Journal of Chromatography, 386 (1987) 159–172
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1063

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ESTERS OF ALIPHATIC AND AROMATIC CARBOXYLIC ACIDS

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SUMMARY

Chromatographic elution data for 29 aliphatic and aromatic carboxylic acids methyl esters, dicarboxylic acids dimethyl esters and esters of acetic acid with different alcohols have been determined, using silica gel as the stationary phase. Twelve mobile phases containing *n*-heptane and/or diethyl ether, 2-propanol and chloroform at different concentrations were used. The analysis of methyl esters of diamantane and adamantane carboxylic acids under the same conditions is discussed and a survey is given of the chromatographic behaviour of 60 compounds. Among all the compounds tested, esters having two functional groups have a longer retention time than esters having only one $COOCH_3$ group. The effects of molecular structure on the chromatographic behaviour are discussed, namely the length of the alkyl chain of the acids, the length of the alkyl chain of the alcohol and the influence of unsubstituted and substituted aromatic rings. A relationship between the measured compounds and polycyclic derivatives has been established. The effects of mobile phase composition on the elution data for the compounds studied are discussed.

INTRODUCTION

Gas chromatography is used for the analysis of methyl esters of fatty acids in many fields of chemistry and pharmacy and in biomedical materials. The analysis of classes of lipids has been carried out by reversed-phase high-performance liquid chromatography (HPLC)[1,2]. Normal-phase HPLC on silica has not often been used; only fatty acid classes and neutral lipids were separated[3]. The aims of this work were (i) to study the liquid chromatographic behaviour of some methyl esters of aliphatic and aromatic carboxylic acids on silica gel with different mobile phases, based on *n*-heptane in combination with 2-propanol, diethyl ether and chloroform, and (ii) to compare the chromatographic behaviour of these compounds and methyl esters of adamantane and diamantane carboxylic acids analysed previously[4].

0021-9673/87/$03.50

EXPERIMENTAL

Apparatus

A Varian 8500 liquid chromatograph with a syringe pump, connected to refractive index detector and an A 25 dual-channel strip-chart recorder, was used (Varian, Palo Alto, CA, U.S.A.). Sample injection was performed by the stop-flow technique, a 5-μl syringe (Hamilton, Bonaduz, Switzerland) was used. A stainless-steel column (250 × 4 mm I.D.) manufactured in our laboratory was filled by the slurry-packing technique with 7.5-μm irregularly shaped silica gel (Silasorb) (Lachema, Brno, Czechoslovakia).

Reagents

Methyl esters were synthesized from related carboxylic acids (Lachema, Brno, Czechoslovakia) by reaction with a solution of diazomethane in diethyl ether. Acetates were commercial products (Lachema). 2-Propanol (analytical-reagent grade) (Lachema) was used without further treatment. *n*-Heptane (Reakhim, Moscow, U.S.S.R.) and diethyl ether (Lachema) were dried over sodium before use and distilled in glass. Chloroform (analytical-reagent grade) (Lachema) was shaken with a 20% solution of sodium hydroxide and then with distilled water, dried over phosphorus pentoxide and distilled through a glass perforated-plate column with exclusion of moisture.

Mobile phases

The mobile phases were mixed by weight using the degassed components. The

TABLE I

MOBILE PHASE COMPOSITIONS

No.	*Components (%, w/w)*	
1	*n*-Heptane	2-Propanol
a	99.9	0.1
b	99.8	0.2
c	99.7	0.3
d	99.5	0.5
2	*n*-Heptane	Diethyl ether
a	97	3
b	95	5
c	90	10
3	*n*-Heptane–diethyl ether (97:3)	2-Propanol
a	99.95	0.05
b	99.9	0.1
c	99.8	0.2
4	*n*-Heptane–chloroform (80:20)	2-Propanol
a	99.8 (70:30)	0.2
b	99.8	0.2

following mobile phases were used: *n*-heptane–2-propanol, *n*-heptane–diethyl ether, *n*-heptane–diethyl ether–2-propanol and *n*-heptane–chloroform–2-propanol. The compositions are given in Table I.

Procedure

Retention data for esters of aliphatic and aromatic carboxylic acids were determined under the same conditions as for the methyl esters of diamantane and adamantane carboxylic acids[4]. The temperature was 20 ± 1°C and the flow-rate of the mobile phase was 30 ml/h. The column was stabilized prior to measurement by washing with fresh mobile phase for 10 h. The column activity was checked before starting

TABLE II

RETENTION DATA

Mobile phase components: a = *n*-heptane; b = diethyl ether.

Compound	*No. of C atoms*	*Mobile phase components (%)*					
		a = 97, b = 3		*a = 95, b = 5*		*a = 90, b = 10*	
		t_R *(s)*	*k'*	t_R *(s)*	*k'*	t_R *(s)*	*k'*
Methyl formate	2	478	3.80	368	2.70	286	1.87
Methyl acetate	3	727	6.30	507	4.09	338	2.40
Methyl propionate	4	462	3.64	340	2.42	241	1.42
Methyl butyrate	5	420	3.22	305	2.07	218	1.19
Methyl valerate	6	398	2.99	290	1.92	208	1.09
Methyl caproate	7	377	2.78	273	1.74	199	1.00
Methyl enanthate	8	352	2.53	262	1.63	190	0.91
Methyl caprylate	9	345	2.46	256	1.57	188	0.89
Dimethyl malonate	5	–	–	2318	22.28	1095	9.99
Dimethyl succinate	6	–	–	–	–	1210	11.14
Dimethyl glutarate	7	–	–	–	–	1213	11.18
Dimethyl adipate	8	–	–	–	–	1349	12.54
Dimethyl pimelate	9	–	–	–	–	1262	11.67
Dimethyl suberate	10	–	–	–	–	1125	10.29
Dimethyl sebacate	12	–	–	–	–	859	7.63
Ethyl acetate	4	614	5.16	433	3.34	286	1.87
Propyl acetate	5	547	4.49	378	2.80	256	1.57
Butyl acetate	6	502	4.04	349	2.50	225	1.26
Amyl acetate	7	446	3.48	324	2.25	223	1.24
Isoamyl acetate	7	472	3.74	334	2.36	227	1.28
2-Ethyl-1-hexyl acetate	10	364	2.65	263	1.64	190	0.91
Cyclooctyl acetate	10	476	3.78	331	2.33	222	1.23
Methyl benzoate	8	356	2.58	266	1.67	201	1.02
Dimethyl phthalate	10	–	–	2590	25.00	1112	10.17
Dimethyl isophthalate	10	1549	14.55	917	8.20	478	3.80
Dimethyl terephthalate	10	1344	12.49	778	6.81	421	3.23
o-Toluic acid methyl ester	9	286	1.87	224	1.25	177	0.78
p-Toluic acid methyl ester	9	370	2.71	275	1.77	205	1.06
Methyl phenylacetate	9	676	5.78	465	3.67	300	2.01

the measurements, then several times during the analyses, and again after completion of the measurements by injecting a solution of dimethyl diamantane-1,6-dicarboxylate in benzene. The dead volume of the column was determined by measuring the retention time of isooctane. Retention data were determined on chromatograms obtained by injecting solutions of the compounds in benzene.

RESULTS AND DISCUSSION

The value of retention times (t_R) and capacity factors (k') are given in Tables II–V.

TABLE III

RETENTION DATA

Mobile phase components: a = *n*-heptane; b = 2-propanol.

Compound	*No. of C atoms*	*Mobile phase components (%, w/w)*							
		a=99.9, b=0.1		*a=99.8, b=0.2*		*a=99.7, b=0.3*		*a=99.5, b=0.5*	
		t_R	k'	t_R	k'	t_R	k'	t_R	k'
Methyl formate	2	–	–	355	2.56	284	1.85	262	1.63
Methyl acetate	3	–	–	428	3.30	321	2.23	248	1.49
Methyl propionate	4	456	3.58	278	1.79	217	1.17	177	0.78
Methyl butyrate	5	405	3.06	248	1.49	194	0.95	159	0.60
Methyl valerate	6	376	2.77	233	1.34	184	0.84	152	0.52
Methyl caproate	7	–	–	216	1.17	170	0.71	144	0.45
Methyl enanthate	8	297	1.98	206	1.07	167	0.68	139	0.39
Methyl caprylate	9	324	2.25	210	1.11	167	0.67	139	0.40
Dimethyl malonate	5	–	–	2113	20.22	1292	11.97	680	5.83
Dimethyl succinate	6	–	–	2563	24.73	1325	12.30	643	5.45
Dimethyl glutarate	7	–	–	2484	23.94	1309	12.14	603	5.05
Dimethyl adipate	8	–	–	2802	27.13	1369	12.74	588	4.90
Dimethyl pimelate	9	–	–	2455	23.65	1175	10.80	504	4.06
Dimethyl suberate	10	–	–	1934	18.42	956	8.60	432	3.34
Dimethyl sebacate	12	–	–	1669	15.76	712	6.15	344	2.46
Ethyl acetate	4	600	5.02	354	2.55	257	1.58	203	1.04
Propyl acetate	5	455	3.57	289	1.90	223	1.23	173	0.73
Butyl acetate	6	407	3.08	268	1.69	207	1.08	162	0.63
Amyl acetate	7	365	2.66	247	1.48	190	0.90	152	0.53
Isoamyl acetate	7	386	2.88	251	1.52	194	0.94	156	0.56
2-Ethyl-1-hexyl acetate	10	307	2.08	212	1.13	167	0.68	138	0.39
Cyclooctyl acetate	10	390	2.92	257	1.58	193	0.94	153	0.54
Methyl benzoate	8	295	1.96	206	1.07	169	0.70	143	0.43
Dimethyl phthalate	10	–	–	1754	16.61	939	8.43	465	3.67
Dimethyl isophthalate	10	–	–	805	7.08	329	2.30	221	1.22
Dimethyl terephthalate	10	–	–	482	3.84	308	2.09	205	1.06
o-Toluic acid methyl ester	9	257	1.58	180	0.81	152	0.52	131	0.31
p-Toluic acid methyl ester	9	325	2.26	217	1.17	174	0.75	144	0.44
Methyl phenylacetate	9	–	–	319	2.84	235	1.36	179	0.80

As in the previous paper[4], the main factors affecting adsorption of the investigated esters and diesters are discussed: (1) the number of $COOCH_3$ groups; (2) the number of carbon atoms; (3) the position of $COOCH_3$ groups on the aromatic ring; and (4) the substitution of the alkyl group.

Effect of the number of $COOCH_3$ groups

For the 29 esters analysed, it was found that under all conditions all diesters have longer retention times than any of the monoesters. Including the methyl esters of diamantane and adamantane analysed previously[4] under the same conditions, the diesters with functional groups close to bulky substituents have shorter retention

TABLE IV

RETENTION DATA

Mobile phase components: a = *n*-heptane–diethyl ether (97:3); b = 2-propanol.

Compound	*No. of C atoms*	*Mobile phase components (%, w/w)*					
		a = 99.95, b = 0.05		*a = 99.9, b = 0.1*		*a = 99.8, b = 0.2*	
		t_R	k'	t_R	k'	t_R	k'
Methyl formate	2	361	2.62	302	2.03	249	1.50
Methyl acetate	3	506	4.08	388	2.90	292	1.93
Methyl propionate	4	339	2.41	273	1.74	213	1.14
Methyl butyrate	5	304	2.05	242	1.43	191	0.92
Methyl valerate	6	284	1.86	227	1.28	181	0.82
Methyl caproate	7	269	1.70	218	1.19	174	0.75
Methyl enanthate	8	257	1.58	207	1.08	168	0.69
Methyl caprylate	9	250	1.51	203	1.04	164	0.65
Dimethyl malonate	5	–	–	1328	12.34	764	6.67
Dimethyl succinate	6	–	–	1404	13.10	816	7.20
Dimethyl glutarate	7	–	–	1424	13.30	822	7.25
Dimethyl adipate	8	–	–	1494	14.00	841	7.44
Dimethyl pimelate	9	–	–	1645	15.52	794	6.97
Dimethyl suberate	10	–	–	1470	13.76	715	6.17
Dimethyl sebacate	12	–	–	1086	9.90	573	4.75
Ethyl acetate	4	433	3.34	321	2.22	245	1.46
Propyl acetate	5	378	2.80	290	1.91	219	1.20
Butyl acetate	6	351	2.52	265	1.66	204	1.05
Amyl acetate	7	325	2.27	249	1.50	193	0.94
Isoamyl acetate	7	317	2.19	248	1.49	192	0.93
2-Ethyl-1-hexyl acetate	10	265	1.66	207	1.08	166	0.67
Cyclooctyl acetate	10	330	2.31	251	1.52	192	0.92
Methyl benzoate	8	258	1.59	207	1.08	171	0.72
Dimethyl phthalate	10	2394	23.04	1354	12.59	721	6.24
Dimethyl isophthalate	10	855	7.58	527	4.30	332	2.34
Dimethyl terephthalate	10	730	6.33	452	3.54	298	1.99
o-Toluic acid methyl ester	9	217	1.18	181	0.82	152	0.53
p-Toluic acid methyl ester	9	265	1.66	214	1.14	171	0.72
Methyl phenylacetate	9	440	3.42	316	2.17	233	1.34

TABLE V

RETENTION DATA

Mobile phases components: mixture of 99.8% of (a) *n*-heptane–(b) chloroform and 0.2% of 2-propanol.

Compound	*No. of C atoms*	*Mobile phase components (%, w/w)*			
		a = 80, b = 20		*a = 70, b = 30*	
		t_R	k'	t_R	k'
Methyl formate	2	274	1.75	286	1.87
Methyl acetate	3	311	2.12	320	2.22
Methyl propionate	4	232	1.33	253	1.54
Methyl butyrate	5	201	1.02	225	1.26
Methyl valerate	6	191	0.92	211	1.11
Methyl caproate	7	174	0.75	189	0.90
Methyl enanthate	8	166	0.67	178	0.78
Methyl caprylate	9	163	0.64	178	0.78
Dimethyl malonate	5	1154	10.58	1247	11.52
Dimethyl succinate	6	1314	12.19	1438	13.43
Dimethyl glutarate	7	1304	12.10	1562	14.69
Dimethyl adipate	8	1398	13.04	1574	14.81
Dimethyl pimelate	9	1151	10.55	1420	13.25
Dimethyl suberate	10	1046	9.50	1180	10.84
Dimethyl sebacate	12	786	6.89	878	7.82
Ethyl acetate	4	264	1.65	296	1.98
Propyl acetate	5	224	1.25	248	1.49
Butyl acctate	6	213	1.14	226	1.27
Amyl acetate	7	191	0.92	194	0.95
Isoamyl acetate	7	187	0.88	199	0.99
2-Ethyl-1-hexyl acetate	10	172	0.73	173	0.74
Cyclooctyl acetate	10	203	1.04	199	0.99
Methyl benzoate	8	179	0.80	187	0.88
Dimethyl phthalate	10	956	8.60	998	9.02
Dimethyl isophthalate	10	358	2.59	385	2.86
Dimethyl terephthalate	10	323	2.24	365	2.66
o-Toluic acid methyl ester	9	158	0.59	169	0.69
p-Toluic acid methyl ester	9	185	0.86	193	0.93
Methyl phenylacetate	9	242	1.43	235	1.36

times than basic methyl esters with a minimum number of carbon atoms. Depending on the mobile phase compositions, methyl formate, acetate, and eventually propionate and butyrate have longer retention times than the least retained dimethyladamantyl malonates.

Effect of the number of carbon atoms

In general, the retention times of the methyl esters of aliphatic carboxylic acids decrease with increasing chain length. The first member of this group, methyl formate, is an exception, its retention time being shorter than that of methyl acetate. This behaviour is approximately the same for all mobile phases tested. The relation-

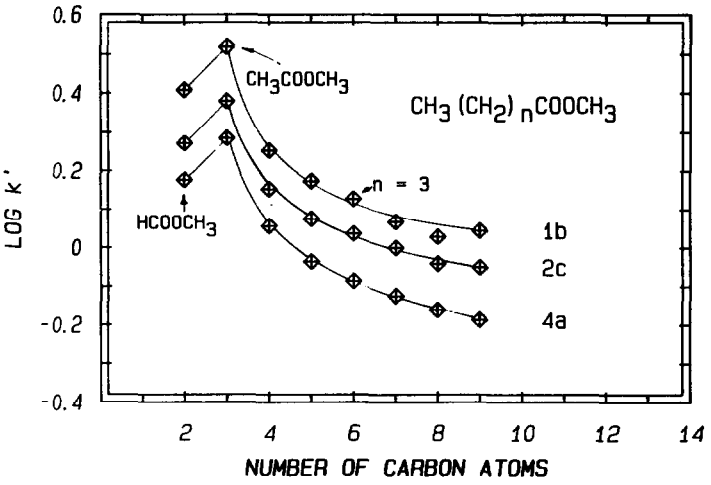


Fig. 1. Plot of log k' *vs.* number of carbon atoms for methyl esters of aliphatic acids. Mobile phases: see Table I.

ship between log k' and the number of carbon atoms in methyl carboxylates of aliphatic acids with three different mobile phases is shown in Fig. 1.

As the alkyl chain of the alcohols in the acetates becomes longer, the retention time decreases. This relationship is shown in Fig. 2 for four different mobile phases.

The different effects of increasing the number of carbon atoms on the chromatographic behaviour of both methyl esters of aliphatic acids and acetates are shown in Fig. 3. A change in the number of carbon atoms in diesters of aliphatic acids does not influence the chromatographic behaviour very much. When mobile phases of lower elution strength are used, the compound with eight carbon atoms (dimethyl adipate) has the longest elution time. Compounds with a larger number of

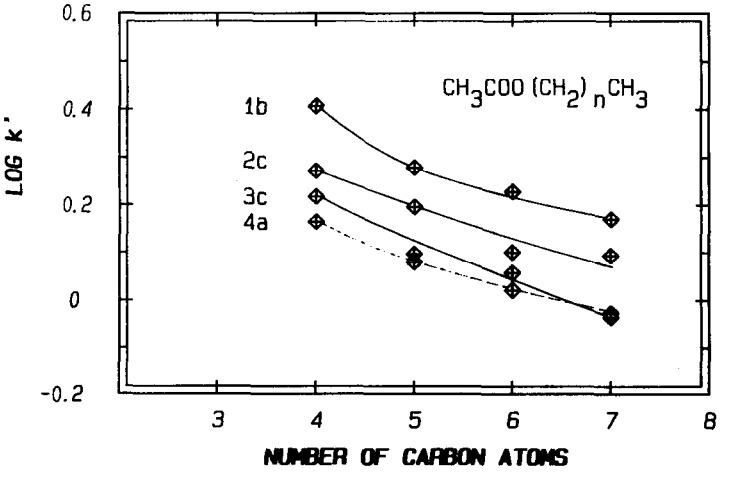


Fig. 2. Plot of log k' *vs.* number of carbon atoms for acetates. Mobile phases: see Table I.

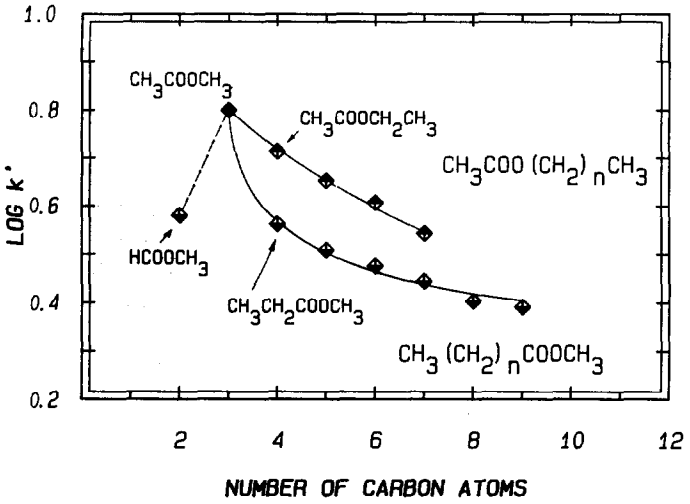


Fig. 3. Comparison of log *k'* *vs.* number of carbon atoms for methyl esters of aliphatic acids and acetates. Mobile phase: *n*-heptane–diethyl ether (97:3), w/w).

carbon atoms have shorter retention times owing to the elongation of the aliphatic chain, similar to the monomethyl esters. The reduction in retention times in dimethyl esters with a smaller number of carbon atoms is due to the fact that dimethyl esters are not able to occupy the ideal conformation of both functional groups of the molecule for adsorption. When using the mobile phases *n*-heptane–diethyl ether and *n*-heptane–diethyl ether–2-propanol, certain changes occur (see Fig. 4).

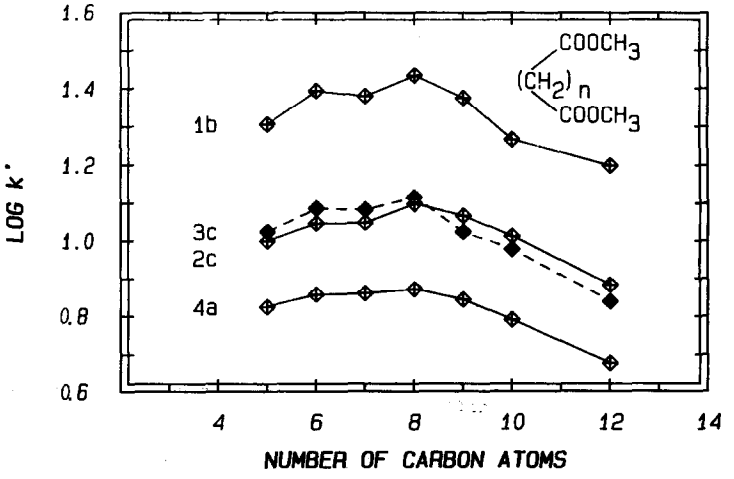


Fig. 4. Plot of log *k'* *vs.* number of carbon atoms for dimethyl esters of aliphatic acids. Mobile phases: see Table I.

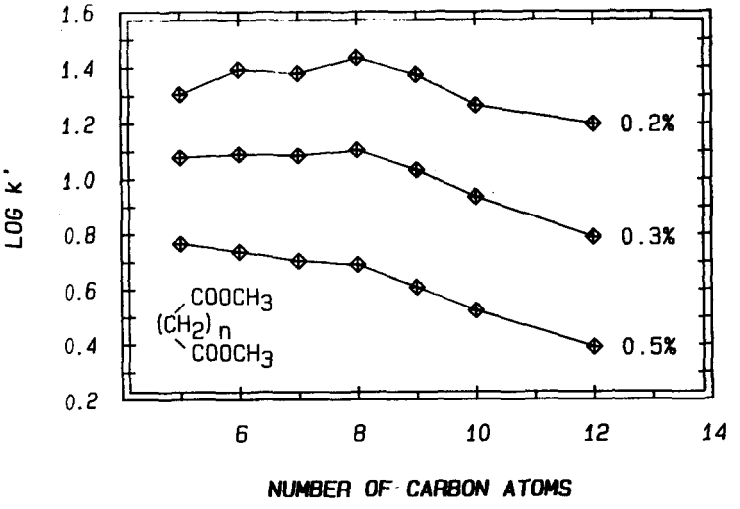
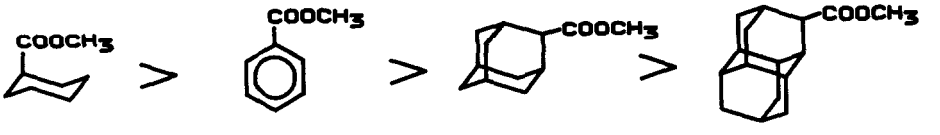


Fig. 5. Change of log k' *vs.* number of carbon atoms with concentration of 2-propanol in the *n*-heptane–2-propanol mobile phase.

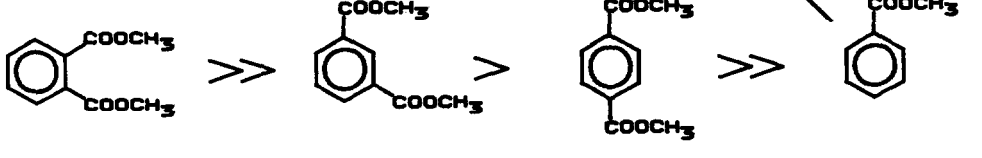
With increase in the elution strength of the mobile phase, the discussed dependence of log k' on the number of carbon atoms is changed. For the mobile phase with the highest elution strength the value of log k' decreases monotonously with increasing number of carbon atoms (see Fig. 5).

Although the influence of the carbon skeleton, *i.e.* aromatic ring and polycyclic molecule on the chromatographic behaviour of methyl esters is slightly different, methyl benzoate can be located between methylcyclohexyl carboxylate and methyl adamantane-2-carboxylate:



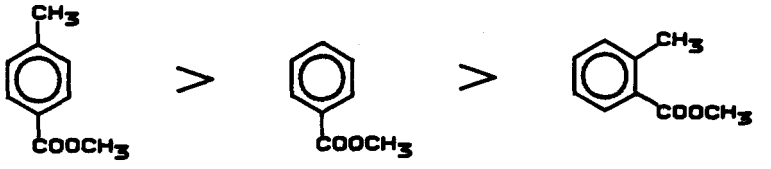
Position of $COOCH_3$ groups on the aromatic ring

Among dimethyl phthalates, a compound with two functional groups, allowing the strongest simultaneous adsorption of both groups, *i.e.* dimethyl phthalate, has by far the longest retention time. The retention times of dimethyl isophthalate and dimethyl terephthalate are diminished with increasing distance between two functional groups in the following order:



Substitution of the alkyl group

The introduction of an alkyl group into methyl esters of polycyclic carboxylates decreased the retention time compared with that of the parent molecule[4]. On the other hand, the introduction of a methyl group into methyl benzoate in the *para* position increases the retention time relative to that of the parent molecule owing to the inductive effect of this methyl group. Introduction into the *ortho* position decreases the retention time as a result of steric hindrance. The methyl esters of *o*- and *p*-toluic acids are eluted in the following order compared with methyl benzoate:



Effect of mobile phase composition

When mobile phases based on *n*-heptane and/or 2-propanol and diethyl ether are used, *i.e.*, *n*-heptane–2-propanol, *n*-heptane-diethyl ether and *n*-heptane–diethyl ether–2-propanol, the change in the capacity factor (k') with change in the elution strength of the mobile phase can be described by the equation[5]

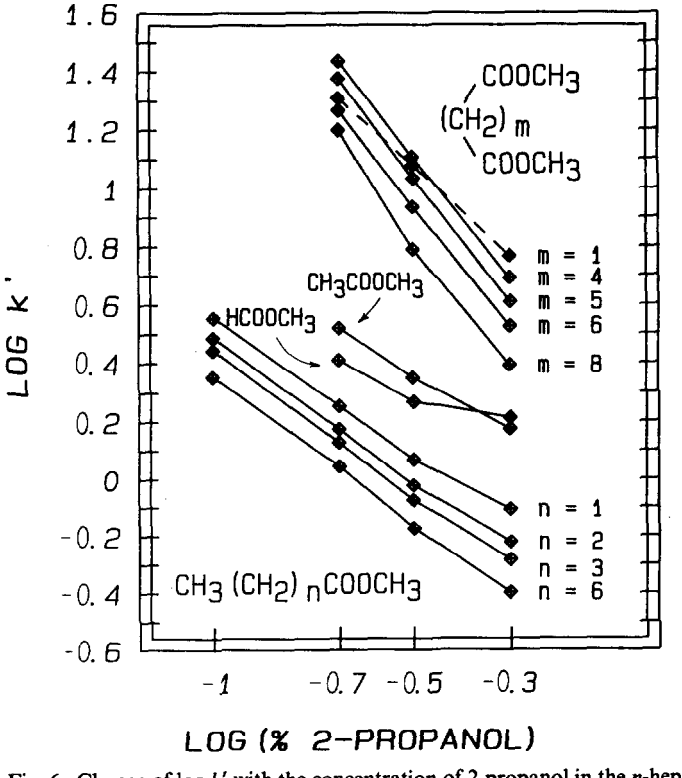


Fig. 6. Change of log k' with the concentration of 2-propanol in the *n*-heptane–2-propanol mobile phase.

$$\log k' = A - n \log c \quad (1)$$

where c is concentration of the more polar component in the two-component mobile phases or the most polar component (2-propanol) in the three-component mobile phase and n and A are constants. Fig. 6 illustrates the relationship between log k' and the log (concentration of 2-propanol in n-heptane). There are linear dependences for aliphatic monomethyl carboxylates and dimethyl dicarboxylates. Exceptional behaviour was observed for methyl formate. The lines expressing the dependence for dimethyl dicarboxylates with a larger number of methylene groups are steeper than those for monomethyl esters. However, dimethyl dicarboxylates with a shorter aliphatic chain do not show such a steep dependence. Acetates have similar dependences of log k' on log (concentration of 2-propanol), such as the aliphatic monomethyl carboxylates. Fig. 7 shows similar dependences for aromatic mono- and dimethyl esters. The majority of compounds show linear dependences in such a way that for compounds with a larger retention the dependence is steeper. Dimethyl isophthalate and methyl phenyl acetate are exceptions, the dependence of log k' on log (2-propanol concentration) not being linear. In contrast to that the behaviour observed with the aforementioned mobile phases, when an increase in concentration of the more polar component of the mobile phase led to a decrease in retention, changes in the chlo-

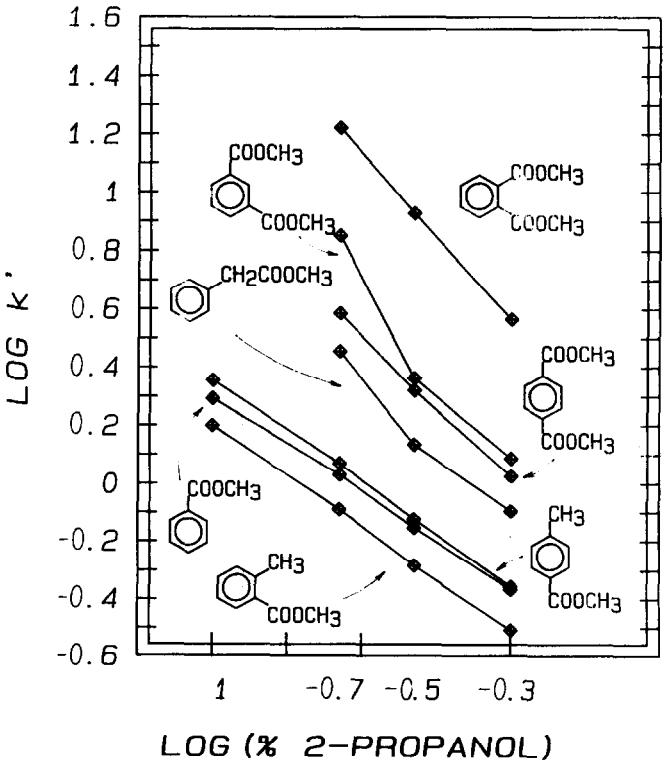


Fig. 7. Change of log k' with the concentration of 2-propanol in the n-heptane–2-propanol mobile phase.

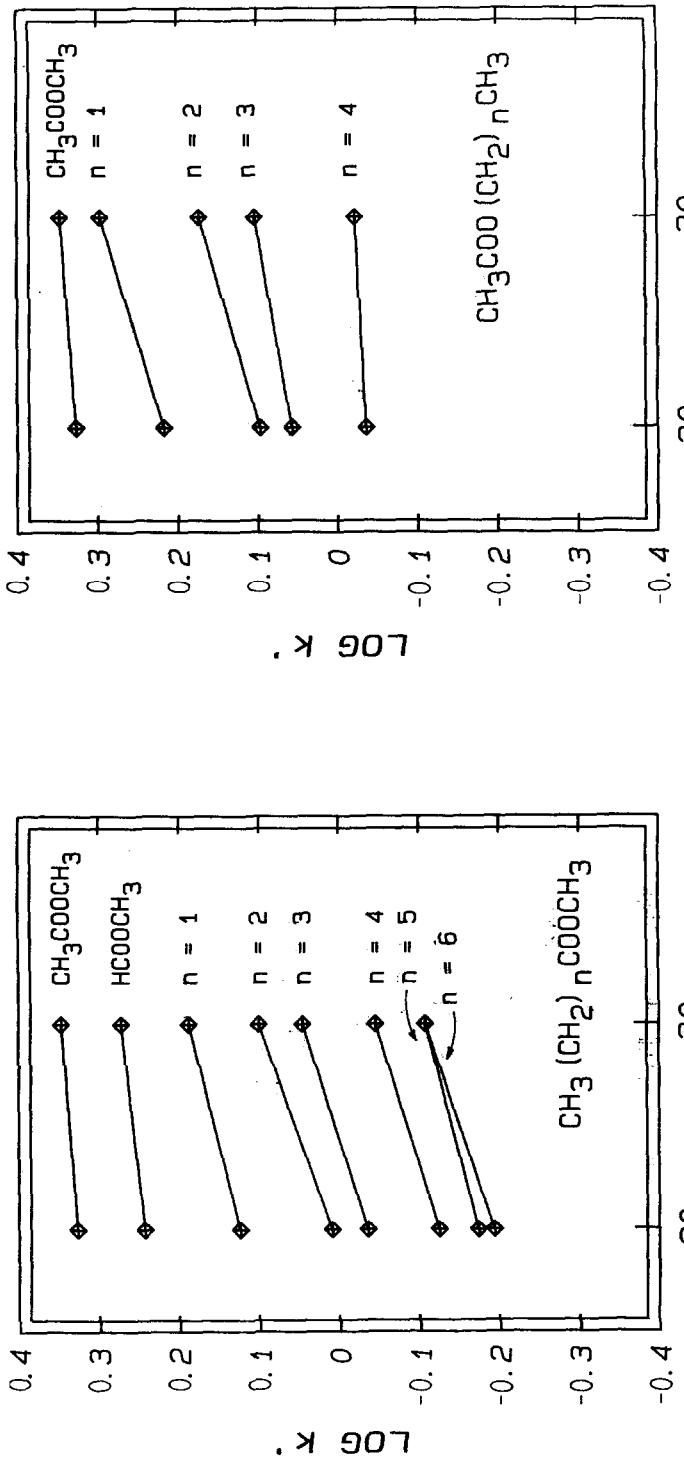


Fig. 8. Change of log k' with the concentration of chloroform in the *n*-heptane–chloroform–2-propanol mobile phase for methyl esters of aliphatic acids.

Fig. 9. Change of log k' with the concentration of chloroform in the *n*-heptane–chloroform–2-propanol mobile phase for acetates.

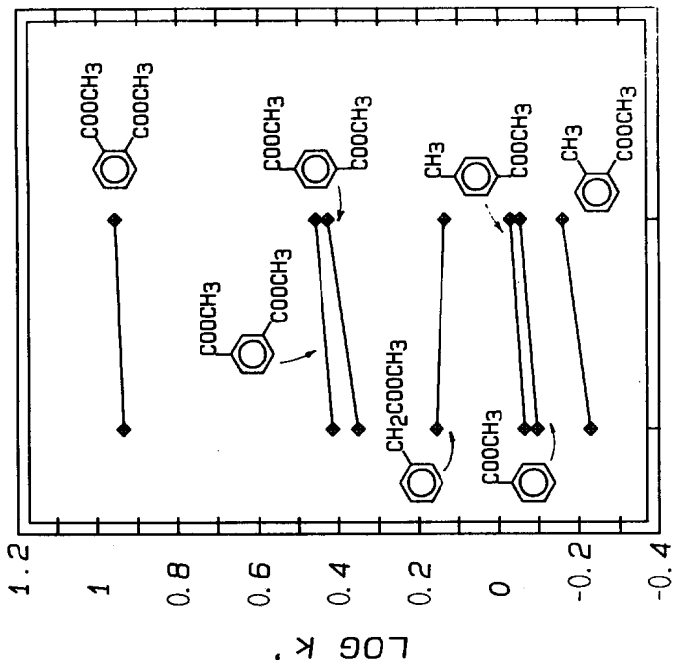


Fig. 10. Change of log k' with the concentration of chloroform in the *n*-heptane–chloroform–2-propanol mobile phase for dimethyl esters of aliphatic acids.

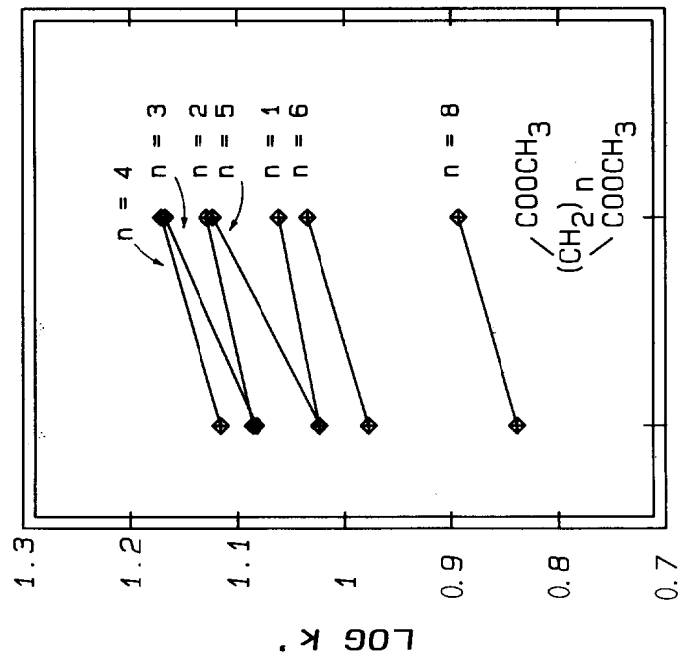


Fig. 11. Change of log k' with the concentration of chloroform in the *n*-heptane–chloroform–2-propanol mobile phase for esters of aromatic acids.

roform concentration in the mobile phase have a different effect on the retention of the compounds analysed.

Fig. 8 shows the dependence of log k' on the concentration of chloroform in the mobile phase for aliphatic methyl esters. The same dependence for acetates is shown in Fig. 9. The value of $\Delta(\log k')/\Delta$(concentration) decreases with increasing retention time for aliphatic monomethyl esters. On the other hand, for the acetates this value increases. An exception is methyl acetate. The same dependence for dimethyl dicarboxylates can be seen in Fig. 10. For compounds with an even number of methylene groups in the molecule and for malonic acid the value of $\Delta(\log k')/\Delta$(concentration) is lower than that for compounds with an odd number of methylene groups. Finally, the values of $\Delta(\log k')/\Delta$(concentration) are the smallest or negative for aromatic mono- and dimethyl esters. Fig. 11 shows the dependence of log k' on chloroform concentration in the mobile phase for the last mentioned compounds.

There are changes in the elution order of some aromatic and polycyclic esters as a result of increases in elution strength. When *n*-heptane–2-propanol containing 0.1% of propanol is used as a mobile phase, the elution time of methyl benzoate is shorter than that of methyl adamantane-1-carboxylate. When the concentration of 2-propanol is raised to 0.3%, the retention times become equal. Finally, the retention time of methyl benzoate becomes longer when 0.5% 2-propanol in *n*-heptane is used.

REFERENCES

1 M. I. Aveldano, M. Van Rollins and L. A. Horrocks, *J. Lipid Res.*, 24 (1983) 83.
2 C. R. Scholfield, *J. Am. Oil Chem. Soc.*, 52 (1975) 36.
3 J. G. Hamilton and K. Comai, *J. Lipid Res.*, 25 (1984) 1142.
4 J. Kříž, L. Vodička and J. Burda, *J. Chromatogr.*, 352 (1986) 427.
5 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 207.