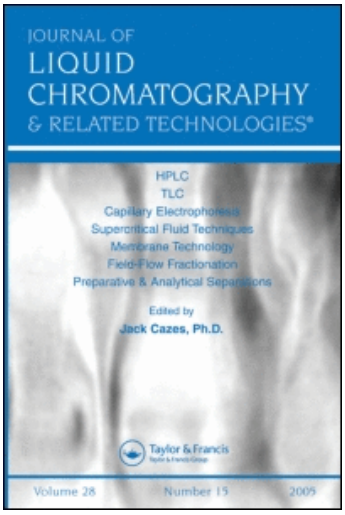

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Separation of N-Containing Species by Means of a New Versatile Ion Interaction RP-HPLC Methodology

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SEPARATION OF N-CONTAINING SPECIES BY MEANS OF A NEW VERSATILE ION INTERACTION RP-HPLC METHODOLOGY

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ABSTRACT

A new reverse phase HPLC methodology is presented for the separation of nitrites, nitrates, aliphatic and aromatic amines contained in the same mixture.

The same basic methodology can be made very versatile by properly varying the experimental chromatographic conditions. Fundamentally, the proposed technique makes use of reverse phase RP-18 columns and of two interaction reagents ,namely octylaminium salicylate and octylaminium ortho-phosphate. Conductometric and spectrophotometric (both direct and indirect) detections are employed.

Through the appropriate choice of mobile phase, kind of detection and detection wavelength, the methodology makes possible the separation and the definite identification of N-containing species, both inorganic and organic.

The method can be very usefully employed in analysis both of drinking water and of waste water.

INTRODUCTION

Previous investigations performed in this laboratory have been devoted to the analysis of amines and anions. By means of the ion interaction reverse phase HPLC technique, new methodologies have been worked out by employing and comparing different interaction reagents and different reverse phase stationary phases [1-6]. Reversed phase columns C-8 and C-18 are used as the stationary phase and the ion interaction reagent used as the mobile phase is generally an ammonium or aminium salt whose anion can be both organic and inorganic. Under these conditions a sort of dynamic functionalization of the stationary phase can be postulated. The results are in good agreement with a model which hypothizes a mechanism in which a process of double-layer ionic adsorption is associated with a step in which electrostatic forces are acting.

It has been shown by different authors [2,6,7-13] that when using this chromatographic technique, retention processes depend on many experimental conditions. These can therefore be suitably varied as a function of the mixture to be separated. Several variants can be so derived from the same basic methodology.

In this paper a method is presented which, through a series of chromatographic runs, allows the evaluation and the interseparation of nitrites, nitrates, aliphatic and aromatic amines contained in the same mixture.

EXPERIMENTAL

Apparatus.

Analyses were carried out by using both a Varian LC 5000 chromatograph (equipped with a Vista 401 Data System and a UV 100 spectrophotometric detector) and a Merck-Hitachi Lichrograph chromatograph mod. L-6200 (equipped with a Merck-Hitachi Model D-2500 Chromato-Integrator and UV/UV-Vis Detector L-4200). A conductometric Wescan 213A detector was also employed.

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

Chemicals and Reagents

Ultra pure water from Millipore Milli-Q was used for the preparation of solutions.

Octylamine was a "Fluka" analytical grade reagent; salicylic acid and all other reagents were "C.Erba" analytical grade chemicals.

Chromatographic Conditions.

Different commercial reverse phase columns, namely a RP-18 Merck Hibar Lichrosorb 5 μm and a RP-18 Merck Hibar Lichrospher 5 μm , 250 x 4.0 mm, were employed.

The solutions to be used as eluents, namely octylaminium salicylate and octylaminium o-phoshate, were prepared,as previously described [1-6], by dissolving the weighed amount of the amine in ultra pure water and by adjusting the pH value of the solutions to 6.4 ±0.4 through additions of salicylic or o-phosphoric acid. In these pH conditions, taking into account the acidic formation constant, octylamine is present in the aminium form and the composition of the eluents so prepared is not exactly stoichiometric. For simplicity, however, the eluents to be used as the mobile phase are mentioned henceforth as ocytlaminium salicylate and octylaminium o-phosphate respectively. In order to condition the chromatographic system properly, eluent must be allowed to flow through the column until a stable baseline signal is obtained. Generally stabilization times of about one hour are necessary. The eluent solutions need to be freshly prepared every three days.

The reproducibility of measurements was very good for successive analyses in the same conditions of eluent preparation

and column conditioning, while little lower for different eluent preparations .For the sake of the most general validity,the average data and the estimates of standard deviation listed in table I were calculated for different preparations.

Betweeen different uses, the column must be regenerated by flowing a water- methanol mixture 1/1 v/v (overnight,flow-rate = 0.1 ml/min). By adopting this treatment,no particular decay of the column life is observed with respect to its use in other chromatographic techniques.

RESULTS AND DISCUSSION

It has been previously shown that the ion interaction reagent reverse phase HPLC chromatographic technique allows good separation of both inorganic and organic anions, as well as of amines[1-6]. The mechanism through which an anion or an amine are retained is somewhat different even if both species give rise, when injected, to ion pairs able to be adsorbed onto the surface of the stationary phase. The anions form the adsorbable species with the aminium ion of the interaction reagent. Amines, in turn, give rise to ion pairs with the anion of the interaction reagent. As concerns the releasing process, it has been proven

that amines elute in the same form under which they were retained, i.e. as the aminium salts formed with the anion of the eluent.

In this study concerning the separation of N-containing species (both anions and amines), two ion interaction reagents have been shown to be of particular interest, namely octylaminium salicylate and octylaminium ortho-phosphate.

Regarding detection, both conductometric and spectrophotometric detections were employed. When using salicylate as counter anion, direct and indirect spectrophotometric detections at 254 nm were employed, owing to the absorptivity value of salicylate (ε = 308 1 $mol^{-1}cm^{-1}$) at this wavelength.

The following figures demonstrate how different chromatographic conditions can usefully be employed for the separation of different analytes contained in the same matrix.

Figure 1 shows the chromatogram recorded (with the use of a Merck Hibar Lichrosorb RP-18 5 μm as stationary phase, 0.0050 M octylaminium salicylate flowing at a rate of 0.70 ml/min as mobile phase and UV detection at λ =254 nm) for a mixture containing aliphatic and aromatic amines, namely : methylamine, ethylamine, propylamine, butylamine, 1,4-phenylendiamine,

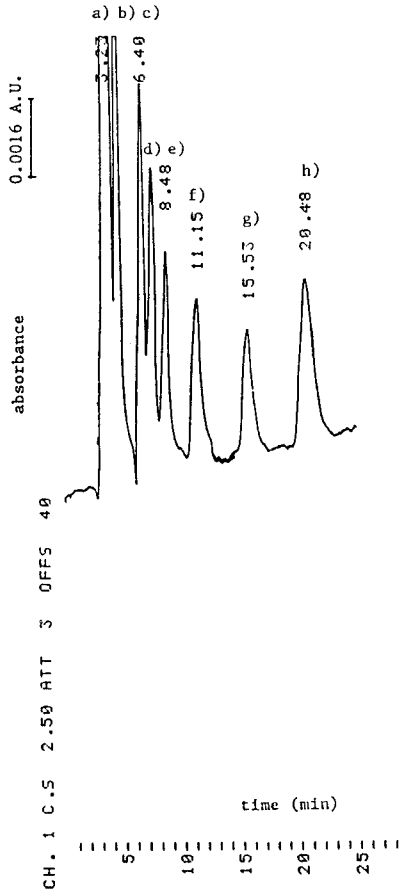

FIGURE 1. Separation of a mixture of : a) methylamine (25.0 ppm), ethylamine (25.0 ppm), propylamine (25.0 ppm), butylamine (25.0 ppm) , b) 1,4-phenylendiamine, c) pentylamine (25.0 ppm), d) 1,3-phenylendiamine e) 2-phenylethylamine (10.00 ppm), f) 1,2-phenylendiamine (2.0 ppm), g) 3-phenylpropylamine (10.0 ppm), h) aniline (5.0 ppm)
Stationary phase : Merck Hibar Lichrosorb RP-18 , 5 μm
Ion Interaction Reagent : 0.0050 M octylaminium salicylate
Flow-rate : 0.7 ml/min ; 100 μl injected
Detection : UV λ= 254 nm

pentylamine, 1,3-phenylendiamine , 3-phenylpropylamine and aniline.

As already mentioned, in these conditions both aliphatic and aromatic amines give rise (when injected) to ion pairs with the salicylate anions of the eluent , under which form they are also eluted. Spectrophotometric detection was employed, the eluates being characterized by positive values of absorbance due to the contributions of both salicylate and amines ; at 254 nm the major contribution is that of salicylate.

Figure 2 shows the separation obtained with the use of a Merck Hibar Lichrospher RP-18 5 μm column and 0.0050 M octylaminium salicylate (flow-rate = 0.8 ml/min) for a mixture containing aliphatic and aromatic amines (propylamine, n-butylamine, benzylamine and pentylamine) together with nitrites and nitrates. Conductometric detection was employed: all the species contained in the mixture are characterized by non null equivalent conductivity values and therefore give rise to positive signals, permitting the simultaneous evaluation of anions and amines .

Figure 3 ,in turn, reports the analysis obtained for the same mixture (as in figure 2) when using spectrophotometric

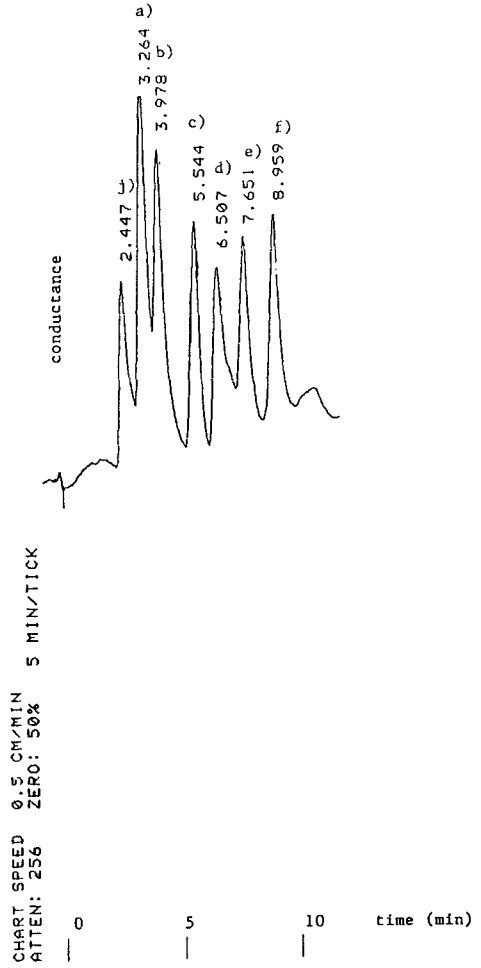

FIGURE 2. Separation of a mixture of : a) propylamine, b) n-butylamine, c) benzylamine ,d) pentylamine,e) nitrites, f) nitrates. j) injection peak
Stationary phase : Merck Hibar Lichrospher RP-18 , 5 µ m
Ion Interaction Reagent : 0.0050 M octylaminium o-phosphate
Flow-rate : 0.8 ml/min ; 100 µl injected.
Conductometric detection.

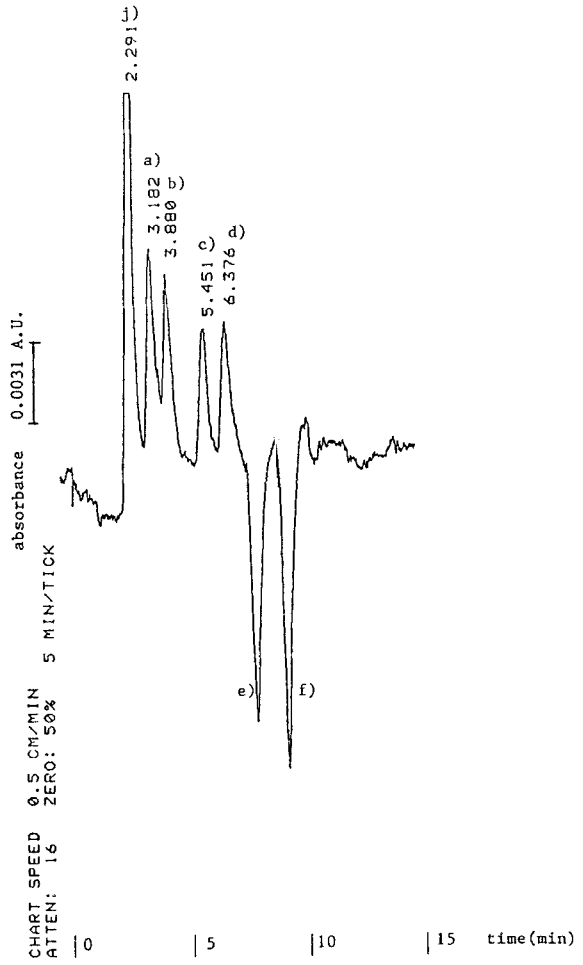

FIGURE 3. Separation of the mixture as in figure 2. Chromatographic conditions as in figure 2. Spectrophotometric detection : λ = 254 nm.

detection at 254 nm, all other conditions being constant. This chromatographic run allows the identification in the mixture of nitrites and nitrates which - being characterized at this wavelength by practically null adsorptivity values - can be detected indirectly as negative peaks .

A further separation step can be achieved by varying the hetaeron anion of the eluent, together with the detection wavelength. By the use of octylaminium ortho-phosphate as ion interaction reagent and by means of spectrophotometric detection at 210 nm, it is possible to obtain the separation between aliphatic and aromatic amines. In these conditions only aromatic amines are characterized by high adsorptivity values.

Figure 4 shows , as an example, the separation that can be achieved for 1,3-phenylendiamine (0.50 ppm), 1,2-phenylendiamine (1.00 ppm) and aniline (1.00 ppm) even in the presence of methylamine, ethylamine, n-butylamine, propylamine and pentylamine at a concentration of 50.0 ppm each.

The use of these conditions of eluent (0.0050 M octylaminium ortho-phosphate) and detection (spectrophotometric, λ = 210 nm) also allows the separation between aliphatic amines (which do not adsorb) and nitrites and nitrates ,which on the contrary do.

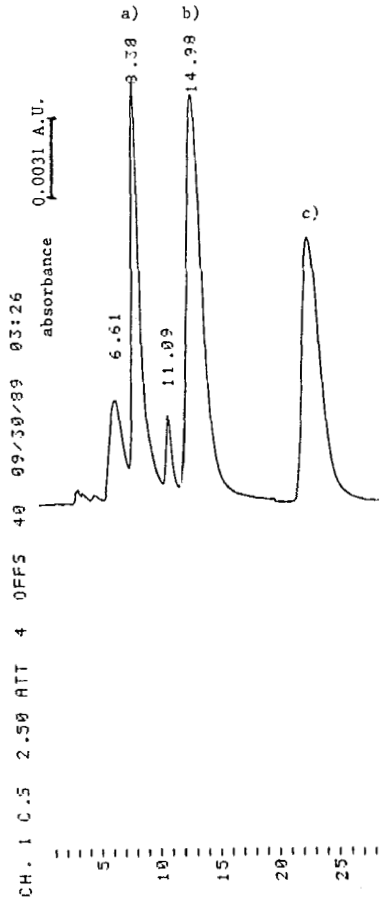

FIGURE 4. Separation of a mixture of methylamine(50.0 ppm), ethylamine (50.0 ppm),propylamine (50.0 ppm), butylamine (50.0 ppm), pentylamine (50.0 ppm), and a) 1,3-phenylendiamine (0.50 ppm), b) 1,2-phenylendiamine (1.00 ppm) and c) aniline (1.00 ppm).
Stationary phase : Merck Hibar Lichrosorb RP-18 5 μm
Ion Interaction Reagent : 0.0050 M octylaminium o-phosphate
Flow-rate : 0.7 ml/min ; 100 μl injected
Detection : UV λ= 210 nm

TABLE I

Retention Times (min) for Typical Amines and Anions.
Stationary Phase : Merck Hibar Lichrospher RP-18 5 μm.
Flow-rate : 0.8 ml/min

	0.0050 M Octylaminium Salicylate	0.0050 M Octylaminium o-Phosphate
propylamine	3.3 ±0.3	2.7 ±0.2
n-butylamime	3.5 ±0.3	3.0 ±0.3
1,4-phenylendiamine	4.3 ±0.3	5.2 ±0.4
benzylamine	5.5 ±0.3	4.9 ±0.3
n-pentylamine	6.5 ±0.4	5.5 ±0.4
chlorides	5.5 ±0.3	16.0 ±0.5
nitrites	7.7 ±0.4	12.5 ±0.4
nitrates	9.0 ±0.3	16.9 ±0.5

Table I reports, for comparison purposes , the retention data for some typical amines and anions obtained with the interaction reagents octylaminium salicylate and octylaminium ortho-phosphate, all other conditions being constant.

As mentioned , the estimates of standard deviations listed in table I are based on at least four determinations and for different eluent preparations.

It is worth underlining that ,by the use of the two interaction reagents,comparable retention times are generally obtained when the analytes are amines (both aliphatic and aromatic) ,whilst relevant differences can be observed in the analysis of nitrites, nitrates and all the anions investigated.

This different behaviour agrees with the retention mechanism already proposed [1,3,6] for the interaction reagent chromatographic technique. The competing equilibria which take place between compounds of similar structure surely play an important role in retention. In particular, the competition equilibria experienced by both the injected amine and the amine portion of the eluent towards the hetaeron anion have to be considered.

It is worth noticing to this end that the chemical properties of the hetaeron anion do not play any role in the retention of amines whilst they affect heavily the retention of anions (as can be seen from the data of table I).

In conclusion, the examples mentioned demonstrate how the same basic methodology can be rendered more and more versatile through suitable variations in some characterizing parameters in order to fit different separation problems. The adjustment of the basic technique to new separation problems can be more advantageously carried out as mechanisms which govern retention become better known. Experimental design studies are in progress in order to obtain useful information relating to different variable parameters.

We believe that the methodology proposed in this papers for the identification and separation of different species containing nitrogen can advantageously contribute to solving environmental problems such as urban and industrial waste waters . Good levels of accuracy, reproducibility and sensitivity (of the order of 50 ppb for aromatic amines) can be achieved.

Furthermore, the proposed methodology presents the great advantage of not requiring any preparation ,derivatization or pretreatment of the sample aside from a suitable (0.45 μ m) filtration process.

This represents not only a saving in time but also gives an exceptional advantage when dealing with environmental samples, in which any pretreatment procedure could modify the natural speciation equilibria.

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