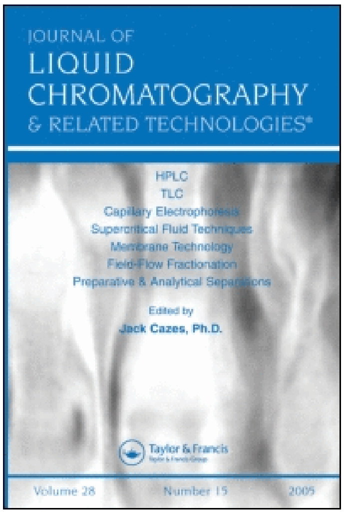

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N-PYRROLYL DERIVATIVE ACIDS CHROMATOGRAPHY

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ABSTRACT

A determination is made on the effects of the variation of pH,methanol %,contents of tetrabutylammonium salts, ionic strength and counterion nature of the mobile phase quaternary ammonium salt,on the retention of a series of analgesics formed by N-pyrrolyl derivative acids through a column constituted by 10 µ irregular particles of octadecyl silica.

The results obtained are applied to mixture separation of said acids.

INTRODUCTION

A series of acids containing the N-pyrrol group in their molecule (in most cases in carbon 2) have been synthetized (1) (2), at our Departament.

These acids are interesting due to the analgesic and plaquetary antiaggregating action they have (3).The acids studied here are :

(I).- 3-(phenyl)-2-(1-|1H|pyrrolyl) propanoic

(II).- 3-(4-hydroxyphenyl)-2-(1-|1H|pyrrolyl) propanoic

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(III).- 3-(4-fluoro phenyl)-2-(1-|1H|pyrrolyl) propanoic
(IV).- 3-(4-chloro phenyl)-2-(1-|1H|pyrrolyl) propanoic
(V).- 2-(1-|1H|pyrrolyl) ethanoic
(VI).- 2-phenyl-2-(1-|1H|pyrrolyl) ethanoic
(VII).- 2-|4-(1-|1H|pyrrolyl)|phenyl ethanoic
(VIII).- 2-(2-fluoro phenyl)-2-(1-|1H|pyrrolyl) ethanoic
(IX).- 2-(4-chloro phenyl)-2-(1-|1H|pyrrolyl) ethanoic
(X).- 2-(1-|1H|pyrrolyl) benzene carboxilic
(XI).- 4-(1-|1H|pyrrolyl) benzene carboxilic

The parameters corresponding to the mobile phases which have the greater influence on the retention of the product chromatographied by stationary phase are usually: pH,solvent organic quantity,quaternary ammonium salt concentration,nature of the counterion used and the ionic strength of the mobile phase. The first three being the most important in this case.

This work tries to quantitatively determine the influence that every mentioned factor has on the retention of a series of analgesics formed by N-pyrrolyl derivatives acids, which passes through the octadecyl-silica column, with the purpose of disposing of sufficient data in order to establish analytical methods for said compounds by means of HPLC.

MATERIAL AND METHODS

Reagents

The acids studied here have been synthetized and purified at our Departament and their purity ranges between 89.9 % of acid (IX) and 99.8 % of acid (XI).All ofthem have been characterized by UV,IR and ^{1}H-RMN.

The dissolutions of tetrabutylammonium,chloride,sulphate, acetate and phosphate salts,have been prepared from tetrabutylammonium bromide dissolutions (TBA-Br),passing them through "Amberlite IRA-400" resin,previously conditioned with the corresponding anion.

Instruments

A chromatograph, made up of a "Laboratory Data Control" (LCD) Constametric II pump, has been used. "Rheodyne" Mod. 7210 injection system, constant injection volume 20 µl. "Waters" Mod.441 (229nm) detector. "Sargent Welch" Mod. XKR recorder. The chromatographic column used was 100 mm long and 8 mm in inner diameter, µ-Bondapack C-18 inside a radial compression "Z-Module" "Waters".

Chromatographic Procedure

In all cases, the injection volume has been 20 µl and the concentration 0.05 mg/ml. The flow was 2 ml/min. The work was carried out at room temperature. The determination of dead volume (V_o) has been made on the basis of samples unretained by the column under our working conditions. (V_o) value for our system is 3 ml. The different mobile phase used were prepared by using pH phospate buffer suitable in each case and methanol at the percentage desired. The ionic strength has been estabilished by adding the necessary quantity of KBr. When working with mobile phases containing TBA^+, the column has been previously conditioned by passing it through the mobile phase for 1-2 hours which is used later before starting a series of tests. After working with one of TBA^+ salts and before beginning work with another one, the column is washed with water-methanol at 50 %, a volume equal to 20 times that of the column.

RESULTS AND DISCUSSION

Influence of Mobile Phase pH

To carry out the study of this influence, the work has been done with mobile phases constituted by 0.05 M phosphate buffer with different pH and methanol in the proportion of (70/30.v/v). The pH of the different mobile phases being : 2.5 ; 3.5 ; 5.0 ; 6.0 and 7.0 .

The acids studied are weak. Their retention by the column must be in close relationship with the form in which they are found, dissociated or undissociated.

Consequently mobile phase pH plays an important rôle in the chromatographic process. Equation I, (4) allows us to determine the capacity factors of both the dissociated (k'_{-1}) and the undissociated (k'_o) forms of every acid studied

$$k' = \frac{k'_o + k'_{-1} \dfrac{k'_a}{|H_3O|^+}}{1 + \dfrac{k_a}{|H_3O|^+}} \qquad \text{(Equation I)}$$

once the pK_a (5) of each, under our working conditions, (Table 1) and the mobile phase pH is known, and the values of capacity factors (k') are experimentally determined.

In Fig. 1, the points represent the experimental values of capacity factor (k') at the different working pH and the continuous lines have been obtained from these experimental data applied to equation I, by the nonlinear minimun square method, developed according to a program (6) applied to a microcomputer.

The sigmoid curves obtained show a good concordance between theoretical previsions proceeding from equation I and the experimental results, observing in all cases that as pH values are increased, the capacity factor values drastically decrease.

Are shown in table 1 the values obtained through equation I for (k'_o) and (k'_{-1}) in it, it is clearly seen that, as expected, the capacity factors of undissociated forms (k'_o) are much higher than those of the dissociated forms (k'_{-1}). The relationship (k'_o / k'_{-1}) is about five in most cases except with acid (IV) in which said relationship has a value of seventeen, which shows the enormous polarity difference between the dissociated and undissociated form of this acid. The (k'_o) and (k'_{-1}) values of the acids : (III), (V), (X) and (XI) have not been determined since their retention by the column is very high.

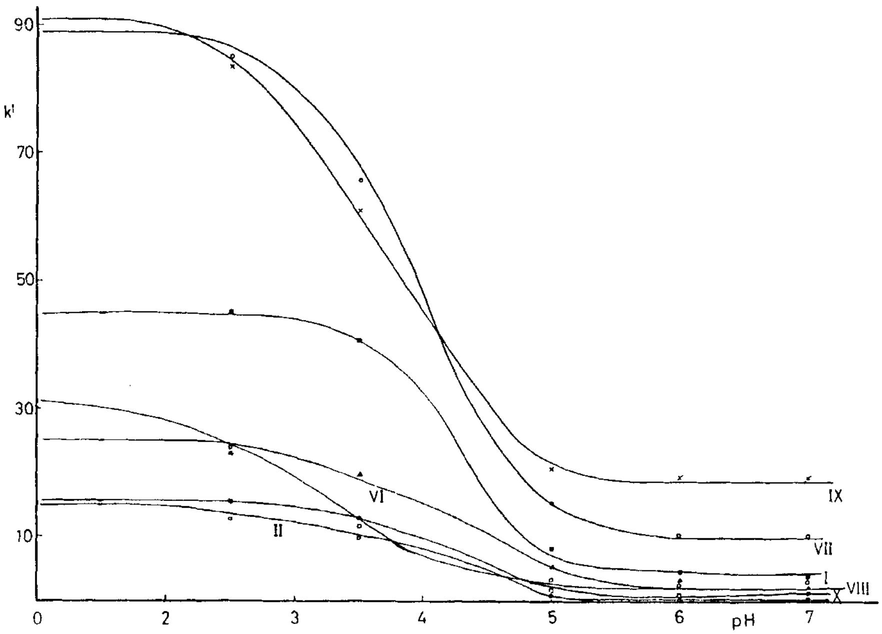

FIGURE 1. Effect of variation pH on the capacity factor of N-pyrrolyl derivatives acids was studied. The points represent the experimental values of capacity factor at the different working pH, and the continuous lines have been obtained from these experimental data applied to equation I. Column: μ-Bondapack C-18. Mobile phases 0.05 M phosphate buffer, methanol (70/30, v/v), the pH of different mobile phases being: 2.5; 3.5; 5.0; 6.0 and 7.0 .

Influence of Methanol Contents

Mobile phases composed of 0.05 M phosphate buffer, pH = 7.0 and methanol in proportions : 0 ; 10 ; 20 ; 30 ; 40 and 50 % (v/v) have been used to carry out the study of this influence (7). The representation of (k') values experimentally obtained, contrasted with the % of methanol contained in mobile phase is shown in Fig.2. It can be observed that (k') values drastically increase in all cases when the % of methanol decreases in the mobile phase,

TABLE 1

Values of pK_a and Capacity Factors of both the Dissociated and Undissociated Forms of every Acids Studied.

Compound	pK_a	k'_o	k'_{-1}
(I)	3.86	25.3	4.32
(II)	3.74	14.7	1.53
(III)...........	4.07	--	--
(IV)	3.83	15.4	0.83
(V)	3.85	--	--
(VI)	3.74	89.3	11.3
(VII)...........	4.57	45.7	8.16
(VIII)..........	3.20	31.1	5.89
(IX)	3.64	93.8	18.7
(X)	3.68	--	--
(XI)	5.51	--	--

but mainly in those acids showing a higer polar character such as acids (VIII) and (IX).

Influence of TBA^+ Concentration

This influence (8),(9) has been determined by working with mobile phases constituted of 0.05 M phosphate buffer pH = 7.0 and methanol in the proportion (60/40,v/v) and the quantity of tetrabutylammonium bromide (TBA-Br) necesary in each case to obtain the concentrations : 0 ; 1×10^{-4} ; 6×10^{-4} ; 1×10^{-3} ; 1×10^{-2} and 5×10^{-2} M. The ionic strength of all mobile phases has been kept constant at 0.1 M value, by adding KBr. In Fig.3,the values of the capacity factor obtained on varying the concentration of (TBA-Br) are represented,having observed that generally the presence of this salt remarkably increases and, in most cases the value of the capacity factor (k'), especially in those acids showing a higer hydrophobic character,also the presence of aromatic groups

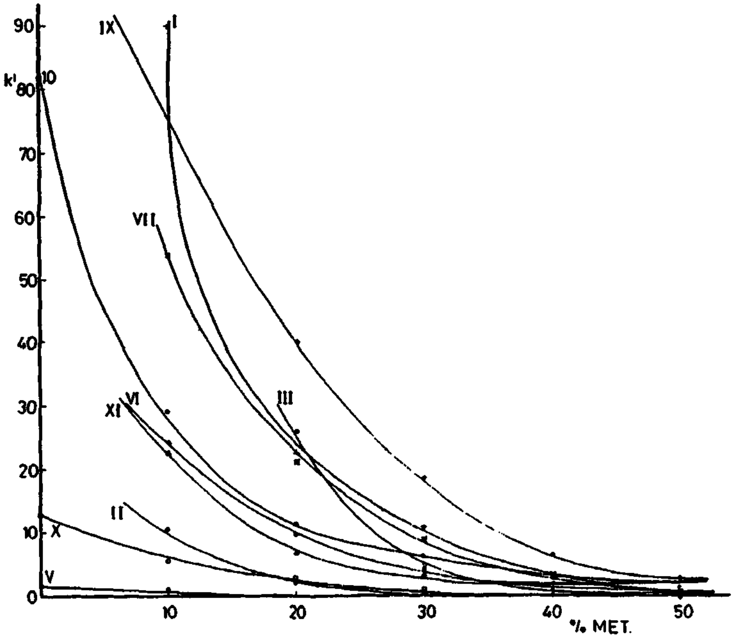

FIGURE 2. Effect of variation % methanol contents in mobile phase on the capacity factor of N-pirrolyl derivatives acids studied. Column: μ-Bondapack C-18. Mobile phases:0.05M phosphate buffer,pH=7.0 and methanol in proportions:0;10;20;30;40 and 50 % (v/v).

as well as the introduction of halogens in said ring has been found to make that effect more remarkable.

In the acids,which due to their scarce hydrophobicity have a little interaction whit ($k' \simeq 0$) apolar stationary phase in absence of (TBA-Br) it is observed that they are much more retained when mobile phases containing (TBA-Br) are used,which is interesting since it can be used to make the chromatographic separation of these acids.

Influence of the Ionic Strength Variation

To study the ionic strength on the anion retention of the acids studied in octadecyl-silica stationary

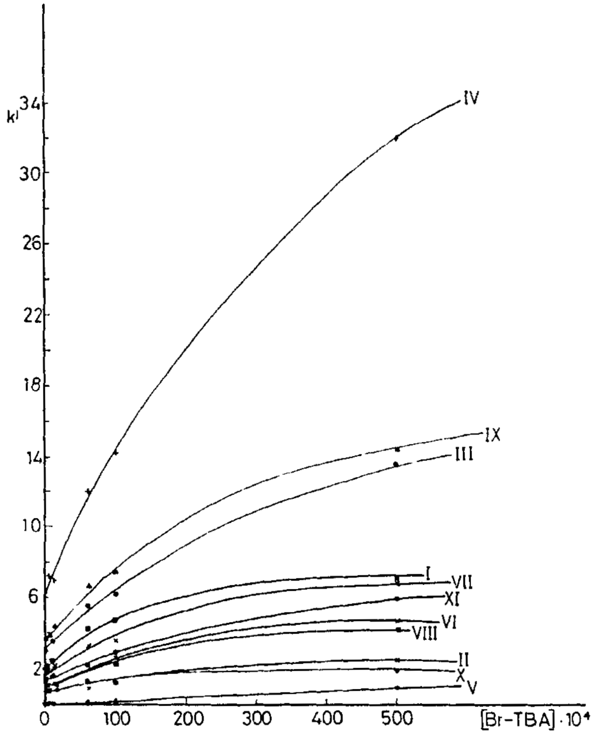

FIGURE 3. Effect of tetrabutylammonium bromide concentration in mobile phases on the capacity factor of N-pyrrolyl derivatives acids was studied. Column: μ-Bondapack C-18.Mobile phases :0.05; M phosphate buffer pH.7.0,methanol (60/40,v/v) concentrations of TBA-Br: $1x10^{-4}$;$6x10^{-4}$;$1x10^{-3}$ $6x10^{-3}$;$1x10^{-2}$ and $5x10^{-2}$ M. Ionic strength of all mobile phases 0.1M.

phases,mobile phases have been prepared which are constituted of mixtures of 0.005 M phosphate buffer,pH = 7.0 and methanol (60/40,v/v) containing 1.7×10^{-3} M of (TBA-Br) and ionic strength : 0.01 ; 0.03 ; 0.05 ; 0.06 ; 0.08 and 0.1 M. obtained by the addition of KBr.

The representation of capacity factors depending on the ionic strength of mobile phases is shown in Fig. 4. It can be seen that positive slope (straight) lines,or zero in some cases,are obtained. As it can be seen in

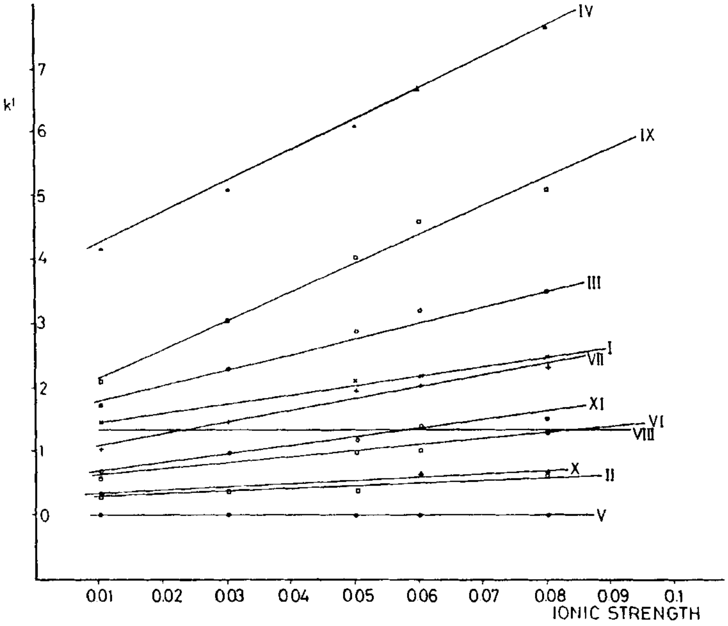

FIGURE 4. Effect of ionic strength of mobile phase on the factor capacity.Column: µ-Bondapack C-18 Mobile phases:0.005M phosphate buffer,pH.7.0, methanol(60/40,v/v),concentration of TBA-Br 1.7×10^{-3} M,ionic strength:0.01;0.03;0.05;0.06; 0.08 and 0.1 M.

Fig.4. the influence of the ionic strength on the factor capacity is very poor or non existent in acids : (II) , (V) ,(VIII) and (X).However,in all the other acids and especially in : (III) , (IV) and (IX),the increase of the ionic strength exerts a remarkable influence on the retention of these acids in the stationary phase.

Influence of the Counterion Variation

To determine this influence mobile phases have been used which are constituted of one of the following salts: bromide,chloride,sulphate,acetate and tetrabutylammonium phosphate,at a concentration of 1×10^{-3} M,the ionic strength being adjusted to 0.025 M in every mobile phase

TABLE 2

Values of Capacity Factor of each Acid when Varying the Counterion of Quaternary Ammonium Salt.

Compounds	TBA-Cl	TBA-Br	TBA-SO_4	TBA-$OOCCH_3$	TBA-PO_4
(I)	5.00	4.33	4.66	3.66	--
(II)	1.66	1.50	2.00	1.50	1.33
(III)	9.00	7.66	10.7	9.00	8.00
(IV)	23.0	18.7	27.3	29.0	29.0
(V)	0.00	0.00	0.00	0.00	0.00
(VI)	2.66	2.33	2.66	2.33	2.33
(VII)	4.66	4.00	4.33	3.66	4.33
(VIII)	2.66	2.33	2.33	2.00	2.33
(IX)	9.00	7.66	8.00	6.33	7.66
(X)	1.66	1.33	2.00	1.33	1.33
(XI)	1.33	1.33	1.50	1.33	1.50

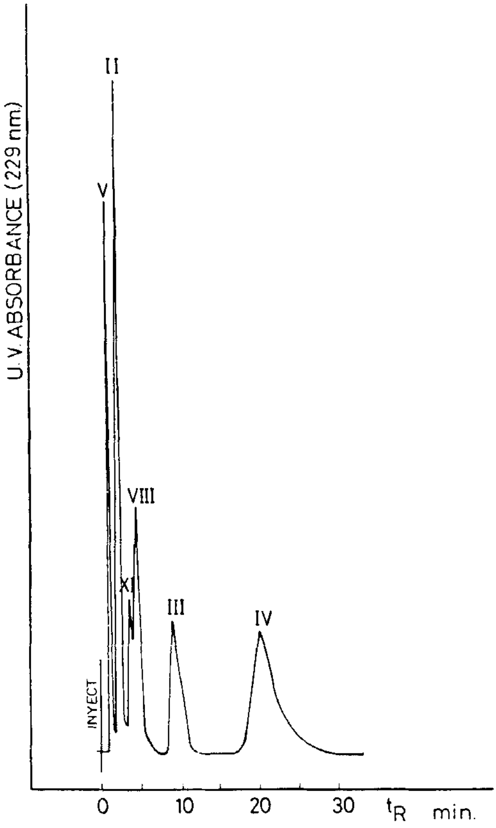

FIGURE 5. Chromatographic separation of six N-pyrrolyl derivatives acids was studied.Column: µ-Bondapack C-18.mobile phase: 0.01 M phosphate buffer pH=7.0 methanol (60/40,v/v),concentration of TBA-Br 0.017 M,and ionic strength 0.1 .

by adding the potassium salt of the corresponding anion. The final pH of every mobile phase is 7.0 ,being adjusted in each case by adding the necessary quantity of the corresponding acid to each anion.

Published material has described cases in which the nature of counterion exerts an important influence on capacity factors (10). However ,the results obtained by us Table 2. show that these are on important differences of capacity factor of each acid when varying the counterion of quaternary ammonium salt.

The poor influence exerted by the counterion used, as well as the little influence of the ionic strength on the value of capacity factor for every acid studied seems to indicate that the chromatographic interactions in this kind of chromatography in the presence of quaternary ammonium salts, proceeding in this case,from an ion pair formation mechanism,rather than an ionic interchange mechanism.

Applications

The results obtained from the chromatographic study allows us to the select the most appropriate chromatographic conditions to analyse by HPLC the acids studied,both in pure aqueous dissolutions and in biological liquids.For instance, a chromatographic separation of six of these acids is obtained by the use of the mobile phase formed by mixing 0.01 M phosphate buffer,pH = 7.0 ,0.017 M tetrabutylammonium bromide and 0.1 ionic strength,with methanol in the proportion (60/40,v/v).The chromatogram obtained is shown in Fig.5.

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