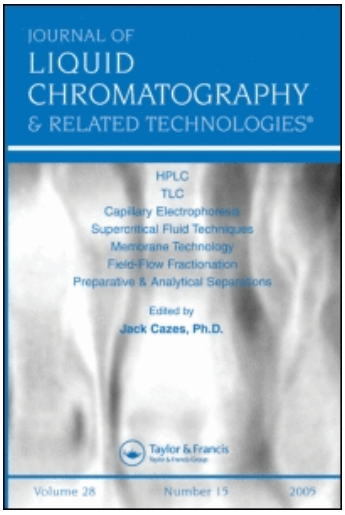

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THE RETENTION OF SOME ORGANIC ACIDS IN ION-PAIR HPLC SYSTEMS

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ABSTRACT

The retention behaviour of some organic acids (N-phenylamides of benzoylacetic acid, phenolic acids and analgesic drugs) as model substances was investigated in reversed phase systems consisting of octadecyl silica (ODS) as a column packing material eluted with the buffer-methanol mixtures containing low concentrations of cetyltrimethylammonium bromide (cetrimide), tetrabutylammonium chloride (TBA-Cl), tetraethylammonium chloride (TEA-Cl) and di(2-ethylhexyl) orthophosphoric acid (HDEHP). The chain length of the n-alkyl group of the ion-pair reagent and the content

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of a modifier in the eluent contribute to retention. Correlation between log k´ and log P and biological activity of N-phenylamides was analysed.

INTRODUCTION

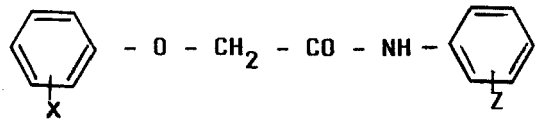
The retention behaviour of some organic acids: N-phenylamides of benzoylacetic acid, phenolic acids and analgesic drugs was investigated in reversed-phase ion-pair chromatography (RP-IPC). Most drugs and related biologically active substances are present mainly in an ionized form in the aqueous solutions. HPLC analysis of these compounds is difficult with reversed-phase columns due to poor resolution; formation of ion pairs with suitable hydrophobic counterions (for acids quaternary alkylammonium compounds) give the possibility of increased selectivity, improved peak symmetry and sensitivity needed for good separation and for quantitative determination. In one of the theories small polar ion pair reagents react with the ionized solutes forming neutral ion pairs, in the other an active ion-exchange surface is produced where long chain, non-polar anions or cations are adsorbed by the hydrophobic stationary phase (1-7). The optimization of the retention and selectivity for the investigated compounds was carried out by changing pH, concentration of methanol and concentration of the ion-pairing reagent in the mobile

phase. Phosphate buffer solutions at lower pH values prevent ionization of carboxyl groups of these compounds reducing their solubility in the mobile phase and increasing their retention time. Di(2-ethylhexyl)orthophosphoric acid (HDEHP-relatively strong acid, pK_A = 1.3) was also used as a modifier to study the retention behaviour of N-phenylamides (8,9). N-phenylamides owing to their biological activity can be used in studies of quantitative structure - activity relationships (QSAR) (1,10,11). The experimental results indicate that quaternary alkylammonium compounds are suitable as ion-pairing reagents for the chromatographic separation of the investigated compounds in the systems with octadecyl silica (ODS) as the column packing material or on HPTLC precoated RP-18 plates.

EXPERIMENTAL

Column HPLC was carried out using a liquid chromatograph (produced by the Institute of Physical Chemistry of the Polish Academy of Sciences, Warsaw) equipped with a 200 ml syringe pump, a 5 µl injector valve and a UV detector (254 nm). The reference sample concentration for HPLC was about 0.1 mg/ml in the eluent. Stainless steel columns 100 x 3.8 mm or 250 x 4 mm were packed with 5 µm or 10 µm ODS (Polish Reagents, Lublin). The appropriate amounts of ion-pairing reagent per 100 cm^3 of the buffer-

TABLE 1. The log P values and pharmacological data of investigated compounds:



No	X	Z	Inhibition of synthetase of prostaglandins ID_{50}(log μmol)	Strength of binding to albumin ID_{50} (log μmol)	log P
1	4-Me	2-COOH	1.58	2.30	2.863
2	4-Me	3-COOH	2.70	3.67	2.863
3	-	2-COOH	2.04	2.78	2.863
4	2-Me	2-COOH	2.70	2.65	2.863
5	2-Me	3-COOH	2.70	3.56	2.863
6	3-Me	2-COOH	2.70	2.40	2.863
7	4-Me	2-COOH, 4Cl	0.28	1.40	3.610
8	4-Me	2-COOH, 6Cl	2.12	2.75	3.610
9	4-Me	2-COOH, 4Br	0.00	1.34	3.819
10	4-Me	2-COOH, 4Me	0.94	2.00	3.390
11	4-Me	2-COOH, 6Me	2.70	2.80	3.390
12	4-Cl	2-COOH, 4Cl	0.04	1.40	3.863
13	4-Me	2-COOH, $5NO_2$	-	-	2.854
14	2-Me	4-COOH	-	-	2.863
15	4-Cl	2-COOH	-	-	3.091

methanol mixtures were added. The void volume was determined by injection of pure methanol. The mobile phase was passed through the column until constant retention of a reference solute was obtained. The flow rate was 1.2 $ml.min^{-1}$. All measurements were carried out at the ambient temperature, 25 $\pm$ 1°C.

High performance thin-layer chromatography was carried out in sandwich chambers with a glass distributor of the eluent, using 10 x 10 cm precoated HPTLC plates of RP-18 F_{254} (E.Merck, Darmstadt, FRG). 1 µl samples of 0.1% w/v solutions of the solutes in methanol were spotted 1 cm from the edge and eluted over a distance of 8.5 cm. The spots of the compounds were localized under UV light at 254 nm and at 366 nm. Chromatographic conditions are given in figure legends or in the text. The N-phenylamides of benzoylacetic acid were synthesized in the Department of Pharmaceutical Chemistry of the Medical Academy of Cracow and their biological activities were determined in the Department of Pharmacology of the Medical Academy of Cracow.

RESULTS AND DISCUSSION

Fig.1 and Fig.2 represent the plots of capacity factors of N-phenylamides of benzoylacetic acid as a function of pH of 0.01 M phosphate buffer used in the mobile phase containing 0.2% of cetrimide and 70% of

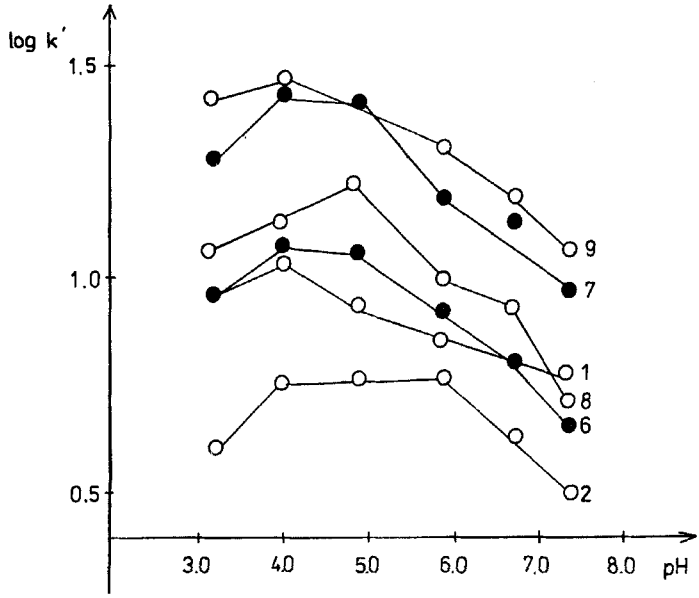


FIGURE 1. Plots of log k´ vs.pH of the mobile phase containing 70% methanol, 0.2% cetrimide and 0.01M phosphate buffer. Column 10 x 3.8 cm dp 5 µm ODS. For notation see Table 1.

methanol. The buffer pH stated is that measured in the undiluted buffer and not in the final eluent. These investigations are the continuation of the previous ones (1). A stronger retention of N-phenylamides is observed at low pH values; the highest increase of retention and best selectivity of separations is observed at buffer solution of pH ca.4. The hydrophobic compounds having two atoms of chloride or chlorine and

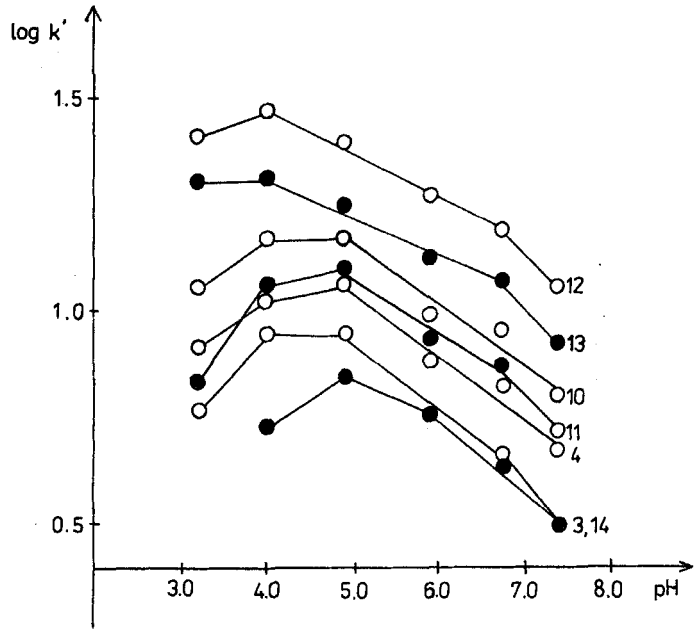


FIGURE 2. As in FIGURE 1

bromine in position 4 in the molecule give the longest retention times.

Fig.3 shows the plot of the capacity factors of the amides as a function of concentration of methanol in the mobile phase containing 0.16% v/v of HDEHP and phosphate buffer of pH 7.17. The effect of the solvent composition in these systems (water or buffer solution + organic modifier) is frequently described by the semi-empirical equation log k´ = constant + n(% water)(12,13). The plots sometimes cross (changing of the sequence of

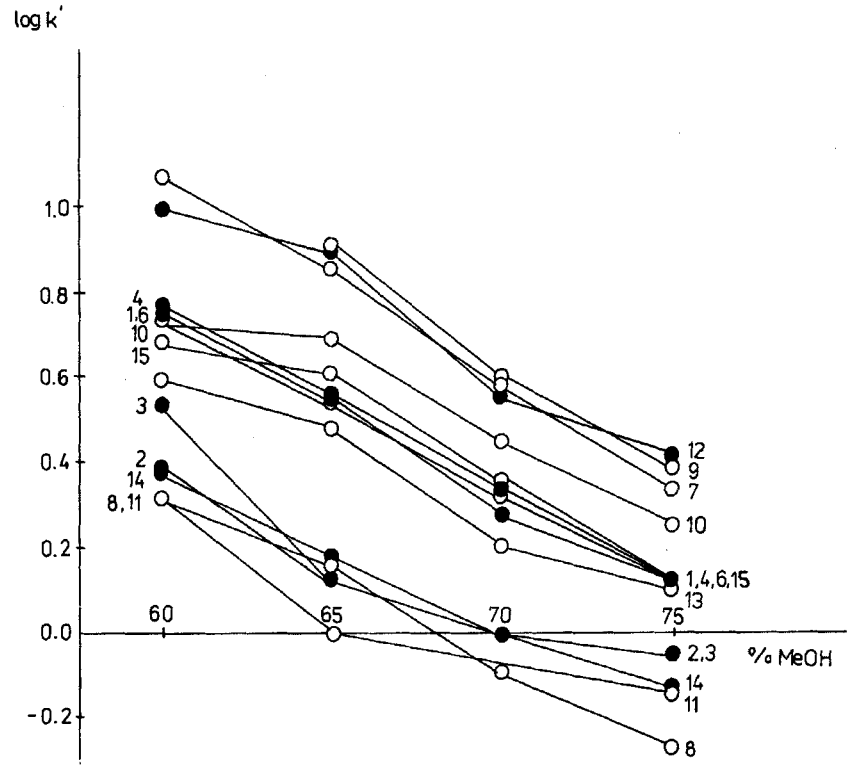


FIGURE 3. Log k´ vs.% methanol in the mobile phase containing 0.16% v/v HDEHP and 0.005M phosphate buffer, pH 7.17. Column 250 x 4 mm, dp 10 µm, ODS. For notation see Table 1.

the solutes). The experimental data (log k´) of some separated compounds obtained in this system for 65% methanol were correlated with log P (partition coefficients calculated from Rekker´s hydrophobic fragmental constant (14) and with biological activity (eqs.3 and 4) expressed by the inhibition of synthetase

of prostaglandins (a) and the strength of binding to albumin (b) according to Collander's equation (15):

$$-\log C = b \log k' + a \qquad (1)$$

where C is the molecular concentration of the compound producing an equivalent biological effect. Equations for correlations log P - log k' and log k' - biological activity are:

$$\log P = 2.46 \log k'_{(HDEHP)} + 1.58;\ n=8;\ r=0.978 \qquad (2)$$

$$-\log C_a = 5.85 \log k'_{(HDEHP)} - 5.27;\ n=6;\ r=0.897 \qquad (3)$$

$$-\log C_b = 3.28 \log k'_{(HDEHP)} - 4.29;\ n=6;\ r=0.968 \qquad (4)$$

n denostes the number of compounds, r - the regression coefficient. The relatively high correlation coefficient shows a good analogy between log $k'_{(HDEHP)}$ - log P and biological activity. The positive slopes indicate the importance of the hydrophobicity of compounds in the quantitative structure - activity relationships.

Good separation of the phenolic acids was obtained for tetraethylammonium chloride (TEA-Cl) as an ion-pairing reagents (Fig.4). Linear relationships decreasing with methanol content, mainly parallel, indicate constant selectivity ($\log \alpha = \Delta \log k'$) for pairs of solutes. The linear relationships $R_M = f(\varphi)$ (φ is the volume fraction of modifier) permit to extend the range of measurable data by extrapolation of the experimental data to the 0% of the modifier. Log k' obtained for 0% modifier may provide better characteristics of the

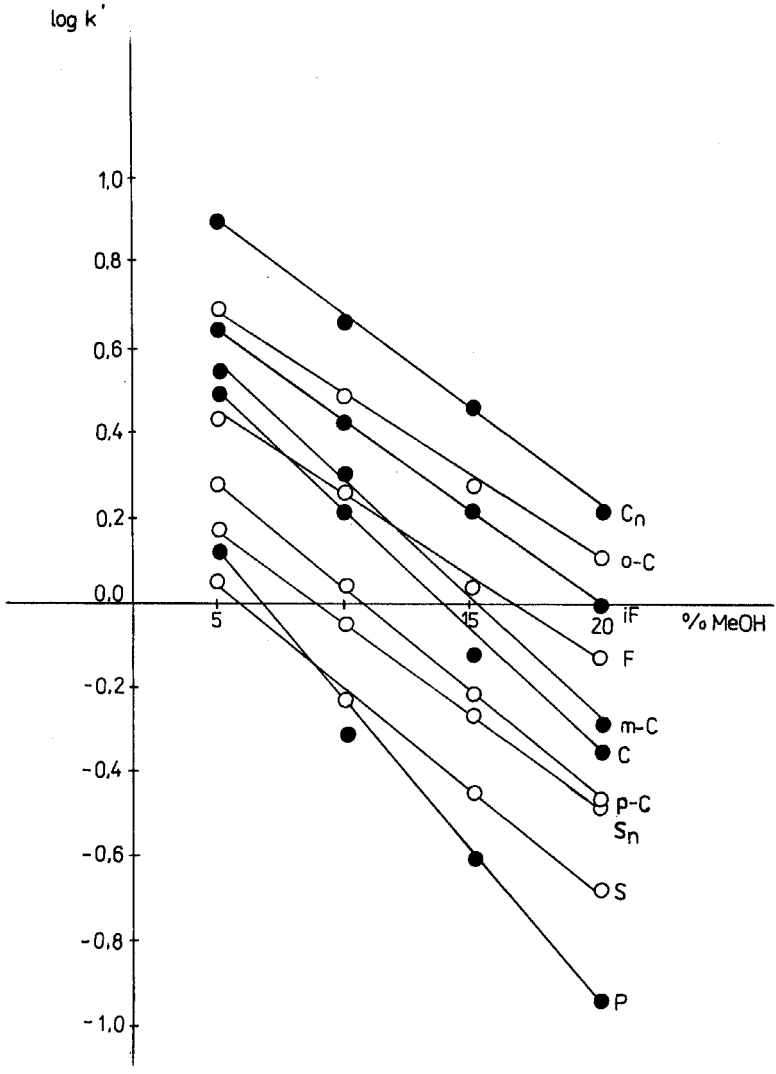


FIGURE 4. Plots of log k´ (for phenolic acids) vs.% methanol in the mobile phase containing 0.01M TEA-Cl and 0.005M phosphate buffer of pH 7.17. Column as in FIGURE 3. For notation see Table 2.

TABLE 2. Investigated compounds

No	Phenolic acids	Symbol	Formula
1	Cinnamic acid	Cn	Cinnamic acid
2	Ortho-Coumaric acid	o-C	2-Hydroxycinnamic acid
3	Meta-Coumaric acid	m-C	3-Hydroxycinnamic acid
4	Para-Coumaric acid	p-C	4-Hydroxycinnamic acid
5	Caffeic acid	C	3,4-Dihydroxycinnamic acid
6	Ferulic acid	F	4-Hydroxy-3-methoxy-cinnamic acid
7	Isoferulic acid	iF	3-Hydroxy-4-methoxy-cinnamic acid
8	Sinapinic acid	Sn	3,5-Dimethoxy-4-hydroxy-cinnamic acid
9	Protocatechuic acid	P	3,4-Dihydroxybenzoic acid
10	Gentisic acid	G	2,5-Dihydroxybenzoic acid
11	Syryngic acid	S	4(Dihydroxy-3,5-dimethoxy-benzoic acid

DRUGS:

No		Symbol	Formula	Source
12	Apranax	Ap	(+)6-Methoxymethyl-2-naphthalene acetic acid	Laroche,F.
13	Polopiryna	As	Acetylsalicylic acid	Polfa,Pl.
14	Ibuprofen	I	α-p-Isobutylphenyl propionic acid	Polfa,Pl.
15	Mefacit	M	N-(2,3-xylyl)-anthraniline acid	Polfa,Pl.
16	Metindol	Md	1-(4-Chlorobenzoyl)-5-methoxy-2-methylnidole-3-acetic acid	Polfa,Pl.
17	Nevigramon	N	1,4-Dihydro-1-ethyl-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid	Chinoin, H.
18	Profenid	P	m-Benzoylhydratropic acid	Specia,F.
19	Voltaren	V	o-(2,6-Dichloro-aniline)phenyl - acetic acid	Geigy,F.

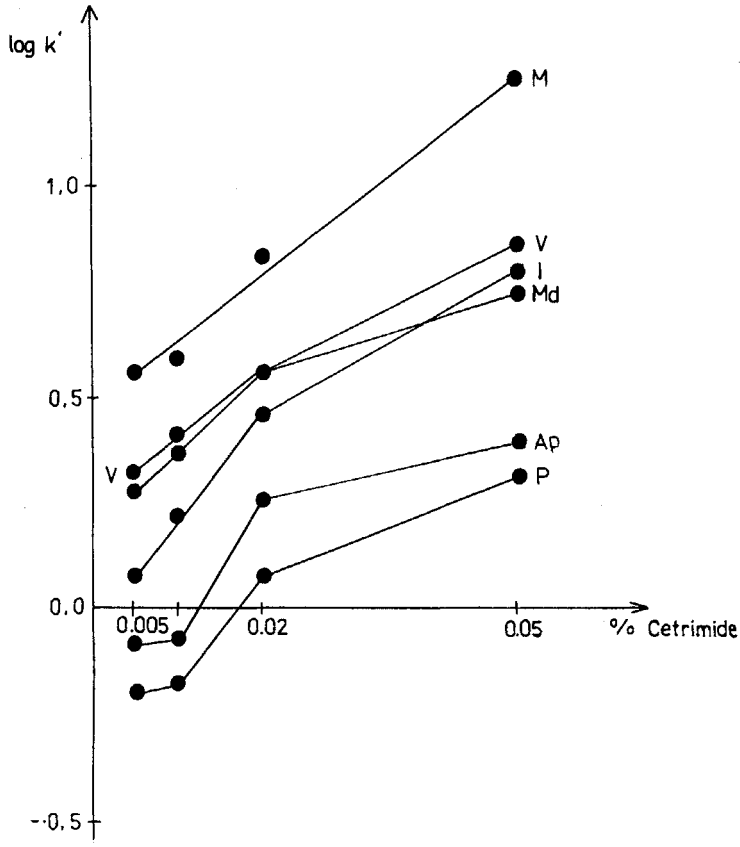


FIGURE 5. Log k´ (for drugs) vs.% cetrimide in the mobile phase containing 70% methanol and 0.05M phosphate buffer, pH 7.38. Column 10 x 3.8 cm, dp 10 µm ODS. For notation see Table 2

lipophilicity of the solutes for the given ion-pair system and permits to evaluate the influence of ion-pair reagent in the buffer solution on retention of the investigated compounds.

The experimental results show that the tetraethyl-ammonium chloride is a suitable ion-pair reagent for the

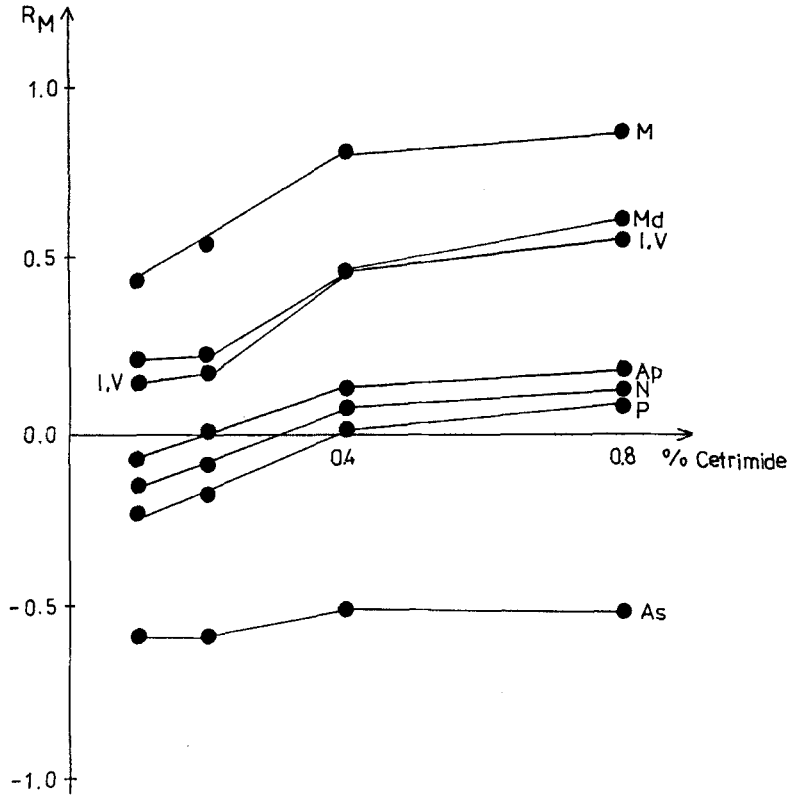


FIGURE 6. R_M vs% cetrimide in the mobile phase containing 50% methanol and 0.006M phosphate buffer, pH 7.38. Stationary phase: HPTLC - RP-18 plates.

chromatographic separation of phenolic acids. Good separations and symmetrical peaks indicate the good efficiency of RP-IPC system used and the obtained data can be employed as taxonomic markers in the chemotaxonomic identification of plant species.

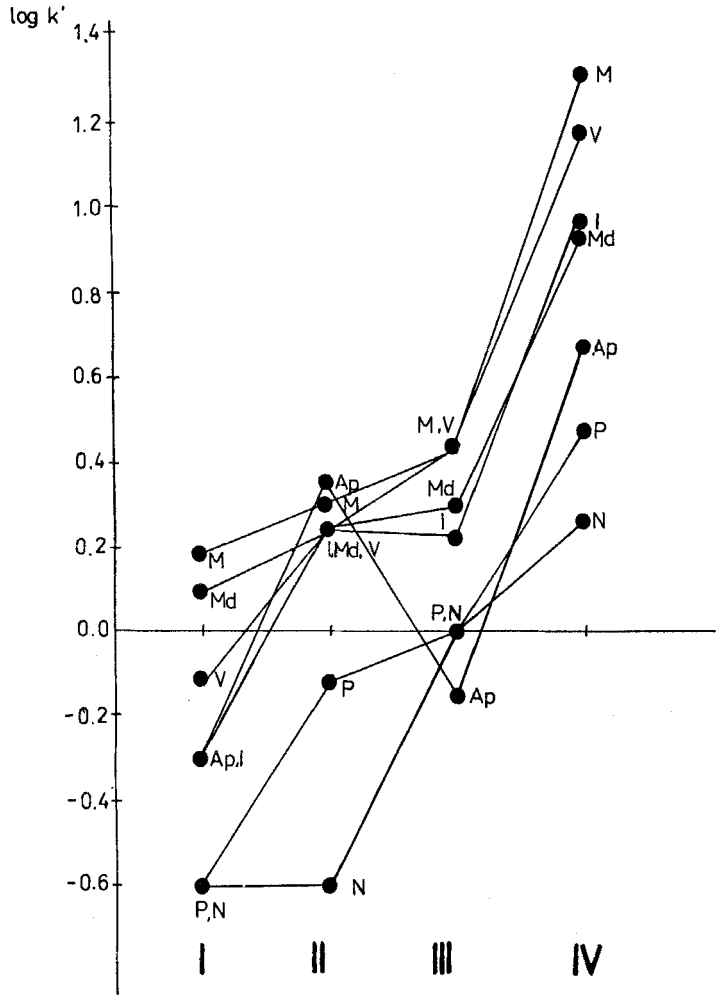


FIGURE 7. Log k´ values of some drugs for the systems:
I. 70% methanol, 0.005M phosphate buffer (pH ▫ 7.4)
II. 70% methanol, 0.005M phosphate buffer (pH ▫ 7.4) and 0.01M tetrabutylammonium chloride (TBA-Cl)
III. 70% methanol, 0.01M phosphate buffer (pH = 7.4) and 0.0055M tetraethylammonium chloride (TEA-Cl)
IV. 50% methanol, 0.01M phosphate buffer (pH = 4.38) and 0.0055M cetyltrimethylammonium bromide.
In all I-IV systems column ODS 100 mm x 3.8 mm, dp = 5 µm. For notation see Table 2.

The other group of the investigated organic acids included spasmolytic and analgesic drugs (Table 2). Figs.5,6 illustrate the retention parameters vs % cetrimide in the eluent obtained by thin-layer (HPTLC) and column chromatography (HPLC). An increase in the cetrimide concentration results in an increase of retention. The highest increase of retention was observed at low concentration of cetrimide. Good results were obtained both in TLC and in column chromatography: only slightly better separation for Metindol, Voltaren and Ibuprofen in HPLC was obtained.

Fig.7 illustrates the selectivity of the systems containing various ion-pair reagents. The best results were obtained for cetrimide. The comparison of these systems shows the effect of the ion-pair reagent on retention and permits to choose optimum conditions for separation of respective compounds.

REFERENCES

1. Bieganowska, M., Petruczynik, A. and Gadzikowska, M., (J.Chromatogr.). in press.

2. Bidlingmeyer, B.A., J.Chromatogr., 18, 525 (1980).

3. Hansen, S.H. and Helboe, P., J.Chromatogr., 285, 53 (1984).

4. Gloor, R. and Johnson, E.L., J.Chromatogr.Sci., 15, 413 (1974).

5. Melander, W.R. and Horvath, Cs., in M.T.W.Hearn (Editor), Ion Pair Chromatography, Marcel Dekker, New York, 1985.

6. Schoenmakers, P.J., Billiet, H.A.H. and de Galan, L., J.Chromatogr., 218, 261 (1981).

7. Bieganowska, M. and Soczewiński, E.,J.Chromatogr., 18, 99 (1984).

8. Dzido, T., J.Chromatogr., 439, 257 (1988).

9. Eksborg, S., Lagerström, P.O., Modin, R. and Schill, S., J.Chromatogr., 83, 99 (1973).

10. Hansch, C. and Leo, A., Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley-Interscience, New York, 1979.

11. Brauman, T., J.Chromatogr.Chromatogr.Revs., 373, 191 (1986).

12. Karger, B.L., Le Page, J.N. and Nobuo Tanaka, in Cs.Horvath (Editor), High Performance Liquid Chromatography, Vol.1, Academic Press, New York 1980, pp.113-205.

13. Soczewiński, E. and Wachtmeister, C.A., J.Chromatogr., 7, 331 (1962).

14. Rekker, R.F., The Hydrophobic Fragmental Constant, Elsevier, New York, 1977.

15. Collander, R., Acta Chim.Scand., 5, 774 (1951).