CHROM. 15,115

CHROMATOGRAPHIC BEHAVIOUR OF 4'-DIMETHYLAMINOAZO-BENZENE-4-SULPHONYL DERIVATIVES OF AMINES IN LIQUID–SOLID SYSTEMS

J. JUSIAK* and E. SOCZEWIŃSKI

Department of Inorganic and Analytical Chemistry, Medical Academy, ul. Staszica 6, 20081 Lublin (Poland)

(First received November 3rd, 1981; revised manuscript received June 15th, 1982)

SUMMARY

4'-Dimethylaminoazobenzene-4'-sulphonyl (dabsyl) derivatives of monoalkyl- and dialkylamines were chromatographed on thin layers of silica or octadecyl silica. Silica layers were eluted with heptane solutions of methyl ethyl ketone, dioxane and diisopropyl ether; satisfactory separations of dialkylsulphonamide compounds were obtained, however, the R_F values of dabsyl derivatives of C_8–C_{12} monoalkylamines were practically identical. The reversed-phase system, water + methanol (80–100%)–ODS-silica was selective for monoalkyl as well as dialkyl derivatives.

INTRODUCTION

Amines and amino acids are frequently derivatized to 5-dimethylaminonaphthalene-1-sulphonyl (Dns) or 4'-dimethylaminoazobenzene-4-sulphonyl (dabsyl) compounds[1-11] whose detection limits are much lower than those of the parent compounds. The derivatives are less strongly absorbed on silica than aliphatic amines and amino acids so that lower eluent strengths are sufficient to secure an appropriate range of capacity ratios; moreover, the derivatives form series of solutes of analogous molecular structure suitable for the study of structural effects in liquid–solid chromatography. Therefore, their chromatographic behaviour was systematically investigated for frequently used systems of the type heptane + polar modifier/silica and water + polar modifier/octadecyl silica.

EXPERIMENTAL

Single dabsyl derivatives of primary and secondary aliphatic amines were prepared by mixing 1 ml of a methanol solution containing 10 μmoles of amine with 2.5 ml of an acetone solution of 25 μmoles of dabsyl chloride (synthesized in this Department[12]) containing 0.5 ml of 0.1 M aqueous NaHCO$_3$. The mixture was heated in a water-bath to 70°C for 10 min; the dark red colour of dabsyl chloride changed to orange.

For thin-layer chromatography (TLC) on silica (Si 60 GF 254; E. Merck,

TABLE I

hR_F VALUES OF DABSYL-AMINES FOR VARIOUS CONCENTRATIONS OF POLAR MODIFIER

Diluent = n-heptane; adsorbent, silica.

Modifier	%	Diethyl	Diisopropyl	Diisobutyl	Di-n-hexyl	Di-n-decyl	Octyl	Nonyl	Decyl	Dodecyl
Dioxane	10	27	26	38	49	61	24	21	25	19
	20	34	32	51	62	80	41	36	40	38
	40	75	72	72	91	95	65	64	69	74
	50	89	85	85	95	100	93	86	88	89
	70	95	100	100	100	–	100	95	100	100
	90	100	–	–	–	–	–	–	–	–
Methyl-ethyl ketone	10	25	31	49	59	66	27	26	31	27
	20	48	47	63	70	82	52	51	52	48
	40	82	81	91	93	93	83	92	94	90
	50	86	87	100	100	100	90	95	100	95
	70	98	100	–	–	–	95	100	–	100
	90	100	–	–	–	–	100	–	–	–
Di-iso-propyl ether	10	7	12	19	25	30	4	5	6	7
	20	16	21	35	37	42	10	9	8	9
	40	35	40	55	60	66	25	26	31	30
	50	39	44	60	70	70	31	34	39	39
	70	54	56	68	78	78	49	54	55	56
	90	64	65	78	84	87	59	69	74	77
	100	67	68	82	87	89	66	72	77	80

TABLE II

hR_F VALUES OF DABSYL-AMINES IN THE SYSTEM WATER + METHANOL–ODS-SILICA

% Methanol	Diethyl	Diisopropyl	Diisobutyl	Di-n-hexyl	Di-n-decyl	Octyl	Nonyl	Decyl	Dodecyl
100	61	69	51	37	14	58	52	50	43
95	46	58	29	17	5	36	28	25	16
90	28	42	14	11	–	21	14	11	7
85	23	33	12	–	–	13	10	–	–

Darmstadt, G.F.R.), the dabsyl-amines were extracted with three 2-ml portions of benzene and the combined extract evaporated to 2 ml; 5-μl samples were spotted. For TLC on high-performance TLC (HPTLC) precoated plates (RP-18, E. Merck), 1-μl samples of the reaction mixture were applied without prior purification.

Sandwich BN chambers with glass distributers were used[13]; for silica systems the samples were spotted behind the eluent front to eliminate demixing effects.

RESULTS AND DISCUSSION

The TLC data summarized in Tables I and II are plotted in Figs. 1–3 as R_M values (equivalent to log capacity factor, k', values in the technique used) at various concentrations of the polar modifier, on a logarithmic scale for silica and on a proportional scale for ODS-silica. The scales were chosen by taking into account that for polar adsorbents linear relationships are frequently obtained for the logarithmic coordinate system[14–16]
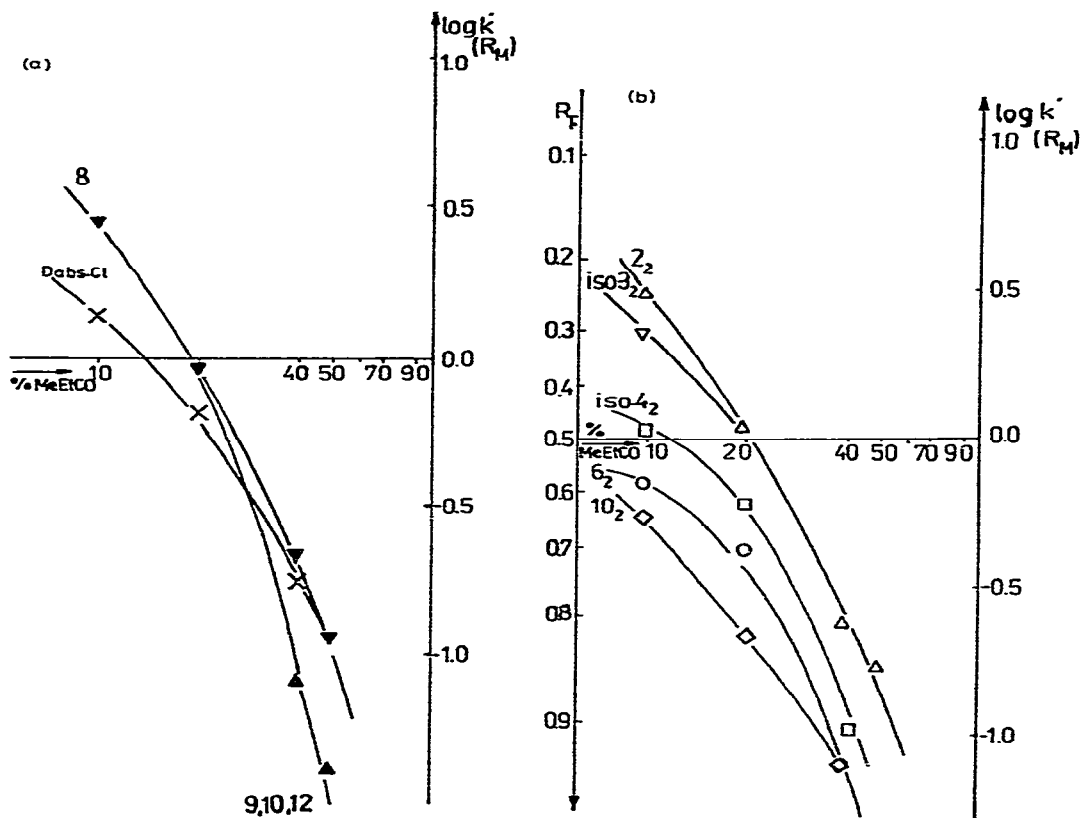

Fig. 1. log k' values of dabsyl derivatives of monoalkyl (a) and dialkyl (b) amines plotted against vol. % of methyl ethyl ketone (MeEtCO) in the eluent (logarithmic scale). Diluent, heptane; adsorbent, silica. Dabs-Cl = Dabsyl chloride. The numbers denote alkyl radicals, subscript "2" indicates secondary amine (e.g., 10 = n-decyl amine; 10_2 = di-n-decyl amine; iso-3_2 = diisopropyl amine).
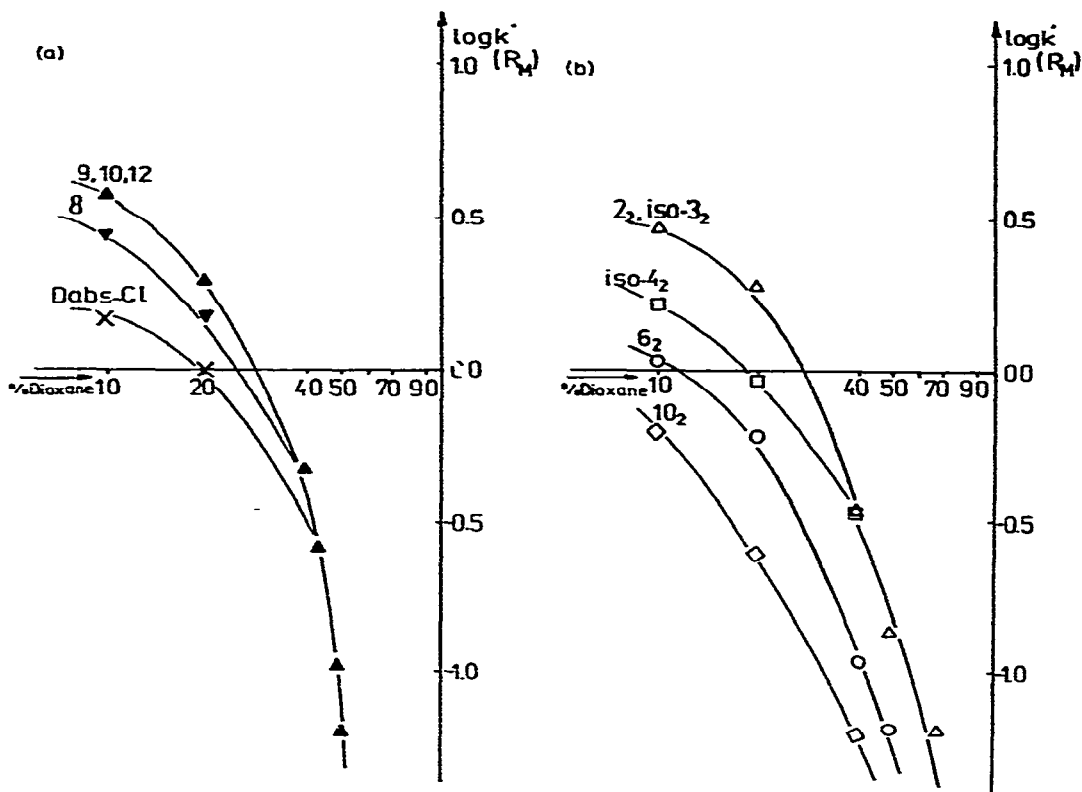
Fig. 2. As in Fig. 1; polar modifier = dioxane.

$$\log k'_{d \to mod} = \log k'_{mod} - a \log \varphi_{mod} \tag{1}$$

while for non-polar adsorbents and aquoorganic diluents[14,15,17–19]

$$\log k'_{w \to mod} = \log k'_w - S \varphi_{mod}$$

where a and S are constants ($S = \log k'_w - \log k'_{mod}$), φ denotes the volume fraction ($\varphi = 0.01 \times$ vol. %) and the indices d, mod and w denote respectively a non-polar diluent (e.g., heptane), polar modifier and water. For analogous liquid–liquid chromatography systems the two typical relationships were reported earlier[15,20,21]

The structure of the dabsyl derivatives is represented in Fig. 4. It can be seen that there are three adsorption centres in the molecules: the weakly adsorbed azo group and N,N-dimethylamino group and a strongly polar sulphonamido group. Although the $(N(CH_3)_2$ group can contribute to adsorption, especially at low concentrations of the polar solvent, the main effect is undoubtedly due to the sulphonamido grouping which probably can be attached by three hydrogen bonds to the silanol groups. (In crystalline silica the silanol groups are arranged in a triangular pattern[22]).

The dimethylaminoazobenzene moiety is identical for all compounds in Figs. 1–3. The differences in adsorption affinity are related to the alkyl sulphonamide
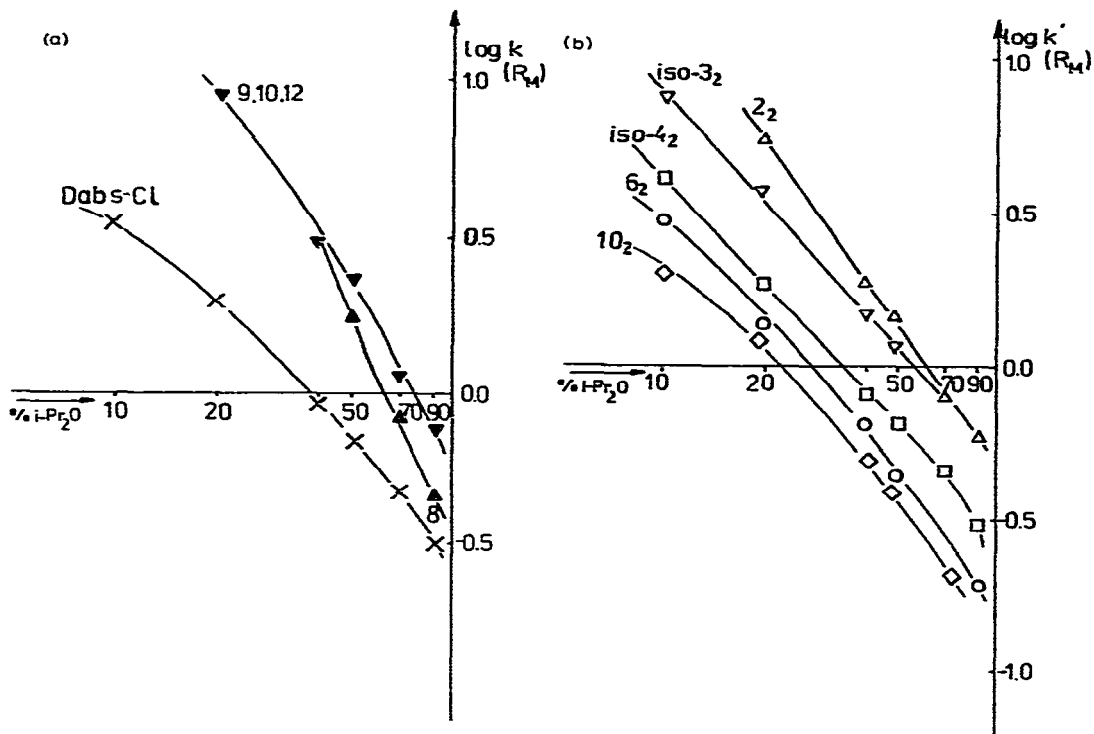
Fig. 3. As in Fig. 1; polar modifier = diisopropyl ether.

grouping. It can be seen (Figs. 1a–3a) that for monoalkyl derivatives the effect of chain length is negligible for the higher homologues investigated (C_8–C_{12}). The plots all fall into one of two types: parabolic for dioxane systems and approximately linear when methylethyl ketone or diisopropyl ether are used as polar modifiers.

For dialkyl derivatives the effect of chain length is more pronounced and selectivity for the homologues is much greater; although the series of dialkylamines derivatized is incomplete, it can be seen that neighbouring homologues can easily be separated. The steric effect of the two alkyl groups is thus much more pronounced than for a single alkyl chain.

Since very poor separation of monoalkyl dabsyl derivatives was obtained when silica was used as adsorbent, in the second series of experiments octadecyl silica was employed; the plates were eluted with water–methanol mixtures. The results are presented in Fig. 5; it can be seen that in accordance with previous reports[14,15,17–19] the plots are linear on the semilogarithmic scale. The monoalkyl homologues are well separated (Fig. 5a) and also for dialkyl derivatives the selectivity is greater than for plain silica, the sequence of spots being reversed as is usual for reversed-phase systems. The results confirm the value of reversed-phase adsorbents for TLC purposes[23].

The estimated detection limit for dabsyl amines on RP-18 silica HPTLC plates was about 25 pmoles (Fig. 6a). The product of hydrolysis of excess of the reagent, dabsyl hydroxide, migrated with the solvent front; another spot of high R_F was due to the product of reaction of dabsyl chloride with methanol.
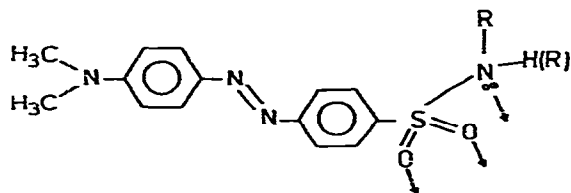
Fig. 4. Structure of dabsyl derivatives: atoms of the main interacting group involved in hydrogen bonding are indicated by arrows.
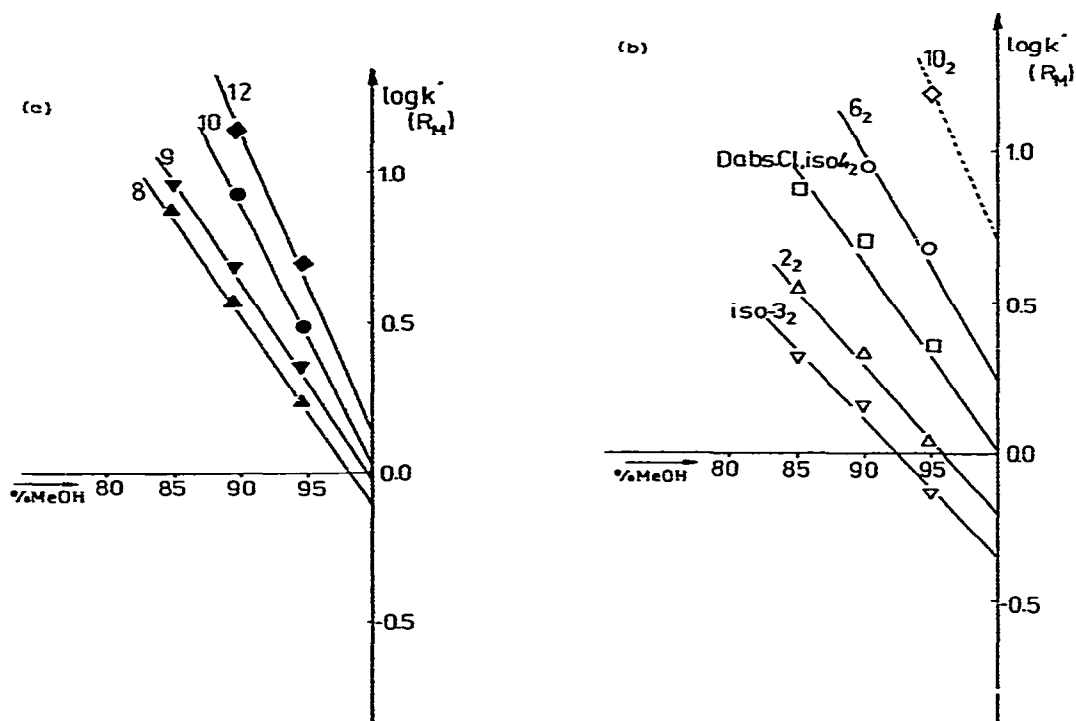

Fig. 5. log k' values of dabsyl derivatives of monoalkyl (a) and dialkyl (b) amines plotted against concentration of methanol (MeOH) in the eluent (second component = water). Adsorbent, ODS-silica.
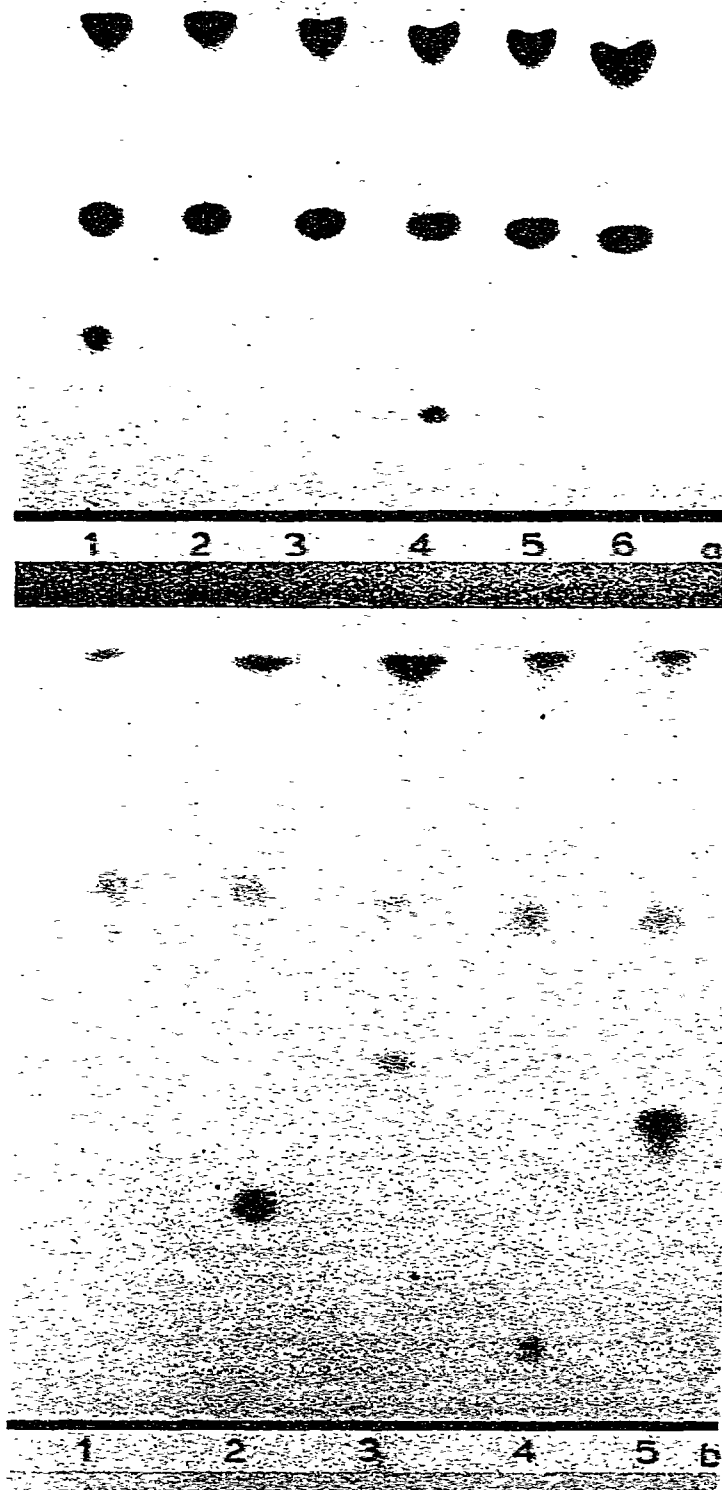
Fig. 6. a, Detection limits for dabsyl derivatives of nonylamine (1–3; 50, 25 and 12.5 pmoles, respectively) and n-decylamine (4–6, as before). b, Selectivity of separation on HPTLC RP-18 plates eluted with methanol–water (19:1). 1 = Dabsyl chloride and methanol; 2 = di-n-hexylamine; 3 = nonylamine; 4 = di-n-decylamine; 5 = n-decylamine.

REFERENCES

1 N. Seiler and M. Wiechmann, in A. Niederwieser and G. Pataki (Editors), *Progress in Thin Layer Chromatography and Related Methods*, Vol. 1, Ann Arbor–Humphrey Sci. Publ., Ann Arbor, London, 1970.
2 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, NY, 2nd ed., 1979, p. 734.
3 J. F. Lawrence and R. W. Frei, *Chemical Derivatization in Liquid Chromatography*, Elsevier, Amsterdam, 1976.
4 J.-K. Lin and J.-Y. Chang, *Anal. Chem.*, 47 (1975) 1634.
5 J. Lammens and M. Verzele, *Chromatographia*, 11 (1978) 376.
6 J. K. Lin and C. H. Wang, *Clin. Chem.*, 26 (1980) 543.
7 J. K. Lin and C. C. Lai, *Anal. Chem.*, 52 (1980) 630.
8 C. C. Lai and J. K. Lin, *CA*, 94 (1981) 585.
9 W. Gołkiewicz and T. Wolski, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 115.
10 H. Szumilo and T. Wolski, *Chem. Anal (Warsaw)*, 26 (1981) 455.
11 J.-K. Lin and C.-C. Lai, *J. Chromatogr.*, 227 (1982) 369.
12 T. Wolski, W. Gołkiewicz and A. Rompala, *Chem. Anal. (Warsaw)*, 25 (1980) 583.
13 E. Soczewiński and T. Wawrzynowicz, *Chromatographia*, 11 (1978) 446.
14 E. Soczewiński and J. Jusiak, *Chromatographia*, 14 (1981) 23.
15 E. Soczewiński, *J. Liquid Chromatogr.*, 3 (1980) 1781.
16 L. R. Snyder and H. Poppe, *J. Chromatogr.*, 184 (1980) 363.
17 L. R. Snyder, J. W. Dolan and J. R. Gant, *J. Chromatogr.* 165 (1979) 3; and refs. 17–34 therein.
18 M. Bieganowska and E. Soczewiński, *J. Chromatogr.*, 205 (1981) 451.
19 W. R. Melander and Cs. Horváth, in Cs. Horváth (Editor), *HPLC —Advances and Perspectives*, Vol. 2, Academic Press, New York, 1980, p. 113.
20 E. Soczewiński and C. A. Wachtmeister, *J. Chromatogr.*, 7 (1962) 311.
21 E. Soczewiński and G. Matysik, *J. Chromatogr.*, 32 (1968) 458.
22 G. E. Berendsen and L. de Galan, *J. Liquid Chromatogr.*, 1 (1978) 403.
23 L. C. Sander, R. L. Sturgeon and L. R. Field, *J. Liquid Chromatogr.*, 4 (Suppl. 1) (1981) 63.