Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1087 (2005) 64–76
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Effects of modifiers in subcritical fluid chromatography on retention with porous graphitic carbon

C. West, E. Lesellier*

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

Available online 14 April 2005

Abstract

The effect of different modifiers in subcritical fluid chromatography (SubFC) on interactions between solute and porous graphitic carbon (PGC) and between solute and carbon dioxide-modifier mobile phases was studied by the use of linear solvation energy relationships (LSERs). This study was performed to allow efficient optimization of the composition of the carbon dioxide-modifier mobile phase in regard of the chemical nature of the solutes to be separated. With all modifiers tested (methanol, ethanol, *n*-propanol, isopropanol, acetonitrile, tetrahydrofuran and hexane), the solute/stationary phase interactions are greater than the solute/mobile phase ones. Dispersion interactions and charge transfer between electron donor solute and electron acceptor PGC mainly explain the retention on this surface, whatever the modifier. These interactions are quite constant over the range of modifier percentage studied (5–40%). For acidic compounds, the retention variation is mainly related to the change in the basic character of mobile and stationary phase due to the variation of modifier percentage. Changes in eluting strength are mostly related to adsorption of mobile phase onto the PGC with methanol and acetonitrile, and to the increase of dispersion interactions between the solute and the mobile phase for other modifiers. Relationships between varied selectivities and solvation parameter values have been studied and are discussed in this paper.

Keywords: Porous graphitic carbon; Subcritical fluid chromatography; Carbon dioxide-modifier mobile phases; Linear solvation energy relationship

1. Introduction

In subcritical and supercritical fluid chromatography (SubFC and SFC), numerous parameters influence the quality of separation of solutes. Naturally, temperature and outer column pressure are common parameters used to modify retention, through density variations or through the amount of CO_2 adsorbed onto the stationary phase [1–4]. Another way to change retention and adjust selectivity is the addition of organic modifier to the CO_2 mobile phase. This addition changes the properties of both the mobile and the stationary phase, and generally reduces the retention.

The organic solvent changes the polarity of the mobile phase, and also its density, more particularly close to the critical point [5]. However, in packed column subcritical fluid chromatography, with fluid density above $0.6\,g\,cm^{-3}$, the variation of the fluid eluting strength depends principally on the volume fraction of modifier [6].

Different interpretations of the modifier's action have been suggested. The results obtained when adding solvatochromic dye Nile Red to the mobile phase are often contradictory. This is due to the polarity of the probe used as molecules of the polar modifier aggregate about the probe forming a polar cluster in a non-polar bulk [7,8]. Nevertheless, studies on the transition energy $Et_{(NT)}$ show that the variation of retention depends more on the change of polarity of the mobile phase than on the density of the modified fluid. This is confirmed by studies of eluting strength on ODS phases, based on the methylene selectivity [9]. Density effects may be important in comparing various mobile phases only when no other character of the mobile phase is different [5].

However, these approaches do not account for the changes of polarity of the stationary phase caused by the adsorption of modifier, a phenomenon that also takes part in the variations of retention. Indeed, the proportion of modifier in the mobile

* Corresponding author. Tel.: +33 1 69336131; fax: +33 1 69336048.
E-mail address: eric.lesellier@iut-orsay.fr (E. Lesellier).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.03.104

phase also influences the amount and nature of the fluids adsorbed onto the stationary phase [9–12].

On bonded silica phases, the adsorption of organic solvent modifies the polarity of the stationary phase [9,13], and masks the residual silanols [14–16]. Generally, a high variation of retention factors is observed between 0 and 5% of modifier, due to partial deactivation of residual silanols on ODS phases [15,16], or to the dynamic covering of the silica surface, particularly when using ethan-1,2-diol [17]. Extensive adsorption of methanol onto the bonded phase also decreases the dipole–dipole interactions. This adsorption was reported by Lockmuller and Mink [14], when Strubinger et al. [10] indicate that the adsorbed layer on ODS was nearly 25% methanol even though the mobile phase contained only 2% methanol.

In the case of polymeric stationary phases, modifier adsorption leads to the swelling of the polymeric phase [10,18,19]. This adsorption "dilutes" the stationary phase and changes its interaction properties.

On any type of stationary phase, aggregation of the modifier at the chromatographic interface induces different surface chemistries: the stationary phase loses parts of its original characteristics and takes on characteristics typical of the modifier due to the latter's preferential adsorption [5].

Porous graphitic carbon (PGC) is a chromatographic support with unique adsorption properties that are very different from other traditional reversed-phase supports. Its structural and chemical stability allow its use in supercritical mobile phase conditions. For a given separation, the choice of an appropriate mobile phase system requires a detailed understanding of what controls retention in SubFC on PGC.

Only few works have been carried out in SFC with PGC. Engel and Olesik [20] demonstrated the use of PGC as a stationary phase in SFC. Addition of modifier to the mobile phase was shown to lower the retention and improve peak shapes. In another paper [21], they studied the effect of a small percentage (1%) of modifier on solvent strength, using solvatochromic parameters to rationalize the effect of the organic modifiers they tested. At this small percentage, solvent strength was shown to depend on the ability of the modifier to adsorb on the PGC and thus compete with the solute for adsorption sites, regardless of the primary adsorption mechanism of the solute. Polarity, molecular size and basicity of the organic solvent were shown to control retention.

Quantitative structure–retention relationships (QSRRs) allow for rationalization of differences in analytical retention in various chromatographic systems in terms of intermolecular interactions involving the solute and the stationary and mobile phases [22,23]. In particular, the linear solvation energy relationship (LSER), using Abraham's parameters [24], has been widely used with this object [25]. The retention of selected probes can be related through this relationship to specific interactions by the following equation:

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

In this equation, capital letters represent the solute descriptors, related to particular interaction properties, while lower case letters represent the system constants, related to the complimentary effect of the phases on these interactions. c is a constant, depending on specific column parameters such as porosity. E is the excess molar refraction (calculated from the refractive index of the molecule) and 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 $cm^3\,mol^{-1}/100$. The system constants (c, e, s, a, b, v), obtained through a multilinear regression of the retention data for a certain number of solutes with known descriptors, reflect the magnitude of difference for that particular property between the mobile and stationary phases. Thus, if a particular coefficient is numerically large, then any solute having the complimentary property will interact very strongly with either the mobile phase (if the coefficient is negative) or the stationary phase (if the coefficient is positive). Consequently, the coefficients also reflect the system's relative selectivity towards that particular molecular interaction.

Thus, retention mechanisms have been studied on ODS [16], cyano [26,27], DVB-C18 [5,28], and PDMS [18,19] stationary phases in SFC using this relationship. These studies led to suggest that selection of a modifier could be made in a rational manner, to either promote or suppress a particular type of molecular interactions. They indicate that modified mobile phases may be compared relative to their regression coefficients to establish a relative order of selectivities towards specific types of interactions.

After a previous study with methanol as modifier in SubFC [29], this paper compares the effect of seven different modifiers added in high proportions (varied from 5 to 100%) on the interaction changes on PGC. LSERs were used in a systematic study of the influence of the nature and proportion of modifier added to carbon dioxide. The results were also correlated to solvatochromic parameters of modifiers or to other relationships such as methylene or hydroxyl selectivity and eluting strength.

2. Experimental

2.1. Chemicals

Solvents used were HPLC grade methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF), *n*-propanol (nPrOH) (Carlo Erba, Milan, Italie), ethanol (EtOH) (VWR Prolabo, Val-de-Fontenay, France), isopropanol (iPrOH) (SdS, Peypin, France) and hexane (HXN) (J.T. Baker). Carbon dioxide was provided by Alphagaz (Bois d'Arcy, France). Table 1 indicates the Kamlet and Taft solvatochromic parameters [27,30–32] of the chosen organic modifiers, along with their molecular volume. The solvents were chosen so as to provide a wide range of size, polarity, hydrophobic-

Table 1
Solvent properties for mobile phase modifiers

Modifier	π^*	α	β	V
MeOH	0.60	0.93	0.62	0.2050
EtOH	0.54	0.86	0.77	0.3050
nPrOH	0.52	0.84	0.90	0.4020
iPrOH	0.48	0.76	0.95	0.4010
ACN	0.66	0.19	0.31	0.2710
THF	0.58	0.00	0.55	0.4550
HXN	−0.11	0.00	0.00	0.6480

π^*: Bulk phase dipolarity/polarizability, α: bulk phase hydrogen bond acidity, β: bulk phase hydrogen bond basicity, V: McGowan's characteristic volume for one molecule of solvent. Values are from reference [33] and calculated from [25].

ity and hydrogen-bonding ability. As we use high concentration of modifiers, we indicated the modifiers' solvatochromic parameters, linked to the bulk solvent properties. It should be noted that the mobile phases used were all mixtures of carbon dioxide and one of these solvents, so the solvatochromic parameters given for pure organic solvents only give a relative indication of the properties of the mixed mobile phases.

The series of test analytes were taken as previously designed [29]. Forty-nine compounds (see Table 2), benzene and naphthalene derivatives, all commercially available, were obtained from a range of suppliers. Solutions of these compounds were prepared in methanol. The solute descriptors used in the solvation parameter model were taken from several sources [33–37] and are summarized in Table 2. The series of test compounds has been selected by observing the requirements of a good QSRR analysis. The compounds were chosen so as to provide a uniform distribution of each descriptor within a wide enough space and absence of cross-correlation among the descriptors was checked, indicating that the descriptors are close to orthogonality.

Additionally, for eluotropic strength measurements, benzene-alkanes with alkyl chains varying from 7 to 15 carbons were also used.

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-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 (modifier and CO_2) were mixed, the fluid was introduced into a dynamic mixing chamber PU 4046 (Pye Unicam, Cambridge, United Kingdom) connected to a pulsation damper (Sedere supplied by Touzart et Matignon, les Ulis, France). 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, Palo Alto, USA), 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 (Tokyo, Japan, supplied by Prolabo, Fontenay-sous-Bois, France). 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 column was Hypercarb porous graphitic carbon (100 mm × 4.6 mm i.d., 5 μm) provided by Thermo-Hypersil Keystone.

2.3. Chromatographic conditions

Samples were chromatographed using carbon dioxide with 5–100% (v/v) modifier. Total flow through the system was 3.0 mL min^{-1}. Since the purpose of the present study is to investigate the effect of modifiers, all of the experiments were performed at constant CO_2 outlet pressure and temperature. Column temperature was maintained at 25 °C (subcritical for all mobile phase compositions). Back pressure was maintained at 150 bar. Inlet pressure varied among the different mobile phase compositions between 175 and 200 bar.

Application of LSER methodology to subcritical systems is subject to some important assumptions. A first assumption is that the measurement of the void volume will not affect the regression results, since void volume depends on fluid density. Blackwell and Stringham [5] report a study on void volume change indicating that the regression intercept (c) is the only system constant significantly affected by its measurement.

A second assumption is that the pressure drop across the column will not affect the regression coefficient. In the same paper, Blackwell and Stringham evaluated the influence of backpressure on the system constants and indicate that the regression intercept again was the only system constant significantly affected by this parameter.

Consequently, subcritical conditions 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 [6].

2.4. Retention factors

Retention factors (k) were determined using the relationship:

$$k = \frac{t_r - t_0}{t_0} \quad (2)$$

Table 2
Chromatographic solutes and LSER descriptors

	Compound	*E*	*S*	*A*	*B*	*V*
1	Benzene	0.610	0.52	0.00	0.14	0.7164
2	Toluene	0.601	0.52	0.00	0.14	0.8573
3	Ethylbenzene	0.613	0.51	0.00	0.15	0.9982
4	Propylbenzene	0.604	0.50	0.00	0.15	1.1391
5	Butylbenzene	0.600	0.51	0.00	0.15	1.2800
6	Pentylbenzene	0.594	0.51	0.00	0.15	1.4209
7	Hexylbenzene	0.591	0.50	0.00	0.15	1.5620
8	Aniline	0.955	0.94	0.26	0.50	0.8162
9	Benzoic acid	0.730	0.90	0.59	0.40	0.9317
10	*N*,*N*-Dimethylaniline	0.957	0.84	0.00	0.47	1.0980
11	Anisole	0.708	0.75	0.00	0.29	0.9160
12	Phenylethan-1-ol	0.784	0.83	0.30	0.66	1.0570
13	Benzyl alcohol	0.803	0.87	0.39	0.56	0.9160
14	Benzaldehyde	0.820	1.00	0.00	0.39	0.8730
15	Acetophenone	0.818	1.01	0.00	0.48	1.0139
16	Benzonitrile	0.742	1.11	0.00	0.33	0.8711
17	Nitrobenzene	0.871	1.11	0.00	0.28	0.8906
18	Chlorobenzene	0.718	0.65	0.00	0.07	0.8288
19	Bromobenzene	0.882	0.73	0.00	0.09	0.8910
20	Phenol	0.805	0.89	0.60	0.30	0.7751
21	*o*-Chlorophenol	0.853	0.88	0.32	0.31	0.8980
22	*o*-Aminophenol	1.110	1.10	0.60	0.66	0.8750
23	2,5-Dimethylphenol	0.840	0.79	0.54	0.37	1.0570
24	2,6-Dimethylphenol	0.860	0.79	0.39	0.39	1.0570
25	3,4-Dimethylphenol	0.830	0.86	0.56	0.39	1.0570
26	Eugenol	0.946	0.99	0.22	0.51	1.3540
27	Resorcinol (1,3-dihydroxybenzene)	0.980	1.00	1.10	0.58	0.8340
28	Phloroglucinol (1,3,5-trihydroxybenzene)	1.355	1.12	1.40	0.82	0.8925
29	Naphthalene	1.340	0.92	0.00	0.20	1.0854
30	α-Naphtol	1.520	1.05	0.61	0.37	1.1441
31	β-Naphtol	1.520	1.08	0.61	0.40	1.1440
32	Nitronaphthalene	1.600	1.51	0.00	0.29	1.2596
33	1-Methylnaphthalene	1.344	0.90	0.00	0.20	1.2260
34	2-Methylnaphthalene	1.304	0.92	0.00	0.20	1.2260
35	Biphenyl	1.360	0.99	0.00	0.26	1.3242
36	Benzophenone	1.447	1.50	0.00	0.50	1.4810
37	Methyl benzoate	0.733	0.85	0.00	0.48	1.0726
38	Ethyl benzoate	0.689	0.85	0.00	0.46	1.2140
39	Propyl benzoate	0.675	0.80	0.00	0.46	1.2260
40	Butyl benzoate	0.668	0.80	0.00	0.46	1.4953
41	*o*-Cresol	0.840	0.86	0.52	0.46	0.9160
42	*m*-Cresol	0.822	0.88	0.57	0.34	0.9160
43	*p*-Cresol	0.820	0.87	0.57	0.31	0.9160
44	*o*-Nitrophenol	1.045	1.05	0.05	0.37	0.9490
45	*m*-Nitrophenol	1.050	1.57	0.79	0.23	0.9490
46	*p*-Nitrophenol	1.070	1.72	0.82	0.26	0.9490
47	*o*-Xylene	0.663	0.56	0.00	0.16	0.9980
48	*m*-Xylene	0.623	0.52	0.00	0.16	0.9980
49	*p*-Xylene	0.613	0.52	0.00	0.16	0.9980

E: Excess molar refraction, *S*: dipolarity/polarizability, *A*: hydrogen bond acidity, *B*: hydrogen bond basicity, *V*: McGowan's characteristic volume. Values are from references [33–37].

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. The relative standard deviation of the retention factor, in each mobile phase condition before data collection, was measured on six injections of butylbenzene and was always inferior to 0.3%. Then capacity factor data were typically collected as single measurements under each set of chromatographic conditions. However, replicate measurements were routinely collected to assess their repetability within a day.

2.5. Data analysis

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. However, in all cases, sufficient solutes were included in the model to give statistically meaningful model results. Multiple linear regression analysis and statistical tests were performed using the program SuperANOVA (Abacus Concept). The quality of the fits was estimated using the overall correlation coefficient (R), standard error in the estimate (SD) 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 0.1%, were eliminated from the model.

The fits were all of reasonable quality, R ranging from 0.953 to 0.976, standard error of estimate varying from 0.124 in high modifier proportions to 0.225 in lower modifier proportions. These values are of the same order as those obtained by Lepont et al. in HPLC on PGC [38]. Besides, Al-Haj et al. [39] indicated that, for partitioning chromatography, R should be close to 0.99 and the standard error less than 0.25. However, the mechanism involved here is not a partitioning mechanism, therefore, we consider our results to be reasonably good. Naturally, in addition to goodness of fit, the coefficients must make chemical sense. Values of the system constants were both large and significantly larger than their uncertainty, therefore amenable to interpretation.

Additionally, similar residual plots were observed at all mobile phase compositions. This indicates that particular deviations are not random experimental errors but due to the inability of the model to completely account for retention variations among the solutes.

Some deviations can be explained by the fact that the molecular volume fails to correctly model the contact surface area for the dispersive interaction of angular or staggered (i.e. not flat) molecules with the graphite surface [29,38] and generally overestimates their retention. As we had mentioned it before, in adsorption interactions, only the portion of the solute that actually contacts the surface is important. Therefore, the MacGowan volume may overestimate the dispersive interactions and is not a perfect measure of dispersive energy between the solute and the PGC surface [34]. A cross-sectional area of the molecule would be more appropriate.

Besides, the solvation parameter model uses descriptors characteristic of the neutral form of the molecule. It has been shown that ionic compounds experience additional retention on PGC [40]. Thus, in the case of ionisable compounds such as benzoic acid, the possible ionization of the molecule may explain the underestimation of chromatographic retention. The use of acidic additives is hoped to improve this situation.

3. Results and discussion

3.1. Eluotropic strength

For each modifier, the percentage was varied from 5 to 100.

Since the eluotropic strength scale is a relative one, it is necessary to choose a reference. Generally, in liquid chromatography, water is the reference solvent because it has the lowest eluotropic strength and therefore allows the obtaining of positive values. Results obtained in SubFC have shown that eluotropic strength is higher than in liquid chromatography with water [9]. Moreover, choosing water as a reference will allow the comparison to the scales established by others.

Eluotropic strength ε° can be calculated from the equation defined by Snyder for adsorption chromatography on polar surfaces [41]:

$$\log \alpha_{CH_2}(H_2O) - \log \alpha_{CH_2}(\text{solvent}) = V_0 \times \varepsilon^\circ \quad (3)$$

where $\alpha_{CH_2}(H_2O)$ is the methylene selectivity value of H_2O acting as the mobile phase, α_{CH_2}(solvent) the methylene selectivity value of the studied mobile phase and V_0 is the volume of a CH_2 group.

This calculation method for eluotropic strength was retained for this study. Consequently, this scale is based only on dispersive interactions as α_{CH_2} depends on the transfer energy variation of the solute from the stationary to the mobile phase due to the methylene group.

The logarithms of retention factors k of members of the homologous series vary linearly with the number of methylene groups [42]. Therefore, $\log \alpha_{CH_2}$(solvent) was obtained by calculating the slope of this relationship:

$$\log k_n = n \times \log \alpha_{CH_2} + \log \rho \quad (4)$$

where $\log k_n$ is the retention factor of a benzene-alkane, n is the number of carbon atoms in the alkyl chain (varied from 4 to 15) and $\log \rho$ represents the specific interaction of the residue of the molecule isolated from the alkyl chain.

In good agreement with Kaur's study on the retention of homologous series on PGC [43], tetrahydrofuran was found stronger than hexane and methanol was found slightly stronger than acetonitrile, which is contrary to what is observed on ODS stationary phases. As shown by Gaudin et al. [44], n-propanol was found to be intermediate between methanol and tetrahydrofuran. Ethanol and isopropanol were found to be slightly weaker than n-propanol.

Different behaviours can be noted when the percentage of modifier is varied (see Fig. 1).

In methanol and acetonitrile modified mobile phases, the variation of eluotropic strength is significant between 5 and 10% modifier. As mobile phase polarity increases with the percentage of modifier, cavity energy increases and the solute–mobile phase dispersion interactions decrease. This should normally lead to an increase in retention. Therefore, the observed variation is probably due to deactivation of the stationary phase by modifier adsorption.

With lower dielectric constant modifiers such as ethanol, n-propanol, isopropanol and hexane, the solvent alkyl chains favour dispersion interactions between solute and mobile phase. For these modified mobile phases, the increase of dispersion interactions in the mobile phase are important between 5 and 60% modifier [45]. These variations become less significant between 60 and 100% modifier. Thus, for al-

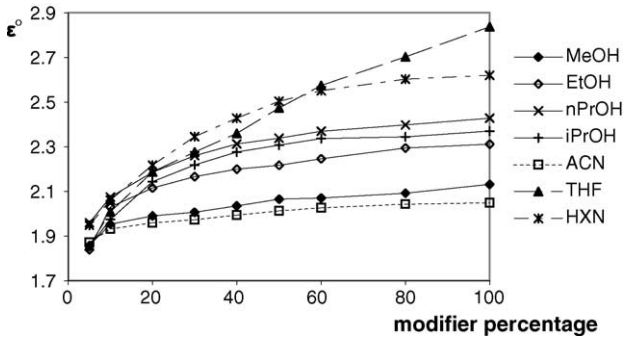

Fig. 1. Variation of eluotropic strength with the percentage of modifier for each organic solvent tested. Eluotropic strengths were measured according to Eqs. (3) and (4), based on the retention factors of benzene-alkanes with alkyl chains varying from 6 to 15 carbons.

cohols, as a general rule, the bigger the volume of the organic solvent (see Table 1), the wider the variation of the eluting strength when the percentage is varied.

In tetrahydrofuran, the eluting strength increases continually between 5 and 100% modifier. This is in accordance with Knox and Kaur's study [46] indicating that THF is a very strong eluent on carbon phases.

3.2. Retention behaviour

When $\log k$ versus $\log k$ plots (so-called κ–κ plots) of the retention data measured on the same column with different mobile phases are linear with unit slope, the retention behaviours are called homoenergetic [47] because of the similar physico-chemical basis of the retention in the two chromatographic systems. Compounds not falling on this straight line indicate that the overall retention mechanism is different.

In Fig. 2a–c, the retention factors measured in hexane-modified mobile phases were plotted against the retention factors measured in methanol-modified mobile phases (at 5, 20 and 40% modifier). In order to illustrate different chromatographic behaviours, we separated polar and non-polar solutes. The polar compounds (represented by white triangles) are compounds 8–44 in Table 2; the non-polar compounds (represented by black diamonds joined by a regression line) are alkylbenzenes with carbon number in the alkyl chain ranging from 0 to 10. Note that, at small modifier percentages (5%), polar species are more retained in methanol than in hexane, comparatively to non-polar species, while at high modifier percentages (40%), it is the contrary. However, from these plots, it is not clear whether the differences come from variations of behaviours of the polar or non-polar species, or both.

To provide a more precise understanding of these variations and a clearer comparison between different modifiers, we grouped the compounds in families, according to their functionalities (polar or non-polar) and to their residue (benzenic or naphthenic). Fig. 3 represents the average retention factor of each family of compounds, at each modifier percentage (varying from 5 to 40%), in hexane, plotted against the

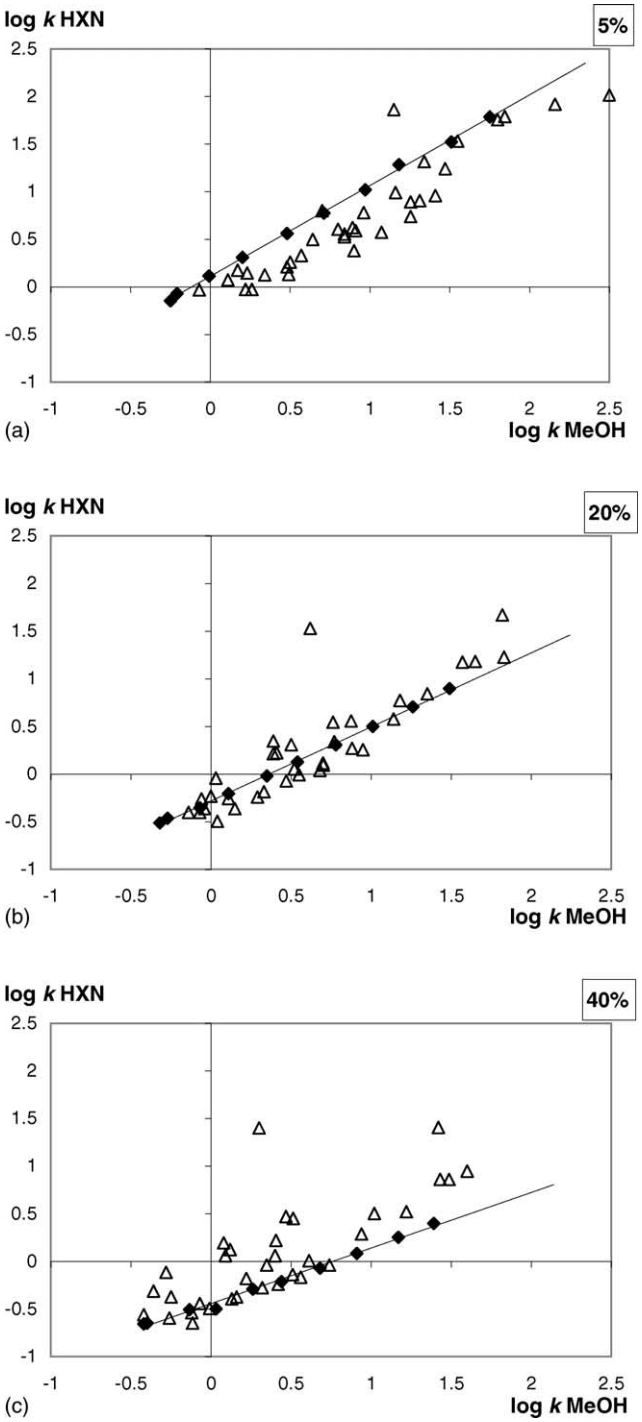

Fig. 2. Plot of $\log k$ on in hexane-modified mobile phases vs. $\log k$ in methanol-modified mobile phases. Compositions: (a) 5% modifier; (b) 20%; (c) 40%. Black diamonds represent non-polar solutes (alkylbenzenes with carbon number in the alkyl chain ranging from 0 to 10); white triangles represent polar solutes (solutes 8–46 in Table 2).

average retention factor of this same family, at each modifier percentage, in methanol. Each curve represents a particular type of compounds.

On such a plot, if both modifiers had the same influence on the retention of a particular type of compounds, the point

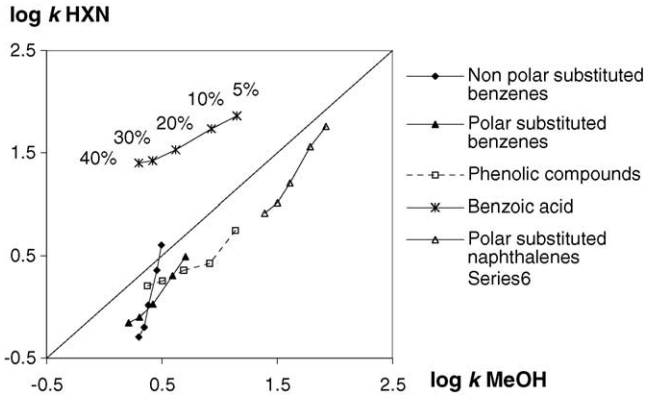

Fig. 3. Plot of average retention for different families of solutes in hexane vs. methanol-modified mobile phases. Non-polar substituted benzenes are compounds 1–7 and 47–49 in Table 2; polar substituted benzenes are compounds 8, 10–19 and 37–46; phenolic compounds are compounds 20–28; polar substituted naphthalenes are compounds 30–32.

representing the average retention would fall on the straight line. If hexane favoured elution, it would fall below the line; if methanol favoured elution, it would fall above.

The increase in modifier percentage favouring the elution, therefore decreasing the retention factors, each curve can be read from right to left to understand the variations with mobile phase composition. If the retention behaviours in both modifiers were similar, the curve representing a family of compounds would be parallel to the straight line while different behaviours would induce curves forming an angle with this line.

First of all, we note that most compounds have higher retention factors in methanol modified mobile phases than in hexane-modified mobile phases (the points are below the straight line). The solute–mobile phase dispersion interactions are greater in hexane than in methanol, as indicated by the eluting strength (see Fig. 1). This could explain the low retention factors. The unique exception to this retention rule is benzoic acid, probably forming dimeric species in hexane, therefore being more retained. For this reason, benzoic acid was not included in the "polar substituted benzenes" group.

Secondly, it can be noted that the curve representing the polar substituted benzenic species (black triangles) and the polar substituted naphthenic species (white triangles) are parallel, the latter being an exact translation of the former towards higher retention factor values. This suggests that the variations in chromatographic behaviour are rather linked to the functionality and that the addition of an aromatic ring, apart from increasing retention, does not modify the retention behaviour when varying the chromatographic conditions. It would be interesting to compare aliphatic and aromatic compounds in the same manner and see if the aromatic character has any influence on the chromatographic behaviour.

Thirdly, the curve representing non-polar substituted benzenic species (black diamonds) and polar substituted benzenic species (black triangles) are not parallel. This observation validates the choice of all-aromatic compounds, as we might have expected their behaviour to be dominated by their common portion but this is clearly not the case. Besides, this corroborates the observations above-mentioned: varying the percentage induces different behaviours on different types of compounds, depending on the nature of the modifier:

(1) The non-polar benzenic species curve shows that, if non-polar solutes have nearly identical retention factors in small concentrations of hexane and methanol, this is no longer the case when the percentage of modifier is increased, the retention factors being then lower in hexane than in methanol. This is due to the greater variation of dispersion interactions in the mobile phase when increasing the percentage of modifier in hexane than in methanol, as noted when observing the eluting strength in Fig. 1.
(2) For polar solutes, the difference in retention factors is nearly constant, the curve being somewhat parallel to the straight line. In other words, for these compounds the difference in behaviour of hexane and methanol is nearly constant when varying the percentage of modifier. This indicates that interactions other than dispersion interactions must be involved in the retention behaviour.

From these observations, we can conclude that the variations observed in Fig. 2 are predominantly due to changes in the mechanism for retention of the non-polar compounds.

Among polar substituted benzenes, phenolic species (white squares) show a different behaviour. When varying the percentage of modifier between 10 and 40%, we observe that, if elution is favoured by hexane-modified mobile phase at small percentages, it is no longer the case at high percentages where the retention factors are nearly the same. Thus, even if the elution of phenolic compounds is favoured by the increase of modifier percentage, the decrease of retention is higher in methanol than in hexane. This particular behaviour could be linked to the major influence of hydrogen-bond donating ability of these solutes, hexane and methanol showing very different behaviours as far as this property is concerned (see β values in Table 1). Indeed, when increasing the percentage of modifier, methanol-modified mobile phases display increasing hydrogen-bond accepting abilities, favouring the elution of acidic compounds, contrary to hexane-modified mobile phases. Benzoic acid, showing a similar trend and being a strong hydrogen-bond donor, confirms this hypothesis.

Other $\log k$–$\log k$ plots, using the same group-averaged capacity factors, were realised in order to compare the behaviours of the varied organic solvents used as modifiers. They are not shown here but the conclusions are the following:

(1) When comparing alcohols used as modifiers (methanol, ethanol, *n*-propanol and isopropanol), the retention decreased with increasing alkyl chain. Additionally, the curves were essentially parallel to the straight line indicating identical chromatographic behaviours of the dif-

ferent types of compounds when varying the mobile phase composition.
(2) Acetonitrile is very similar to methanol.
(3) The variations of retention factors in tetrahydrofuran when increasing its percentage in the mobile phase are more important than in any other modifier. This seems to indicate that the eluting strength evaluated through the values of methylene selectivity is valid to explain the retention behaviour of not only non-polar but also polar substituted compounds, in this chromatographic system.

3.3. LSER studies

3.3.1. Model description

The dominant contributions to retention are the dispersion interaction term (v) and the excess molar refractivity term (e). The electron-donating ability of the stationary and mobile phases (a) also has a high influence at low percentages of modifier. In Fig. 4a–d, the system constants c, e, a and v are plotted against modifier percentage.

Acidity of the modifier (b) appears to have no influence on retention, as indicated by Engel and Olesik at 1% modifier [21]. This is also corroborated by the studies of Bush and Eckert [48] on solid–fluid equilibria in supercritical CO_2. They showed that, even though CO_2 can act as a Lewis acid, the b coefficient is insignificant to explain the solubility of solutes.

3.3.1.1. The c term. c is a constant. Although it may contain some additional information, the constant is assumed to be essentially related to the phase ratio contribution to retention.

As we had previously noticed for methanol-modified mobile phases, the c term increases between 5 and 40% modifier percentage for all the alcohol modifiers but remains constant in acetonitrile, tetrahydrofuran and hexane (see Fig. 4a).

When the percentage of modifier is increased [6]:

(1) the volume of the mobile phase increases, due to changes in the mobile phase density;
(2) the volume of the stationary phase may increase, due to the adsorption of the mobile phase. As a matter of fact, Strubinger et al. [10] indicated that the adsorption of CO_2 with methanol on a stationary phase is cooperative, not competitive. In other words, the total amount of adsorbed mobile phase is increased by the addition of modifier.

Thus, an increasing c constant indicates that the phase ratio $V_{stationary}/V_{mobile}$ increases, that is to say, $V_{stationary}$ (the volume of stationary phase) increases more than V_{mobile} (the volume of mobile phase). In acetonitrile, tetrahydrofuran and hexane modified mobile phases where c remains constant, the variations of $V_{stationary}$ and V_{mobile} probably compensate.

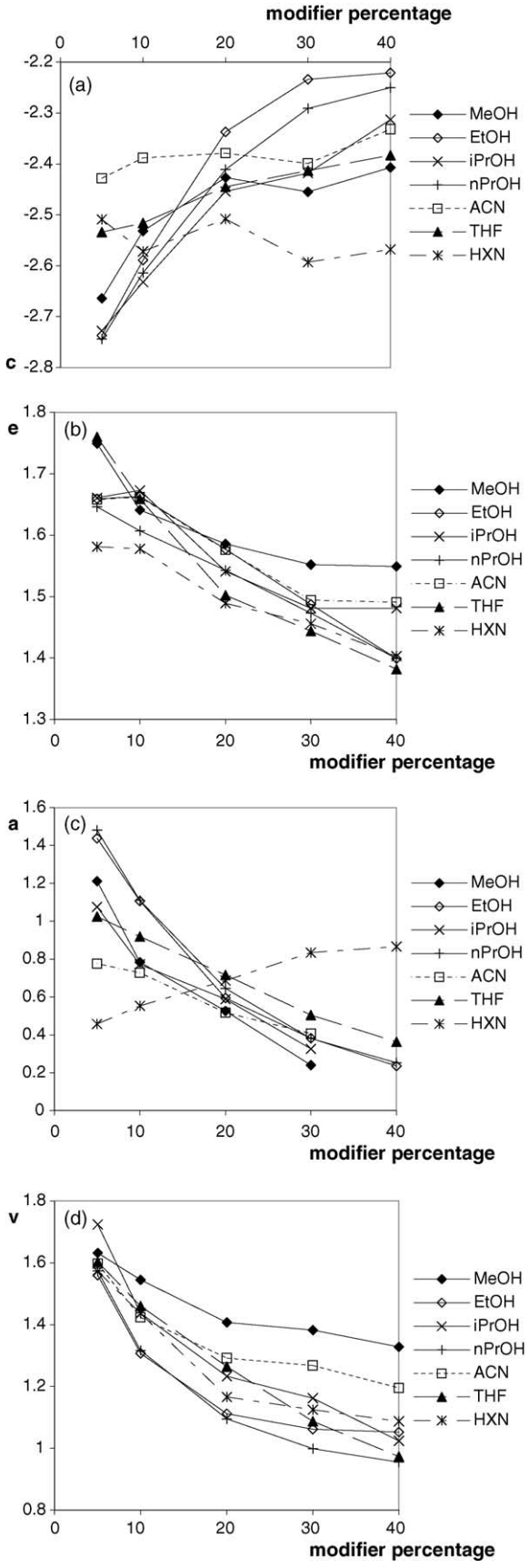

Fig. 4. Variations of the LSER coefficients as a function of mobile phase composition (a) the c constant (regression intercept); (b) the e coefficient (excess molar refraction); (c) the a coefficient (hydrogen-bond donating); (d) the v coefficient (dispersion).

3.3.1.2. The v coefficient. Assuming that the cavity energy is strongly reduced by the use of fluid of low cohesivity as carbon dioxide, v represents the difference in dispersion interactions between the solute and the stationary phase on the one hand, the solute and the mobile phase on the other hand:

$$v = v_{\text{stationary}} - v_{\text{mobile}} \quad (5)$$

The fact that it is positive indicates that the stationary phase is dominant over the mobile phase with respect to this property.

The v coefficient decreases for all mobile phase when the percentage of modifier is increased (Fig. 4d). In SFC, with low density fluid, the addition of organic modifier to carbon dioxide increases the fluid density, i.e. the eluotropic strength of the mobile phase. However, as described elsewhere [9], in SubFC working with higher fluid density, the polar modifier 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 also consider a decrease in the solute/stationary phase dispersion interactions, induced by the sorption of methanol or acetonitrile onto the PGC surface.

For the higher modifier percentages in the mobile phase, variations of the stationary phase composition probably become less important than the variations of the mobile phase composition. In particular, at 40% modifier, we compared v obtained with methanol, ethanol and *n*-propanol, the chain length being the unique structural difference between these solvents. The longer the alkyl chain and, jointly, the weaker the polarity of the molecule, the higher the dispersion interactions in the mobile phase (v_{mobile} increases), the smaller the global v coefficient.

3.3.1.3. 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. e represents the following:

$$e = e_{\text{stationary}} - e_{\text{mobile}} \quad (6)$$

Again, this coefficient being positive indicates that the stationary phase is dominant over the mobile phase towards this particular type of interactions (Fig. 4b).

In acetonitrile and hexane modified mobile phases, the e coefficient does not vary significantly. This means that, when increasing the percentage of modifier in the mobile phase, the variations of charge-transfer interactions between the solutes and the mobile phase and the solute and the stationary phase compensate. Additionally, we can notice that, at low modifier percentages, hexane shows the lowest values of e. As e_{mobile} is necessarily small in hexane-modified mobile phase, hexane being unable to establish charge-transfer interactions, the low values of e can only be due to low charge-transfer interactions between the solute and the stationary phase when PGC is covered with hexane.

For the five other modifiers, when the percentage of modifier is increased, e decreases. As we had explained it for methanol modified mobile phases, when increasing the proportion of modifier, 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, the CO_2 and the modifier get adsorbed onto the PGC surface and function as components of the stationary phase. The modifier adsorbed, physically blocking the PGC surface, reduces the stationary phase's ability to establish charge-transfer interactions, therefore decreasing $e_{\text{stationary}}$. As indicated by Strubinger et al. [10], binary subcritical mobile phases exhibit gross compositional heterogeneity at interfaces, resulting in the modifier being present at many times the bulk concentration, thus having a tremendous effect on the stationary phase character. In other words, modifier is preferentially adsorbed onto the surface and the resulting coefficient describes the difference between the mobile phase and a modifier-rich stationary phase. It is quite evident here that this phenomenon should be considered to explain that the variation of $e_{\text{stationary}}$ is more important than the variation of e_{mobile}, resulting in a decrease of the coefficient e.

There is only little difference in the excess molar refraction term of the stationary phase among the various adsorbed modifiers.

3.3.1.4. The a coefficient. The a term is related to the H-bond donating ability (HBD) of the solute. Conversely, it describes the difference in H-bond accepting ability (HBA) between the mobile and stationary phases. It shows lower values than v and e but varies strongly with the modifier percentage (Fig. 4c). For all modifiers but hexane, it decreases rapidly and even becomes no more statistically sound in 40% methanol, isopropanol and acetonitrile. In hexane modified mobile phases, on the contrary, a increases continually between 5 and 40% modifier. The a coefficient represents the following:

$$a = a_{\text{stationary}} - a_{\text{mobile}} \quad (7)$$

Carbon dioxide acts is a weaker Lewis base than the modifiers used, apart from hexane. Therefore, when increasing the percentage of modifier in the mobile phase, a_{mobile} (representing the basic character of the mobile phase) increases, leading to a decrease of a. However, the Lewis basicity of the graphite surface remains higher than the one of the mobile phase (a is positive), possibly due to the high adsorption of the modifier at the surface.

Although supercritical carbon dioxide is similar to hexane in respect to its polarity, it is significantly different from hexane in its ability to Lewis acid–base pair and hydrogen-bond [49]. Thus, CO_2 is a Lewis base with proton acceptor selectivity properties. Therefore, in hexane modified mobile phases, the interpretation is the reverse to the preceding.

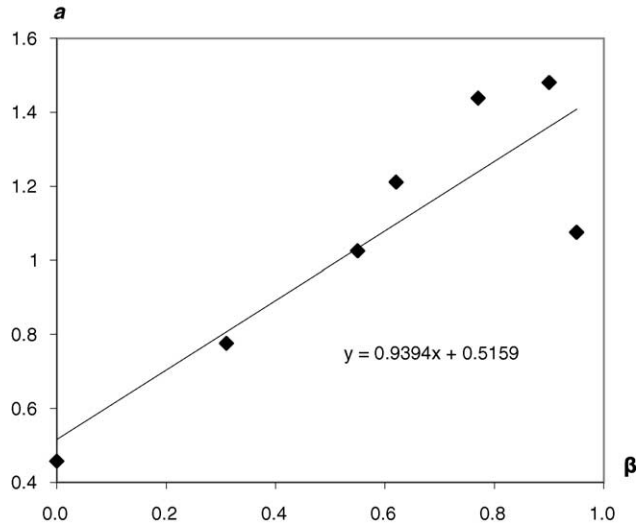

Fig. 5. Coefficient a measured at 5% modifier with all seven modifiers, plotted against the basic character (β) of the organic modifier.

These variations of a can be related to the basic properties of the modifiers, according to Kamlet and Taft solvatochromic parameters. At any modifier percentage, a plot of a obtained with a given modifier, versus β, representing the basic character of the organic solvent used as modifier, shows a reasonable correlation. For instance, the plot of the a coefficient measured at 5% modifier for the seven modifiers, against their basic character β, is plotted in Fig. 5. However, the slope of the regression line varies greatly when the modifier percentage is increased, as can be noticed in Fig. 6, where the slope of the regression line is plotted against modifier percentage. At 5% modifier (see Fig. 5), the slope of this curve is positive, indicating that, the higher the basicity of the organic solvent, the higher the a coefficient. This suggests that the addition of a basic modifier in small proportions increases the stationary phase basicity more than the mobile phase basicity. The modifier adsorbed onto the stationary phase controls the retention. In this respect, modifiers with strong hydrogen-bond accepting ability actively contribute to the stationary phase's hydrogen-bond accepting ability and induce high retention of hydrogen-donor solutes. For instance, as confirmed by its low β solvatochromic basicity parameter (0.31), acetonitrile is known to be a relatively weak eluent toward H-donor solutes. When it gets adsorbed onto the PGC surface, the global basicity of the stationary phase is lower than with tetrahydrofuran or with the alcohols, as indicated by the low a coefficient.

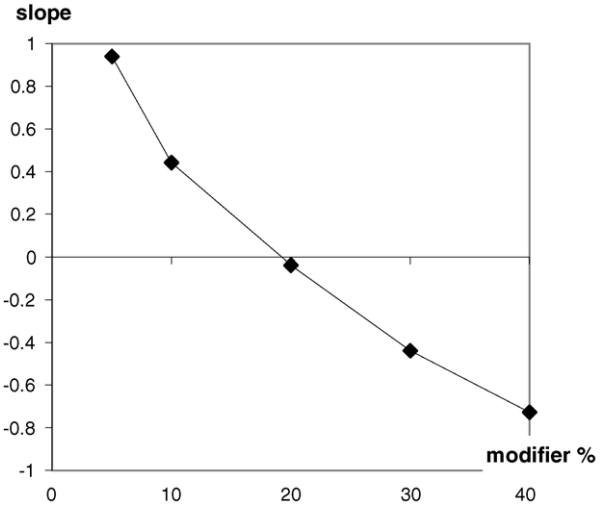

Fig. 6. Slope of the a vs. β curve plotted against modifier percentage.

This is in good agreement with the results of Engel and Olesik [21] indicating lowered solvent strength when using basic modifiers at small percentages (1%).

When increasing the percentage of modifier in the mobile phase, the slope of the a versus β plot decreases, equals zero at 20% modifier then becomes negative at higher percentages, as can be seen in Fig. 5, where the slope of this curve is plotted against the modifier percentage.

Therefore, at high modifier proportions, the higher the basicity of the organic solvent, the smaller the a coefficient. Modifiers with strong hydrogen-bond accepting ability actively contribute to the mobile phase's hydrogen-bond accepting ability and favour the elution of hydrogen-donor solutes.

We can conclude from this study that, at small modifier percentages, the characteristics of the modifier-rich stationary phase control the retention of acidic compounds, while at high modifier percentages, the characteristics of the modifier-rich mobile phase control the elution.

Generally, the observations made with methanol modified mobile phases are also valid with ethanol, *n*-propanol, isopropanol, tetrahydrofuran and acetonitrile mobile phases, hexane inducing somewhat different results. The charge–transfer and dispersion interactions govern the retention while the basic character of the stationary and mobile phases contributes to retention, particularly when the modifier is in small proportions.

3.3.2. Model use

The various mobile phase modifiers may be compared in terms of selectivities towards specific types of solutes. A modifier inducing a large x coefficient, being either positive or negative, will tend to be more selective, with respect to that particular type of interaction than a modifier with a small coefficient. As a matter of fact, Eq. (8) deduced from Eq. (1) relates the logarithm of the selectivity between two compounds to their difference in descriptor values:

$$\log \alpha = e\Delta E + s\Delta S + a\Delta A + b\Delta B + v\Delta V \quad (8)$$

Therefore, to enhance the separation of compounds differing in their X property, one should choose the conditions where the x coefficient is the most appropriate. For good selectivity, it is desirable that, in addition to a large x coefficient, the other coefficients have little influence on the separation. Most of the time, multiple interactions are established

so selectivity is a matter of degree. Naturally, changing the modifier to enhance selectivity is not always the best choice. Sometimes, increasing or decreasing the proportion of the chosen modifier is an easier means of retention control.

A first example is the separation of homologous series, differing only in volume. Indeed, 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 for these compounds is only related to dispersion interaction modifications [50]. As described elsewhere in HPLC [34,51–52], *v* increases linearly with the methylene selectivity in SubFC. Therefore, when optimizing the separation of this type of compounds, one should choose the conditions where the *v* coefficient is the highest. For instance, methanol and acetonitrile at any percentage, or any other modifier at a small percentage would be suitable. Furthermore, analysis time must be considered and, in this respect, higher percentages are often more desirable.

In the same manner as *v* was seen to be a good indicator of methylene selectivity, *a* is quite well correlated to the hydroxyl selectivity, as can be seen in Fig. 7. The hydroxyl selectivity was determined plotting the retention factors of phenol, resorcinol (1,3-dihydroxybenzene) and phloroglucinol (1,3,5-trihydroxybenzene) against the number of hydroxyl groups. Indeed, as indicated in a previous paper [29], these three meta-substituted phenols are perfectly aligned on such a plot. Thus, the hydroxyl selectivity $\log \alpha_{OH}$ is taken as the slope of the regression line between the three compounds. Although *E* and *V* also vary between these three compounds, the difference in retention is principally related to hydrogen-bond donating ability. Other selectivities between couples of compounds differing in a hydroxyl group (such as toluene–cresol, xylene–dimethylphenol, nitrobenzene–nitrophenol, naphthalene–naphtol) were also considered in like manner. In the case of nitrobenzene-*o*-nitrophenol where the –OH group is involved in an intramolecular interaction with the nitro group, the difference in acidity (ΔA) is nearly equal to zero (see Table 2). Therefore, the selectivity is not linked to the basicity of the chromatographic system (*a*). In any other case, the selectivities appeared to increase linearly with the coefficient *a*. Thus, Eq. (9) reads:

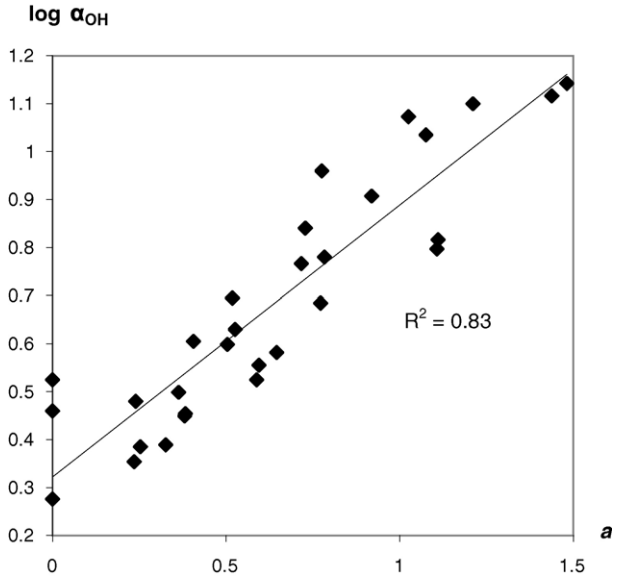

Fig. 7. Logarithm of the –OH selectivity measured with phenol, resorcinol and phloroglucinol, vs. the *a* coefficient, measured in all mobile phases tested.

Table 3
Study of selectivities between compounds differing of a hydroxyl group, following Eq. (9)

R–OH	R–H	ΔA	*g*	R^2
3,4-Dimethylphenol	*o*-Xylene	0.56	0.67	0.967
2,4-Dimethylphenol	*m*-Xylene	0.53	0.61	0.977
2,6-Dimethylphenol	*m*-xylene	0.39	0.44	0.927
2,5-Dimethylphenol	*p*-Xylene	0.54	0.61	0.980

$$\log \alpha_{OH} = ga + i \quad (9)$$

Therefore, *a* can be considered as a good indicator of hydroxyl selectivity.

Furthermore, considering Eqs. (8) and (9), the slope (*g*) is related to the difference in acidity of the two compounds considered (ΔA).

In Table 3, four couples of structurally similar compounds differing in a hydroxyl group (all xylenes-dimethylphenols) are presented, along with the difference in their *A* coefficient (ΔA), the slope (*g*) and the determination coefficient (R^2) of the regression line, according to Eq. (9). Thus, the slope of the $\log \alpha_{OH} = f(a)$ relationship (*g*) is clearly related to the difference in *A* of the two compounds considered.

Several conclusions can be drawn from these observations:

(1) The increase of acidity linked to the addition of an –OH group varies from one couple of compounds to another.
(2) Compounds differing of an –OH group are better separated with chromatographic systems inducing a large *a* coefficient.
(3) Compounds presenting a small difference in acidity require the highest *a* coefficients, obtained with low modifier percentages.

Similar relationships can be observed between a given coefficient and compounds differing primarily in the complimentary property. For instance, adding an aromatic ring induces an increased E value and naturally an increase in volume. Therefore, the appropriate equation would be the following:

$$\log \alpha_{\Phi} = g_e e + g_v v + i \quad (10)$$

This is illustrated by the selectivity between nitrobenzene and nitronaphthalene. A multiple linear regression of $\log \alpha_{\Phi}$ against *e* and *v* shows a good correlation ($R^2 = 0.943$). As the difference in excess molar refraction ($\Delta E = 0.73$) is twice the difference in volume ($\Delta V = 0.37$), the coefficient associated to charge transfer interactions ($g_e = 1.03$) is twice the

coefficient associated to dispersion interactions ($g_v = 0.50$). Thus, good aromatic selectivity (α_Φ) is obtained with mobile phases inducing primarily high *e* coefficients and, to a lesser extent, high *v* coefficients.

These examples illustrate the effective use that can be made of the LSERs in order to evaluate the potential of a chromatographic system for a given separation problem.

Moreover, selection of a mobile phase modifier to vary the different interactions in order to achieve maximum resolution cannot be done regardless of column efficiency and peak asymmetry.

Peak asymmetry variations with the modifier percentage were not significant.

Alcohols, particularly isopropanol, generally induced better efficiencies for most compounds.

4. Conclusion

This study provides us a greater understanding of the effects of adding various modifiers to the supercritical mobile phase with porous graphitic carbon. It has been shown that mobile phase composition may be adjusted so as to favour particular separations, taking into account analysis time requirements. For strongly retained compounds, the use of at least 20% THF, hexane, or alcohol with long alkyl chain is suggested to reduce the analytical duration. High amounts of methanol allow rapid elution of acidic compounds.

The effect of the modifier was shown to depend not only on the nature but also on the proportion of modifier. These effects are related to mobile phase–solute interaction modifications as well as modifier adsorption onto the stationary phase. With methanol and acetonitrile modifiers, the eluotropic strength variations depend on these two phenomena. For the other modifiers, these variations mostly depend on changes of dispersion interactions in the mobile phase.

Dispersion and charge transfer interactions rule the retention of most compounds. For acidic compounds, the basicity of the modifier controls the retention changes. In this case, the behaviour of hexane is opposite to the others.

The major source of band broadening arises from non ideal interactions leading to peak asymmetry, for acidic or basic compounds in particular. The use of acidic or and/or basic additives may improve the peak profiles for such compounds and shall be investigated with the varied modifiers.

Finally, LSER is an efficient way to study with accuracy complex chromatographic systems such as subcritical fluid chromatography with carbon dioxide-modifier mobile phases on porous graphitic carbon.

References

[1] J.R. Strubinger, J.F. Parcher, Anal. Chem. 61 (1989) 951.
[2] J.F. Parcher, J.R. Strubinger, J. Chromatogr. 479 (1989) 251.
[3] J.R. Strubinger, H. Song, J.F. Parcher, Anal. Chem. 63 (1991) 98.
[4] T.A. Berger, Chromatographia 37 (1993) 645.
[5] J.A. Blackwell, R.W. Stringham, Chromatographia 46 (1997) 301.
[6] E. Lesellier, K. Gurdale, A. Tchapla, Chromatographia 55 (2002) 555.
[7] T.A. Berger, J.F. Deye, Anal. Chem. 62 (1990) 1181.
[8] J.F. Deye, T.A. Berger, A.G. Anderson, Anal. Chem. 62 (1990) 615.
[9] K. Gurdale, E. Lesellier, A. Tchapla, Anal. Chem. 71 (1999) 2164.
[10] J.R. Strubinger, H. Song, J.F. Parcher, Anal. Chem. 63 (1991) 104.
[11] K. Gurdale, E. Lesellier, A. Tchapla, J. Chromatogr. A 866 (2000) 241.
[12] E. Lesellier, K. Gurdale, A. Tchapla, J Chromatogr. A 975 (2002) 335.
[13] D.M. Heaton, K.D. Bartle, A.A. Clifford, M.S. Klee, T.A. Berger, Anal. Chem. 66 (1994) 4253.
[14] C.H. Lochmüller, L.P. Mink, J. Chromatogr. 471 (1989) 357.
[15] J.G.M. Janssen, P.J. Schoenmakers, C.A. Cramers, J. High Resolut. Chromatogr. 12 (1989) 645.
[16] D. Pyo, W. Li, M.L. Lee, J.D. Werkwerth, P.W. Carr, J. Chromatogr. A 753 (1996) 291.
[17] S. Cocks, R.M. Smith, Anal. Proc. 29 (1992) 93.
[18] J. Weckwerth, P. Carr, Anal. Chem. 70 (1998) 1404.
[19] D. Pyo, H. Kim, J.H. Park, J. Chromatogr. A 796 (1998) 347.
[20] T.M. Engel, S.V. Olesik, Anal. Chem. 62 (1990) 1554.
[21] T.M. Engel, S.V. Olesik, Anal. Chem. 63 (1991) 1830.
[22] R. Kaliszan, J. Chromatogr. A 656 (1993) 417.
[23] R. Kaliszan, Anal. Chem. 64 (1992) 619A.
[24] M.H. Abraham, A. Ibrahim, A.M. Zissimos, J. Chromatogr. A 1037 (2004) 29.
[25] C.F. Poole, S.K. Poole, J. Chromatogr. A 965 (2002) 263.
[26] J.A. Blackwell, R.W. Stringham, J.D. Werckwerth, Anal. Chem. 69 (1997) 409.
[27] G.O. Cantrell, R.W. Stringham, J.A. Blackwell, J.D. Werckwerth, P.W. Carr, Anal. Chem. 68 (1996) 3645.
[28] J.A. Blackwell, R.W. Stringham, J. High Resolut. Chromatogr. 20 (1997) 631.
[29] C. West, E. Lesellier, A. Tchapla, J. Chromatogr. A 1048 (2004) 99.
[30] M.J. Kamlet, R.W. Taft, J. Am. Chem. Soc. 98 (1976) 377.
[31] R.W. Taft, M.J. Kamlet, J. Am. Chem. Soc. 98 (1976) 2886.
[32] M.J. Kamlet, J.L. Abboud, R.W. Taft, J. Am. Chem. Soc. 99 (1977) 6027.
[33] 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.
[34] M. Reta, P.W. Carr, P.C. Sadek, S.C. Rutan, Anal. Chem. 71 (1999) 3484.
[35] S.K. Poole, C.F. Poole, J. Chromatogr. A 845 (1999) 381.
[36] S.K. Poole, S. Patel, K. Dehring, H. Workman, J. Dong, J. Chromatogr. B 793 (2003) 265.
[37] R. Kaliszan, M.A. van Straten, M. Markuszewski, C.A. Cramers, H.A. Claessens, J. Chromatogr. A 855 (1999) 455.
[38] C. Lepont, A.D. Gunatilleka, C.F. Poole, Analyst 126 (2001) 1318.
[39] M.A. Al-Haj, R. Kaliszan, A. Nasal, Anal. Chem. 71 (1999) 2976.
[40] Q.H. Wan, P.N. Shaw, M.C. Davies, D.A. Barrett, J. Chromatogr. A 697 (1995) 219.
[41] L.R. Snyder, in: J.C. Giddings, R.A. Keller (Eds.), Principles of Adsorption Chromatography, Chromatogr. Sci. Series, Marcel Dekker, New York, 1968.
[42] H.J. Möckel, A. Braedikow, H. Melzer, G. Aced, J. Liq. Chromatogr. 14 (1991) 2477.
[43] B. Kaur, LC–GC Int. 3 (3) (1990) 41.
[44] K. Gaudin, P. Chaminade, A. Baillet, J. Chromatogr. A 973 (2002) 61.
[45] E. Lesellier, A.M. Krstulovic, A. Tchapla, Chromatographia 36 (1993) 275.

[46] J.H. Knox, B. Kaur, in: P. Brown, R. Hartwick (Eds.), High Performance Liquid Chromatography, Wiley, New York, 1989 (Chapter 4).
[47] D. Corradini, K. Kalghatgi, C. Horváth, J. Chromatogr. 728 (1999) 225.
[48] D. Bush, C.A. Eckert, Fluid Phase Equilib. 150–151 (1998) 479.
[49] J.H. Phillips, R.J. Robey, J. Chromatogr. 465 (1989) 177.
[50] P.W. Carr, J. Li, A.J. Dallas, D.I. Eikens, L.C. Tan, J. Chromatogr. A 656 (1993) 113.
[51] A. Sándi, M. Nagy, L. Szepesy, J. Chromatogr. A 893 (2000) 215.
[52] P.T. Jackson, M.R. Schure, T.P. Weber, P.W. Carr, Anal. Chem. 69 (1997) 416.