

CHROM. 14,881

HIGH-BACK-PRESSURE LIQUID CHROMATOGRAPHY

IV. STUDIES ON THE RETENTION BEHAVIOUR OF AROMATIC COMPOUNDS

TOYOHIDE TAKEUCHI* and DAIDO ISHII

Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya-shi, 464 (Japan)

(Received March 8th, 1982)

SUMMARY

The retention behaviour of solutes on various stationary phases was studied by micro-high-performance liquid chromatography using liquefied solvents as the mobile phase. An octadecylsilylated stationary phase discriminates between coplanar and non-planar aromatic hydrocarbons in the normal-phase system using isobutane as the mobile phase. The retention of non-planar aromatic hydrocarbons was smaller than that of coplanar compounds, whereas the retention of aromatic compounds was generally dependent on their ring number in the mode employing dimethyl ether as the mobile phase and porous polystyrene polymer as the stationary phase. Binary solvent elution was examined for the separation of polar compounds using aromatic amines and phthalates as test solutes.

INTRODUCTION

Micro-scale liquid chromatography (LC) has given rise to some interest in high-performance LC (HPLC) as it not only saves expensive stationary and mobile phases but also facilitates direct coupling of LC and mass spectrometry. Micro-HPLC, in which 0.25–0.50 mm bore packed columns are used, has also been investigated in the last 5 years. The flow-rates employed in micro-HPLC are very small (1–10 $\mu\text{l}/\text{min}$), leading to the possibility of using low-boiling solvents as the mobile phase¹ in addition to the above advantages.

In previous work¹, lower alkanes such as butane and propane were successfully employed as the mobile phase in micro-HPLC. The whole chromatograph (including the pumping, injection, column and detection parts) was kept at a pressure higher than the vapour pressure of a particular substance by operating the back-pressure pump, consisting of a gas-tight syringe and a weight. When porous silica gel is used as the stationary phase, the retention of polynuclear aromatic hydrocarbons (PAHs) increases with a decrease in the carbon number of alkanes, leading to an increase in the resolution of solutes. These liquefied solvents have low viscosities, which results in the attainment of higher column efficiencies.

The above technique has been applied to open-tubular micro-capillary LC². Numbers of theoretical plates greater than 100,000 were attained on wall-coated columns within reasonable times, owing to the low viscosity of liquefied alkanes.

This paper describes additional work on mobile and stationary phases for high-back-pressure LC and also considers the retention behaviour of solutes.

EXPERIMENTAL

The apparatus was the same as in previous work¹ except in the experiment using the binary solvent mobile phase. The apparatus was assembled from a pump, a guard column, a micro-valve injector, a separation column, a UV detector, a back-pressure pump and a gas cylinder.

Two flow pumps were employed for binary solvent elution, as shown in Fig. 1. Each gas was individually liquefied and mixed through a home-made T-piece consisting of stainless-steel tubing. The mixing ratio of two solvents was varied by changing the flow-rates of two pumps. Flow-rates could be selected stepwise.

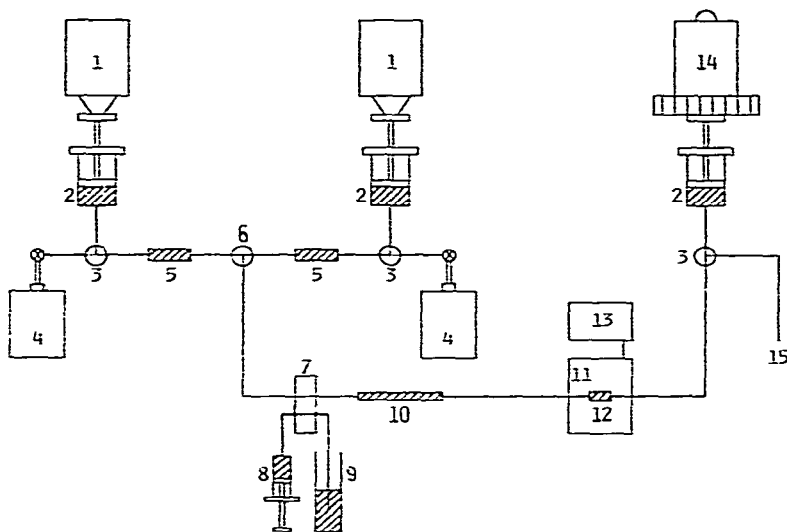


Fig. 1. Diagram of apparatus. 1, Micro Feeder; 2, gas-tight syringe; 3, three-way valve; 4, gas cylinder; 5, guard column (packed with silica gel); 6, T-piece; 7, micro-valve injector; 8, sample; 9, waste reservoir; 10, separation column; 11, UV detector; 12, micro flow cell; 13, recorder; 14, weight; 15, drain.

The packings examined and their characteristics are listed in Table I. All of the packings except TSK-GEL G 1000H were slurried in acetonitrile and packed into a fused silica tube (*ca.* 10 cm × *ca.* 0.25 mm I.D.) as in previous work³. After the packing, dichloromethane and then *n*-hexane were passed through the prepared columns in order to condition them before use. TSK-GEL G 1000H was slurried in diethyl ether and packed by the same method. Fused silica tubing was selected as the column material as higher column efficiencies were expected to be obtained, according to the results of previous work^{3,4}.

PAHs, phthalates and aromatic amines were selected as test solutes. The struc-

TABLE I
PACKINGS AND THEIR CHARACTERISTICS

Packing	Supplier	Functional group	Particle diameter (μm)	Shape*
Develosil 100-5	Nomura Chemical	Silica gel	5	S
Spherisorb 5S	Phase Separations	Silica gel	5	S
SS-05	JASCO	Silica gel	5	S
Nucleosil 10NH ₂	Machery, Nagel & Co.	Aminoalkylsilyl	10	S
LiChrosorb NH ₂	Merck	Aminoalkylsilyl	10	I
SN-01	JASCO	Aminoalkylsilyl	10	I
Develosil Phenyl	Nomura Chemical	Phenylsilyl	7	S
JASCO Phenyl	JASCO	Phenylethylsilyl	5	S
Nucleosil Phenyl	Machery, Nagel & Co.	Phenylethylsilyl	7	S
Develosil ODS	Nomura Chemical	Octadecylsilyl	5	S
SC-01	JASCO	Octadecylsilyl	5	I
LiChrosorb RP-18	Merck	Octadecylsilyl	5	I
Spherisorb 10R	Phase Separations	Octadecylsilyl	10	S
Develosil TMS	Nomura Chemical	Trimethylsilyl	7	S
Develosil C ₈	Nomura Chemical	Octylsilyl	5	S
TSK-GEL G 1000H	Toyo Soda	Styrene-divinyl-benzene	5	S

* S = spherical, I = irregular.

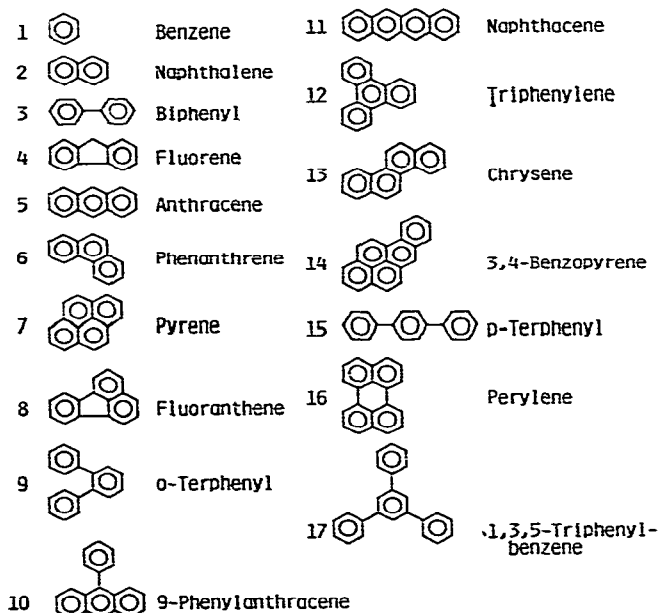


Fig. 2. Structures of PAHs used.

tures of the PAHs used are illustrated in Fig. 2. Standard gases were purchased from Tokyo Chemical Industry (Tokyo, Japan). The boiling points of isobutane, *n*-butane, *cis*-2-butene and dimethyl ether are -11.6 , -0.5 , $+3.7$ and -24.8°C , respectively. Other reagents were purchased from Tokyo Chemical Industry or Wako (Osaka, Japan).

All experiments were carried out at ambient temperature (20 – 25°C).

RESULTS AND DISCUSSION

Silica gel and modified silica gel packings were examined in the normal-phase mode using isobutane as the mobile phase. It has been reported that unreacted or unshielded silanol groups exist on the surface-modified silica gel and work as active adsorptive sites in normal-phase LC⁵⁻⁹. Accessible unreacted or unshielded silanol groups increase with increasing molecular cross-sectional area of the modifier⁸.

Table II lists the retentions of PAHs on silica gel and modified silica gel packings using isobutane as the mobile phase. Capacity factors, k' , were calculated assuming that isooctane was unretained on each column. Silica gel (Si) columns retain PAHs more than the other columns examined. PAHs are also retained on octadecylsilyl (ODS), phenylsilyl (P), phenylethylsilyl (PE) and aminoalkylsilyl (NH_2) columns, but they are not retained on trimethylsilyl (TMS) and octylsilyl (C_8) columns. Although the details of the structures of the packings are not clear, the above dif-

TABLE II
RETENTION OF PAHs

Mobile phase: isobutane.

Column	k'							
	1*	2*	3*	5*	7*	13*	14*	17*
Develosil 100-5	0.59	1.48	2.51	3.38	4.12	8.47	9.91	20.1
Spherisorb 5S	0.50	1.29	2.24	3.19	3.91	8.32	9.84	20.3
SS-05	1.11	3.22	6.68	8.74	10.4	25.3	29.8	> 56
Nucleosil NH_2	0.18	0.77	0.95	2.30	3.72	7.51	11.2	6.03
LiChrosorb 10 NH_2	0.19	0.67	0.83	1.86	2.78	5.33	7.39	4.74
SN-01	0.20	0.77	0.91	2.12	3.18	6.38	9.05	5.15
Develosil Phenyl	0.07	0.16	0.18	0.30	0.38	0.49	0.64	0.43
JASCO Phenyl	0.10	0.20	0.25	0.39	0.53	0.70	0.97	0.62
Nucleosil Phenyl	0.25	0.65	0.87	1.49	2.10	3.49	4.69	4.21
Develosil ODS	0.09	0.15	0.15	0.39	0.61	0.86	1.50	0.26
SC-01	0.10	0.21	0.21	0.62	1.01	1.82	3.61	0.29
LiChrosorb RP-18	0.10	0.27	0.27	0.67	1.05	1.84	3.52	0.39
Spherisorb 10R	0.26	0.55	0.64	1.18	1.76	2.90	4.39	2.22
Develosil TMS	0	0	0	0	0	0	0	0
Develosil C_8	0	0	0	0	0	0	0	0

* The numbers refer to the compounds in Fig. 2.

ference is due to the presence or absence of silanol groups on the modified surface. The retention of PAHs is strongly dependent on residual silanol groups. A high coverage shields the underlying silica gel from any appreciable interaction with PAHs for the short-chain bonded phases, TMS and C₈, leading to non-retention. Thus, bonded short-chain alkyl groups in TMS and C₈ do not interact with the solutes. These results agree with those reported previously⁵⁻⁹.

Typical chromatograms are shown in Fig. 3. The difference in selectivity is appreciable.

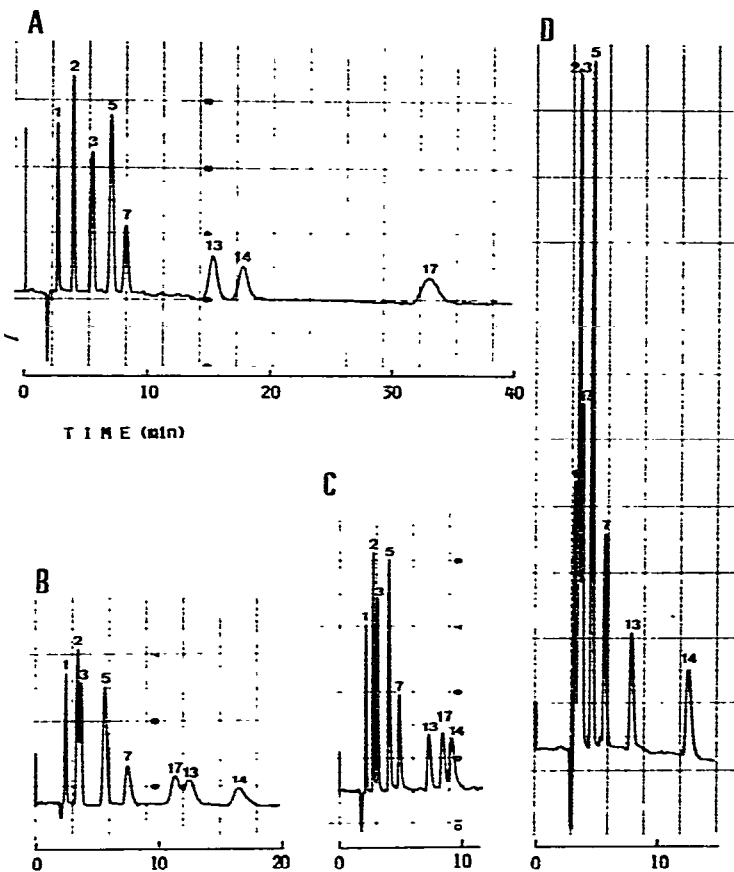


Fig. 3. Separations of PAHs on silica gel and modified silica gel columns. Columns: (A) Spherisorb 5S; (B) LiChrosorb 10NH₂; (C) Nucleosil Phenyl; (D) SC-01 (each 10 cm × 0.25 mm I.D.). Mobile phase: isobutane. Flow-rates: 2.8 μ l/min for (A), (B) and (C); 1.4 μ l/min for (D). Sample: the numbers refer to compounds in Fig. 2. Wavelength of UV detection: 254 nm.

The retention order of PAHs for each column is shown in Table III. The elution order is dependent on the modifier and nearly the same selectivity is observed for similar columns. The selectivity differs greatly between Si and ODS columns.

Figs. 4-6 show plots of $\log k'$ on a P column *versus* $\log k'$ on an Si column, $\log k'$ on an ODS column *versus* $\log k'$ on an Si column and $\log k'$ on an ODS column

TABLE III
ELUTION ORDER OF PAHs

Mobile phase: isobutane.

Column	Elution order							
	1*	2*	3*	5*	7*	13*	14*	17*
Develosil 100-5	1	2	3	4	5	6	7	8
Spherisorb 5S	1	2	3	4	5	6	7	8
SS-05	1	2	3	4	5	6	7	8
Nucleosil NH ₂	1	2	3	4	5	7	8	6
LiChrosorb 10NH ₂	1	2	3	4	5	7	8	6
SN-01	1	2	3	4	5	7	8	6
Develosil Phenyl	1	2	3	4	5	7	8	6
JASCO Phenyl	1	2	3	4	5	7	8	6
Nucleosil Phenyl	1	2	3	4	5	6	8	7
Develosil ODS	1	2	2	5	6	7	8	4
SC-01	1	2	2	5	6	7	8	4
LiChrosorb RP-18	1	2	2	5	6	7	8	4
Spherisorb 10R	1	2	3	4	5	7	8	6

* The numbers refer to the compounds in Fig. 2.

versus $\log k'$ on a P column, respectively. The retention of each solute on P and ODS columns is smaller than on an Si column. It is clear that the retention of rotatable solutes relative to coplanar solutes on an ODS column is smaller than that on P and Si columns. In order to illustrate this, the retentions of further solutes on ODS and Si columns were measured and the results are shown in Fig. 7. Plots of the retentions of rigid coplanar solutes lie on a straight line, whereas plots of all the rotatable solutes deviate from the line. For *o*-terphenyl and 1,3,5-triphenylbenzene with great steric

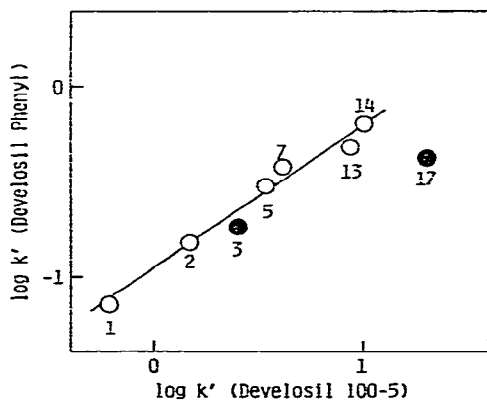


Fig. 4. $\log k'$ on a P column versus $\log k'$ on an Si column. Mobile phase: isobutane. Sample: the numbers refer to compounds in Fig. 2.

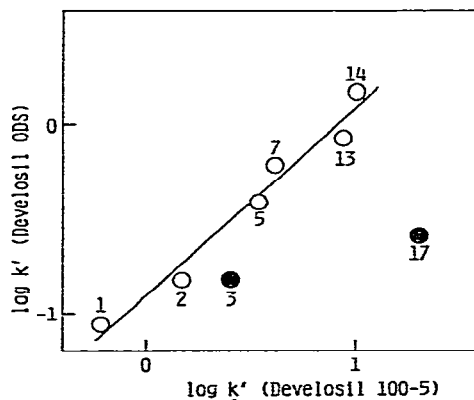


Fig. 5. $\log k'$ on an ODS column versus $\log k'$ on an Si column. Operating conditions as in Fig. 4.

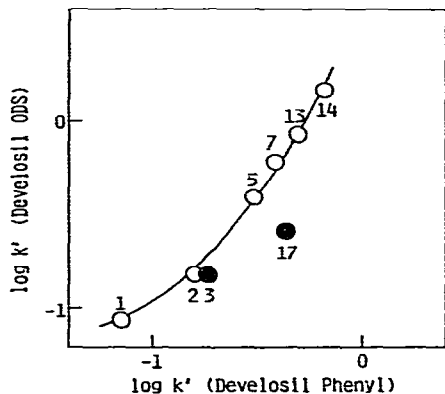


Fig. 6. $\log k'$ on an ODS column versus $\log k'$ on a P column. Operating conditions as in Fig. 4.

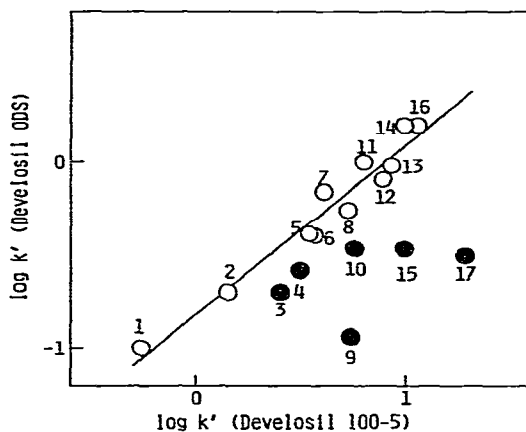


Fig. 7. $\log k'$ on an ODS column versus $\log k'$ on an Si column for seventeen PAHs. Operating conditions as in Fig. 4.

hindrance, the plots deviate considerably from the straight line. The magnitude of the deviation from the straight line can indicate the degree of deviation from the plane of the solutes. It should be noted that fluorene, with sp^3 hybrid-orbital hydrogen atoms, is discriminated from coplanar solutes. Therefore, the octadecyl groups prevent the non-planar solutes from reaching and interacting with the underlying silica gel surface and act only as a surface deactivator on coplanar solutes.

Some results on the interaction between solutes and ODS stationary phase have been reported. Kikta and Grushka⁵ examined the retention behaviour of phenones on modified Corasil columns in the normal-phase system. They found that as the length of alkyl side-chain increases, k' decreases. The decrease in k' may be due to the fact that the bulkier phenones are sterically hindered from interacting with the underlying silanol groups on the Corasil surface.

In the reversed-phase mode, Tanaka *et al.*¹⁰ reported that rigid planar aromatic compounds are preferentially retained by ODS stationary phase in methanol as the mobile phase.

Further examination of the interaction of solutes with other modified silica gel packings has not been carried out.

cis-2-Butene was examined as the mobile phase. The solvent strength of *cis*-2-butene is so great that PAHs were retained on the Si column only slightly. Fig. 8 demonstrates separations of *cis*- and *trans*-stilbene on a Spherisorb SS column employing isobutane, *n*-butane and *cis*-2-butene as the mobile phase. Fig. 9 shows the separation of naphthalene and 1,3,5-triphenylbenzene, the k' values of which are much smaller than those obtained with isobutane. Alkenes such as *cis*-2-butene will be effective for the elution of strongly retained solutes.

Dimethyl ether has a polarity intermediate between that in the reversed-phase and in the normal-phase mode. Aromatic compounds were not retained on an ODS column when dimethylether was used as the mobile phase, whereas they were retained on a polystyrene column (TSK-GEL G 1000H). The retention of aromatic compounds is due to the interaction between the aromatic rings of the solute and the

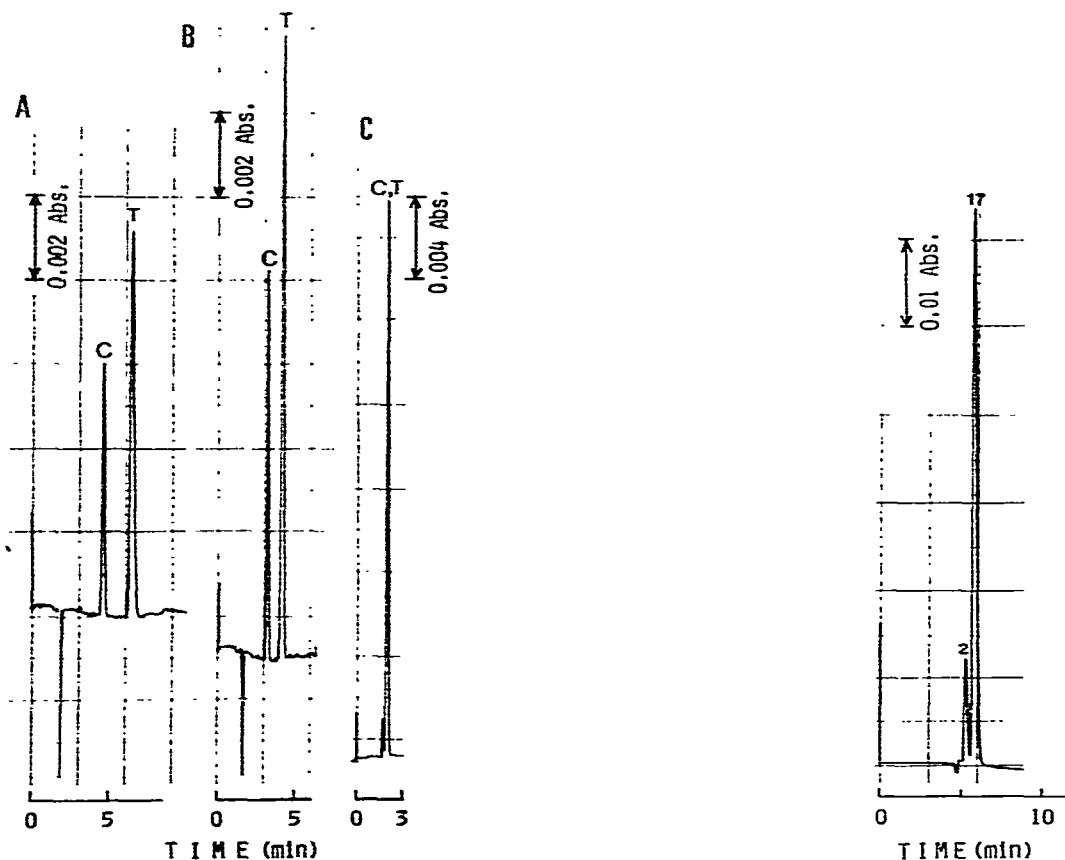


Fig. 8. Separations of stilbene isomers on a Spherisorb 5S column. Column: 9.7 cm \times 0.26 mm I.D. Mobile phases: (A) isobutane; (B) *n*-butane; (C) *cis*-2-butene. Flow-rate: 2.8 μ l/min. Sample: *cis*-stilbene (C) and *trans*-stilbene (T). Wavelength of UV detection: 280 nm.

Fig. 9. Separation of PAHs on a Spherisorb 5S column using *cis*-2-butene as the mobile phase. Column: 9.7 cm \times 0.26 mm I.D. Flow-rate: 2.8 μ l/min. Sample: naphthalene and 1,3,5-triphenylbenzene. Wavelength of UV detection: 254 nm.

benzene rings of the stationary phase. Thus, the retention of a solute increases with increasing number of aromatic rings in the solute.

Fig. 10 shows separations of aromatic amines and PAHs on a G 1000H column employing dimethyl ether as the mobile phase. As expected, the retention was primarily dependent on the number of aromatic rings in the solute molecule, solutes with more rings eluting later. The position of an amino group caused no appreciable change in the retention, *e.g.*, α - and β -naphthylamine could not be resolved in this mode.

Fig. 11 shows the retention of PAHs on a G 1000H column. The retention of a solute is primarily dependent on the number of aromatic rings in the molecule. A solute with more aromatic rings generally gives a larger k' value. The structure of a solute also affects the retention; thus, rotatable solutes give smaller k' values than coplanar solutes with the same number of aromatic rings. Solute with greater steric

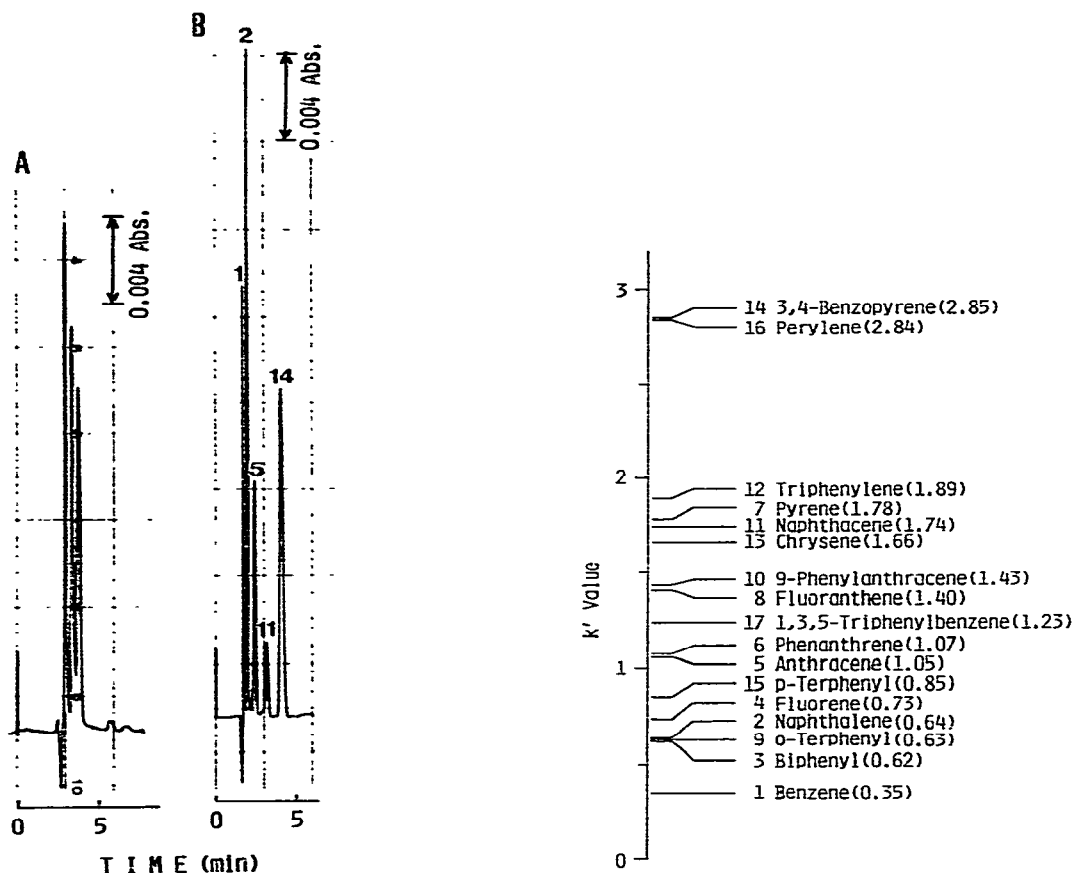


Fig. 10. Separations of aromatic compounds on a G 1000H column using dimethyl ether as the mobile phase. Column: 10.3 cm \times 0.26 mm I.D. Flow-rate: (A) 1.4 μ l/min; (B) 2.8 μ l/min. Sample: (A) aniline, α -naphthylamine and N-phenyl- α -naphthylamine, eluted in that order; (B) PAHs; the numbers refer to compounds in Fig. 2. Wavelength of UV detection: (A) 235 nm; (B) 265 nm.

Fig. 11. Retention of PAHs on a G 1000H column. Column: 10.3 cm \times 0.26 mm I.D. Mobile phase: dimethyl ether. Sample: the numbers refer to compound in Fig. 2.

hindrance elute before solutes with smaller steric hindrance, resulting from the decrease in the interaction with the stationary phase due to the rotation of aromatic rings, but they give larger k' values than solutes with fewer aromatic rings, except *o*-terphenyl. It is assumed that the benzene rings in *o*-terphenyl rotate considerably.

Non-aromatic five- or six-membered rings in a solute also increases the k' values. The degree of increase in k' due to a non-aromatic five-membered ring is less than that due to an aromatic six-membered ring, while a non-aromatic six-membered ring is equivalent to an aromatic six-membered ring. As demonstrated in Fig. 11, coplanar fluoranthene elutes between coplanar three-ring compounds and coplanar four-ring compounds, whereas the k' value of perylene is nearly the same as that of 3,4-benzopyrene.

For the analysis of polar solutes, the solvent strength should be adjusted so as to elute them in a reasonable time. An eluent including a polar solvent is generally

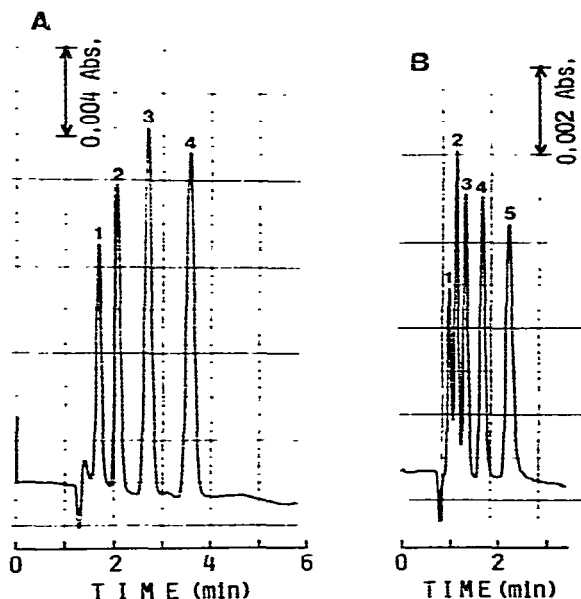


Fig. 12. Binary-solvent separations of phthalates and N-alkylanilines. Column: Spherisorb 5S, 10.4 cm \times 0.25 mm I.D. Mobile phase: (A) isobutane-dimethyl ether (80:20); (B) isobutane-dimethyl ether (94:6). Flow-rate: (A) 3.47 μ l/min (2.78 μ l/min + 0.69 μ l/min); (B) 5.90 μ l/min (5.56 μ l/min + 0.35 μ l/min). Sample: (A) 1, di-2-ethylhexyl phthalate; 2, di-*n*-butyl phthalate; 3, diethyl phthalate; 4, dimethyl phthalate; (B) 1, N,N-diethylaniline; 2, N,N-dimethylaniline; 3, N-*n*-butylaniline; 4, N-ethylaniline; 5, N-methylaniline. Wavelength of UV detection: 235 nm.

employed in such a case. Binary solvent elution was examined by employing dimethyl ether as the modifier. The apparatus shown in Fig. 1 was employed in this system.

Fig. 12 shows separations of phthalates and N-alkylanilines on a Spherisorb 5S column by binary solvent elution. The retention of solutes could be changed by changing the mobile phase composition. The mobile phase composition was varied by changing the flow-rates of the two pumps. Thus, the composition could be varied not continuously but stepwise. The reproducibility of retention in binary solvent elution was poor, owing to the pulsation of the pumps. The relative standard deviation of the retention of dimethyl phthalate in Fig. 12A was *ca.* 6% for 25 measurements.

REFERENCES

- 1 T. Takeuchi, Y. Watanabe, K. Matsuoka and D. Ishii, *J. Chromatogr.*, 216 (1981) 153.
- 2 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 240 (1982) 51.
- 3 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 213 (1981) 25.
- 4 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 238 (1982) 409.
- 5 E. J. Kikta and E. Grushka, *Anal. Chem.*, 48 (1976) 1098.
- 6 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 142 (1977) 213.
- 7 H. Hemetsberger, M. Kellermann and H. Ricken, *Chromatographia*, 10 (1977) 726.
- 8 P. Roumeliotis and K. K. Unger, *J. Chromatogr.*, 149 (1978) 211.
- 9 N. Tanaka, H. Goodell and B. L. Karger, *J. Chromatogr.*, 158 (1978) 233.
- 10 N. Tanaka, K. Sakagami and M. Araki, *J. Chromatogr.*, 199 (1980) 327.