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ION-PAIR PHASE SYSTEMS FOR THE SEPARATION OF CARBOXYLIC ACIDS, SULPHONIC ACIDS AND PHENOLS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY*

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SUMMARY

Liquid–liquid systems consisting of long-chain aliphatic amines as the stationary phase and aqueous solutions of acids as the mobile phase for the separation of acidic compounds by high-pressure liquid chromatography are described.

Ion-pair formation and liquid–liquid distribution are involved in the distribution of acids in such phase systems. The influence of the type of amine, pH, type and concentration of the eluent acid, temperature and addition of methanol to the aqueous mobile phase on the retention and selectivity was investigated.

The suitability of the phase systems for the separation of carboxylic and sulphonic acids and the unique possibilities of adjusting retentions were demonstrated with test mixtures of acidic compounds. Some applications of the phase systems in the analysis of aromatic carboxylic acids in clinical and forensic chemistry are considered.

INTRODUCTION

The separation of very polar compounds such as sulphonic and carboxylic acids is, despite the potential of modern liquid chromatography, limited mainly to ion-exchange chromatography[1–3]. In about the last 2 years, however, ion-pair partition systems, previously applied mainly to inorganic compounds[4–7], have also been used succesfully for the separation of ionizable organic compounds[8–16]. Reversed-phase adsorption chromatography has also been shown to be valuable for the separation of acidic compounds[17]. Previously it was shown that liquid–liquid systems consisting of long-chain aliphatic amines and dilute aqueous acids, applied for many years to the separation of metal ions[4–7], are very suitable for the separation of carboxylic and sulphonic acids by high-pressure liquid chromatography (HPLC)[15]. Recently, a new type of liquid chromatography, the so-called soap chromatography,

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has been introduced, which involves ion-pair formation between ionizable organic substances and anionic or cationic detergents added to the mobile phase[18,19].

In this paper, we describe a study of the parameters that influence the retention behaviour of acidic compounds in liquid–liquid systems consisting of long-chain aliphatic amines as the stationary phase and dilute aqueous acids as the mobile phase.

EXPERIMENTAL

Apparatus

All liquid chromatographic experiments were carried out on a high-pressure liquid chromatograph (Siemens SP 200, Siemens, Karlsruhe, G.F.R.) using UV detection (Zeiss PM 2 DLC, Zeiss, Oberkochen, G.F.R.) and a high-pressure injection valve (Valco CV-6-UHPa). In order to resist the acidic medium all feed-lines were constructed from stainless-steel 316 tubes and Swagelok couplings.

Two types of columns were used: (i) stainless steel columns (length 150 mm, I.D. 2.8 mm) and (ii) custom-made straight thick-walled glass columns (length 150 mm, I.D. 3.0 mm). Both types of columns were connected to the injection port and detector by means of Swagelok zero dead volume couplings. In all experiments a stainless-steel pre-column (50 × 1 cm I.D.) was installed in front of the injection port in order to prevent loss of stationary phase.

The wavelength in all experiments was adjusted to 235 nm for perchloric acid and 254 nm for trichloroacetic acid as the mobile phase.

Materials

The long-chain aliphatic amines used were tri-*n*-octylamine (TOA), tri-*n*-heptylamine (THPA), tri-*n*-hexylamine (THA), tri-*n*-pentylamine (TPA), dioctylamine (DOA), diheptylamine (DHPA), dihexylamine (DHA) and octylamine (OA) (Fluka, Buchs, Switzerland). Tetraheptylammoniumchloride (THPAC) was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.) and Amberlite LA-2 from Rohm and Haas (Brussels, Belgium).

The solid supports were (i) diatomite earth (Kieselguhr, Merck, Darmstadt, G.F.R.), ground and classified to a particle size range of 5–7 μm by means of an air classifier (Alpine MZR, Augsburg, G.F.R.), and (ii) low-surface-area silica (Sperosil XOC 005, Rhône-Poulenc, Neuilly sur Seine, France), ground and classified to a particle size range of 4–6 μm.

The other chemicals used were 70% (w/w) perchloric acid and sulphuric acid (Merck); trichloroacetic acid (TCA) (Merck); and sulphonic and carboxylic acids and phenols (Merck and Fluka).

Procedures

The capacity ratio (k_i') of a component i for a given column was derived from its retention time (t_{Ri}) and that of an unretained compound (t_{R0}) using the following relationship:

$$k_i' = \frac{t_{Ri} - t_{R0}}{t_{R0}} = K_i q$$

where K_i = overall distribution constant and q = phase ratio. For pairs of compounds, the selectivity factor (r_{ji}) was derived from their capacity ratios:

$$k'_j/k'_i = r_{ji}$$

In all the experiments, formamide was used as a non-retarded compound.

Two procedures were used to prepare the columns:

(i) The glass columns were dry-packed with coated diatomite earth or low-surface-area silica. Small portions of coated support were placed in the glass tube and compressed with a PTFE- topped plunger.

(ii) The metal columns were packed by a slurry technique. The column was connected to a metallic mixing vessel, with a magnetic stirrer inside, which was filled with a diluted slurry of coated low-surface-area silica (*ca.* 5%, w/w) in the eluent. The slurry was pumped into the vertically positioned column with a forced liquid flow up to a pressure of 500 atm.

In order to determine the phase ratio on the given columns, a number of statically measured overall distribution constants[20] of some standard compounds were correlated with their retention times on the column. The phase ratio was calculated from the slope of the linear regression.

Aqueous acidic solutions were prepared by diluting a weighed amount of the standard solutions (or solids) with doubly distilled water. The molarity of the solutions was determined by titration with aqueous borax solutions and the pH with a pH meter (Radiometer, Copenhagen, Denmark).

To adjust the anion concentration of the eluent at a certain pH, the sodium salt was added. The pH of the eluent was adjusted with sodium hydroxide or phosphate buffers.

All eluents were saturated with the stationary phase. The samples were dissolved in the mobile phase and 10 μl of these solutions (*ca.* 1 μg/ml) were injected with an injection valve into the top of the column.

RESULTS AND DISCUSSION

Many studies on the mechanism of distribution of inorganic and organic compounds in ion-pair systems have been described[4,5,7,15,16,21] since Smith and Page[22] first applied liquid ion exchangers to the extraction of acids.

In order to discuss the results presented later, the expression for the distribution constant of organic acids as derived by Kraak and Huber[15] is very helpful. The overall distribution constant, K_X, of an acid HX in phase systems consisting of long-chain aliphatic amines as the stationary phase and dilute aqueous acids as the mobile phase can be expressed as the sum of two individual distribution mechanisms. In eqn. 1 the first term (ΔK_{X1}) describes the liquid–liquid distribution and the second term (ΔK_{X2}) the ion-pair formation:

$$K_X = \Delta K_{X1} + \Delta K_{X2} \quad (1)$$

where

$$\Delta K_{X1} = K_{HX} \cdot \frac{1}{(1 + 1/K_1 [H^+]_m)}$$

$$\Delta K_{X2} = K_2 C \cdot \frac{1}{([A^-]_m + 1/K_3\,[H^+]_m)(1 + K_1\,[H^+]_m)}$$

K_{HX} = partition coefficient of the undissociated acid HX;
K_1 = formation constant of the acid HX;
K_2 = ratio of the formation constants of the ion pairs BHX and BHA (selectivity coefficient);
K_3 = formation constant of the ion pair BHA;
C = overall amine concentration;
m = aqueous phase.

Eqn. 1 describes the dependence of the overall distribution constant, K_X, of an acid HX on the pH and anion concentration of the aqueous phase.

The capacity ratios of compounds measured on the same column are proportional to their overall distribution constant, the phase ratio being the proportionality constant. The selectivity factor of pairs of compounds characterizes the ability of the phase system to distribute those compounds differently between the two phases and is given by the ratio of their capacity ratios.

For many acidic compounds of different classes the effects of pH, anion concentration, type of amine, type of anion, temperature and addition of methanol to the eluent on their capacity ratios and the selectivity factors for pairs of compounds were investigated in this work on columns with long-chain aliphatic amines as the stationary phase and dilute aqueous acids as the mobile phase.

Influence of pH and anion concentration on the capacity ratio

The dependence of the overall distribution constant on the pH and anion concentration, $[A^-]_m$, of the aqueous phase is described by eqn. 1. The first term (ΔK_{X1}) approaches the value K_{HX} = constant at $1 \gg 1/K_1[H^+]_m$ and declines to zero at $1 \ll 1/K_1[H^+]_m$ (see Fig. 1a). At a constant anion concentration, the second term (ΔK_{X2}) declines to zero at low and high pH, with a maximum at the value $\frac{1}{2}\log(1/K_1K_3[A^-]_m)$.

The shape of the curve that describes the dependence of ΔK_{X2} on pH is determined by the relative magnitude of the formation constants, K_1 and K_3 (see Fig. 1b). The shape of the curve reflecting the overall distribution constant (K_X) as a function of pH is determined by the extent to which ΔK_{X1} and ΔK_{X2} contribute to the overall curve (Fig. 1c).

The dependence of K_X on the anion concentration, $[A^-]_m$, is described by the second term, ΔK_{X2}. The extent to which the anion concentration influences the overall distribution constant depends mainly on the relative magnitudes of ΔK_{X1} and ΔK_{X2} under the chosen conditions.

A linear relationship between the overall distribution constant of an acid HX and the reciprocal of the anion concentration will exist if $[A^-] \gg 1/K_3[H^+]_m$. In this instance the slope is $K_2\,C/1 + K_1[H^+]_m$ and the intercept is ΔK_{X1}.

In order to investigate the effect of pH and anion concentration, TOA was chosen as the stationary phase and aqueous perchloric acid as the mobile phase. The capacity ratio was measured at pH 1.5 and 5.0 at different perchlorate concentrations for different acids. At high pH a phosphate buffer was added to the eluent. No remarkable change of the capacity ratios was caused by the addition of phosphate, as is

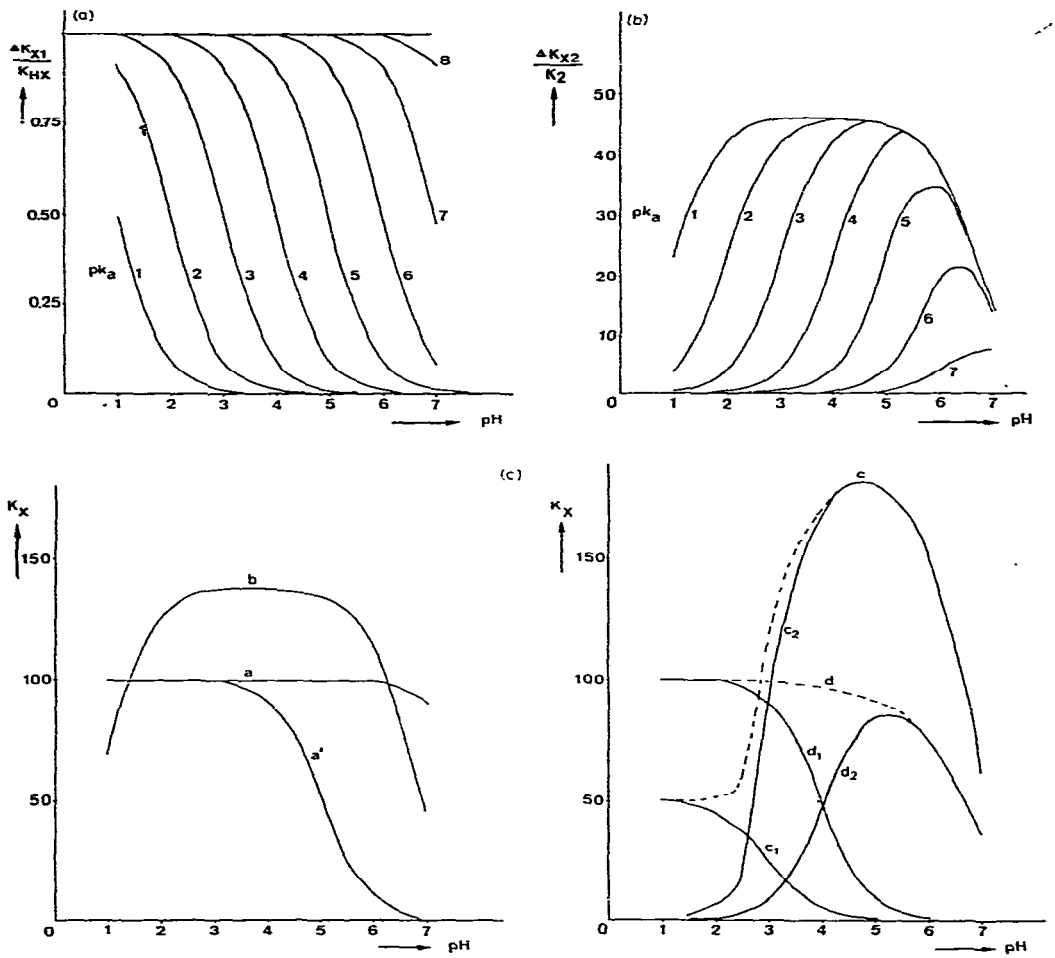

Fig. 1. Effect of pH and pK_a on (a) the liquid–liquid distribution and (b) ion-pair formation of acidic compounds. p$K_a = -\log K_1$. (c) Characteristic combinations of liquid–liquid distribution and ion-pair formation as function of pH. Curves: (a) Very weak acid; only liquid–liquid distribution; (a') weak acid; only liquid–liquid distribution; (b) very strong acid; only ion-pair formation; (c) weak acid; liquid–liquid distribution (c_1) and ion-pair formation (c_2) ($\Delta K_{X1} < \Delta K_{X2}$); (d) weak acid; liquid–liquid distribution (d_1) and ion-pair formation (d_2) ($\Delta K_{X1} > \Delta K_{X2}$).

shown in Table I. The effect of anion concentration on the capacity ratio at low and high pH is shown by the results in Table II. It can be seen that ion-pair formation is dominant for sulphonic acids ($K_1 > 1$) at pH 1.5 and 5.0, while liquid–liquid distribution dominates for carboxylic acids ($K_1 \approx 10^4$) and phenols ($K_1 \approx 10^{10}$) at pH 1.5. At pH 5.0, however, the slope of the linear regression of k_i' *versus* $[ClO_4^-]^{-1}$ for carboxylic acids indicates ion-pair formation for all of the compounds examined. From the intercept, however, it must be concluded that liquid–liquid distribution still occurs for a number of carboxylic acids.

Some acids, such as benzoic acid, display a smaller capacity ratio at pH 5.0 than at pH 1.5. This result can be explained by the fact that the ion-pair formation

TABLE I

INFLUENCE OF PHOSPHATE BUFFER ON k_i'

Compound	k_{i1}'*	k_{i2}'*
Phenol	1.07	1.11
4-Nitrophenol	3.65	3.72
Benzoic acid	0.69	0.73
4-Nitrobenzoic acid	1.80	1.86
2-Hydroxybenzoic acid	5.30	5.43
Benzenesulphonic acid	0.38	0.39
2-Naphthol-6-sulphonic acid	2.72	2.68

* Phase systems: (1) TOA–0.05 *M* $NaClO_4$, pH = 4.7, q = 0.03. (2) TOA–0.05 *M* $NaClO_4$ + 0.05 *M* KH_2PO_4, pH = 4.7, q = 0.03. Glass column (15 cm).

term (ΔK_{X2}) does not exceed the value of the liquid–liquid distribution (ΔK_{X1}) at its plateau, as is shown for benzoic acid in Fig. 2a. The phase ratio, q, on the column was determined by a linear regression of retention times and statically measured distribution constants of some standard compounds[20]. From the known phase ratio (q) and the value for K_3 ($\approx 10^8$), ΔK_{X1} was calculated from $K_X = \Delta K_{X1} = K_{HX}$ (at low pH) and K_1, while ΔK_{X2} was calculated from the slope of the linear regression of k_i' *versus* $[ClO_4^-]^{-1}$ at high pH.

These procedures for the calculation of ΔK_{X1} and ΔK_{X2} were also used to construct Fig. 2b for 2-hydroxybenzoic acid, a compound that shows an increase in capacity ratio at pH 5.0 compared with pH 1.5.

From the linear regression of k_i' *versus* $[ClO_4^-]^{-1}$ at pH 5.0 for phenol ($K_1 \approx 10^{10}$), only liquid–liquid distribution can be assumed. For 4-nitrophenol ($K_1 \approx 10^7$), however, from the slope of the linear regression a small contribution of ion-pair formation must be assumed at pH 5.0.

The pH and the anion concentration can influence the selectivity factors of

TABLE II

LINEAR REGRESSION OF THE CAPACITY RATIO (k_i') *VERSUS* PERCHLORATE CONCENTRATION IN THE MOBILE PHASE AT pH 1.5 AND 5.0 FOR PHENOLS, CARBOXYLIC ACIDS AND SULPHONIC ACIDS AT 25°

$pK_a = -pK_1$. a = intercept; b = slope; r = regression coefficient.

Compound	pK_a	*pH = 1.5*			*pH = 5.0*		
		a	b	r	a	b	r
Phenol	9.9	1.096	−0.002	—	1.081	0.001	—
4-Nitrophenol	7.15	4.425	−0.006	—	3.032	0.025	0.9968
2,5-Dihydroxybenzoic acid	2.97	0.236	−0.002	—	−0.003	0.026	0.9998
2,4-Dihydroxybenzoic acid	—	0.522	−0.002	—	−0.009	0.038	0.9999
Benzoic acid	4.19	0.842	−0.001	—	0.052	0.030	0.9991
4-Nitrobenzoic acid	3.41	1.140	−0.005	—	0.007	0.093	0.9999
2-Hydroxybenzoic acid	2.97	1.745	−0.001	—	−0.031	0.253	0.9998
4-Methylbenzoic acid	—	2.621	−0.001	—	0.323	0.079	0.9994
Cinnamic acid	—	3.608	−0.005	—	0.524	0.128	0.9998
Benzenesulphonic acid	0.70	−0.004	0.019	0.9999	−0.002	0.018	0.9990
4-Methylbenzenesulphonic acid	—	0.010	0.062	0.9993	0.012	0.059	0.9994
1-Naphthol-4-sulphonic acid	—	0.005	0.167	0.9996	−0.036	0.168	0.9994

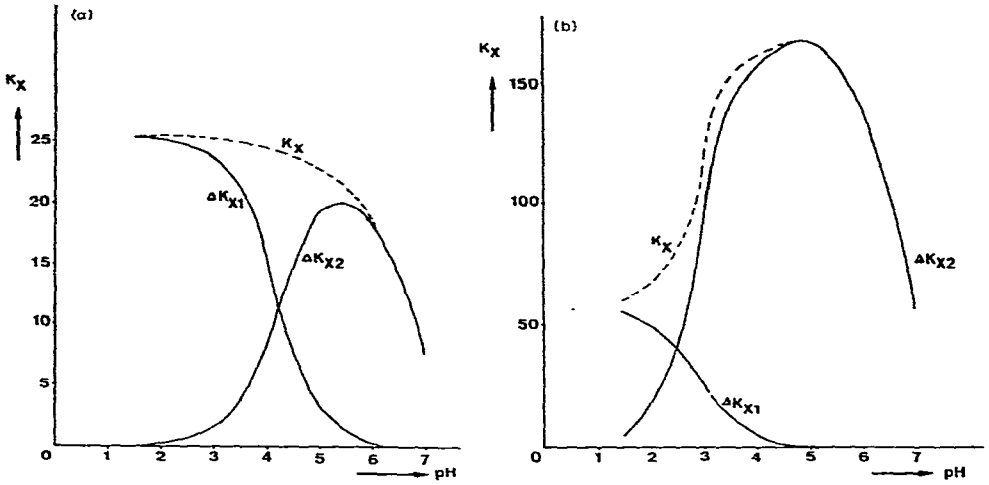

Fig. 2. Overall distribution constant of (a) benzoic acid and (b) 2-hydroxybenzoic acid as a function of pH, constructed from experimentally measured ΔK_{X1} and ΔK_{X2} values. Phase system: TOA–0.05 *M* ClO_4^- + phosphate buffer.

pairs of compounds considerably, as can be seen from the differences in the slopes of the linear regression.

Influence of the type of amine on k_i'

The influence of the type of amine on the overall distribution constant of an acid is given by eqn. 1. Under the same experimental conditions, *i.e.*, the same volume phase ratio and eluent composition, the overall distribution constant can differ because of a change in the partition coefficient, K_{HX}, the selectivity coefficient, K_2, the formation constant, K_3, and the overall amine concentration, C. A change in K_{HX} and/or K_2 can influence both the capacity ratios and the selectivity factors. The constant C influences only the absolute value of the capacity ratios if the second term is dominant. However, under conditions where both terms contribute significantly to the distribution, a change in C can result in a change in the selectivity factors. A change in K_3 results in a shift of the pH at which the second term reaches its maximum value.

In order to determine the influence of the type of amine, some aliphatic amines were tested for their applicability as stationary phase in combination with aqueous perchloric acid as the mobile phase. For various reasons several amines could not be used as stationary phases: DOA, DHPA and THPAC form solid salts with perchloric acid and the sample compounds, which precipitate in the eluent; TPA is easily stripped from the column owing to its low viscosity; and OA has too high a solubility in the aqueous phase. Only four aliphatic amines (TOA, THPA, THA and Amberlite LA-2) were found to be suitable as stationary phase in combination with aqueous perchloric acid as the mobile phase.

For some aromatic acids, including phenols, the capacity ratios and selectivity factors were determined on columns coated with these four amines with aqueous perchloric acid (0.05 *M*) solutions of pH 1.5 and 5.5 as the eluent. The results of these measurements are given in Table III.

The results for the secondary amine Amberlite LA-2 are incomplete because

TABLE III

DEPENDENCE OF THE CAPACITY RATIOS (k'_i) AND SELECTIVITY FACTORS (r_{ji}) OF PHENOLS, CARBOXYLIC ACIDS AND SULPHONIC ACIDS ON THE ALKYL CHAIN OF THE AMINE STATIONARY PHASE WITH AQUEOUS PERCHLORIC ACID OF pH 1.5 AND 5.5 AS THE MOBILE PHASE AT 25°

Compound	*0.05 M ClO_4^- (pH 1.5)*								*0.05 M ClO^- + 0.056 M Na_2HPO_4 (pH 5.5)*					
	TOA		*THPA*		*THA*		*Amberlite LA-2*		*TOA*		*THPA*		*THA*	
	k'_i	r_{ji}	k'_i	r_{ji}	k'_i	r_{ji}	k'_i	r_{ji}	k'_i	r_{ji}	k'_i	r_{ji}	k'_i	r_{ji}
4-Methoxymandelic acid	0.04	–	0.03	–	0.07	–			0.24	–	0.26	–	0.24	–
4-Hydroxyphenylacetic acid	0.05	1.25	0.05	1.66	0.11	1.57			0.04	6.00*	0.10	2.60*	0.06	4.00*
4-Aminosalicylic acid	0.08	1.60	0.13	2.60	0.23	2.09			0.44	11.00	0.54	5.40	0.82	13.60
3,4-Dihydroxycinnamic acid	0.14	1.75	0.19	1.46	0.36	1.56			0.14	3.14	0.14	3.86*	0.20	4.10*
2-Acetylbenzoic acid	0.17	1.21	0.20	1.05	0.30	1.20*			0.24	1.71	0.22	1.57	0.24	1.22
3,4-Dimethoxybenzoic acid	0.40	2.35	0.48	2.40	0.72	2.40			0.20	1.20*	0.16	1.37*	0.26	1.08
4-Methoxyphenylacetic acid	0.41	1.02	0.49	1.02	0.70	1.03*			0.56	2.80	0.52	3.25	0.60	2.31
4-Hydroxy-3-methoxycinnamic acid	0.71	1.73	0.94	1.92	1.44	2.06	0.38	–	0.54	1.04*	0.44	1.18*	0.72	1.20
4-Hydroxycinnamic acid	0.76	1.07	1.03	1.09	1.54	1.07	0.29	1.31*	0.54	1.00	0.60	1.36	0.80	1.11
3-Hydroxycinnamic acid	0.77	1.01	0.99	1.04*	1.55	1.00	0.30	1.03	0.80	1.48	0.82	1.37	1.00	1.25
3-(4-methoxyphenyl)propanoic acid	1.03	1.34	1.24	1.25	1.67	1.08	0.76	2.53	1.28	1.60	1.12	1.36	1.34	1.34
2-Hydroxycinnamic acid	1.16	1.13	1.58	1.27	2.53	1.51	0.52	1.46*	1.66	1.30	1.60	1.43	2.00	1.49
2,4-Dimethoxybenzoic acid	1.51	1.30	1.67	1.06	2.25	1.12*	1.51	2.90	1.14	1.46*	0.92	1.74*	1.16	1.72*
3,4-Dimethoxycinnamic acid	1.55	1.03	1.94	1.16	3.07	1.36	1.22	1.24*	1.32	1.16	1.18	1.28	1.54	1.33
4-Methoxycinnamic acid	4.23	2.73	4.86	2.50	6.59	2.15	3.35	2.75	4.14	3.14	3.68	3.12	4.28	2.78
2-Benzoylbenzoic acid	5.84	1.38	7.29	1.50	10.4	1.57	3.56	1.06	19.2	4.64	13.7	3.73	20.3	4.74
2-Diphenylcarboxylic acid	11.6	1.98	13.4	1.84	16.8	1.62	7.48	2.10	34.0	1.77	26.9	1.96	27.5	1.35

2,4-Toluenedisulphonic acid	0.09	–	0.10	–	0.24	–			0.06	–	0.08	–	0.10	–
2-Toluenesulphonic acid	1.09	12.11	1.03	10.30	1.14	4.75			1.08	18.00	1.00	12.5	1.20	12.0
2-Chlorobenzenesulphonic acid	1.12	1.03	1.15	1.12	1.32	1.16	2.67		1.14	1.05	1.06	1.06	1.36	1.13
2,4-Dimethylbenzenesulphonic acid	3.49	3.12	3.08	2.67	3.19	2.42			3.46	3.03	3.14	2.96	3.28	2.41
2-Iodobenzenesulphonic acid	3.54	1.01	3.45	1.12	3.85	1.20	6.64	–	3.56	1.03	3.32	1.06	4.02	1.23
3-Chlorobenzenesulphonic acid	3.54	1.00	3.49	1.01	4.05	1.05	17.0	2.56	3.48	1.02*	3.38	1.02	3.98	1.01*
Indane-4-sulphonic acid	6.75	1.91	6.09	1.74	6.96	1.72			6.78	1.95	6.26	1.85	7.06	1.77
2,3,4-Trimethylbenzenesulphonic acid	8.64	1.28	7.71	1.27	8.24	1.18			8.52	1.26	7.94	1.27	8.28	1.17
4-Isopropylbenzenesulphonic acid	9.50	1.10	9.64	1.25	11.2	1.36			9.96	1.17	9.46	1.19	11.2	1.35
3-Iodobenzenesulphonic acid	10.6	1.11	10.5	1.09	12.7	1.13			10.2	1.03	10.2	1.08	12.5	1.12
3,4,5-Trimethylbenzenesulphonic acid	11.3	1.06	10.1	1.04*	11.5	1.10*			11.2	1.09	10.2	1.00	11.7	1.07*
4-*tert.*-Butylbenzenesulphonic acid	24.5	2.18	22.1	2.20	25.6	2.22			21.9	1.96	21.5	2.10	25.6	2.18
3,4-diethylbenzenesulphonic acid	25.5	1.04	23.4	1.06	26.4	1.03			26.0	1.19	23.6	1.10	27.4	1.07
Benzosuberone-4-sulphonic acid	39.9	1.56	36.7	1.56	41.8	1.58			40.4	1.55	37.5	1.59	43.6	1.59
9,10-Anthraquinone-2-sulphonic acid	73.4	1.84	70.8	1.93	88.1	2.11			74.9	1.85	73.7	1.96	87.9	2.01
1,2-Dihydroxybenzene	0.17	–	0.20	–	0.28	–			0.22	–	0.26	–	0.26	–
Phenol	1.01	5.94	1.14	5.70	1.31	4.68	0.47		1.14	5.18	1.24	4.76	1.38	5.31
4-Methylphenol	3.01	2.98	3.26	2.86	3.51	2.68			3.40	2.98	3.58	2.89	3.76	2.72
4-Ethylphenol	8.57	2.85	9.16	2.81	9.64	2.75			9.36	2.75	10.2	2.85	10.5	2.79
4-Propylphenol	30.4	3.55	32.2	3.52	33.1	3.34			35.2	3.76	37.4	3.67	36.6	3.49
2-Hydroxynaphthalene	34.5	1.13	39.2	1.22	45.0	1.36			39.3	1.12	45.4	1.21	50.8	1.39
4-*tert.*-butylphenol	45.1	1.31	48.3	1.23	50.5	1.12			53.1	1.35	56.5	1.24	56.2	1.11

* Successive capacity ratios are reversed.

of the very high capacity ratio and the very poor theoretical plate height, which severely restrict its use as a stationary phase in HPLC. They are given as an illustration of the effect of the class of amine and the chain length on the capacity ratio.

At low pH (Table III, pH 1.5) the influence of the chain length of the amine on k_i' is large for carboxylic acids but less pronounced for phenols and sulphonic acids. For sulphonic acids the capacity ratios show a minimum value for THPA at a constant volume phase ratio. However, a gradual decrease in the capacity ratios with decreasing chain length of the amine is found if the overall amine concentration, C, in the ΔK_{X2} term of eqn. 1 is kept constant. This result indicates a decrease in the selectivity coefficient, K_2, for ionized compounds with decreasing chain length of the amine stationary phase. The same behaviour for ionizable compounds has been found by other workers using liquid anion exchangers in extraction chromatography[21,22,24,25]. According to Pierotti *et al.*[23], for phenols a linear relationship $\log k_i' = a + bn$ is found, where n is the number of methylene groups in the amine and a and b are constants. For carboxylic acids, however, such a relationship was not found, probably owing to a slight contribution of ion-pair formation at low pH. The increase in the capacity ratios of phenols and carboxylic acids with decreasing chain length of the amine agrees with the results of Pierotti *et al.* [23].

At pH 5.5 (Table III), for carboxylic acids liquid–liquid distribution and ion pair formation occur. Compared with the capacity ratios at pH 1.5, the values for carboxylic acids decrease for THA in most instances and changes irregularly for TOA and THPA. This result can be explained by the fact that the decrease in the first term, ΔK_{X1}, is or is not compensated for by the increase in the second term, ΔK_{X2}, due to the increased pH. In most instances the behaviour of the capacity ratio of carboxylic acids agrees with the results for sulphonic acids because ion-pair formation dominates. For sulphonic acids no significant change in the capacity ratios at high pH compared with low pH is found, as would be expected owing to the domination of ion-pair formation within this pH range. The larger values of the capacity ratios of phenols at higher pH must be attributed to the increase in the ionic strength of the mobile phase, caused by the addition of a phosphate buffer which promotes the solubility of phenols in the amine phase.

From Table III it can be seen that in many instances the selectivity factors of successively eluted compounds arranged within a group can vary significantly with the type of amine involved.

Influence of temperature on k_i' and r_{ji}

The influence of temperature on the k_i' value of an acid HX is determined by the temperature dependence of the partition coefficient, K_{HX}, and the formation constants, K_1, K_2 and K_3, and to a minor extent by the change in phase ratio.

Usually the liquid–liquid distribution constant and the formation constants K_1 and K_3 decrease with increasing temperature, whereas the change in the selectivity factor, K_2, can be irregular. The effect of temperature on the capacity ratios of different acids was investigated for three types of amines (TOA, THPA and THA) in combination with perchloric acid at low pH (*ca.* 1.5) and high pH (*ca.* 5.5). Some serious stripping problems occur with the phase systems at higher temperatures: at low pH TOA and THPA can be used up to 65° and THA up to 45°, and at high pH only TOA could be used up to 45°.

From the results (Table IV), three effects can be recognized when the temperature is increased: (i) the capacity ratios of sulphonic acids (except anthraquinone-2-sulphonic acid) increase at low and high pH; (ii) the capacity ratios of carboxylic acids decrease at low pH and increase at high pH; and (iii) the capacity ratios of phenols decrease at low and high pH. This behaviour can be explained by means of eqn. 1.

For phenols, liquid–liquid distribution is dominant at low and high pH, and the temperature dependence of the overall distribution constant, K_X, is determined by the temperature dependence of the partition coefficient, K_{HX}, which usually decreases with increasing temperature. For carboxylic acids, liquid–liquid distribution is dominant at low pH and ion-pair formation at high pH. At low pH, k_i' decreases owing to a decrease in the partition coefficient, K_{HX}, with increasing temperature.

At high pH, ion-pair formation is dominant and the dependence of the overall distribution constant, K_X, is determined mainly by the influence of temperature on the selectivity coefficient, K_2 (*i.e.*, the effect of temperature on the formation constants of the ion pairs BHA and BHX). According to Table IV, the selectivity coefficients of carboxylic acids increase with the temperature at high pH.

For sulphonic acids, ion-pair formation is dominant at low and high pH and the change in the selectivity coefficient with increasing temperature determines the effect on k_i'. As for carboxylic acids at high pH, the capacity ratios (*i.e.* selectivity coefficient) of sulphonic acids increase with increasing temperature at low and high pH.

The effect of temperature on the selectivity factors is considerable in most instances and can be even more pronounced if simultaneously both liquid–liquid distribution and ion-pair formation contribute significantly to the distribution. Further, an increase in temperature has a very favourable effect on the theoretical plate height, especially if ion-pair formation is dominant[20,26].

Influence of addition of methanol to the mobile phase on k_i' and r_{ji}

The addition of methanol changes the polarity of the aqueous and the stationary phase[27,28]. It influences the partition coefficient, K_{HX}, and the formation constants, K_1, K_2 and K_3, and consequently the overall distribution constant, K_X. For various carboxylic acids, sulphonic acids and phenols the capacity ratios were determined on a column containing TOA as the stationary phase and mixtures of methanol and water, 0.05 *M* in perchloric acid, as the mobile phase.

In order to check that no stationary phase was stripped from the column as a result of the addition of methanol, the capacity ratios of some standard compounds were measured before and after the experiments. No loss of liquid stationary phase was found up to a methanol concentration of 40% (v/v).

The dependence of k_i' on the methanol content is shown in Fig. 3. For carboxylic acids and phenols, liquid–liquid distribution is dominant and it can be seen that the capacity ratio decreases with increasing methanol content. This behaviour agrees with the results usually obtained in liquid–liquid phase systems when the polarity difference between the two phases decreases[23]. Under the chosen conditions, ion-pair formation is the dominating process for sulphonic acids. The effect of the addition of methanol on the capacity ratio of sulphonic acids is different from that for carboxylic acids and phenols, which could be expected because of the different distribution mechanisms. The effect of methanol on the chemical equilibria, however, is very complex and therefore difficult to predict.

TABLE IV

INFLUENCE OF TEMPERATURE ON THE CAPACITY RATIOS (k_i') AND SELECTIVITY FACTORS (r_{ji}) OF PHENOLS, CARBOXYLIC ACIDS AND SULPHONIC ACIDS FOR THE PHASE SYSTEMS TOA, THPA AND THA–0.05 *M* $HClO_4$ AT pH 1.5 AND 5.5

Compound	*0.05 M $HClO_4$ (pH 1.5)*							
	TAO						*THPA*	
	25°		*45°*		*65°*		*25°*	
	k_i'	r_{ji}	k_i'	r_{ji}	k_i'	r_{ji}	k_i'	r_{ji}
3,4-Dihydroxycinnamic acid	0.14	—	0.10	—	0.06	—	0.19	—
2-Acetylbenzoic acid	0.17	1.21	0.16	1.60	0.13	2.16	0.20	1.05
3,4-Dimethoxybenzoic acid	0.40	2.35	0.34	2.12	0.26	2.00	0.48	2.40
4-Methoxyphenylacetic acid	0.41	1.02	0.35	1.03	0.28	1.08	0.49	1.02
4-Hydroxy-3-methoxycinnamic acid	0.71	1.73	0.54	1.54	0.36	1.28	0.94	1.92
4-Hydroxycinnamic acid	0.76	1.07	0.55	1.02	0.36	1.00	1.03	1.09
3-Hydroxycinnamic acid	0.77	1.01	0.55	1.00	0.36	1.00	0.99	1.04*
3-(4-methoxyphenyl)propanoic acid	1.03	1.34	0.87	1.58	0.66	1.83	1.24	1.25
2-Hydroxycinnamic acid	1.16	1.13	0.81	1.07*	0.53	1.24*	1.58	1.27
2,4-Dimethoxybenzoic acid	1.51	1.30	1.16	1.43	0.85	1.60	1.67	1.06
3,4-Dimethoxycinnamic acid	1.55	1.03	1.20	1.03	0.85	1.00	1.94	1.16
4-Methoxycinnamic acid	4.23	2.73	2.95	2.46	1.93	2.27	4.86	2.50
2-Benzoylbenzoic acid	5.84	1.38	4.34	1.47	2.94	1.52	7.29	1.50
2-Diphenylcarboxylic acid	11.6	1.98	8.45	1.95	5.49	1.87	13.4	1.84
2,4-Toluenedisulphonic acid	0.09	—	0.25	—	0.64	—	0.10	—
2-Toluenesulphonic acid	1.09	12.1	1.55	6.20	2.17	3.39	1.03	10.3
2-Chlorobenzenesulphonic acid	1.12	1.03	1.63	1.05	2.30	1.06	1.15	1.12
2,4-Dimethylbenzenesulphonic acid	3.49	3.12	4.61	2.83	6.27	2.73	3.08	2.67
2-Iodobenzenesulphonic acid	3.54	1.01	4.63	1.01	5.98	1.05*	3.45	1.12
3-Chlorobenzenesulphonic acid	3.54	1.00	4.42	1.05*	5.58	1.07*	3.49	1.01
Indane-4-sulphonic acid	6.75	1.91	8.02	1.81	10.0	1.80	6.09	1.74
2,3,4-Trimethylbenzenesulphonic acid	8.64	1.28	11.1	1.38	14.2	1.41	7.71	1.27
4-Isopropylbenzenesulphonic acid	9.50	1.10	12.9	1.16	15.4	1.09	9.64	1.25
3-Iodobenzenesulphonic acid	10.6	1.11	12.1	1.07*	13.8	1.20*	10.5	1.09
3,4,5-Trimethylbenzenesulphonic acid	11.3	1.06	13.1	1.08	15.3	1.11	10.1	1.04*
4-*tert.*-Butylbenzenesulphonic acid	24.5	2.18	29.8	2.28	36.0	2.34	22.1	2.20
3,4-Diethylbenzenesulphonic acid	25.5	1.04	30.2	1.01	35.4	1.01*	23.4	1.06
Benzosuberone-4-sulphonic acid	39.9	1.56	46.3	1.53	51.4	1.45	36.7	1.56
9,10-Anthraquinone-2-sulphonic acid	73.4	1.84	61.9	1.34	54.7	1.06	70.8	1.93
1,2-Dihydroxybenzene	0.17	—	0.15	—	0.12	—	0.20	—
Phenol	1.01	5.94	0.84	5.60	0.65	5.42	1.14	5.70
4-Methylphenol	3.01	2.98	2.41	2.37	1.78	2.74	3.26	2.86
4-Ethylphenol	8.57	2.85	6.82	2.83	4.91	2.76	9.16	2.81
4-Propylphenol	30.4	3.55	23.6	3.46	15.6	3.18	32.2	3.52
2-Hydroxynaphthalene	34.5	1.13	21.1	1.12*	12.2	1.28*	39.2	1.22
4-*tert.*-Butylphenol	45.1	1.31	35.1	1.66	23.4	1.92	48.3	1.23

* Successive capacity ratios are reversed.

At least Fig. 3 shows that the addition of methanol affects the capacity ratio of monosulphonic acids to a lesser extent than those of carboxylic acids and phenols. 1-Naphthol-3-sulphonic acid, however, behaves more like a phenol, probably be-

								0.05 M $HClO_4$ + 0.056 M Na_2HPO_4 (pH 5.5)			
				THA				*TOA*			
45°		*65°*		*25°*		*45°*		*25°*		*45°*	
k'_i	r_{ji}	k'_i	r_{ji}	k'_i	r_{ji}	k'_i	r_{ji}	k'_i	r_{ji}	k'_i	r_{ji}
0.15	—	0.10	—	0.36	—	0.21	—	0.14	—	0.20	—
0.19	1.27	0.17	1.70	0.30	1.20*	0.25	1.19	0.24	1.71	0.42	2.10
0.41	2.16	0.35	1.73	0.72	2.40	0.58	2.32	0.20	1.20	0.30	1.40*
0.41	1.00	0.34	1.03*	0.70	1.03*	0.56	1.03*	0.56	2.80	0.82	2.73
0.68	1.66	0.49	1.44	1.44	2.06	0.97	1.73	0.54	1.04*	0.68	1.21*
0.70	1.03	0.47	1.04*	1.54	1.07	0.98	1.01	0.54	1.00	0.66	1.03*
0.70	1.00	0.47	1.00	1.55	1.00	1.00	1.02	0.80	1.48	0.92	1.39
0.99	1.41	0.77	1.64	1.67	1.08	1.28	1.28	1.26	1.57	1.70	1.85
1.05	1.07	0.69	1.12*	2.53	1.51	1.59	1.24	1.64	1.30	1.70	1.00
1.28	1.22	0.96	1.39	2.25	1.12*	1.66	1.04	1.12	1.46*	1.26	1.35*
1.44	1.12	1.08	1.12	3.07	1.36	2.03	1.22	1.30	1.16	1.50	1.19
3.32	2.31	2.21	2.05	6.59	2.15	4.34	2.14	4.10	3.15	4.42	2.95
5.19	1.56	3.57	1.62	10.4	1.57	7.17	1.65	19.0	4.64	23.5	5.32
9.26	1.78	6.23	1.75	16.8	1.62	11.5	1.61	33.6	1.77	38.1	1.62
0.28	—	0.61	—	0.24	—	0.36		0.06	—	0.18	—
1.49	5.32	2.09	3.42	1.14	4.75	1.56	4.33	1.08	18.0	1.54	8.55
1.65	1.11	2.29	1.09	1.32	1.16	1.86	1.19	1.14	1.05	1.56	1.01
4.34	2.63	5.91	2.58	3.19	2.42	4.48	2.41	3.46	3.03	4.62	2.96
4.50	1.04	5.68	1.04*	3.85	1.20	4.92	1.10	3.56	1.03	4.44	1.04*
4.31	1.04*	5.42	1.05*	4.05	1.05	5.02	1.02	3.48	1.02*	4.10	1.08*
7.53	1.75	8.95	1.65	6.96	1.72	8.26	1.64	6.78	1.95	7.50	1.83
10.1	1.34	12.7	1.41	8.24	1.18	10.3	1.24	8.52	1.26	10.3	1.38
12.1	1.20	15.1	1.19	11.2	1.36	11.5	1.12	9.96	1.17	12.5	1.21
11.6	1.05*	13.1	1.15*	12.7	1.13	—		10.2	1.03	11.0	1.14*
11.9	1.03	13.7	1.04	11.5	1.10*	—		11.2	1.09	12.5	1.14
27.0	2.27	31.5	2.30	25.6	2.22	—		21.9	1.96	26.1	2.09
28.8	1.07	34.8	1.10	26.4	1.03	—		26.0	1.19	28.8	1.10
42.0	1.46	46.5	1.34	41.8	1.58	—		40.4	1.55	44.2	1.53
59.7	1.42	51.2	1.10	88.1	2.11	—		74.9	1.85	54.1	1.22
0.17	—	0.14	—	0.28	—	0.22		0.22	—	0.18	—
0.91	5.35	0.70	5.00	1.31	4.68	1.03	4.68	1.14	5.18	0.90	5.00
2.54	2.79	1.88	2.69	3.51	2.68	2.70	2.62	3.40	2.98	2.72	3.02
7.04	2.77	5.01	2.66	9.64	2.75	7.28	2.70	9.36	2.75	7.86	2.89
23.6	3.35	16.6	3.31	33.1	3.34	24.0	3.30	35.2	3.76	26.9	3.42
22.7	1.04*	13.2	1.26*	45.0	1.36	26.8	1.12	39.3	1.12	23.8	1.13*
35.8	1.58	25.1	1.90	50.5	1.12	35.9	1.34	53.1	1.85	41.3	1.74

cause of the formation of an intramolecular hydrogen bond between the hydroxyl and the sulphonate groups.

A completely different dependence of the capacity ratio on the methanol con-

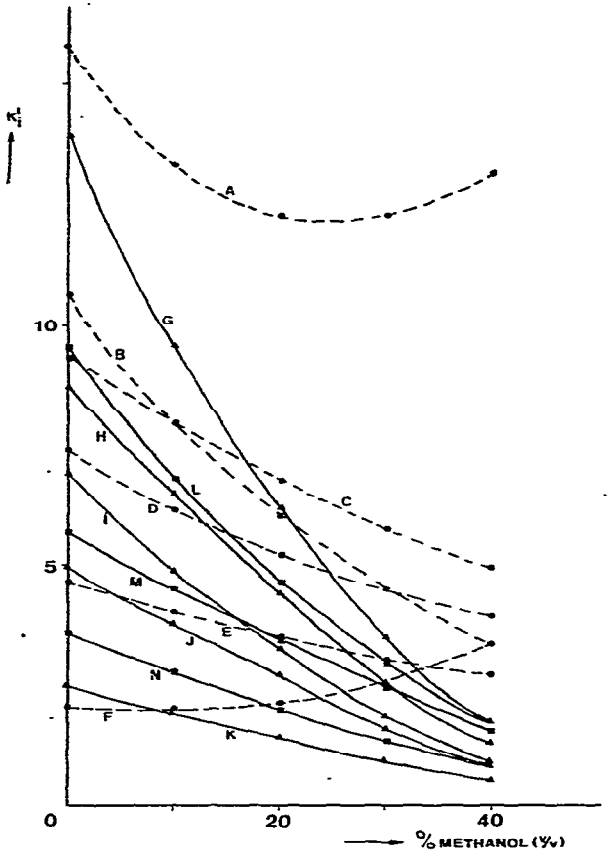

Fig. 3. Influence of methanol on the capacity ratio, k_i'. Mobile phase: 0.05 *M* $HClO_4$ + methanol, pH = 1.5. Stationary phase: TOA (4%, w/w) on Kieselguhr; glass column (15 cm). ●, Sulphonic acids: (a) 1-naphthol-3,8-disulphonic acid; (b) 1-naphthol-3-sulphonic acid; (c) 2,3,4-trimethylbenzenesulphonic acid; (d) indane-4-sulphonic acid; (e) 1-naphthol-5-sulphonic acid; (f) 2-naphthol-3,6-disulphonic acid. ▲, Carboxylic acids: (g) 2-diphenylcarboxylic acid; (h) 2-methoxycinnamic acid; (i) 2-benzoylbenzoic acid; (j) 4-methoxycinnamic acid; (k) benzilic acid. ■, Phenols: (l) 2,5-dimethylphenol; (m) 2-nitrophenol; (n) 4-methylphenol.

tent is found for disulphonic acids. For 2- naphthol-3,6-disulphonic acid k_i' is constant at low and increases at high methanol contents, while for 1-naphthol-3,8-disulphonic acid k_i' decreases at low and increases again at high methanol contents. From batch experiments, the methanol content of the eluent was determined by gas chromatography and the results are given in Table V. Up to a methanol content of 5% (v/v) in the eluent the percentage of methanol in the amine phase is larger, whereas above 5% (v/v) of methanol, the amine phase contains less methanol. This effect might be the reason why, for the very polar disulphonic acids, which contain two charges on their molecules, the solvation in the mobile phase becomes less effective compared with the amine phase. This effect promotes ion-pair formation and increases the capacity ratio.

According to Fig. 3, the addition of methanol seems to be a very suitable means of adjusting the retention and selectivity for the group of acids including phenols.

TABLE V

METHANOL UPTAKE BY THE STATIONARY PHASE (TOA) AS A FUNCTION OF THE METHANOL CONTENT OF THE ELUENT

Methanol in TOA (%, v/v)	*Methanol in eluent (%, v/v)*
2.2	2.0
4.8	5.0
6.0	10.0
16.0	30.0
25.0	40.0

Influence of the nature of the acid added to the mobile phase on k_i' and r_{ji}

The nature of the eluent acid (HA) can influence the overall distribution constant, K_X, of an acid HX in different ways. It can affect: (i) the nature of the organic phase and hence the partition coefficient, K_{HX}, and (ii) the selectivity factor, K_2, and the formation constant, K_3. In order to investigate the influence of the type of eluent acid, sulphuric, hydrochloric, methanesulphonic and trichloroacetic acid were tested for their ability to serve as the competing acid in combination with TOA. Sulphuric, hydrochloric and methanesulphonic acid, however, could not be used in practice as the solubilities of the amine salts in the mobile phase were found to be too large, which results in unstable columns (*N.B.*, it is generally known that the solubility of perchlorates is relatively low). The solubility of the amine could be suppressed by using

TABLE VI

INFLUENCE OF THE NATURE AND CONCENTRATION OF THE COUNTER ION ON THE CAPACITY RATIO (k_i') AND SELECTIVITY FACTOR (r_{ji})

Compound	*0.05 M $HClO_4$*		*0.05 M TCA*		*0.075 M TCA*		*0.1 M TCA*	
	k_i'	r_{ji}	k_i'	r_{ji}	k_i'	r_{ji}	k_i'	r_{ji}
4-Methoxyphenylacetic acid	0.41	—	2.10	—	1.55	—	1.26	—
Benzoic acid	0.85	2.07	5.12	2.44	3.62	2.33	2.99	2.37
3-(4-Methoxyphenyl)propanoic acid	1.03	1.21	5.14	1.00	3.83	1.06	3.09	1.03
4-Nitrobenzoic acid	1.05	1.02	8.25	1.60	5.52	1.44	4.12	1.33
2-Hydroxycinnamic acid	1.16	1.10	23.1	2.80	12.1	2.19	7.74	1.88
2,4-Dimethoxybenzoic acid	1.51	1.30	1.59	14.5*	1.36	8.90*	1.26	6.14*
Salicylic acid	1.69	1.12	16.9	10.6	11.2	8.23	8.34	6.62
Benzenesulphonic acid	0.43	—	0.14	—	0.08	—	0.07	—
1-Naphthol-3,6-disulphonic acid	0.44	1.02	0.08	1.75*	0.02	4.00*	0.01	7.00*
2-Chlorobenzenesulphonic acid	1.12	2.54	0.45	18.1	0.28	14.0	0.20	20.—
4-Toluenesulphonic acid	1.25	1.12	0.44	3.30*	0.31	1.11	0.22	1.10
2-Naphthol-3,6-disulphonic acid	2.04	1.63	0.28	1.57*	0.08	3.88*	0.03	7.33*
2,4-Dimethylbenzenesulphonic acid	3.49	1.71	1.58	5.64	0.98	12.2	0.73	24.3
1-Naphthol-5-sulphonic acid	4.56	1.31	5.86	3.71	2.48	2.53	1.40	1.92
1-Naphthol-3-sulphonic acid	11.1	2.43	9.36	1.60	4.15	1.67	2.30	1.64
1-Naphthol-3,8-disulphonic acid	14.5	1.31	2.59	3.61*	0.69	6.01*	0.26	8.85*
Phenol	1.01	—	4.94	—	3.45	—	2.71	—
2-Methylphenol	3.34	3.31	14.35	2.90	10.41	3.02	8.10	2.99
2-Nitrophenol	5.15	1.54	7.73	1.86*	6.40	1.63*	5.97	1.36*

* Successive capacity ratios are reversed.

very large acid concentrations ($> 1\ M$), but this led to serious corrosion of the chromatographic apparatus.

Apart from perchloric acid, only trichloroacetic acid was found to be useful at acid concentrations $\geqslant 0.05\ M$. Compared with 0.05 M perchloric acid, a completely different behaviour of the acetic compound was found for the phase system TOA–TCA, as can be seen from Table VI.

The considerable change in selectivity on changing from perchlorate to trichloroacetate as the counter ion might be explained as follows. It is known that acids such as perchloric acid form polymolecular complexes with TOA of the general formula $(TOA \cdot HClO_4)_n$. Trichloroacetic acid, however, forms preferentially the monomolecular complex TOA·TCA·TCA[29]. The presence of a second TCA molecule bound via hydrogen bonds to the TOA·TCA molecule possibly causes a different distribution mechanism to partition only for acidic molecules such as those of carboxylic acids and phenols. The relatively weakly bound second TCA molecule can be replaced with a sample molecule HX. This exchange mechanism can be expressed by

$$(TOA \cdot TCA \cdot TCA)_s + (HX)_m \leftrightarrows (TOA \cdot TCA \cdot HX)_s + (TCA)_m$$

where the subscript s refers to the stationary phase and m to the mobile phase. This

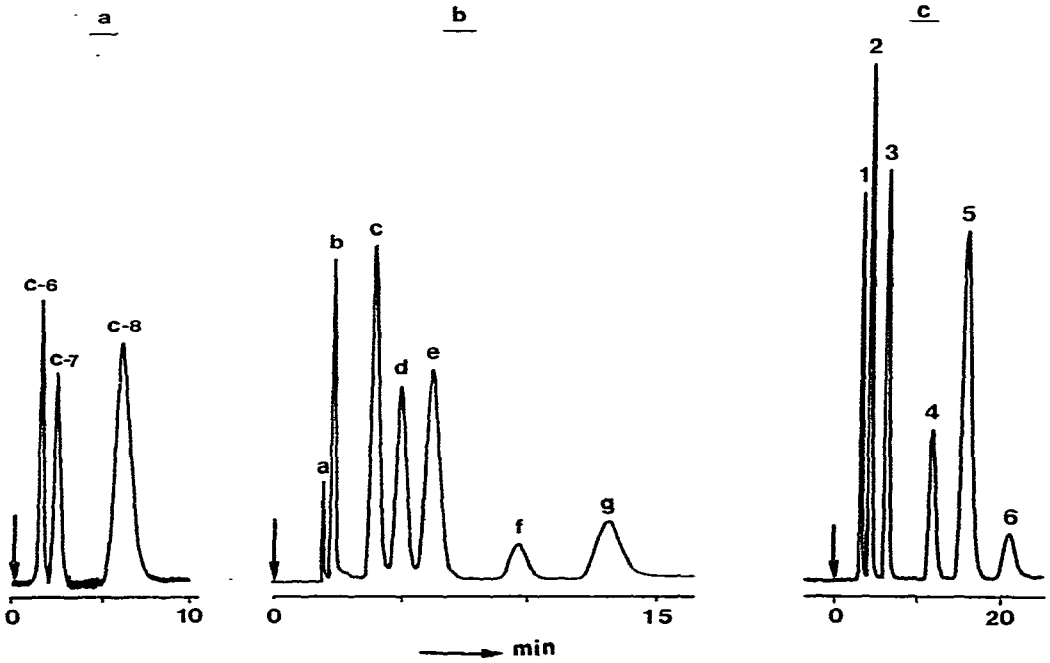

Fig. 4. Separation of closely related acidic compounds. (a) Alkylsulphonic acids. (b) a, Unretained; b, 3,4-dihydroxycinnamic acid; c, 3-hydroxycinnamic acid; d, 2-hydroxycinnamic acid; e, 3,4-dimethoxycinnamic acid; f, cinnamic acid; g, 1-methoxycinnamic acid. (c) 1, 4-hydroxyphenylacetic acid; 2, 2-acetylbenzoic acid; 3, 4-methoxyphenylacetic acid; 4, 3-(4-methoxyphenyl)propanoic acid, 5, 2,4-dimethoxybenzoic acid; 6, benzilic acid. Stationary phase: 4% (w/w) TOA on Kieselguhr. Mobile phase: 0.05 M $HClO_4$, pH 1.5. Glass column (15 cm).

equilibrium shows that a reciprocal linear relationship must exist between the overall distribution constant of the acid HX and the TCA concentration in the mobile phase. This relationship is indeed found for carboxylic acids and phenols, as can be seen from Table VI.

For mono- and disulphonic acids, the situation is completely different. Under the chosen conditions the sulphonic acids are present as ions and ion-pair formation therefore dominates. If perchloric acid is replaced with trichloroacetic acid, for some compounds a considerable decrease in the capacity ratio occurs while for others, such as naphtholsulphonic acids, only minimal changes are observed. However, all sulphonic acids show a stronger dependence of their capacity ratio on the counter-ion concentration for TCA than for perchloric acid as measured previously[15]. The decrease in the capacity ratio on changing from 0.05 *M* perchloric acid to 0.05 *M* TCA must be caused by the larger formation constant of TOA·TCA·TCA (*ca.* 10^{10}) compared with that of $(TOA \cdot HClO_4)_n$ (*ca.* 10^8). This influences the selectivity factor, K_2, and hence the capacity ratio. The deviating behaviour of 1-naphthol-5-sulphonic acid and 1-naphthol-3-sulphonic acid can probably be attributed to the presence of the hydroxyl group in the molecule, which can influence all distribution mechanisms.

Owing to the presence of two TCA molecules per TOA molecule, the capacity ratios of monosulphonic acids, according to eqn. 1, are proportional to the reciprocal of the square of the TCA concentration. For disulphonic acids, there is a dependence

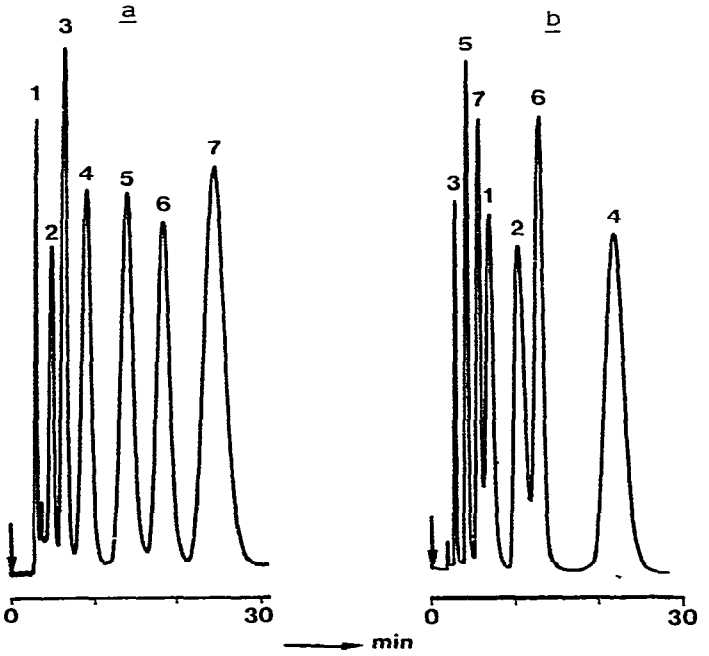

Fig. 5. Influence of the type of anion in the mobile phase on the elution sequence of acidic compounds. Glass column (15 cm). Phase system: (a) 4% TOA on Kieselguhr–0.1 *M* TCA; (b) 4% TOA on Kieselguhr–0.05 *M* $HClO_4$. Peaks: (1) 2-naphthol-3,6-disulphonic acid; (2) 4-methoxyphenylacetic acid; (3) 1-naphthol-3-sulphonic acid; (4) 2,4-dimethylbenzenesulphonic acid; (5) 4-nitrobenzoic acid; (6) 2-nitrophenol; (7) salicylic acid.

on the fourth power of the reciprocal of the TCA concentration. These predictions agree well with the experimental results.

According to Table VI, the type of counter ion used seems to be a powerful means of adjusting retention and selectivity.

Applicability of the phase system in HPLC

The applicability of long-chain aliphatic amines–dilute aqueous acid phase systems in HPLC and the possibilities of influencing the selectivity were demonstrated by a number of separations of test and natural mixtures of acidic compounds.

The separation of alkylsulphonic acids is still a problem. Fig. 4a shows the separation of three alkylsulphonic acids on TOA–perchloric acid using a tensammetric detector[30]. The large increase in capacity ratio with increasing alkyl chain length limits the separation to only three successive alkylsulphonic acids under isocratic conditions. For the resolution of more than three members of this group of compounds in a reasonable time, column switching or gradient step elution has to be applied.

The potential of the amine–diluted acid systems for the separation of closely related compounds such as cinnamic acid and some of its derivatives and for the separation of carboxylic acids of different types is shown in Figs. 4b and 4c.

Figs. 5a and 5b demonstrate the effect of the type of counter ion on the separa-

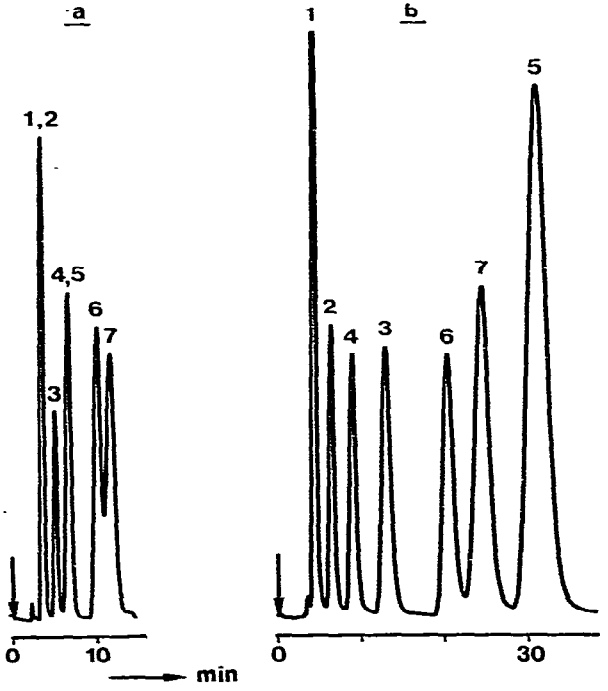

Fig. 6. Effect of pH of the mobile phase on the resolution of a mixture of acidic compounds. pH: (a) 1.5; (b) 5.5. Phase system: 4% TOA on Spherosil XOC–0.05 M ClO_4^-; metal column of length 150 mm, I.D. 3 mm. Peaks: (1) 3,4-dimethoxybenzoic acid; (2) 4-methylphenylacetic acid; (3) 4-nitrobenzoic acid; (4) 2,4-dimethoxybenzoic acid; (5) salicylic acid; (6) 3-methylphenol; (7) 4-nitrophenol.

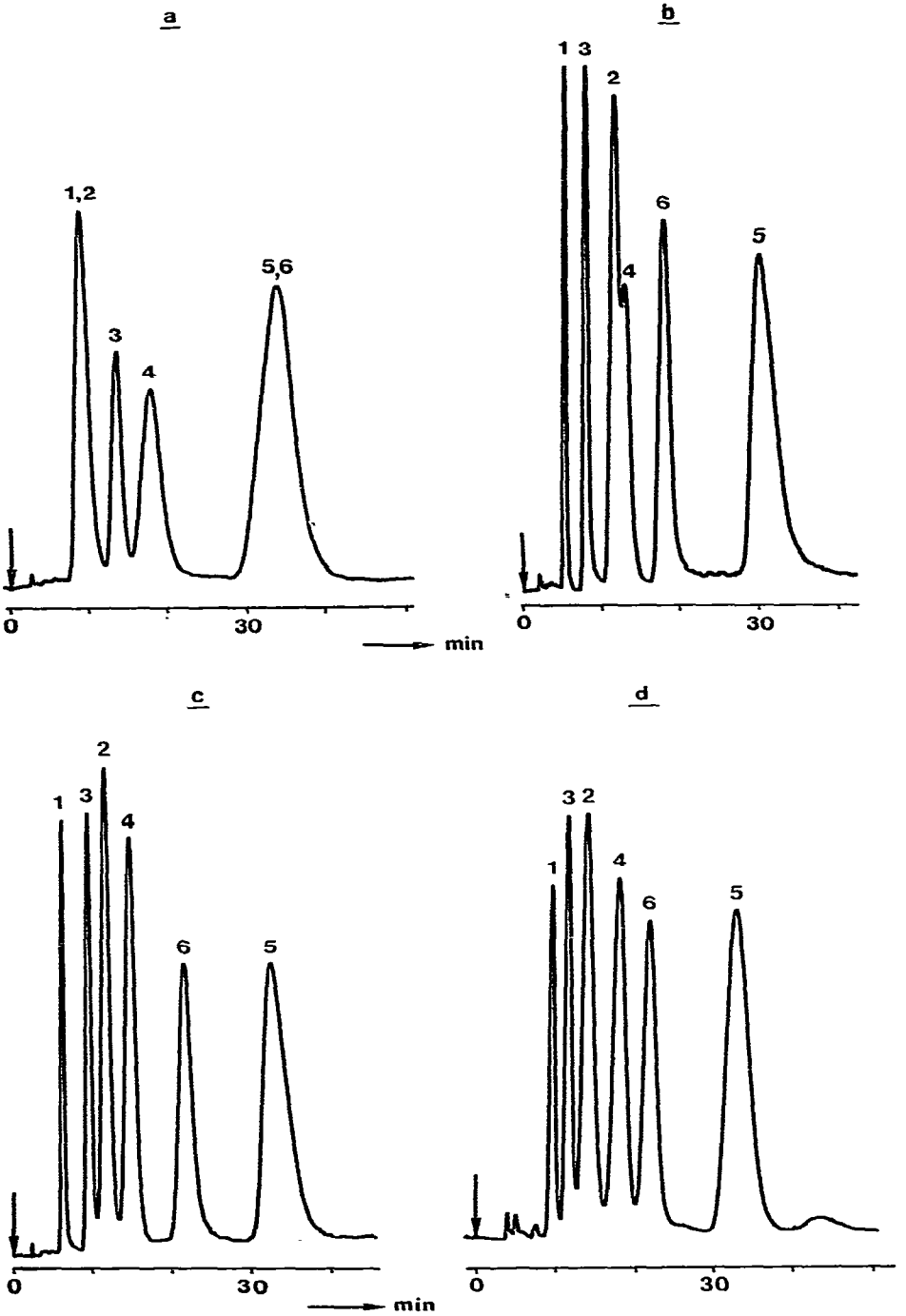

Fig. 7. Influence of temperature and addition of methanol on the resolution of acidic compounds. Metal column (15 cm). Phase system: (a)–(c) 4% TOA on Spherosil XOC–0.05 *M* $HClO_4$; (d) 4% TOA on Spherosil XOC–0.05 *M* $HClO_4$ + 20% (v/v) methanol. Temperature: (a) 25°; (b) 48°; (c) 43°; (d) 25°. Peaks: (1) salicylic acid; (2) 2-naphthol-3,6-disulphonic acid; (3) 4-methylphenol; (4) 1-naphthol-5-sulphonic acid; (5) 2,3,4-trimethylbenzenesulphonic acid; (6) 2,5-dimethylphenol.

tion of a test mixture of phenols and carboxylic and sulphonic acids. A completely different elution sequence is found on changing from TCA (Fig. 5a) to perchloric acid (Fig. 5b).

The effect of pH on the resolution of a mixture of compounds is illustrated in Figs. 6a and 6b. At low pH some pairs of compounds are not resolved; changing the pH from 1.5 to 5.5 not only results in the complete resolution of these pairs of compounds but also changes the elution sequence.

A suitable choice of the column temperature can be very critical in order to obtain a complete resolution of two compounds, as is shown in Figs. 7a, 7b and 7c. At 25° two pairs of compounds are not separated (Fig. 7a), whereas at 48° the resolution is improved but two compounds are still not completely separated (Fig. 7b). The optimal column temperature for this mixture was found to be 43° (Fig. 7c). Apart from the use of temperature as a parameter for adjusting the selectivity for this particular mixture, the addition of methanol to the aqueous phase can also be used to improve the resolution, as is shown in Fig. 7d.

The applicability of the described phase systems to the analysis of compounds of clinical or forensic interest in body fluids or foods is shown in Figs. 8 and 9. Benzoic and sorbic acid have been used as preservatives in foodstuff and juices for

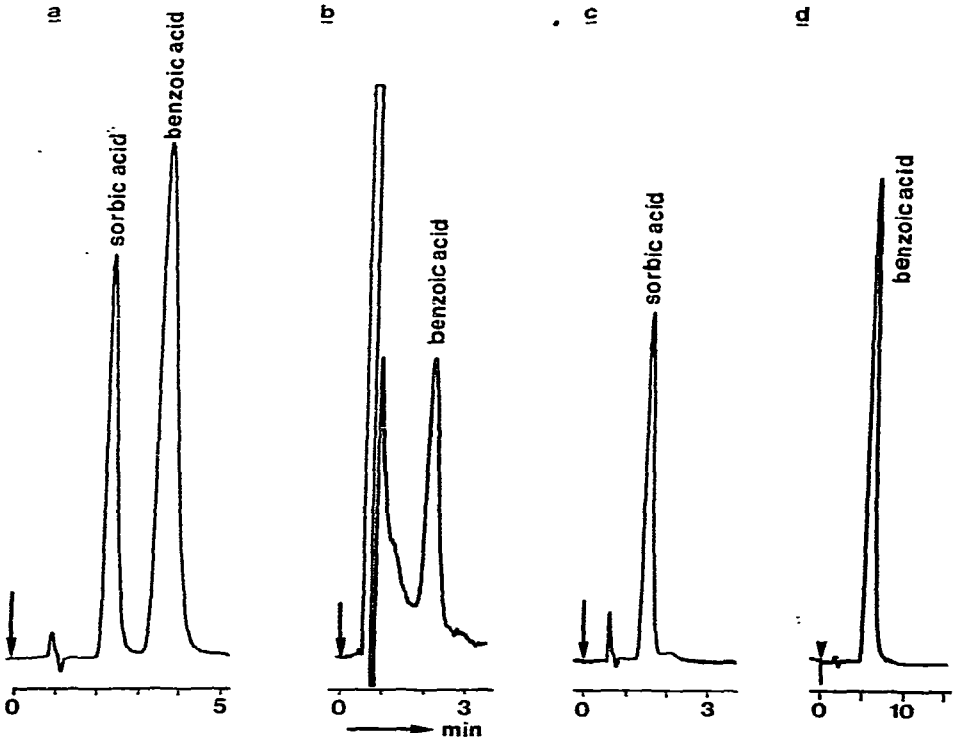

Fig. 8. Analysis of benzoic acid and sorbic acid extracted from foodstuffs and directly in essence. Phase system: 4% TOA on Spherosil XOC–0.1 M TCA; metal column (15 cm). (a) About 0.1 μg of benzoic acid and sorbic acid; (b) current essence containing 1500 ppm of benzoic acid, diluted 40-fold with eluent; (c) 1 g of rye bread containing 100 ppm of sorbic acid was extracted; (d) 1 g of margarine containing 100 ppm of benzoic acid was extracted.

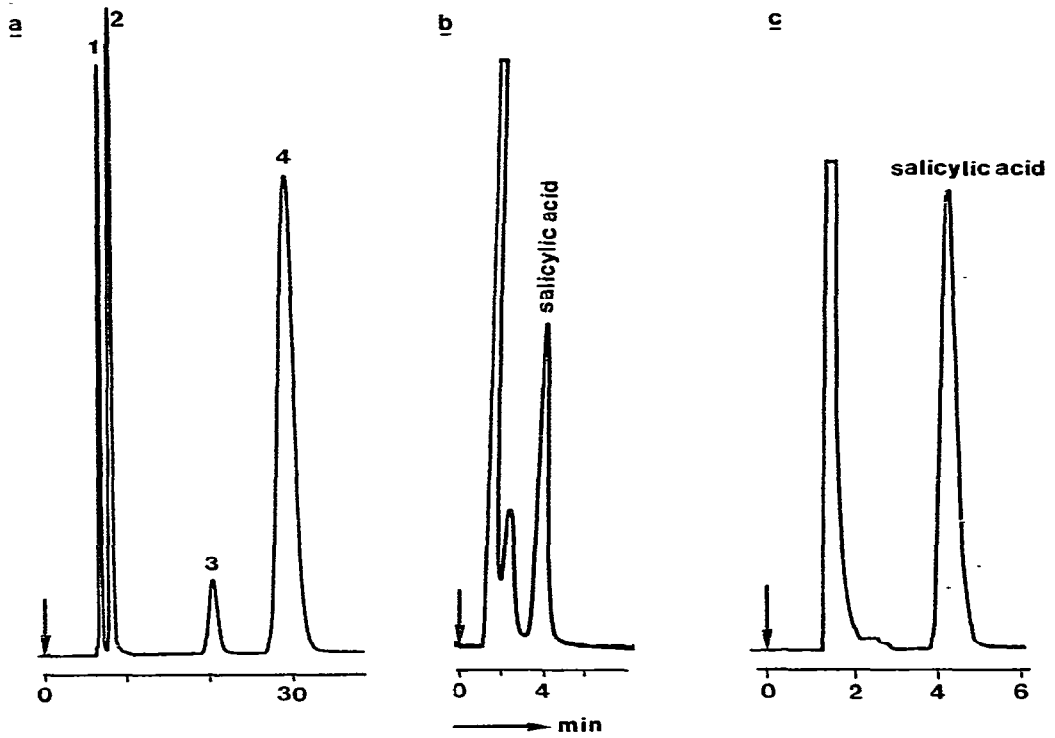

Fig. 9. Analysis of salicylic acid directly, in a urine extract and in deproteinized serum. Phase system: 4% TOA on Kieselguhr–0.25 *M* $HClO_4$; glass column (15 cm). (a) Aspirin tablet containing (1) caffeine, (2) aspirin, (3) salicylic acid and (4) phenacetin. (b) 10 ml of urine was extracted; (c) 0.2 ml of serum containing 50 ppm of salicylic acid was deproteinized.

many years, but in some countries the use of sorbic acid is forbidden or restricted by law. Hence a simple method for the separation and determination of sorbic and benzoic acid in foodstuff is of great value.

Fig. 8a shows the separation of sorbic and benzoic acid using a TOA–TCA system. Sorbic acid is commonly used as a preservative in rye bread and Fig. 8c shows the analysis of sorbic acid extracted from rye bread, which is simple because of the low background. The determination of benzoic acid in current juice essence by direct injection of a 40-fold diluted solution is shown in Fig. 8b. Benzoic acid is commonly added to margarine, and Fig. 8d shows the potential of the TOA–TCA system for the analysis of this compound extracted from Dutch margarine.

The analysis of an aspirin tablet is shown in Fig. 9a. The analysis of salicylic acid in plasma and urine is of importance in determining the relationship between concentration and therapeutic effects. Figs. 9b and 9c show the determination of salicylic acid in urine and plasma, respectively. For plasma, direct injection of deproteinized diluted plasma was applied. No changes in the column properties were found after 2000 serum injections, which illustrates the stability of the phase system[31]. With directly injected urine the background was found to be too high and extraction was necessary in order to improve it.

CONCLUSIONS

Phase systems consisting of long-chain aliphatic amines as the stationary phase and aqueous solutions of acids as the mobile phase are very promising for the separation of acidic compounds such as carboxylic acids, sulphonic acids and phenols. The variety of parameters available for adjusting the retention and the selectivity on the one hand and the stability of the phase system on the other favours its use in practical applications.

Future work will be devoted to the selectivity of long-chain aliphatic amines with optically active centres for the separation of optical isomers and to the suitability of long-chain aliphatic acids as stationary phases for the separation of bases.

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