Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1048 (2004) 99–109
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Retention characteristics of porous graphitic carbon in subcritical fluid chromatography with carbon dioxide–methanol mobile phases

C. West*, E. Lesellier, A. Tchapla

Groupe de Chimie Analytique de Paris Sud, LETIAM (EA 3343), IUT d'Orsay, Plateau du Moulon, 91400 Orsay, France

Received 29 April 2004; received in revised form 2 July 2004; accepted 2 July 2004

Abstract

Numerous relationships usually used in high-performance liquid chromatography (HPLC) for describing the retention on porous graphitic carbon (PGC) have been applied in subcritical fluid chromatography, with CO_2–methanol mobile phases. As reported in HPLC, octanol–water partition coefficient failed to fit the retention, whereas satisfactory results were obtained with the sum of partial negative charges. A better fit was reached by using the solvation parameter model, allowing a better understanding of the interactions developed between the solute, the stationary and the mobile phases. Results show that the dominant contribution to retention was given by the polarizability (*E*) and the volume (*V*), while the hydrogen-bond basicity (*B*) was not selected in the retention model, whatever the methanol content. The increase in methanol percentage favours the retention decrease, mainly through the volume for hydrophobic compounds, and through the hydrogen-bond acidity for polar compounds.

Keywords: Porous graphitic carbon; Subcritical fluid chromatography; Solvation parameter model; Mobile phase composition

1. Introduction

Porous graphitic carbon (PGC) is a chromatographic support with unique adsorption properties that are very different from other traditional reversed-phase supports. It was made commercially available at the end of the 1980s under the trade name Hypercarb [1].

At the molecular level, it is composed of flat sheets of hexagonally arranged carbon atoms (about 10^5 atoms per sheet) [2]. Hydroxyl, carbonyl and carboxylate groups are expected to be present at the edge of the graphite sheets but they are considered to be insignificant in the retention mechanism [3].

The earlier studies performed in high-performance liquid chromatography (HPLC) considered PGC to be a perfect reversed-phase support because of the hydrophobicity of its surface and of the absence of free silanol groups.

The planar surface allows a close proximity of the molecular surface of the solute, leading to a high steric selectivity. Besides, due to this planarity, the dispersive interactions between non-polar compounds and PGC are favoured providing higher methylene selectivity than on ODS phases [4]. Moreover, the chemical stability of PGC allows its use with highly acidic or basic mobile phases.

It soon became evident that the order of retention on PGC was not only governed by the order of solute hydrophobicity as more polar compounds can be more strongly retained than less polar ones [5,6]. Jackson and Carr showed that any polar functional group added to the benzene ring, regardless of its electron-donating or -withdrawing nature, induces an increase in retention [7]. This increase in retention was explained by the polarizability of the carbon network due to the overlapping of the hybridized orbitals, allowing dipole type and electron lone pair donor–acceptor interactions.

Consequently, the retention by PGC in HPLC with aqueous–organic eluents is generally explained by three factors:

* Corresponding author. Tel.: +33 1 6933 6131; fax: +33 1 6933 6048.
E-mail address: west@iut-orsay.fr (C. West).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.07.010

(i) Hydrophobic effect, driving the analyte out of the aqueous mobile phase [3].
(ii) London type dispersive interactions with the stationary phase (called hydrophobic adsorption [5]).
(iii) Interaction of polarizable or polarized functional groups in samples with the graphite surface, particularly strong when the stereochemistry of the molecule forces the polar group to be close to the surface [2,8]. This effect is called PREG (polar retention effect on graphite).

In order to better identify the structural factors of the solute affecting retention, and to explain the retention variations when changing the mobile phase composition, quantitative structure–retention relationships (QSRRs) can be used [9]. Among the varied models that have been applied to retention on PGC, some of them, using numerous descriptors not related to Gibbs free energy, are not interpretable in physical terms and not very informative. This is the case with the solvatochromic model, based on the measurement of spectral energy differences. This model is often confused with the solvation parameter model.

The latter has been very successful in describing chromatographic behaviours and various processes in which a solute is distributed between two phases [10,11]. It describes retention in terms of the difference in solute–stationary phase and solute–solvent interactions. A descriptor value is attributed to each type of interaction.

Using Abraham parameters [12–16], the classical equation used in HPLC is:

$$\log k = c + eE + sS + aA + bB + vV \quad (1)$$

where k is the solute retention factor.

In this equation, capital letters represent the solute descriptors, while lower case letters represent the system constants. The system constants are obtained by multiple linear regression analysis for a certain number of solutes with known descriptors. c is a solute-independent constant, characteristic of the column studied. E is the excess molar refraction (calculated from the refractive index of the molecule) and it models polarizability contributions from n and π electrons; S is the solute dipolarity/polarizability; A and B are the solute overall hydrogen-bond acidity and basicity; V is the McGowan characteristic volume in units of $cm^3\,mol^{-1}/100$. V is used to describe both the endoergic cavity formation process and the exoergic dispersion interactions.

For gas chromatography, V is replaced by $\log L^{16}$, the partition coefficient of the solute between the gaseous phase and hexadecane [17–19]. This coefficient neglects the cavity energy and the dispersive interactions between the solute and the mobile phase, thanks to the low density of the gaseous phase [20].

Lepont et al. used the model to account for the retention mechanism of neutral organic compounds on PGC with methanol–water mobile phases [21]. As observed with ODS phases, the mobile phase induces a large positive v value (due in part to the unfavourable cavity formation into the hydro–organic mobile phase) and a negative b value (due to the acidic character of water).

However, as the solvation parameter model was developed as a partition model and not as an adsorption model, it was deficient for predicting the retention properties of angular molecules, due to the planar surface. Other studies also demonstrated that predictions based on solute shapes on varied stationary phases failed [14]. However, for the identification of the most informative structural descriptors and the study of the variations of the constants following the analytical conditions changes, this model provides accurate information.

The properties of supercritical fluids allow the improvement of chromatographic separations of numerous solutes, by reducing the analysis time. Because carbon dioxide acts as a non-polar solvent, the addition of more polar modifiers (methanol, acetonitrile) is often required to increase the solute's solubility into the mobile phase. The understanding of retention with modified supercritical phases has been investigated by linear solvation energy relationship (LSER) models, with different stationary phases: cyano [22,23], PDMS [20,24], RP-C_{18} [25–27] and various other phases [28]. However, only few works have been carried out with PGC [29] and the detailed effects of the increase of modifier were not investigated.

The purpose of this paper is to study the retention behaviour of non-ionised organic compounds and the PGC surface in subcritical fluid chromatography (SubFC) with methanol-modified carbon dioxide mobile phases. Relationships between the retention factors and the octanol–water partition coefficients and with the negative charge excess of the solutes will be investigated. Then, the solvation parameter model will be used to identify precisely the interactions established and to determine the dominant contributions to retention. The results are compared to those obtained in high-performance liquid chromatography with methanol–water mobile phases.

2. Experimental

2.1. Chemicals

The solvent used was HPLC-grade methanol (Carlo Erba, Milan, Italy). Carbon dioxide was provided by Alphagaz (Bois d'Arcy, France).

Fifty-one test compounds (see Table 1), benzene and naphthalene derivatives, were obtained from a range of suppliers. Solutions of these compounds were prepared in methanol.

2.2. Chromatographic system

Chromatographic separations were carried out using equipment manufactured by Jasco (Tokyo, Japan, supplied by Prolabo, Fontenay-sous-Bois, France). Two model 980-

Table 1
n-Octanol–water partition coefficients (log *P*), excess negative charges ($\sum q^-$) and solute descriptors (*E*, *S*, *A*, *B*, *V*)

Compound	log *P*	$\sum q^-$	*E*	*S*	*A*	*B*	*V*
Benzene	2.061		0.610	0.52	0.00	0.14	0.7164
Toluene	2.588		0.601	0.52	0.00	0.14	0.8573
Ethylbenzene	3.820		0.613	0.51	0.00	0.15	0.9982
Propylbenzene	4.350		0.604	0.50	0.00	0.15	1.1391
Butylbenzene	4.880		0.600	0.51	0.00	0.15	1.2800
Pentylbenzene	5.410		0.594	0.51	0.00	0.15	1.4209
Hexylbenzene	5.520		0.591	0.50	0.00	0.15	1.5620
Aniline	1.032	−0.172	0.955	0.94	0.26	0.50	0.8162
Benzoic acid	1.793	−1.019	0.730	0.90	0.59	0.40	0.9317
Isophthalic acid	1.670	−1.672					
1,3-Benzenedicarboxylic acid							
Trimesic acid	1.150	−2.409					
1,3,5-Benzenetricarboxylic acid							
N,*N*-Dimethylaniline	2.278		0.957	0.84	0.00	0.47	1.0980
Anisole	2.155		0.708	0.75	0.00	0.29	0.9160
Phenylethan-1-ol	1.332	−0.267	0.784	0.83	0.30	0.66	1.0570
Benzyl alcohol	1.100	−0.474	0.803	0.87	0.39	0.56	0.9160
Benzaldehyde	1.500	−0.411	0.820	1.00	0.00	0.39	0.8730
Acetophenone	1.746		0.818	1.01	0.00	0.48	1.0139
Benzonitrile	1.681		0.742	1.11	0.00	0.33	0.8711
Nitrobenzene	1.808		0.871	1.11	0.00	0.28	0.8906
Chlorobenzene	2.808		0.718	0.65	0.00	0.07	0.8288
Bromobenzene	3.017		0.882	0.73	0.00	0.09	0.8910
Phenol	1.543	−0.352	0.805	0.89	0.60	0.30	0.7751
o-Chlorophenol	1.960		0.853	0.88	0.32	0.31	0.8980
o-Aminophenol			1.110	1.10	0.60	0.66	0.8750
2,5-Dimethylphenol			0.840	0.79	0.54	0.37	1.0570
2,6-Dimethylphenol			0.860	0.79	0.39	0.39	1.0570
3,4-Dimethylphenol			0.830	0.86	0.56	0.39	1.0570
Eugenol			0.946	0.99	0.22	0.51	1.3540
Resorcinol	0.800	−1.205	0.980	1.00	1.10	0.58	0.8340
1,3-Dihydroxybenzene							
Phloroglucinol	0.400	−1.390	1.355	1.12	1.40	0.82	0.8925
1,3,5-Trihydroxybenzene							
Naphthalene	3.180		1.340	0.92	0.00	0.20	1.0854
α-Naphthol	2.980		1.520	1.05	0.61	0.37	1.1441
β-Naphthol			1.520	1.08	0.61	0.40	1.1440
Nitronaphthalene	3.200		1.600	1.51	0.00	0.29	1.2596
1-Methylnaphthalene	3.870		1.344	0.90	0.00	0.20	1.2260
2-Methylnaphthalene	3.860		1.304	0.92	0.00	0.20	1.2260
Biphenyl	4.040		1.360	0.99	0.00	0.26	1.3242
Benzophenone	3.180		1.447	1.50	0.00	0.50	1.4810
Methylbenzoate	2.157		0.733	0.85	0.00	0.48	1.0726
Ethylbenzoate	2.640		0.689	0.85	0.00	0.46	1.2140
Propylbenzoate	3.170		0.675	0.80	0.00	0.46	1.2260
Butylbenzoate	3.700		0.668	0.80	0.00	0.46	1.4953
o-Cresol	2.047		0.840	0.86	0.52	0.46	0.9160
m-Cresol	2.047		0.822	0.88	0.57	0.34	0.9160
p-Cresol	2.047		0.820	0.87	0.57	0.31	0.9160
o-Nitrophenol	1.267		1.045	1.05	0.05	0.37	0.9490
m-Nitrophenol	1.267		1.050	1.57	0.79	0.23	0.9490
p-Nitrophenol	1.267		1.070	1.72	0.82	0.26	0.9490
o-Xylene	3.092		0.663	0.56	0.00	0.16	0.9980
m-Xylene	3.092		0.623	0.52	0.00	0.16	0.9980
p-Xylene	3.092		0.613	0.52	0.00	0.16	0.9980

PU pumps were used, one for carbon dioxide and a second for the modifier. Control of the mobile phase composition was performed by the modifier pump. The pump head used for pumping the carbon dioxide was cooled to −2 °C by a cryostat (Julabo F10c, Seelbach, Germany, supplied by Touzart et Matignon, les Ulis, France). When the two solvents (methanol and CO_2) were mixed, the fluid was introduced into a dynamic mixing chamber PU 4046 (Pye Unicam, Cambridge, UK) connected to a pulsation damper (Sedere supplied by Touzart et Matignon). The injector valve was supplied with a 20 μL loop (model 7125 Rheodyne, Cotati, CA, USA).

The columns were thermostated by an oven (Jetstream 2 Plus, Hewlett-Packard, Palo Alto, USA), regulated by a cryostat (Haake D8 GH, Karlsruhe, Germany). The detector was a UV–vis HP 1050 (Hewlett-Packard), with a high-pressure resistant cell. The detection wavelength was 254 nm. After the detector, the outlet column pressure was controlled by a Jasco 880-81 pressure regulator (supplied by Prolabo). The outlet regulator tube (internal diameter 0.25 mm) was heated to 80 °C to avoid ice formation during the CO_2 depressurization.

Chromatograms were recorded using the AZUR software (Datalys, France). The chromatographic columns were Hypercarb porous graphitic carbon (100 mm × 4.6 mm i.d., 5 μm) provided by Thermo-Hypersil Keystone and a Kromasil octadecyl bonded silica column (250 mm × 4.6 mm i.d., 5 μm) provided by TSP-Shandon, Les Ulis, France.

2.3. Chromatographic conditions

All compounds were injected under the following operating conditions:

HPLC: flow rate, 1 mL min^{-1}; column temperature, 25 °C; mobile phase composition, methanol–water (90:10).
SubFC: flow rate, 3 mL min^{-1}; column temperature, 25 °C (subcritical for all mobile phase compositions); outlet column pressure, 15 MPa; modifier percentage, 5, 10, 20, 30 and 40%.

Subcritical conditions (with $T < 31$ °C) were chosen to reduce any density variations of the mobile phase related to the addition of modifier. In these conditions, it has been shown using ODS stationary phases that retention was only ruled by the modifier percentage [30].

2.4. Retention factors

Retention factors (k) were determined using the relationship:

$$k = \frac{t_r - t_0}{t_0}$$

where t_r is the solute retention time, determined using the peak maximums (even when tailing did occur, for some of the acidic and basic derivatives) and t_0 is the hold-up time measured on the first negative peak due to the unretained dilution solvent.

2.5. Data analysis

Multiple linear regression analysis and statistical tests were performed using the program SuperANOVA (Abacus Concept). The solute descriptors used in the solvation parameter model were taken from several sources [14,31–38] and are summarized in Table 1, along with octanol–water partition coefficients (log P) and negative charge excess ($\sum q^-$) values [39].

3. Results and discussion

3.1. log k = f(log P) relationship

In Fig. 1, log k values of mono- and disubstituted benzenes on ODS and PGC phases in SubFC [CO_2–MeOH (90:10)] are plotted against log P values obtained from Rekker [40]. The trends are very similar to those generally obtained in RPLC [5,6]. As pointed out by Kaliszan [9], perfect correlations between retention factors and octanol–water partition coefficients can only be obtained when structurally similar compounds are used, which is the case here. Fairly good linearity is observed with the ODS phase, indicating that the dispersion interaction plays a major role in determining the retention on this stationary phase in SubFC, as in HPLC. All points are close to the straight line connecting the plots for alkylbenzenes.

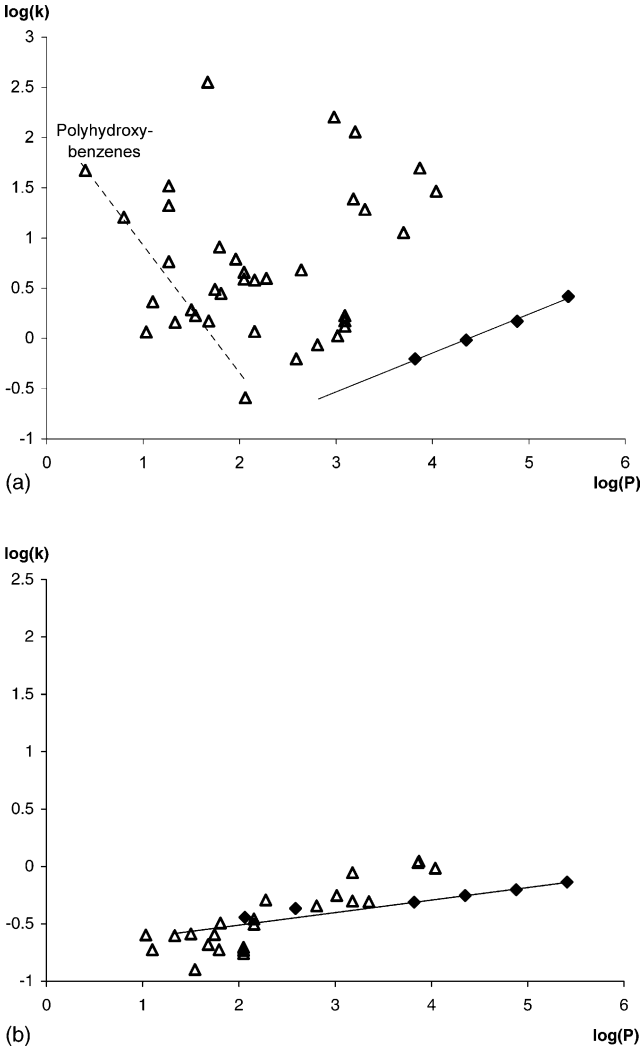

Fig. 1. Variation of log k for all solutes vs. octanol–water partition coefficient. Column: Hypercarb (a), Kromasil C_{18} (b); eluent: CO_2–MeOH, (90:10). Full lines drawn through points for n-alkylbenzenes; broken line drawn through phenol, 1,3-di- and 1,3,5-trihydroxybenzene.

On the carbon phase, all points are above the straight line indicating that any polar substituent causes an increase in retention relative to organic–aqueous liquid–liquid partition. In other words, as reported in HPLC, dispersion interaction is not the unique interaction governing retention on PGC in SubFC. The PREG effect is also observed with subcritical fluid as mobile phase.

Moreover, it is interesting to note that *meta*-substituted polyhydroxybenzenes (phenol, resorcinol and phloroglucinol) are perfectly aligned (correlation coefficient is equal to 0.9991). This is in good accordance with the results published by Hennion et al.: a linear relationship is observed in HPLC for mono- and disubstituted in positions 1 and 3, and trisubstituted in positions 1, 3 and 5 [6].

A plot of $\log k$ against the number of hydroxyl groups is also perfectly linear (see Fig. 2), whatever the composition of the mobile phase (from 5 to 40% methanol). This means that any additional *meta*-hydroxyl causes an equal increase in retention.

The slopes of these regression lines are related to the hydroxyl selectivity. The increase in methanol percentage decreases this selectivity.

It would have been interesting to check this point with carboxylic groups (benzoic, isophthalic and trimesic acids) but the trisubstituted acid is strongly retained in SubFC and was never eluted.

3.2. $\log k = f(\sum q^-)$ relationship

Different parameters have been suggested to evaluate charge repartition in the molecules. A promising submolecular polarity parameter was introduced by Kaliszan [41]: the parameter Δ is the largest difference molecular local dipole in a molecule. Hennion et al. proposed a simplest parameter noted $\sum q^-$, equal to the sum of partial negative charges [6]. They observed that $\log k$ values of polar substituted benzenes measured in water plotted against $\sum q^-$, showed good linearity. $\sum q^-$ is representative of the sum of partial dipoles existing in a molecule. A molecule having several electronegative atoms possesses important partial dipoles; therefore, the sum of partial negative charges is high. $\sum q^-$ was chosen, preferably to an overall dipole moment as the latter can be near zero in the case of some symmetrical molecule, while these molecules behave as polar solutes. Hence, submolecular polarity parameters such as $\sum q^-$, describe specific intermolecular interactions more accurately.

In Fig. 3, $\log k$ values of some substituted benzenes on PGC in SubFC are plotted against $\sum q^-$ values [39] calculated with MOPAC software. Fairly good linearity is observed at any methanol percentage in the subcritical mobile phase. (The curves were not all represented here for clarity reasons.) The absolute value of the slope decreases when the percentage of methanol in the mobile phase is increased. This indicates that two compounds having close values of $\sum q^-$ will be better resolved when the percentage of methanol in the mobile phase is low.

To compare SubFC and HPLC mobile phases with PGC, we looked for identical eluotropic strength compositions. We selected MeOH–water (90:10) among several tested compositions to compare it to CO_2–MeOH (90:10). These two mobile phases provide an identical methylene selectivity for a homologous series of alkylbenzenes, that is to say, the slopes in plots of $\log k$ versus carbon number (ranging from 4 to 10) are very close. The use of methylene selectivity in SFC to compare eluotropic strength of mobile phases was suggested by Smith [42].

For these compositions, the negative charge excess selectivity is higher in SubFC than in HPLC as the slope is higher (see Fig. 3). Consequently, polar compounds having different $\sum q^-$ should be better resolved in SubFC than in HPLC.

This specific effect of the subcritical mobile phase can also be observed through Fig. 4, where the retention factors measured in SubFC where plotted against the retention

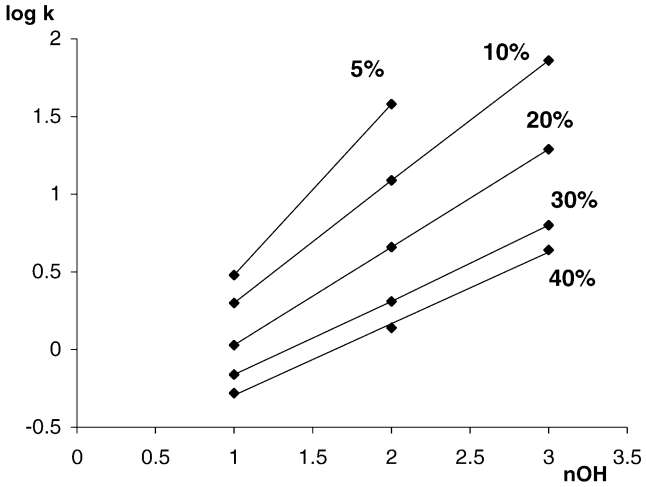

Fig. 2. Variation of $\log k$ vs. the number of hydroxyl groups on benzene for different methanol percentages.

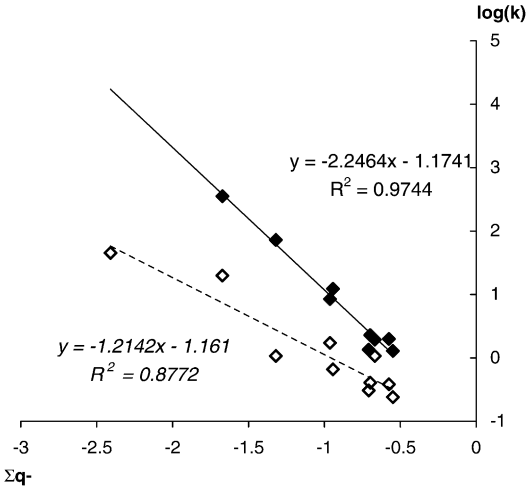

Fig. 3. Variation of $\log k$ for 10 solutes vs. sum of partial negative charges. Column: Hypercarb. Full line: SubFC, CO_2–MeOH (90:10); broken line: HPLC, MeOH–water (90:10).

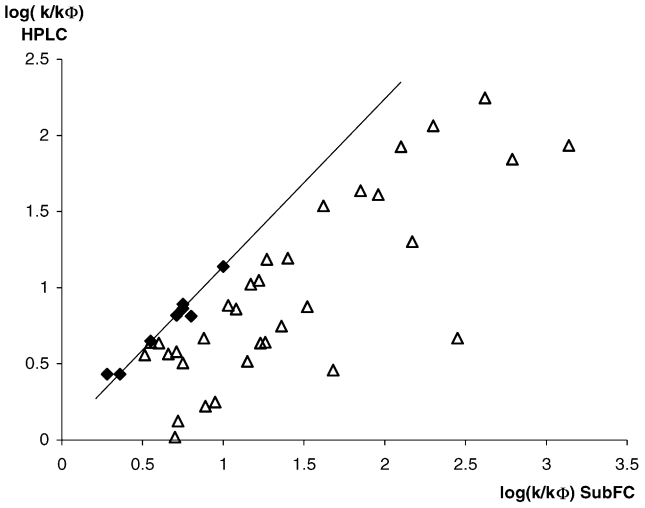

Fig. 4. Variation of the normalised retention logarithm in HPLC vs. the normalised retention logarithm in SubFC.

Table 2
Cross-correlation matrix for solute descriptors (r^2)

	e	*s*	*a*	*b*	*v*
e	1	−0.680	0.000	0.210	−0.324
s		1	−0.283	−0.289	0.073
a			1	−0.284	0.445
b				1	−0.258
v					1

factors measured in HPLC. To eliminate any phase ratio contribution [14,23], the retention factors were divided by the retention factor for benzene, chosen as reference compound. All points representing polar compounds are below the line joining the alkylbenzenes which confirms that all polar compounds are more strongly retained in SubFC than in HPLC, probably because of weaker polar interactions between solute and supercritical fluid.

3.3. The solvation parameter model

Judging by the temperature and pressure used and the modifier percentages, the subcritical fluid more closely resembles a liquid than a gas. Hence, we chose to use the molecular volume *V* instead of the partition coefficient log L^{16}. This choice is opposed to that of other authors having worked with lower supercritical mobile phase reduced densities (lower than 0.5) [20].

The system constants for each mobile phase composition were obtained by multiple linear regression analysis for the measured retention factors, as some mobile phases failed to elute all the analytes. To obtain chemically meaningful coefficients, the solute parameters must be varied over a wide range. Consequently, the probe solute set was carefully chosen to have a uniform distribution of each descriptor within a chosen space (see Fig. 5). However, the *A* descriptor is distributed in a narrower range as the number of solutes with significant hydrogen-bond acidity is limited. Besides, other experiments were carried out in HPLC on ODS stationary phases, with methanol–water mobile phases, using the same set of compounds. The system constants obtained matched the results obtained by Poole and Poole in similar chromatographic conditions [10]. This corroborates the validity of our set of compounds.

Absence of cross-correlation among the descriptors was checked (see Table 2). Graphs of the residuals (difference between the experimental and predicted log *k* values) plotted against the values of each individual descriptor showed no correlation.

The quality of the fits was estimated using the overall correlation coefficient (*R*), standard error in the estimate (S.D.) and Fischer *F*-statistic. A few outliers were eliminated from the set as their residuals were too high. Descriptors that were not statistically significant, with a confidence interval of 5%, were eliminated from the model.

The system constants and statistics are summarized in Table 3. The fits, although worse than the fits obtained with ODS stationary phases, are reasonably good and provide chemically sound information on the retention mechanisms for PGC. The statistics are similar to those reported elsewhere in HPLC on PGC [21].

3.4. Accuracy of the model

The solvation parameter model does not contain any term for shape selectivity. The molecular volume fails to correctly

Table 3
System constants and model fit statistics

Column	Mobile phase	MeOH *c* (%)	*e*	*s*	*a*	*b*	*v*	*n*	*R*	S.D.	*F*
PGC	Subcritical fluid	5	−2.664 (0.195)	1.749 (0.123)		1.211 (0.112)		1.632 (0.169)	43	0.963	0.200	165.3
		10	−2.532 (0.179)	1.641 (0.112)		0.783 (0.106)		1.545 (0.158)	46	0.959	0.192	159.8
		20	−2.472 (0.156)	1.586 (0.097)		0.526 (0.092)		1.407 (0.139)	44	0.965	0.165	178.2
		30	−2.455 (0.138)	1.552 (0.088)		0.240 (0.082)		1.382 (0.121)	42	0.969	0.144	196.6
		40	−2.407 (0.146)	1.549 (0.088)				1.328 (0.127)	43	0.961	0.158	241.4
	Liquid	90	−2.405 (0.212)	1.021 (0.179)	0.411 (0.189)		−1.029 (0.239)	1.713 (0.191)	37	0.940	0.195	60.3
Kromasil	Subcritical fluid	10	−0.778 (0.051)	0.664 (0.040)	−0.571 (0.065)	−0.436 (0.037)	−0.344 (0.078)	0.411 (0.052)	34	0.996	0.043	619.5

n is the number of solutes considered in the regression, *R* is the multiple correlation coefficient, S.D. is the standard estimate error, *F* is Fischer's statistic and the numbers in brackets represent 95% confidence limits.

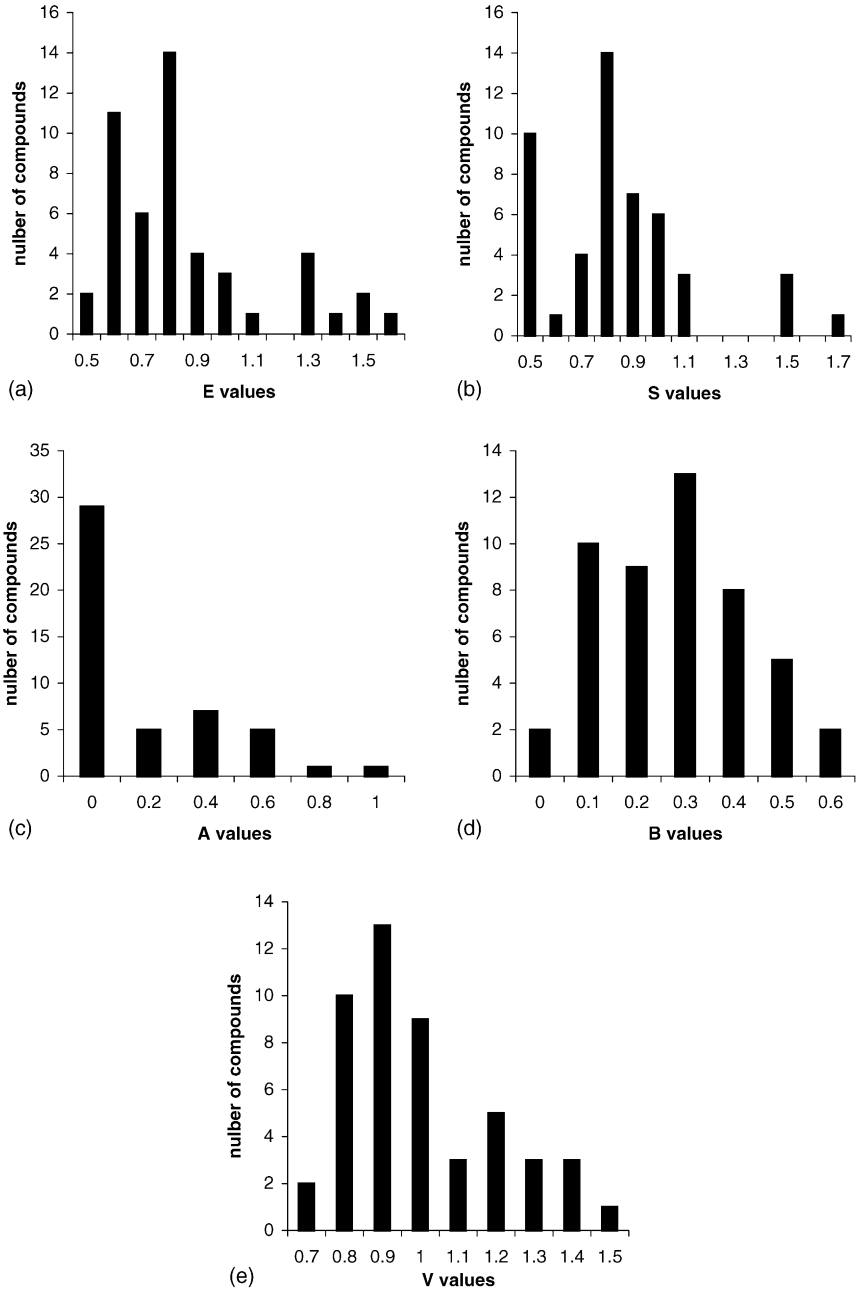

Fig. 5. Distribution of descriptor values.

model the contact surface area for the dispersive interaction of angular molecules with the graphite [14]. For instance, the parameters for ethylbenzene and *p*-xylene are almost identical. Therefore, according to the solvation parameter model theory, the selectivity for this pair of solutes should be close to 1. However, the experimental values indicate that the "flat" isomer (*p*-xylene) is more retained than the angular one (ethylbenzene). Despite that, because of their low residuals these compounds have been kept in the set. Besides, benzophenone was systematically eliminated from the regressions as its experimental retention factor was far too low, compared to the calculated one.

On the other hand, close examination of the residuals showed that, regardless of the mobile phase composition, any particular compound would always deviate in the same manner from the model, that is to say, its residual would always be either positive or negative and with the same relative amplitude, indicating that particular deviations are not random experimental errors. In particular, for homologous series such as alkylbenzenes and alkylbenzoates (see Fig. 6), we noted that, the longer the alkyl chain, the smaller the residual (except for toluene, which fits well on the flat carbon surface).

Comparing the residual graph to that obtained with the ODS column, we noted that the pattern was quite different.

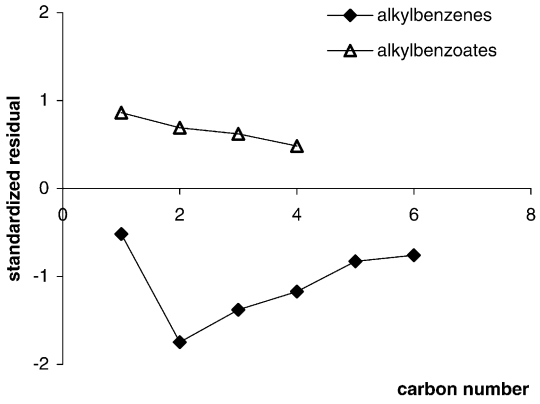

Fig. 6. Plots of average standard residuals vs. carbon number in homologous series. The residual for each solute at each composition is standardized by dividing the residual by the standard deviation. Chromatographic conditions: CO_2–MeOH (60:40).

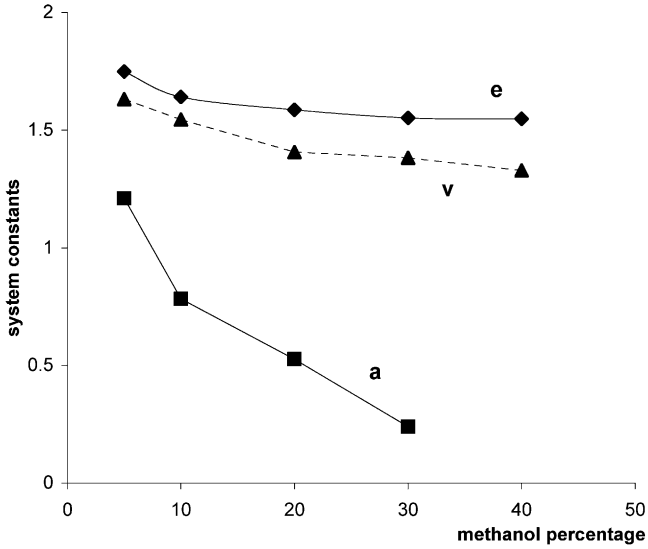

Fig. 7. Variation of the system constants with CO_2–MeOH mobile phase composition (%, v/v) for PGC.

In other words, if the descriptors fail to perfectly describe the behaviour of some compounds, it is due to the particular nature of PGC and the adsorption mechanism.

Adding a new descriptor may improve the fit but another bulk descriptor would obviously be correlated to the molecular volume, which is against the basic requirements of QSRRs [41]. Replacing the molecular volume by a contact surface area may also improve the fit and reduce the residuals for angular molecules. However, as we wish to compare the results with those reported elsewhere, we choose to use the molecular volume.

3.5. Evolution of the system constants with the proportion of modifier

The system constants (e, v, s, a, b) reflect the difference in solvation properties in the two phases. System constants with a positive sign indicate that the characterized interaction is more favourable for the stationary phase than for the mobile phase and leads to an increase in retention. Consequently, system constants also reflect the system's relative selectivity towards a particular molecular interaction.

First, the c term is negative and weaker than those obtained with ODS stationary phases. The values obtained (around -2.5) are in agreement with those reported by Lepont et al. using a PGC column in HPLC [21]. Because c is a constant, characteristic of the phase ratio of the studied column, it underlines the lower surface area of PGC ($120\,m^2\,g^{-1}$) with regards to the bonded silica one.

In SubFC, all the other selected system constants are positive (see Fig. 7). This means that all type of interactions considered are more favourable for the stationary phase than for the mobile phase. The values decrease when the percentage of methanol in the mobile phase is increased. Thus, when the percentage of methanol is increased, the retention decreases.

The dominant contributions to retention are the dispersion interaction term (v) and the excess molar refractivity term (e). This indicates that PGC is particularly selective towards analytes differing primarily in their molecular volume and in their ability to interact with the stationary and mobile phase through π or n electron pairs.

3.5.1. The v coefficient

It represents the difference in hydrophobicity between the stationary phase and the mobile one:

$$v = v_{\text{stationary}} - v_{\text{mobile}}$$

Each term has to be dissected into two terms, a cavity and a dispersive term. However, because of the solid nature of PGC, no cavity needs to be formed to insert the solute which is simply adsorbed onto the flat surface. Because of the less important cohesive energy of carbon dioxide (apolar fluid) in comparison with the one of hydro–organic liquids used in HPLC, the cavity term of the mobile phase is weak and can also be neglected. Consequently, the v coefficient is rather related to the dispersion interactions [25] and the values of v on PGC with subcritical phases are lower than with hydro–organic phases [21].

The v coefficient slightly decreases when the percentage of methanol in the mobile phase is increased.

In HPLC, whatever the stationary phase, an increase of the methanol percentage favours the dispersion interactions between the solute and the mobile phase [10,21]. In SubFC, with low-density fluid, the addition of methanol to carbon dioxide increases the fluid density, i.e. the eluotropic strength of the mobile phase [24,25]. However, as described elsewhere [43], in SubFC working with higher fluid density, the methanol addition mainly increases the mobile phase polarity, i.e. decreases the dispersion interaction between the solute and the mobile phase (v_{mobile} decreases). Consequently, to explain a decrease in the v coefficient, one should consider a decrease in the solute–stationary phase dispersion interactions. This decrease is induced by the sorption of methanol onto

the PGC surface, increasing the stationary phase polarity. In HPLC, lower values of v are observed with aromatic phases in regard to aliphatic ones. A stronger sorption of methanol into the aromatic phase is suggested to explain this behaviour [14].

Moreover, in a homologous series, the *E*, *S*, *A* and *B* descriptors are nearly constant, only the *V* descriptor varies significantly. Consequently, the difference of retention in a homologous series is only related to dispersion interaction modifications. As described elsewhere in HPLC [13], v increases linearly with the methylene selectivity, showing that this coefficient is a good indicator of methylene selectivity in SubFC with methanol as modifier.

3.5.2. The e coefficient

The excess molar refraction term (e) is related to charge transfer, reflecting the interaction between the electronic excess of the solute (π and n electrons) and the surface of PGC or the mobile phase.

Bassler et al. demonstrated PGC to behave as an electron-pair acceptor for substituted aromatic solutes capable of n-donation, which indicates that the electronic density at the surface of PGC is locally low [8]. Besides, Lim and co-workers showed that inorganic oxo-anions were retained on PGC [44,45]. Elfakir et al. [46] and Takeuchi et al. [47] also observed total retention of oxo-anions and inorganic anions possessing no hydrophobic function such as halides, suggesting again that the PGC was an electron-pair acceptor.

Hanai, modelling the electronic density at the surface of graphitic carbon, showed that the free electrons of an extended aromatic molecule tend to localize at the edge of the plans [2]. This indicates that electron density is low at the centre and high at the edge. This is in good agreement with a high excess molar refractivity term reflecting molecular interactions realised at the centre of the PGC surface.

The e coefficient decreases very slowly when increasing the percentage of methanol in the mobile phase. e represents the following:

$$e = e_{\text{stationary}} - e_{\text{mobile}}$$

Since the refractive index of methanol (1.329) is higher than that of liquid carbon dioxide (1.195), when increasing the percentage of methanol, the refractive index of the mobile phase increases. Therefore, the ability of the mobile phase to interact with n and π electrons is reduced, meaning that e_{mobile} decreases, which cannot explain the variation of e.

Besides, some methanol is adsorbed onto the stationary phase, also reducing its ability to establish charge-transfer interactions, meaning that $e_{\text{stationary}}$ decreases. Hence, the variation of e follows the variation of $e_{\text{stationary}}$.

As for the methanol–water mobile phase, the refractive index of water (1.333) is slightly higher than that of methanol [14]. This little difference explains why excess molar refractivity is almost constant over a large range of methanol percentage (from 10 to 100%) when PGC is used as stationary phase in HPLC. The greater difference between methanol and carbon dioxide induces a greater excess molar refractivity variation in SubFC (from 1.74 to 1.55; between 5 and 40% of methanol in carbon dioxide).

3.5.3. The a coefficient

The a term shows lower values than the two previous ones (v, e) but varies strongly with the modifier percentage. It is related to the H-bond donating ability of the solute, in other words with the electron-donating ability of the mobile and stationary phases. Merly et al. report the selective retention of metallic cations on a PGC column, with acidified aqueous mobile phases [48]. They suggest a charge-transfer interaction between the electronic cloud of the graphite and available orbitals of the metal ion centres.

The a term decreases very rapidly and is no more statistically sound when the percentage of methanol is higher than 30. This is corroborated by the fact that the retention factors for acidic solutes tend do decrease very rapidly between 5 and 20% of methanol, then slower between 20 and 40%. The a coefficient represents the following:

$$a = a_{\text{stationary}} - a_{\text{mobile}}$$

Carbon dioxide acts as a Lewis acid [20] while methanol, due to the hydroxyl group is a Lewis base. Therefore, when increasing the percentage of methanol in the mobile phase, a_{mobile} (representing the basic character of the mobile phase) increases, leading to a decrease of a. However, until 30% of methanol into the mobile phase, the Lewis basicity of the graphite surface is higher than the one of the mobile phase (a is positive), possibly due to the great electronic density at the edge of graphitic carbon [2].

Furthermore, a good correlation appears between a and the hydroxyl group number of substituted benzenes. a increases linearly with the −OH selectivity, showing that the weaker the basicity of the mobile phase, the greater the Lewis acid interactions of solutes.

3.6. Comparison of SubFC and HPLC results

As expected, the constant term c, related to the phase ratio, is almost identical in HPLC and SubFC because the same column is used. In HPLC, the three main contributions to retention are the dispersion interaction term and the excess molar refraction term, as in SubFC, but also the H-bond acceptor term. The latter indicates that the system is particularly selective towards analytes differing in their hydrogen-bond accepting ability (see Table 3).

3.6.1. The v coefficient

The v term in HPLC is close to the one obtained in SubFC because the two mobile phases have been chosen to have identical eluotropic strength based on the methylene selectivity. *V* is a combination of the endoergic cavity term and the exoergic dispersion interaction term. The latter always prevail over the former as well in SubFC [17] as in non-aqueous HPLC. However, when hydro–organic phases are

used in HPLC, the cavity formation in the mobile phase is not negligible explaining that v_{mobile} is higher than in SubFC [21].

3.6.2. The e and s coefficients

The *e* term is higher in SubFC than in HPLC, which means that the differences in the charge-transfer interactions between the solute and the stationary phase and between the solute and the mobile phase are greater in SubFC than in HPLC. Once again, when comparing methanol–water (90:10) to CO_2–methanol (90:10), the refraction index of water and methanol are higher than the one of CO_2. Then, e_{mobile} in SubFC is higher than e_{mobile} in HPLC. However, as *e* is higher in SubFC than in HPLC, necessarily $e_{stationary}$ is higher in SubFC than in HPLC. This is probably due to a greater adsorption of the liquid mobile phase onto the surface of PGC, reducing its ability to establish charge-transfer interactions.

The *s* term is positive in HPLC but not statistically significant in SubFC. However, the polarity of the liquid mobile phase being higher than that of the subcritical phase, s_{mobile} is higher in HPLC than in SubFC, meaning that the liquid mobile phase establishes more dipole–dipole type interactions with the solutes than the subcritical mobile phase.

Then, necessarily, $s_{stationary}$ is higher in HPLC than in SubFC, meaning that the stationary phase covered with liquid mobile phase establishes more dipole–dipole type interactions with the solutes than the stationary phase covered with subcritical mobile phase.

3.6.3. The a and b coefficients

Comparing the system constants obtained with isoeluotropic strengths, we notice that the main difference resides in the presence or absence of the H-bond terms. The *a* term, reflecting stationary and mobile phases H-bond accepting ability, is present and positive in the model calculated in SubFC but not statistically sound in HPLC, as reported previously with the same liquid mobile phase composition [21]. On the other hand, the *b* term, reflecting the stationary and mobile phases H-bond donating ability, is present and negative in the model calculated in HPLC but never statistically sound in SubFC, whatever the mobile phase composition. This is chemically sound as the H-bond donor and acceptor characters of the HPLC mobile phase are higher than the SFC mobile phase ones.

The *b* coefficient represents the following:

$$b = b_{stationary} - b_{mobile}$$

$b_{stationary}$ represents the hydrogen-bond donor ability of the PGC and is obviously zero. In SubFC, carbon dioxide cannot act as a hydrogen-bond donor. Adding methanol to the carbon dioxide probably increases b_{mobile} but, as the methanol adsorbs onto the stationary phase, $b_{stationary}$ increases as well and, globally, *b* is equal to zero. In HPLC, b_{mobile} is more important as both methanol and mainly water have H-bond donor ability and, globally, *b* is negative.

4. Conclusions

The solvation parameter model was successfully applied to describe retention on PGC in SubFC. The increase of methanol in the mobile phase reduces the retention by decreasing the *v*, *e* and *a* coefficients. Relationships between the methylene selectivity and the *v* coefficient, and between the hydroxyl selectivity and the *a* coefficient, were also reported. On the other hand, because the hydrogen-bond basicity is not selected as a pertinent descriptor in SubFC, no relationship takes place between the *b* and the *v* coefficients as it generally does in HPLC, as well by using ODS than PGC stationary phases. This is probably due to the lack of water in the carbon dioxide modified subcritical mobile phase. This lack of water also explains the lower value of the *v* coefficient in SubFC, because the unfavourable process of separating solvent molecules to provide a cavity for the solute is strongly reduced in carbon dioxide–methanol.

References

[1] J.H. Knox, M.T. Gilbert, UK Patent 7 939 449; J.H. Knox, M.T. Gilbert, US Patent 4 263 268.
[2] T. Hanai, J. Chromatogr. A 989 (2003) 183.
[3] P. Ross, J.H. Knox, Adv. Chromatogr. 37 (1997) 73.
[4] J. Kříž, E. Adamcová, J.H. Knox, J. Hora, J. Chromatogr. A 663 (1994) 151.
[5] N. Tanaka, K. Kimata, K. Hosoya, H. Miyanishi, T. Araki, J. Chromatogr. A 656 (1993) 265.
[6] M.-C. Hennion, V. Coquart, S. Guenu, C. Sella, J. Chromatogr. A 712 (1995) 287.
[7] P.T. Jackson, P.W. Carr, J. Chromatogr. A 958 (2002) 121.
[8] B.J. Bassler, R. Kaliszan, R.A. Hartwick, J. Chromatogr. 461 (1989) 139.
[9] R. Kaliszan, J. Chromatogr. A 656 (1993) 417.
[10] C.F. Poole, S.K. Poole, J. Chromatogr. A 965 (2002) 263.
[11] M.A. Al-Haj, R. Kaliszan, A. Nasal, Anal. Chem. 71 (1999) 2976.
[12] A. Sándi, L. Szepesy, J. Chromatogr. A 818 (1998) 1.
[13] A. Sándi, M. Nagy, L. Szepesy, J. Chromatogr. A 893 (2000) 215.
[14] M. Reta, P.W. Carr, P.C. Sadek, S.C. Rutan, Anal. Chem. 71 (1999) 3484.
[15] M. Rosés, D. Bolliet, C.F. Poole, J. Chromatogr. A 829 (1998) 29.
[16] K. Valkó, M. Plass, C. Bevan, D. Reynolds, M.H. Abraham, J. Chromatogr. A 797 (1998) 41.
[17] M.H. Abraham, G.S. Whiting, R.M. Doherty, W.J. Shuely, J. Chromatogr. 518 (1990) 329.
[18] W. Kiridena, W.W. Koziol, C.F. Poole, J. Chromatogr. A 932 (2001) 171.
[19] M.H. Abraham, C.F. Poole, S.K. Poole, J. Chromatogr. A 842 (1999) 79.
[20] J.D. Weckwerth, P.W. Carr, Anal. Chem. 70 (1998) 1401.
[21] C. Lepont, A.D. Gunatillaka, C.F. Poole, Analyst 126 (2001) 1318.
[22] J.A. Blackwell, R.W. Stringham, J.D. Weckwerth, Anal. Chem. 69 (1997) 409.
[23] G.O. Cantrell, R.W. Stringham, J.A. Blackwell, J.D. Weckwerth, P.W. Carr, Anal. Chem. 68 (1996) 3645.
[24] D. Pyo, H. Kim, J.H. Park, J. Chromatogr. A 796 (1998) 347.
[25] D. Pyo, W. Li, M.L. Lee, J.D. Weckwerth, P.W. Carr, J. Chromatogr. A 753 (1996) 291.
[26] J.A. Blackwell, R.W. Stringham, Chromatographia 46 (1997) 301.
[27] J.A. Blackwell, R.W. Stringham, J. High Resolut. Chromatogr. 20 (1997) 631.

[28] D.M. Heaton, K.D. Bartle, A.A. Clifford, M.S. Klee, T.A. Berger, Anal. Chem. 66 (1994) 4253.
[29] T.M. Engel, S.V. Olesik, Anal. Chem. 63 (1991) 1830.
[30] E. Lesellier, K. Gurdale, A. Tchapla, Chromatographia 55 (2002) 555.
[31] S.K. Poole, C.F. Poole, J. Chromatogr. A 845 (1999) 381.
[32] M.H. Abraham, H.S. Chadha, R.A.E. Leitao, R.C. Mitchell, W.J. Lambert, R. Kaliszan, A. Nasal, P. Haber, J. Chromatogr. A 766 (1997) 35.
[33] S.K. Poole, S. Patel, K. Dehring, H. Workman, J. Dong, J. Chromatogr. B 793 (2003) 265.
[34] R. Kaliszan, M.A. van Straten, M. Markuszewski, C.A. Cramers, H.A. Claessens, J. Chromatogr. A 855 (1999) 455.
[35] J. Li, J. Chromatogr. A 982 (2002) 209.
[36] A. Sandi, M. Naguy, L. Szepezy, J. Chromatogr. A 893 (2000) 215.
[37] M.D. Trone, M.G. Khaledi, J. Chromatogr. A 886 (2000) 245.
[38] K. Valkonetbal, J. Chromatogr. A 797 (1998) 41.
[39] V. Coquart, Ph.D. thesis, Université Paris 6, France, 1993.
[40] R.F. Rekker, The Hydrophobic Fragmental Constant, Elsevier, Amsterdam, 1977.
[41] R. Kaliszan, Anal. Chem. 64 (1992) 619A.
[42] R.M. Smith, Anal. Chem. 56 (1984) 256.
[43] K. Gurdale, E. Lesellier, A. Tchapla, Anal. Chem. 71 (1999) 2164.
[44] M.F. Emery, C.K. Lim, J. Chromatogr. 479 (1989) 212.
[45] G. Gu, C.K. Lim, J. Chromatogr. 515 (1990) 183.
[46] C. Elfakir, P. Chaimbault, M. Dreux, J. Chromatogr. A 829 (1998) 193.
[47] T. Takeuchi, T. Kojima, T. Miwa, J. High Resolut. Chromatogr. 23 (10) (2000) 590.
[48] C. Merly, B. Lynch, P. Ross, J.D. Glennon, J. Chromatogr. A 804 (1998) 187.