COMPARISON OF THE SELECTIVITY OF CYANO-BONDED SILICA STATIONARY PHASES IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

The selectivities of three brands of cyano-bonded silica stationary phase (CPS-Hypersil, Spherisorb-CN and Ultrasphere cyano) have been compared for a series of test compounds using a range of methanol–phosphate buffer (pH 7.0) and acetonitrile–phosphate buffer (pH 7.0) eluents. The retentions were expressed as retention indices using the alkyl aryl ketone scale. Considerable differences were found on elution with the different eluents and between the different brands of stationary phases. The retentions were also compared to separations on ODS-Hypersil.

INTRODUCTION

The majority of separations carried out by reversed-phase high-performance liquid chromatography (HPLC) use an octadecylsilyl-(ODS), or other alkyl-bonded silica stationary phase. However, all these materials have relatively similar selectivity properties although there are differences in the overall retentive capacity between phases with different alkyl chain lengths. There are also often small differences in relative retentions on different brands of nominally equivalent packing materials. As a consequence, because changing brands or the length of the carbon chain will have relatively little effect on selectivity, almost all optimisation strategies in liquid chromatography concentrate on the influence of changing the pH or organic modifier in the mobile phase. The only exceptions are with analytes, such as basic drugs, which are partially retained by specific interactions with residual silanol groups on the surface of the silica. These interactions can vary considerably between brands and chain length, because of differences in the chemistry of the bonding reaction and of the protection afforded to the column surface.

However, other column materials are available for reversed-phase chromatography, which have different selectivity properties so that analytes containing different functional groups will show different relative retentions and thus potentially could improve or alter the separation of the components of a mixture. These stationary

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phases are starting to attract considerable interest and include cyano- and amino-bonded silicas[1] and polymeric materials, such as polystyrene–divinylbenzene[2] and the very specific chiral phases. The cyano-bonded columns are of particular interest as it is reported that they can be used for both normal- and reversed-phase separations[3]. Much work has been carried out by De Smet *et al.*[4] who have suggested that a single cyano-bonded column used with eluents prepared from a limited group of mobile phases, dichloromethane, tetrahydrofuran (THF), acetonitrile, *n*-hexane, and water, can separate a wide range of drug compounds. They have subsequently examined in detail the separations using methanol–buffer eluents[5]. The effects of eluent pH, ionic strength and proportion of organic modifiers on the separation of a series of acidic, neutral, and basic drugs were examined using a LiChrosorb CN column. In most cases the behaviour of the drugs to these parameters closely resembled effects observed on ODS-bonded columns although the selectivities differed. Other workers have suggested that in reversed-phase chromatography the primary retention of cyanopropyl-bonded columns is similar to a short chain alkyl-bonded silica[1]. In their studies Massart and his co-workers initially used a Micropak-CN column[3] but subsequently changed to a LiChrosorb CN column[4], which was reported to have better selectivity.

The retention properties in reversed-phase chromatography of individual cyano-bonded columns have been examined previously and compared with other types of stationary phases. Cooper and Lin[6] studied the retention of the simple test compounds phenol, aniline and nitrobenzene relative to toluene. They demonstrated an increase in stationary phase polarity in the order octyl-, phenyl-, to cyano-bonded silica. In a similar study Moats and Leskinen[7] compared cyano-bonded silica, polymeric and silica columns over a wide composition range of aqueous acetonitrile eluents for the separation of penicillins. Pietrogrande *et al.*[8] have compared the separation of benzodiazepines on octadecyl-, phenyl- and cyano-bonded silica and have related the capacity factors to octanol–water partition coefficients. Zorbax-CN has been compared with alkyl- and phenyl-bonded Zorbax phases for the separation of phenylthiohydantoin amino acid derivatives[9] and a range of retention test compounds[10].

However, each of these studies has only examined a single brand of cyano-bonded column. As noted earlier differences can be found between different brands of ODS-bonded silica and in previous studies from these laboratories it has been possible to make direct comparisons between phases by using a set of column test compounds[11,12]. The retentions of the test compounds were determined relative to the alkyl aryl ketone retention index scale[13,14] in a similar manner to the methods of McReynolds and Rohrschneider for the comparison of stationary phases in gas–liquid chromatography. This approach has the advantage that because the retentions are calculated by interpolation between the retention index standards, the values are insensitive to differences in the overall retention capacity of the column or to small changes in the experimental conditions.

In the present study the retentions of the standard test compounds have been used to compare three different brands of cyano-bonded silica using methanol–buffer and acetonitrile–buffer as eluents. The results have also been compared with the selectivity on ODS-Hypersil in the same eluents.

EXPERIMENTAL

Chemicals and reagents

Standard alkyl aryl ketones (acetophenone, propiophenone, butyrophenone, and valerophenone) and column test compounds, toluene, nitrobenzene, N-methylaniline, methyl benzoate, 2-phenylethanol, and *p*-cresol were laboratory grade from a number of suppliers. Methanol and acetonitrile were HPLC grade and disodium hydrogenphosphate, and sodium dihydrogenphosphate for buffers were analytical reagent grade from FSA Laboratory Suppliers (Loughborough, U.K.).

High-performance liquid chromatography

HPLC separations were carried out using a Kontron LC 410 pump and a Pye-Unicam PU 4020 variable-wavelength detector set at 254 nm. The samples (10 μl) were injected, using a Rheodyne 7125 valve with a 20-μl loop, onto the column which was surrounded by jacket through which was circulated water at 30°C from a thermostated bath. The peaks were recorded using a Spectra-Physics 4270 integrator.

The cyano-bonded columns were CPS-Hypersil 5 μm (Batch No. 12/1423, Shandon Southern, Runcorn, U.K.) (100 × 5 mm I.D.), Spherisorb-CN 3 μm (Batch 19/205, Phase-Separations, Queensferry, U.K.) (150 × 5 mm I.D.) and Ultrasphere cyano 5 μm (Batch 3UEC122N, Beckman) (250 × 4.6 mm I.D.).

Methods

The analytes as dilute solutions in methanol–water were separated using methanol–pH 7 buffer or acetonitrile–pH 7 buffer in different proportions as the mobile phase. The pH 7.0 buffer was prepared using disodium hydrogenphosphate (0.6850 g) and sodium dihydrogenphosphate (0.7882 g) in water (500 ml). A sample of 10% aqueous sodium nitrate (5 μl) was used to determine the column void volume.

The capacity factors were calculated from the mean retention time of triplicate injections. The retention indices were calculated from log k' (capacity factor) of the analytes as reported earlier[13] by comparison with the linear least squares relationship between log k' of the alkyl aryl ketones and their carbon number × 100.

RESULTS AND DISCUSSION

The retention times of the alkyl aryl ketones and column test compounds, (nitrobenzene, toluene, 2-phenylethanol, *p*-cresol, and N-methylaniline, and methyl benzoate) were measured on each of three cyano-bonded columns (CPS-Hypersil, Ultrasphere cyano, and Spherisorb-CN) using 10–40% methanol–pH 7.0 buffer and 10–30% acetonitrile–pH 7.0 buffer as eluents. The first four column test compounds had been selected previously to be representative of the different interactions that can occur on a column[8]. N-Methylaniline was included to test for interactions with acidic uncapped silanols on the silica surface. Methyl benzoate has previously been found to have a retention index value on ODS-silica columns that was virtually independent of the brand of stationary phase and of the eluent composition over a wide range and can be used as a secondary standard. Buffered eluents were used to ensure that the effects of the underlying silanols on the silica surface and the degree of ionisation of *p*-cresol and N-methylaniline would be constant throughout the study. The peak shapes on all

TABLE I

CAPACITY FACTORS OF ALKYL ARYL KETONES AND TEST COMPOUNDS ON CPS-HYPERSIL COLUMN

Methanol–phosphate buffer (pH 7.0) and acetonitrile–phosphate buffer (pH 7.0) are used as mobile phases.

Compound	Capacity factors						
	% Methanol				% Acetonitrile		
	10	20	30	40	10	20	30
Retention index standards							
Acetophenone	2.57	1.80	1.19	0.81	2.28	1.74	1.31
Propiophenone	4.56	2.95	1.80	1.19	3.99	2.90	1.69
Butyrophenone	8.34	5.00	2.85	1.74	7.27	4.86	2.47
Valerophenone	17.7	9.54	4.87	2.72	14.74	8.84	3.74
Test compounds							
2-Phenylethanol	1.59	1.19	0.95	0.66	1.62	1.28	0.79
N-Methylaniline	1.74	1.37	1.06	0.80	2.03	1.77	1.27
p-Cresol	2.11	1.70	1.20	0.86	2.20	1.74	1.08
Nitrobenzene	2.55	2.05	1.61	1.20	2.86	2.53	1.66
Toluene	3.51	2.74	2.04	1.46	4.04	3.68	2.14
Methyl benzoate	4.05	2.77	1.83	1.22	3.75	2.76	1.54
Void volume (min)	2.03	2.07	2.10	2.12	1.94	1.92	1.98

TABLE II

CAPACITY FACTORS OF ALKYL ARYL KETONES AND TEST COMPOUNDS ON SPHERI-SORB-CN COLUMN

Methanol–phosphate buffer (pH 7.0) and acetonitrile–phosphate buffer (pH 7.0) are used as mobile phases.

Compound	Capacity factors						
	% Methanol				% Acetonitrile		
	10	20	30	40	10	20	30
Retention index standards							
Acetophenone	2.20	1.32	0.87	0.66	1.33	0.92	0.70
Propiophenone	3.11	1.87	1.13	0.77	1.89	1.21	0.89
Butyrophenone	4.98	2.69	1.49	0.91	2.68	1.60	1.12
Valerophenone	8.10	3.98	2.02	1.10	4.02	2.17	1.44
Test compounds							
2-Phenylethanol	0.85	0.43	0.45	0.45	0.76	0.60	0.49
N-Methylaniline	1.21	0.88	0.64	0.64	1.08	0.87	0.71
p-Cresol	0.80	0.61	0.45	0.44	0.77	0.62	0.51
Nitrobenzene	1.83	1.29	0.90	0.73	1.32	0.99	0.77
Toluene	1.39	1.15	0.84	0.70	1.39	1.14	0.94
Methyl benzoate	2.01	1.39	0.93	0.71	1.50	1.07	0.78
Void volume (min)	2.37	2.44	2.42	2.33	2.40	2.30	2.31

TABLE III

CAPACITY FACTORS OF ALKYL ARYL KETONES AND TEST COMPOUNDS ON ULTRA-SPHERE CYANO COLUMN

Methanol–phosphate buffer (pH 7.0) and acetonitrile–phosphate buffer (pH 7.0) are used as mobile phases.

Compound	Capacity factors						
	% Methanol				% Acetonitrile		
	10	20	30	40	10	20	30
Retention index standard							
Acetophenone	2.99	1.65	1.03	0.76	1.50	1.10	0.82
Propiophenone	5.09	2.47	1.35	0.95	2.23	1.55	1.07
Butyrophenone	8.66	3.71	1.84	1.18	3.33	2.24	1.40
Valerophenone	–	5.75	2.58	1.53	5.26	3.18	1.85
Test compounds							
2-Phenylethanol	1.24	0.84	0.63	0.51	0.87	0.76	0.60
N-Methylaniline	1.55	1.00	0.76	0.62	1.10	1.02	0.84
p-Cresol	1.33	0.92	0.62	0.52	0.94	0.83	0.67
Nitrobenzene	2.48	1.53	1.03	0.81	1.42	1.26	0.99
Toluene	2.17	1.50	1.02	0.82	1.58	1.53	1.23
Methyl benzoate	3.57	2.06	1.24	0.86	1.90	1.23	1.00
Void volume (min)	2.69	2.72	2.66	2.71	2.61	2.60	2.59

three columns were symmetrical and suggested that silanol interactions were minimal. This would be expected as at pH 7.0, N-methylaniline should not be protonated and thus would behave as a neutral analyte. In most cases the column efficiencies were significantly higher in the acetonitrile–buffer eluents than the methanol–buffer eluents [*i.e.*, nitrobenzene on CPS-Hypersil $N = 1950$ with methanol–buffer (10:90) and $N = 3400$ with acetonitrile–buffer (10:90)].

Using the retention times and the column void volumes measured with an aqueous sodium nitrate solution the capacity factors on the three columns were calculated (Tables I–III). The capacity factors varied considerably, for example valerophenone on elution with methanol–buffer (20:80) had capacity factors of 3.98, 5.75, and 9.54 on the different columns. These differences confirmed the difficulty of making direct comparisons between columns using just capacity factors. Overall, the k' values are much shorter than those for the same analytes on ODS columns and are even shorter than those measured previously on the short alkyl-bonded SAS-Hypersil column [with methanol–water (30:70) as the eluent: butyrophenone, $k' = 20.6$ and nitrobenzene, $k' = 4.93$)[13]].

In each case there was a linear relationship between the logarithm of the capacity factors of the alkyl aryl ketones and their carbon numbers (log k' = carbon number ketones × slope + constant) (Table IV) with correlations comparable to the results from studies on ODS columns[13,15]. In their study, De Smet and Massart[5] found that over all their drug compounds, there was a reasonable relationship between size, expressed as carbon number, and retention on the LiChrosorb CN column, although in methanol–buffer (30:70) all the drug compounds with fewer than 10 carbon atoms were eluted with k' values less than 1.

TABLE IV

CORRELATION BETWEEN CARBON NUMBER OF ALKYL ARYL KETONES AND LOG k' DIFFERENT CYANO-BONDED SILICA COLUMNS

Compound	% Methanol				% Acetonitrile		
	10	20	30	40	10	20	30
CPS-Hypersil							
Correlation	0.998	0.998	0.998	0.999	0.999	0.999	0.999
Slope × 10³	2.8	2.4	2.0	1.7	2.7	2.3	1.7
Spherisorb-CN							
Correlation	0.999	0.999	0.999	0.998	0.999	0.999	0.999
Slope × 10³	2.1	1.6	1.2	0.74	1.6	1.2	1.0
Ultrasphere cyano							
Correlation	0.999	0.999	0.998	0.998	0.999	0.999	0.999
Slope × 10³	2.3	1.8	1.3	1.0	1.8	1.5	1.2

Using the linear relationships for the ketones on the present columns the retention indices of the column test compounds were calculated (Table V). In each case the change in retention indices with composition was much smaller than the differences in capacity factors. However, particularly with the eluents containing the higher proportions of organic modifier all the retention times were very short ($k' < 1.5$) and the slopes of the curves for the ketones standards were very flat [methanol–water (30:70) eluent: CPS-Hypersil, slope = $2.00 \cdot 10^{-3}$; Spherisorb-CN, slope $1.2 \cdot 10^{-3}$ and Ultrasphere cyano, slope $1.3 \cdot 10^{-3}$]. These contrast with the much higher methylene selectivity in the same eluent on the SAS-Hypersil column (slope $3.35 \cdot 10^{-3}$)[13]. On the cyano-bonded columns the capacity factors of the test compounds in these eluents were also low so that even very small differences in retention times would cause significant variations in retention indices (I). This was reflected in a poor repeatability of retention indices based on individual separations, for example successive individual injections of nitrobenzene [in methanol–water (40:60), average $k' = 0.81$] gave I values of 802, 840 and 854 on the Ultrasphere cyano column. Differences in the I values of up to 30 units are therefore probably not significant for analytes with these low retentions, although normally retention indices can be determined with a precision of better than 5 units (standard deviation) even over a prolonged period[16]. There are noticeable differences in the selectivities of the separations with the two organic modifiers and in particular N-methylaniline and toluene were relatively more highly retained with the acetonitrile containing eluents.

The separations on two of the columns, Spherisorb-CN and Ultrasphere cyano, were relatively similar with differences in the retention indices in most cases of less than 30 units. However, the CPS-Hypersil column was markedly different and the retention indices with this column, particularily for p-cresol and 2-phenylethanol, differed by over 100 units from the other two brands. However, according to the manufacturers all three columns have similar carbon loadings (3.5, 3.9 and 4.4%) and have been prepared from monofunctional cyanopropylsilane reagents. The differences between brands were larger than observed earlier for the same test compounds on different

TABLE V

RETENTION INDICES OF TEST COMPOUNDS ON THREE CYANO-BONDED STATIONARY PHASES AND COMPARISON WITH ALKYL-BONDED HYPERSIL COLUMNS

Methanol–phosphate buffer (pH 7.0) and acetonitrile–phosphate buffer (pH 7.0) are used as mobile phases.

Compound	Retention indices						
	% Methanol				% Acetonitrile		
	10	20	30	40	10	20	30
CPS-Hypersil							
2-Phenylethanol	730	732	746	752	750	746	709
N-Methylaniline	744	757	782	800	787	807	830
p-Cresol	774	795	808	817	800	804	789
Nitrobenzene	804	830	871	900	842	873	898
Toluene	854	882	921	950	898	942	961
Methyl benzoate	877	884	899	905	886	889	877
Spherisorb-CN							
2-Phenylethanol	616	499	574	579	651	653	651
N-Methylaniline	692	693	698	782	748	781	807
p-Cresol	604	596	574	562	654	663	662
Nitrobenzene	782	797	817	864	801	829	841
Toluene	723	765	793	843	815	877	921
Methyl benzoate	802	817	826	850	836	857	846
Ultrasphere cyano							
2-Phenylethanol	635	640	647	632	672	699	688
N-Methylaniline	678	683	706	714	729	779	809
p-Cresol	649	661	642	644	692	722	726
Nitrobenzene	766	785	807	833	790	839	869
Toluene	741	779	804	835	816	895	953
Methyl benzoate	834	855	869	856	860	834	873
ODS-Hypersil[15,a]							
2-Phenylethanol			773	778	752	735	713
N-Methylaniline			776	787	783	799	828
p-Cresol			797	800	790	784	776
Nitrobenzene			813	829	820	842	869
Toluene			957	985			996
Methyl benzoate			901	916	885	882	886
SAS-Hypersil[13]							
2-Phenylethanol			747				
p-Cresol			779				
Nitrobenzene			816				
Toluene			892				
Methyl benzoate			895				

[a] Values are for methanol–phosphate buffer (pH 8.5)[17] except for methyl benzoate, methanol–water[13].

brands of ODS-silicas. Particularly noticeable in the present study was the variation in the retention index value of methyl benzoate, which ranged from $I = 846$ to 877 with acetonitrile–buffer (30:70). In contrast, on six different brands of ODS-bonded silicas it had only varied between $I = 881$ to 891 with acetonitrile–water (50:50)[8].

The changes in the retention indices of the column test compounds with eluent composition also differed on the different brands (Table V). With increasing proportions of methanol the retention indices of all the column test compounds increased on the CPS-Hypersil column, whereas on the Spherisorb-CN column the indices of *p*-cresol and 2-phenylethanol decreased but those of the other test compounds increased. On the Ultrasphere cyano column the retention indices of compounds containing hydroxyl groups were relatively constant (Fig. 1).

With acetonitrile containing eluents, *p*-cresol and 2-phenylethanol decreased markedly with increasing modifier on the CPS-Hypersil columns but showed only small changes on the other two columns. The other test compounds, toluene, nitrobenzene, N-methylaniline and methyl benzoate all increased on all three columns.
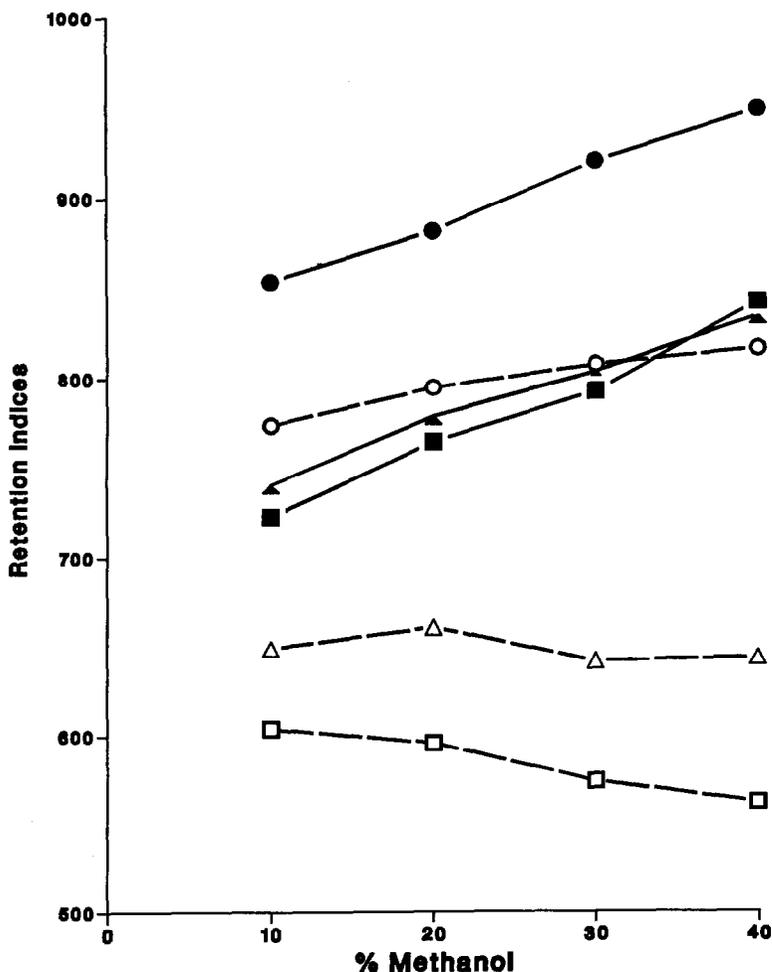
Fig. 1. Comparison of the changes in the retention index with methanol percentage on three cyano-bonded silica columns for toluene (solid symbols) and *p*-cresol (open symbols). Columns; ● and ○, CPS-Hypersil; ■ and □, Spherisorb-CN; ▲ and △, Ultrasphere cyano.

Again there is a marked difference in the I values on the CPS-Hypersil column, which were higher than the other two columns.

The retention indices can also be compared with the retention indices of these test compounds on ODS-Hypersil and SAS-Hypersil measured in earlier studies in these laboratories using the same or closely similar mobile phase[13,15,17] (Table V). As expected there were often differences between the selectivity of the bonded phases suggesting that changing columns could offer considerable optimisation potential. However, in methanol–water (30:70) the separation on the CPS-Hypersil column was quite similar to that on the SAS-Hypersil column (for example on CPS- and ODS-Hypersil: 2-phenylethanol, $I = 746$ and 747, respectively; p-cresol, $I = 808$ and 779; and toluene, $I = 921$ and 892) whereas the retention indices on the other two cyano columns were very different (Spherisorb-CN and Ultrasphere cyano: 2-phenylethanol, $I = 574$ and 647, respectively; p-cresol, $I = 574$ and 642, toluene, $I = 793$ and 804). This suggested that a specific cyano interaction with the analytes might not be significant on the CPS-Hypersil column. This lack of an interaction agrees with the comments that cyano-bonded columns in reversed-phase can effectively act as short alkyl-silanes[1] and the finding that in normal phase separations a cyano-bonded column usually behaves as a weakly retentive silica column with few specific interactions[18].

However, the results from the Spherisorb-CN and Ultrasphere cyano columns suggest that the differences between columns may reflect marked variations in interactions and possibly specific cyano effects. This would agree with other workers who have suggested that more specific interactions can take place if the length of the alkyl chain carrying the cyano group enables it to screen residual silanols on the surface of the silica[19].

CONCLUSION

The selectivity of different brands of cyano-bonded silica varies considerably, which may make the transfer of methods between brands difficult. The effects of changing the organic modifier or its proportion in the mobile phase also causes different effects on the relative retention with the different brands.

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