

Influence of shape parameters on the separation of tetrachlorodibenzo-*p*-dioxin isomers on reversed-phase columns

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ABSTRACT

The retention orders of tetrachlorodibenzo-*p*-dioxin (TCDD) isomers on a monomeric and a polymeric ODS column were determined by RP-HPLC. It is demonstrated that the drastic alteration of the retention order with variation of the stationary phase can be explained by a variable influence of steric effects on the retention. The length-to-breadth ratios of TCDDs were calculated and correlated with retention data. The polymeric ODS phase proved to be superior to the monomeric phase with respect to selectivity. The enhanced selectivity is attributed to an enhancement of the shape selectivity of the stationary phase. The retention order is also strongly dependent on the mobile phase.

INTRODUCTION

In recent years, high-performance liquid chromatography (HPLC) has been used by several groups in clean-up procedures for the determination of polychlorodibenzofurans (PCDFs) and polychlorodibenzo-*p*-dioxins (PCDDs) in different matrices. Both normal- and reversed-phase (RP) columns and combinations of the two modes have been used [1-7]. RP-HPLC has also been employed for the separation of individual isomers of reference compounds [8].

In this work, we studied systematically the separation of thirteen commercially available tetrachlorodibenzo-*p*-dioxin (TCDD) isomers on octadecyl-silica gel (ODS) columns of different surface coverages.

Wise and May [9] reported the dependence of selectivity on the surface coverage with octadecyl groups for polycyclic aromatic hydrocarbons (PAHs) separated on ODS columns from various suppliers. Wise *et al.* [10] demonstrated the relationship between the retention on polymeric ODS phases and the shape parameters of PAHs, especially the length-to-breadth ratio (L/B).

The aim of this work was to examine whether the same relationship can be used to explain the different retention orders of TCDD isomers on a polymeric ODS phase compared with monomeric ODS phases with lower surface coverages.

EXPERIMENTAL

Chemicals

All solvents were of HPLC grade (Rathburn, Walkerburn, UK). Eleven of the isomers were obtained from Cambridge Isotope Labs. (Woburn,

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MA, USA) as solutions in nonane (50 ± 5 ppm). 1478-, 1236-, 1239-, 1267-, 1378-, 1289- and 2378-TCDD were received as individual isomers; 1237/1238- and 1368/1379-TCDD were received as mixtures of two isomers. The concentrations of the two isomers in the delivered standard mixtures differed considerably. By comparison of the intensity ratios and the retention orders on a PONA gas chromatographic capillary column (50 m \times 0.2 mm I.D.; 5.5 μ m) (Hewlett-Packard, Palo Alto, CA, USA) we succeeded in assigning the peaks of the 1368–1379-TCDD mixture unambiguously (conditions: HP 5970B mass-selective detector with HP 5890A gas chromatograph; injection mode, splitless; temperature programme, 130°C (0.5 min), 130–230°C at 30°C/min, 230–315°C at 1°C/min; carrier gas, helium). 1237-TCDD and 1238-TCDD were assigned according to their shape parameters. 1234-TCDD and 1278-TCDD were received as crystals from Cambridge Isotope Labs. In Fig. 1a the numbering of dioxin substituents is presented according to IUPAC nomenclature.

Apparatus

An HP 1050 liquid chromatograph with a quaternary gradient pump and autoinjector was used in

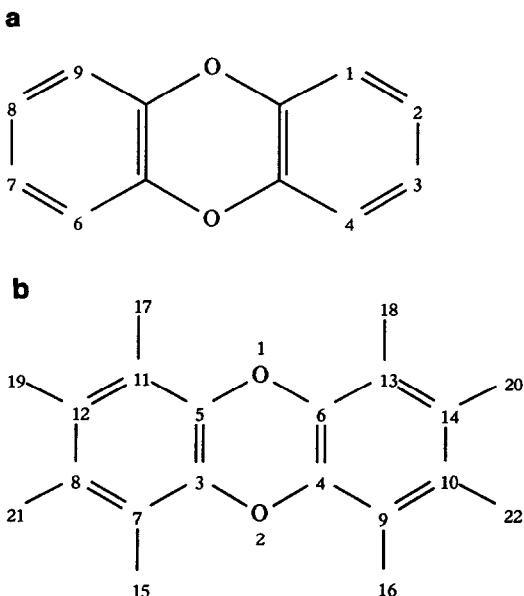


Fig. 1. (a) Numbering of dioxin substituents. (b) Numbering employed for quantum mechanical calculations.

conjunction with a LDC Spectromonitor III spectrophotometric detector. The temperature of the column was controlled by a laboratory-made copper jacket and a water-bath. A PC-based laboratory data system (Hewlett-Packard, Vectra Series) was used to record, store process and plot the data.

Columns

The relative retention indices for one of the monomeric columns (ODS1) were taken from O'Keefe *et al.* [5], who employed two series-connected 250 mm \times 6.2 mm I.D. Zorbax ODS (DuPont Instruments Division, Wilmington, DE, USA) columns. With a given absolute retention time for 2378-TCDD of 15.5 min and a calculated dead time of 6.4 min, we evaluated the capacity factors of the analytes.

We employed a second monomeric column (ODS2): Spherisorb ODS-2, particle diameter 5 μ m (250 mm \times 4.6 mm I.D.). As a polymeric column (ODS3) we used a 250 mm \times 4.6 mm I.D. Vydac 201TP (Separations Group, Hesperia, CA, USA) column (particle diameter 5 μ m).

Chromatographic conditions

All retention data were obtained under isocratic and isothermal (25°C) conditions. The eluents were premixed [ODS2, acetonitrile–water (88:12, v/v) or methanol; ODS3, acetonitrile–water (80:20, v/v)]. The flow-rate was 1 ml/min in all instances. The UV detector was operated at 235 nm with a response time of 1 s. Retention times were measured with solutions of the TCDD isomers and the standards benz[*a*]anthracene and benzo[*b*]chrysene in 2-propanol.

The standard solutions in nonane were diluted with 2-propanol. For mixtures the nonane was evaporated before dissolving the standard in 2-propanol in order to avoid peak broadening or distortion due to the solvent effects of the sample solution. The injection volume was generally 10 μ l, containing 2–6 ng of each isomer. For a mixture of all TCDD isomers the injection volume was 7 μ l, containing 2–5 ng of each isomer.

The measurements with ODS1 were carried out with methanol at 2 ml/min (40°C). Details are given in ref. 5.

Calculation of length-to-breadth ratios

The geometry of the molecules was optimized by the semi-empirical method Austin Model 1 (AM1) with the software package AMPAC, run on an IBM 3090 computer in the CIRCE at Orsay (France). AM1 is a method that is well adapted to large organic molecules [11]. Initial input geometries were created in the form of a *Z*-matrix, involving internal coordinates in which each atom is related to previously specified atoms by a bond length, an angle and a dihedral angle. Each initial geometry was subsequently fully optimized by AM1.

The numbering scheme employed for the input of internal coordinates is given in Fig. 1b. Optimization was started with the following bond lengths: O–O, 0.296 nm; C–O, 0.137 nm; C–C, 0.140 nm; C–H, 0.110 nm; and C–Cl, 0.170 nm. The dihedral angles were taken into the optimization. The starting values were 0° or 180°, respectively, in all instances. The starting values for the bond angles corresponded to an idealized benzene ring.

The cartesian coordinates of the optimized molecule structure were employed to evaluate the length-to-breadth ratio (*L/B*) with in-house software on a desk-top microcomputer (Tandy 3000 NL). The programme calculates the rectangle enclosing the analyte with maximized *L/B*, according to the method of Wise *et al.* [10], with respect to the Van der Waals radii of the outer atoms of the molecule. The Van der Waals radii were taken from ref. 12.

Retention indices

The retention indices were calculated according to Popl *et al.* [13] using the following equation:

$$\log I_R = \log I_n + \frac{\log R - \log R_n}{\log R_{(n+1)} - \log R_n} \quad (1)$$

where *R* represents the corrected retention time of the solute (retention time minus the void volume multiplied by the flow-rate) and *n* and *n* + 1 represent standards with lower or higher retention times, respectively. The standards were assigned the following values (log *I_n*): benzene 1, naphthalene 2, phenanthrene 3, benz[*a*]anthracene 4 and benzo[*b*]chrysene 5. The void volume was approximated by the retention volume of the non-retained solute acetone (solution in acetonitrile).

RESULTS AND DISCUSSION

Characterization of bonded phases

The two ODS columns examined were characterized according to the method of Sander and Wise [14] by a test mixture of benzo[*a*]pyrene (BaP), phenanthro[3,4-*c*]phenanthrene and tetrabenzonaphthalene (TBN). Following their classification scheme, the Vydac 201TP is polymeric like ($\alpha_{\text{TBN/BaP}} = 0.49$) and the Spherisorb ODS-2 monomeric like ($\alpha_{\text{TBN/BaP}} = 1.83$). $\alpha_{\text{TBN/BaP}}$ is defined as $k'_{\text{TBN}}/k'_{\text{BaP}}$. By Sander and Wise Zorbax ODS was classified as a monomeric-like material.

Retention of TCDDs on OD-silica gels

In Table I the recalculated capacity factors *k'* obtained by O'Keefe *et al.* [5], the capacity factors for the monomeric and the polymeric ODS columns employed in our studies, the relative retention indices (RRI) (retention time relative to the absolute retention time of 2378-TCDD) for all three partition systems and the retention indices (log *I_R*) for ODS2 and ODS3 are given. The retention times of TCDD isomers on the ODS2 column were measured with pure methanol and with acetonitrile–water (88:12) as mobile phases.

The comparison of the capacity factors shows clearly that the ODS3 column is more selective than the other two ODS columns. Only one co-elution was observed with the ODS3 column (1368- and 1378-TCDD), the capacity factors ranging between 2.83 and 8.48. The ODS2 phase, however, with acetonitrile–water as mobile phase, exhibited several co-elutions and the capacity factors were in the range 3.31–5.06. The selectivity of the ODS2 column is also strongly dependent on the mobile phase. Replacement of acetonitrile–water with pure methanol leads to a drastic alteration of the retention order. The selectivity of the ODS1 column can be compared with that of the ODS2 column. The slight differences in retention order can be attributed to minor variations in the nature of the stationary phase.

Comparison of the relative retention indices defined by O'Keefe *et al.* [5] shows another characteristic difference between the three ODS phases. With methanol as mobile phase 2378-TCDD is eluted from ODS1 and ODS2 as one of the analytes which elute at an average position. With acetonitrile–water

TABLE I

CAPACITY FACTORS (k'), RELATIVE RETENTION INDICES (RRI) AND RETENTION INDICES ($\log I_R$) OF TCDD COMPOUNDS FOR THREE ODS COLUMNS

TCDD isomer	H_f (kcal/mol)	L/B	ODS1 ^a		ODS2 ^b		ODS2 ^c			ODS3 ^d			
			k'^e	RRI ^{e,f}	k'	RRI ^f	$\log I_R$	k'	RRI ^f	$\log I_R$	k'	RRI ^f	$\log I_R$
1234	-12.24	1.35	1.74	1.132	2.31	1.087	4.855	4.08	0.839	4.970	3.34	0.458	4.298
1236	-14.31	1.49	1.46/1.49 ^g	1.016/1.027 ^g	1.98	0.976	4.687	4.03	0.830	4.953	3.13	0.435	4.270
1237	-16.71	1.63	1.44	1.077	1.98	0.976	4.687	4.46	0.902	>5	4.38	0.567	4.431
1238	-16.69	1.59	1.44	1.077	1.98	0.976	4.687	4.46	0.902	>5	3.95	0.522	4.381
1239	-14.13	1.47	1.46/1.49 ^g	1.016/1.027 ^g	1.93	0.961	4.659	3.86	0.802	4.900	3.88	0.514	4.371
1246	-13.01	1.35	1.38	0.983	— ^h	—	—	—	—	—	—	—	—
1247	-15.45	1.47	1.45	1.013	—	—	—	—	—	—	—	—	—
1248	-15.48	1.40	1.45	1.013	—	—	—	—	—	—	—	—	—
1249	-12.99	1.29	1.38	0.983	—	—	—	—	—	—	—	—	—
1267	-14.79	1.68	1.07	0.853	1.50	0.819	4.391	3.39	0.725	4.741	2.71	0.391	4.203
1268	-15.74	1.55	1.31/1.41 ^g	0.953/0.997 ^g	—	—	—	—	—	—	—	—	—
1269	-13.37	1.35	1.02	0.833	—	—	—	—	—	—	—	—	—
1278	-17.12	1.66	1.32	0.956	1.75	0.903	4.5534	4.09	0.839	4.971	3.62	0.487	4.336
1279	-15.57	1.59	1.31/1.41 ^g	0.953/0.997 ^g	—	—	—	—	—	—	—	—	—
1289	-14.56	1.60	1.07	0.853	1.53	0.829	4.410	3.31	0.713	4.713	2.99	0.420	4.251
1368	-16.70	1.48	1.66	1.100	2.24	1.064	4.821	4.86	0.967	>5	3.79	0.505	4.364
1369	-14.35	1.28	1.30/1.33 ^g	0.950/0.963 ^g	—	—	—	—	—	—	—	—	—
1378	-18.10	1.59	1.59	1.068	2.10	1.039	4.784	4.90	0.974	>5	4.77	0.608	4.470
1379	-16.57	1.59	1.66	1.100	2.17	1.039	4.784	4.86	0.967	>5	4.67	0.607	4.472
1469	-12.01	1.17	1.02	0.833	—	—	—	—	—	—	—	—	—
1478	-15.83	1.34	1.30/1.33 ^g	0.950/0.963 ^g	1.75	0.903	4.557	3.92	0.812	4.920	2.83	0.404	4.221
2378	-19.54	1.84	1.42	1.000	2.05	1.000	4.724	5.06	1.000	>5	8.48	1.000	4.743

^a Stationary phase, Zorbax ODS; column dimensions, 500 mm × 6.2 mm I.D.; mobile phase, methanol.^b Stationary phase, Spherisorb ODS-2; column dimensions, 250 mm × 4.6 mm I.D.; mobile phase, methanol.^c Stationary phase, Spherisorb ODS-2; column dimensions, 250 mm × 4.6 mm I.D.; mobile phase, acetonitrile–water (88:12, v/v).^d Stationary phase, Vydac 201TP; column dimensions, 250 mm × 4.6 mm I.D.; mobile phase, acetonitrile–water (88:12, v/v).^e Data from ref. 5.^f Retention time relative to absolute retention time of 2378-TCDD (30.3 min).^g Alignment is not unambiguous.^h — = Not determined.

2378-TCDD is eluted the last from ODS2 and on ODS3 it is well resolved from all the studied TCDD isomers. Possibly the selectivity of ODS3 for TCDDs makes this partition system well suited to separate 2378-TCDD, the most toxic TCDD isomer, from a large excess of the other TCDD isomers prior to analysis by GC–MS or another appropriate method. In Fig. 2 the chromatogram of the thirteen TCDD isomers on ODS3 is shown. 2378-TCDD is perfectly baseline resolved from all the other TCDD isomers within 35 min.

Further, a shift concerning the range of $\log I_R$ is observed on comparing ODS2 to ODS3. For ODS1 no data are available that allow the evaluation of

$\log I_R$. With acetonitrile–water as mobile phase, six of the examined isomers elute from ODS2 after benzo[*b*]chrysene, whereas from ODS3 all isomers elute between benz[*a*]anthracene and benzo[*b*]chrysene. This observation is in agreement with the results of other workers [10], who found that ODS columns from various manufacturers not only provide different efficiencies for PAHs but also different selectivities and retention characteristics. They also observed a dependence of $\log I_R$ of PAHs on the surface coverage with ODS groups and on $\alpha_{TBN/BAp}$. The dependence of $\log I_R$ on the mobile phase was not reported, however.

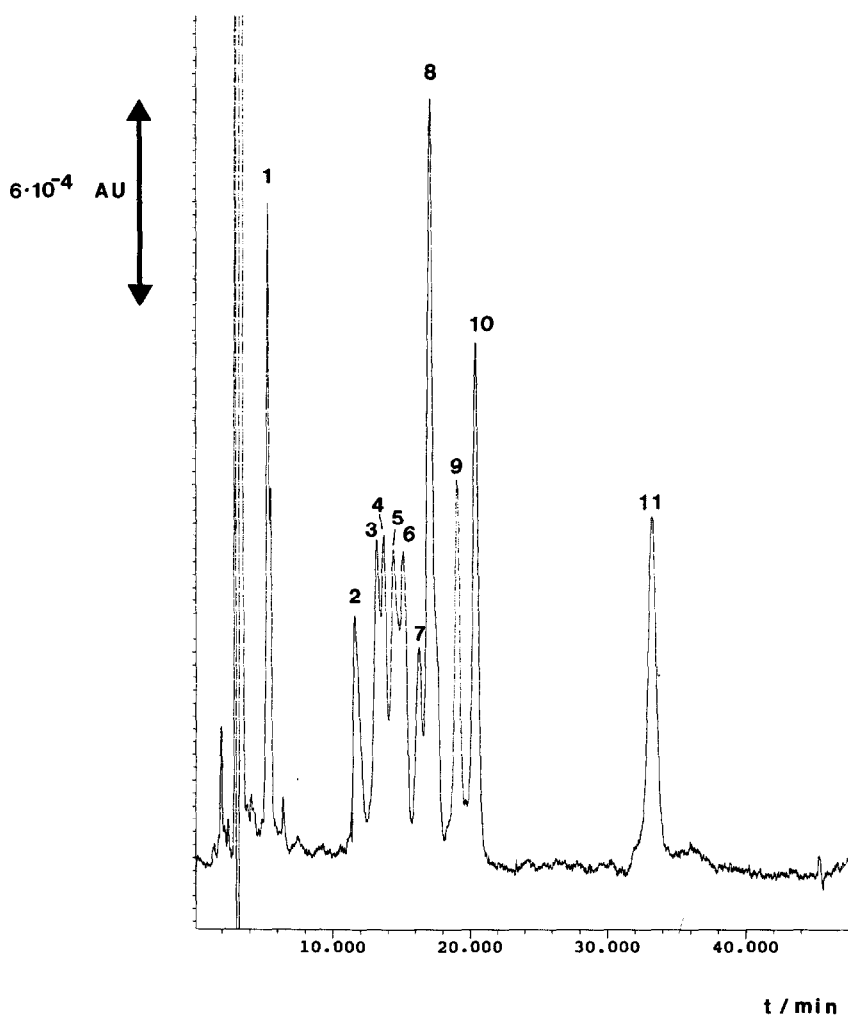


Fig. 2. Separation of TCDD isomers on ODS3. Stationary phase, Vydac 201TP; mobile phase, acetonitrile–water (80:20, v/v); flow-rate, 1 ml/min; detector wavelength, 235 nm; temperature, 25°C. Peaks: 1 = solvent; 2 = nonane; 3 = 1267-TCDD; 4 = 1478-TCDD; 5 = 1289-TCDD; 6 = 1236-TCDD/1234-TCDD; 7 = 1278-TCDD; 8 = 1368-TCDD/1239-TCDD/1238-TCDD; 9 = 1237-TCDD; 10 = 1379-TCDD/1378-TCDD; 11 = 2378-TCDD.

Calculation of geometric parameters

Wise and co-workers [10] found a relationship between shape parameters, particularly the calculated length-to-breadth ratio (L/B), of PAHs and alkylated derivatives and their retention indices on polymeric ODS phases. The parameter L/B was introduced by Radecki *et al.* [15] in quantitative structure–retention relationship studies to explain the retention order of PAH isomers separated on nematic liquid crystal GC phases and by Lamparc-

zyk *et al.* [16] to describe the bioactivity of PAHs. Wise *et al.* [17] found an analogy between the retention order of PAH isomers separated on liquid crystal phases of the smectic type by GC and their retention order on polymeric-type ODS phases employed in RP-HPLC.

The PCDDs constitute a class of compounds with a rigid fused-ring structure, similar to the class of PAHs. It is not yet clear whether all the TCDD isomers exhibit in solution a folded or a near-planar

structure. In the following text a folded structure of TCDDs is defined as a structure with a distortion angle $\beta > 10^\circ$; β is given by the angle between the planes defined by the two benzene rings.

2378-TCDD has a near-planar structure in the crystal lattice [18]. Graininger *et al.* [19] calculated the ether linkage (COC) bond angles for the 22 TCDD isomers from infrared data (studies in the gas phase). The average bond angles range from near planar (115.1°) for 2378-TCDD to approximately tetrahedral for isomers with aromatic rings of low electron-withdrawing capacity. However, it must be stated that dibenzo-*p*-dioxin, with aromatic rings of low electron-withdrawing capacity, also exhibits a near-planar structure in the crystal lattice [20].

We found by quantum mechanical calculation that the energetic difference between a folded and a near-planar structure (closed-shell molecules) for TCDDs is extremely small. The heat of formation (H_f) calculated by AMPAC was -14.20 kcal/mol for 1238-TCDD with a distortion angle β of 14.5° and -14.13 kcal/mol for a planar structure (1 kcal = 4.184 kJ). With 1247-TCDD we obtained $H_f = -15.45$ kcal/mol for the planar and $H_f = -15.52$ kcal/mol for the folded ($\beta = 14.2^\circ$) structure. With 1478-TCDD the energetic difference was even less: $H_f = -15.88$ kcal/mol ($\beta = 14.9^\circ$), $H_f = -15.83$ kcal/mol ($\beta = 0.0^\circ$).

Therefore, we assumed that a near-planar structure can be readily achieved by all the TCDD isomers and might be the preferred structure when the analyte is sorbed on ODS-silica gel. The near-planar structures were employed for the calculation of the optimized L/B values.

The calculated ratios and the data obtained for H_f (near-planar structures) are listed in Table I. The thermodynamic stability follows the general trend that isomers with substituents in lateral positions are more stable than isomers with substituents in an α -position to one of the oxygen atoms. 2378-TCDD with substituents only in lateral positions is thermodynamically the most stable TCDD isomer ($H_f = -19.54$ kcal/mol), and 1469-TCDD with substituents only in α -positions is the least stable isomer ($H_f = -12.01$ kcal/mol). The differences in the H_f values are significant, in order to reflect the stability of these isomers in the environment, and might be useful to explain substitution patterns of TCDD isomers in natural samples.

The values for L/B range from 1.17 to 1.84. The TCDDs clearly differ in their geometric parameters. It can be deduced that 2378-TCDD can be easily separated from the other isomers if the retention mechanism is purely shape controlled.

Correlation of capacity factors with length-to-breadth ratios

A direct correlation of L/B with the retention indices ($\log I_R$) of the TCDD isomers on the polymeric phase (ODS3), in analogy with the work of Wise *et al.* [10], gave only unsatisfactory results ($R = 0.60$). This means that L/B is a parameter that does not independently predict the retention index of TCDDs on polymeric ODS phases. In contrast to the PAHs, the TCDDs have large dipole moments ($\mu = 0.02$ – 2.80 D), owing to the presence of chlorine atoms [21], whereas the dipole moments of PAHs are of the order of $\mu = 0.01$ D, and there is little variation among the values [22]. However, the overall dipole moment is not a parameter that can be used to describe all polar features of a molecule in quantitative structure–retention relationship (QSRR) studies of separations by RP-HPLC [23]. In spite of the limited validity of the overall dipole moment as a descriptor of the polarity of a molecule, it can be deduced that differences in polarity are much smaller with PAHs than with TCDDs.

For a classical RP-HPLC system a submolecular polarity parameter (maximum excess charge difference) was used together with other parameters as a significant descriptor of retention data [24]. Sarna *et al.* [25] pointed out that elution from an RP column with an aqueous mobile phase reflects the hydrophobicity of a compound, in addition to its organic phase–water partition coefficient. They determined octanol–water partition coefficients for a series of PCDDs and PCDFs experimentally by RP-HPLC.

We assume that for the separation of TCDDs by RP-HPLC, differences in the polarity of the solutes cannot be neglected in QSRR studies. The influence of polarity can be superimposed by steric effects (quantitatively expressed by the shape parameter L/B), and this shape parameter gains more influence with increasing surface coverage with ODS groups making the organic layer thicker and denser. Obviously, the mobile phase also influences to a great extent the shape selectivity of the stationary phase.

In order to verify our assumptions, we correlated

TABLE II

RESULTS OF THE REGRESSION ANALYSIS

a, b, c = Regression coefficients; r = partial correlation coefficient; P = probability; R = multiple correlation coefficient.

Correlation	a	b	c	F	P (%)	R	$r(\log k'_2)$	$r(L/B)$
$\log k'_2 = \log k'$ (ODS2) ^a	-0.872	1.371	0.800	18.51	0.05	0.887	0.817	0.865
$\log k'_2 = \log k'$ (ODS2) ^b	-0.987	1.538	0.727	21.63	0.03	0.901	0.840	0.865
$\log k'_2 = \log k'$ (ODS1)	-1.015	1.495	0.428	21.41	0.03	0.900	0.839	0.714

^a Mobile phase: methanol.

^b Mobile phase: acetonitrile-water (88:12, v/v).

the logarithms of the capacity factors ($\log k'_1$) of ODS3 with those of the columns with less surface coverage with ODS groups ($\log k'_2$) according to the equation

$$\log k'_1 = a + b \log k'_2 + cL/B \quad (2)$$

The variable $\log k'_2$ serves as a polarity descriptor. The results of the three equations are presented in Table II. In all instances the variables $\log k'_2$ and L/B are significant descriptors of $\log k'_1$. The partial correlation coefficients show that the shape parameter L/B contributes strongly to the predictive ability of the three equations.

Fig. 3 shows the plots of experimentally deter-

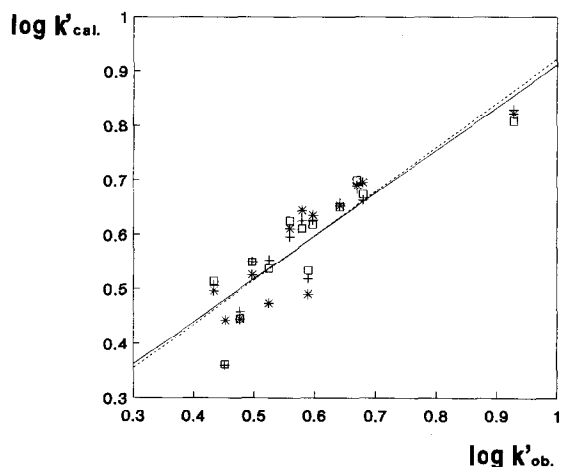


Fig. 3. Plot of logarithms of calculated capacity factors ($\log k'_{cal.}$) vs. logarithms of observed capacity factors ($\log k'_{ob.}$) on ODS3. \square = [$k'_2 = k'$ (ODS1)]; $+$ = [$k'_2 = k'$ (ODS2), mobile phase = methanol]; $*$ = [$k'_2 = k'$ (ODS2), mobile phase = acetonitrile-water (80:20, v/v)].

mined $\log k'$ (on ODS3) vs. predicted $\log k'$. The plots show no clustering and uniform error variances.

CONCLUSIONS

The results of the correlation analysis suggest that the observed differences in the selectivity of ODS columns can be attributed to an increasing influence of steric effects on the retention of analytes with increasing surface coverage of the stationary phase. Also, the mobile phase influences to a great extent the selectivity of ODS phases. The exhibited differences in selectivity with variations of the mobile phase can also be attributed to a variable influence of steric effects on the retention.

L/B can be used as a shape parameter to examine the influence of the geometry of the analytes. The relatively low multiple correlations show that L/B does not totally express the variance of steric parameters. It must be kept in mind that L/B is a two-dimensional parameter. If molecules deviate from a planar structure, this will strongly influence the retention behaviour.

Wise and May [9] have shown that polymeric ODS phases are superior to monomeric ODS phases with respect to the selectivity in the separation of PAHs. We found the same superiority in the separation of TCDDs. 1237-1238-TCDD and 1368-1379-TCDD, which co-elute on the monomeric phases, can be baseline separated on the polymeric ODS3 column.

This enhanced selectivity can be understood as an enhancement of the shape selectivity of the stationary phase, due to an organic layer with higher

density compared with the organic layers of monomeric ODS phases. The influence of the mobile phase is not fully understood, however.

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