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REVERSED-PHASE ION-PAIR CHROMATOGRAPHY OF NAPHTHALENEACETIC ACID DERIVATIVES WITH WATER AND AN ORGANIC MODIFIER AS THE MOBILE PHASE

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SUMMARY

The retention behaviour of three naphthaleneacetic acid derivatives on two hydrophobic supports, LiChrosorb RP 8 and Spherisorb ODS, has been studied with a mixture of phosphate buffer (pH 7) and methanol or acetonitrile as the mobile phase. The addition of quaternary ammonium compounds to the mobile phase increases the capacity factors, indicating a mixed retention mechanism that includes ion-pair partition. Constants for ion-pair extraction and ion-pair formation in the mobile phase have been derived.

By gas chromatographic measurements, it was found that LiChrosorb RP 8 adsorbs about 0.18 ml of methanol per gram after equilibration with phosphate buffer (pH 7)–methanol (60:40, v/v) as the mobile phase.

The effects of the support and the composition of the mobile phase on efficiencies, asymmetry and selectivity factors are discussed.

INTRODUCTION

The mechanism of retention in chromatography with chemically bonded phases has been discussed in several papers during the last few years. Horváth *et al.*[1], using a thermodynamic approach based on the solvophobic theory evaluated by Sinanoğlu[2], views the chromatographic process as a reversible association of the solute with the hydrocarbon ligand of the bonded phase. One conclusion is that this type of chromatography is dominated by interactions between the eluent and the stationary phase and the solute and the eluent and that the solvation of the solute solely accounts for the relative retention, which is similar to the findings of Locke[3]. Several other studies indicate, however, that this might be a simplification.

Kikta and Grushka[4] found that the retention mechanism on alkyl-bonded stationary phases is a complex function of the solute, the solvent, the chain length of the bonded phase and the amount of the bonded phase. Hemetsberger *et al.*[5] reported a linear relationship between the capacity ratio and the extent of coverage by the bonded phase, while the selectivity depended on the chain length of the alkyl group

and only minor effects were observed by changing the composition of the mobile phase. Karch *et al.*[6] found that the selectivity was optimal with long bristles (C_{18}) but that the speed of analysis increases with decreasing length of the bristle. The retention was also influenced by the carbon content of the support, the pore size distribution and the total porosity of the support.

Locke[7] claimed that the "brush"-type bonded supports act as weak adsorbents, while with polymerized silicones, *e.g.*, Permaphase packings, partitioning of solutes can occur between two liquid phases because the solvent inside the gel has a different thermodynamic activity than the solvent in the interstitial volume (*cf.*, Kirkland[8]).

The mobile phase in reversed-phase chromatography is most often a mixture of an aqueous phase and an organic modifier and, as pointed out by Pryde[9], there will be a region near the surface of the support that has a significantly different composition from that of the bulk mobile phase. Recently, some workers[10–12] have made observations indicating ion-pair distribution in systems of this kind.

Reversed-phase ion-pair chromatography has mainly been performed by coating a hydrophobic support with a water-immiscible solvent, *e.g.*, 1-pentanol[13–17] or butyronitrile[17], and eluting with an aqueous solution of the counter ion. This paper describes studies of the reversed-phase ion-pair chromatography of carboxylates in systems containing organic solvents that are freely soluble in water.

EXPERIMENTAL

Apparatus

Photometric measurements were performed on a Zeiss PMQ II spectrophotometer with 10-mm quartz cells.

The liquid chromatograph consisted of the following components: a Milton Roy mini-pump pulse damper (LDC 711-47): an injection valve (Altex Model 905-19 syringe loading sample injector with a sample volume up to 100 μl or an Altex Model 905-23 injection valve equipped with a loop filler port, Model 905-29); a precision-bore stainless-steel separation column (length 100 mm, I.D. 3.2 mm and O.D. 1/4 in.); a Waters Model 440 UV detector operated at 254 nm with a 10-μl flow cell; and a Linear Model 252 recorder. The column end-fittings were modified Swagelok® connections.

For gas chromatographic determinations, a Perkin-Elmer F-11 chromatograph equipped with a 5.3 m × 3 mm column containing 15% Carbowax on Celite 545, 60–100 mesh, and a flame-ionization detector were used. The column and injector temperatures were 90° and 150°, respectively, and the flow-rate of the carrier gas (nitrogen) was 40 ml/min.

Chemicals

The chromatographic supports were LiChrosorb RP 8 (particle size 10 μm), obtained from Merck (Darmstadt, G.F.R.), and Spherisorb ODS (particle size 10 μm), obtained from Phase Separations (Queensferry, Great Britain).

Naproxen (6-methoxy-α-methyl-2-naphthaleneacetic acid), 6-methoxy-2-naphthaleneacetic acid (MNA) and 6-hydroxy-α-methyl-2-naphthaleneacetic acid (HNP) were kindly supplied by Syntex (Maidenhead, Great Britain). Tetraethyl-, tetrapropyl- and tetrabutylammonium hydrogen sulphate were supplied by Hässle (Möln-

dal, Sweden), and were used after recrystallization, checking of purity and quantitative determination according to Gustavii *et al.*[18]. Anhydrous diethyl ether from May & Baker (Dagenham, Great Britain) and *n*-hexane (für die Spektroskopie) (Merck) were used in the extractions. Methanol (zur Analyse) (Merck) was used for chromatography. All other chemicals were of analytical-reagent grade.

Determination of molar absorptivities

The molar absorptivities of the acids were determined both on the Zeiss spectrophotometer and on the Waters Model 440 detector, where registrations of the response were made during pumping solutions of different concentrations at a flow-rate of 0.8 ml/min. The values found are reported in Table I.

TABLE I

MOLAR ABSORPTIVITIES

Wavelength: 254 nm. Flow-rate using Waters Model 440: 0.8 ml/min.

Photometer	*Substance*	*Concentration (mole/l)*	*Molar absorptivity* $\times 10^{-3}$
Zeiss PMQ II	Naproxen	$(5–20)\cdot10^{-5}$	4.31
	MNA	$(5–20)\cdot10^{-5}$	4.21
Waters 440	Naproxen	$(4.34–43.4)\cdot10^{-6}$	4.08
	MNA	$(2.31–23.1)\cdot10^{-6}$	3.97

Chromatographic technique

The separation column was packed by the balanced-density slurry technique[19] with 1,1,2,2-tetrabromoethane–tetrachloroethylene (30:70, w/w) as the dispersion medium at an initial pressure of 400–500 bar. The packed column was washed with 250 ml of *n*-hexane, 100 ml of methylene chloride and finally 100 ml of methanol before applying the mobile phase. Equilibrium was obtained after the passage of 10–20 interstitial volumes (V_m). V_m was 0.58 ml for a 100-mm column and 0.83 ml for a 150-mm column with LiChrosorb RP 8 as the support. It was determined by injecting the non-retained potassium dichromate solute. The solvent reservoir, the column and the detector were maintained at a constant temperature of $25 \pm 0.3°$ in an incubator.

Determination of methanol adsorbed on the support

A column packed with LiChrosorb RP 8 was equilibrated with a mobile phase [phosphate buffer (pH 7)–methanol (60:40, v/v)] for more than 4 h. It was then eluted with about 90 ml of dimethylformamide, after washing the pump and the connections carefully with the same solvent. The eluate was collected in a 100.0-ml flask containing 250 μl of ethanol (internal standard). After dilution to 100.0 ml with dimethylformamide, an aliquot (1 μl) was injected into the gas chromatograph. The evaluation was made from a standard graph constructed from peak-height ratios of known concentrations of methanol and the internal standard. The concentration of methanol in the mobile phase was determined with the same technique.

The amount of methanol adsorbed on the support was calculated by compensating for the content of he solvent in the interstitial volume.

A test of the gas chromatographic procedure gave a recovery of 99.9% and a relative standard deviation of 0.73% ($n = 6$).

RESULTS AND DISCUSSION

The chromatographic studies of the naphthaleneacetic acid derivatives were performed on LiChrosorb RP 8 or Spherisorb ODS with mobile phases of buffers (pH 7) mixed with methanol, acetonitrile or tetrahydrofuran. Symmetrical quaternary alkylammonium ions were added to the mobile phase when retention as ion pairs was desired.

In partition chromatography, the capacity factor, k', of a sample X is proportional to its distribution ratio, D:

$$k'_X = DV_s/V_m \tag{1}$$

where V_s is the volume of stationary phase and V_m is the interstitial volume. By distribution as acid (HX), the distribution ratio is given by

$$D = k_{d(HX)}a_{H+}(a_{H+} + k'_{HX})^{-1} \tag{2}$$

where $k_{d(HX)}$ is the distribution coefficient of HX and k'_{HX} is the acid dissociation constant. Ion-pair distribution gives

$$D = E''_{QX}\,[Q] \tag{3}$$

where [Q] is the concentration of the counter ion in the aqueous phase and E''_{QX} is the conditional extraction constant defined by

$$E''_{QX} = C_{QXst}C_{Qm}{}^{-1}C_{Xm}{}^{-1} \tag{4}$$

where C_{QXst} is the total concentration of X present as ion pair in the stationary phase and C_{Qm} and C_{Xm} are the total concentrations of the respective components in the mobile phase.

These equations, however, are not directly applicable to the actual system where the organic solvent is miscible with water in all proportions and will not form an easily defined stationary phase. The properties of the hydrophobic surface of the solid phase will, under these circumstances, be of considerable importance (*cf.*, Karch *et al.*[6]).

Support

Chromatographic results obtained with naproxen on one C_8 support (LiChrosorb RP 8) and one C_{18} support (Spherisorb ODS) are given in Table II. There is a large difference in efficiency, LiChrosorb RP 8 giving a 5–7 times lower HETP and more symmetrical peaks than Spherisorb ODS. The tailing on Spherisorb is, in fact, more severe than the asymmetry factor indicates, as shown in Fig. 1, it may be due to residual silanol groups as suggested by Knox and Jurand[10].

Addition of tetrapropylammonium ion to the mobile phase increased the

TABLE II

INFLUENCE OF THE SUPPORT

Mobile phase: phosphate buffer (pH 7) (ionic strength = 0.1)–methanol (60:40, v/v). Sample: Naproxen. Flow-rate: 1.5 mm/sec.

Support	*[TPA] (mole/l)*	*k'*	*HETP (μm)*	*Asymmetry factor (A_s)*
Spherisorb ODS	—	2.18	350	1.30
(10 μm)	$5 \cdot 10^{-2}$	8.35	390	1.45
LiChrosorb RP 8	—	4.28	67	1.13
(10 μm)	$5 \cdot 10^{-2}$	8.83	54	1.14

capacity factors, indicating that ion-pair distribution affects the retention. The effect is more pronounced on Spherisorb than on LiChrosorb.

Organic solvent

The choice of organic solvents intended as modifiers was limited to those freely soluble in the aqueous phase, *e.g.*, alcohols, nitriles and cyclic ethers of low molecular weight. Methanol, acetonitrile and tetrahydrofuran were used in this study.

Some chromatographic results obtained with methanol and acetonitrile as modifiers and LiChrosorb RP 8 as support are given in Tables III and IV. The HETP is less than 80 μm in both systems but the peaks show a slightly higher tendency for tailing to occur with acetonitrile. The selectivity factor increases by 20–30% with a decrease in acetonitrile content from 20 to 10%. The addition of a quaternary

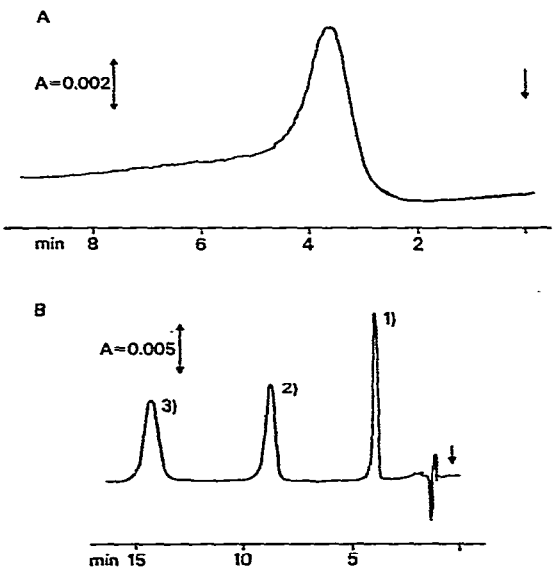

Fig. 1. Spherisorb ODS and LiChrosorb RP 8 as supports. (a) Support: Spherisorb ODS (10 μm). Mobile phase: phosphate buffer (pH 7)–methanol (60:40, v/v) containing 0.05 *M* tetrapropylammonium ion. Naproxen, 176 ng. b) Support: LiChrosorb RP 8 (10 μm). Mobile phase: phosphate buffer (pH 7)–methanol (60:40, v/v) containing 0.025 *M* tetrapropylammonium ion. 1 = HNP, 139 ng; 2 = MNA, 137 ng; 3 = Naproxen, 176 ng.

TABLE III

CHROMATOGRAPHIC RESULTS WITH METHANOL AS THE MODIFIER

Support: LiChrosorb RP 8 (10 μm). Mobile phase: phosphate buffer (pH 7) (ionic strength = 0.1)–methanol (60:40, v/v) + tetrapropylammonium hydrogen sulphate (TPA), neutralized. Flow-rate: 1.5 mm/sec. Mean results from three chromatographic runs.

Sample	*[TPA]·10² (mole/l)*	*k'*	*α**	*HETP (μm)*	*A_s*
Naproxen	—	4.28	1.69	67	1.13
MNA	—	2.53	2.72	65	1.18
HNP	—	0.93		75	1.28
Naproxen	1	6.60	1.66	59	1.15
MNA	1	3.97	2.61	61	1.12
HNP	1	1.52		64	1.19
Naproxen	2.5	7.94	1.67	57	1.06
MNA	2.5	4.75	2.61	64	1.06
HNP	2.5	1.82		62	1.20
Naproxen	5	8.83	1.64	54	1.14
MNA	5	5.37	2.61	64	1.06
HNP	5	2.06		67	1.07

* α = selectivity factor (k'_{II}/k'_{I}).

ammonium compound does not influence the HETP or asymmetry to a significant extent. The selectivity is also unchanged in the methanol-containing system while some slight changes are obtained in the acetonitrile system.

The capacity factors show a strong dependence on the content of modifier. An illustration is given in Fig. 2, which shows the relationship between the logarithm of the capacity factor of naproxen and the percentage of methanol with Spherisorb ODS as the support. Similar straight-line relationships have been found for indole compounds[19], alcohols and phenols[20] and aromatic hydrocarbons[21].

TABLE IV

CHROMATOGRAPHIC RESULTS WITH ACETONITRILE AS THE MODIFIER

Support: LiChrosorb RP 8 (10 μm). Mobile phase: phosphate buffer (pH 7) (ionic strength = 0.1)–acetonitrile + tetrabutylammonium hydrogen sulphate (TBA), neutralized. Flow-rate: 1 ml/min. Mean results from two chromatographic runs.

Acetonitrile concentration (%, v/v)	*Sample*	*[TBA]·10² (mole/l)*	*k'*	*α*	*HETP (μm)*	*A_s*
10	Naproxen	—	34.6	2.01	61	1.38
	MNA	—	17.2	3.44	54	1.15
	HNP	—	5.00		57	1.26
20	Naproxen	—	4.19	1.58	78	1.39
	MNA	—	2.66	2.89	70	1.26
	HNP	—	0.92		63	1.53
	Naproxen	1	20.4	1.48	65	1.57
	MNA	1	13.8	3.40	58	1.24
	HNP	1	4.05		65	1.83
	Naproxen	4	23.2	1.40	73	1.37
	MNA	4	16.5	3.11	65	1.33
	HNP	4	5.32		77	1.61

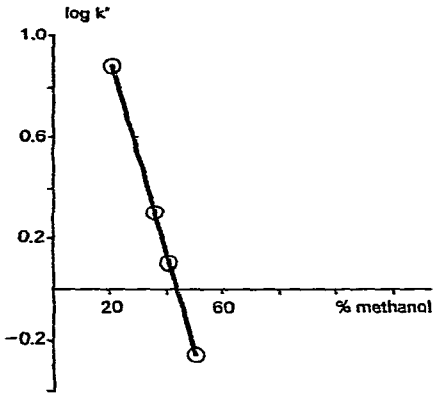

Fig. 2. Capacity factor with methanol as modifier in the mobile phase. Support: Spherisorb ODS (10 μm). Mobile phase: phosphate buffer (pH 7)–methanol. Sample: Naproxen.

The carboxylic acids have about the same retention times with 20% of acetonitrile, 30% of tetrahydrofuran and 40% of methanol, which seems to reflect that the hydrogen-accepting ability decreases in the order acetonitrile > tetrahydrofuran > methanol (*cf.*, Tijssen *et al.*[22]). The retention might depend at least partly on the distribution of the acids to an adsorbed stationary phase rich in the organic solvent, but an estimation of such an effect is impossible owing to unknown properties of this phase.

Addition of a quaternary ammonium compound increases the capacity factor in the presence of acetonitrile and methanol, which indicates ion-pair distribution (eqns. 1 and 3). With tetrahydrofuran as the modifier, the ion-pair effect is not so evident, as shown in Table V. These results are, however, in good accordance with previous studies, which have shown that non-polar solvents have a low ability to solvate ion pairs[23] while alcohols and nitriles are efficient extraction media (*e.g.*, Fransson *et al.*[17]). The deviating effect of tetrahydrofuran is a further indication of the existance of an adsorbed stationary phase that is rich in the organic solvent and to which partition of solutes can occur[9–12].

The amount of methanol adsorbed to LiChrosorb RP8 was determined as described under Experimental. It was found that the support adsorbs 0.18 ml/g (mean of two experiments: 0.176 and 0.194 ml/g), which is 2–3 times less than the amount of pentanol that is spontaneously adsorbed on a similar support from a saturated

TABLE V

CAPACITY FACTORS WITH TETRAHYDROFURAN AS THE MODIFIER

Support: Spherisorb ODS (10 μm). Mobile phase: phosphate buffer (pH 7) (ionic strength = 0.1)–tetrahydrofuran (70:30 v/v) + tetraethylammonium hydrogen sulphate (TEA), neutralized. Sample: Naproxen.

[TEA]·10² *(mole/l)*	*k'*
—	0.7
1	0.7
10	1.0

aqueous solution[16]. According to the manufacturer, LiChrosorb RP 8 has a specific surface area of 300 m^2/g, giving a coverage of methanol corresponding to 14.8 $\mu mole/m^2$.

The ion-pair effect

The effect of ion-pair distribution shown in Table III with methanol as the modifier is further illustrated in Fig. 3. It shows that addition of tetrapropylammonium (TPA) ion gives an increase in k' without changing the separation selectivity, as $\Delta \log k'$ (= $\log \alpha$) remains constant. The increase in the capacity factors levels off with increasing concentration of TPA. This is not in accordance with eqn. 1, but this equation is valid only under the assumption that no side-reactions occur. The effect observed here might be the result of association reactions in the mobile phase (*cf.*, Lagerström[24]).

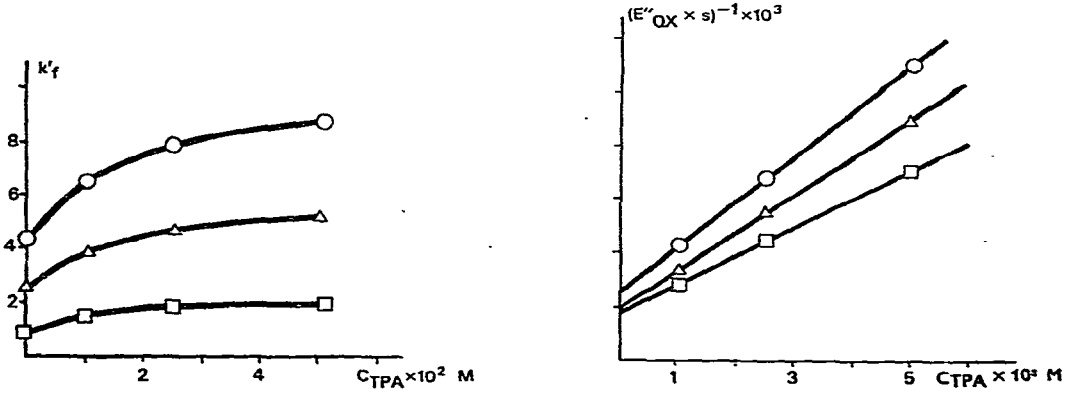

Fig. 3. Capacity factor with TPA in the mobile phase. Support: LiChrosorb RP 8 (10 μm). Mobile phase: phosphate buffer (pH 7)–methanol (60:40, v/v) + TPA. ○, Naproxen; △, MNA; □, HNP.

Fig. 4. Determination of extraction and ion-pair formation constants. Conditions and symbols as in Fig. 3. Scales on ordinate: ○, 0–10; △, 0–50; □, 0–25.

Assuming that the retention is due partly to ion-pair extraction and partly to some other effect, adsorption or acid distribution, which is independant of the presence of the ion-pair reagent, the following retention equation is valid:

$$k' = k'_0 + E''_{QX} C_{Qm} V_s/V_m \tag{5}$$

where k'_0 represents the ion-pair independent retention. Setting $V_s/V_m = s$, we obtain

$$E''_{QX} s = (k' - k'_0) C_{Qm}^{-1} \tag{6}$$

Association reactions that are dependent on the concentration of the counter ion can either be ion-pair formation in the mobile phase or a dimerization or polymerization of the quaternary ammonium compound in the same phase. Associations of symmetrical quaternary alkylammonium compounds in aqueous systems have not been observed in the actual concentration range studied. The following equation is valid, assuming ion-pair formation in the mobile phase:

$$(E''_{QX} s)^{-1} = (E'_{QX} s)^{-1} + k_a (E'_{QX} s)^{-1} C_{Qm} \tag{7}$$

where E''_{QX} is the stoichiometric extraction constant ($= [QX]\ [Q]_m^{-1}[X]_m^{-1}$), k_a is the ion-pair formation constant ($= [QX]_m[Q]_m^{-1}[X]_m^{-1}$) and $C_{Qm} = [Q]_m$ is the initial concentration of the quaternary ammonium compound, since it is present in large excess.

Plots of $(E''_{QX}s)^{-1}$, calculated from chromatographic data according to eqn. 6, against the concentration of the quaternary ammonium compound in the mobile phase are shown in Fig. 4. The straight-line relationships indicate that eqn. 7 is valid. Extraction and association constants obtained from intercepts and slopes are given in Table VI.

TABLE VI

ION-PAIR FORMATION AND EXTRACTION CONSTANTS

Support: LiChrosorb RP 8 (10 μm). Mobile phase: phosphate buffer (pH 7) (ionic strength = 0.1)–methanol (60:40, v/v) + tetrapropylammonium hydrogen sulphate (TPA), neutralized. $C_{TPA} \cdot 10^2$ = 1–5 mole/l. Amounts injected: Naproxen, 176 ng; MNA, 137 ng; HNP, 139 ng.

Anion	*Log* k'_a	*Log* E'_{QX}*	*Log* E_{QX} *(pentanol)***
Naproxen	1.81	3.51	2.9
MNA	1.78	3.28	2.6
HNP	1.81	2.90	2.3

* E'_{QX} is calculated with the assumption that $V_s = 0.1$ ml.

** E_{QX} is estimated from chromatographic data for 4-methoxyphenyl acetate with TBA as counter ion and pentanol as stationary phase[18], with compensation for structural differences according to Leo *et al.*[27].

Ion-pair formation in aqueous buffer solutions between some aromatic carboxylates and quaternary ammonium compounds has been reported[24,25]. In those instances, the association constants were of the magnitude 5–10, *i.e.*, smaller than those found here, but the difference seems reasonable considering the lower polarity of the medium used in this study, which should favour associations[26].

The extraction constant is a measure of the extracting ability of the stationary phase, which should have different thermodynamic properties from the bulk liquid mobile phase. A comparison of the constants obtained with estimated constants based on data from reversed-phase ion-pair chromatography with pentanol as the stationary phase is given in Table VI. There is a remarkably good agreement with almost identical $\Delta \log E_{QX}$ values, *i.e.*, identical selectivity between the found and estimated constants, thus giving further evidence to support the hypothesis that a partition mechanism is at least partly responsible for the retention in systems of this kind.

With acetonitrile as the modifier (Table IV) only two different concentrations of the quaternary ammonium compound (TBA) were used. Assuming a straight-line relationship in this system also, the following constants are obtained: $\log E'_{QX} = 4.9$, 4.6 and 3.9 and $\log k_a = 2.6$, 2.4 and 2.2 for naproxen, MNA and HNP, respectively (s is assumed to be the same as in the methanol system). The selectivity seems to be higher with acetonitrile than with methanol (*cf.*, Tables III and IV).

Some results obtained with a higher content of methanol in the mobile phase (55%) are reported in Table VII. The ion-pair effect is also evident in this system; a five-fold increase in the concentration of TPA increases the capacity factor 1.3-fold

TABLE VII

CHROMATOGRAPHIC RESULTS WITH METHANOL (55%) AS THE MODIFIER

Support: LiChrosorb RP 8 (10 μm). Mobile phase: phosphate buffer (pH 7) (ionic strength = 0.1)–methanol (45:55, v/v) + tetrapropyl- or tetrabutylammonium hydrogen sulphate, neutralized. Flow-rate: 1.9 mm/sec. Mean results from two chromatographic runs.

Sample	*Q*	*[Q]·10²* *(mole/l)*	*k'*	*α*	*HETP* *(μm)*	A_s
Naproxen	TPA	1	1.41	1.45	77	1.19
MNA			0.97	2.26	71	1.19
HNP			0.43		73	1.29
Naproxen		5	1.88	1.45	67	1.15
MNA			1.30	2.24	71	1.13
HNP			0.58		74	1.11
Naproxen	TBA	1	2.44	1.44	71	1.12
MNA			1.70	2.24	74	1.18
HNP			0.76		56	1.26
Naproxen		5	3.22	1.42	75	1.13
MNA			2.27	2.23	75	1.05
HNP			1.02		74	1.15

for all three substances, which is of exactly the same magnitude as with 40% of methanol in the mobile phase. The same change with the more hydrophobic tetrabutylammonium has a greater effect on the capacity factor, with an increase of about 1.7-fold in all instances.

An increase in methanol content from 40 to 55% decreased the selectivity factors by about 20%, while the HETP and the asymmetry factors were of the same magnitude.

In ion-pair chromatography skewed peaks can be obtained due to dissociation of the ion pair in the less polar phase, giving tailing in reversed-phase systems. The dissociation increases in more polar media, *e.g.*, alcohols, but it decreases if other dissociated ion pairs containing the same counter ion are also present in the less polar phase[28,29]. The good symmetry obtained in this study indicates either that buffer anions are co-extracted with the counter ion or that the stationary phase has a rather non-polar character.

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