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ALKYL ARYL KETONES AS A RETENTION INDEX SCALE WITH ACETONITRILE OR TETRAHYDROFURAN CONTAINING ELUENTS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

ROGER M. SMITH*, GRACE A. MURILLA* and CHRISTINA M. BURR
Department of Chemistry, Loughborough University of Technology, Loughborough, Leics. LE11 3TU (U.K.)

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SUMMARY

Alkyl aryl ketones can be used as retention index scale standards in a wide range of eluents comprising different proportions of acetonitrile–pH 7 buffer or tetrahydrofuran–water. In each case, there is a linear relationship between the carbon number of the alkyl aryl ketones and the logarithm of their capacity factors. This scale can be used to calculate the retention indices of test compounds, which were less dependent on small changes in the eluent composition than the capacity factors.

INTRODUCTION

Although high-performance liquid chromatography (HPLC) can be very reproducible on a single set of equipment and column, the reproducibility of the retentions of analytes obtained in different laboratories or on different occasions is often very poor. This has limited the application of HPLC to qualitative analyses because it is often difficult to reliably compare results obtained in different laboratories for identification purposes or to compare the properties of columns or mobile phases. Although retentions are conventionally reported as capacity factors (k') [calculated as $k' = (t_R - t_0)/t_0$], these are very susceptible to small changes in the operating conditions, column temperature, and eluent composition such as might occur between operators or laboratories. They are also very sensitive to the exact value used for the column void volume (t_0) and although a number of different methods have been proposed for the determination of this value, these often give different values[1] and frequently reports do not define the method used.

Relative capacity factors compared to an internal standard are a more reproducible method to record retentions but different laboratories may base results on different standard compounds and prevent direct comparison.

Retentions based on Kováts retention indices have been widely used in gas–liquid chromatography (GLC) because they are more reproducible than direct reten-

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* Present address: Government Chemist Department, Nairobi, Kenya.

tion time measurements but so far similar concepts have have not gained wide acceptance in HPLC. The first proposals, based on the linear relationship between log k' and carbon number for the members of a homologous series were made by Baker and Ma[2], who suggested that the alkan-2-ones could be used as the basis of a retention index scale in methanol–buffer and acetonitrile–buffer eluents. Subsequently Smith[3] proposed that the alkyl aryl ketones could be used as the basis of a retention index scale and would be easier to detect. The application of this scale has been tested in a series of studies aimed at improving the reproducibility of retention values for drug analyses of forensic interest[4–7]. The results with local anaesthetics and barbiturates have confirmed that retention indices are a much more robust and reproducible method for recording retentions than capacity factors but that they are susceptible to changes in selectivity caused by changes in the stationary phase. Their value has been demonstrated in a interlaboratory collaborative study of the identification of barbiturates by HPLC[8].

In an extension of this work a set of column test compounds was selected, whose retention indices could be used to characterise the selectivity of different stationary phases and eluents, in a similar manner to the use of McReynolds or Rohrschneider constants in GLC[9,10]. These were then used to compare the differences in the selectivity of different isoeluotropic binary[10] and ternary[11] eluents containing methanol, acetonitrile, and tetrahydrofuran.

However, most of these studies of the applications of the alkyl aryl ketone retention index scale have been carried out using methanol–aqueous buffer eluents. In the initial studies, it was shown that the retention indices of test compounds were largely unchanged over a wide range of methanol proportions but some changes were noted at high or low levels[3]. However, no corresponding study has been carried out of the effect of changing the proportions of other organic modifiers on the retention and selectivity properties although Baker examined acetonitrile–buffer systems using the alkan-2-one scale[2].

The present study is a detailed examination of the applicability of the retention indices based on alkyl aryl ketones to mobile phases containing a range of proportions of acetonitrile or tetrahydrofuran. One aim of this study was to determine the robustness of the retention indices to small changes in the composition of the organic modifier and thus their applicability for use in interlaboratory studies with these eluents. This has subsequently been tested by a detailed examination of the reproducibility of retention indices for the identification of the thiazide diuretic drugs using a acetonitrile–1% aqueous acetic acid eluent[12].

EXPERIMENTAL

Chemicals and eluents

Reference samples of alkyl aryl ketones (acetophenone, propiophenone, butyrophenone, and valerophenone) and test compounds (toluene, nitrobenzene, 2-phenylethanol, N-methylaniline, methyl benzoate, and *p*-cresol) were laboratory reagent grade from a range of different suppliers.

Acetonitrile and tetrahydrofuran (THF) were HPLC-grade and disodium hydrogen orthophosphate and potassium dihydrogen phosphate were reagent grade from Fisons Scientific Apparatus (Loughborough, U.K.).

Buffer solutions pH 7.0 were prepared by adding disodium hydrogen orthophosphate (0.50 g) and potassium dihydrogen phosphate (0.301 g) to water and making up to 1 l.

HPLC equipment

HPLC separations were carried out using a Pye-Unicam 4010 pump and Altex 153 fixed-wavelength detector set at 254 nm. The samples (10 μl) were injected using a Rheodyne 7125 valve onto a 100 × 5 mm I.D. column packed either with Hypersil ODS 5-μm (batch 6/868 from Shandon Southern Products, Runcorn, U.K.) or Spherisorb ODS 5-μm (Batch 19/35, Phase Separations, Queensferry, U.K.). The columns were enclosed in a circulating water jacket at 30°C. The column void volume was determined by injecting a solution of aqueous sodium nitrate (12.16 mg/ml).

Calculations

Capacity factors (k') were calculated as $k' = (t_R - t_0)/t_0$ in which t_R and t_0 are the retention times of the sample and the column void volume, respectively.

The retention indices were calculated as described previously using a least square correlation of the log k' against the carbon number × 100 for the alkyl aryl ketones[3].

RESULTS AND DISCUSSION

Although previous studies using the alkyl aryl ketones have examined isoelutropic eluents containing acetonitrile or tetrahydrofuran no systematic study has so far been carried out to determine the effect of the proportion of these organic components in the eluents on the retention indices of typical analytes.

Acetonitrile–pH 7.0 buffer eluents

The retention times of the alkyl aryl ketones (acetophenone, propiophenone, butyrophenone, and valerophenone) and a set of analytes were determined over a range of proportions from 10:90 to 100:0 of acetonitrile–pH 7.0 phosphate buffer on a Hypersil ODS column. To improve reproducibility all the studies were carried out at constant temperature. The analytes included the column test compounds, toluene, 2-phenylethanol, *p*-cresol, and nitrobenzene, used in previous studies to measure the selectivity changes of different column materials and ternary eluents[9,10]. Two additional compounds, N-methylaniline and methyl benzoate, were also examined. In earlier studies with methanol–buffer or water eluents, the retention index value of methyl benzoate was found to be very consistent irrespective of the composition and this compound appeared to reflect the same selectivity effects as the alkyl aryl ketones[3,10]. This consistency has also enables this compound to be used as a secondary standard in estimating retention indices from previously published data of separations in methanol–water[7,13] and it was of interest to determine if this role could be extended to other organic modifiers. N-Methylaniline was included as it has been reported to be susceptible to interactions with free silanol groups although in previous studies, using ternary[11] and methanol–pH 7.0 buffer eluents[14], no specific interactions were observed.

At each eluent composition the column void volume was determined using a solution of sodium nitrate. Although this method has been reported to be susceptible to changes in the concentration of the standard and of the ionic-strength of the eluent[1], in previous studies it has been found to give reproducible results. Calculations have also shown that except for compounds with very short retentions the retention indices are virtually independent of small differences in the values of the column void volume. The capacity factors for the alkyl aryl ketones and the test compounds were calculated from the retention times (Table I). These showed the expected large changes with the proportion of acetonitrile emphasising their sensitivity to even small changes in eluent composition.

For each proportion of acetonitrile, there was a linear correlation between the logarithm of the capacity factors and the carbon numbers of the homologous alkyl aryl ketones (Table II) (Fig. 1). These were used to calculate the retention indices of the test compounds in each eluent (Table III). The changes in the retention indices with the proportion of acetonitrile are much smaller than for the capacity factors (Fig. 2) and are comparable to those found previously for methanol–water eluents[3]. For most of the compounds the values were virtually constant over limited changes in the eluent composition, such as those that might be expected to occur in the preparation of a specified eluent in different laboratories. The greatest deviations were found with the more polar compounds, *p*-cresol and 2-phenylethanol, which will interact more by hydrogen bonding. Some deviations occurred with very high or low proportions of the organic modifier. At high acetonitrile proportions, the very short retention times probably contributed to the uncertainties as any errors in measurement would be more significant. The retention indices of methyl benzoate from 10 to 80% acetonitrile (*RI* = 880–887) were virtually constant and within this range it could again be used as a secondary standard. These values were very similar to

TABLE I

CAPACITY FACTORS (k') OF ALKYL ARYL KETONES AND COLUMN TEST COMPOUNDS ON ELUTION WITH DIFFERENT PROPORTIONS OF ACETONITRILE

Column, Hypersil ODS; eluent, acetonitrile–phosphate buffer (pH 7.0).

Compound	*k'*									
	Acetonitrile (%)									
	10	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*	*100*
Alkyl aryl ketones										
Acetophenone	20.67	9.74	3.81	2.26	1.41	0.90	0.59	0.38	0.27	0.23
Propiophenone	62.93	26.51	8.64	4.46	2.44	1.47	0.88	0.54	0.34	0.27
Butyrophenone	–	–	18.01	8.08	3.93	2.23	1.23	0.72	0.42	0.31
Valerophenone	–	–	–	15.00	6.41	3.42	1.73	0.97	0.54	0.37
Test compounds										
2-Phenylethanol	12.47	5.12	1.97	1.19	0.79	0.51	0.35	0.23	0.21	0.20
p-Cresol	18.46	8.30	3.21	1.84	1.12	0.70	0.43	0.27	0.22	0.21
Methyl benzoate	53.06	22.02	7.58	3.95	2.21	1.35	0.80	0.50	0.34	0.27
N-Methylaniline	17.07	9.68	4.81	2.89	1.76	1.12	0.68	0.42	0.30	0.26
Nitrobenzene	25.73	14.90	6.61	3.63	2.05	1.23	0.71	0.43	0.29	0.23
Toluene	–	–	17.82	8.63	4.85	2.50	1.35	0.80	0.48	0.34

TABLE II

CORRELATION OF LOG CAPACITY FACTORS OF ALKYL ARYL KETONES WITH CARBON NUMBER IN ELUENTS CONTAINING ACETONITRILE

Conditions as Table I. For the equation log k' = a (carbon number × 100) + b.

	Acetonitrile (%)									
	10	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*	*100*
Correlation coefficient	*	*	0.9995	0.9996	0.9995	0.9990	0.9979	0.9991	0.9969	0.9807
Slope ($\times 10^3$)	4.83	4.53	3.37	2.73	2.18	1.91	1.56	1.35	1.01	0.69
Intercept	−2.55	−2.49	−2.11	−1.82	−1.59	−1.57	−1.47	−1.49	−1.38	−1.20

* Only two ketones examined therefore retention indices calculated by direct interpolation.

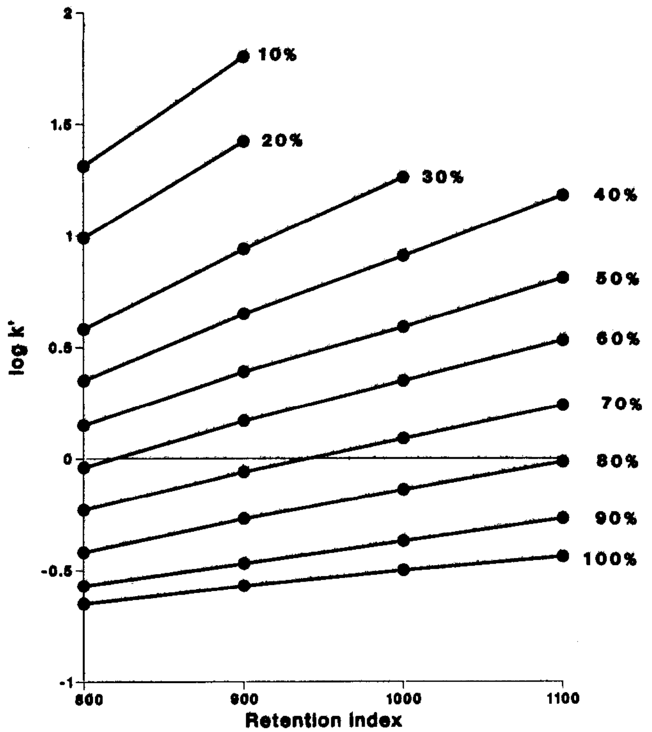

Fig. 1. Comparison of capacity factors of alkyl aryl ketones with retention indices in different proportions of acetonitrile–phosphate buffer (pH 7.0).

TABLE III

RETENTION INDICES OF TEST COMPOUNDS IN DIFFERENT PROPORTIONS OF ACETONITRILE

Column, Hypersil ODS; eluent, acetonitrile–phosphate buffer (pH 7.0).

Compound	*Retention index*									
	Acetonitrile (%)									
	10	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90**	*100*
2-Phenylethanol	752	735	713	695	680	666	653	628	695	719
p-Cresol	790	784	776	763	751	736	710	681	715	749
Methyl benzoate	885	882	886	886	886	887	880	885	903	907
N-Methylaniline	783	799	828	834	841	844	836	827	850	882
Nitrobenzene	820	842	869	872	871	866	848	833	834	805
Toluene	—	—	996	1010	1021	1026	1029	1037	1052	1050

* Buffer solution diluted five fold to prevent precipitation.

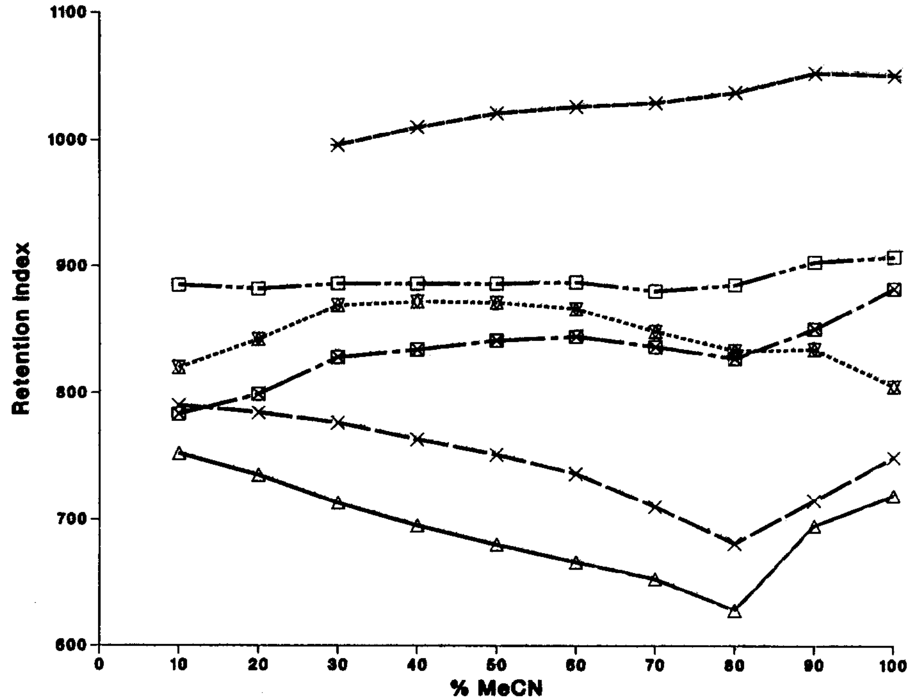

Fig. 2. Retention indices of test compounds in different proportions of acetonitrile–phosphate buffer (pH 7.0); △, 2-phenylethanol; X, *p*-cresol; □, methyl benzoate; ☒, N-methylaniline; ------, nitrobenzene; ✱, toluene.

that found previously for methyl benzoate with acetonitrile–water (50:50) eluents (*RI* = 889)[10] but as expected were different to those found with methanol–water eluents, typically *RI* = 905[3]. As expected from the earlier study[11], N-methylaniline behaved as a neutral compound and showed no specific interaction with the stationary phase.

Schoenmakers *et al.*[15] have proposed that as the volume fraction of organic modifier is changed, there is a relationship between φ and the capacity factor, $\log k' = \log k_0 - S\varphi$, in which S is the slope and $\log k_0$ is the intercept equivalent to a water eluent. Often this relationship is curved and a more correct representation would be a quadratic expression, but in most cases the simple linear relationship is sufficient accurate for practical use. Over a wide range of samples, it has also been found that there is also a relationship between S and the values of $\log k_0$ such that $S = p + q \log k_0$. Thus as the proportion of organic modifier decreases the retentions of all analytes increase and the selectivity remains nearly constant. These results were examined for methanol–buffer in earlier studies of separation of the barbiturates. If the full set of samples and column test standards were compared a reasonable correlation was observed but if only compounds with different functional groups were examined then virtually no correlation was obtained[4].

Using the results for the capacity factors (Table I) between $k' = 1$ and 10 the values of S and $\log k_0$ were calculated for the samples in the present study (Table

TABLE IV

RELATIONSHIP BETWEEN PROPORTION OF ACETONITRILE AND CAPACITY FACTORS

Calculated as in ref. 15. Based on capacity factors between 1 and 10.

Compound	*log k_0*	*S*
Acetophenone	1.478	2.74
Propiophenone	1.691	2.57
Butyrophenone	1.969	2.70
Valerophenone	2.182	2.76
2-Phenylethanol	1.173	2.65
p-Cresol	1.433	2.85
Methyl benzoate	1.612	2.50
N-Methylaniline	1.408	2.31
Nitrobenzene	1.543	2.44
Toluene	2.343	3.44
Correlation coefficient	0.6327	
Slope	0.527	

IV). Clearly the range of values for S is very limited and the correlation with log k_0 was very poor. These results were not unexpected as a similar limited range of S had been found by Schoenmakers *et al.*[15] with this organic modifier and in a comparison of 31 compounds had only found a correlation of 0.06. In contrast to the previous study, there was not a systematic change in S along the homologous series of alkyl aryl ketones. However, because the relationships between log k' and φ were very curved in the present work, an arbitrary range of capacity factors had been used to determine the slopes and thus these results might not be a good test of the relationship. In a study over a more limited range of 50–90% acetonitrile, Jandera[16] has demonstrated a linear relationship between log k' and φ and in this case for each homologous series of compounds there was a linear relationship between S and log k_0.

Tetrahydrofuran–water systems

A corresponding series of experiments was carried out using different proportions of tetrahydrofuran-water as the eluent with a Spherisorb ODS column. In order to avoid very short retention times a more limited range of eluent compositions was used. The eluent was not buffered as N-methylaniline was not included in the test samples, because in the previous study it had been found to show no specific interactions. The capacity factors (Table V) were calculated and again there was a linear relationship for the homologous ketones for each eluent (Table VI, Fig. 3), which were used to determined the retention indices (Table VII, Fig. 4). For some of the analytes the retention indices changed systematically with the proportion of modifier. The effect was greater than with acetonitrile or methanol but the variation was much smaller than the changes in capacity factors. Compared to the alkyl aryl ketones, the retentions of relatively non-polar compounds such as toluene increased steadily with increased proportion of THF whereas for polar analytes, such as *p*-cresol, the values decreased markedly. Only the index values for the medium polarity compounds re-

TABLE V

CAPACITY FACTORS *(k')* OF ALKYL ARYL KETONES AND COLUMN TEST COMPOUNDS IN DIFFERENT PROPORTIONS OF TETRAHYDROFURAN

Column, Spherisorb ODS; eluent, tetrahydrofuran–water at 30°C.

Compound	*k'*				
	Tetrahydrofuran (%)				
	20	*30*	*40*	*50*	*60*
Alkyl aryl ketones					
Acetophenone	8.36	4.06	1.85	1.36	0.84
Propiophenone	21.38	8.63	3.10	2.11	1.12
Butyrophenone	50.82	16.33	4.62	2.93	1.38
Valerophenone	128.97	31.16	6.81	4.00	1.66
Test compounds					
2-Phenylethanol	6.21	2.95	1.31	0.97	0.60
p-Cresol	19.19	7.81	2.51	1.70	0.87
Methyl benzoate*	(20.07)	(7.74)	–	(1.70)	(1.07)
Nitrobenzene	23.74	10.19	3.24	2.14	1.10
Toluene	65.69	22.54	5.99	3.84	1.77

* Determined in a separate series of separations.

mained virtually constant. It therefore appears that with this organic modifier retention indices might be more sensitive to eluent composition than with other modifiers although the high eluent strength of THF means that it also exerts a proportionally greater effect on the capacity factors.

The values of the retention indices particularily for *p*-cresol were markedly different than those in acetonitrile–buffer solutions reflecting the differences in the selectivities of the eluents. The retention values in THF–water are similar to those observed in earlier comparisons of isoeluotropic solvents mixtures and ternary eluents[10,11].

The retention values for methyl benzoate were measured on a separate occasion from the other test compounds and this study gave similar but slightly different

TABLE VI

CORRELATION OF LOG CAPACITY FACTORS OF ALKYL ARYL KETONES WITH CARBON NUMBER IN ELUENTS CONTAINING TETRAHYDROFURAN

For the equation $\log k' = a$ (carbon number × 100) + b.

	Tetrahydrofuran (%)				
	20	*30*	*40*	*50*	*60*
Correlation coefficient	0.9999	0.9992	0.9973	0.9966	0.9948
Slope ($\times 10^3$)	3.943	2.931	1.867	1.54	0.977
Intercept	−2.23	−1.72	−1.21	−1.08	−0.85
Standard deviation	0.008	0.0154	0.0176	0.0164	0.0130

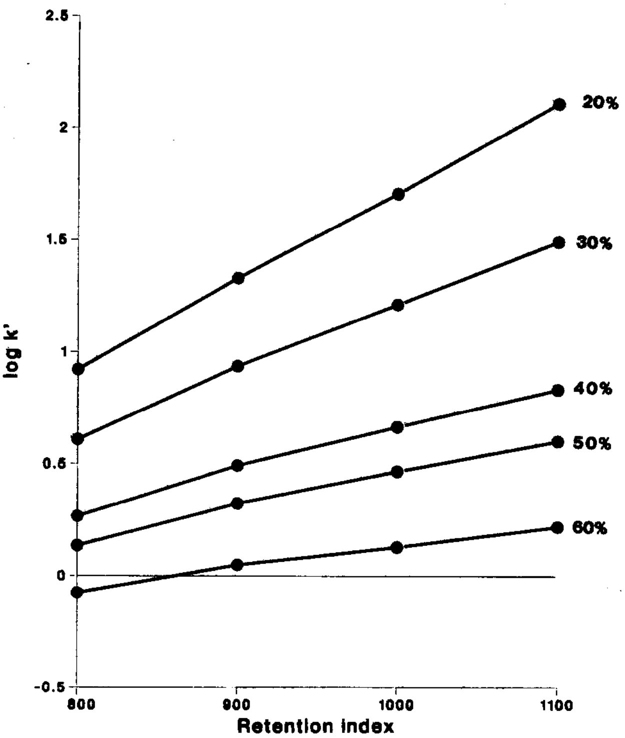

Fig. 3. Comparison of capacity factors of alkyl aryl ketones with retention indices in different proportions of tetrahydrofuran–water.

retention times for the ketones, emphasising the problems of direct intralaboratory reproducibility even under controlled conditions with temperature thermostating. The retention index values of methyl benzoate (*RI* = 883–901) showed a greater variation than previously, although the value was comparable to that determined

TABLE VII

RETENTION INDICES OF COLUMN TEST COMPOUNDS IN ELUENTS CONTAINING DIFFERENT PROPORTIONS OF TETRAHYDROFURAN

Column, Spherisorb ODS; eluent, tetrahydrofuran–water at 30°C.

Compound	*Retention index*				
	Tetrahydrofuran (%)				
	20	*30*	*40*	*50*	*60*
2-Phenylethanol	766	748	711	693	639
Methyl benzoate	901	896	–	892	883
p-Cresol	891	892	862	851	801
Nitrobenzene	914	931	921	917	907
Toluene	1026	1049	1064	1081	1119

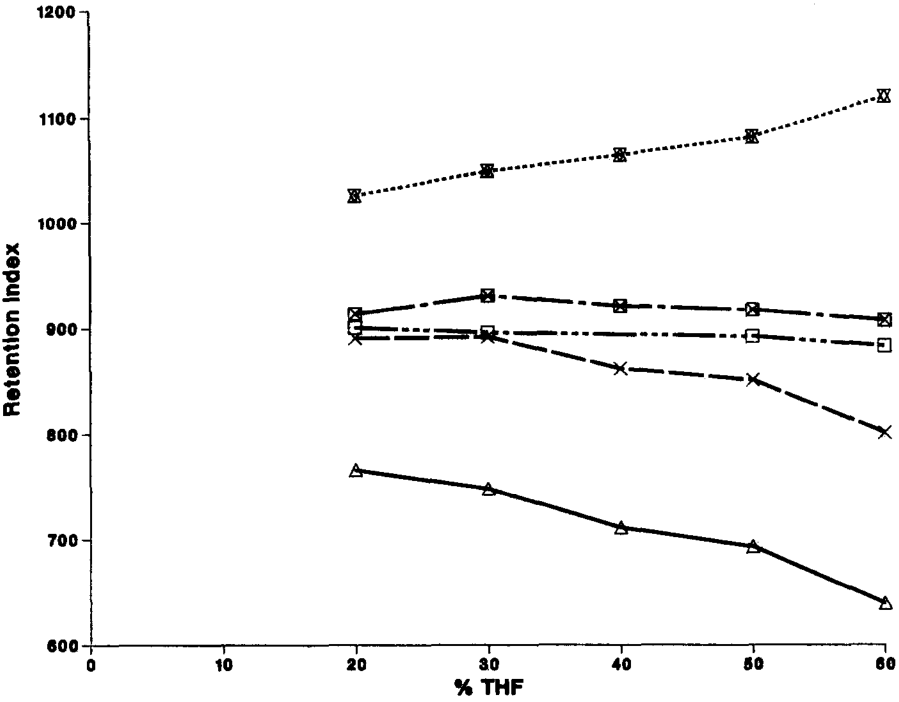

Fig. 4. Retention indices of test compounds in different proportions of THF–water; △, 2-phenylethanol; X, *p*-cresol; □, methyl benzoate; ☒, nitrobenzene; ------, toluene.

TABLE VIII

RELATIONSHIP BETWEEN PROPORTION OF TETRAHYDROFURAN AND CAPACITY FACTORS OF SAMPLES

Calculated as in ref. 15 based on capacity factors in Table V omitting 40% tetrahydrofuran.

Compound	*log k_0*	*Slope*
Acetophenone	1.385	2.47
Propiophenone	1.908	3.03
Butyrophenone	2.433	3.88
Valerophenone	2.975	4.67
2-Phenylethanol	1.262	2.51
p-Cresol	1.926	3.35
Methyl benzoate	1.894	3.20
Nitrobenzene	2.027	3.35
Toluene	2.563	3.91
Correlation coefficient	0.9876	
Slope	1.266	
Intercept	−0.790	

previously for THF–water (40:60, v/v) at room temperature. (*RI* = 888)[10].

Again the relationship between the proportion of organic modifier and capacity factors was examined (Table VIII). The correlations were good, except for the capacity factors measured with 40% THF, which showed a similar discrepancy for each compound. These values were therefore omitted from the calculations. However, as can be seen in the slope of the alkyl aryl ketones and the retention indices of the test compounds the values from 40% THF appeared to be internally consistent. The slopes calculated from the remaining proportions of THF, showed a much greater range of values than were found with acetonitrile containing eluents and systematic changes in S were found for the homologous ketones. There was a reasonable correlation (0.98) between S and log k_0 for the analytes, comparable to the results found by Schoenmakers *et al.*[15]

It appeared that, as the proportion of THF was altered, there were significant changes in the selectivity of the column with greater separations of polar and nonpolar compounds at higher values of φ. Retention indices might therefore be more susceptible in this system to the conditions of the separation. However, when the retention indices of the column test compounds were determined previously on different ODS columns, using THF–water (40:60) as the eluent, much smaller differences were observed between different makes of column packing material, than with the isoeluotropic eluents containing methanol or acetonitrile[10]. Thus for THF containing eluents the retention indices appear to be dependent on the eluent composition but more independent of the make of ODS-bonded silica gel.

These observations could be important in optimisation procedures as the addition of different proportions of THF to an isoeluotropic mixture should markedly alter relative retentions and selectivity and the effect will probably be more systematic than changes in acetonitrile.

CONCLUSIONS

Retention indices of analytes can be calculated using the alkyl aryl ketone scale for eluents containing wide proportions of either acetonitrile or THF. With both modifiers the values are quite robust over small changes in eluent composition but deviate more at low or high concentrations of modifier and the effect of variations in the eluent are smaller for retention indices than for capacity factors. The greatest deviations were for compounds with much higher or much lower polarities than the reference compounds.

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REFERENCES

1 H. Engelhardt, H. Müller and B. Dreyer, *Chromatographia*, 19 (1984) 240.
2 J. K. Baker and C.-Y. Ma, *J. Chromatogr.*, 169 (1979) 107.

3 R. M. Smith, *J. Chromatogr.*, 236 (1982) 313.
4 R. M. Smith, T. G. Hurdley, R. Gill and A. C. Moffat, *Chromatographia*, 19 (1984) 401.
5 R. M. Smith, T. G. Hurdley, R. Gill and A. C. Moffat, *Chromatographia*, 19 (1984) 407.
6 R. M. Smith, T. G. Hurdley, R. Gill and A. C. Moffat, *J. Chromatogr.*, 355 (1986) 75.
7 R. M. Smith, T. G. Hurdley, R. Gill and A. C. Moffat, *LC, GC, Mag. Liq. Gas Chromatogr.*, 4 (1986) 314.
8 R. Gill, A. C. Moffat, R. M. Smith and T. G. Hurdley, *J. Chromatogr. Sci.*, 24 (1986) 153.
9 R. M. Smith, *J. Chromatogr.*, 236 (1982) 321.
10 R. M. Smith, *Anal. Chem.*, 56 (1984) 256.
11 R. M. Smith, *J. Chromatogr.*, 324 (1985) 243.
12 R. M. Smith, G. A. Murilla, T. G. Hurdley, R. Gill and A. C. Moffat, *J. Chromatogr.*, 384 (1987) 259.
13 R. M. Smith, in preparation.
14 R. M. Smith, T. G. Hurdley, R. Gill and A. C. Moffat, *J. Chromatogr.*, 351 (1986) 259.
15 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.
16 P. Jandera, *J. Chromatogr.*, 314 (1984) 13.