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Separation of homologous aromatic alcohols and carboxylic acids by packed column supercritical fluid chromatography

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Abstract

The retention behaviour of the members of homologous series of polar analytes on supercritical fluid chromatography has been investigated on different stationary phases; octadecylsilyl- amino- and cyano-bonded silicas and polystyrene divinylbenzene. The changes in the relative retentions of homologous phenylalkanols and phenylalkanoic acids with changes in temperature and pressure of the mobile phases were determined. The results suggested that in each case there was a mixed mode of separation in which the retention could be related to a combination of the vapour pressure of the analytes and of polarity interactions, whose importance increased with the polarity of the stationary phase.

1. Introduction

Many of the early studies of the retention of analytes in supercritical fluid chromatography (SFC) were carried out using relatively non-polar stationary phases in capillary columns and the work usually concentrated on the relationship between pressure and/or temperature and the solute solubility in the mobile phase [1]. It was found that the retention of homologues, such as the alkyl benzenes [2] and alkyl aryl ketones [3], increased with carbon number and that there was little change in selectivity with temperature [2]. Although, it was recognised that interactions with the stationary phase played a part in retention, this was frequently regarded as a problem and considerable effort was devoted to preparing inert surfaces free from strong interactions [1].

In contrast, the effect of the stationary phase has been studied more closely in packed column supercritical fluid chromatography. Upnmoor and Brunner [4,5] examined the separation of xanthines, fat-soluble vitamins and carboxylic acids on a range of different columns and suggested that, with carbon dioxide as the eluent, the separations were determined by a normal-phase type mechanism. Retentions were influenced primarily by interactions between the analytes and polar silanol groups on the surface

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of the silica gel. If methanol was added as a modifier, it reduced retentions for both bonded and non-bonded silica stationary phases confirming that alkyl-bonded chains on the surface played little part in retention, other than to shield some silanol groups from the analytes.

Recently the use of packed columns in SFC has been reviewed by Taylor and Chang [6], Petersen [7], Schoenmakers et al. [8] and by Poole et al. [9]. These reviews noted the significance of silanol interactions and that as a consequence numerous studies have been devoted to devising phases in which the silanols are fully masked, or to investigating the potential of non-silica based polymeric stationary phases. A different approach has been to use modified eluents in which a polar additive, such as methanol, was included in the mobile phase. This was considered to interact preferentially with the active sites on the stationary phase. Alternatively the effective area of silica can be reduced by using pellicular beads or wide-pore materials [10]. In most of these studies, the aim has been to reduce or to eliminate the normal-phase type interactions rather than use the interactions to increase the selectivity of the separation.

At constant density, eluent strength on both types of columns is considered to be dependent on the solubility of the analyte in the mobile phase [11]. Solubility differences between analytes are governed mainly by their vapour pressure and only secondarily by solute–solvent interaction with the supercritical phase. However, under conditions of constant eluent pressure, the significance of the volatility changed with temperature. On increasing the temperature the separation passed initially through a "LC-like region" in which the density and hence the solubility strength of the eluent decreased and hence retention increased. At higher temperatures retentions decreased as analyte volatility became more important and the separations occurred in a "GC-like region". Berger has recently emphasised that these changes do not imply a change from one mechanism of retention to another but that both mechanisms are always operating and it is the sum of these effects that is observed [12]. The influence of vapour pressure even on solid analytes was observed in an earlier separation of three sterols [13] by SFC. Although they had negligible volatilities, they were still eluted in the order of their vapour pressures.

Thermodynamic models of retention in SFC, such as those described by Schoenmakers [14] and more recently the detailed treatment based on a lattice–fluid model by Martire and Boehm [15] (Eq. 1) describe retention as combination of a mobile phase contribution $[\mathrm{F}(T_\mathrm{R}, \rho_\mathrm{R})]$ dependent on the reduced temperature and density of the eluent and an analyte–stationary phase interaction, which can be expressed as the retention factor (k^0) at zero density corresponding to ideal gas chromatography. This latter term is closely related to vapour pressure and for a homologous series increases systematically with chain length corresponding to increased retention [16].

$$\ln k = \ln k^0 + \mathrm{F}(T_\mathrm{R}, \rho_\mathrm{R}) \qquad (1)$$

These changes with chain length are reflected in direct solubility studies. *n*-Heptane and *n*-dodecane were miscible in liquid carbon dioxide at 22–24°C. but *n*-hexadecane is only 5% soluble and *n*-tetracosane 1–2% soluble [17].

Despite many studies of the effect of the physical and stationary phase effects on retention in SFC, little work has systematically compared the effects of analyte structure or the presence of different functional groups on the retention. Wheeler and McNally [18] compared the retention of various compounds of agricultural interest in capillary packed column SFC with retention on reversed-phase HPLC. However, as with many similar studies the analytes had different sizes, polarities and ionisation differences so that the influence of the individual effects could not easily be isolated. King and Friedrich [19] examined the potential of solubility parameter theory to predict solubilities and hence retention in SFC. They found a reasonable correlation between the reduced solubility parameter $\Delta = \delta_1 / \delta_2$ and retention. The solubility parameter of the mobile phase (δ_1) was derived from standard equations. The solubility parameter for the analyte (δ_2) was calculated using a method proposed by Fedors [20] as the ratio of the energy of vaporisation (ΔE_v) and the corresponding molar volume. Each of these values was derived by the accumulation of terms for the carbon skeleton and functional groups. Low values of δ_2 and hence high values of the reduced solubility parameter Δ corresponded to non-polar analytes and could be correlated with low retentions on SFC.

Previously, we have examined the separation of homologous analytes on packed columns by SFC. When the relatively non-polar alkyl aryl ketones or alkylbenzenes were examined on an octadecylsilyl(ODS)-bonded silica column, there was a linear relationship between log k of the homologues and their carbon number [21]. A similar general relationship was also found for a PS-DVB column, which lacks silanol groups [22]. Although these relationships resembled those found for similar homologues on reversed-phase high-performance liquid chromatography (HPLC) and could have been attributed to a "hydrophobic" effect, this comparison would probably be misleading and a better model would be with the influence of analyte volatility in gas–liquid chromatography (GLC). Supercritical carbon dioxide has a low polarity with relatively weak intermolecular forces so that the size of the analyte should not have the same importance as in reversed-phase chromatography.

However, when the alkyl aryl ketones were examined on a polar cyano-bonded silica column material by SFC, the retentions of the smaller and more polar homologues increased with decreasing carbon number, although the higher homologues showed a linear relationship between log k and carbon number [23]. A similar effect was observed on cyano-bonded columns from different manufacturers. The separations were compared with normal-phase liquid chromatographic separations on the same columns and from the results it was proposed that the retentions of the alkyl aryl ketones in SFC were determined by the summation of a volatility effect, which increased the retentions systematically with carbon number, and a normal-phase polar–polar interaction between the analytes and active sites on the stationary phase. This latter effect became less important with increasing carbon number but was dominant for the smallest homologues.

The present study extends the examination of the relationship between chain length and retention on polar and non-polar stationary phases by packed column SFC to determine the influence of different functional groups. Homologous phenylalkanols and phenylalkanoic acids are separated on polar and non-polar stationary phases to compare the relative effects of polar interactions and analyte volatility. Little previous work has examined the influence of carbon chain length or carbon skeleton on the retention of analytes containing the same functional groups. By examining homologues in which the polarity or ionisation of any functional group is the same the polarity effects can be normalised and the differences caused by size and methylene selectivity can be compared.

2. Experimental

2.1 Chemicals and samples

The phenylalkanols, phenylalkanoic acids and alkyl aryl ketones were of laboratory grade from a range of supplies. Carbon dioxide was laboratory grade from British Oxygen Company and solvents were HPLC grade from Fisons Scientific Equipment.

2.2 Equipment

The supercritical fluid separations were carried out using a JASCO system (Japanese Spectroscopic Company, Tokyo, Japan), consisting of a 880 PU pump with cooled pump head for the delivery of carbon dioxide at 2 ml min^{-1}, a PU-980 pump for the delivery of modifier into a SP8500 dynamic mixer (Spectra Physics), an 860-CO column oven, an 875-UV ultraviolet detector fitted with a high-pressure flow cell and a 880-81 back-pressure regulator. The chromatograms were recorded using Jones JCL6000 chromatographic data system software on an Elonex 386SX computer. Samples (5 μl) were injected using a 7125 Rheodyne valve (Cotati, CA, USA) fitted with a 20-μl loop.

The separations were carried out on a cyano Capcell column (4.6 × 150 mm, Shiseido, Yokohama, Japan), a Spherisorb ODS-B column (5 μm, 4.6 × 250 mm, Phase Separations, Queensferry, UK), aminopropyl-Spherisorb (5 μm, 4.6 × 250 mm, Phase Separations), or a PLRP-S column (5 μm, 4.6 × 160 mm, Polymer Laboratories, Church Stretton, UK).

3. Results and discussion

Two series of homologues, the phenylalkanols from benzyl alcohol to 5-phenylpentanol and the phenylalkanoic acids from benzoic acid to 5-phenylhexanoic acid were chosen to represent different polar interactions. These analytes and phenol were chromatographed on both nominally non-polar, ODS-silica and PS-DVB columns, and on polar cyano- and amino-bonded silica columns. Similar pressures and hence densities of carbon dioxide were used on each column to permit comparisons. Under selected conditions the results were also compared with the separations of the alkyl aryl ketones.

3.1 ODS-Silica column

Although a ODS-bonded silica column should behave as a non-interactive non-polar stationary phase, because of the number of uncapped silanol groups that are present on the silica surface, it will still possess significant polar activity. This is clearly reflected in many SFC studies as the addition of even very low proportions of methanol as a modifier usually has a significant effect on retention.

In the present study, a base-deactivated silica, Spherisorb ODS-B, was chosen as a typical low-silanol alkyl-bonded silica column. The retentions of the phenylalkanols on elution with carbon dioxide at about 150 bar and 60°C showed a systematic increase with increasing chain length except for the longest member, phenylpentanol, which decreased slightly (Table 1). These changes along the homologous series can be correlated with increases in the vapour pressures of the analytes: benzyl alcohol, b.p. 204°C; 2-phenylethanol, 219°C; 3-phenylpropanol, 235°C [24]. Phenol was more rapidly eluted than the phenylalkanols in agreement with its lower boiling point (182°C) [24]. All the retention times were reduced markedly on the addition of 1.0% *v/v* methanol as a modifier (Table 1) and little difference was then found between the homologous phenylalkanols suggesting that at least part of the original retention was due to interactions with the stationary phase.

Table 1
Retention of phenylalkanols on ODS-bonded silica column

Compound	Retention factor	
Pressure (bar)	155	162
Modifier (% methanol)	0.0	1.0
Phenol	2.11	-
Benzyl alcohol	2.48	1.72
2-Phenylethanol	3.76	1.66
3-Phenylpropanol	4.33	1.92
4-Phenylbutanol	5.32	2.18
5-Phenylpentanol	5.08	2.26

Conditions: column, ODS-B Spherisorb; eluent, carbon dioxide; flow rate, 2 ml min^{-1}; temperature, 60°C, column pressure as given.

Under similar elution conditions, the phenyl weak alkanoic acids showed a very different behaviour and no elution occurred with carbon dioxide alone. A similar problem was found for benzoic acid on ODS-silica by Smith and Sanagi [21]. Geiser et al. [25] found that it was often necessary to add water to the mobile phase to obtain good peak shapes for the free fatty acids. Nomura et al. were able to elute the fatty acids with carbon dioxide alone but only from a specially prepared highly endcapped column material [26].

On the addition of 1.96% *v/v* methanol as a modifier, most of the acids were eluted. However, they had virtually the same retention times, except phenylacetic acid, which was much more highly retained, and benzoic acid, which was totally retained. (Table 2). On further increasing the proportion of methanol to 4.76%, the re-

Table 2
Retention of phenylalkanoic acids on ODS-bonded silica column.

Compound	Retention factor	
Pressure (bar)	156	156
Modifier (% methanol)	1.96	4.76
Benzoic acid	retained	5.70
Phenylacetic acid	20.77	6.15
Phenylpropanoic acid	10.78	3.31
Phenylbutanoic acid	9.39	2.74
Phenylpentanoic acid	10.19	2.95
Phenylhexanoic acid	10.31	3.02

Conditions as in Table 1. None of the acids were eluted with carbon dioxide alone.

tentions decreased and benzoic acid was eluted. The retentions of phenylpropanoic acid to phenylhexanoic acid were again very similar (Table 2) but the shorter acids were again more highly retained. It appeared that there was a strong interaction between the carboxylic acids and the stationary phase and this effect was strongest for benzoic and phenylacetic acids. For the higher homologues the similarity of their retention times suggested that under these conditions the polarity effects were dominant and that the influence of the analyte vapour pressure was much smaller. This can be correlated with their boiling points, which were much higher than the phenylalkanols (benzoic acid, b.p. 249°C; phenylacetic acid, 248°C and phenylpropanoic acid 279°C [24]) and hence they would have lower volatilities.

The increased retention of benzoic and phenylacetic acids could also be because they were partially ionised, however, although they are the stronger acids their pK_a values of 4.19 and 4.28, respectively, are not very different from those of the larger homologues, phenylpropanoic acid, 4.37, and phenylbutanoic acid, 4.76 [27]. Berger and Deye [28] have reported that for a number of strong acids the inclusion of an acidic additive to the modifier, such as citric, trifluoroacetic or dichloroacetic acid, improved the retention and peak shapes. The added acid apparently acted by ion suppression of the analytes. However, under their conditions benzoic acid was virtually unretained in both the absence and presence of modifier so that the potential influence of ionisation on weak acids was unclear.

The separations of both these homologous series were markedly different from the separation of the alkyl aryl ketones at 153 bar and 60°C (Fig. 1). For the same carbon number the retentions of the ketones were much shorter reflecting their lower polarities even though the boiling points of the ketones and alcohols were similar: acetophenone, b.p. 202°C; propiophenone, 218°C and butyrophenone, 220°C [24]. The ketones showed a nearly linear relationship between carbon number and logarithm of the retention factor with a very different slope to the alcohols. Thus even though this is a well-capped column, polarity effects are apparently dominating the separation of polar analytes.

The solubility parameters for these sets of analytes were calculated using the methods of Fedors [20]. The resulting δ_2 values (Table 3) decreased systematically with carbon chain length and were smaller for the alkyl aryl ketones than for the phenylalkanols or phenylalkanoic acids. For any eluent conditions, the

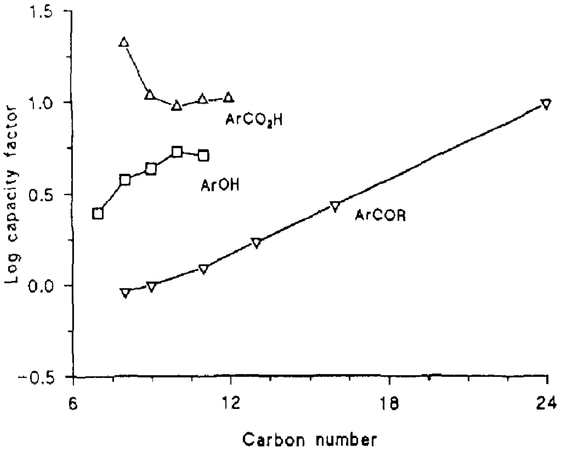

Fig. 1. Comparison of retention factors of homologues on an ODS-bonded silica column at 60°C. Compounds: ▽ = alkyl aryl ketones with carbon dioxide as eluent at 153 bar; □ = phenylalkanols with carbon dioxide at 155 bar; △ = phenylalkanoic acids with carbon dioxide plus 1.96% methanol at 156 bar.

Table 3
Solubility parameters (δ_2) for homologues

Carbon number	Solubility parameter (δ_2)		
	Alkyl aryl ketones	Phenylalkanoic acids	Phenylalkanols
7		11.93	12.78
8	9.90	11.53	12.27
9	9.76	11.21	11.88
10	9.65	10.95	11.56
11	9.56	10.74	11.29
12	9.48	10.56	11.07

Values calculated according to Fedors [20].

reciprocals of these solubility parameters will be directly related to the corresponding reduced solubility Δ values so that greater solubility (and hence shorter retentions) would be predicted for the alkyl aryl ketones than the other groups. This is in agreement with the present study. However, the changes in the solubility parameters with increased chain length predicts that the solubility should increase and hence retentions should decrease with carbon number. As already noted the opposite change is observed for homologues in SFC and retention increases with chain length (Fig. 1). The experimental changes also agree with the reported changes in solubility in supercritical fluid noted earlier for the alkylbenzenes [17].

The changes in solubility parameters were also compared with a recent report of the separation of homologues on a diol column using normal-phase conditions [29]. In that case there was a systematic decrease in retention factors with chain length in agreement with the predictions: *n*-alkanols on elution with hexane changed from ethanol $k = 3.40$ to dodecanol $k = 2.41$; alkan-2-ones using hexane–diethyl ether 99.2:0.8; from pentan-2-one, $k = 2.82$, to 2-undecanone, $k = 2.37$; and similar changes were observed for homologous methyl and ethyl esters.

Thus the results from this study appear to agree with the model proposed by Martire and Boehm [15] in which the solubility of sample in a supercritical fluid depends on a solute–stationary phase interaction. This is represented by a k^0 term for the retention in an eluent with zero density, which would be directly related to volatility (or carbon number of a homologue). The validity of this approach is further confirmed by a recent report by Wang et al. which reported a linear relationship between retention values and boiling points of homologues in SFC [30].

3.2 PS-DVB columns

In order to determine the extent of the effect of the residual silanol groups on the ODS-silica columns, the homologues were separated on a polystyrene-divinylbenzene (PS-DVB) column at 151 bar and 60°C (Table 4). The retentions of the phenylalkanols were similar to those on the ODS column under similar conditions and the alcohols were again eluted with a systematic increase in retention with increasing carbon number from benzyl alcohol to phenylpentanol. Surprisingly, phenol was now more highly retained than benzyl alcohol even though they have similar boiling points. This effect is probably caused by π–π interactions between the phenolic ring and the aromatic polystyrene stationary phase and similar interactions have been reported for naphthols and PS-DVB columns [31].

However, on adding methanol as modifier to the mobile phase there was a much smaller effect than on the ODS-bonded silica column. Increasing proportions of methanol, 1.23%, 2.76% and 4.76%, caused a systematic decrease in retention

Table 4
Retention of phenylalkanols on PS-DVB column

Compound	Retention factor							
Pressure (bar)	151	151	152	153	157	207	247	153
Temperature (°C)	40	60	60	60	60	60	60	80
Modifier (% methanol)	0.0	0.0	1.23	2.76	4.76	0.0	0.0	0.0
Phenol	3.13	3.79	2.57	2.57	1.44	2.74	2.01	4.72
Benzyl alcohol	2.13	2.87	2.10	-	1.39	1.55	1.45	4.22
2-Phenylethanol	2.36	3.37	2.37	1.97	1.48	1.44	1.53	4.97
3-Phenylpropanol	3.37	5.19	3.08	3.02	1.83	2.54	2.05	7.64
4-Phenylbutanol	4.10	6.44	3.82	3.45	2.34	3.04	2.46	10.08
5-Phenylpentanol	4.72	7.67	4.60	4.11	2.57	3.57	2.74	13.16

Conditions: column, PL-RPS; eluent, carbon dioxide plus modifier as indicated; flow rate, 2 ml min^{-1}.

for all the analytes. For example, the retention of 5-phenylpentanol changed from $k = 7.67$ to 4.60 to 4.11 to 2.57. In contrast, on the ODS-bonded silica column (Table 1), the addition of 1.0% modifier reduced the retention factor for 5-phenylpentanol from $k = 5.08$ to 2.26. The smaller change with modifier for the PS-DVB column suggested that the effect of the methanol was to alter the solubilising properties of the eluent rather than by altering strong surface interactions as with the surface silanols on the silica based column.

If the column temperature was changed from 40° to 60° to 80°C, which would decrease the density of the carbon dioxide from 0.79 to 0.60 to 0.43 g ml^{-1}, the retentions of the phenylalkanols increased (Table 4) suggesting that the separations were being carried out in the "LC region" of the temperature vs. retention relationship [2] in which the decrease in eluent density is more important than the increase in analyte volatility. The corresponding effect was observed on increasing the column pressure from 151 bar to 207 bar and then to 247 bar at 60°C (Table 4). The reductions in the retentions of each of the alcohols can be attributed to the increases in the density of the eluent from 0.60 to 0.73 and to 0.79 g ml^{-1}.

The differences in retention on comparison with the ODS-bonded silica column were equally marked for the phenylalkanoic acids. None of the acids was eluted with carbon dioxide alone but the addition of even a small proportion of methanol (1.23%) was sufficient to elute all the acids (Table 5). Unlike the ODS-bonded silica column, the acids showed a marked increase in retention with carbon number corresponding to the changes in boiling points. Thus it appeared that there was only a small polar interaction with the stationary phase and the elution of the analytes was primarily determined by their relative volatilities. Further addition of methanol decreased the retention times in each case but caused no change in relative retentions (Table 5). Taylor and Chang [6] described a separation by Yang which showed a similar relationship between chain length and retention for the separation of the free fatty acids on a PS-DVB column.

Table 5
Retention of phenylalkanoic acids on PS-DVB column. Conditions: as Table 4; temperature, 60°C. None of the acids were eluted with carbon dioxide alone

Compound	Retention factor		
Pressure (bar)	156	169	163
Modifier (% methanol)	1.23	2.44	4.76
Benzoic acid	4.52	2.43	1.65
Phenylacetic acid	4.46	2.41	1.52
Phenylpropanoic acid	4.89	2.75	1.69
Phenylbutanoic acid	6.12	3.33	2.05
Phenylpentanoic acid	7.21	4.01	2.52
Phenylhexanoic acid	8.88	5.46	2.90

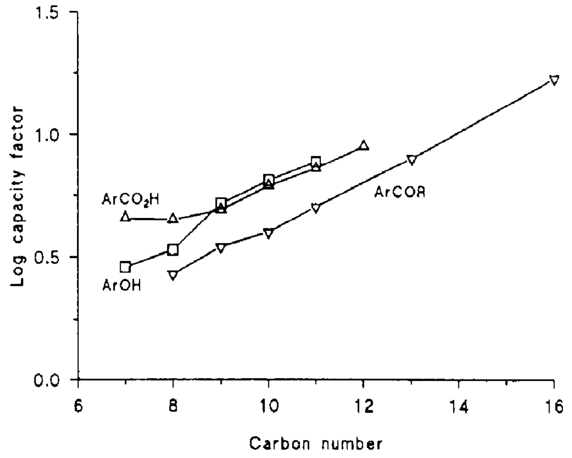

Fig. 2. Comparison of retention factors of homologues on a PS-DVB column at 60°C. Compounds: ▽ = alkyl aryl ketones with carbon dioxide as eluent at 147 bar; □ = phenylalkanols with carbon dioxide as eluent at 151 bar; △ = phenylalkanoic acids with carbon dioxide plus 1.23% methanol as eluent at 156 bar.

The retentions of the phenylalkanols were compared with the separation of a series of homologous alkyl aryl ketones (Fig. 2) under similar conditions with carbon dioxide as the eluent and with the phenylalkanoic acids in the presence of 1.25% methanol. The alcohols and ketones with similar boiling points, such as benzyl alcohol and acetophenone, had almost identical retentions suggesting that vapour pressure was playing a major part in the solubility of the analyte in the mobile phase. In each case the slope of the relationship between log *k* and carbon number for the higher homologous was similar suggesting that in each case there was a common systematic methylene increment corresponding to the changes in volatility. For the smaller acids there were deviations from linearity so that some polarity effects may still be present.

3.3 Cyano-bonded silica column

From earlier studies [23], it would be expected that analytes would experience stronger polar interactions on a cyano-bonded column. The separation of the homologues was therefore examined using a cyano-Capcell stationary phase. This material is coated with a polymeric layer and should show little silanol activity [32,33].

Under similar conditions to those used to achieve separations on the ODS-bonded silica and PS-DVB columns, the phenylalkanols were eluted with shorter retention times (Table 6). This suggests that in this case the retention may be resulting from the very few remaining free silanol groups rather than from interactions with the cyano groups themselves. In a previous study large differences between cyano-bonded columns were observed which could be attributed to variation in silanol activity [23]. Reports from HPLC studies also suggest that the retentive activity of cyano-bonded silica columns results primarily from uncapped silanol groups [34]. The retentions increased if the column temperature was raised to 80°C but decreased if the pressure was raised to 201 bar (Table 6), which can be attributed to changes in the density of the eluent. Under these conditions phenol was more highly retained than both benzyl alcohol and 2-phenylethanol, in contrast to its more rapid elution on ODS-bonded silica, suggesting that in the present case its higher polarity was more significant than its higher volatility and that it was interacting more strongly with the stationary phase.

The alkyl aryl ketones were only weakly retained on this column [23]. The difference between the two series of homologues (Fig. 3) suggested that for compounds with similar boiling points, e.g propiophenone, $k = 0.55$ com-

Table 6
Retention of phenylalkanols on cyano-bonded silica column

Compound	Retention factor			
Pressure (bar)	156	156	157	201
Temperature (°C)	40	60	80	60
Phenol	1.42	1.65	2.61	1.04
Benzyl alcohol	1.27	1.43	1.99	0.86
2-Phenylethanol	–	1.59	2.38	0.93
3-Phenylpropanol	1.99	2.19	3.30	1.24
4-Phenylbutanol	2.12	2.38	3.93	1.32
5-Phenylpentanol	2.13	2.58	4.36	1.33

Conditions: column, cyano-Capcell; eluent, carbon dioxide; flow rate, 2 ml min^{-1}.

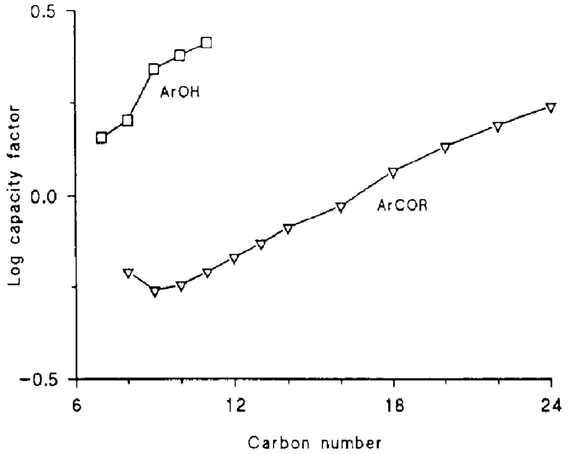

Fig. 3. Comparison of retention factors of homologues on cyano-bonded silica phase at 60°C with carbon dioxide as eluent. Compounds: ∇ = alkyl aryl ketones at 156 bar [data from Ref. 23]; □ = phenylalkanols. Conditions as in Table 6.

pared to 2-phenylethanol, $k = 1.59$, the higher polarity of the phenylalkanols significantly increased their retention. As with the ODS-bonded silica column the slopes of the two sets of homologues differed and the phenylalkanols showed a greater change with increasing chain length. Unlike the other two columns, it was not possible to elute the phenylalkanoic acids even on the addition of methanol to the mobile phase. Again this suggest the presence of strong polar–polar interactions with this stationary phase. This would agree with the observation by Karlsson et al. [35] using cyanosilicone-coated capillary columns that increasing the proportion of methanol in the eluent (up to 20%) decreased the retention of benzoic acids and sharpened the peak shape. Like Berger and Deye [28] they also examined the effect of the addition of citric acid to the eluent but it had little influence on retention or peak shape. However, the cyano stationary phase was unstable and degraded markedly over a period of three weeks use so the effective chemical nature of the column material used in the study is uncertain.

3.4 Amino bonded silica

A brief study was also carried out to examine the retention of the phenylalkanols on an amino-propyl-bonded silica column. Addition of methanol (0.5%) as a modifier was required to elute the phenylalkanols and the retention factors showed a typical polar effect with benzyl alcohol being retained more than 2-phenylethanol (Table 7). However, unlike the separations on the cyano-bonded column, the retentions then increased markedly with chain length so than analyte vapour pressure effects were important. The phenylalkanoic acids were not eluted even with a modified eluent.

Table 7
Retention of phenylalkanols on aminopropyl bonded silica column

Compound	Retention factor
Benzyl alcohol	8.59
2-Phenylethanol	7.66
3-Phenylpropanol	10.14
5-Phenylpentanol	12.06

Conditions: column, S5NH$_2$-Spherisorb; eluent, carbon dioxide plus 0.5% methanol; flow rate, 2 ml min^{-1}; temperature, 60°C; pressure, 150 bar.

In order to separate the effects of the amino groups and underlying silanols, a trial run was also carried out using an amino-bonded vinyl alcohol copolymer column but problems were experienced and it appeared that the column was not suitable for SFC work. Although a study on this type of column has recently been reported by Berger and Deye, the results were not good [36].

4. Conclusion

It has often been claimed that the primary influence on retention in SFC is the solubility of the analyte in the supercritical fluid mobile phase and it has frequently been demonstrated that this model can explain changes in retention with the temperature and pressure of the eluent. However, few studies have examined the influence of analyte structure on retention. The present work has demonstrated the inadequacy of the concept that the solubility in supercritical fluids can be solely defined by using reduced solubility parameters in an analogy of solubility effects in organic solvents. This method predicts that higher members of a homologous series would be less polar and hence they would be more soluble in supercritical carbon dioxide leading to shorter retentions.

However, the retention of homologues generally increases with chain length. The present studies have shown that the volatility of the analyte, which decreases with increasing chain length, plays a significant contribution, and can be used to predict the changes in the retention of homologues. This is in agreement with theoretical studies on retention in SFC which include an element for the retention factor in ideal gas chromatography. In addition the effect of the different stationary phases on the homologous series provides evidence that stationary phase interactions also play an important role.

Thus separations in SFC are controlled primarily by two factors. Polar–polar interactions are present similar to those found in normal-phase chromatography. Secondly, there is a vapour pressure effect which causes the larger less volatile members of homologous series to be more highly retained by reducing their solubility in the mobile phase.

The nature of the stationary phase in the SFC column determines which of these effects is the more important. On non-polar PS-DVB columns, in which polar–polar interactions are minimal, volatility is important, although π–π interactions can have an effect. Under these conditions the principal effect of a modifier is to increase the solubility of the analytes in the eluent. On a ODS-silica column volatility plays a role but polar interactions with the underlying silanols have a more significant effect which can be largely eliminated by the addition of even small amounts of a polar modifier. In contrast, on a cyano-bonded silica, polarity effects dominate and volatility plays only a small part in determining retention. Even significant additions of modifier may not be sufficient to eliminate strong polar interactions, such as those with carboxylic acid groups. However, this group of analytes may also be partially ionised.

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