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Retention behaviour of polycyclic aromatic hydrocarbons on a liquid-crystal bonded phase in reversed-phase liquid chromatography

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ABSTRACT

The liquid chromatographic characteristics of polycyclic aromatic hydrocarbons on a recently reported liquid-crystal bonded phase were evaluated. The results clearly indicated that the material has a strong molecular planarity recognition capability because of its ordered bonded-phase structure. The recognition capability was dependent on mobile phase composition and column temperature. The behaviour can be explained by a so-called 'slot-like' structure used to describe retention of polycyclic aromatic hydrocarbons on polymeric octadecylsilica phases.

INTRODUCTION

It is necessary to have good separation and analytical methods for the recognition of molecular planarity and shape of polycyclic aromatic hydrocarbons (PAHs), because their physicochemical properties and biological activities are closely related to their molecular structures [1–3].

The most common method of PAH separation is reversed-phase liquid chromatography (RPLC) with chemically bonded octadecylsilica (ODS) phases as the universal choice of stationary phase. Commercially available ODS phases can be basically divided into two types, depending on the bonding chemistry: one is polymeric, synthesized from polyfunctional silanes, and the other is monomeric, based on monofunctional silanes as the starting material. The retention behaviour of PAHs has been reported to depend on the type of ODS stationary phases [4–10], and the conclusion is that polymeric phases are better able to recognize molecular planarity in PAHs. Planar molecules are retained longer than non-planar molecules with polymeric phases because of the "slot-like" structure on the surface, as described by Sander and Wise

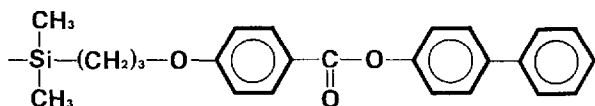


Fig. 1. Chemical structure of the liquid-crystal bonded phase.

[4–7]. Solid-state cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (CP-MAS-NMR) clearly reveals that the reduced molecular freedom of alkyl chains on the polymeric phase increases molecular planarity recognition [11]. It can be concluded that very ordered phases should have stronger molecular planarity recognition capability.

Extending the investigations of several previous publications [8–14], we evaluated a liquid-crystal bonded phase the structure of which is shown in Fig. 1 [15,16]. The phase has a very ordered structure in the normal temperature range and one can expect it to have strong molecular planarity recognition power. In this paper, we will describe the retention behaviours of PAHs with the liquid-crystal stationary phase in RPLC.

EXPERIMENTAL

Synthesis of (4-(allyloxy)benzoyl)biphenyl [17]

To a two-neck 250-ml round-bottom flask equipped with a 50-cm reflux condenser, a PTFE septum with a nitrogen line, a magnetic stirring bar and an oil bath were added, under nitrogen, 4.93 g of 4-phenylphenol dissolved in 50 ml of dry pyridine. Next, 6.13 g of 4-(allyloxy)benzoyl chloride was added with stirring. The mixture was stirred for 3 h at 25°C and then heated to 60°C for an additional 2 h. After the reaction mixture had cooled to 25°C, 200 ml of acidic (20% HCl) deionized water was added. The resulting precipitate was collected by vacuum filtration and washed with 200 ml of cold deionized water. The crude product was recrystallized from an acetone–ethanol (1:1) mixture yielding 8.45 g (88%, m.p. 138–140°C).

Synthesis of silane reagent and silica bonding [15,16]

The (4-(allyloxy)benzoyl)biphenyl (11.8 mmol) dissolved in 20 ml of dry toluene was added to a two-neck 100 ml round-bottom flask equipped with a 50-cm reflux condenser, a PTFE septum with a nitrogen line, a magnetic stirrer and an oil bath. Next, 11.8 mmol of dimethylchlorosilane were added to the reaction flask while stirring and purging with nitrogen. After 5 min, 8 mg of hexachloroplatinic acid were added. The reaction mixture was heated to 65°C and stirred for ten days under nitrogen. Then 20 ml of freshly distilled toluene were added followed by 6.5 g of silica (Nucleosil 300-10) and 0.5 ml of dry pyridine. The reaction mixture was stirred for ten days at 40°C under nitrogen. The solid was then collected and washed with 60 ml of toluene followed by 60 ml of ethanol. The wash procedure was repeated six times.

RPLC measurements

The synthesized liquid-crystal phase was packed into a fused-silica capillary of 200 mm × 0.52 mm I.D. by a slurry technique. For comparison two commercially

available stationary phases, TSK ODS-120T (Tosoh, Tokyo, Japan), representative of polymeric ODS, and Capcell ODS 120SG (Shiseido, Yokohama, Japan), representative of monomeric ODS, were also packed into the same size of capillaries.

The microcolumn LC system consisted of a microfeeder MF-2 pump (Azuma Electric, Tokyo, Japan), a Rheodyne 7513 injector (Cotati, CA, USA) and a Jasco Uvidec 100-III UV detector set at 254 nm. Mobile phases were pure methanol and mixtures of methanol and water, and the typical flow-rate was 2 $\mu\text{l}/\text{min}$. The column temperature was controlled by a home-made oven. The chromatographic measurements were performed at least in triplicate. The retention data used for the evaluation were mean values of these experimental data. The peak of dichloromethane, which was the sample solvent, was used to measure the column dead volume.

PAHs used for the evaluation were commercially available (Tokyo Kasei, Tokyo, Japan) except tetrabenzonaphthalene (TBN) and phenanthro[3,4-*c*]phenanthrene (PhPh), which were synthesized in the laboratory of Chevron Research and Technology Company (Richmond, CA, USA).

RESULTS AND DISCUSSION

To understand the basic chromatographic characteristics of the synthesized liquid-crystal bonded phase the separation of various PAHs was performed. The data obtained are summarized in Table I, in which the retention data with ODS-120T and Capcell ODS are also tabulated for comparison. The data for the liquid-crystal phase are shown in Fig. 2, in which $\log k'$ (capacity factor) values are plotted as a function of F number using 100% methanol as the mobile phase. A high linear correlation between $\log k'$ and F number was found [12] for the retention behaviour of PAHs with monomeric ODS phases in aqueous RPLC. The F number is defined by Schabron *et al.* [18] as follows: $F = (\text{number of double bonds}) + (\text{number of primary and secondary carbons}) - 0.5$ (for a non-aromatic ring). Fig. 2 clearly shows that planar PAHs are on the line, but the retention of non-planar PAHs such as *o*-terphenyl, PhPh and TBN shows a negative deviation from the line. This means that the phase

TABLE I
RETENTION DATA OF PAHs WITH THREE DIFFERENT BONDED PHASES
Mobile phase: methanol.

Solute	Capacity factor (k') on stationary phase		
	Liquid crystal	ODS-120T	Capcell ODS
Benzene	0.02	0.06	0.12
Naphthalene	0.09	0.16	0.21
Anthracene	0.47	0.38	0.41
Pyrene	0.53	0.66	0.59
<i>o</i> -Terphenyl	0.15	0.25	0.36
Triphenylene	0.72	0.75	0.61
BaP	2.48	1.81	0.80
TBN	2.12	1.94	1.86
PhPh	0.79	0.88	1.17

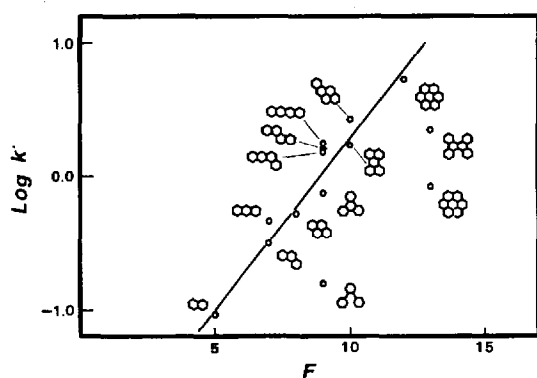


Fig. 2. Relationship between $\log k'$ and F number with the liquid-crystal stationary phase. Mobile phase, methanol, flow-rate, 2 $\mu\text{l}/\text{min}$.

has a strong planarity recognition capability. Planar solutes are retained longer, while non-planar solutes are eluted faster. Another interesting trend is the positive deviation from this line for the retention of rod-like PAHs. Although more detailed investigation will be required, it can be said that the liquid-crystal phase used in this study also has a shape selectivity for PAHs, as found by Chang *et al.* [19] in capillary supercritical fluid chromatography with a liquid-crystal stationary phase.

In order to examine in more detail the planarity recognition capability of the liquid-crystal phase, the separation factor of *o*-terphenyl and triphenylene was evaluated with the three phases shown in Table I, and the values are summarized in Table II. This factor has been confirmed by Tanaka and co-workers [20,21] and Jinno *et al.* [14] as a good indicator of the planarity recognition capability of the stationary phases in RPLC. Generally, polymeric ODS phases give a value of about 2–3, and monomeric phases a value of about 1–2 in aqueous mobile phase environments, although it has been observed that these values are larger when pure methanol is used as the mobile phase. The values reported in Table II are, therefore, in agreement with previous results. The value of the liquid-crystal phase is 4.8, and it suggests that this phase has very strong planarity recognition capability.

Mobile phase composition is one of major factors influencing the planarity recognition capability of the stationary phases in RPLC. The data summarized in

TABLE II

SEPARATION FACTORS BETWEEN *o*-TERPHENYL AND TRIPHENYLENE, AND BETWEEN TBN AND BaP

Stationary phase	Separation factors	
	Triphenylene/ <i>o</i> -terphenyl	TBN/BaP
Liquid crystal	4.80	0.85
ODS-120T	3.00	1.07
Capcell ODS	1.69	2.33

TABLE III

RETENTION DATA OF *o*-TERPHENYL AND TRIPHENYLENE WITH DIFFERENT MOBILE PHASE COMPOSITIONS

Temperature: room temperature.

Mobile phase composition (methanol-water)	Capacity factor (k')		Separation factor
	<i>o</i> -Terphenyl	Triphenylene	
100:0	0.15	0.72	4.80
95:5	0.24	1.12	4.67
90:10	0.38	1.72	4.53
85:15	0.64	2.74	4.28
80:20	1.01	4.21	4.17

Table III indicate the effect of the water content in the mobile phase on the separation factor of *o*-terphenyl and triphenylene. For clarification the separation factors are plotted as a function of the water content in Fig. 3. It appears that increasing the water content in the mobile phase results in a decrease in the separation factor. The results can be explained as follows: In an aqueous environment, *o*-terphenyl will be more solvated by methanol. This makes the solute bulkier, so that the planarity difference between triphenylene and *o*-terphenyl will be smaller with increasing water content in the mobile phase.

Another measure of stationary phase planarity recognition in RPLC which has been proposed by Sander and Wise [6,7] is the retention ratios of benzo[*a*]pyrene (BaP), TBN and PhPh. These values are also calculated from the data in Table I and shown in Table II. These data also indicate strongly that the liquid-crystal phase has a greater capacity to recognize PAH molecular planarity.

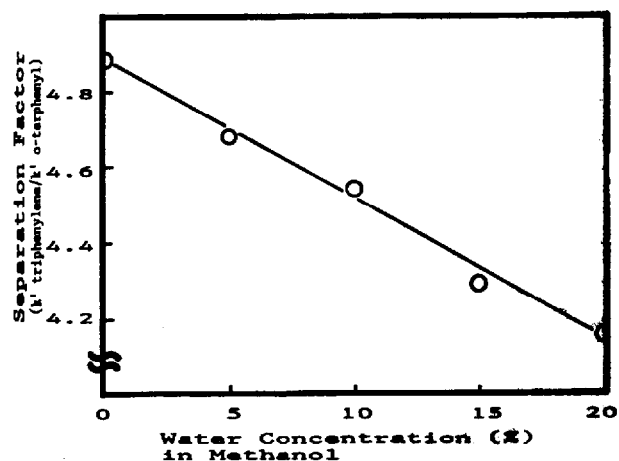


Fig. 3. Separation factor of *o*-terphenyl and triphenylene versus mobile phase compositions. Column temperature, room temperature.

TABLE IV

RETENTION DATA OF BaP, TBN AND PhPh WITH DIFFERENT MOBILE PHASE COMPOSITIONS

Temperature: room temperature.

Mobile phase composition (methanol-water)	Capacity factor (k')			Separation factor TBN/BaP
	BaP	TBN	PhPh	
100:0	2.48	2.12	0.79	0.85
95:5	4.18	3.83	1.32	0.92
90:10	6.99	7.11	2.21	1.02
85:15	12.00	12.90	3.71	1.08
80:20	19.80	23.37	6.21	1.18

Sander and Wise proposed [6,7,22] column selectivity based on the separation factor between BaP and TBN ($k'_{\text{TBN}}/k'_{\text{BaP}}$). They reported that monomeric ODS phases give values about 1.5–2, and polymeric ODS phases give a value less than 1 in aqueous mobile phase. As shown in Table II, the liquid-crystal phase gives a value of 0.85 in 100% methanol as the mobile phase. Therefore, the phase is quite similar to the typical polymeric ODS phases and has a strong planarity recognition capability.

For the separation factors between BaP and TBN the effect of the mobile phase

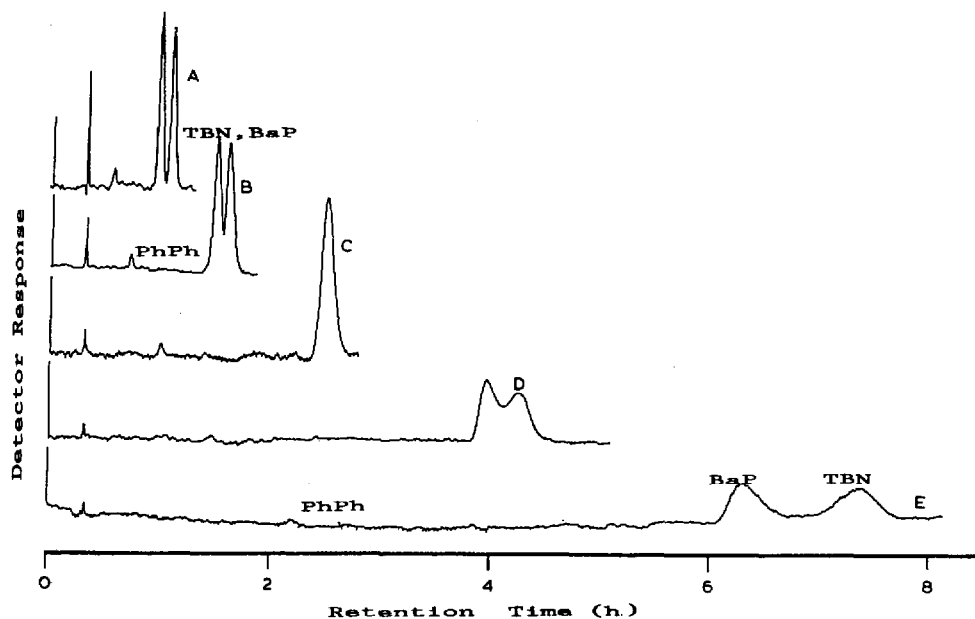


Fig. 4. Chromatograms for the separation of BaP, TBN and PhPh with different mobile phase (methanol-water) compositions. (A) 100:0; (B) 95:5; (C) 90:10; (D) 85:15; (E) 80:20. Column temperature, room temperature; flow-rate, 2 $\mu\text{l}/\text{min}$; detection, UV at 254 nm.

TABLE V

SEPARATION FACTORS BETWEEN *o*-TERPHENYL AND TRIPHENYLENE WITH DIFFERENT COLUMN TEMPERATURES

Mobile phase: methanol-water (80:20).

Column temperature (°C)	Capacity factor (k')		Separation factor
	<i>o</i> -Terphenyl	Triphenylene	
0	1.94	8.82	4.55
27	1.03	4.44	4.31
40	0.74	3.04	4.11
50	0.66	2.56	3.88
60	0.59	2.21	3.75
70	0.56	2.01	3.59

composition was also evaluated. The results are summarized in Table IV. Increasing the water content in the mobile phase increases the separation factor. This means that the phase is changing to a monomer-like from a polymer-like structure, and this fact is in good agreement with that discussed above for the separation factors between *o*-terphenyl and triphenylene. In Fig. 4, the chromatograms clearly indicate the effect of the mobile phase composition on the elution order of BaP and TBN with the liquid-crystal stationary phase.

Temperature is also one of the major factors influencing the molecular recog-

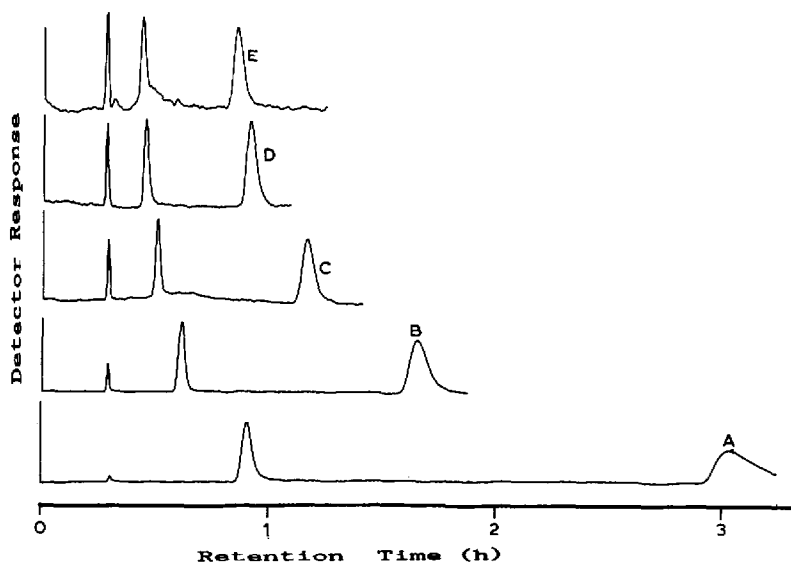


Fig. 5. Chromatograms for the separation of *o*-terphenyl and triphenylene with different column temperatures. (A) 0°C; (B) 27°C; (C) 40°C; (D) 60°C; (E) 70°C. Mobile phase, methanol-water (80:20); flow-rate, 2 μ l/min; detection, UV at 254 nm.

niton capability of the stationary phase. In our previous work [8] and the work of Sander and Wise [6,22], increasing the temperature caused a decrease in the planarity recognition capability of polymeric ODS phases. Solid-state CP-MAS-NMR studies explained this fact by revealing that an increase in temperature caused the polymeric ODS phase to change from a solid-like to liquid-like structure [8]. This kind of behaviour would also be expected of the liquid crystal phase because it also undergoes a thermal phase change. Therefore, the separation factors between *o*-terphenyl and triphenylene were measured at various column temperatures. The results are tabulated in Table V. The corresponding chromatograms are illustrated in Fig. 5. An increase of the column temperature not only reduces the retention time but also causes a decrease in the separation factor between *o*-terphenyl and triphenylene. Fig. 6, in which the separation factors are plotted as a function of temperature, shows these effects clearly. A decrease of temperature increases the planarity recognition capability of the liquid-crystal phase in the same manner as polymeric ODS phases. It is possible to explain this fact by the same theoretical interpretation of the stationary phase structure change with the temperature. The orderliness of the bonded phase increases with the decrease of the temperature, and this orderliness or rigidity of the bonded phase offers higher planarity recognition capability. Although we did not measure the solid-state NMR spectra of the liquid-crystal phase at different temperatures, a situation similar to that which has been found in the polymeric ODS phase [8] may be present in this phase structure.

It was indicated in the above discussions that there are some similarities between polymeric ODS phases and the liquid-crystal phase in their planarity recognition capability. The similarity is better seen in the correlation matrix shown in Table VI, in which cross-correlations among the retention data sets obtained for the three different phases in Table I are summarized. The similarity between ODS-120T and the liquid-crystal phase is much higher than other correlations, and there is also a low correlation between Capcell and the liquid crystal phase. It is clear that the

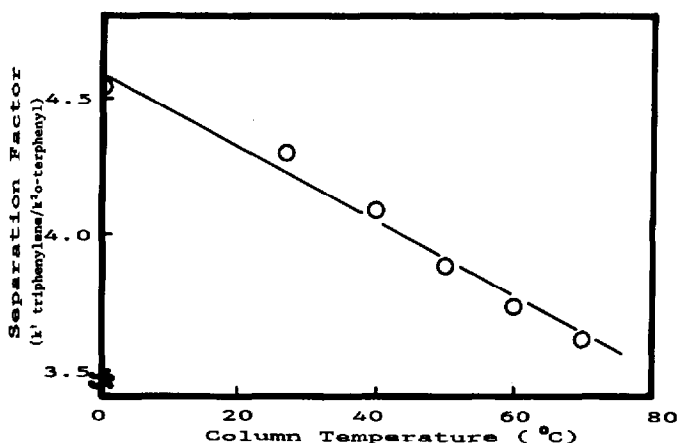


Fig. 6. Temperature dependency of the separation factors between *o*-terphenyl and triphenylene. Mobile phase, methanol-water (80:20); flow-rate, 2 μ l/min.

TABLE VI

CORRELATION MATRIX OF THE RETENTION DATA WITH THREE DIFFERENT STATIONARY PHASES IN TABLE I

Mobile phase: methanol.

Stationary phase	Liquid crystal	ODS-120T	Capcell ODS
Liquid crystal	—		
ODS-120T	0.982	—	
Capcell ODS	0.740	0.844	—

liquid-crystal phase is very similar to the polymeric ODS phases and dissimilar to monomeric phases. This similarity is mainly due to the orderliness of the bonded-phase structures. This explains that the strong planarity recognition capability of the liquid-crystal phase is due to its ordered structure.

In conclusion, the liquid-crystal phase, like typical polymeric ODS phases, possesses a strong planarity recognition power, but its ability to recognize PAHs is much higher than that of polymeric ODS phases because its orderliness can be assumed to be tighter and more rigid than that of polymeric ODS phases, although more details should be known by structure studies of the liquid-crystal phase with spectroscopic measurements. This is currently being investigated in our laboratory.

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REFERENCES

- 1 R. G. Harvey (Editor), *Polycyclic Hydrocarbons and Carcinogenesis* (ACS Symposium Series, No. 283), American Chemical Society, Washington, DC, 1986.
- 2 I. A. Smith, G. D. Berger, P. G. Seybold and M. P. Serve, *Cancer Res.*, 38 (1978) 2968.
- 3 G. H. Loew, J. Phillips, J. Wong, L. Hiedmeland and G. Pack, *Cancer Biochem. Biophys.*, 2 (1978) 113.
- 4 L. C. Sander and S. A. Wise, *Anal. Chem.*, 56 (1984) 504.
- 5 L. C. Sander and S. A. Wise, *J. Chromatogr.*, 316 (1984) 163.
- 6 L. C. Sander and S. A. Wise, *Anal. Chem.*, 59 (1987) 2309.
- 7 L. C. Sander and S. A. Wise, *LC-GC*, 8 (1990) 378.
- 8 K. Jinno, T. Ibuki, N. Tanaka, M. Okamoto, J. C. Fetzer, W. R. Biggs, P. R. Griffiths and J. M. Olinger, *J. Chromatogr.*, 461 (1989) 209.
- 9 K. Jinno, T. Nagoshi, N. Tanaka, M. Okamoto, J. C. Fetzer and W. R. Biggs, *J. Chromatogr.*, 392 (1987) 75.
- 10 K. Jinno, S. Shimura, N. Tanaka, K. Kimata, J. C. Fetzer and W. R. Biggs, *Chromatographia*, 27 (1989) 285.
- 11 K. Jinno, *J. Chromatogr. Sci.*, 27 (1989) 729.
- 12 K. Jinno, S. Shimura, J. C. Fetzer and W. R. Biggs, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 673.
- 13 K. Jinno, S. Shimura, J. C. Fetzer and W. R. Biggs, *Poly. Arom. Comp.*, 1 (1990) 151.
- 14 K. Jinno, K. Yamamoto, H. Nagashima, T. Ueda and K. Itoh, *J. Chromatogr.*, 517 (1990) 193.

- 15 J. Pesek and T. Cash, *Chromatographia*, 27 (1989) 559.
- 16 J. Kohler, *Chromatographia*, 21 (1986) 573.
- 17 M. A. Apfel, H. Finkelmann, G. M. Janini, R. J. Laub, B. H. Luhman, A. Price, W. L. Roberts, T. H. Shaw and C. A. Smith, *Anal. Chem.*, 57 (1985) 651.
- 18 J. F. Schabron, R. J. Hurtubise and H. F. Silver, *Anal. Chem.*, 49 (1977) 2253.
- 19 H. C. K. Chang, K. E. Markides, J. S. Bradshaw and M. L. Lee, *J. Chromatogr. Sci.*, 26 (1988) 280.
- 20 K. Kimata, I. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki and N. Tanaka, *J. Chromatogr. Sci.*, 27 (1989) 721.
- 21 N. Tanaka, K. Sakagami and M. Araki, *J. Chromatogr.*, 199 (1980) 327.
- 22 L. C. Sander and S. A. Wise, *Anal. Chem.*, 61 (1989) 1749.