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SEPARATION OF IONIC DRUG SUBSTANCES BY SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

The use of ion-pairing principles in supercritical fluid chromatography (SFC) is demonstrated. Using this technique, the scope of SFC can be extended to include ionic and ionizable compounds. The possibilities of ion-pair techniques and the advantages of SFC over high-performance liquid chromatography (HPLC) are discussed. The influence of different ion-pairing reagents on the selectivity was elucidated for a wide range of pharmaceutical compounds. The dependence of efficiency and selectivity on the eluent density was investigated, leading to an optimization strategy for cationic and anionic substances including matching ion-pair reagents, the stationary phase and density programming. It was demonstrated that owing to the combined influences on selectivity of the ion-pair type and the density, together with the high efficiency of SFC, optimized separations are obtained faster by SFC than by HPLC. The limitations of ion-pair SFC are mainly due to the solubility of ion-pairing reagents in the carbon dioxide–modifier mixtures. The solubility of typical ion-pairing reagents under different SFC conditions was investigated.

INTRODUCTION

One of the fundamental problems with supercritical fluid chromatography (SFC) is that strongly polar and ionic compounds are often separated with long elution times and tailing peaks. For polar substances the addition of polar modifiers such as methanol or acetonitrile may improve the separation. However, for ionic compounds, which include most pharmaceuticals and compounds of biological interest, this method often fails.

This work demonstrates that the application of ion-pairing principles, which have already been used for a considerable time in high-performance liquid chromatography (HPLC), leads to improvements in the selectivity and versatility of SFC for polar compounds. A good overview of ion-pair chromatography, including straight-phase systems, was given by Schill *et al.*[1]. Recently we have shown[2] the separation of enantiomeric 1,2-α-amino alcohols as diastereomeric ion pairs. It was demonstrated that the ion-pair principle works in SFC and that the use of carbon dioxide as a mobile phase gives advantages over HPLC with respect to resolution and efficiency.

The aim of this study was to extend the use of ion-pair principles in SFC to the

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analysis of cationic and anionic organic compounds. The influence on the selectivity of different ion-pair reagents, including sodium heptanesulphonate, dimethyloctylamine, tributylamine and citric acid, on various straight-phase columns was investigated. A problem with ion-pair SFC (IPSFC) is the limited solubility of ion-pairing agents in carbon dioxide–modifier mixtures, and systematic measurements of solubility limits in supercritical carbon dioxide–modifier mixtures were investigated[3].

EXPERIMENTAL

Apparatus

The SFC equipment has been described previously[2]. The set-up for the determination of the solubility limits, consisting of an extraction part and a detection part, has been presented recently[3].

Chemicals

Carbon dioxide (48 grade) was obtained from Carba Gas (Basle, Switzerland), acetonitrile and methanol from Rathburn (Walkerburn, U.K.), dimethyloctylamine (DMOA), triethylamine (TEA), trioctylamine (TOA), tributylamine (TBA), tetrabutylammonium bromide (TBABr), sodium heptanesulphonate monohydrate (HSNa) and the acidic test compounds benzoic acid and *p*-hydroxybenzoic acid from Fluka (Buchs, Switzerland) and citric acid (CiA), salicylic acid and tropic acid from Merck (Darmstadt, F.R.G.). All other test compounds, *i.e.*, the 1,2-α-amino alcohols, Isradipin and its by-products, bromocriptine, Spirapril (ACE inhibitor) and its degradation and by-products were supplied by Sandoz (Basle, Switzerland) (structures are shown in Fig. 1). Spirapril is a product in co-development with Schering (Phough, U.S.A.).

Eluents

The ion-pair reagents were dissolved in the polar modifiers (methanol or acetonitrile) and mixed with liquid carbon dioxide. The amount of modifier in the carbon dioxide was regulated by the flow-rate of the modifier pump. The quoted concentrations of the ion-pairing agents refer to their concentrations in the modifier solution.

Columns

The cyano-bonded phase CS-MP Spheri 5, the diol-bonded phase OH-MP Spheri 10 and the silica phase SS-MP Spheri 5 were obtained from Brownlee Labs. (Santa Clara, CA, U.S.A.). The column dimensions were 100 mm × 4.6 mm I.D. The diol phase LiChrospher 100 Diol was purchased from Merck (250 mm × 4 mm I.D. column) and the cyano phase Spherisorb CN (100 mm × 4 mm I.D. column) from Stagroma (Wallisellen, Switzerland).

RESULTS AND DISCUSSION

Because of the more favourable mass transfer kinetics in SFC, owing to the high diffusivity and low viscosity, SFC gives a better separating performance than HPLC[4]. This leads to better resolution in SFC than in HPLC, especially at high linear

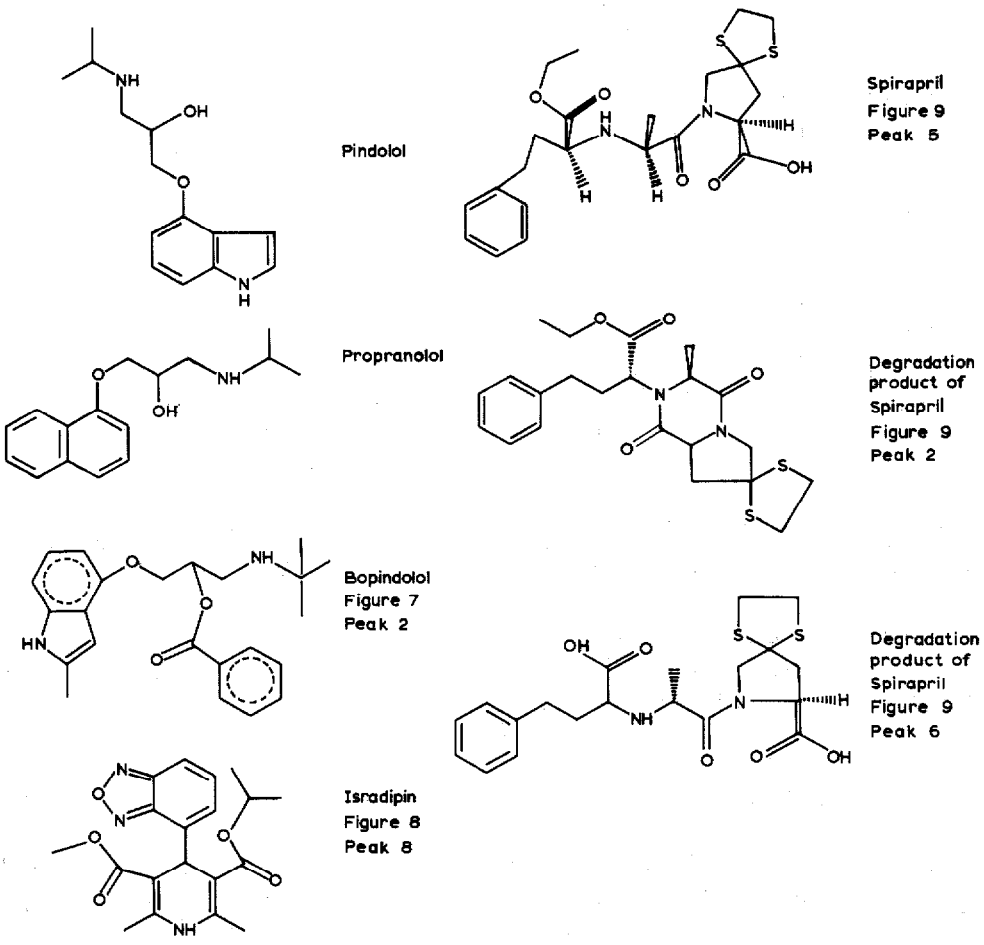

Fig. 1. Structures of test compounds.

velocities[2], as shown for the diasteromeric separation of racemic propranolol[2]. These advantages are also illustrated in Fig. 2 for the separation of Isradipin and ten by-products. In HPLC more than double the analysis time is required.

In SFC the retention and selectivity depend strongly on the density of the fluid and pressure and temperature changes are therefore important factors in the optimization process. A change of eluent in HPLC with a straight phase needs between 300 and 2000 column volumes for equilibrium, whereas pressure changes in SFC, which affect retention in the same way, need only a few seconds[5]. Because of the high diffusivities under supercritical conditions, modifier changes in SFC need only 10–30 column volumes. Owing to the faster establishement of chromatographic conditions, optimizations in SFC can be achieved in a very short time. The use of pressure gradients to optimize separations of mixtures having a broad range of polarity is possible, resulting in greater sensitivity and shorter analysis times, as shown in Fig. 2.

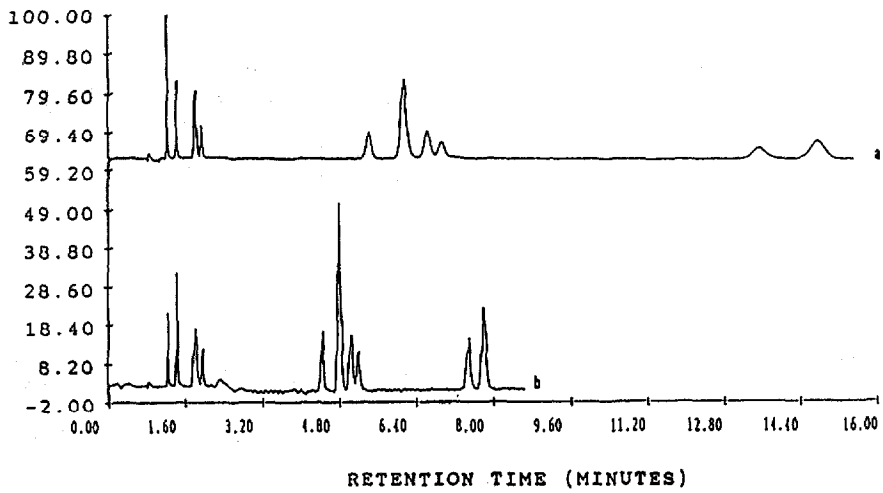

Fig. 2. Separation of Isradipin and its by-products using SFC. (a) Isocratic (carbon dioxide; 4% methanol; 50°C, 128 bar); (b) pressure gradient (conditions as in a, but after 2.2 min a pressure gradient from 128 to 305 bar within 5 min, then 1 minute at 305 bar. Column, silica Spheri 5, 5 μm (400 mm × 4.6 mm I.D.).

Possibilities of the ion-pair technique

When working with straight phases and assuming a liquid chromatographic system with unlimited capacity, the capacity ratio (k') is given by

$$k'_x = \frac{1}{K_{ex}^{(QX)}\,[Q^+]}\,\frac{V_s}{V_m} \qquad (1)$$

where K_{ex}^{QX} is the extraction constant, $[Q^+]$ the counter ion concentration and V_s and V_m are the volumes of the stationary and mobile phase, respectively[1]. Referring to eqn. 1, the retention of an ionic compound is governed by the concentration of the counter ion and the extraction constant, the latter depending on the nature of the organic phase, on the pH, on the nature of the counterion and on the concentration and nature of competing ions. Hence the use of ion-pair reagents gives various possibilities for optimizing the separation of polar and ionic compounds as ion pairs with non-polar eluents on straight phases.

Ion-pair SFC

In ion-pair SFC, the advantages of SFC can be combined with the potential for selectivity of the ion-pair technique. This technique has been applied to the diastereomeric separation of racemic mixtures of 1,2-α-amino alcohols[2,6] and of basic drug compounds including indole derivatives and acidic compounds such as phenolates and carboxylic acids.

Solubility of ion-pairing reagents

The solubility limit of the ion-pairing reagents was investigated by an on-line determination of the equilibrium concentration by UV detection after the establishment of equilibrium between a solid ion pair and a supercritical solvent[3]. Fig.

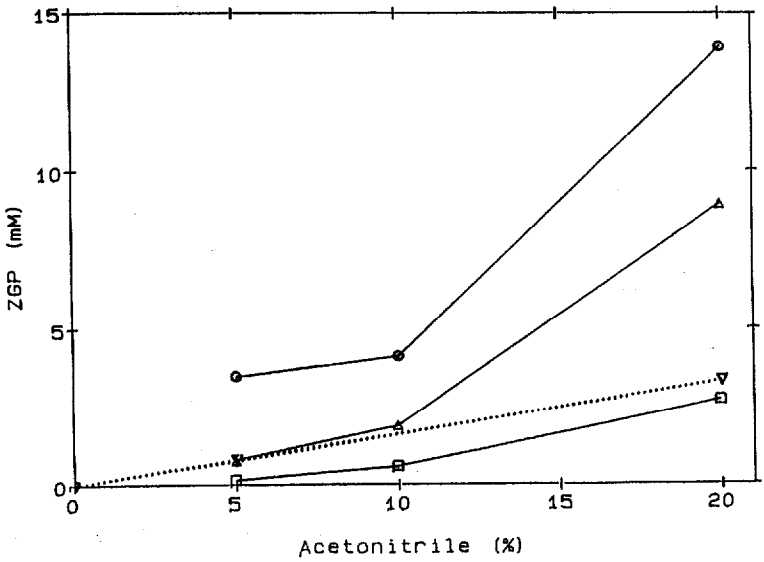

Fig. 3. Solubility of N-benzoxycarbonylglycyl-L-proline (ZGP) in carbon dioxide and acetonitrile on the proportion of modifier. ○, 300 bar, 80°C; △, 300 bar, 60°C; ▽, 250 bar, 40°C; □, 300 bar, 20°C.

3 demonstrates that with increasing amount of modifier the solubility strongly increases. Under isobaric conditions the solubility increases with increasing temperature, despite the decrease in density. This effect is enhanced with increasing amount of modifier. Under isothermal conditions the solubility increases with increasing pressure, as shown in Fig. 4. Hence the solubility depends on the nature of the ion-pair reagent. For all investigated ion-pair reagents we found a sufficient

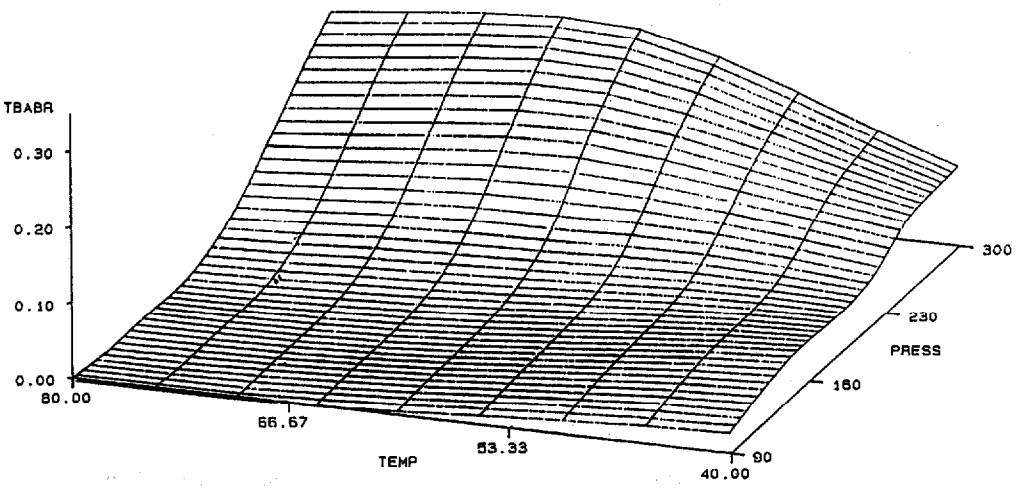

Fig. 4. Solubility of TBABr (m*M*) as a function of temperature (°C) and pressure (bar) of a mixture of carbon dioxide and acetonitrile.

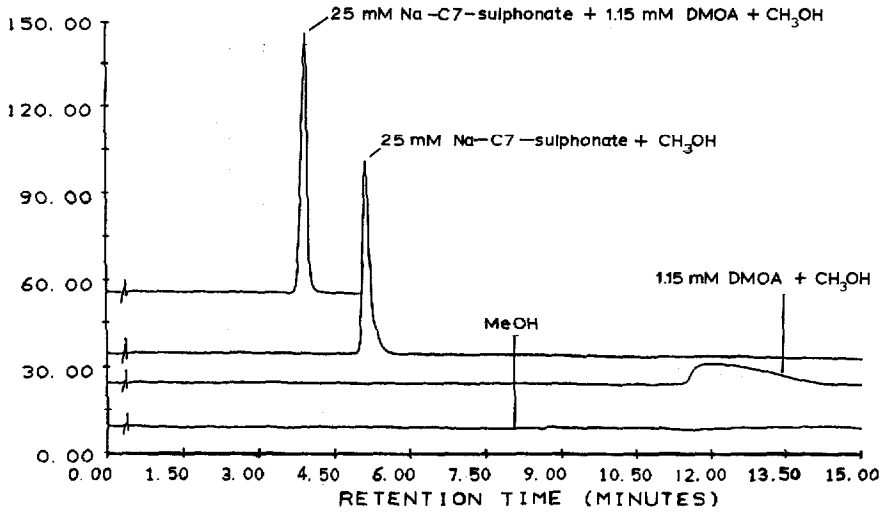

Fig. 5. Supercritical elution of propranolol with ion-pair modifiers in carbon dioxide. Column, cyano, 5 μm (100 mm × 4.6 mm I.D.) 60°C, 225 bar.

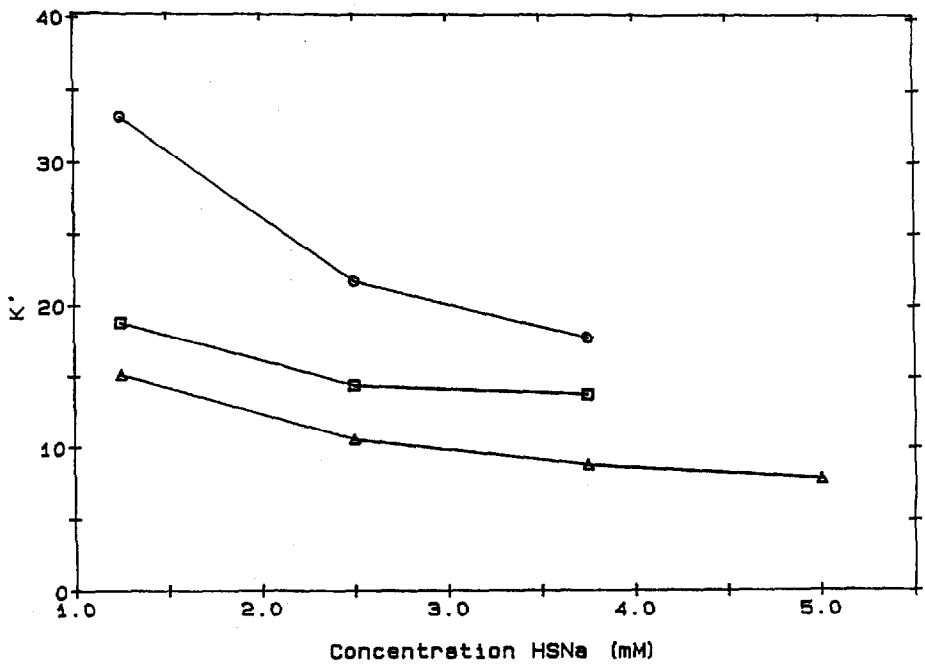

Fig. 6. Dependence of the retention on the counter ion (HSNa) concentration. Conditions: carbon dioxide–20% methanol–1.15 m*M* DMOA; 60°C, 225 bar. Column, cyano, 5 μm (100 mm × 4.6 mm I.D.). □, Bopindolol; ○, precursor; △, propranolol.

solubility in both carbon dioxide–acetonitrile and –methanol mixtures. As a rule of thumb, at least 5% of methanol or 10% of acetonitrile is necessary in order to obtain a sufficient solubility for typical concentrations of the ion-pair reagent to achieve a significant degree of ion pairing. Additionally, the density must be maintained above 0.5 g/cm^3.

Separation of cationic compounds

Cationic organic compounds of pharmaceutical interest such as indole derivatives and aliphatic amines are often not eluted in supercritical carbon dioxide even with the addition of large amounts of polar modifiers such as methanol. In addition to their insufficient solubility in supercritical fluids, strong interactions with the silica surface

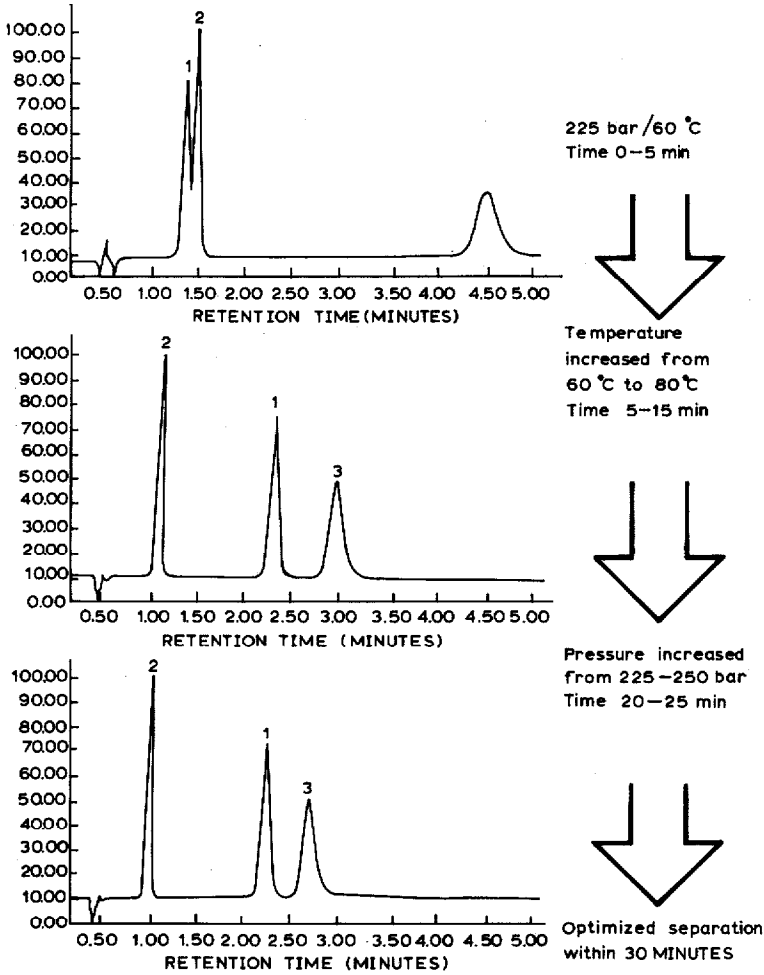

Fig. 7. Optimization of the separation of Bopindolol (2), its precursor (3) and benzoic acid (1) as a degradation product within 30 min. Conditions: carbon dioxide–20% methanol–20 m*M* TBA–20 m*M* acetic acid; flow-rate, 4 ml/min. Column, diol, 10 μm (100 mm × 4.6 mm I.D.).

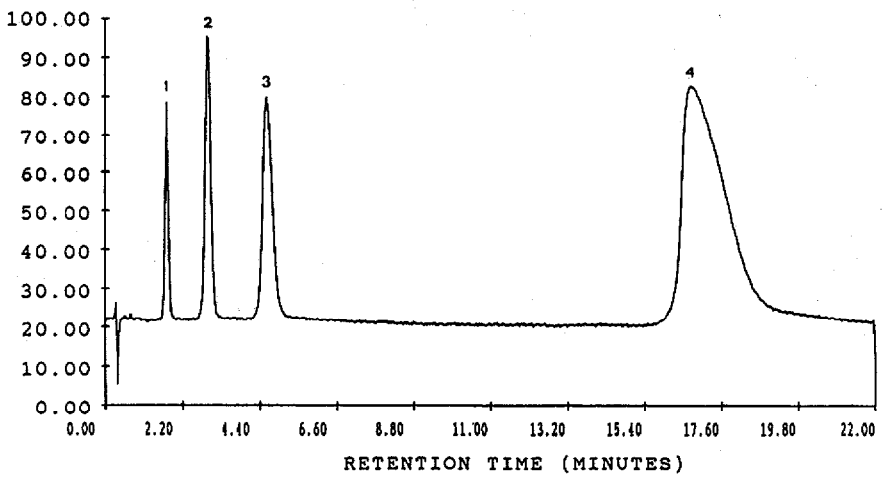

Fig. 8. Separation of acidic compounds on a diol phase. Conditions: carbon dioxide–20% methanol–20 m*M* TBA–20 m*M* acetic acid–20 m*M* CiS; 60°C, 225 bar. Column, diol, 10 μm (100 mm × 4.6 mm I.D.). (1) Benzoic acid; (2) *p*-hydroxybenzoic acid; (3) tropic acid; (4) salicylic acid.

even of chemically modified material are responsible for this behaviour. The use of anionic counter ions such as HSNa and competing ions such as DMOA is necessary. Fig. 5 demonstrates the influence of the methanol modifier and the counter and competing ions on the elution. In accordance with eqn. 1, increasing the amount of HSNa results in a decrease in the retention times (Fig. 6) of several 1,2-α-amino alcohols. Concentrations of ion pairing agents between 1 and 4 m*M* lead to successful separations. Under these conditions most cationic compounds are eluted with good peak shapes and short retention times on cyanophases. Diol phases are also suitable for the separation of cationic compounds, as is demonstrated by Fig. 7. These offer the additional advantage of being able to separate anionic (acidic) compounds which are not retarded on either cyano or silica phases.

Separation of anionic compounds

Carboxylic acids and phenolates are well separated on diol phases. As a counter ion TBA and as a competing ion acetate (OAc) were used. For larger carboxylic acids with more complex structures the addition of citric acid is necessary in order to obtain reasonable elution times and good peak shapes (Figs. 8 and 9). As already mentioned, mixtures of anionic and cationic compounds (Fig. 7) are best separated on diol phases. On cyano phase acids such as benzoic acid are not retained. Hence the best choice of the initial conditions, when starting an optimization of the separation of ionizable compounds, is to use a diol-phase and TBA and OAc in methanol as ion-pairing agents.

Influence of density

Selectivity and retention strongly depend on the density of the supercritical fluid. The density can be changed rapidly by varying the pressure or temperature. When using poorly soluble ion-pair reagents a temperature increase would sometimes be

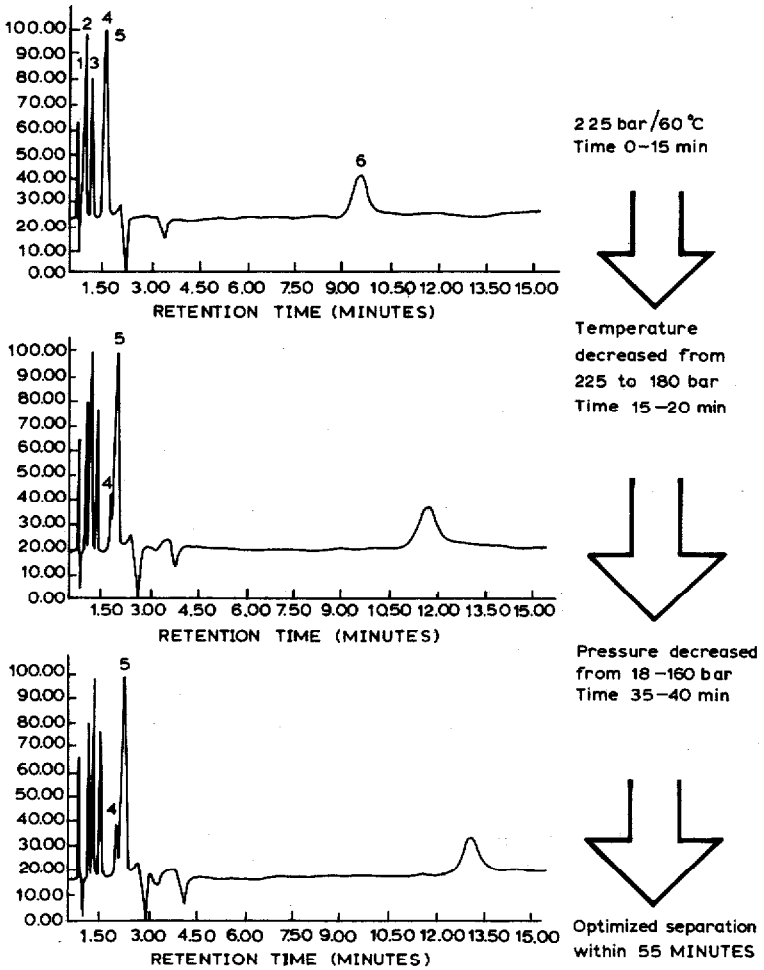

Fig. 9. Optimization of Spirapril, its by-products and degradation products. Conditions: carbon dioxide–20% methanol–20 m*M* TBA–20 m*M* acetic acid–20 m*M* CiS. Column, diol 10 μm (100 mm × 4.6 mm I.D.). (1–4) By-products of Spirapril; (5) Spirapril; (6) diacid of Spirapril.

preferred to a pressure decrease, because at constant density the solubility of ion-pair reagents (Fig. 4) increases with increasing temperature. In Fig. 10 the dependence of the capacity factor on the outlet pressure is demonstrated. This effect can be exploited for the fast optimization of a separation, as shown in Figs. 7 and 9. Starting with the standard condition on a diol phase (carbon dioxide + 20% methanol, 20 m*M* TBA, 20 m*M* OAc), one can obtain an optimized separation within 30 min by changing only the temperature. The same is valid for the separation of Spirapril together with its by-products and degradation products.

A decrease in pressure results in an additional separation of peak 4. The establishment of equilibrium take place immediately[5]. A further benefit of the influence of the density on the retention is the applicability of pressure gradients,

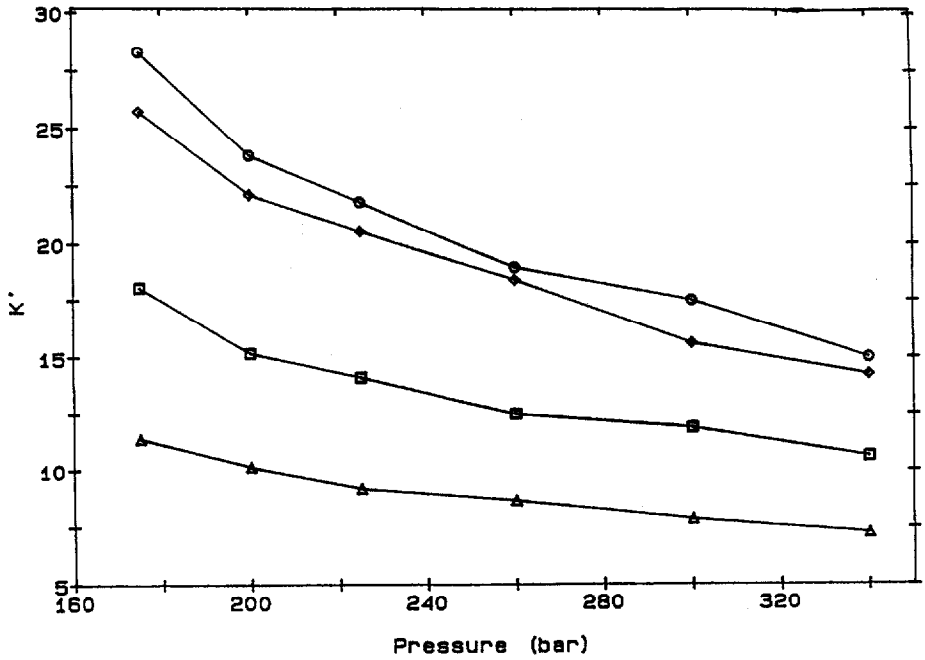

Fig. 10. Dependence of the retention of 1,2-α-amino alcohols on the outlet pressure. Conditions: carbon dioxide–20% methanol–2.5 m*M* HSNa–1.15 m*M* DMOA; 60°C. Column, cyano, 5 μm (100 mm × 4.6 mm I.D.). □, Bopindolol; ○, precursor; ◇, pindolol; △, propranolol.

necessary for a sensitivity- and time-optimized separation of complex mixtures. Hence the isocratic separation of Isradipin and its by-products can be improved by using a pressure gradient (Fig. 2).

CONCLUSION

The ion-pair principle works successfully on straight phases in SFC. The solubility of ion-pairing agents in supercritical fluids is sufficient to achieve a significant ion-pair effect. By the introduction of ion-pairing agents in SFC, the method can be extended to a wide range of cationic and anionic organic compounds such as pharmaceuticals. SFC gives a better separating performance than HPLC. Thermodynamic equilibration for both pressure and for modifier changes are achieved much more rapidly than eluent changes in straight-phase HPLC. Pressure gradients can be applied. The combination of the benefits of SFC with the high potential with respect to selectivity of ion-pair chromatography results in rapid optimizations of the separation of ionic and ionizable compounds and in the application of pressure gradients in ion-pair chromatography. The introduction of the ion-pair principle in capillary SFC gives a powerful tool with better efficiency and more possibilities for controlling selectivity.

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