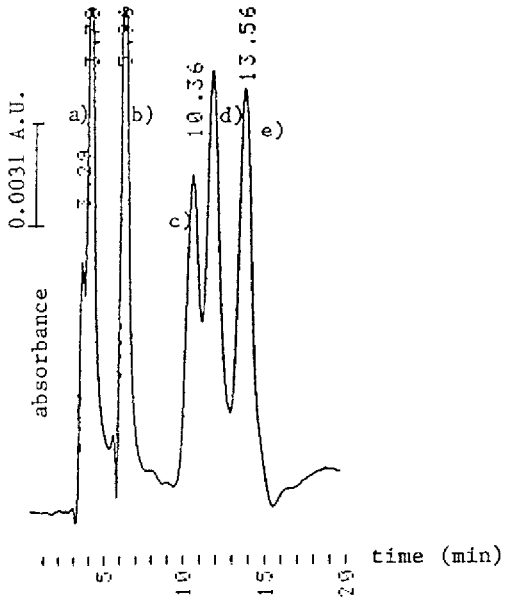

Fig. 1. Separation of a mixture of (a) ethylamine, (b) propylamine, (c) *tert.*-butylamine, (d) *sec.*-butylamine and (e) *n*-butylamine (50.0 ppm each). Injection volume: 100 μl. Stationary phase: LiChrospher RP-18 (5 μm). Ion-interaction reagent: pentylamine salicylate (0.0050 *M*). Flow-rate: 1.0 ml min^{-1}. Spectrophotometric detection at 254 nm.

through the mechanisms that explain retention[5–7] in this chromatographic technique. The eluent flowing through the stationary phase is adsorbed on the column as an ion pair, modifying the original packing and inducing a so-called "dynamic functionalization" of the column. It is generally accepted that, when active sites are still available on the stationary phase, the extent of "functionalization" increases with increase in the analytical concentration of the ion-interaction reagent and with increasing length of its alkyl chain. Greater "functionalization" generally involves increased interactions between the analyte and the stationary phase so that, if other effects do not intervene, greater retention times are to be expected. This is the situation observed for anions.

The behaviour of amines, on the other hand, is only apparently different and can be explained as follows. When amines are injected, the species that forms between the amine and the anion of the eluent is physico-chemically very similar to the ion pair of the eluent already adsorbed on the stationary phase and so is able to compete with it for the stationary phase.

These considerations also explain why only amines characterized by alkyl chains shorter than that of the eluent can be analysed. Those with longer chains do not elute in reasonable times, probably because they are more retained on the column than the "functionalizing" eluent itself.

It is concluded that the more similar are the chain lengths, the more comparable are the affinities for the stationary phase and the greater is the competition. It follows

that retention increases as the difference in the alkyl chain lengths of the analyte and the eluent decreases and *vice versa*.

These considerations and the data in Tables I–IV permitted the optimum chromatographic conditions for the separation of mixtures of interest to be established.

Figs. 2 and 3 show typical chromatograms recorded for a mixture containing propylamine, *n*-butylamine, benzylamine, nitrites and nitrates. The conditions were LiChrospher RP-18 (5 μm) as stationary phase and octylamine salicylate flowing at 0.8 ml/min as the ion-interaction reagent, with conductometric detection (Fig. 2) and spectrophotometric detection at 254 nm (Fig. 3). Owing to the molar absorptivity of eluent at this wavelength [$\varepsilon_{salicylate} = (3.08 \pm 0.02) \cdot 10^2$ l cm^{-1} mol^{-1}], nitrites and nitrates, which are transparent, appear as negative peaks and can be easily identified and resolved from amines, which under these conditions give positive peaks. It is worth remembering that transparent amines can also be detected spectrophotometrically, owing to the formation of ion pairs, *i.e.*, the corresponding aminium salicylates. According to the retention mechanism, in this form amines are retained and eluted so that the observed absorbance is due to high absorptivity of the salicylate anion.

The comparison between the behaviour of eluents has so far concerned the lipophilic cationic portion. In order to evaluate the possible role of the anion of the eluent, the use of octylamine orthophosphate was examined with constant stationary phase characteristics. Some typical retention times are given in Table IV.

As can be observed, with respect to the use of octylamine salicylate (Table I), greater retentions of anions were obtained and almost the same retentions for amines.

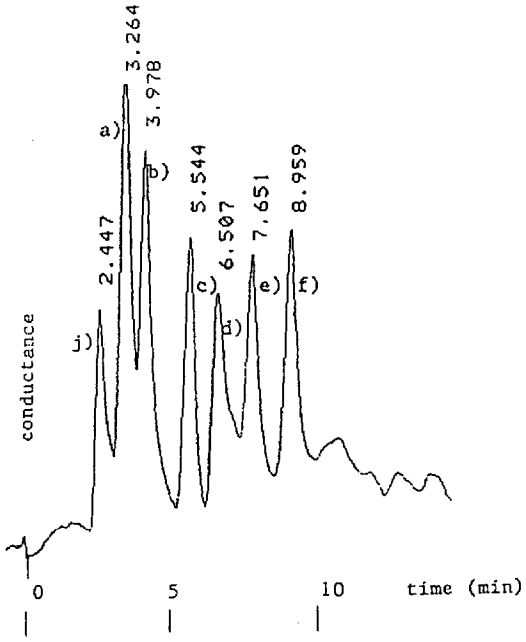

Fig. 2. Separation of a mixture of: (a) propylamine, (b) *n*-butylamine, (c) benzylamine, (d) pentylamine, (e) nitrites and (f) nitrates. Concentrations in the mixture: amines 50.0 ppm each, nitrite and nitrates 10.0 ppm each. Injection volume: 100 μl. Stationary phase: LiChrospher RP-18 (5 μm). Ion-interaction reagent: octylamine salicylate (0.0050 *M*). Flow-rate: 0.8 ml min^{-1}. Conductometric detection.

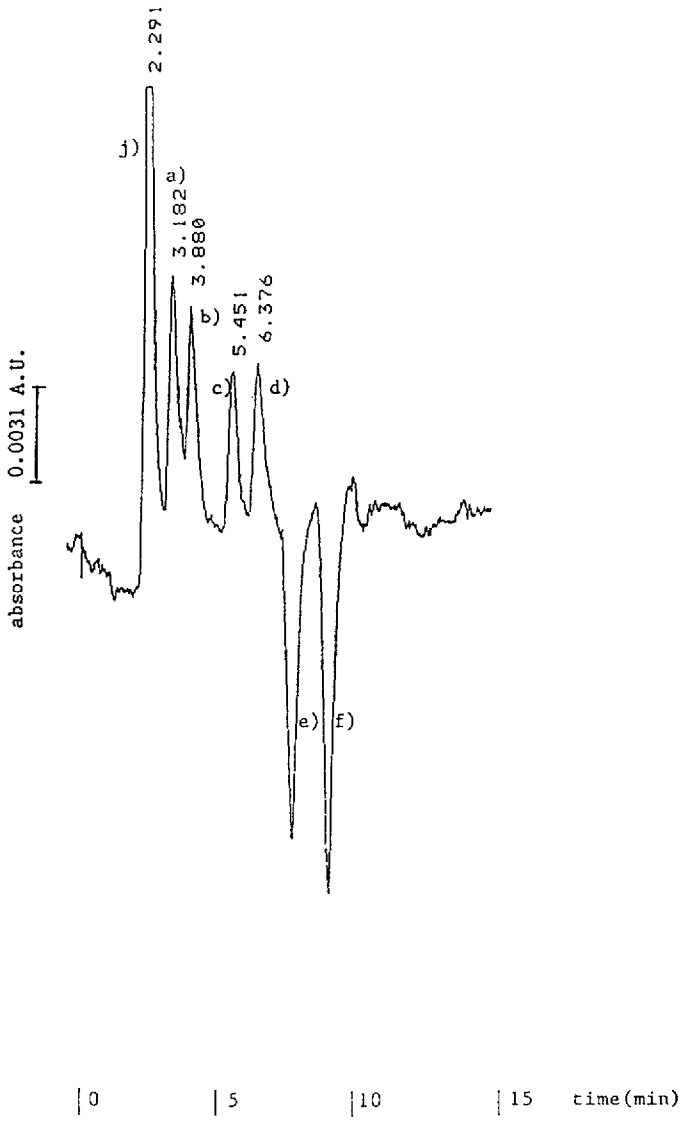

Fig. 3. Separation of the mixture as in Fig. 2, with 50.0 ppm of each components in the mixture. Conditions as in Fig. 2 except spectrophotometric detection at 254 nm.

This result agrees well with the described mechanism, according to which injected amines form an ion pair with the anion of the eluent and in this form are adsorbed and retained through direct interactions with the stationary phase. The results confirm that these interactions would mainly interest the aminium portion rather than its counter anion.

The sensitivity is about the same order for amines and nitrates with octylamine salicylate as eluent, with both conductometric and UV detection (Figs. 2 and 3),

whereas with the use of octylamine ortho-phosphate as eluent with detection at 210 nm, sensitivities about a 1000-fold greater are obtained for nitrites and nitrates with respect to amines, as shown in Fig. 4, which is a chromatogram recorded with a mixture containing 100.0 ppm each of butylamine, pentylamine and hexylamine, 0.20 ppm of nitrites and 0.20 ppm of nitrates. These results, in addition to demonstrating the exceptionally good sensitivity in the analysis of nitrites and nitrates, agree with the retention mechanism concerning amines. The low sensitivity in their analysis corresponds well with the low molar absorptivity at 210 nm shown by the investigated amines, the ortho-phosphate which forms an ion pair with the amine being almost transparent.

The high absorptivities of nitrites and nitrates under these conditions can find applications of practical interest, *e.g.*, in samples of environmental interest such as drinking, river and waste waters. The determination of nitrites and nitrates is in fact possible in the presence of all those potential interferents which do not absorb at 210 nm or are characterized by greater retentions.

The high concentration of chlorides in sea water makes the evaluation of other species generally very difficult. It was shown (Fig. 5) that, by using octylamine phosphate as the ion-interaction reagent and with spectrophotometric detection at 230

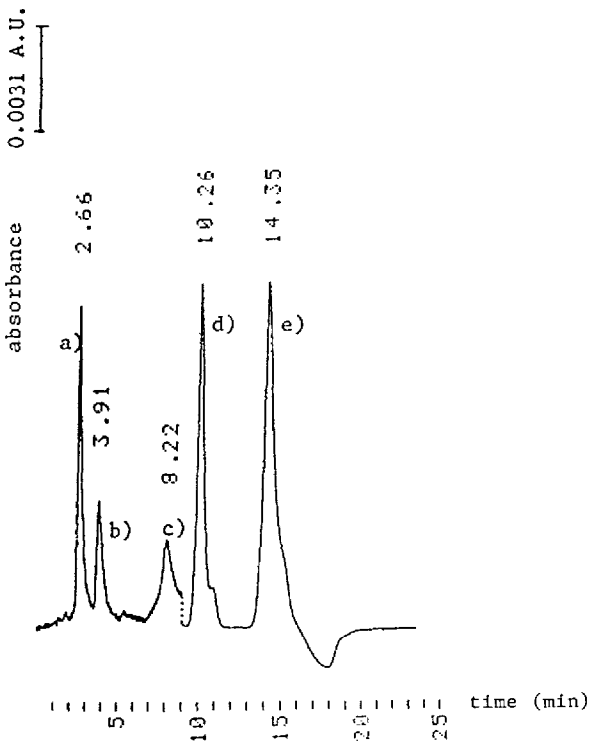

Fig. 4. Separation of a mixture of (a) n-butylamine (100 ppm), (b) *n*-pentylamine (100 ppm), (c) n-hexylamine (100 ppm), (d) nitrites (0.20 ppm) and (e) nitrates (0.20 ppm). 100 μl injected. Stationary phase: LiChrospher RP-18 (5 μm). Ion-interaction reagent: octylamine orthophosphate (0.0050 *M*). Flow-rate: 1.0 ml min^{-1}. Spectrophotometric detection at 210 nm.

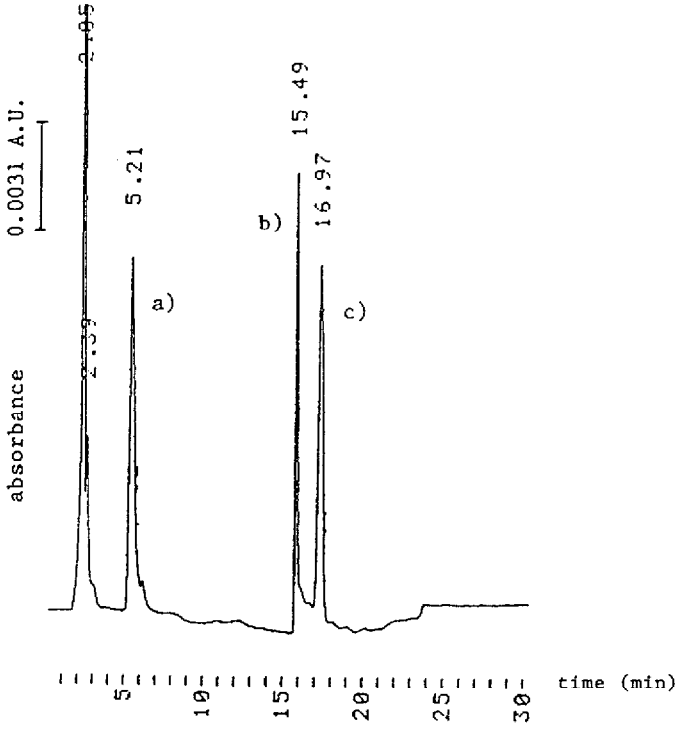

Fig. 5. Chromatogram of Venice lagoon water with 0.50 ppm of 1,4-phenylenediamine added. Peaks: (a) added 1,4-phenylendiamine; (b) unidentified; (c) nitrates (0.20 ± 0.05 ppm). Stationary phase: LiChrospher RP-18 (5 μm). Ion-interaction reagent: octylamine orthophosphate (0.0050 *M*). Flow-rate: 0.8 ml min^{-1}. Spectrophotometric detection at 230 nm.

nm, it is possible to determine in sea water all the species characterized by high molar absorptivities, even in the presence of such a large amount of chlorides, which are transparent at this wavelength. The same analysis with conductometric detection shows only a very large positive peak due to chlorides, which precludes any separation in the retention time window between 2 and 16 min.

It was observed that chlorides when present in so high amounts as in sea water (even if not detected spectrophotometrically) can shift the retention times of anions characterized by similar retention towards times much higher than those shown by the same anions in standard aqueous solutions. Accurate studies of matrix effects are necessary in order to perform a reliable and complete analysis of lagoon water for the accurate identification and determination of nitrites, iodides and bromides, which are probably responsible for the peak at about 15 min in Fig. 5. In contrast, the peak at about 17 min was identified as corresponding to nitrates, which were also determined. Their concentration was 0.20 ± 0.05 ppm.

The determination of aromatic amines in lagoon water is possible under these conditions. Fig. 5 shows the chromatogram recorded for a sample from Venice lagoon to which 0.50 ppm of 1,4-phenylendiamine had been added. This addition did not cause any shift or modification of the pre-existing signals already discussed.

In conclusion, the proposed method for the simultaneous determination of amines, nitrites and nitrates seems to be very advantageous. In particular, the use of octylamine orthophosphate offers the advantage of exceptionally good sensitivity for nitrite and nitrate anions. The accuracy and reproducibility are more than satisfactory and the analysis times are short. In contrast to other proposed techniques[8], no derivatization or pretreatment procedures are required.

REFERENCES

1 M. C. Gennaro, *J. Liq. Chromatogr.*, 10 (1987) 3347.
2 M. C. Gennaro, *J. Chromatogr.*, 449 (1988) 103.
3 M. C. Gennaro and P. L. Bertolo, *J. Chromatogr.*, 473 (1989) 433.
4 M. C. Gennaro and E. Marengo, *Chromatographia*, 25 (1988) 603.
5 B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, 18 (1980) 525.
6 W. E. Hammers, C. N. M. Aussems and M. Janssen, *J. Chromatogr.*, 360 (1986) 1.
7 J. Stahlberg, *J. Chromatogr.*, 356 (1986) 231.
8 K. K. Verma and K. K. Stewart, *Anal. Chim. Acta*, 214 (1988) 207.