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RESOLUTION OF ENANTIOMERIC AMIDES ON A PIRKLE-TYPE CHIRAL STATIONARY PHASE

A COMPARISON OF SUBCRITICAL FLUID AND LIQUID CHROMATOGRAPHIC APPROACHES

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SUMMARY

Subcritical and supercritical fluid chromatography (SubFC and SFC) have been evaluated for the resolution of an homologous series of enantiomeric amides. The solutes were the 2-naphthoyl amides of an homologous series of amines, ranging from 2-aminobutane to 2-aminooctane, and the *p*-methyl-, *p*-methoxy- and *p*-chlorophenylamides of 2-aminoheptane. The chiral stationary phase (CSP) used was the covalent form of (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine. In liquid chromatography (LC) the mobile phase comprised hexane–2-propanol–acetonitrile (97:3:0.5) at a flow-rate of 2 ml/min and temperatures of 20–35°C. In SFC, the mobile phases were various mixtures of carbon dioxide and polar modifiers, such as alcohols, chloroform and water. For the best conditions in LC, the chiral resolution, α, increased through the homologous series from $\alpha = 1.03$ for the amide derived from 2-aminobutane to $\alpha = 1.11$ for the 2-aminooctane amide. The values of α observed for the π-basic amides of 2-aminoheptane (*p*-methyl and *p*-methoxy) were greater than that observed for the π-acidic amide (*p*-chloro), *i.e.*, $\alpha = 1.08$ *versus* 1.04. The selectivities, resolutions and efficiencies obtained by LC and SubFC were similar. These results indicate that the mechanism of chiral recognition is the same in LC and SubFC and that the methods should be interchangeable. The actual analysis time for SubFC was significantly shorter than that required for LC: as short as 2 min for the 2-aminooctane amide, whereas LC takes over 10 min under the best conditions.

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INTRODUCTION

In the past few years, there has been much interest in supercritical fluid chromatography (SFC)[1–4]. This is due, in part, to the fact that SFC offers a number of advantages over liquid chromatography (LC), such as shorter analysis time, cheaper solvents and the use of detectors akin to those for gas chromatography. The resolution and selectivity achieved by each approach appear to be equivalent.

While most of the initial work with SFC has involved adsorption or partition-type stationary phases, Mourier *et al.*[5] have reported the resolution of some enantiomeric phosphine oxides on a Pirkle-type chiral stationary phase (CSP). While this paper was in preparation, Hara *et al.*[6] reported the chiral resolution of 2-amino acid derivatives with supercritical carbon dioxide on a CSP N-formylvaline bonded silica gel.

The results in refs. 5 and 6 suggest that SFC and LC are interchangeable in chiral chromatography. We have investigated this assumption, using an homologous series of enantiomeric amides and a Pirkle-type CSP. The solutes were the 2-naphthylamides of an homologous series of amines, ranging from 2-aminopropane to 2-aminooctane. The *p*-methyl-, *p*-methoxy- and *p*-chlorophenylamides of 2-aminoheptane were also tested. The CSP was the covalent form of (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine. For LC we used hexane, modified with a polar solvent, such as 2-propanol, as the mobile phase, while in subcritical fluid chromatography (SubFC) the mobile phases consisted of mixtures of subcritical carbon dioxide and various polar modifiers.

The retentions, selectivities, efficiencies and resolutions were determined for each solute in LC and SubFC. Differences due to alterations in one of the chromatographic parameters, *e.g.*, the structure of the mobile-phase modifiers, temperature, were also investigated.

EXPERIMENTAL

The apparatus for SFC has been described previously[7]. The carbon dioxide, kept in a container with an eductor tube, was passed into a Model 303 pump (Gilson, Villiers-le-Bel, France) through an ethanol cooling bath. The pump head was cooled in order to improve pump efficiency. The inlet adaptor and cooling jacket were laboratory-made. The polar modifiers were added with a second Gilson pump and mixed with carbon dioxide in a Gilson mixer (Model 802). A constant-temperature water-bath provided temperature control for the column.

A Polychrom 9060 diode-array detector (Varian, Palo Alto, CA, U.S.A.) set at 234 nm was used without modifications at pressures up to 300 bar. Pressure was monitored by a manual back-pressure regulator (TESCOM, Model 26-1700; G.E.C. Composants, Asnières, France) connected in-line after the detector and maintained at 35°C by a water-bath. All results were recorded with a Shimadzu CR3A integrator (Touzart et Matignon, Vitry-sur-Seine, France). The standard operating conditions were: average pressure, 230 bar; temperature, 25°C; average carbon dioxide flow-rate, 4 ml/min at -15°C.

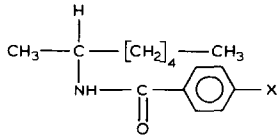
For LC, we used a modular liquid chromatograph, equipped with a Model 4270 integrator, a Model 8440 UV–VIS detector, set at 234 nm, and a 8700 solvent-

TABLE I

FORMULAE OF AMIDES TESTED

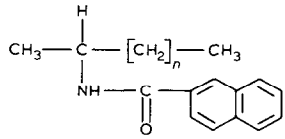
Amides derived from 2-aminoheptane.

X = CH_3, OCH_3, Cl



Amides derived from 2-aminopropane to 2-aminooctane.

n	Symbol
0	A
1	B
2	C
3	D
5	F



delivery system (Spectra-Physics, Santa-Clara, CA, U.S.A.). The standard operating conditions were: flow-rate, 2 ml/min; temperature, 25°C.

A stainless-steel, Pirkle covalent column (25 cm × 4.6 mm I.D.) (J. T. Baker, Phillipsburgh, NJ, U.S.A.) was packed with 5-μm spherical particles of aminopropylsilica, modified with (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine.

The synthesis of the amide samples has been described elsewhere[8]. The solutes are listed in Table I.

Carbon dioxide was N 45-grade (99.995% pure) (Air Liquide/Alphagaz, Paris, France); methanol, 2-propanol, *tert.*-butanol, 2-butanol and *n*-butanol were of analytical grade, *n*-hexane was LiChrosolv grade. All were obtained from Merck (Darmstadt, F.R.G.); acetonitrile and chloroform were of analytical grade, from Prolabo (Paris, France).

RESULTS AND DISCUSSION

Retention

A difference in the relative retention between LC and SubFC has been observed. For solutes F, D and C (Table I), the retention order is reversed in the two methods (Table II, Fig. 1). For example, in Fig. 1, $k'_{2F} < k'_{2D} < k'_{2C}$ in LC (left, curve a) but $k'_{2C} < k'_{2D} < k'_{2F}$ in SubFC (left, curve c), where the 2-propanol content of the mobile phase is 5% in each case. However, it should be mentioned that the results in Table II were obtained with two different columns for LC and SubFC, whereas the results in Fig. 1 were obtained with the same column.

Moreover, the k'_2 values are greater in SubFC; *i.e.*, the elution strength of carbon dioxide–2-propanol (95:5) is lower than that of hexane–2-propanol (95:5). This is somewhat surprising: it is well known that the polarity of carbon dioxide varies with its density, but it is always greater than that of hexane, particularly at densities close to 1 g/ml[9]. It is possible that polar modifiers interact more strongly with carbon dioxide than with hexane and, consequently, they are less able to dissolve the solutes. In addition, the difference between $k'_{2_{SubFC}}$ and $k'_{2_{LC}}$ increases with the

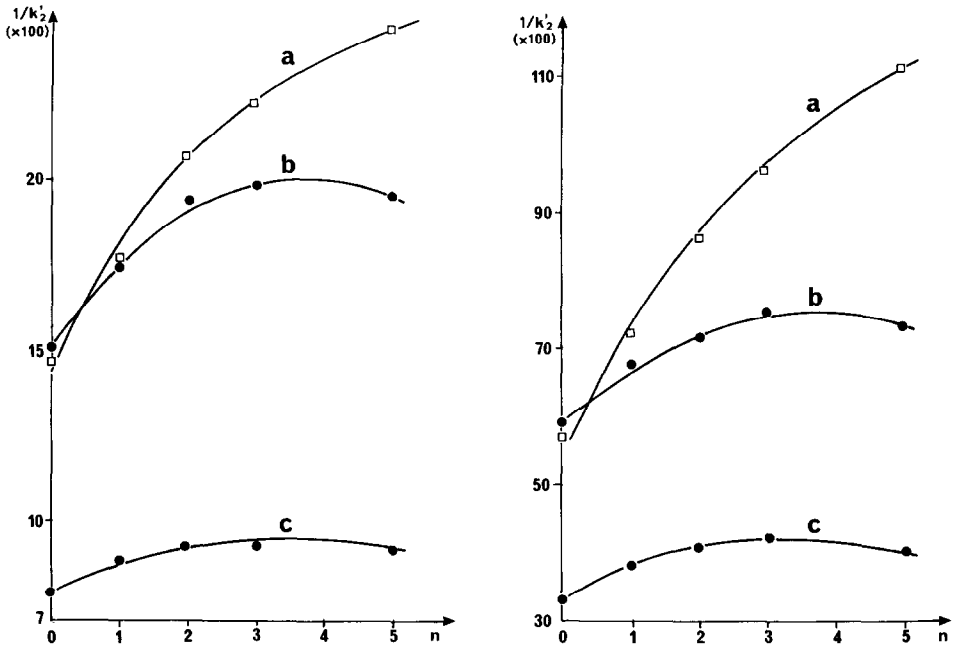


Fig. 1. Reciprocal of the capacity factor, k'_2, *vs.* chain length, n, for two stationary phases. Left: Pirkle-type phase. Right: aminopropyl phase. (a) LC: flow-rate, 2 ml/min; hexane–2-propanol (95:5); temperature, 35°C. (b) SubFC: flow-rate, 3 ml/min; average pressure, 230 bar; temperature, 25°C; mobile phase, carbon dioxide–2-propanol (90:10, w/w). (c) As (b) except for mobile phase, carbon dioxide–2-propanol (95:5, w/w). For n, see Table I.

TABLE II

COMPARISON OF RETENTION DATA FOR RACEMIC AMIDES RESOLVED ON THE (*R*)-N-(3,5-DINITROBENZOYL)PHENYLGLYCINE STATIONARY PHASE IN LC AND SubFC

k'_2 = capacity factor of the second eluted enantiomer; α = selectivity between the enantiomers; R_s = resolution; t_{anal} = analysis time (min) (retention time of the last eluted enantiomer). SubFC: flow-rate, 4.3 ml/min; temperature, 25°C; average column pressure, 230 bar. LC: flow-rate, 2 ml/min; temperature, 20°C. Detection, 234 nm; proportions of solvents, w/w.

Solute	*Hexane–2-propanol–acetonitrile (97:3:0.5)*					*Carbon dioxide–2-propanol (90:10)*				
	k'_2	α	N	R_s	t_{anal}	k'_2	α	N	R_s	t_{anal}
F	6.66	1.11	8550	2.08	15.75	5.18	1.09	7950	1.51	4.73
D	7.71	1.09	957	1.82	18.00	5.17	1.07	7600	1.22	4.72
C	8.35	1.06	11 120	1.30	20.5	5.09	1.05	8300	0.90	4.67
B	9.82	1.03	9985	0.67	24.9	5.42	1.01	–	–	4.92
CH_3	3.54	1.07	8820	1.20	10.2	1.87	1.06	9153	0.89	2.20
OCH_3	7.58	1.08	4869	1.02	19.1	2.62	1.065	5725	0.83	2.77
Cl	2.65	1.04	–	–	8.12	1.73	1.01	–	–	2.10

hydrophobic character of the solute: for instance, $(k'_{2_{SubFC}} - k'_{2_{LC}})$ increases from 5.76 for $n = 0$ (solute A) to 6.9 for $n = 5$ (solute F), which means that the solubility of the methylene groups of the amides is lower in carbon dioxide than in hexane. These two facts favour a smaller k'_2 in LC.

The above results, obtained with an aminopropyl column and a Pirkle-type CSP, were of similar magnitude. They will be discussed elsewhere but needed to be mentioned here as a difference in behaviour between hexane and subcritical carbon dioxide with polar modifiers.

In LC, the number of methylene groups, n, determines the hydrophobicity of the solutes. As n increases, the affinity of the solute for the mobile phase increases, resulting in a decreased k'_2. If the only parameter were hydrophobicity, $1/k'_2$ should be linearly dependent on n (ref. 8). The non-linearity observed in Fig. 1 points to the fact that other interactions occur, in addition to hydrophobic interactions.

Selectivity

Tables II and III summarize the results obtained by LC and SubFC with various polar modifiers. Greater stereoselectivities were obtained with LC but the analysis time was longer. Another comparison between the two methods, obtained with

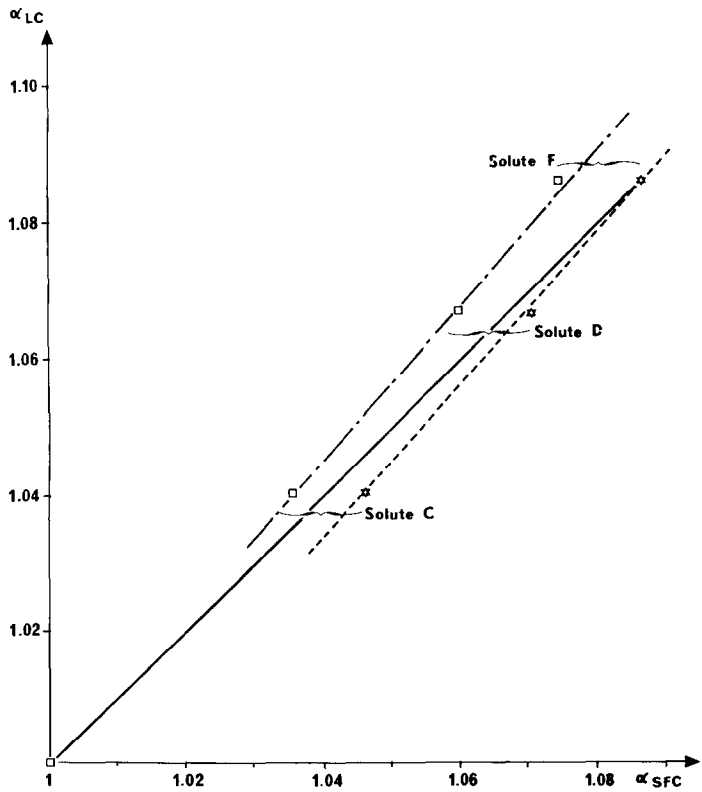


Fig. 2. Selectivity in LC, α_{LC}, *versus* selectivity in SubFC, α_{SFC}, for various solutes. LC operating conditions as in Fig. 1. In SubFC, mobile phases: ✡, carbon dioxide–2-propanol (95:5, w/w), $k'_{SubFC} = 2k'_{LC}$; □, carbon dioxide–2-propanol (90:10, w/w), $k'_{SubFC} = k'_{LC}$. For solute identification, see Table I.

TABLE III

RETENTION DATA OBTAINED IN SubFC FOR RACEMIC AMIDES ON THE (*R*)-N-(3,5-DINITROBENZOYL)PHENYLGLYCINE STATIONARY PHASE

Symbols and operating conditions as in Table II.

Solute	*Carbon dioxide–2-propanol–chloroform (95:3:2)*				*Carbon dioxide–2-propanol–acetonitrile (90:8.2:1.8)*				*Carbon dioxide–methanol (95:5)*				*Carbon dioxide–2-propanol–water (95:4.75:0.75)*			
	k'_2	α	N	R_s	k'_2	α	N	R_s	k'_2	α	N	R_s	k'_2	α	N	R_s
F	4.63	1.08	8200	1.36	3.85	1.08	8037	1.36	4.34	1.06	9500	1.17	7.20	1.08	10 100	1.58
D	4.58	1.07	8186	1.14	4.01	1.07	7753	1.12	4.53	1.05	9370	0.93	7.28	1.06	9890	1.25
C	4.73	1.05	7561	0.8	4.05	1.05	6875	0.71	4.65	1.03	9950	0.65	7.35	1.06	9275	1.12
B	No separation				No separation				No separation				8.08	1.01	–	–
CH_3	1.65	1.07	9000	0.91	1.57	1.06	14 000	1.10	1.69	1.04	7850	0.54	2.50	1.05	9320	0.90
OCH_3	2.45	1.05	7650	0.78	2.11	1.05	4200	0.56	2.2	1.03	6210	0.44	3.84	1.05	9200	0.90

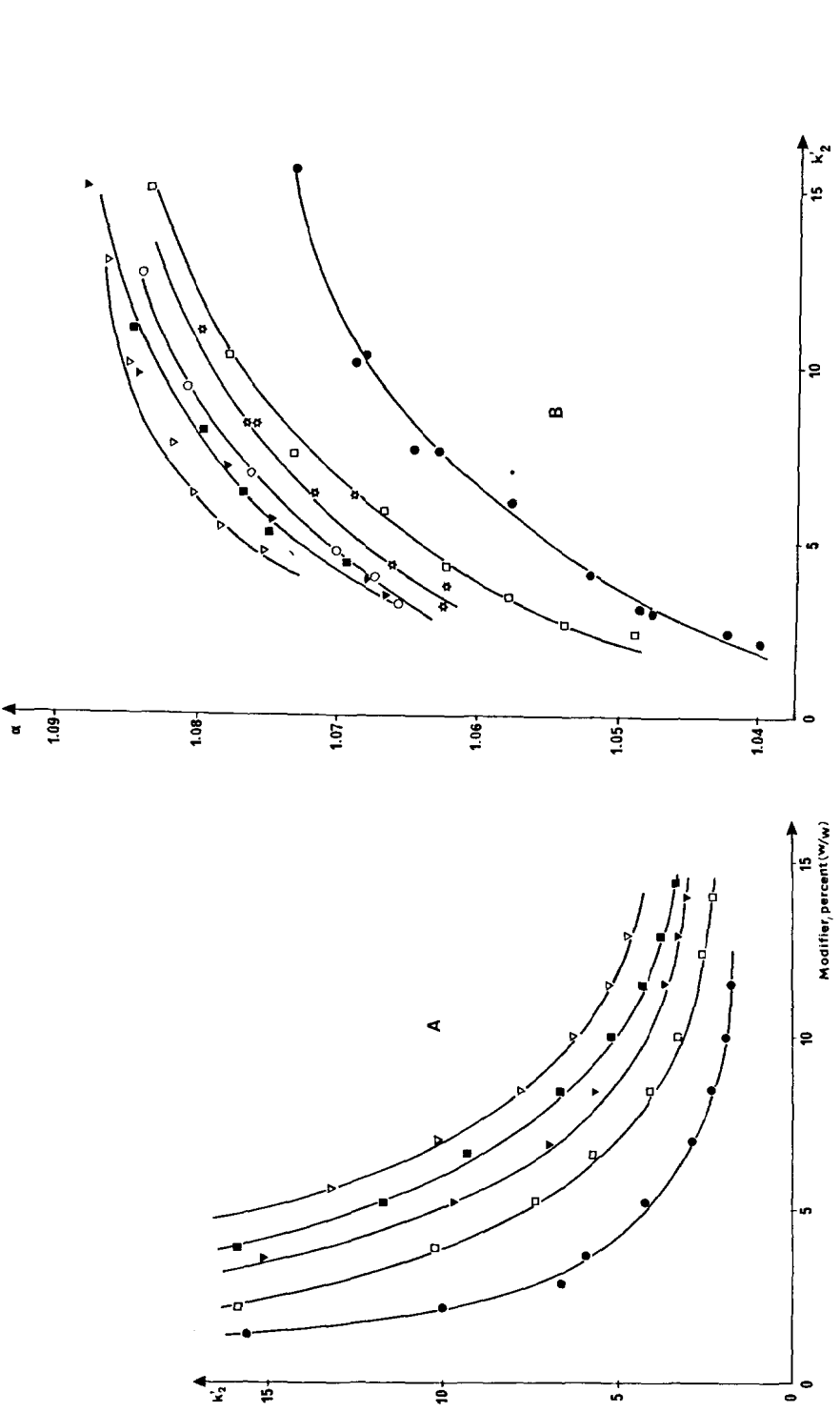


Fig. 3. Influence of various alcohols in SubFC. (A) Influence of the alcohol concentration on the capacity factor, k'_2, for solute F. Average pressure: 230 bar. Temperature: 25°C. Detection: 234 nm. Flow-rate: 3 ml/min. Modifiers: methanol (●); 2-propanol (▼); *n*-butanol (□); 2-butanol (■); *tert.*-butanol–2-butanol (50:50) (▽). (B) Change in selectivity, α, with capacity factor, k'_2, for various alcohol concentrations in the mobile phase (see A). Symbols and operating conditions as in (A) except for 2-propanol–methanol (90:10) (✡) and 2-propanol–methanol (98:2) (○).

the same column, is presented on Fig. 2. When the mobile phases were adjusted so that the capacity factors were the same in both methods (Fig. 2, □), the stereoselectivities obtained by LC were slightly greater than by SubFC. When the capacity factors were higher in SubFC (Fig. 2, ✡), the stereoselectivities obtained by SubFC were slightly greater than by LC. Not only was the stereoselectivity similar in both methods, but the enantiomeric elution order was the same. This indicates that the method of chiral recognition is the same.

A chiral recognition model for these solutes has been described[8,10]. In this model, all the attractive interactions are located on a single bond in both the solute and the CSP, *i.e.*, the amide bond[10–12]. Attractive interactions responsible for the formation of diastereomeric solute–CSP complexes are the dipole stacking, hydrogen bonding and π–π interactions. The chiral discrimination is governed by steric fit on the CSP: as the length of the alkyl chain becomes greater, the steric bulk at the chiral centre increases, resulting in greater selectivity.

Influence of various alcohols. With polar solutes and a polar CSP, it is necessary to add polar modifiers to hexane and subcritical carbon dioxide. We used various alcohols in the mobile phase. The influence of the nature of the alcohol on selectivity has been studied in SubFC. Analogous studies in LC have been carried out by Pescher *et al.*[13] on phosphine oxides. The retention and selectivity of various alcohols for solute F are presented in Fig. 3. It appears that:

(a) For alcohols of similar steric conformations, *e.g.*, straight-chain alcohols, the elution order is governed by the polarity, P', according to Rohrschneider and as defined by Snyder[14–16].

(b) For alcohols with the same P' but different steric configuration at the OH groups such as *n*-butanol and 2-propanol ($P' = 3.9$), it is necessary to take into account the steric hindrance of the alcohol moiety. *n*-Butanol as the polar modifier gives lower k'_2 values than 2-propanol (Fig. 3).

(c) At constant k'_2, selectivities are greater for alcohols with large steric hindrance close to the OH moiety. While it is impossible to add pure *tert.*-butanol to carbon dioxide (this alcohol is a solid at room temperature), the best selectivities were obtained with mixtures of *tert.*-butanol and 2-butanol (Fig. 3, ▽). 2-Propanol and 2-butanol gave similar selectivities, since the two alcohols are sterically similar (Fig. 3, ▼, ■).

All of these results confirm a two-point interaction model between the Pirkle-type CSP and alcohols in LC, as described by Pescher *et al.*[13]. The alcohols may interact by means of two hydrogen bonds with the amide group of the chiral moiety. Because small alcohols are attached more strongly to the chiral phase, it is more difficult for solutes to displace them. The overall effect is a shift in the solute–CSP/solute–mobile phase equilibrium towards the mobile phase, resulting in a smaller number of interactions between the solute and the CSP, lower retention and, at constant k', lower selectivity. These results are valid for SubFC and LC.

As will be shown later, the efficiency decreases from methanol to *tert.*-butanol, but is compensated by an increase in stereoselectivity.

Influence of pressure. For the same retentions, the selectivity, α, does not vary significantly with the average column pressure (Fig. 4). This suggests that carbon dioxide does not interact with the chiral moiety of the stationary phase during the separation process or, more probably, it is easily displaced by the amides and alco-

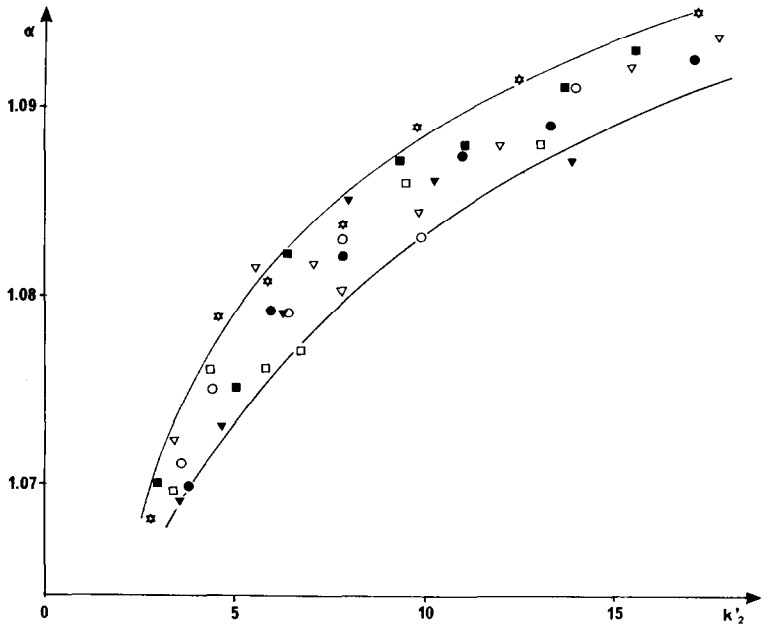


Fig. 4. Change in selectivity, α, with capacity factor, k'_2, for solute F at various average column pressures in SubFC. Mobile phase: carbon dioxide–2-propanol (alcohol concentrations between 3 and 15%, w/w); flow-rate, 4 ml/min. Temperature: 25°C. Detection: 234 nm. Pressures, P (bar): 135 (●); 155 (▽); 175 (■); 196 (✡); 222 (▼); 238 (○); 258 (□).

hols, which are much more polar. Increased values of k'_2 were observed at lower pressure. This phenomenon is often explained by the solubility of solutes in the mobile phase, depending on fluid density[17,18]. However, at high alcohol concentrations, these variations are less important, because the role of carbon dioxide in the solubilization process becomes smaller.

Influence of temperature. Temperature is an important parameter in LC[19], in subcritical and supercritical fluid chromatography. The fundamental equation for chromatographic retention is

$$\ln k_i^0 = \ln \varphi + \frac{\Delta S_i^0}{R} - \frac{\Delta H_i^0}{RT} \tag{1}$$

where φ is the phase ratio, and ΔH_i^0 and ΔS_i^0 are enthalpy and entropy changes, respectively, associated with the retention process of solute i. In LC, an increase in T results in a decrease in k'. In SubFC and SFC, a linear dependence is observed only if the fluid density is kept constant (a decrease in T will result in an increase in the fluid density). From eqn. 1 we can write

$$\ln \alpha = \frac{(\Delta S_2^0 - \Delta S_1^0)}{R} - \frac{(\Delta H_2^0 - \Delta H_1^0)}{RT} \tag{2}$$

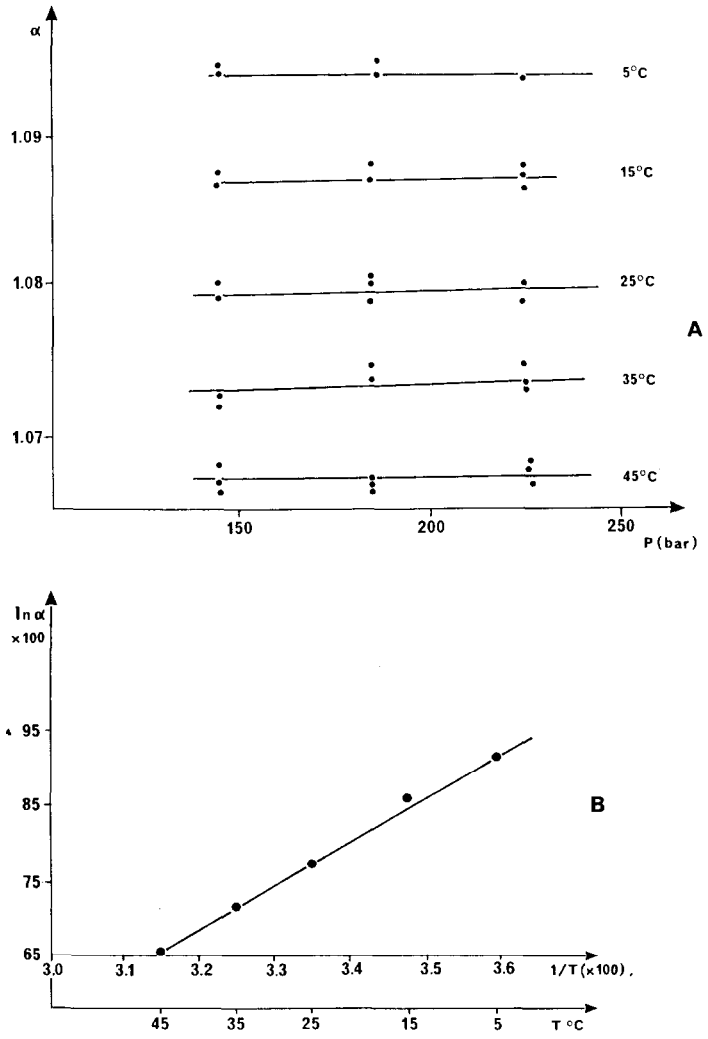


Fig. 5. (A) SubFC and SFC. Influence of temperature, T, and average column pressure, P, on selectivity, α, for solute F. Mobile phase: carbon dioxide–2-propanol (93:7, w/w); flow-rate, 4 ml/min. Detection: 234 nm. (B) SubFC. Logarithm of selectivity, ln α, *versus* the reciprocal of the temperature, $1/T$. Details as in (A) except average column pressure, 225 bar.

where the subscripts 1 and 2 refer to the enantiomers eluted first and second. Eqn. 2 predicts a linear relationship between ln α and $1/T$. This is clearly established by the results presented in Fig. 5. The best selectivities were obtained, as predicted, at low temperature. The value of the slope in Fig. 5B was calculated to be $\Delta(\Delta H_i^0) = -0.48$ kJ/mol. It reflects the differences in affinity of both enantiomers for the CSP. This low value is reflected in the difficulties in separating these amides.

Efficiency

The resolution of polar solutes requires the addition of polar modifiers to

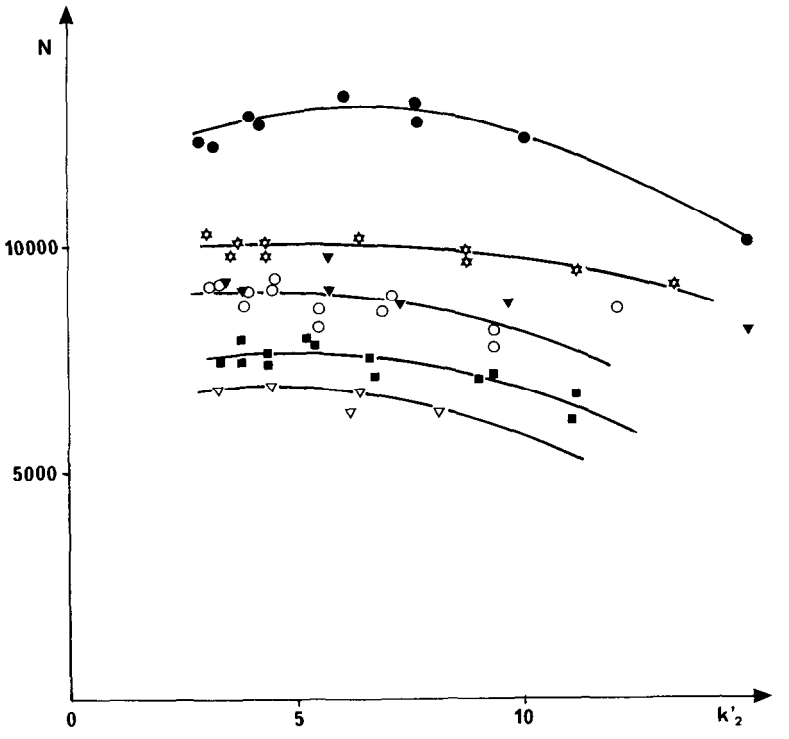


Fig. 6. Efficiency, N, *vs.* capacity factor, k'_2, for solute F and different alcohols in SubFC. Various modifier concentrations in the mobile phase (between 5 and 15%, w/w); average column pressure, 230 bar; temperature, 25°C; detection, 234 nm; flow-rate, 3 ml/min; modifiers, see Fig. 3.

carbon dioxide. The influence of the nature and concentration of the polar modifier on the efficiency was studied.

Influence of various alcohols. As shown in Fig. 6, the lower the polarity of the alcohol, the greater is the value for the reduced plate height, h. There are a number of possible explanations for this including:

(a) The viscosity of large alcohols is greater than that of small ones: $\eta(25°C) = 1.9$ cP for 2-propanol and 0.53 cP for methanol. Thus, the diffusion of solutes will be faster in a carbon dioxide–methanol mixture than in a carbon dioxide–2-propanol mixture at the same alcohol concentration.

(b) An higher percentage by weight is needed for larger alcohols than for the lower alcohols to obtain a constant k'. The viscosity increases, while the solute diffusion coefficient, D_m, decreases.

(c) The kinetics of hydrogen-bonding interactions between the solute and larger alcohols may be slower due to steric hindrance.

(d) The solvation shells around solute molecules increases with larger alcohols, and this lowers the diffusion rate.

The addition of small amounts of water to 2-propanol (5%, w/w, to avoid problems with solubility in carbon dioxide[20–22]) gives usual effects on the retention (Fig. 7) and a surprising effect on the efficiency (Fig. 8). These phenomena have

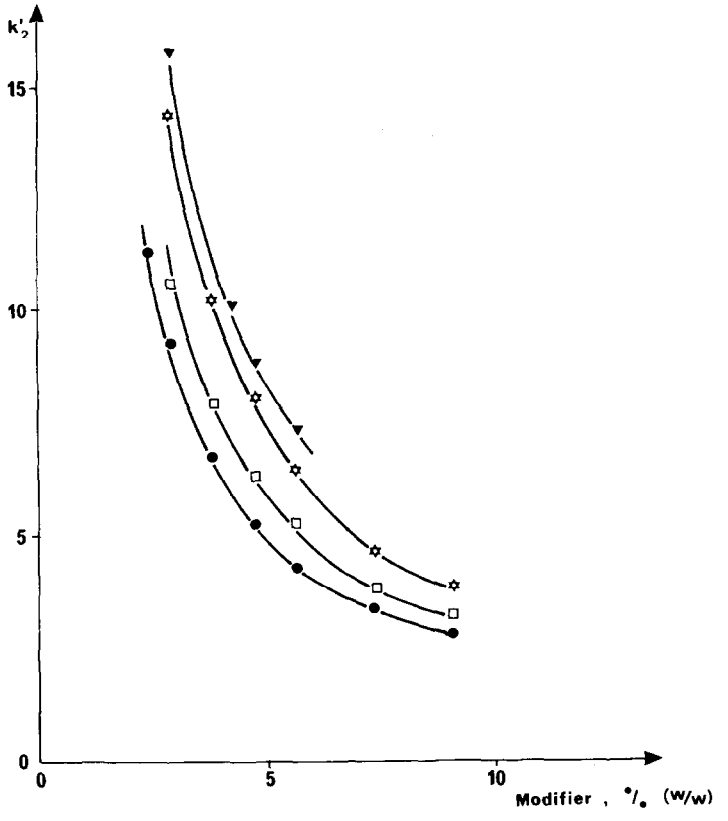


Fig. 7. Influence of the water content of 2-propanol on the capacity factor, k'_2, for solute F in SubFC. Average column pressure, 230 bar; temperature, 25°C; detection, 234 nm; mobile phase, carbon dioxide–2-propanol–water with various concentrations of modifier (between 5 and 15%, w/w); flow-rate, 4 ml/min. Water content in 2-propanol: 1% (▼); 2% (✡); 4% (□); 5% (●).

previously been observed in SFC for phosphine oxides[5], where greater efficiency and selectivity were obtained. With our solutes, at constant k'_2, an unchanged selectivity but higher efficiency was observed for all alcohol–water mixtures than for pure alcohols. The influence of water remains unclear; it cannot be explained only by variations of the mobile phase viscosity. Possible explanations are the modification of the solvation shells of the solute and the fact that water covers polar silanol sites. Recent results[23], obtained in capillary SFC, support the conclusion that drastic changes observed with packed columns are only due to surface and stationary phase modifications. This conclusion can hardly explain the increase in selectivity for phosphine oxides and, at the same time, the constant selectivity for amides, when water is added to the mobile phase.

Influence of temperature. Column temperature is a parameter that has received little attention so far. Recently, some experiments have been conducted in LC with chiral phases[24,25]. The stability of diastereomeric complexes increases when the temperature decreases, resulting in an increased value of α, as we have previously seen

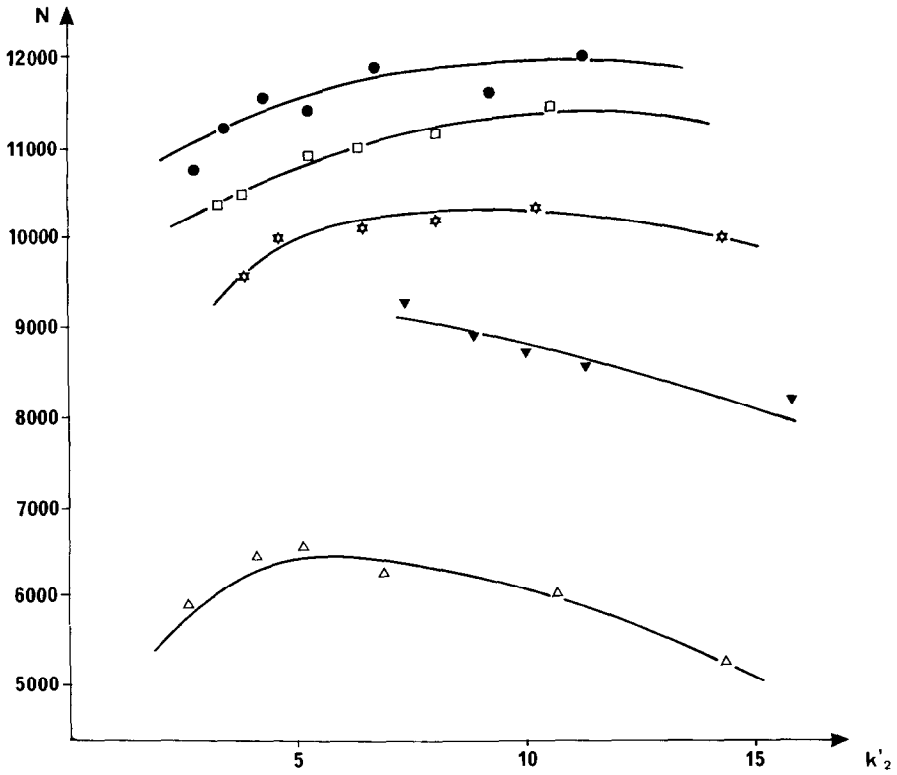


Fig. 8. Influence of the water content of 2-propanol on the efficiency, N, in SubFC. Conditions as in Fig. 7.

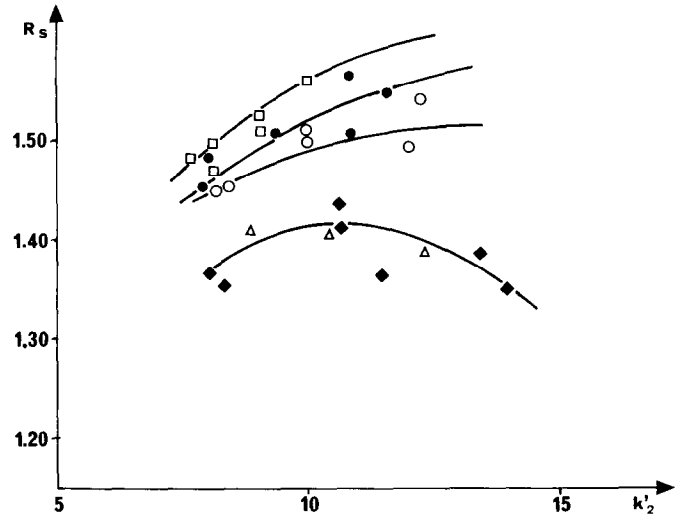


Fig. 9. Resolution, R_s, between the enantiomers of solute F at various temperatures. The variations of k'_2 are obtained by changing the average column pressure (k'_2 increases when P decreases). Mobile phase: carbon dioxide–2-propanol (93:7, w/w); flow-rate, 4 ml/min. Detection: 234 nm. Temperatures: 45°C (◆); 35°C (○); 25°C (□); 15°C (●); 5°C (△).

in Fig. 5. However, the reduction in mass-transfer rates due to higher viscosities at low temperature reduces column efficiency and tends to offset somewhat the expected gain in resolution[13,25]. These results are valid for subcritical and supercritical fluid chromatography; Fig. 9 shows that the optimum resolution is observed at 25°C.

Resolution

Resolution is the most important objective in chromatography. At constant analysis time, the resolution values remain independent of the nature of the alcohol as shown in Fig. 10. However, when water is added to the polar modifier, there is an increase in efficiency and, consequently, better resolution (Fig. 11).

Resolution per unit of time

Density, viscosity and diffusion coefficient are important parameters in chromatography. The viscosity in supercritical and subcritical carbon dioxide is lower than the usual values for liquid phases. Consequently, the solute diffusion coefficient, D_m, is always greater in SubFC and SFC than in LC.

With the same packed column and solute, it has been shown that the ratio

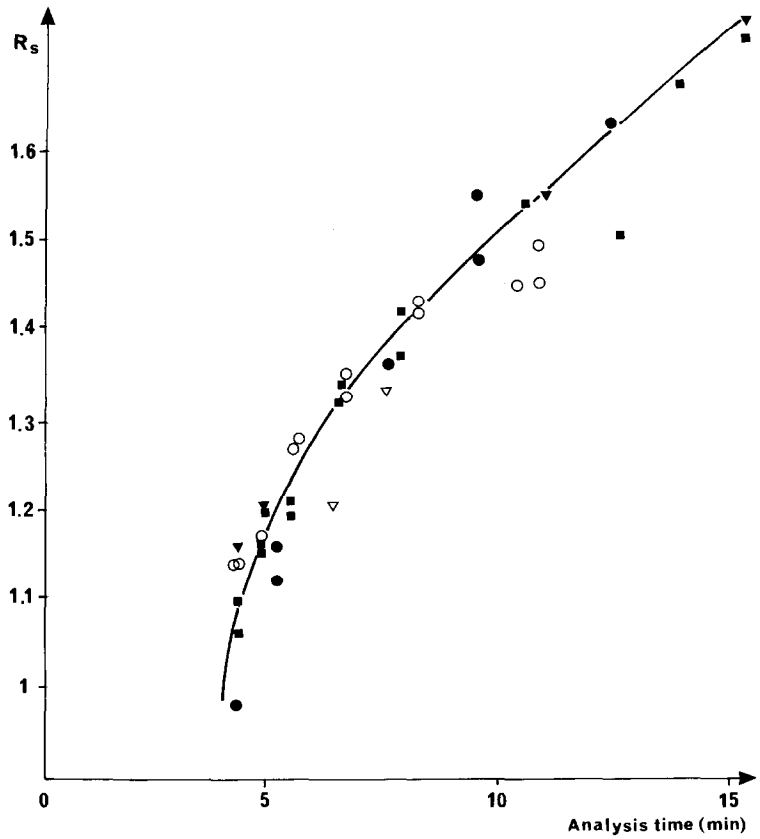


Fig. 10. Resolution, R_s, between the enantiomers of solute F *vs.* analysis time for various alcohols (symbols as in Fig. 3). Operating conditions as in Fig. 3.

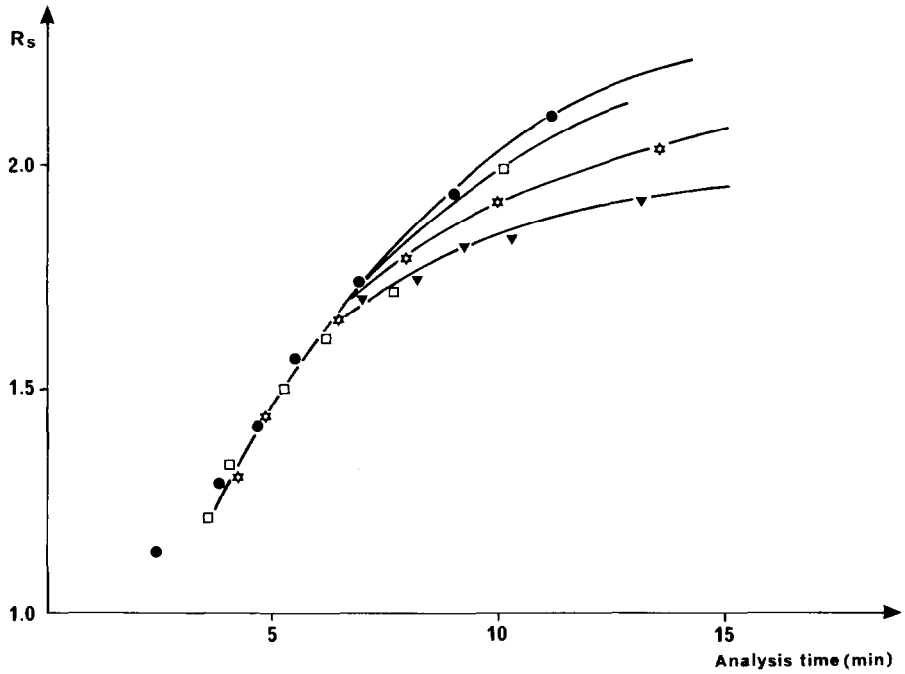


Fig. 11. Influence of the water content of 2-propanol on the resolution, R_s, of the enantiomers of solute F. Symbols and operating conditions as in Fig. 7.

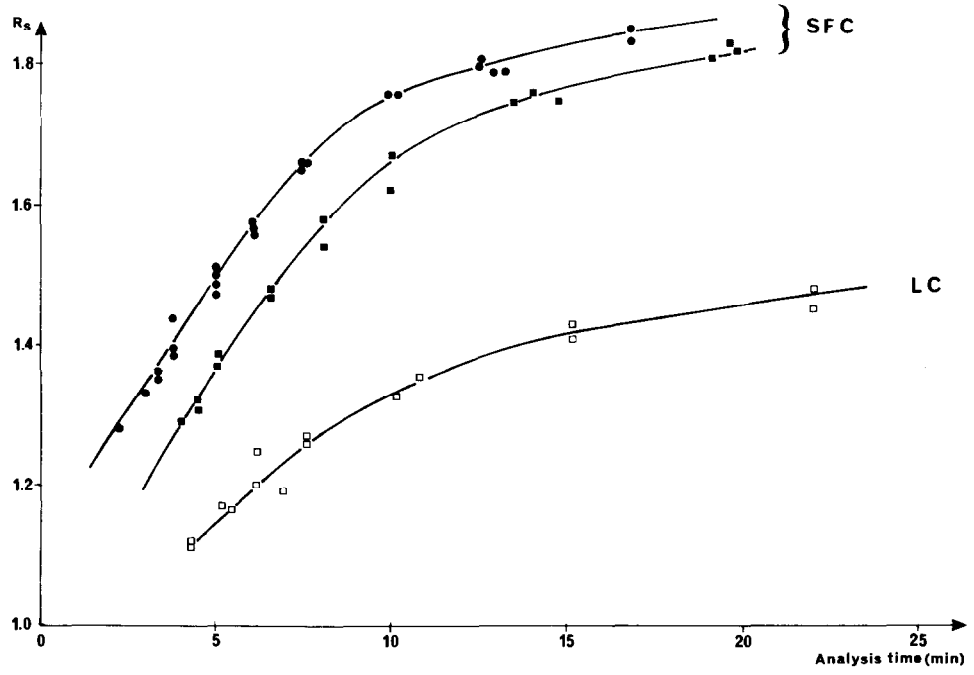


Fig. 12. Comparison between LC and SubFC. Resolution per unit of time of the enantiomers of solute F. LC (□): mobile phase, hexane–2-propanol (95:5); temperature, 35°C; detection, 234 nm; flow-rate, between 0.7 and 3.5 ml/min. SubFC (■): mobile phase, carbon dioxide–2-propanol (95:5, w/w); average column pressure, 230 bar; temperature, 25°C; detection, 234 nm; flow-rate, between 2 and 7 ml/min. (●) As (■), except for mobile phase, carbon dioxide–2-propanol–water (95:4.8:0.2).

D_m(SFC)/D_m(LC) can be calculated by using the Van Deemter curves at the minimum reduced plate height[26]. The same values of h_{min} are obtained with both LC and SFC, but the corresponding optimum velocity is always 5–10 times greater with supercritical carbon dioxide. This result was expected since the optimum linear velocity is inversely proportional to the particle diameter and proportional to the diffusion coefficient.

The D_m values are high even when a polar modifier, such as methanol, is added to carbon dioxide. However, D_m decreases when the viscosity of the mobile phase increases. Sassiat *et al.*[27] have shown that no discontinuity in the benzene diffusion coefficient occurs between pure carbon dioxide and pure methanol at 150 bar and 40°C. In the usual range of methanol concentrations (< 10%, w/w), the supercritical state is maintained and D_m for benzene is at least 4 times higher than for pure methanol at 20°C.

If the efficiency per unit of time or the resolution per unit of time is considered instead of the overall efficiency or resolution, then subcritical fluid chromatography is preferable to LC, as shown in Fig. 12. Short analysis times (< 4 min) are obtained with SubFC without significant loss of resolution, in spite of a greater retention in SubFC than in LC. With similar retention in both methods, differences in the analysis times can be greater[5].

CONCLUSION

Very similar retentions, stereoselectivities and efficiencies are obtained in subcritical fluid chromatography and in liquid chromatography for the resolution of enantiomeric amides on a Pirkle-type CSP. In agreement with conclusions for the resolution of phosphine oxides[5], this suggests that both methods can be used interchangeably. However, the actual analysis times for SubFC are significantly shorter than those with LC, indicating that when time is the key factor, SubFC is more advantageous. For preparative purposes this advantage is quite important: SubFC and SFC can provide high output per unit of time and an easy elimination of the eluent.

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