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Separation of substituted aromatic isomers with porous graphitic carbon in subcritical fluid chromatography

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Abstract

The ability of porous graphitic carbon (PGC) to separate structural isomers has been reported in high-performance liquid chromatography (HPLC). This paper presents studies carried out in subcritical fluid chromatography (SubFC). Various polar and nonpolar modifiers were added to the carbon dioxide mobile phase, in proportions ranging from 5 to 40%. The effects of both the nature and the percentage of the modifier on aromatic isomer separations were studied. Two types of selectivity behaviour appear. The first one, related to steric recognition, is due to the number of contact points between the compounds and the flat surface of PGC. In this case, retention orders are often identical to that reported in HPLC. The second is related to the favourable interaction between the polar moieties of the solutes and the stationary phase. In this case, the retention and selectivity strongly depend on the mobile phase composition. Thus, the separations obtained are greatly enhanced, compared to those obtained in HPLC. The retention and selectivity variations observed when the composition of the mobile phase is changed are discussed based on linear solvation energy relationships (LSERs). Practical applications are presented, namely benzene, toluene, ethylbenzene and xylenes (BTEX) and flavour molecules separations.

Keywords: Supercritical fluid chromatography; Linear solvation energy relationship; Porous graphitic carbon; Substituted aromatic; Isomers

1. Introduction

The separation of isomers is a topic of a great interest as isomeric species generally have different biological activities. However, the substituent position on an aromatic ring (*ortho*, *meta*, *para*) or the double bond conformation, or the non aromatic cycle conformation (*cis*, *trans*), induces few differences in interactions between the analytes and the mobile phase or in boiling temperature between isomers. Shape selectivity, when it occurs, arises from interactions in the stationary phase [1], which implies that the isomeric solutes establish different interactions with the stationary phase. Consequently, such separations require especially suited chromatographic system.

In liquid chromatography, this kind of separation first depends on the physical state of the stationary phase, solid or liquid like. In reversed-phase liquid chromatography with solvated bonded octadecyl chains (ODS), the retention is governed by a partition retention mechanism. In this case, solutes are surrounded by the alkyl chains, and generally, conformational changes can not provide the required energetic differences inducing retention variations. However, with large and rigid compounds, such as polynuclear aromatic hydrocarbons (PAHs) [2] or carotenoid pigments [3], the increase in rigidity of the bonded chains, related to a decrease in temperature, favours the separation between planar and non planar or between linear and bent compounds. Thus, some type of isomeric separation is possible on highly ordered polymeric ODS.

Alumina and amorphous silica have geometrically heterogeneous surfaces, which are far from flat at the atomic level but can distinguish molecules on the basis of specific effects, such as dipole and H-bonding interactions [4]. Thus, isomeric separations of polar compounds are possible on these stationary phases.

With solid adsorbents, such as porous graphitic carbon (PGC) and carbon-clad zirconia [5], interactions take place on a flat and rigid solid surface. For compounds which do not possess any functionality group to undergo interactions other than dispersion forces (hydrocarbonaceous compounds), a steric recognition is

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mainly based on the number of contact points between solute and adsorbent [6]. Enhanced dispersion interactions and overlap of π electrons, achieved for planar aromatic solute molecules, is a critical factor of retention, therefore, a critical factor for separation on PGC [4,7]. For instance, the increase in planarity of cyclohexanes induces a greater retention on PGC [8]. Satisfactory separations were also reported for butyl- and nonylphenol polyethoxylate (NPE) isomers [9]. As expected, the retention of the more branched isomers is lower than that of the linear isomers.

Moreover, any type of functional group, be it polar or not, causes an increase in retention on PGC, making it different from both the polar adsorbents (alumina and silica gel) and the nonpolar bonded phases (ODS). This makes PGC highly suitable for the separation of both polar and nonpolar species.

The nature of the polar substituents (such as OH, NO_2, COOH, NH_2 or halogens), their effects on the electron density distribution in the benzene ring, their ability to create intra- or intermolecular interactions, such as hydrogen bonding, all influence charge transfer, dipole–dipole, acidic or basic interactions between the solutes and the stationary and mobile phases. Therefore, PGC is especially suited for separation of di-*ortho*, mono-*ortho* and non-*ortho* polychlorinated biphenyls (PCBs). Ortho substitutions reduce the planarity of the two rings, as the dihedral angle formed by the two aromatic rings depends on the number of *ortho* substituents [10–12].

Moreover, PGC displays an energetically homogeneous surface [6]. However, despite this surface homogeneity that would simplify the retention phenomenon (as compared to heterogeneous bonded silica), understanding of the retention order of isomers can be unclear, due to the numerous types of interactions occurring on PGC.

Additionally, understanding is further complicated by the fact that the authors, having presented isomeric separations on PGC, used very different liquid mobile phases from one paper to another, thus leading to different retention orders between isomers [6,13–17].

An insight into the retention mechanism occurring in any chromatographic system can be brought by studies based on linear solvation energy relationships (LSER). Using Abraham parameters [18], the classical equation used in high-performance liquid chromatography (HPLC) is:

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

where k is the solute retention factor.

In this equation, capital letters represent the solute descriptors, related to particular interaction properties, while lower case letters represent the system constants, related to the complementary 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, the solute dipolarity/polarizability; A and B, the solute overall hydrogen-bond acidity and basicity; V, 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. Consequently, the coefficients also reflect the system's relative selectivity towards that particular molecular interaction. This model was recently applied in subcritical fluid chromatography (SubFC) with PGC [19,20].

The use of supercritical fluids as a mobile phase is indeed expected to improve the separation efficiency, thanks to increased diffusion coefficients. Wen and Olesik [21] showed that the addition of low amounts of CO_2 to a methanol–water mobile phase created a buffered mobile phase in SFC, thus altering the retention of acidic and basic isomers without modifying their retention order. Obviously, such modifications of mobile phase properties could be dramatically enhanced by the use of super/or subcritical mobile phases, with greater carbon dioxide amounts mixed with different organic solvents used as modifiers. Additionally, the varied nature of modifiers that can be added to carbon dioxide could provide different separations on PGC [20,22], and the LSER could help in understanding isomeric separations.

This paper presents the study of isomer selectivities of di- and tri-substituted aromatic compounds in SFC with PGC. The influence of mobile phase composition on retention order and separation were investigated. The results presented in our two previous papers [19,20] were used to support many discussions in the current paper. Besides, some practical applications of isomeric separation on PGC in SubFC are presented.

2. Experimental

2.1. Chemicals

Solvents used as modifiers were HPLC grade methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF), ethanol (EtOH) (VWR Prolabo, Val-de-Fontenay, France), *n*-propanol (nPrOH) (Carlo Erba, Milan, Italy), isopropanol (iPrOH) (SdS, Peypin, France) and hexane (HXN) (J.T. Baker). Carbon dioxide was provided by Alphagaz (Bois d'Arcy, France). The solvents were chosen, as previously discussed [20], so as to provide a wide range of size, polarity, hydrophobicity and hydrogen-bonding ability, therefore, inducing a wide variety of interactions with the solutes.

The series of isomer families were chosen, in the same manner, so as to provide a wide variety of substituents. The isomeric systems chosen were selected for simplicity, to provide some insight as to the retention mechanism, and not for any analytical importance. Differently substituted benzenic species, all commercially available, were studied (see Table 1). Solutions of these compounds were prepared in methanol. For each isomeric group, solutions of both the individual isomers and a mixture of the isomers were prepared. The solute descriptors used in the solvation parameter model were taken from several sources [23–25] and are summarized in Table 1.

Additionally, for application demonstrations, toluene, ethylbenzene, propylbenzene, linalool, nerol, geraniol, camphor and menthone were also used.

Table 1
Chromatographic solutes and LSER descriptors

	Compound	*E*	*S*	*A*	*B*	*V*
1	*o*-Xylene	0.663	0.56	0.00	0.16	0.9980
2	*m*-Xylene	0.623	0.52	0.00	0.16	0.9980
3	*p*-Xylene	0.613	0.52	0.00	0.16	0.9980
4	*o*-Cresol	0.840	0.86	0.52	0.30	0.9160
5	*m*-Cresol	0.822	0.88	0.57	0.34	0.9160
6	*p*-Cresol	0.820	0.87	0.57	0.31	0.9160
7	2,3-Dimethylphenol	0.850	0.85	0.52	0.36	1.0569
8	2,4-Dimethylphenol	0.840	0.80	0.53	0.39	1.0569
9	2,5-Dimethylphenol	0.840	0.79	0.54	0.37	1.0569
10	2,6-Dimethylphenol	0.860	0.79	0.39	0.39	1.0569
11	3,4-Dimethylphenol	0.830	0.86	0.56	0.39	1.0569
12	3,5-Dimethylphenol	0.820	0.84	0.57	0.36	1.0569
13	*o*-Isopropylphenol	0.822	0.79	0.52	0.44	1.1978
14	*m*-Isopropylphenol	0.811	0.92	0.55	0.46	1.1978
15	*p*-Isopropylphenol	0.791	0.89	0.55	0.38	1.1978
16	*o*-Chlorophenol	0.853	0.88	0.32	0.31	0.8975
17	*m*-Chlorophenol	0.909	1.06	0.69	0.15	0.8975
18	*p*-Chlorophenol	0.915	1.08	0.67	0.20	0.8975
19	*o*-Nitrophenol	1.045	1.05	0.05	0.37	0.9490
20	*m*-Nitrophenol	1.050	1.57	0.79	0.23	0.9490
21	*p*-Nitrophenol	1.070	1.72	0.82	0.26	0.9490
22	*o*-Nitrobenzylalcohol	1.059	1.11	0.45	0.65	1.0900
23	*m*-Nitrobenzylalcohol	1.064	1.35	0.44	0.64	1.0900
24	*p*-Nitrobenzylalcohol	1.064	1.39	0.44	0.62	1.0900
25	*o*-Nitrotoluene	0.866	1.11	0.00	0.28	1.0320
26	*m*-Nitrotoluene	0.874	1.10	0.00	0.25	1.0320
27	*p*-Nitrotoluene	0.870	1.11	0.00	0.28	1.0320
28	*o*-Methylacetophenone	0.780	1.00	0.00	0.51	1.1550
29	*m*-Methylacetophenone	0.806	1.00	0.00	0.51	1.1550
30	*p*-Methylacetophenone	0.842	1.00	0.00	0.52	1.1550

E, excess molar refraction; *S*, dipolarity/polarizability; *A*, hydrogen bond acidity; *B*, hydrogen bond basicity; *V*, McGowan's characteristic volume; values from references [18–20].

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 the other 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, UK) 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, CA, 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, except for flavours that were detected at 210 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 (Les Ulis, France).

2.3. Chromatographic conditions

Samples were chromatographied using carbon dioxide with 5–40% (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 on the isomer separations, all 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.

Methylacetophenones, nitrobenzyl alcohols, isopropylphenols and nitrotoluenes were studied in methanol-modified mobile phases, while the other isomer groups were chromatographed in all seven modifiers.

2.4. Retention factors

Retention factors (k) were determined using the relationship:

$$k = \left(\frac{t_r - t_0}{t_0}\right)$$

where t_r is the solute retention time, determined using the peak maximums (even when tailing did occur) and t_0, the hold-up time measured on the first negative peak due to the unretained dilution solvent. All isomer solutes were injected separately to determine retention order in each mobile phase condition, and then the isomer mixtures were injected.

3. Results and discussion

3.1. Elution order

At low percentages of the modifier (below 20%), the separation was shown [20] to be dominated by the interactions between the solutes and the stationary phase covered with adsorbed modifier. With 10% methanol in the mobile phase, all isomeric mixtures elutes in the same order, apart from xylene isomers.

As reported in HPLC [26], xylene isomers are eluting in *meta*, *para*, *ortho* order. An identical elution order was reported in HPLC on carbon-clad zirconia [5], which displays a retention behaviour very similar to that of PGC [27].

The lower retention of *meta*-xylene is related to the smaller number of contact points between this isomer and the flat surface of porous graphitic carbon (three contact points, as opposed to four contact points for the *para*- and *ortho*-isomers) [26].

The greater retention of *ortho*-xylene in comparison to *para*-xylene could be due to the greater value of the E descriptor (charge transfer) of the former, inducing a higher charge-transfer interaction. Other studies on carbon surfaces suggested that the electrons of *ortho*-isomer are more able to induce a significant dipole in the carbon surface, whereas the electronic distribution in the *para*-isomer is more symmetric, leading to smaller induced dipoles [5].

The higher retention of *ortho* substituted compounds ('*ortho* effect') is also observed with alumina and silica gel in HPLC [4].

The elution order of all other isomeric mixtures is *ortho*, *meta*, *para*. Interaction between polar groups and the carbon surface are thought to be particularly strong [5]. When approaching PGC, the aromatic plane of the solute would tend to interact with the planar graphite surface, with the polar side of the solute orientated towards the surface. Thus, the polar aromatic solute would not align in a parallel manner to the graphite surface, but rather be orientated at a certain angle. Any alteration in a molecule that decreases the degree to which the polar group can contact the carbon surface weakens retention: when steric hindrance of the polar group is large (as is the case with *ortho*-methyl and *ortho*-isopropyl groups), it cannot contact the carbon surface as effectively: steric crowding around the polar group decreases retention. Thus, as congestion around the polar group decreases, retention increases.

Additionally, some intramolecular hydrogen bonding interactions have to be considered for *ortho*-nitrophenol and chlorophenol reducing the acidity of these compounds, therefore, largely decreasing their retention, compared to their *meta*- and *para*-isomers.

Consequently, in most cases, the selectivity between *meta*- and *ortho*-isomers is greater than the selectivity between *para*- and *meta*-isomers.

With larger amounts of the modifier in the mobile phase, the effect of the stationary phase on the selectivity is lessened as the interactions between the solutes and the mobile phase dramatically increase, modifying the separation. In this case, elution orders can be altered. For instance, the elution order of cresols is changed from *ortho*, *meta*, *para* to *meta*, *ortho*, *para* when the proportion of the modifier is increased from 5 to 40%. The solvation of the *meta* substituted phenolic compound is probably higher than that of the *ortho*-isomer, due to steric crowding caused by the methyl group, thus leading to reduced retention of the former.

3.2. Variation of retention with the proportion of modifier

In our previous paper [20], we showed that retention on PGC could be expressed by a reduced form of Eq. (1):

$$\log k = c + eE + aA + vV \quad (3)$$

The *s* and *b* coefficients were found not significant in the chromatographic conditions studied. Additionally, when the concentration of the modifier in the mobile phase was increased, all three coefficients remaining (*e*, *a* and *v*) were shown to decrease, the decrease in *a* values being larger than the decrease in *e* and *v*. Hexane-modified mobile phases induced different variations as the *a* coefficient increased with the percentage of hexane in the mobile phase.

As expected from the LSER model, the increase in the modifier percentage reduces the retention factor for all studied compounds.

For non acidic compounds, such as xylenes, methylacetophenones, nitrotoluenes and *o*-nitrophenol, these retention variations are mainly related to the decrease in *e* and *v* val-

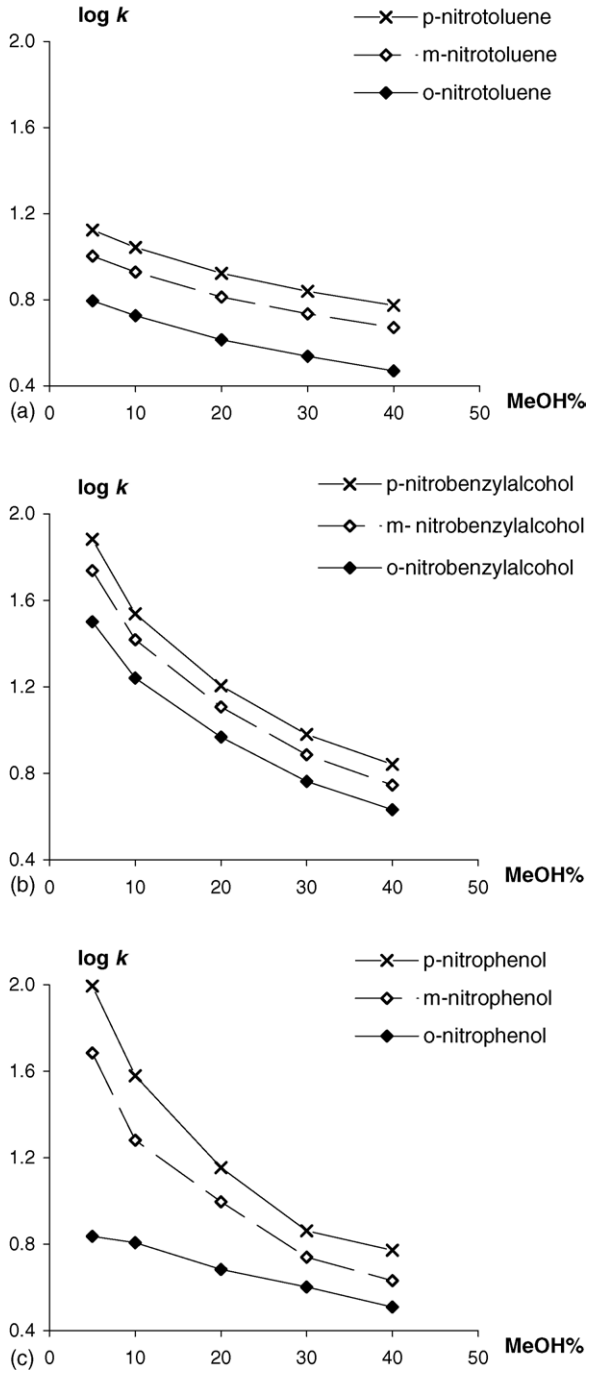

Fig. 1. Retention behaviour of representative isomeric solutes in methanol-modified mobile phases: (a) *o*-, *m*-, *p*-nitrotoluenes; (b) *o*-, *m*-, *p*-nitrobenzyl alcohols; (c) *o*-, *m*-, *p*-nitrophenols. Conditions: Hypercarb 100 mm × 4.6 mm, dp = 5 μm, 25 °C, back pressure 15 MPa.

ues, due to solvent adsorption onto the stationary phase, which reduces the solute/stationary phase interactions (see Fig. 1a). For acidic compounds (phenols and benzylic alcohols), the change in mobile phase basicity, due to the increase in the modifier content, explains the large retention variation observed (see Fig. 1b). Different behaviours are observed depending on the nature of the modifier, according to our previous results [20]. Namely, acetonitrile induces smaller retention decrease than the alcohols, while hexane induces a retention increase.

Thus, in one group of isomers, the three compounds can present a different behaviour, depending on their H-bond donating ability. For instance, *ortho*-nitrophenol is not acidic, due to intramolecular hydrogen bonding between the hydroxyl and nitro groups, whereas the *meta*- and *para*-isomers are acidic. Therefore, the retention variation of the *ortho*-isomer when the modifier percentage is increased is lower than that of the two other isomers (see Fig. 1c).

3.3. Variation of selectivity with the percentage of modifier

Eq. (4) deduced from Eq. (1), relates the logarithm of the selectivity between two compounds to their difference in descriptor values:

$$\log \alpha = e\nabla E + s\Delta S + a\Delta A + b\Delta B + v\Delta V \quad (4)$$

As only coefficients e, a and v were found to be significant in the chromatographic conditions considered in this study, a reduced form of Eq. (4) can be used:

$$\log \alpha = e\Delta E + a\Delta A + v\Delta V \quad (5)$$

As isomeric species are considered, the volume is identical between two compounds, and Eq. (5) reduces to Eq. (6):

$$\log \alpha = e\Delta E + a\Delta A \quad (6)$$

Consequently, except with hexane, when the modifier percentage is increased, the selectivity factors between two isomeric compounds decreases, whatever be the nature of the substituents. This is consistent with other observations on shape selectivity in SFC [28]. Stronger mobile phases reduce the interaction of solutes with the stationary phase and, since shape selectivity is a stationary phase phenomenon, an increase in solvent strength decreases shape selectivity.

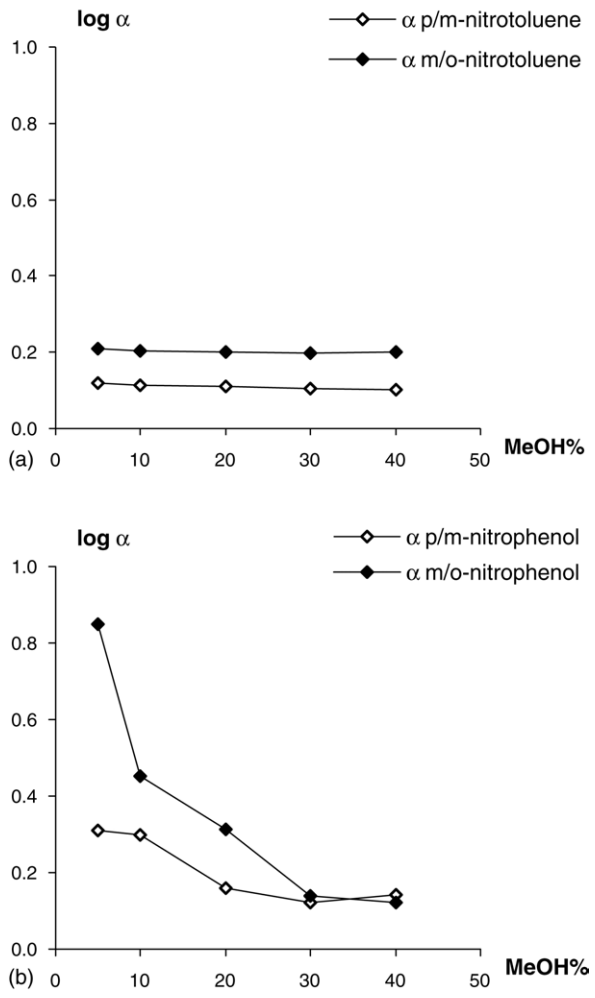

Fig. 2. Logarithm of selectivity factors plotted against methanol percentage in the mobile phase: (a) *m*/*o* and *p*/*m*-nitrotoluenes; (b) *m*/*o* and *p*/*m*-nitrophenols. Experimental conditions as in Fig. 1.

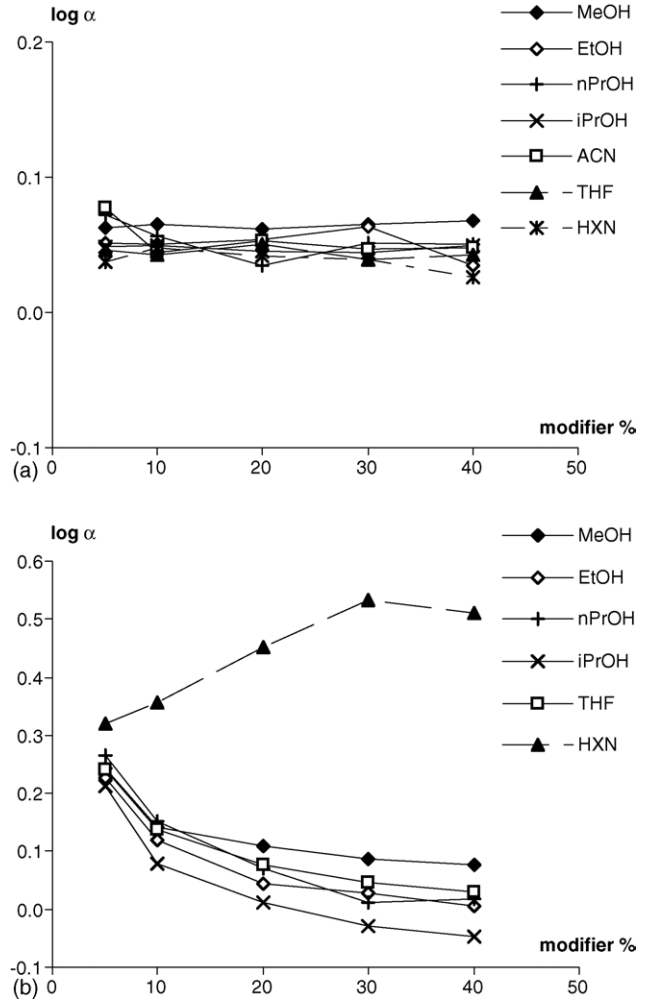

Fig. 3. Logarithm of selectivity factors plotted against modifier percentage in the mobile phase. (a) *p*/*m*-xylenes; (b) 2,4/2,6-nitrophenols. Experimental conditions as in Fig. 1.

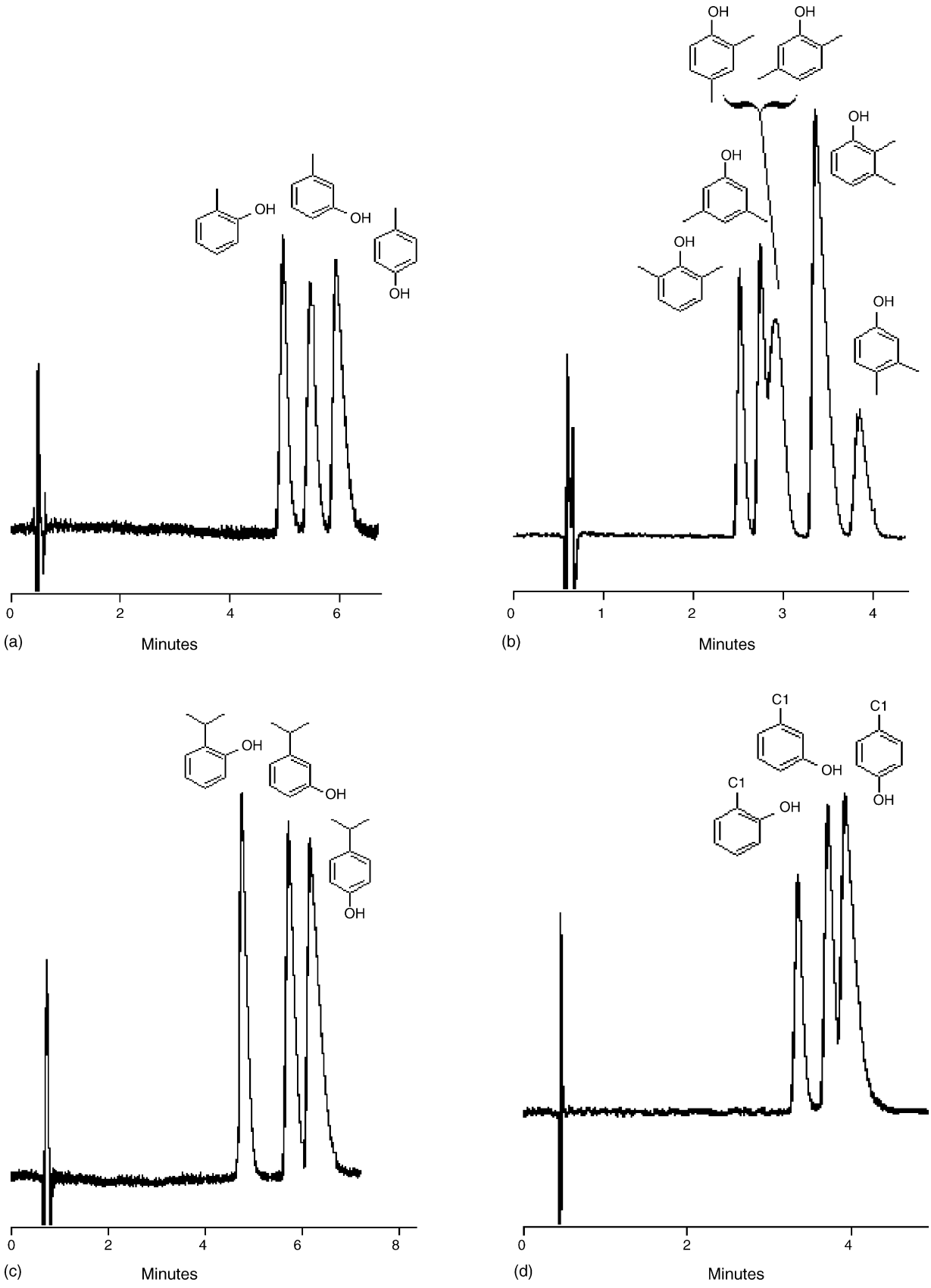

Fig. 4. Chromatograms of isomeric mixtures: (a) cresols with 7% methanol; (b) dimethylphenols with 20% *n*-propanol; (c) isopropylphenols with 5% *n*-propanol; (d) chlorophenols with 15% methanol; (e) nitrophenols with 40% ethanol; (f) nitrobenzyl alcohols with 40% methanol; (g) nitrotoluenes with 40% methanol; (h) methylacetophenones with 40% methanol. Experimental conditions as in Fig. 1.

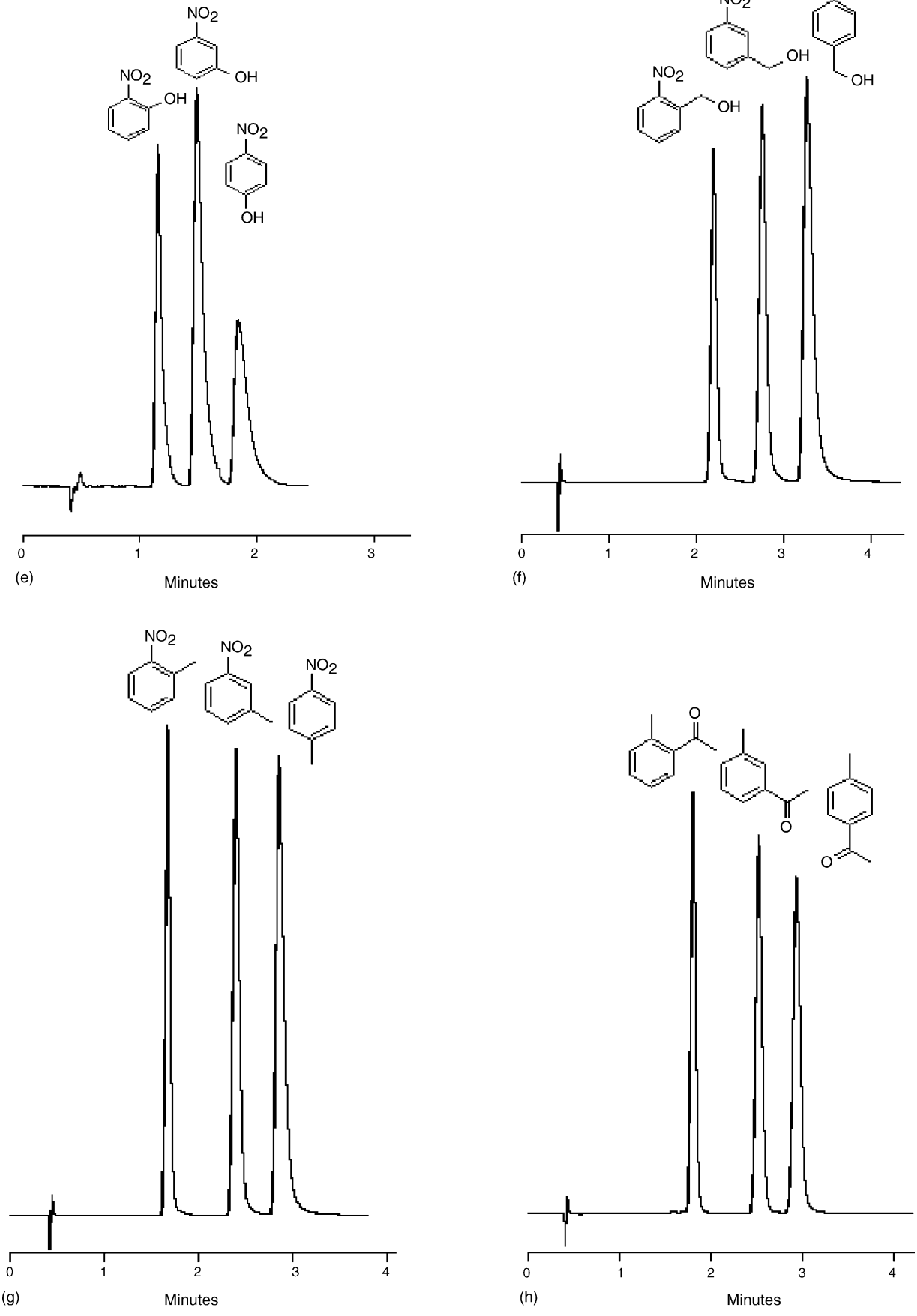

Fig. 4. (*Continued*).

When the difference in acidity between two isomers is small (or equal to zero), the variation of the selectivity factor is only related to the variations of the *e* coefficient. Thus, the selectivity variation with the percentage of the modifier is very small, as evidenced in Fig. 2a, where the selectivity variations between nitrotoluene isomers are represented.

When the difference in acidity is large, as is the case for nitrophenols (Fig. 2b), the increase in modifier percentage in the mobile phase, leading to a large decrease in the *a* coefficient, induces a large variation of the selectivity factor.

3.4. Variation of selectivity with the nature of modifier

As previously shown, the *e* coefficient does not vary greatly when the nature of the modifier is changed. Thus, significant changes in selectivity factors are not expected for isomeric compounds having close acidity values. This is evidenced by Fig. 3a, where the logarithm of selectivity factor between *para*- and *meta*-xylenes is plotted against modifier percentage, for the seven modifiers tested. The composition of the mobile phase is seen to have no influence on such a separation.

On the other hand, the *a* coefficient was shown to depend greatly on the mobile phase composition. Thus, separations of isomeric solutes having different acidic properties are expected to depend on the nature of the modifier used. For instance, 2,6-dimethylphenol, with its double-*ortho* substitution, is less acidic than 2,4-dimethylphenol, due to different steric crowding of the phenolic group, as evidenced by the values of their *A* coefficients (0.39 as opposed to 0.53, see Table 1). Consequently, the separation of these two isomeric solutes is strongly dependent on the nature and percentage of the modifier, as shown in Fig. 3b. For all modifiers but hexane, the selectivity is better at low percentages. If the proportion of the modifier needs to be increased to reduce analysis time, a modifier maintaining a high selectivity can be chosen.

3.5. Choice of appropriate separation conditions

Considering all these preliminary investigations, appropriate mobile phase conditions were determined for each isomeric mixture. The best separations obtained are presented in Fig. 4.

Cresol isomers were perfectly separated with a low amount (7%) of methanol in the mobile phase (Fig. 4a), while a perfect separation of the six dimethylphenol isomers was never reached. However, the use of *n*-propanol as a modifier, inducing better column efficiency and peak symmetry, led to the chromatogram presented in Fig. 4b, where five peaks are visible.

Perfect baseline separation was never reached for *meta*- and *para*-isomers of chloro- and isopropylphenols (Fig. 4c and d). Again, the use of *n*-propanol as a modifier led to sharper peaks.

For nitro-substituted compounds (Fig. 4e–g) and methylacetophenone (Fig. 4h) isomer families, satisfactory separations were obtained even with high proportions of the modifier. This is due to the fact that an extended π system dramatically increases retention on PGC [14,29].

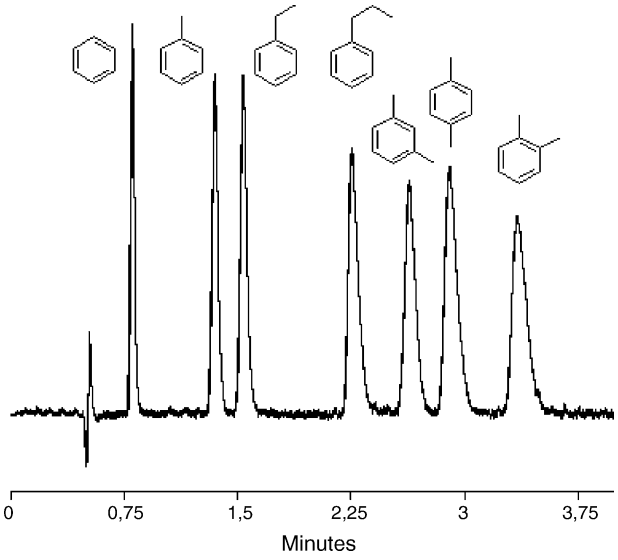

Fig. 5. BTEX separation with pure carbon dioxide as a mobile phase. Experimental conditions as in Fig. 1.

3.6. Applications

3.6.1. BTEX separation

A perfect separation of benzene, toluene, ethylbenzene, propylbenzene and the three xylene isomers (BTEX) is obtained on PGC with pure carbon dioxide as a mobile phase (Fig. 5). Baseline resolution was never achieved between the *para*- and *ortho*-xylenes even when the smallest proportion of the modifier was added to carbon dioxide. This can be observed in Fig. 6, where the selectivity factors between the critical pairs (ethylbenzene/toluene, *para*/*meta*-xylenes, *ortho*/*para*-xylenes) is plotted against methanol percentage in the mobile phase. One percent of methanol in the mobile phase is shown to have a dramatic effect on the selectivity between *para*- and *ortho*-xylene.

We believe that the modifier adsorbed onto the stationary phase reduces the possibility of close contact between the solutes and the PGC surface. The energy of solute–adsorbent interactions is very much dependent upon the distance between the

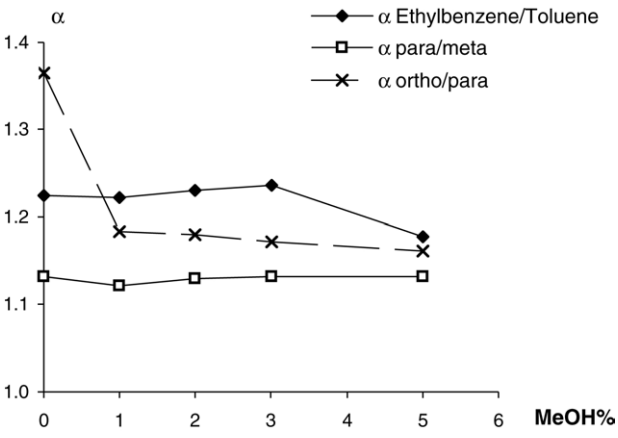

Fig. 6. Selectivity factors plotted against methanol percentage in the mobile phase. Ethylbenzene/Toluene; *para*/*meta*-xylene; *ortho*/*para*-xylene. Experimental conditions as in Fig. 1.

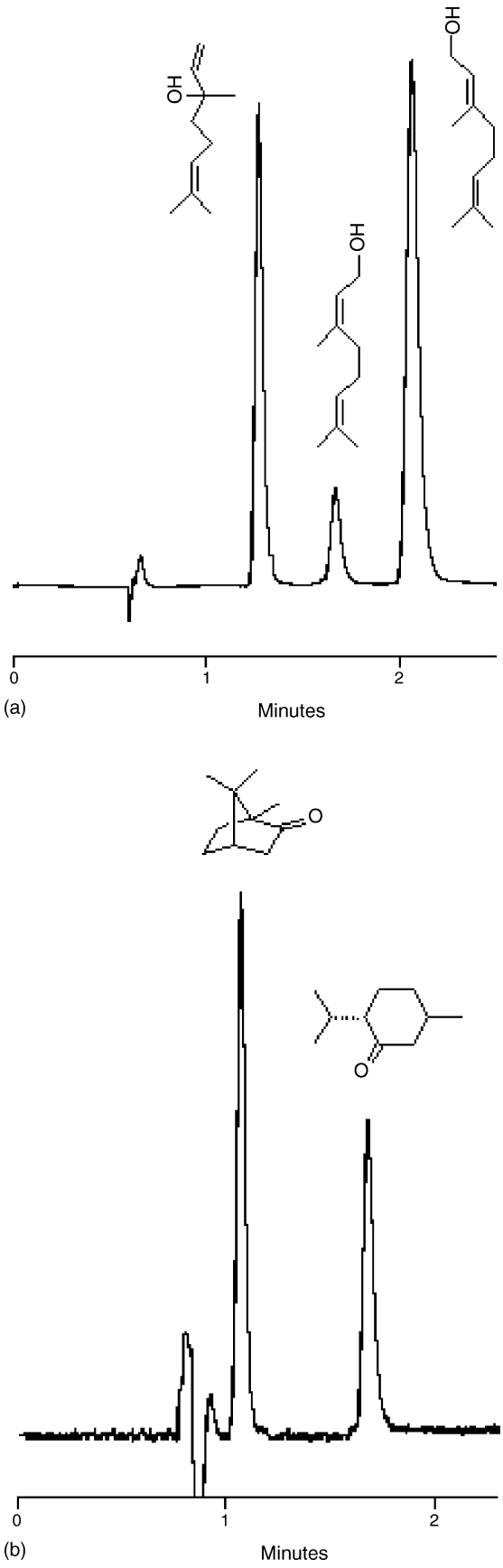

Fig. 7. (a) Separation of linalool, nerol and geraniol (*cis* and *trans* isomers), obtained with 10% ethanol; (b) separation of camphor and menthone obtained with 3% ethanol. Experimental conditions as in Fig. 1.

surface and the force centers in the solute molecule [6]. With pure carbon dioxide mobile phase, the close proximity of the molecular surface of a solute and the stationary phase, made possible by mutual steric compatibility, is a critical factor for retention to be favourable, leading to pronounced steric selectivity. This would also explain why such a separation was never achieved in liquid mobile phases as the participation of not only the solid stationary phase but also the organic solvents residing on the stationary phase is important to effect separation in practice.

3.6.2. Flavours

The separations presented in Fig. 7 demonstrate that PGC is equally good for other types of isomerisms. For these separations, ethanol was used as a modifier, as is generally the case for flavours.

The position of the hydroxyl group in linalool (first compound eluted in Fig. 7a) is less favourable to a close contact with the carbon surface than that of nerol and geraniol, at the end of the aliphatic chain. Nerol and geraniol are *cis* and *trans* isomers and are also very well separated.

In the same manner, close contact of the camphor molecule with the PGC surface is not possible, due to its bridged structure, while the more planar menthone shows enhanced steric compatibility, as evidenced by its longer retention time.

4. Conclusion

The use of carbon dioxide and organic modifiers in SFC is very well suited to the separation of aromatic isomers on PGC. The solvation parameter model is relevant to understand selectivity variations between aromatic isomers, when the composition of the mobile phase is varied.

Very good separations were obtained for most isomeric mixtures, apart from the more challenging dimethylphenol mixture.

Beyond these simple isomer separations, further work will be carried out to investigate the separation of more complicated isomeric mixtures, either by increasing the column length or by coupling PGC with other stationary phases allowing complementary shape selectivity.

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