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Alkylbenzenes as a retention-index scale in reversed-phase high-performance liquid chromatography

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Abstract

Retention in high-performance liquid chromatography is very susceptible to small changes in the stationary- and mobile-phase composition and in the operating conditions. One of the methods to minimise these effects is by using a retention index. In this study, alkylbenzene homologs from toluene to decylbenzene have been used as a retention-index scale in reversed-phase high-performance liquid chromatography. Three different stationary phases, octylsilyl(RP-8)-silica, octadecylsilyl(ODS)-silica and PLRP-S poly(styrene–divinylbenzene) (PS-DVB) polymer have been used with aqueous methanol and acetonitrile as the liquid phase. The application of this retention-index scale was studied using a number of model compounds of different polarities. The alkylbenzenes are readily detected with a UV detector and give linear relationships between $\log k'$ and the number of carbon atoms. On RP-8, ODS, and PLRP-S columns, a retention scale based on alkylbenzenes showed poor coverage of the less retained polar compounds but it can potentially be used as an alternative method for measuring the retention of relatively non-polar compounds.

1. Introduction

Retention in HPLC is susceptible to changes in stationary- and mobile-phase composition as well as in the operating conditions. The capacity factor, k' , has been used to express retention and it eliminates effects due to differences in eluent flow-rate and column size. However, it is still sensitive to small changes in mobile-phase composition and it is difficult to obtain inter-laboratory reproducibility even when using the same column material. For interlaboratory or intralaboratory comparison of chromatographic systems, it is therefore necessary to have a means of expressing retentions that is largely independent of column efficiency and operating con-

ditions such as variation in solvent composition, temperature and flow-rate.

To record chromatographic retention, a number of retention-index methods have been developed based on comparison of the retention of the analyte retention with that of a series of standards. A retention-index scale in reversed-phase HPLC based on alkyl-arylketones has been suggested by Smith [1]. This method has been successfully applied to the analysis of pharmaceuticals using reversed-phase HPLC [2] and packed-column supercritical fluid chromatography [3]. Bogusz and Aderjan [4] reported an improved retention-index scale performance using 1-nitroalkanes where the compounds cover the retention range for the majority of toxicologically relevant substances. Retention indices used in re-

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versed-phase HPLC including *n*-alkanes, alkylbenzenes, alkan-2-ones, esters and polycyclic aromatic hydrocarbons have been compared and reviewed [5–8].

The selection of a retention-index scale in HPLC is based on a number of factors [2]: the compounds should have strong chromophores, should be inert, not readily ionized, readily available, and they should elute with retention times similar to those of the analytes of interest. In this study, a retention-index scale based on alkylbenzenes has been investigated for reversed-phase HPLC using different mobile-phase systems. Alkylbenzenes are stable compounds that are readily commercially available. They have a high UV absorbance at a wavelength of 254 nm, and thus are easily detected by a UV detector and favourable for HPLC analysis.

A number of reports have described the use of alkylbenzenes as standards for the characterization of selectivity by Jandera [9,10] but the retention indices were not calculated. Using thin-layer chromatography, Bruggeman et al. [11] determined the retention indices of polyaromatic hydrocarbons compared to *n*-alkylbenzenes. These workers suggested that this retention method could also be used in HPLC. Apart from the study by Bruggeman et al. [11], little work on the use of alkylbenzenes as a retention-index scale in HPLC has been reported.

The present paper investigates the use of alkylbenzenes as a retention-index scale in reversed-phase HPLC using octylsilyl(PR-8)-silica, octadecylsilyl(ODS)-silica, and PLRP-S poly(styrene-divinylbenzene) (PS-DVB) stationary phases with aqueous methanol and acetonitrile mobile phases to determine the effect of the organic component of the eluent on the selectivity of the separation and the linearity of the retention characteristics. Test compounds having different functional groups were used in order to examine the diversity of the retention-index scale.

2. Experimental

2.1. Chemicals

The alkylbenzenes used were from a Polysci-

ence kit (Polyscience Corporation, IL, USA) and the test compounds were laboratory grade from a range of suppliers. Methanol and acetonitrile were HPLC grade from Merck (Darmstadt, Germany). Double distilled water was obtained from the laboratory and the solvents were filtered through a 0.45- μ m polypropylene membrane before use.

2.2. Equipment

The samples were separated on three different columns, i.e. a 250 \times 4.6 mm I.D. column packed with LiChrosorb RP-8 10 μ m particles (Merck, Darmstadt, Germany), a 250 \times 4.6 mm I.D. Shim-pack ODS 5 μ m column (Shimadzu, Kyoto, Japan) or a 150 \times 4.6 mm I.D. column packed with PLRP-S poly(styrene-divinylbenzene) 5 μ m particles (Polymer Laboratory, Church Stretton, UK). A Shimadzu LC-6A pump (constant-flow mode) was used and the analytes were detected at 254 nm using either a Shimadzu SPD-6A UV detector or a GOW-MAC UV detector (Madison, NJ, USA). Peaks were recorded using a Waters 746 Data Module (Millipore, USA). Aliquots (10 μ l) of the samples were injected using a Rheodyne 7125 injection valve with a 20- μ l sample loop. The column temperature was maintained at 30°C using a water jacket.

2.3. Standard test mixtures

Alkylbenzene standard solutions were made up using 20 μ l of each standard in 5 ml methanol and diluted twice in methanol–water (50:50, v/v) before use. The test compounds were examined at 5–10 μ l in 5 ml methanol. A solution of sodium nitrate (0.15 g in 25 ml of methanol–water, 80:20) was used as the column void-volume marker.

2.4. Procedure

Samples of alkylbenzenes and test compounds in methanol were injected in triplicate onto the column. The samples were eluted using methanol–water or acetonitrile–water mixtures with different compositions at a flow-rate of 1

ml min⁻¹ and detected using a sensitivity between 0.08 and 0.04 AUFS. Capacity factors were calculated as $k' = (t_R - t_o)/t_o$ where t_R is the retention time of triplicate runs and t_o is the column void-volume.

Retention indices of the compounds based on alkylbenzenes were calculated by plotting $\log k'$ against carbon number $\times 100$ for the standards to a linear correlation using a least squares routine and then interpolating the $\log k'$ values of each of the compounds.

3. Results and discussion

3.1. Retention on the RP-8 stationary phase

The retention behaviour of the test compounds was examined using a RP-8 column. The log of the capacity factor of members of homologous

series possesses a simple relationship to the carbon number: $\log k' = aC_n + b$. Using varying compositions of the methanol–water mixture, the k' values of alkylbenzenes and the test compounds were calculated (Table 1). From the k' values of alkylbenzenes, a retention-index scale was derived based on a plot of $\log k'$ against the number of carbon atoms $\times 100$ (toluene $I = 700$) (Fig. 1). The homologs show a close correlation between $\log k'$ and carbon number $\times 100$. The slope and intercept of the curves changed with the percentage of methanol in the solvent and correlations, r , of better than 0.9991 were obtained. From the regression equations, the retention-index values of the compounds were determined.

The capacity factors of the test compounds decreased markedly with increasing percentage of methanol (Table 1). In contrast, the retention-index values of the compounds were largely

Table 1
Capacity factors and retention indices of alkylbenzenes and test compounds on an RP-8 column with different methanol composition

Compound	Capacity factor, k'					Retention index, I					
	Methanol (%)					Methanol (%)					
	40	50	60	70	80	40	50	60	70	80	Nominal
<i>Standards</i>											
Toluene	8.16	3.93	1.80	0.95	0.53	703	705	708	716	726	700
Ethyl benzene	16.62	7.15	2.76	1.29	0.67	794	792	789	793	806	800
Propyl benzene	38.96	14.12	4.59	1.85	0.86	903	896	888	888	895	900
Butyl benzene	–	29.04	7.85	2.75	1.12	–	1004	996	988	992	1000
Hexyl benzene	–	–	22.64	6.09	1.98	–	–	1202	1189	1193	1200
Octyl benzene	–	–	–	14.27	3.64	–	–	–	1408	1403	1400
Decyl benzene	–	–	–	–	6.82	–	–	–	–	1621	1600
<i>Test compounds</i>											
Benzamide	0.72	0.33	0.28	0.22	0.20	395	332	349	340	392	
<i>p</i> -Cresol	2.26	1.16	0.63	0.38	0.26	539	519	506	482	480	
Benzaldehyde	2.08	1.10	0.61	0.38	0.28	530	512	497	482	504	
Acetophenone	2.72	1.28	0.73	0.47	0.33	565	537	533	535	553	
Nitrobenzene	3.30	1.73	0.92	0.54	0.36	589	581	578	571	585	
Methylbenzoate	5.52	2.52	1.13	0.57	0.38	653	637	618	589	609	
Benzophenone	18.60	6.38	2.18	0.98	0.54	809	779	748	725	730	
Biphenyl	47.55	15.78	8.44	1.83	0.86	929	914	892	885	891	
Slope ($\times 10^{-3}$)	3.40	2.91	2.20	1.69	1.24						
Intercept	-1.48	-1.46	-1.31	-1.23	-1.17						
Correlation, r	0.9987	0.9992	0.9991	0.9989	0.9991						

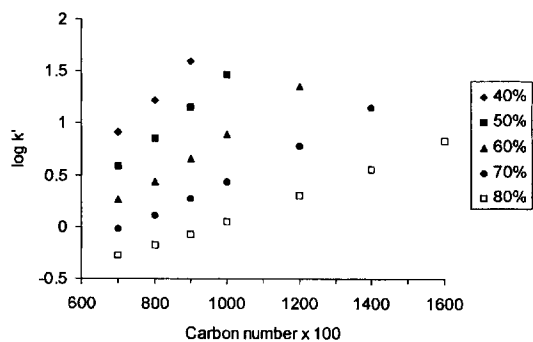


Fig. 1. Log k' versus retention index (carbon number \times 100) of alkylbenzenes on a RP-8 column using different percentages of methanol as eluent.

unaffected by the composition of the mobile phase (Fig. 2). The retention-index values of the test compounds showed a small but general decrease with increasing proportion of methanol in the solvent. Benzaldehyde and *p*-cresol showed a change in elution order at a methanol content higher than 70%—i.e. *p*-cresol was eluted before benzaldehyde. Among the test compounds examined, only benzophenone and biphenyl were within the range of the alkylbenzene retention-index scale.

A similar study was also carried out using acetonitrile–water mixtures as the eluent. The capacity factors of the test compounds decreased with increasing acetonitrile content in the solvent (Table 2). It was noted that with this eluent system, the k' values were generally smaller than those obtained using methanol. As expected,

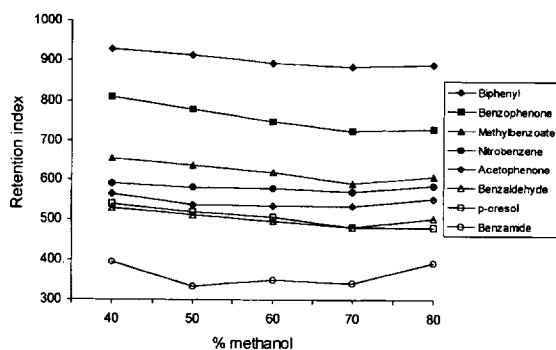


Fig. 2. Variation of retention index values of test compounds on a RP-8 column using different percentages of methanol as eluent.

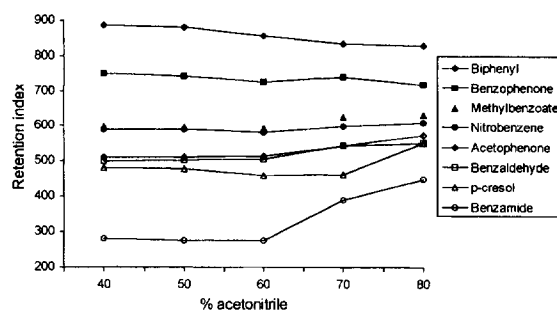


Fig. 3. Variation of retention index values of test compounds on a RP-8 column using different percentages of acetonitrile as eluent.

there was a close correlation between log k' and $100 \times$ carbon number. The slope and intercept of the curves changed with the percentage of methanol in the solvent and correlations of better than 0.9980 were obtained. From the regression equations, the retention-index values of the compounds were determined.

Although, as before, the capacity factors were greatly affected by changes in the acetonitrile concentration in the mobile phase, there was generally only a slight variation in the retention indices of the test compounds with a variation in the acetonitrile composition (Table 2 and Fig. 3). Benzamide and *p*-cresol showed increased retention at high percentages of acetonitrile (>70%) suggesting differences in selectivity for these more polar compounds. Except for benzophenone and biphenyl, the retention-index values for the test compounds lie outside the extrapolated range of the alkylbenzene scale. This indicated the limitation of the alkylbenzene scale especially for relatively polar compounds such as drugs.

3.2. Retention on the ODS-silica stationary phase

The retention behaviour of the test compounds was further examined using an ODS column. Using varying compositions of the methanol–water mixture, the k' values of alkylbenzenes and the test compounds were calculated (Table 3). The capacity factors of the test compounds decreased markedly with increasing percentage

Table 2

Capacity factors and retention indices of alkylbenzenes and test compounds on an RP-8 column with different acetonitrile composition

Compound	Capacity factor, k'					Retention index, I					
	Acetonitrile (%)					Acetonitrile (%)					
	40	50	60	70	80	40	50	60	70	80	Nominal
<i>Standards</i>											
Toluene	4.18	2.64	1.26	0.89	0.60	710	709	709	727	736	700
Ethyl benzene	6.50	3.74	1.62	1.07	0.73	794	794	793	798	814	800
Propyl benzene	10.87	5.58	2.18	1.34	0.85	899	897	892	888	881	900
Butyl benzene	17.96	8.39	2.94	1.69	1.09	1000	994	992	977	971	1000
Hexyl benzene	–	19.53	5.59	2.82	1.52	–	1206	1205	1173	1173	1200
Octyl benzene	–	–	–	4.92	2.38	–	–	–	1388	1396	1400
Decyl benzene	–	–	–	8.90	3.85	–	–	–	1620	1632	1600
<i>Test compounds</i>											
Benzamide	0.51	0.46	0.34	0.37	0.35	281	276	276	388	450	
<i>p</i> -Cresol	1.38	1.04	0.59	0.47	0.43	482	478	459	463	551	
Benzaldehyde	1.50	1.16	0.68	0.56	0.43	500	503	505	544	551	
Acetophenone	1.59	1.17	0.70	0.59	0.45	510	510	513	544	573	
Nitrobenzene	2.32	1.62	0.85	0.64	0.48	598	595	593	624	629	
Methylbenzoate	2.44	1.66	0.88	0.68	0.50	589	589	581	597	607	
Benzophenone	5.26	2.99	1.32	0.92	0.60	752	743	726	740	718	
Biphenyl	10.30	5.19	1.97	1.26	0.76	887	880	856	836	830	
Slope ($\times 10^{-3}$)	2.12	1.75	1.31	1.12	0.891						
Intercept	–0.872	–0.820	–0.829	–0.864	–0.859						
Correlation, r	0.9994	0.9994	0.9991	0.9980	0.9980						

of methanol, particularly for the non-polar compounds such as biphenyl. From the k' values of alkylbenzenes, a plot of $\log k'$ vs. carbon number $\times 100$ was constructed and used as a retention-index scale. The homologs showed a close correlation between $\log k'$ and carbon number $\times 100$. The slope and intercept of the curves changed with the percentage of methanol in the solvent and correlations of better than 0.9906 were obtained. From the regression equations, the retention-index values of the compounds were determined.

The retention-index values of benzophenone, methylbenzoate and benzamide were largely unaffected by the different compositions of the mobile phase (Fig. 4). Similar to the results found for the RP-8 column, only benzophenone and biphenyl were within the range of the alkylbenzene retention-index scale whereas the

other test compounds were eluted before toluene, the first member of the homolog series.

Another series of experiments was performed to examine the retention of the test compounds

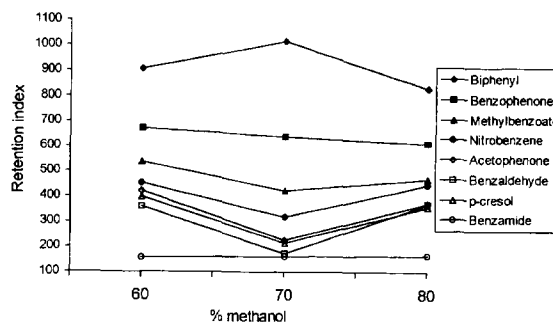


Fig. 4. Variation of retention index values of test compounds on an ODS column using different percentages of methanol as eluent.

Table 3

Capacity factors and retention indices of alkylbenzenes and test compounds on an ODS column with different methanol composition

Compound	Capacity factor, k'					Retention index, I					
	Methanol (%)					Methanol (%)					
	40	50	60	70	80	40	50	60	70	80	Nominal
<i>Standards</i>											
Toluene	–	–	7.7	3.24	1.76	–	–	698	673	706	700
Ethyl benzene	–	–	14.38	5.12	2.63	–	–	805	827	804	800
Propyl benzene	–	–	21.13	6.46	3.65	–	–	898	905	885	900
Butyl benzene	–	–	–	8.72	5.96	–	–	–	1006	1005	1000
Hexyl benzene	–	–	–	15.05	12.75	–	–	–	1189	1192	1200
Octyl benzene	–	–	–	–	30.49	–	–	–	–	1407	1400
Decyl benzene	–	–	–	–	–	–	–	–	–	–	1600
<i>Test compounds</i>											
Benzamide	–	–	0.38	0.27	0.19	–	–	156	161	164	
<i>p</i> -Cresol	–	–	1.49	0.83	0.42	–	–	401	217	356	
Benzaldehyde	–	–	1.19	0.73	0.45	–	–	360	174	369	
Acetophenone	–	–	1.70	0.85	0.45	–	–	424	227	373	
Nitrobenzene	–	–	1.98	1.13	0.61	–	–	453	320	446	
Methylbenzoate	–	–	3.19	1.53	0.75	–	–	539	422	496	
Benzophenone	–	–	6.77	2.92	1.19	–	–	675	639	611	
Biphenyl	–	–	24.84	8.99	2.91	–	–	909	1016	830	
Slope ($\times 10^{-3}$)	–	–	2.20	1.29	1.77						
Intercept	–	–	–0.633	–0.360	–1.00						
Correlation, r	–	–	0.9906	0.9947	0.9994						

on the ODS column using acetonitrile–water mixtures as the mobile phase. It was observed that the capacity factors of the test compounds decreased with increasing acetonitrile content of the solvent (Table 4). It was noted that, as for the RP-8 column, the capacity factors—especially for the less polar compounds—were greatly affected by changes in the acetonitrile concentration in the mobile phase. There was a close correlation between $\log k'$ and $100 \times$ carbon number. The slope and intercept of the curves changed with the percentage of acetonitrile in the solvent and correlations of better than 0.9993 were obtained. From the regression equations, the retention-index values of the compounds were determined. It was found that there were slight variations of the retention indices of biphenyl, benzophenone, nitrobenzene, *p*-cresol and benzamide with variations in the acetonitrile

composition (Table 4 and Fig. 5). Benzaldehyde showed increased retention at high percentages of acetonitrile whereas methyl benzoate showed decreased retention. Except for benzophenone

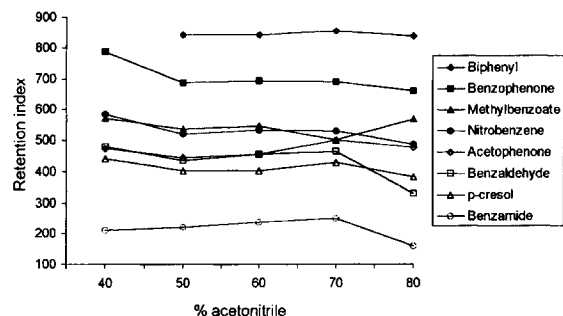


Fig. 5. Variation of retention index values of test compounds on an ODS column using different percentages of acetonitrile as eluent.

Table 4

Capacity factors and retention indices of alkylbenzenes and test compounds on an ODS column with different acetonitrile composition

Compound	Capacity factor, k'					Retention index, I					
	Acetonitrile (%)					Acetonitrile (%)					
	40	50	60	70	80	40	50	60	70	80	Nominal
<i>Standards</i>											
Toluene	14.96	8.20	3.79	2.07	1.24	702	704	709	714	719	700
Ethyl benzene	27.04	13.38	5.54	2.87	1.64	796	795	792	795	796	800
Propyl benzene	53.04	23.54	8.88	4.28	2.36	902	899	897	894	898	900
Butyl benzene	–	41.16	14.05	6.33	3.27	–	1002	998	992	988	1000
Hexyl benzene	–	–	35.83	14.37	6.69	–	–	1204	1195	1187	1200
Octyl benzene	–	–	–	34.03	14.27	–	–	–	1409	1398	1400
Decyl benzene	–	–	–	–	30.87	–	–	–	–	1613	1600
<i>Test compounds</i>											
Benzamide	0.67	0.60	0.44	0.32	0.17	212	221	236	248	160	
<i>p</i> -Cresol	2.84	1.59	0.94	0.63	0.37	441	401	402	429	383	
Benzaldehyde	3.68	1.92	1.21	0.76	0.31	481	436	456	465	330	
Acetophenone	3.50	2.01	1.21	0.87	0.52	473	444	456	499	478	
Nitrobenzene	7.01	3.00	1.69	0.98	0.54	583	518	531	530	488	
Methylbenzoate	6.60	3.29	1.82	1.23	0.73	573	535	546	499	569	
Benzophenone	25.55	7.37	3.51	1.86	1.00	787	684	692	688	659	
Biphenyl	–	17.31	6.88	3.66	1.91	–	842	841	856	839	
Slope ($\times 10^{-3}$)	2.75	2.34	1.96	1.74	1.56						
Intercept	–0.760	–0.734	–0.812	–0.927	–1.03						
Correlation, r	0.9995	0.9998	0.9994	0.9993	0.9994						

and biphenyl, the retention-index values for the test compounds lie outside the extrapolated range of the alkylbenzene scale.

3.3. Retention on the PLRP-S stationary phase

In order to examine the versatility of the retention-index system, the retention behaviour of the test compounds was further examined using a PLRP-S polymer column. Using varying compositions of the methanol–water mixture, the k' values of alkylbenzenes and the test compounds were calculated (Table 5). It was found that the compounds were relatively strongly retained on the column. With methanol concentrations of less than 60%, most of the compounds studied were not eluted within reasonable time. With methanol concentration of 80%, the alkyl benzenes toluene, ethyl benzene and

propyl benzene and all test compounds were eluted. The capacity factors of the test compounds decreased markedly with increasing percentage of methanol. From Table 5, it can be seen that benzophenone and biphenyl were within the range of the retention-index scale.

A similar study was also carried out using acetonitrile–water mixtures as the eluent. It was observed that the compounds were generally less retained on the column than when methanol–water mixtures were used as eluent. The capacity factors of the test compounds decreased with increasing acetonitrile concentration of the solvent (Table 6). The k' values were generally smaller than those obtained using methanol as eluent. There was a close correlation between $\log k'$ and $100 \times$ carbon number. The slope and intercept of the curves changed with the percentage of acetonitrile in the solvent and correla-

Table 5
Capacity factors of alkylbenzenes and test compounds on a PLRP-S column with different methanol composition

Compound	Capacity factor, k'				
	Methanol (%)				
	40	50	60	70	80
<i>Standards</i>					
Toluene	–	–	–	25.01	13.4
Ethyl benzene	–	–	–	46.58	22.11
Propyl benzene	–	–	–	–	36.90
Butyl benzene	–	–	–	–	–
Hexyl benzene	–	–	–	–	–
Octyl benzene	–	–	–	–	–
Decyl benzene	–	–	–	–	–
<i>Test compounds</i>					
Benzamide	–	–	–	0.62	0.37
<i>p</i> -Cresol	–	–	–	3.27	1.53
Benzaldehyde	–	–	–	10.39	5.04
Acetophenone	–	–	–	12.03	5.69
Nitrobenzene	–	–	–	21.37	9.31
Methylbenzoate	–	–	–	25.43	10.26
Benzophenone	–	–	–	–	30.17
Biphenyl	–	–	–	–	31.85

tions of better than 0.9995 were obtained. From the regression equations, the retention-index values of the compounds were determined.

In contrast to the results found for the capacity factors, there were only relatively slight changes in the retention indices of the test compounds with variation of the acetonitrile composition (Table 6 and Fig. 6). Relative to the retention-

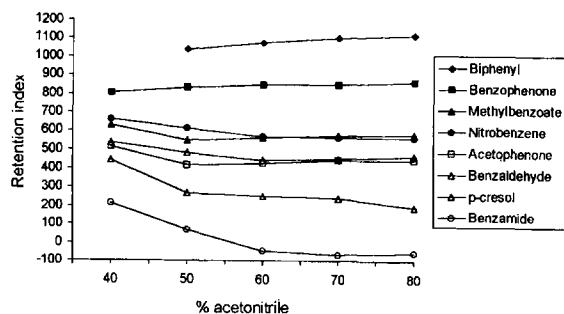


Fig. 6. Variation retention index values of test compounds on a PLRP-S column using different percentages of acetonitrile as eluent.

index scale, benzamide, *p*-cresol, benzaldehyde and nitrobenzene showed slowly decreasing retention with increasing percentages of acetonitrile. As has been found for the RP-8 and ODS column, the retention-index values for the test compounds were outside the extrapolated range of the alkylbenzene scale, except for benzophenone and biphenyl. This indicated the limitation of the alkylbenzene scale especially for relatively polar compounds such as drugs. Nevertheless, the retention index would be useful for non-polar and higher-molecular-mass analytes, such as aromatic hydrocarbons.

3.4. Effect of mobile phase

In general, for all the three columns used in the present work, variations in the concentration of methanol or acetonitrile in the mobile phase result in large variations in the observed retentions of the alkylbenzenes and the test compounds; however, the retention indices are much more consistent. This increased robustness is due to the normalisation of the retention indices to the methylene or carbon-number selectivity which varies uniformly for the alkylbenzenes and, to some extent, for the test compounds. Moreover, polar-group selectivity also varies, giving rise to relatively small changes in the retention indices.

When using acetonitrile–water as the eluent, retention of the compounds was generally lower than when methanol–water was used as the eluent. This indicates the higher elution power of acetonitrile compared to methanol, especially for the elution of the more non-polar compounds.

3.5. Effect of stationary phase

In the course of this study, it was found that very large differences in the retention of the compounds were observed on the RP-8, ODS, and PLRP-S stationary phases. When using methanol–water as the mobile phase, the compounds showed increased retention on the ODS column when compared to the RP-8 column, e.g. with methanol–water (70:30) as the eluent, the capacity factors on the ODS column were $k' =$

Table 6
Capacity factors and retention indices of alkylbenzenes and test compounds on a PLRP-S column with different acetonitrile composition

Compound	Capacity factor, k'					Retention index, I					
	Acetonitrile (%)					Acetonitrile (%)					
	40	50	60	70	80	40	50	60	70	80	Nominal
<i>Standards</i>											
Toluene	23.23	10.65	5.48	3.21	1.89	701	700	700	701	710	700
Ethyl benzene	43.12	17.40	8.21	4.51	2.49	798	802	802	800	803	800
Propyl benzene	82.34	27.02	11.83	6.35	3.30	901	894	893	900	899	900
Butyl benzene	–	45.62	18.52	8.79	4.37	–	1003	1006	995	994	1000
Hexyl benzene	–	–	39.73	18.02	7.69	–	–	1199	1205	1185	1200
Octyl benzene	–	–	–	34.87	14.38	–	–	–	1398	1396	1400
Decyl benzene	–	–	–	–	27.31	–	–	–	–	1613	1600
<i>Test compounds</i>											
Benzamide	0.61	0.51	0.28	0.23	0.20	214	68	–44	–65	–54	
<i>p</i> -Cresol	3.81	1.35	0.92	0.66	0.41	445	271	251	240	192	
Benzaldehyde	8.16	3.73	1.99	1.36	0.91	535	482	445	450	465	
Acetophenone	7.27	2.73	1.85	1.33	0.86	517	417	428	444	445	
Nitrobenzene	18.09	6.97	3.28	1.99	1.23	661	612	571	562	565	
Methylbenzoate	14.9	5.08	3.18	2.10	1.28	631	547	564	577	580	
Benzophenone	43.63	25.20	9.87	5.23	2.97	801	831	848	844	864	
Biphenyl	–	53.71	24.14	12.60	6.34	–	1037	1073	1101	1120	
Slope ($\times 10^{-3}$)	2.75	2.08	1.73	1.49	1.29						
Intercept	–0.560	–0.428	–0.475	–0.535	–0.633						
Correlation, r	0.9995	0.9994	0.9996	0.9997	0.9995						

5.12 for ethylbenzene and $k' = 0.73$ for benzaldehyde, whereas the corresponding values on the RP-8 column were $k' = 1.29$ and $k' = 0.38$, respectively (Tables 1 and 3). The increase in the retention power of the ODS stationary phase corresponds with the increase in the total carbon loading (C%) compared to that of the RP-8 stationary phase. It was also observed that the elution order of the retention-index standards and the test compounds was virtually unchanged although the absolute values of the capacity factors and retention indices varied (Figs. 2 and 4). As the alkyl chain in each case is essentially inert and does not interact with the analytes, the selectivity and relative elution order of the analytes on the RP-8 and ODS columns are rather similar except for analytes that can interact with the free silanol groups.

The test compounds and the alkylbenzene

standards were markedly more retained on the PLRP-S column than on the RP-8 and ODS columns, e.g., with methanol–water (70:30) as the eluent, the capacity factors on the PLRP-S column were $k' = 46.58$ for ethylbenzene and $k' = 10.39$ for benzaldehyde (Table 5). This was as expected since PLRP-S is a non-polar stationary phase with a very high retention capacity.

When acetonitrile–water was used as the mobile phase on the RP-8, ODS and PLRP-S columns, the trends observed were similar to those found when using methanol–water as the eluent. However, both the RP-8 and ODS columns showed slightly increased retentions, e.g., with acetonitrile–water (70:30) as the eluent, the capacity factors on RP-8 column were $k' = 1.07$ for ethylbenzene and $k' = 0.56$ for benzaldehyde, while the corresponding values on the ODS were $k' = 2.87$ and $k' = 0.76$, respectively. The reten-

tions of the compounds on the PLRP-S column were higher than on the PR-8 and ODS columns although the values were significantly lower than those obtained when using the corresponding methanol–water mobile phase.

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