

Retention mechanism in reversed-phase liquid chromatography: specific effects of propiophenyl and multifunctional (cyanopropyl–octadecyl) bonded silicas

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Abstract

Accurate measurements of the retention of homologous series were made on a phenyl-3-propyl-bonded silica and on a multifunctional silica (simultaneously bonded with *n*-octadecyl and cyanopropyl groups). It is shown that the retention of these types of phases is governed by their hydrocarbonaceous part, as on pure *n*-alkyl-bonded silicas. Energetic considerations gained from plots of $\log k'$ vs. $\log k'$ allow one to characterize global effects of an organic modifier on the chromatographic equilibrium. In particular, the influence of π – π interactions on retention is shown. These types of curves enable one also to compare different columns in RPLC. In a general way it is shown that the specific effects are superimposed on the effect of hydrocarbonaceous parts, which is always predominant. The mechanism of insertion of the solute is always valid and multifunctional phases are intermediate between pure alkyl-bonded silica and functionalized phases.

Keywords: Retention mechanism; Multifunctional bonded silica; Propiophenyl bonded silica; Stationary phases, LC; Mobile-phase effects

1. Introduction

Reversed-phase liquid chromatography (RPLC) is one of the most commonly used techniques for the separation of compounds in complex mixtures. The most commonly used phases are alkyl-bonded silicas. There also exist functional alkyl-bonded phases whose main advantage is a selectivity which is different from those of alkyl-bonded silicas, thus allowing separations of solutes of similar structures. This is the

reason why the synthesis and the use of bonded phases containing aromatic moieties such as phenyl groups or derivatives [1–6], cyano groups [7–11], alkylamide groups [12,13] and cyclodextrins [14] have been developed.

Therefore, in order to make better use of these types of phases, it is necessary to characterize the molecular interaction mechanism. The mechanism has been widely studied in the case of alkyl-bonded phases and two theories rationalize it: the solvophobic theory developed by Melander and Horvath [15], who describe a mechanism of association and observe a rather passive role

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to the stationary phase in the retention mechanism, whereas the lattice theory of Martire and Boehm [16] or of Dill [17] and Dorsey and Dill [18] is more in favour of a mechanism of partition (and even adsorption) and considers the stationary phase as an essential component for the retention.

However, in spite of these two different interpretations, they both take into account the solubility of the solutes in the mobile phase and the creation of a cavity in this phase. This cavity is proportional to the solute size, and particularly for homologous series the retention of these homologues increases with increasing carbon number of the alkyl chain of the solute (n) [19,20]. As the free energy change due to cavity formation is proportional to the surface tension of the mobile phase, the retention of the same solute in different aqueous–organic solvent mixtures can be correlated with their surface tensions [15,17]. A certain number of studies has been carried out attempting to establish a correlation between the surface tension of the mobile phase and the retention properties of the solute [15], in particular homologous series [21].

The correlation is not completely perfect. However, it has been demonstrated with many homologous series that it is qualitatively valid to predict their retention behaviour in a mobile phase such as methanol–water and acetonitrile–water on octadecyl-bonded silica [22]. Two exceptions were reported: one occurs when analysing solute with π electrons; the other using tetrahydrofuran (THF)–water as mobile phase [22]. Thus, for solutes with π electrons there is a shift of the crossover of the plots of $\log k'$ vs. percentage of methanol and $\log k'$ vs. percentage of acetonitrile towards high percentages of organic solvent, or a loss of this crossover compared with the crossover observed for the curves obtained with n -alkanes. This was related to the development of π – π interactions between the solute and acetonitrile [22].

We propose here to extend these studies by looking at the effects of the nature of the graft, such as a propiophenyl-bonded phase or a multifunctional phase (silica grafted simultaneously with octadecyl and cyanopropyl chains). With

these phases, solute–mobile phase, solute–stationary phase and mobile phase–stationary phase π – π interactions can exist with acetonitrile and solutes with π electrons [23]. We also investigated whether the particular effect previously observed with THF as organic modifier is always valid with these two kinds of bonded silicas.

In order to discriminate these different cases, we use a graphical representation described for the first time by Melander et al. [24], which permits us to compare the retentions of solutes on different alkyl-bonded silicas. Some other workers have used this to classify different packings [25] and to characterize specific interactions involving the stationary phase [1,26–30].

2. Experimental

2.1. Reagents

Methanol (Carlo Erba, Milan, Italy) and acetonitrile (Merck, Darmstadt, Germany) were of HPLC grade. They were filtered through a 0.5- μ m Millipore filter (Whatman, Hillsboro, OR, USA). Water was of ultra-high quality (18 M Ω cm) from an Elgastat UHQ system (Elga, High Wycombe, UK).

Homologous series ($C_nH_{2n+1}Z$, where $Z = H, Cl, OH, Ph, COOMe$ and $1 \leq n \leq 24$) were obtained from various commercial sources.

2.2. Equipment

The apparatus consisted of a PE Series 410 Bio pump (Perkin-Elmer, Norwalk, CT, USA), a Model 7125 injection valve with a 20- μ l loop (Rheodyne, Cotati, CA, USA), an R 401 refractive index detector (Waters, Milford, MA, USA), a Model 757 UV spectrophotometer detector (254 nm) (Kratos, ABI, Ramsey, NJ, USA) and a DDL 21 light-scattering detector (Eurosep, Cergy St. Christophe, France).

The column temperature was controlled using a Croco-cil oven (Cluzeau, Sainte-Foy-la-Grande, France) thermostated with water by means of a Model HS 40 cryostat (Huber,

Offenburg-Elgersweier, Germany) or a Model UC F10 cryostat (Julabo, Seelbach, Germany, supplied by Touzart et Matignon, Vitry sur Seine, France) with a precision of 0.1°C.

Three columns were used: Hypersil ODS (5 μm), 150 \times 4.6 mm I.D. (Shandon, Runcorn, UK), laboratory packed; Zorbax Phenyl (5 μm), 250 \times 4.6 mm I.D. (DuPont, Wilmington, DE, USA); and Spherisorb Mixte, 150 \times 4.6 mm I.D. (Phase Sep, Deeside, UK).

The eluent flow-rate was 1 ml/min.

2.3. Methods

All retention values are reported in terms of capacity factor, k' . The calculation of this parameter requires the determination of the void volume (or dead volume marker) of the column used. The role of the dead volume marker was discussed previously [20]. In the range of column porosity ($\epsilon_T = 0.5\text{--}0.8$) we have verified that the plots $\log k'$ versus n_c are either a straight line with a break (for all MeOH–water and MeCN–water mixtures investigated) or curved (THF–water mixtures), independently of the value of ϵ_T chosen to determine k' values. This was true independently of the k' values in a range of $\log k'$ such as -0.5 to 1.5 for the first-eluted homologues. Consequently, the knowledge of the true value of dead time was not necessary to develop the following study. Thus, the weighing method was used for this purpose. Each k' value reported is the average from at least three reproducible injections. The accuracy of the measurements and the mathematical treatment of the results were as described previously [31].

Under all the investigated experimental conditions (excepted for THF–water mixtures), it has been reported previously that the linearization of curves of $\log k'$ vs. n_c gives correlation coefficients higher than 0.99. If we consider $\log k'$ vs. $\log k'$ curves, theoretically a minimum of four different members of each homologous series would be considered statistically acceptable to define a linear relationship. Based on the F -test for significance of an additional term in multiple regression a correlation coefficient r^2

higher than 0.99 is required at 95% confidence for four data points.

For all cases studied, the linear correlation coefficients were always higher than 0.99. In some cases, we have only reported the figures for the smaller and higher homologues ($n = 7$, $n = 14$) used to draw the straight line, but calculations were made with at least four homologues for each series.

3. Theoretical

For each solute, the retention is given by the following equation:

$$\log k' = \frac{-\Delta G^\circ}{2.3RT} + \log \phi \quad (1)$$

where ΔG° is the free energy of transfer of a molecule from the mobile phase to the stationary phase and ϕ is the phase ratio.

For a given monofunctional homologue $Z(\text{CH}_2)_n\text{H}$, ΔG° can be decomposed into terminal (due to the terminal heads Z and H), $\Delta G_Z^\circ + \Delta G_H^\circ$, and core (due to the alkyl chain), $\Delta G_{n\text{CH}_2}^\circ$, contributions according to the following equation:

$$\Delta G^\circ = \Delta G_Z^\circ + \Delta G_H^\circ + \Delta G_{n\text{CH}_2}^\circ \quad (2)$$

Moreover, in the case of bonded phases with monomeric behaviour [32], this non-polar contribution $\Delta G_{n\text{CH}_2}^\circ$ can be decomposed into individual contributions of each methylene group of the alkyl chain, provided the chain length is long enough [20,33]. The compilation of the results obtained during the last 10 years on the behaviour of homologous series in RPLC was recently reviewed [22]. Thus the well known linear relationship between $\log k'$ and the number of carbon atoms in homologues is established. It must be noted that the number of methylene units associated with different terminal groups Z are not similar in number. Thus the effective terminal group includes several repeated methylene units before a linear $\log k'$ vs. n_c plot is observed. It is always valid independently of the nature of homologous series tested

provided that $n \geq 6$, using monofunctional alkylated bonded silicas with organic–water mobile phases of different compositions (MeOH or MeCN). In the last case, we can write

$$\Delta G_{n\text{CH}_2}^\circ = n(\Delta G_{\text{CH}_2}^\circ) \quad \text{with } n \geq 6 \quad (3)$$

By combining Eqs. 1–3, one obtains Eq. 4, which has also been reported as Eq. 5 [34], where p corresponds to the contribution of the methylene selectivity ($\log \alpha$) and q represents specific interactions to the retention [35].

$$\log k' = \frac{-[\Delta G_Z^\circ + \Delta G_H^\circ]}{2.3RT} + \log \phi - \frac{n \Delta G_{\text{CH}_2}^\circ}{2.3RT} \quad (4)$$

$$\log k' = q + pn \quad (5)$$

In order to compare the energetics of the phenomenon, either by using the same stationary phase with two different mobile phase compositions or two different stationary phases percolated by the same mobile phase, we considered Eqs. 4 and 5 in the following way.

By expressing $(\log k')_A$ of a solute under conditions A versus $(\log k')_B$ of the same solute under conditions B, the equation of $(\log k')_A$ vs. $(\log k')_B$ for all the homologues of a given series is given by Eq. 6 or 7:

$$\begin{aligned} (\log k')_A = & \frac{(\Delta G_{\text{CH}_2}^\circ)_A}{(\Delta G_{\text{CH}_2}^\circ)_B} (\log k')_B + \frac{(\Delta G_{\text{CH}_2}^\circ)_A}{(\Delta G_{\text{CH}_2}^\circ)_B} \\ & \times \left[\frac{(\Delta G_Z^\circ)_B + (\Delta G_H^\circ)_B}{2.3RT} - (\log \phi)_B \right] \\ & - \frac{(\Delta G_Z^\circ)_A + (\Delta G_H^\circ)_A}{2.3RT} + (\log \phi)_A \quad (6) \end{aligned}$$

$$\log k'_A = \frac{p_A}{p_B} \cdot \log k'_B + \frac{p_A}{p_B} \cdot q_B - q_A \quad (7)$$

Therefore, homologues of a given series should fall on a straight line with a slope which is the ratio of the logarithm of the methylene selectivity under each of the conditions:

$$\frac{p_A}{p_B} = \frac{(\Delta G_{\text{CH}_2}^\circ)_A}{(\Delta G_{\text{CH}_2}^\circ)_B} = \frac{(\log \alpha)_A}{(\log \alpha)_B} \quad (8)$$

According to either the hydrophobic theory or partition theory, the change in $\log k'$ of consecutive members of homologous series ($\log \alpha$) is directly related to the variation of the contact area ΔA between the solutes with the bonded species on silica (or the hydrocarbonaceous area of the solute) when adding a methylene group to the solute chain [15]:

$$\log \alpha = \Delta(\Delta A)(a_s + \gamma) \quad (9)$$

It is also correlated with the properties of the mobile phase since γ corresponds to the surface tension of the bulk solvent and a_s is a characteristic constant of the solvent.

Therefore, the ratio p_A/p_B provides a scale which leads to a comparison of the molecular mechanisms which occur [24].

A linear correlation of the plots $\log k'_A$ vs. $\log k'_B$ with unit slope indicates an homoenergetic process which characterizes an identical retention mechanism under the two conditions A and B. A linear correlation with a slope different from unity indicates a similar but not identical retention mechanism and is termed a homoenergetic process. The absence of correlation indicates a heteroenergetic process and a mixed or different retention mechanism. (This is the case for phases with polymeric behaviour [32] or when tetrahydrofuran is used [21].) If the slope is greater than unity, the conditions A are more hydrophobic than B, which enables one to define a hydrophobic scale, such as the n^* value defined by Czok and Engelhardt [36].

Under the same chromatographic conditions, homologous series with different head groups and similar polarity have close methylene selectivities [37], and one could consider obtaining approximately parallel straight lines for plots of $\log k'$ vs. $\log k'$ drawn for the different series.

For a given homologous series, the intercept with the ordinate cannot be easily interpreted because its expression depends on the phase ratios (see Eq. 6), which change with the column and the mobile phase and are difficult to estimate [35,38–40].

However, if we consider the difference of the intercepts with the ordinate (characterized by

ΔO) between two straight lines corresponding to two homologous series of head groups Z and Z', the phase ratios cancel and the expression of ΔO is given by

$$\Delta O_{(Z'-Z)} = \frac{p_A}{p_B} (q_B^Z - q_B^{Z'}) - (q_A^Z - q_A^{Z'}) \quad (10)$$

Let us consider the alkanes as a reference ($Z = H$) and a series with head group Z' (RZ') such that $\Delta O > 0$. Knowing that the more negative is ΔG° (higher value of q) the more retained the solute is (Eq. 4), we have the case where RZ' has a weaker interaction with the stationary phase (or a stronger one with the mobile phase) than RH under conditions B than conditions A. In other words, there is a global effect of acceleration for the conditions B in comparison with A for all the homologous series for which the straight lines are above that of the alkanes and vice versa for the straight lines below. It has been stated before that q_B and q_A reflect the specific interactions between the head group (Z or Z') and the solvent. The comparison of the values of $(q_B^H - q_B^{Z'})$ and $(q_A^H - q_A^{Z'})$ permits us to demonstrate the specific effect on homologous series RZ' of conditions B compared with the conditions A. For instance, if $(q_A^H - q_A^{Z'})$ is equal to $(q_B^H - q_B^{Z'})$, no specific effect which occurs. However, these specific effects are not only responsible for the positive or negative value of ΔO . When there is a change in methylene selectivity between conditions A and B ($p_A/p_B \neq 1$), a positive or negative value of ΔO could be observed, owing to the value of p_A/p_B , even if identical values of $(q_B^H - q_B^{Z'})$ and $(q_A^H - q_A^{Z'})$ are observed. Hence it must be noted that the effects of either retardation or acceleration reflect two different contributions: one due to the specific effects indicated above and the other due to the non-specific selectivity of methylene groups, p_A/p_B .

Therefore, this study will allow us to discuss specific effects of solvents (when working on the same column with two different mobile phases) or specific effects of phases (the same mobile phase and two different columns).

4. Results and discussion

4.1. Molecular mechanism of retention of functionalized stationary phases

In order to ensure that the two functional bonded phases function in the RPLC mode, we studied the behaviour of different homologous series with organic modifier–water mixtures of various compositions.

For the phenyl-3-propyl-bonded silica, as for pure alkyl-bonded silicas, the plots of $\log k'$ vs. n_c (with $n_c \geq 6$) are linear for mobile phases constituted by MeOH–water mixtures and curved for THF–water mixtures (Fig. 1). For MeCN–water, the plots $\log k'$ vs. n_c seems slightly curved, especially for shorter homologues. This was not confirmed by α vs. n_c curves, which are more sensitive to slight changes in selectivity. As demonstrated previously [20,21], a convex shape of $\log k'$ vs. n_c plots corresponds to a regular decrease in α vs. n_c plots (here this is only the case for THF–water mixtures). In contrast, a variation of $\log k'$ vs. n_c represented by two broken lines exhibits plots of α vs. n_c with two plateaux separated by a drop [20,31]. This latest shape of the curves was always observed for plots in MeOH–water and in MeCN–water mixtures. Thus, as can be seen in Fig. 2 (given for MeCN–water mixtures as an example), the methylene selectivity increases drastically for shorter homologues. From a value of n_c higher than 5–7, the methylene selectivity presents a plateau exactly as observed previously with pure alkyl-bonded silica. Assuming that a phenyl group is equivalent to three methylene groups, the equivalent chain length of phenyl-3-propyl-bonded silica corresponds to a hexyl alkyl chain. Consequently, it could be considered that the enhancement of selectivity observed with phenyl-3-propyl-bonded silica for homologues shorter than six carbon atoms corresponds to the jump observed on the same curve (α vs. n_c) with a pure alkyl-bonded chain. The plateau of selectivity generally observed for values of n_c less than the length of the alkyl-bonded chain is not observed here owing to the small number of

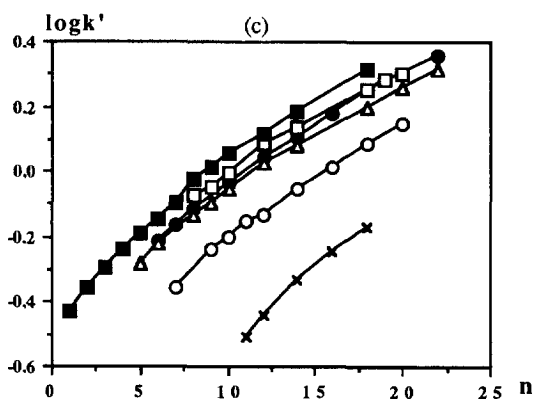
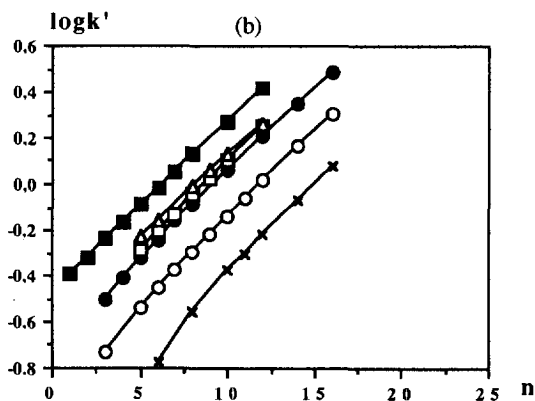
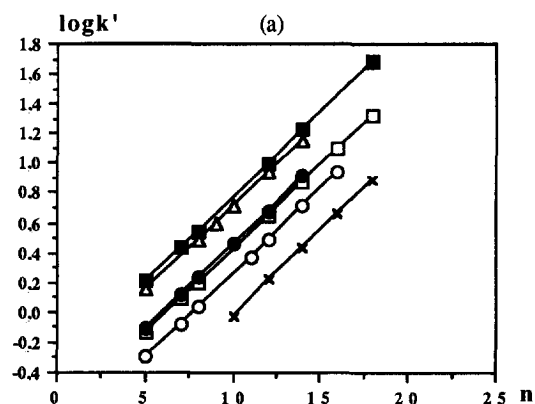


Fig. 1. $\log k'$ vs. n for different homologous series on the propyl-3-phenyl-bonded silica. (a) MeOH–H₂O (80:20); (b) MeCN–H₂O (80:20); (c) THF–H₂O (70:30). ■ = Phenylalkanes; □ = alkanes; △ = benzoates; ○ = methyl esters; ● = chloroalkanes; × = alcohols.

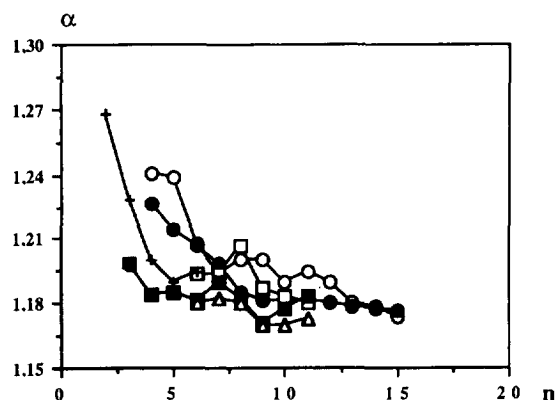


Fig. 2. α vs. n for different homologous series on the propyl-3-phenyl-bonded silica. Mobile phase, MeCN–H₂O (80:20). Symbols as in Fig. 1; + = PhCOR.

homologues which fall before the break and could be used to prove it ($n = 3$). Such a phenomenon was previously observed for a hexyl-bonded silica [20]. In the case of mixed monofunctional bonded silicas, the curves of $\log k'$ vs. n_c are linear and show a double break. Thus a molecular mechanism of insertion proposed elsewhere [22] to account for the results with a C₁₈-bonded silica could also be proposed to account for the results observed with these two functionalized bonded silicas when used with MeOH–water and MeCN–water mixtures.

In a first approximation, whatever the chromatographic conditions, provided that we work with the same stationary phase and the same mobile phase composition, the methylene selectivity can be considered to be identical for all homologous series [37]. Thus, q values (Eq. 5) give a comparative scale of retention for different stationary phases.

As shown in Fig. 3, the relative retention order of the different series studied is not affected by the nature of the three types of bonded silica used in this study with an MeOH–water mobile phase, from 0% to 50% water content. The same phenomenon has been observed using MeCN instead of MeOH [41].

Moreover, a comparative examination of the plots of p vs. percentage of organic modifier (MeOH and MeCN) was made for the three different bonded silicas (Fig. 4). The plots are

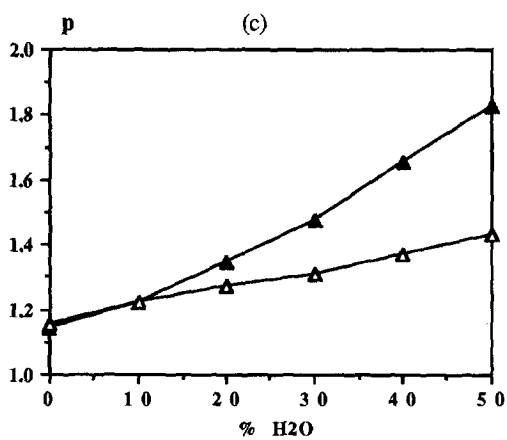
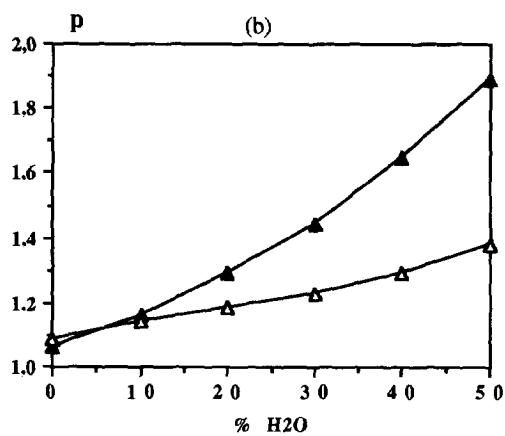
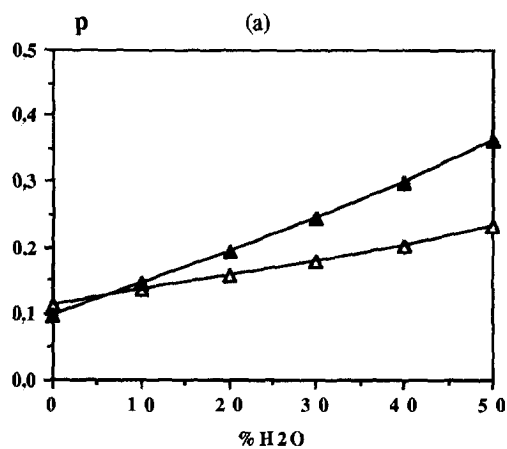
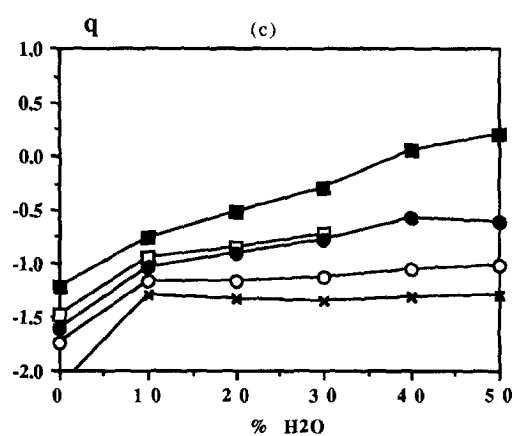
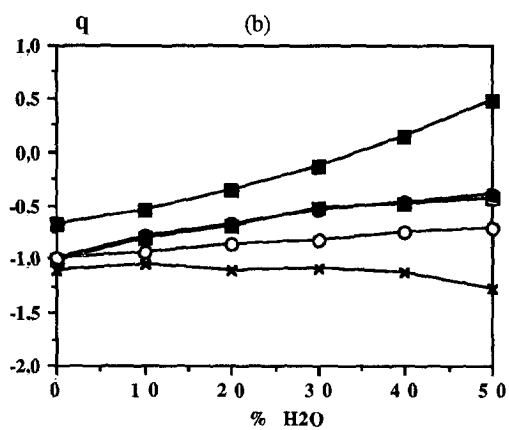
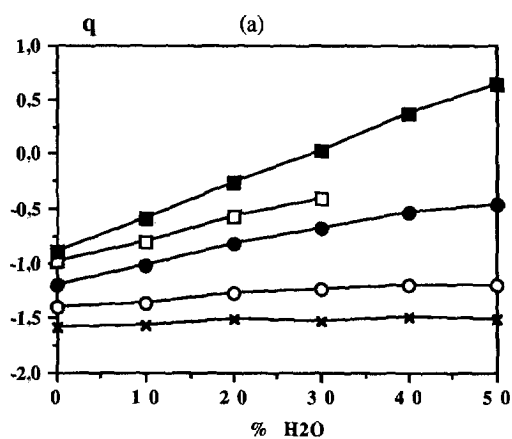


Fig. 3. q vs. percentage of water in the MeOH–H₂O mobile phase for different homologous series. (a) On C₁₈-bonded silica; (b) on propyl-3-phenyl-bonded silica; (c) on multifunctional bonded silica. Symbols as in Fig. 1.

Fig. 4. p vs. percentage of water in the mobile phase. (a) On C₁₈-bonded silica; (b) on propyl-3-phenyl-bonded silica; (c) on multifunctional bonded silica. Mobile phase: \blacktriangle = MeOH–H₂O; \triangle = MeCN–H₂O.

similar, which demonstrates that the global influence of the nature of the organic modifier on the retention is not drastically affected by the insertion of a bonded cyanopropyl group inside a bonded octadecyl chain or by replacing a methyl terminal group with a phenyl terminal group. Hence these two types of phases behave like reversed phases.

Phenyl-3-propyl phase specific effects

Plots of $\log k'$ vs. $\log k'$ were constructed to compare the effects of a phenyl-3-propyl-bonded phase with those of an octadecyl-bonded phase. The results are reported in Table 1 and as an

example in Fig. 5. A linear correlation ($r > 0.99$) was found in each case. The slope given here for the alkanes is always higher than 1.00, which confirms an homoenergetic process of retention between the two phases.

With a pure organic mobile phase, the value of the slope shows that the methylene selectivity on the phenyl-3-propyl-bonded phase is three to four times weaker than that on a C_{18} -bonded phase, which could affect drastically the separation on a propiophenyl-bonded silica (Table 1). This difference decreases if aqueous mixtures are used, leading to a value of 1.58 for the slope. Accordingly, the hydrophobicity difference be-

Table 1
Log k' vs. log k' : stationary phase effect

System	H ₂ O In mobile phase (%)	Z	Organic modifier MeOH		Organic modifier MeCN	
			p_A/p_B	Relative retention effect/ alkanes ^a	p_A/p_B	Relative retention effect/ alkanes ^a
A = C ₁₈ ; B = phenyl-3-propyl	0	H	2.75	(0)	3.83	(0)
		COOMe	3.23	(-)	3.05	(+)
		Ph	3.37	(-)	4.31	(-)
	10	H	2.21	(0)	2.4	(0)
		COOMe	2.03	(-)	2.19	(-)
		Ph	2.20	(-)	2.54	(-)
		OH	2.26	(-)	2.55	(-)
		Cl	2.15	(-)	2.30	(-)
	20	H	1.69	(0)	2.10	(0)
		COOMe	1.71	(-)	1.99	(-)
		Ph	1.68	(-)	2.16	(-)
		OH	1.72	(-)	2.07	(+)
		Cl	1.68	(-)	2.09	(-)
	30	H	1.58	(0)	1.93	(0)
		COOMe	1.53	(-)	1.78	(-)
Ph		1.51	(-)	1.97	(-)	
OH		1.59	(0)	1.95	(+)	
Cl		1.51	(-)	1.98	(-)	
A = C ₁₈ ; B = mixed	0	H	1.41	(0)	1.56	(0)
		COOMe	1.20	(-)	×	×
		Ph	1.63	(-)	1.61	(0)
		OH	×	×	1.96	(-)
		Cl	1.22	(-)	1.58	(0)
A = mixed; B = phenyl-3-propyl	0	H	1.99	(0)	2.51	(0)
		Ph	2.60	(-)	2.68	(-)

^a (0) = No effect; (-) = retardation; (+) = acceleration.

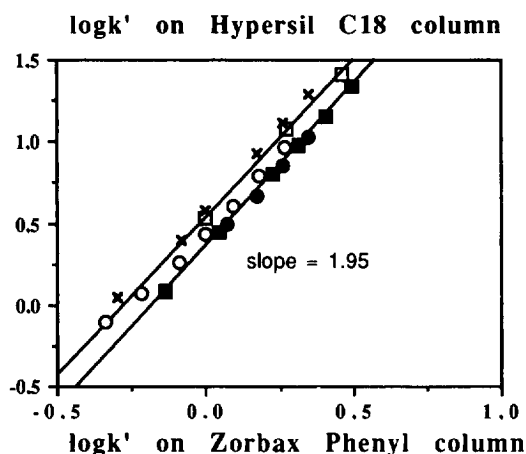


Fig. 5. Log k' on a Hypersil C_{18} column vs. log k' on a Zorbax phenyl column mobile phase, MeCN– H_2O (70:30). Symbols as in Fig. 1. The slope corresponds to the ratio of methylene selectivity obtained for the alkanes.

tween these two phases vanishes in aqueous–organic mixtures containing increasing amounts of water.

In a mobile phase containing methanol, the phenyl-3-propyl-bonded silica leads to a global retentive effect compared with a C_{18} -bonded silica [reported as (–) in Table 1]. Independently of the nature of the series tested, the values of $(q^H - q^{Z'})$ are always smaller on phenyl-3-propyl-bonded silica than on octadecyl-bonded silica (Table 2). The same effect is observed when using acetonitrile instead of methanol, excepted for alcohols, for which a weak effect of acceleration (+) is observed.

Multifunctional phase specific effects

Table 1 compares the multifunctional bonded-phase silica bonded by octadecyl and cyano-

Table 2
Specific effects of homologous series for different chromatographic systems

Z'	H_2O (%)	$(q^H - q^{Z'})_{MeOH}$			$(q^H - q^{Z'})_{MeCN}$		
		Phenyl-3-propyl	Mixed	C_{18}	Phenyl-3-propyl	Mixed	C_{18}
Cl	0	–0.023	0.130	0.213	0.046	0.148	0.250
	10	–0.020	0.076	0.202	0.025	0.108	0.230
	20	–0.026	0.063	0.243	0.037	0.089	0.257
	30	0.008	0.050	0.275	0.020	0.069	0.280
	40	0.007			0.034	0.072	0.267
	50	–0.035			0.073		
Ph	0	–0.334	–0.261	–0.100	–0.093	–0.072	–0.045
	10	–0.247	–0.185	–0.219	–0.149	–0.108	–0.120
	20	–0.329	–0.324	–0.311	–0.203	–0.148	–0.169
	30	–0.408	–0.421	–0.430	–0.245	–0.244	–0.214
	40	–0.638			–0.287	–0.306	–0.254
	50	–0.906			–0.379		
COOMe	0	–0.026	0.248	0.416	0.239	0.185	0.479
	10	0.134	0.208	0.555	–	0.244	0.550
	20	0.165	0.318	0.700	0.269	0.355	0.625
	30	0.282	0.407	0.828	0.353	0.336	0.697
	40	0.257			0.416	0.437	0.764
	50	0.283			0.507		
OH	0		0.654	0.588		–0.292	0.543
	10		0.335	0.745		0.294	0.756
	20		0.483	0.939		0.485	0.955
	30		0.626	1.118		0.573	1.079
	40					0.709	1.195
	50						

propyl chains (Spherisorb ODCN column denoted "Mixed") to the octadecyl chain. In pure MeOH, one observes a global retentive effect of phenylalkanes on the multifunctional bonded silica in comparison with the alkyl phase.

The influence of the nature of the head of the series is not easy to understand. For chlorides and methyl esters the slopes are not parallel to that observed for the alkane, which suggests a homoenergetic process of retention. In acetonitrile these effects vanish: chlorides, alkanes and phenylalkanes give plots of $\log k'$ vs. $\log k'$ very close together. A global retentive effect is just observed for the alcohols.

The comparison of this column with the phenyl-3-propyl-bonded silica column shows a global retentive effect on the phenylalkanes on the phenyl-3-propyl-bonded column in comparison with the multifunctional bonded silica.

All these results indicate a global specific effect of the multifunctional bonded phase towards aromatic solutes, but they also show that this effect is less marked than for the phenyl-3-propyl-bonded silica, as one would expect.

To take an advantage of these π - π effects, we tested the separation of isomeric solutes possessing multiple double bonds. As the retention mechanism on multifunctional bonded silica is by insertion of the solute between the grafted chains, studies of separations of mixed triglycerides with different degrees of unsaturation, differently distributed along the chains, were carried out. Specific interactions occur between a π solute and a mixed bonded phase or phenyl phase [23,42,43], but the optimum separation was obtained with a pure C_{18} -bonded silica [44].

4.2. Effect of mobile phase composition

Solute-mobile phase interactions on alkyl-bonded silica

Before studying the specific effects of the functional bonded silicas, and in order to illustrate the solvent effects, we then considered previously published results [20,21,38].

The results of the examination of the plots of $\log k'$ vs. $\log k'$ on a C_{18} column are given in Table 3 and as an example in Fig. 6. Regardless

of the percentage of water in the mobile phase, there is a global effect of acceleration of MeCN-water mixtures in comparison with MeOH-water mixtures for the phenylalkanes and a weak effect of acceleration for the chloroalkanes. With the methyl esters and the alcohols, a retentive effect is observed in pure MeCN in comparison with pure MeOH, but an acceleration effect is effective as soon as water is added to the mobile phase. It appears that the magnitude of the phenomenon is different for the same head groups in aromatic series [45].

Using propiophenyl-bonded silica, plots $\log k'$ in MeOH-water vs. $\log k'$ in MeCN-water were constructed. As can be seen from Fig. 7 and Table 3, there is a global acceleration effect of MeCN-water mixtures in comparison with MeOH-water mixtures for the alcohols and the methyl esters and a small acceleration effect for the phenyl and chlorinated solutes. Compared with results obtained with an octadecyl-bonded silica, they are qualitatively similar.

Solute-mobile phase interactions

With the aim of elucidating the reason for these different types of behaviour, we have reported in Table 2 the variation of $(q^H - q^{Z'})$ with the composition of mobile phase for the four series tested ($Z' = \text{Ph, OH, COOMe, Cl}$) and for the three columns studied. Owing to the respective values of q^H and q^{Ph} , the difference $(q^H - q^{\text{Ph}})$ is always higher in acetonitrile than in methanol (independently of the percentage of water), showing that there are π - π interactions between MeCN and the phenylalkanes on the three bonded phases. These π - π effects have been discussed elsewhere [21,23,31]. The study of $[(q^H - q^{Z'})_{\text{Phenyl}} - (q^H - q^{Z'})_{C_{18}}]$ for a given mobile phase shows that this difference is always negative, whatever the percentage and the organic solvent (Table 2). Hence there is a specific retentive effect of a propiophenyl-bonded group compared with an octadecyl-bonded group.

The same conclusions could be drawn for methyl esters chromatographed on C_{18} silicas, considering mobile phases with water contents higher than 10%. However, the opposite is

Table 3
Log k' vs. log k' : solvent effect

Column	H ₂ O (%)	Z	p_A/p_B ^a	Relative retention effect/alkanes ^b
C ₁₈	0	H	0.87	(0)
		COOMe	0.87	(-)
		Ph	0.87	(+)
		OH	0.87	(-)
		Cl	0.87	(-)
	10	H	1.07	(0)
		COOMe	1.08	(+)
		Ph	1.07	(+)
		OH	1.08	(+)
		Cl	1.07	(-)
	20	H	1.22	(0)
		COOMe	1.22	(+)
		Ph	1.22	(+)
		OH	1.22	(+)
		Cl	1.22	(+)
30	H	1.36	(0)	
	COOMe	1.38	(+) ≈ (0)	
	Ph	1.37	(+) ≈ (0)	
	OH	1.37	(+)	
Phenyl-3-propyl	10	H	1.18	(0)
		COOMe	1.19	(+)
		Ph	1.35	(+)
		OH	1.24	(+)
		Cl	1.15	(+)
	20	H	1.47	(0)
		COOMe	1.46	(+)
		Ph	1.58	(+)
		OH	1.36	(+)
		Cl	1.41	(+)
	30	H	1.67	(0)
		COOMe	1.79	(+)
		Ph	1.82	(+)
		OH	1.80	(+)
		Cl	1.79	(+)

^a A = MeOH; B = MeCN.

^b See Table 1.

observed with phenyl-bonded silica and the role of this polar head seems unclear. For chlorides, the observation leads to a negligible specific influence of the nature of the solvent for this series (the difference between $q^H - q^{Cl}$ in MeOH and MeCN is always smaller than 0.04 in the range of the mobile phase compositions investigated).

Surprisingly, the difference ($q^H - q^{OH}$) is approximately the same in MeCN as in MeOH

independently of the water content in mobile phase, which seems to indicate that there is no difference in specific interactions for alcohols when they are chromatographed in aqueous MeCN or MeOH.

Mobile phase–stationary phase interactions

As reported in Theoretical section, the change in ΔO values vs. percentage of water is due not only to the difference in ($q^H - q^Z$) values ob-

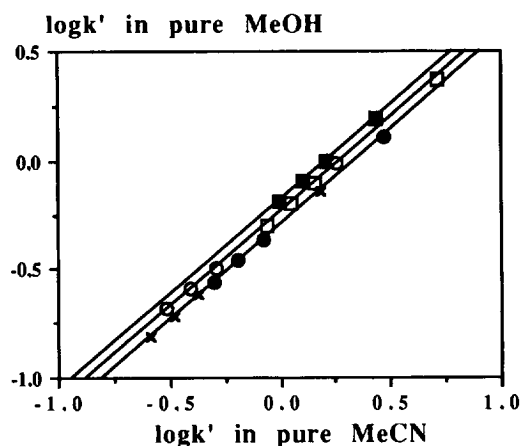


Fig. 6. Log k' in pure MeOH vs. log k' in pure MeCN on Hypersil C_{18} . Symbols as in Fig. 1.

tained under conditions B or A (Table 2), but also to the enhancement of p_A/p_B values. This is correlated with a differential effect of the organic modifier and permit one particularly to explain why ΔO increases with increasing percentage of water in methanol relative to acetonitrile for n -alkanols on C_{18} -bonded silica. The variation of the ratio p_A/p_B versus percentage of water mixed with methanol and acetonitrile is reported in Fig. 8.

In a first step, one could think that this effect is due only to the properties of the bulk mobile phase (see Eq. 9). However, the ratio of the surface tensions of methanol and acetonitrile in mixtures with water, γ_A/γ_B , is always smaller than the p_A/p_B ratio, independently of the amount of water and the bonded phase used (Fig. 8). In the range 0–50% of water, this phenomenon was observed for the three bonded silicas investigated. Between 50 and 100% of water, we have just studied it for C_{18} -bonded silica. Thus a specific effect of the nature of the solvent on the conformation of chain must also be postulated. There is a differential change of $\Delta(\Delta A)$ when using MeCN instead of MeOH as organic modifier (Eq. 9) to explain the difference observed in Fig. 8.

Such a general effect of the nature of the solvent was well demonstrated by Alvarez-Zepeda et al. [40]. They found a difference in

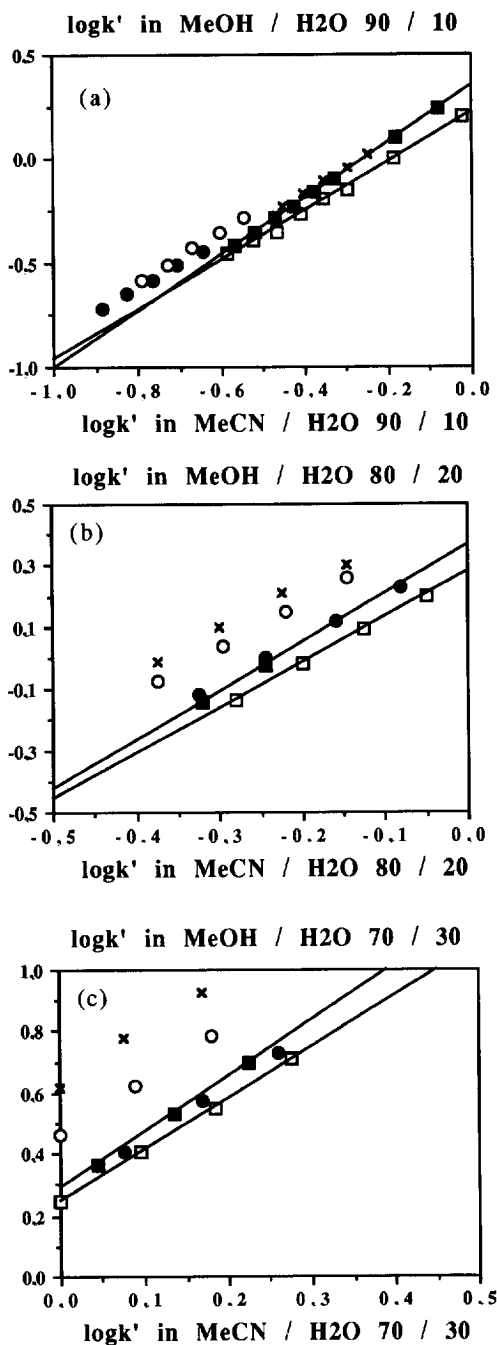


Fig. 7. Log k' vs. log k' on a phenyl column with different mobile phases. (a) MeOH–H₂O (90:10) vs. MeCN–H₂O (90:10); (b) MeOH–H₂O (80:20) vs. MeCN–H₂O (80:20); (c) MeOH–H₂O (70:30) vs. MeCN–H₂O (70:30). Symbols as in Fig. 1.

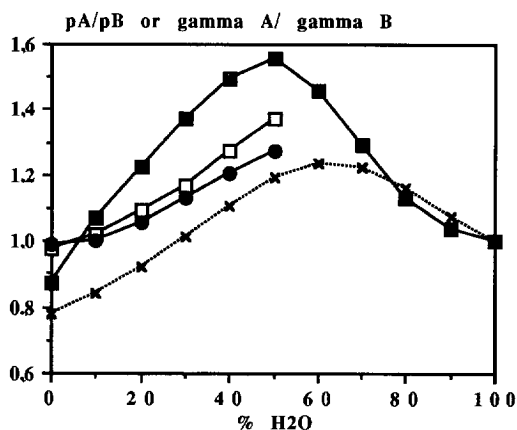


Fig. 8. p_A/p_B and γ_A/γ_B vs. percentage of water for different columns and mobile phases. A = MeOH–H₂O mixtures; B = MeCN–H₂O mixtures. ■ = On C₁₈-bonded silica; □ = on propyl-3-phenyl-bonded silica; ● = on multifunctional bonded silica; × = surface tension of MeOH–H₂O mixtures/surface tension of MeCN–H₂O mixtures.

solvation between MeOH and MeCN in aqueous–organic eluents. Thus, MeCN provides improved solvation for hydrocarbonaceous bonded moieties relative to MeOH. Such an effect occurs on pure alkyl-bonded chains in addition to phenyl-3-propyl-bonded chains or mixed stationary phases.

5. Conclusion

The results of this study indicate that there is a close correlation between the behaviour of the homologous series on phenyl-3-propyl-bonded phases or multifunctional bonded phases, in comparison with that observed on octadecyl-bonded silicas.

Because of its specificity, the stationary phase acts in an important way by interaction of its π electrons with aromatic solutes or within the mobile phase. If we consider the global effect of π – π interactions, the multifunctional bonded phase is intermediate between the pure alkyl-bonded phase and the propiophenyl-bonded phase.

The graphical representation used allows us to characterize specific effects of the three com-

ponents on the chromatographic equilibrium. In addition to the phase and the solvent effect, the graphical representation can be used to illustrate the matrix effect which can influence the retention of some solutes in comparison with others (for example, the presence of metals, which influences the activity, or residual silanols).

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References

- [1] T. Hanai and J. Hubert, *J. Chromatogr.*, 291 (1984) 81.
- [2] W.R. Melander, J.X. Huang, Cs. Horvath, R.W. Stout and J.J. De Stefano, *Chromatographia*, 20 (1985) 641.
- [3] A. Kaibara, M. Hirose and T. Nakagawa, *Chromatographia*, 30 (1990) 99.
- [4] Y.D. Kahie, C. Pietrogrande, M.I. Medina Mendez, P. Reschiglian and F. Dondi, *Chromatographia*, 30 (1990) 447.
- [5] K. Jinno, K. Yamamoto, T. Ueda, H. Nagashima, K. Itoh, J.C. Fetzer and W.R. Biggs, *J. Chromatogr.*, 594 (1992) 105.
- [6] R. Nasuto and L. Kwietniewski, *Chromatographia*, 29 (1990) 597.
- [7] M. De Smet and D.L. Massart, *J. Chromatogr.*, 410 (1987) 77.
- [8] R.M. Smith and S.L. Miller, *J. Chromatogr.*, 464 (1989) 297.
- [9] S.N. Lanin, Yu.S. Nikitin, A.A. Pyatygin and S.M. Staroverov, *Chromatographia*, 27 (1989) 147.
- [10] R.S. Shreedhara Murthy, L.J. Crane and C.E. Bronnimann, *J. Chromatogr.*, 542 (1991) 205.
- [11] N.E. Zhou, C.T. Mant, J.J. Kirkland and R.S. Hodges, *J. Chromatogr.*, 548 (1991) 179.
- [12] C.H. Loesch and W. Simon, *Chromatographia*, 12 (1983) 669.
- [13] B. Buszewski, P. Kasturi, R.K. Gilpin, M.E. Gangoda, M. Jaroniec, *Chromatographia*, 39 (1994) 155.
- [14] K.K. Unger, *Packings and Stationary Phases in Chromatographic Techniques (Chromatographic Science Series, Vol. 47)*, Marcel Dekker, New York, 1990.
- [15] W. Melander and Cs. Horvath, *High Performance Liquid Chromatography (Advances and Perspectives, Vol. 2)*, Academic Press, New York, 1980.

- [16] D.E. Martire and R.E. Boehm, *J. Phys. Chem.*, 87 (1983) 1045.
- [17] K.A. Dill, *J. Phys. Chem.*, 91 (1987) 1980.
- [18] J.G. Dorsey and K.A. Dill, *Chem. Rev.*, 89 (1989) 331, and references cited therein.
- [19] W.R. Melander and Cs. Horvath, *Chromatographia*, 15 (1982) 86.
- [20] A. Tchaplá, H. Colin and G. Guiochon, *Anal. Chem.*, 56 (1984) 621.
- [21] S. Héron and A. Tchaplá, *J. Chromatogr.*, 556 (1991) 219.
- [22] A. Tchaplá, S. Héron, E. Lesellier and H. Colin, *J. Chromatogr. A*, 656 (1993) 81.
- [23] G. Thévenon-Emeric, A. Tchaplá and M. Martin, *J. Chromatogr.*, 550 (1991) 267.
- [24] W.R. Melander, J. Stoveken and Cs. Horvath, *J. Chromatogr.*, 199 (1980) 35.
- [25] B. Walczak, L. Morin Allory, M. Lafosse, M. Dreux and J.R. Chretien, *J. Chromatogr.*, 395 (1987) 183, and references cited therein.
- [26] N. Tanaka, Y. Tokuda, K. Iwaguchi and M. Araki, *J. Chromatogr.*, 239 (1982) 761.
- [27] T. Hanai and J. Hubert, *J. Chromatogr.*, 291 (1984) 81.
- [28] W.T. Cooper and L.Y. Lin, *Chromatographia*, 21 (1986) 335.
- [29] T.H. Dzido and H. Engelhardt, *Chromatographia*, 39 (1984) 51.
- [30] J. Kriz, E. Adamcova, J.H. Knox and J. Mora, *J. Chromatogr. A*, 663 (1994) 151.
- [31] A. Tchaplá, S. Héron, H. Colin and G. Guiochon, *Anal. Chem.*, 60 (1988) 1443, and references cited therein.
- [32] S. Héron and A. Tchaplá, *Chromatographia*, 36 (1993) 11.
- [33] E. Grushka, H. Colin and G. Guiochon, *J. Chromatogr.*, 248 (1982) 325.
- [34] H. Colin and G. Guiochon, *J. Chromatogr. Sci.*, 18 (1980) 54.
- [35] H. Colin, A.M. Krstulovic, M.F. Gonnord, G. Guiochon, Z. Yun and P. Jandera, *Chromatographia*, 17 (1983) 9.
- [36] M. Czok and H. Engelhardt, *Chromatographia*, 27 (1989) 5.
- [37] A. Tchaplá and S. Héron, *J. Chromatogr. A*, 684 (1994) 175.
- [38] Z. Yun, Doctoral Thesis, University of Paris VI, 1982.
- [39] A.M. Krstulovic, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 2438.
- [40] A. Alvarez-Zepeda, B.N. Barman and D.E. Martire, *Anal. Chem.*, 64 (1992) 1778.
- [41] S. Héron, PhD Dissertation, University of Paris VI, 1992.
- [42] A. Chartier, C. Gonnet, D. Morel, J.L. Rocca and J. Serpinet, *J. Chromatogr.*, 438 (1988) 261.
- [43] S. Héron and A. Tchaplá, *Anal. Chem.*, 66 (1994) 114.
- [44] S. Héron, J. Bleton and A. Tchaplá, in E.G. Perkins and J.L. Sébédio (Editors), *New Trends in Lipids and Lipoproteins Analysis*. AOCS Press, Champaign, IL, 1995, Ch. 16.
- [45] P.J. Schoenmakers, *Optimization of Chromatographic Selectivity (Journal of Chromatography Library, Vol. 35)*, Elsevier, Amsterdam, 1986, Ch. 5.