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Retention and selectivity in supercritical fluid chromatography on an octadecylsilyl-silica column

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SUMMARY

The changes in retention and selectivity of a series of model test compounds on an octadecylsilyl-silica column in supercritical fluid chromatography have been examined at different column pressures. The effects of the addition of modifiers to the carbon dioxide eluent have been compared using retention indices based on the alkyl aryl ketone scale. Without modifier, polar hydrogen-bonding analytes, such as alcohols and phenols, were highly retained, but their relative retention times were considerably reduced as the proportion of modifier increased. Aliphatic **amines** appeared to interact with free silanols on the surface on the stationary phase in the absence of modifiers and their retention changed markedly on the addition of small proportions of modifier. However, the relative retentions of more weakly basic aromatic **amines** were unaltered by modifier.

INTRODUCTION

Supercritical fluid chromatography (SFC) has been undergoing a considerable growth in the last few years[1-3] and is now moving from the research to the industrial laboratory as commercial equipment becomes available and as more applications are demonstrated for petrochemicals, agrochemicals and pharmaceuticals and interest is starting to be expressed in its application for official methods[4]. It has considerable potential as an alternative instrumental analytical technique complementing high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC). In particular it can offer a different selectivity to the other techniques and thus may enable difficult separations to be achieved. As with HPLC there is the possibility of altering the composition of the eluent either by using a different supercritical fluid or, more commonly, by the addition of modifiers. However, as yet the mechanisms and selectivity of the retention in single eluents are not fully understood and the influence of the modifier has not yet been examined in detail.

As well as the composition of the mobile phase, the nature of the stationary phase and the operating conditions can have a significant effect on the separation. In order for SFC to be fully accepted, it must be possible to reproduce the conditions of a separation in different laboratories. However, this may be more difficult than in other chromatographic techniques, because the separation power of the eluent is very dependent on the density of carbon dioxide and hence on the temperature, mean pressure, and pressure drop across the column'. Whereas the first of these is easy to define and control, it will be more difficult to accurately reproduce pressures in different laboratories. Even if standardised gauges are used, the mean pressure in the column will depend on the resistance of the column to flow, as well as on the pressure applied by the pump.

In addition, for many analytes it has been found necessary to add a modifier, such as methanol or acetonitrile, to the carbon dioxide eluent in order to obtain reasonable retention times and good peak shapes[6–10]. It is difficult to reproducibly prepare mixed eluents in defined proportions for packed columns using a dual-pump system, because the compressibility of the carbon dioxide means that its mass flow-rate is determined by the pressure and temperature in the pump head, rather than just by the flow-rate setting, whereas the modifier flow should be independent of the pressure'. Related problems in capillary SFC using syringe pumps have been discussed in a study of the separation of sulphonamides[10]. Even the use of premixed cylinders of eluent is not a solution as it is reported that the composition leaving the cylinder changes as the cylinder's contents are consumed[11].

As many of these changes in operating conditions affect all analytes to a similar extent, relative measurements of retention compared to a standard or standards might give better reproducibility and enable results from different instruments or laboratories to be compared. This concept forms the basis of the use of retention indices in GLC and HPLC[12] and in previous work we have examined its application to SFC on polystyrenedivinylbenzene (PSDVB) packed columns' [3] and for the separation of barbiturates[14,15] and benzodiazepines[9] on PS-DVB and octadecylsilyl (ODS)-silica packed columns. On the PS-DVB column it was found that the retention indices of a series of model test compounds were largely independent of the temperature and pressure of the separation but were critically dependent on the modifier proportion. Large relative retention changes with the proportion of modifier were also observed in the studies of the drug compounds on both PS-DVB and ODS-silica based columns.

The present study reports the changes in the selectivity and relative retentions of a set of model aromatic test compounds on an ODS-silica stationary phase with operating conditions and the potential application of retention indices to improve the reproducibility of recorded separations. The different behaviour of the model compounds on the addition of modifiers should also give an indication of the types of interactions that are taking place.

EXPERIMENTAL

Chemicals

n-Alkanes from decane to tetracosane, alkyl aryl ketones from valerophenone to octadecanophenone and test compounds were laboratory grade from a range of suppliers. Carbon dioxide was industrial grade (99.98%) from British Oxygen

Company (Brentford, U.K.) and modifiers were HPLC grade from FSA Scientific Apparatus (Loughborough, U.K.).

Equipment

The separations were carried out using a packed-column **chromatograph**, consisting of a Jasco BIP-1 pump (Hachioji City, Tokyo, Japan) with cooled check valves and pumping head, operating under constant-pressure conditions, a Pye Unicam-XPS pump for the addition of modifier, a Rheodyne (Cotati, CA, U.S.A.) 7125 injection valve with a 20-μl loop and a Pye 104 gas chromatographic oven (Pye Unicam, Cambridge, U.K.). The analytes were detected using the flame ionisation detector on the Pye 104 or at 254 nm with an ACS 750/12 variable-wavelength ultraviolet spectrophotometric detector (Applied Chromatography Systems, **Maccles**field, U.K.) fitted with a high-pressure flow cell. A crimped tube was used as a restrictor. The samples were separated on a 250 x 4.6 mm I.D. column packed with Ultrasphere ODS 5-μm particles (Beckman, High Wycombe, U.K., batch No. T608035) and peaks were recorded using a Hewlett-Packard (Winnersh, U.K.) 3390 integrator.

Method

Samples of the test compounds and retention index standards in methanol were injected onto the column and eluted with supercritical carbon dioxide at 60°C and different applied pressures. Methanol was added as required. A sample of acetone was used as the column void volume marker.

Capacity factors were calculated as k' = $(t_R - t_0)/t_0$ (where t_R is retention time and t_0 column void volume) and the mean of triplicate injections was used in calculations. Retention indices based on the alkyl aryl ketones were calculated as described for HPLC[16] by fitting log k' against carbon number x 100 for the standards to a linear correlation using a least-squares routine and then interpolating the log k values for the test compounds.

RESULTS AND DISCUSSION

The mechanism of retention on bonded-phase columns in SFC is not fully understood, but for a homologous series of compounds the retention increases logarithmically with carbon number in a similar manner to separations by GLC or reversed-phase HPLC[12]. A number of series of homologous organic compounds could potentially be used as retention index scales. The n-alkanes are already widely accepted for GLC, but because of the need for a more polar analyte with a chromophore, the alkyl aryl ketones and alkan-Zones have been used in HPLC[12]. The former have been used as retention index standards in our earlier studies on SFC using a PS-DVB column[13] and the alkan-Zones have been reported to show linear behaviour in SFC on an ODS column". Thus, either set of homologues could potentially be used as a retention index scale. There are also isolated reports on retention being expressed as retention indices in SFC using the *n*-alkanes[18].

When n-alkanes, from **decane** to tetracosane, and alkyl aryl ketones, from valerophenone to octadecanophenone, were examined by SFC on an ODS-silica (Ultrasphere ODS) column (Table I), both sets of compounds were retained with

similar retention times. Comparable retention times were also found for a number of model aromatic test compounds containing different functional groups and for four homologous alkylbenzenes (toluene to n-butylbenzene). Two polar model compounds, benzamide and benzoic acid, were not eluted from the column. Benzylamine was highly retained and was not eluted at 1665 p.s.i. At higher pressures it gave tailing peaks, but a less basic aromatic amine, N-propylaniline, was readily eluted with symmetrical peaks at all the pressures examined. The peaks for the hydroxy

TABLE I

CAPACITY FACTORS OF ALKYL ARYL KETONES, n-ALKANES, ALKYLBENZENES AND TEST COMPOUNDS AT DIFFERENT COLUMN PRESSURES

Conditions: column, Ultrasphere ODS; eluent, carbon dioxide; temperature, 60°C.

Compound	Capacity factor			
	Mean column pressure (p.s.i.)			
	1665	1950	2160	2470
Alkyl aryl ketones				
Valerophenone	2.39	1.43	1.00	0.81
Hexanophenone	2.91	1.69	1.16	0.95
Heptanophenone	3.58	2.02	1.37	1.10
Octanophenone	4.45	2.43	1.62	1.30
Decanophenone	7.04	3.62	2.21	1.84
Dodecanophenone	10.81	5.26	3.25	2.52
Tetradecanophenone	16.95	7.65	4.53	3.47
Hexadecanophenone	26.28	11.10	6.30	4.74
Cktadecanophenone	40.05	15.66	8.57	6.32
n-Alkanes				
Decane	0.97	0.62	0.48	0.43
Dodecane	1.70	1.01	0.77	0.66
Tetradecane	2.83	1.56	1.15	0.97
Hexadecane	4.56	2.37	1.66	1.37
Octadecane	7.20	3.46	2.34	1.90
Eicosane	il.25	4.99	3.25	2.57
Docosane	16.92	7.15	4.43	3.48
Tetracosane	25.56	10.19	6.15	4.68
Alkylbenzenes				
Toluene	0.46	0.34	0.28	0.21
Ethylbenzene	0.64	0.46	0.36	0.28
n-Propylbenzene	0.85	0.59	0.46	0.36
n-Butylbenzene	1.12	0.76	0.58	0.46
Test compounds				
Nitrobenzene	1.02	0.66	0.48	0.36
Benzaldehyde	1.14	0.80	0.56	0.47
Methyl benzoate	1.24	0.82	0.62	0.51
N-Propylaniline	2.43	2.12	1.16	1.00
p-Cresol	2.63	1.71	1.35	1.08
Benzyl alcohol	4.62	2.66	1.84	1.47
Benzylamine		7.01	4.83	3.95

compounds, *p*-cresol and benzyl alcohol, both tailed badly and the retention times varied with sample loading on the column.

These results contrast with reversed-phase (RP) HPLC on an ODS-silica column, where the non-polar alkanes would be much more retained than any of the substituted compounds and the polar hydroxy and amino compounds would be rapidly eluted. However, the present results are similar to those found for SFC on a PS-DVB column[13], although in that case, at similar elution pressures, the analytes were more retained. On the PS-DVB column the first members of the alkyl aryl ketone series (acetophenone to butanophenone) were resolved from the solvent front, whereas on the ODS-silica column they were virtually unretained suggesting a weaker retention by the column. This difference between the relative retention on the ODS-silica and PS-DVB column materials was also observed by Morin *et al.*[17].

There was a linear relationship for both the alkyl aryl ketones and the n-alkanes between log k' and the carbon number (Table II, Fig. 1). The slopes of the two sets of standards are different, suggesting that the methylene increment differs for the two sets of compounds. However, because the n-alkanes can only be detected using a flame ionisation detector, they are impractical for use in the presence of an organic modifier when spectroscopic detection has to be used. Consequently, the alkyl aryl ketones have been adopted as the retention index scale in the rest of the study. When these compounds were examined previously on a PS–DVB column, propiophenone was found to be anomalous and to have a higher retention index $(I = 938$–$960)$ than its nominal value of 900 units[13]. However, as noted above, the smaller alkyl aryl ketones were unresolved in the present study and not included in the set of standards. However, during the examination of the barbiturates, a wider range of ketones was examined individually on the ODS-silica column. On elution with carbon dioxide containing 4.2 and 8.4% methanol propiophenone behaved as expected $(I = 902$ and 888, respectively), but acetophenone was more rapidly eluted $(I = 726$ and $722)$ than its nominal value of 800 units[15].

TABLE II

CORRELATION COEFFICIENTS BETWEEN LOG k' AND RETENTION INDICES (CARBON NUMBER x 100) FOR ALKYL ARYL KETONES AND n-ALKANES

Conditions as in Table I.

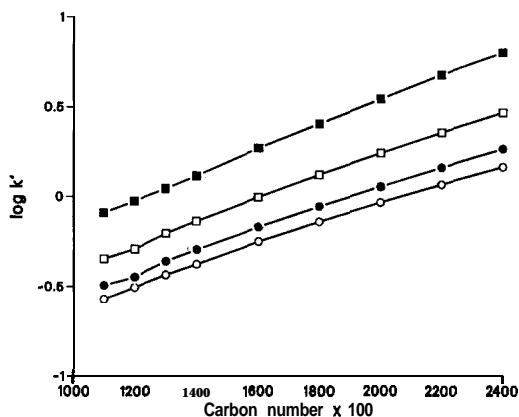| Compound	Mean column pressure (p.s.i.)	Correlation coefficient	Slope x 10^{-4}	Intercept
Alkyl aryl ketones				
Valerophenone-octadecanophenone	1665	0.9999	9.51	-0.676
	1950	0.9999	8.10	-0.742
	2160	0.9998	7.28	-0.808
	2470	0.9998	6.94	-0.855
n-Alkanes				
Decane–tetracosane	1665	0.9986	10.01	-0.980
	1950	0.9984	8.60	— 1.030
	2160	0.9978	7.77	— 1.052
	2470	0.9978	7.31	— 1.059

Fig. 1. Relationship between log k' and carbon number x 100 for alkyl **aryl** ketones on an Ultrasphere ODS column with different proportions of methanol (**%**, w/w) as modifier in the eluent. ■ = 0%; □ = 4.0%; ● = 8.3%; 0 = 12.7% methanol. Column: Ultrasphere ODS 5-μm, 2470 **p.s.i.**, 60°C.

Retention indices

Using the linear relationship for the alkyl aryl ketones (valerophenone to octanophenone) the retention indices of all the analytes were determined (Table III). As expected, all the alkyl aryl ketones gave retention indices close to their nominal values (carbon number x 100).

The retention indices of the model compounds were often markedly different from those on the PS-DVB column[13].Nitrobenzene was much lower (I = 583 at 2470 p.s.i. and 60°C compared to 946 at 2515 p.s.i. on the PS-DVB column). In contrast, the retention of benzyl alcohol was much larger (Z = 1473 compared to **904**), whereas N-propylaniline (I = 1230 compared to 1123) and benzylamine (I = 2089 compared to 1891) were relatively similar on the two columns. Similar differences in relative retentions between these columns were also found by Morin *et al.*[17]. Compared to acetophenone, nitrobenzene had a much higher capacity factor on PRP- 1 (PS-DVB) column suggesting that there are particularly strong π–π interactions which are absent on an ODS column.

The retention indices of the polar compounds on the ODS-silica column were also much larger in SFC than in RP-HPLC. The retention on an Hypersil ODS column with methanol-water (50:50)[16] can be compared with the SFC separation at 1950 p.s.i. (in parenthesis) for the polar analytes; p-cresol, 807 (1203); benzyl alcohol, 720 (1440); and N-propylaniline, 989 (ref. 19) (13 17). In contrast, the retention index values for the less polar compounds were larger on RP-HPLC than on SFC (in parenthesis): nitrobenzene, 843 (690); methyl benzoate, 909 (809); toluene, 1010 (337). These changes emphasize the different selectivities of SFC and RP-HPLC separations. For homologues the two systems show a similar behaviour of increasing retention with molecular size (as in reversed-phase chromatography), but an almost complete reversal of retention characteristics with regard to the polarity of the analytes. Whereas in RP-HPLC polar compounds are rapidly eluted, in SFC with carbon dioxide the polar compounds are retained and in some cases cannot be eluted. This marked change was also observed in the study of the benzodiazepines and a nearly complete reversal of elution order on similar columns was observed in the two techniques'.

TABLE III

RETENTION INDICES OF ANALYTES BASED ON ALKYL ARYL KETONE STANDARDS

Conditions as in Table I. Based on valerophenone to octadecanophenone scale.

Compound	Retention index			
	Mean column pressure (p.s.i.)			
	1665	1950	2160	2470
Alkyl aryl ketones				
Valerophenone	1109	1107	1109	1100
Hexanophenone	1199	1197	1199	1195
Heptanophenone	1294	1293	1295	1291
Octanophenone	1393	1391	1397	1395
Decanophenone	1602	1615	1582	1613
Dodecanophenone	1798	1806	1812	1809
Tetradecanophenone	2004	2006	2009	2008
Hexadecanophenone	2204	2205	2206	2204
Octadecanophenone	2397	2390	2390	2383
n-Alkanes				
Decane	695	657	673	699
Dodecane	954	921	949	972
Tetradecane	1186	1154	1191	1210
Hexadecane	1405	1377	1410	1427
Octadecane	1613	1580	1617	1630
Eicosane	1816	1777	1812	1821
Docosane	2003	1970	1996	2011
Tetracosane	2191	2160	2191	2195
Alkylbenzenes				
Toluene	355	337	348	246
Ethylbenzene	506	496	501	428
n-Propylbenzene	634	628	643	583
n-Butylbenzene	764	765	784	740
Test compounds				
Nitrobenzene	721	690	666	583
Benzaldehyde	772	795	763	753
Methyl benzoate	809	809	826	803
N-Propylaniline	1116	1317	1196	1230
p-Cresol	1163	1203	1288	1281
Benzyl alcohol	1410	1440	1473	1473
Benzylamine	–	1959	2048	2089

Similar large differences between RP-HPLC and SFC were also reported by Wheeler and McNally", who found that compared to substituted benzenes such as nitrobenzamides, polynuclear aromatic compound had much longer retentions on RP-HPLC, but were rapidly eluted by SFC with 5 or 2% methanol in carbon dioxide.

Sensitivity of retention and retention indices to changes in operating conditions
        To be valuable as a source of robust retention data, the retention indices of analytes should be largely independent of the small variations in operating conditions that can occur between laboratories or between replicate separations on the same

column. The marked changes in capacity factors with mean column pressure (Table I) emphasise the importance of pressure as well as temperature in SFC. Although the latter is reasonably reproducible between laboratories, pressure differences from nominated values are likely to be significant.

In contrast, the retention indices of most of the test compounds showed only small variations with the changes in pressure. Some of the apparent differences at 2470 p.s.i. may be due to the uncertainty involved in the measurement of very small retention times and particular problems will be experienced in the accuracy of the extrapolated values lower than 1100 (valerophenone). Even in these cases the changes are only moderate. More significant changes were noted for nitrobenzene, which decreased, and the polar analytes, p-cresol, N-propylaniline and benzylamine, which increased with increasing pressure.

TABLE IV

EFFECT OF PROPORTION OF METHANOL AS A MODIFIER ON THE CAPACITY FACTORS

Conditions: column, Ultrasphere ODS; eluent, carbon dioxide plus methanol; temperature, 60°C; mean column pressure, 2470 p.s.i.; UV detection, 254 nm.

Compound	Capacity factor			
	Methanol (%,w/w)			
	0	4.0	8.3	12.7
Alkyl aryl ketones				
Valerophenone	0.81	0.45	0.32	0.27
Hexanophenone	0.95	0.51	0.36	0.31
Heptanophenone	1.10	0.62	0.44	0.37
Octanophenone	1.30	0.73	0.51	0.42
Decanophenone	1.84	0.99	0.67	0.56
Dodecanophenone	2.52	1.31	0.88	0.72
Tetradecanophenone	3.47	1.73	1.23	0.92
Hexadecanophenone	4.74	2.26	1.45	1.16
Octadecanophenone	6.32	2.92	1.84	1.45
Alkylbenzenes				
Toluene	0.21	0.24	0.23	0.24
Ethylbenzene	0.28	0.29	0.27	0.29
n-Propylbenzene	0.36	0.34	0.31	—
n-Butylbenzene	0.46	0.42	0.34	0.34
Test compounds				
Nitrobenzene	0.34	0.30	0.23	0.19
Benzaldehyde	0.47	0.23	0.18	0.16
Methyl benzoate	0.51	0.29	0.22	0.20
N-Propylaniline	1.00	0.48	0.36	0.30
p-Cresol	1.08	0.31	0.04	0.09
Benzyl alcohol	1.47	0.24	0.15	0.14
Benzylamine	3.95	1.07	0.69	0.54
Benzoic acid	—	0.55	0.38	0.25
Benzamide	—	0.75	0.24	0.13

Effect of the addition of modifiers to the mobile phase

As noted earlier, for many polar compounds peak shapes and retentions in SFC are greatly altered by the addition of small percentages of an organic modifier to the mobile phase. Some authors have suggested that for packed columns this occurs by a dynamic coating of free silanols on the surface of the stationary phase[8,21] as well as changes in the overall elution strength of the eluent. The capacity factors of the alkyl aryl ketones, alkylbenzenes and test compounds were therefore determined using constant pressure and temperature but with increasing proportions of methanol (Table IV) or acetonitrile (Table V) in the mobile phase. In both cases the retention times decreased markedly on the addition of modifier with acetonitrile showing the greater effect. Even with small proportions of methanol, benzamide and benzoic acid could now be eluted with only slight peak tailing. However, these compounds still gave broad tailing peaks with acetonitrile as modifier and their retentions could not be

TABLE V

EFFECT OF PROPORTION OF ACETONITRILE AS A MODIFIER ON THE CAPACITY FACTORS

Conditions: column, Ultrasphere ODS; eluent, carbon dioxide plus acetonitrile; temperature, 60°C; mean column pressure, 2470 p.s.i.; detection at 254 nm. Benzoic acid and benzamide gave broad peaks and retentions could not be measured.

Compound	Capacity factor			
	Acetonitrile (%,w/w)			
	0.0	3.7	7.1	13.4
Alkyl aryl ketones				
Valerophenone	0.81	0.41	0.26	0.19
Hexanophenone	0.95	0.49	0.31	0.23
Heptanophenone	1.10	0.57	0.37	0.27
Octanophenone	1.30	0.66	0.43	0.32
Decanophenone	1.84	0.90	0.57	0.43
Dodecanophenone	2.52	1.20	0.75	0.56
Tetradecanophenone	3.47	1.59	0.94	0.72
Hexadecanophenone	4.74	2.07	1.23	0.91
Octadecanophenone	6.32	2.67	1.55	1.14
Alkylbenzenes				
Toluene	0.21	0.21	0.21	0.19
Ethylbenzene	0.28	0.25	0.25	0.23
n-Propylbenzene	0.36	0.31	—	—
n-Butylbenzene	0.46	0.38	0.30	0.28
Test compounds				
Nitrobenzene	*0.36*	*0.24*	*0.15*	*0.10*
Benzaldehyde	*0.47*	*0.20*	*0.13*	0.10
Methyl benzoate	0.51	0.26	0.18	0.15
N-Propylaniline	1.00	0.50	0.34	0.28
p-Cresol	1.08	0.36	0.18	0.10
Benzyl alcohol	1.47	0.35	0.19	0.10
Benzylamine	3.95	0.25	0.16	0.13

TABLE VI

EFFECT OF PROPORTION OF METHANOL AS A MODIFIER ON THE RETENTION INDICES

Conditions as in Table IV. Based on valerophenone to octadecanophenone scale.

Compound	Retention index			
	Methanol (%, w/w)			
	0.0	4.0	8.3	12.7
Alkyl aryl ketones				
Valerophenone	1100	1083	1080	1065
Hexanophenone	1195	1171	1163	1185
Heptanophenone	1291	1307	1309	1306
Octanophenone	1395	1414	1420	1412
Decanophenone	1613	1623	1627	1631
Dodecanophenone	1809	1821	1824	1825
Tetradecanophenone	2008	2101	2011	2015
Hexadecanophenone	2204	2196	2194	2194
Octadecanophenone	2383	2374	2370	2367
Alkylbenzenes				
Toluene	246	633	823	958
Ethylbenzene	428	771	955	1110
n-Propylbenzene	583	898	1062	—
n-Butylbenzene	740	1031	1127	1249
Test compounds				
Nitrobenzene	583	809	820	797
Benzaldehyde	753	630	651	639
Methyl benzoate	803	771	793	821
N-Propylaniline	1230	1124	1170	1154
p-Cresol	1281	813	493	193
Benzyl alcohol	1473	656	535	503
Benzylamine	2089	1679	1650	1604
Benzoic acid	—	1214	1200	1015
Benzamide	—	1430	849	473

readily measured. The polar hydroxyl-containing analytes gave good peak shapes with both modifiers.

There was again a linear relationship between carbon number and log k' for the alkyl aryl ketones (Fig. 1). The reduced methylene selectivity indicated by the flatter curves with increasing organic modifier content was also noted by Yonker and co-workers[22,23] in studies of alkyl aryl ketones using a capillary column with carbon dioxide containing 2-propanol as a modifier.

Using these results the corresponding retention indices were calculated for the methanol (Table VI) and acetonitrile eluents (Table VII). With this column the retention indices of the alkylbenzenes increased markedly with both modifiers (Figs. 2 and 3). A similar but small shift to higher retention indices was also seen on the PS-DVB column with increased modifier. The retention indices of most of the less polar model compounds, methyl benzoate, nitrobenzene and benzaldehyde, increased slightly or remained relatively constant. At the higher proportions of modifier many of

TABLE VII

EFFECT OF PROPORTION OF ACETONITRILE AS A MODIFIER ON THE RETENTION INDICES

Conditions as in Table V. Based on valerophenone to octadecanophenone.

Compound	Retention index			
	Acetonitrile (%,w/w)			
	0.0	3.7	7.1	13.4
Alkyl aryl ketones				
Valerophenone	1100	1075	1056	1046
Hexanophenone	1195	1192	1187	1179
Heptanophenone	1291	1304	1307	1310
Octanophenone	1395	1405	1421	1424
Decanophenone	1613	1620	1633	1641
Dodecanophenone	1809	1820	1831	1838
Tetradecanophenone	2004	2013	2000	2018
Hexadecanophenone	2204	2197	2196	2190
Octadecanophenone	2383	2374	2369	2355
Alkylbenzenes				
Toluene	*246*	*592*	*906*	*1023*
Ethylbenzene	*438*	*730*	*1033*	*1185*
n-Propylbenzene	*583*	*880*	—	—
n-Butylbenzene	*740*	*1012*	*1163*	*1313*
Test compounds				
Nitrobenzene	*583*	*693*	*634*	601
Benzaldehyde	*753*	*582*	*561*	*573*
Methyl benzoate	*803*	*765*	*587*	*845*
N-Propylaniline	*1230*	*1209*	*1249*	*1326*
p-Cresol	*1281*	*989*	*771*	*594*
Benzyl alcohol	*1473*	*962*	*807*	*587*
Benzylamine	*2089*	*726*	*710*	*749*

the retention times are very small and measurement and calculation uncertainties from 20 to 50 units are estimated to be present in these results, particularly for compounds with short retention times or extrapolated indices.

Of the two basic compounds the retention index of N-propylaniline remained virtually constant, in contrast to benzylamine which suffered an initial large change but remained constant upon further addition. These results suggest that the more basic aliphatic amine group was interacting with free silanol groups on the surface of the silica and that this effect was removed by the initial addition of methanol. Smaller proportions may have the same effect but this could not be tested in the present equipment. This observation agrees with work carried out elsewhere[24]. The subsequent lack of change on increased addition of methanol agrees with the study on the PS-DVB column (which lacks the silanol effect) where the retention indices of both amines were virtually unaltered by the addition of modifier[13].

The retention indices of the other polar compounds, p-cresol, benzyl alcohol, benzamide, and benzoic acid, changed considerably with increasing modifier content.
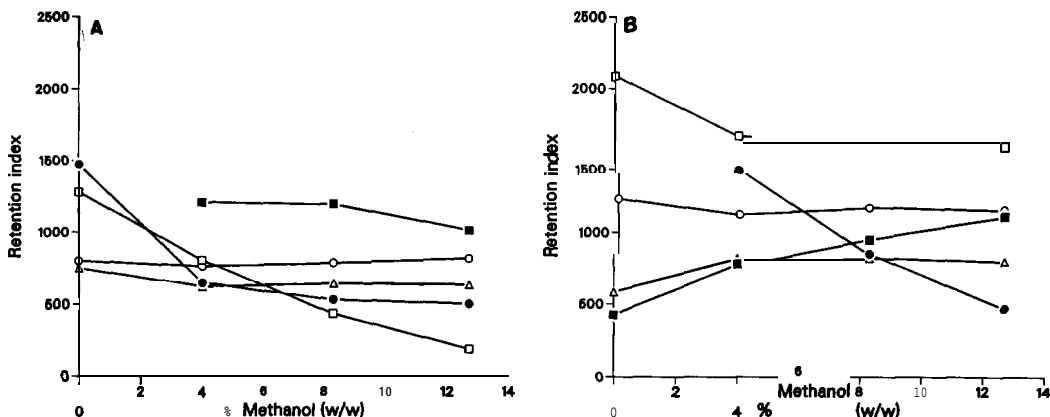
Fig. 2. Effect of the addition of methanol as a modifier on the retention indices (based on alkyl aryl ketones) of test compounds. Conditions: Ultrasphere ODS column; temperature, 60°C; mean pressure, 2470 p.s.i. Compounds: (A): ■ = benzoic acid; □ = pcresol; ● = benzyl alcohol; 0 = methyl benzoate; △ = benzaldehyde. (B): ■ = ethylbenzene; □ = benzylamine; ● = benzamide; 0 = N-propylaniline; A = nitrobenzene.

p-Cresol changed from 1281 to 193 units in methanol. Large changes for these compounds were also noted on the PS-DVB column[13]. These conclusions suggest that these compounds are not interacting strongly with the column materials, but are interacting poorly with the mobile phase and have a low solubility, which is considerably increased by the addition of modifier. The peak shapes also improved considerably with the addition of modifier. Corresponding changes have been noticed by other workers with polar amide pesticides[20] and in our earlier work with benzodiazepines[9] and barbiturates[15].

Except for the silanol interaction of the basic amines, these changes with modifier proportion do not appear to be primarily related to the stationary phase as
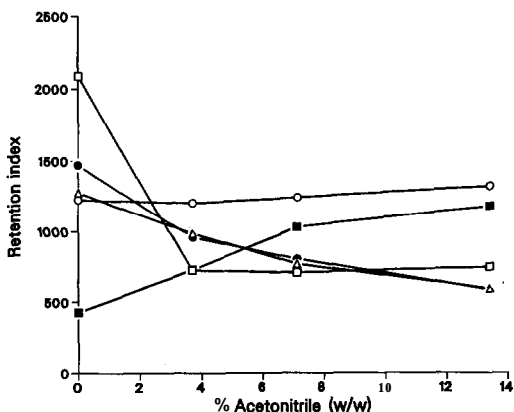

Fig. 3. Effect on the addition of acetonitrile as a modifier on the retention indices (based on alkyl aryl ketones) of test compounds. Conditions as in Fig. 1. Compounds: □ = benzylamine; 0 = N-propyl-aniline; ● = benzyl alcohol; ■ = ethylbenzene; A = p-cresol.

they are similar on both PS-DVB and ODS-silica columns. The changes almost suggest that as the proportion of modifier is increased, the mobile phase is in transition from an SFC- to an HPLC-type system in which low retentions would be expected for the amide, alcohols and acids, although the comparison with the retention indices in methanol-water given earlier suggests an even greater contrast.

CONCLUSIONS

The alkyl aryl ketones can be used as retention index standards for SFC on ODS-columns and can compensate for small changes in the pressure. They can also be used as a comparison scale to study the marked changes in selectivities with the proportion of organic modifiers. Basic **amines** seem to suffer interaction with silanol groups on the stationary phases but aromatic **amines** are unaffected. The influence of silanols is reduced and the elution of polar compounds is improved by the addition of modifiers to the mobile phase.

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REFERENCES

1 R. M. Smith (Editor), **Supercritical Fluid Chromatography (RSC Chromatography Monographs),** Royal Society of Chemistry, London, 1988.
2 B. A. Carpentier and M. R. Sevenants (Editors), **Supercritical Fluid Extraction and Chromatography: Techniques and Applications (ACS Symposium Series, No.** 366), American Chemical Society, Washington, DC, 1988.
3 C. M. White (Editor), **Modern Supercritical Fluid Chromatography,** Hiithig, Heidelberg, 1988.
4 D. E. Knowles, B. E. Richter, M. B. Wygant, L. Nixon and M. R. Anderson, *J. Assoc. Off. Anal.* Chem., 71 (1988) 451.
5 P. J. Schoenmakers and F. C. C. J. G. Verhoeven, *J. Chromatogr., 352 (1986) 315.*
6 A. L. Blilie and T. Greibrokk, **Anal. Chem., 57 (1985) 2239.**
7 J. M. Levy and W. M. Ritchey, **J. High Resolut. Chromatogr. Chromatogr. Commun.,** 8 (1985) 503.
8 B. W. Wright and R. D. Smith, *J. Chromatogr., 355 (1986) 367.*
9 R. M. Smith and M. M. Sanagi, *J. Chromatogr., 483 (1989) 51.*
10 S. Schmidt, L. G. Blomberg and E. R. Campbell, **Chromatographia, 25 (1988) 775.**
11 M. E. P. McNally and J. R. Wheeler, *J. Chromatogr., 447 (1988) 53.*
12 R. M. Smith, **Adv. Chromatogr., 26 (1987) 277.**
13 R. M. Smith and M. M. Sanagi, **Chromatographia, 26 (1988) 77.**
14 R. M. Smith and M. M. Sanagi, *J. Pharm. Biomed. Anal., 6 (1988) 837.*
15 R. M. Smith and M. M. Sanagi, *J. Chromatogr., 481 (1989) 63.*
16 R. M. Smith, *J. Chromatogr., 236 (1982) 313.*
17 Ph. Morin, M. Caude and R. Rosset, *J. Chromatogr., 407 (1987) 87.*
18 B. W. Wright, H. T. Kalinoski and R. D. Smith, **Anal.** Chem., 24 (1987) 304.
19 R. M. Smith, *J. Chromatogr., 351 (1986) 259.*
20 J. R. Wheeler and M. E. McNally, **Fresenius' Z. Anal. Chem.,** 330 (1988) 237.
21 P. J. Schoenmakers, L. G. M. Uunk and P. K. de Bokx, *J.* Chromatogr., 459 (1988) 201.
22 C. R. Yonker and R. D. Smith, **Anal. Chem.,** 59 (1987) 727.
23 C. R. Yonker, D. G. McMinn, B. W. Wright and R. D. Smith, *J. Chromatogr., 396 (1987) 19.*
24 M. Ashraf-Khorassani and L. T. Taylor, in C. M. White (Editor), **Modern Supercritical Fluid Chromatography,** Hiithig, Heidelberg, 1988, p. 115.