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ALKYLARYLKETONES AS A RETENTION INDEX SCALE IN LIQUID CHROMATOGRAPHY*

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SUMMARY

A retention index scale based on the relative retentions of the alkylarylketones (PhCOR) has been evaluated for reversed-phase high-performance liquid chromatography. The standards were readily detected by absorbance at 254 nm and showed a linear relationship between $\log k'$ and carbon number. The retention index values for a number of test compounds were determined using different column materials and solvent systems.

INTRODUCTION

The acceptance of high-performance liquid chromatography (HPLC) in official and standard methods has been hindered by wide variations in the properties of reversed-phase columns with nominally the same composition¹. These differences are caused largely by the different techniques used to prepare the bonded phase and to cap residual silica hydroxy groups². It is therefore difficult to specify elution conditions for a particular type of column, such as ODS-silica, without recommending or defining a manufacturer or grade of stationary phase to be used. However, if the retention performance of a column could be determined and expressed in a readily comparable form, this could be used as in a specification. Any column and conditions satisfying the criteria would then be suitable for a particular separation, as long as the efficiency of the column was also appropriate.

In order to be able to make comparisons between chromatographic systems, it is necessary to have a means of expressing retentions which is largely independent of column efficiency, variations in solvent composition, flow-rate, and temperature. For simple assays the retention of an analyte relative to a single standard can be used but no one compound is suitable in all cases.

A retention index system based on a series of standards covering a range of polarities would avoid many of these problems and could form the basis of a guide to

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column activity. In gas-liquid chromatography (GLC) the Kovats retention index³ based on the retention times of *n*-alkanes has been widely accepted as a reference scale, independent of individual laboratory standards. By also acting as the basis of the Rohrschneider⁴ and McReynolds⁵ constants, the Kovats index has enabled the comparative retention behaviour of different liquid stationary phases to be compared, and for most chromatographers this is its most valuable function. Recent studies aimed at the standardisation of essential oil analysis have extended its use to the determination of *g*-pack values characteristic of the performance of individual columns⁶. However, so far no comparable index system has been adopted for liquid chromatography (LC).

The *n*-alkane scale cannot be adopted directly for LC because of the low polarity and thus limited retention range of the alkanes. They also lack a significant chromophore and would be inconvenient or impossible to detect on most instruments using absorbance detectors. Recently Baker and co-workers proposed a retention index scale based on the alkan-2-ones⁷ and examined the possibility of predicting the index value for a number of pharmaceuticals⁸⁻¹⁰. However, although they have a wide polarity range the alkan-2-ones have only a limited absorbance at 254 nm and the higher homologues are not widely available.

The use of retention index values would also enable the retentions of samples to be more easily compared. The present nearly universal use of capacity factors, while eliminating effects due to differences in column size and eluent flow-rate, cannot overcome the changes caused by small differences in solvent composition, and capacity factors, (k') reported by one laboratory are difficult to reproduce exactly even using the same column material. The trend in recent years to short microparticulate columns has also made the accurate determination of absolute values of $k' = (t - t_0)/t_0$ (where t = retention time and t_0 = retention time of an unretained compound) more difficult because of the uncertainty in the measurement of t_0 values of 1 min or less, compared to retention times of up to 30 min.

In the present paper we report the consideration of alternative retention index standards, which could have wide applicability, and discuss the detailed evaluation of the alkylarylketones as potential standards using different solvent and column systems.

EXPERIMENTAL

Materials

All standard and test compounds were reagent grade. Alkylarylketones used as standards were acetophenone (BDH, Poole, Great Britain), propiophenone (Hopkin and Williams, Great Britain), butyrophenone and valerophenone (Koch-Light, Poole, Great Britain), hexanophenone, heptanophenone and octanophenone (Aldrich, Poole, Great Britain).

Chromatography

Separations were carried out using Shandon columns (10 cm × 5 mm I.D.) which had been slurry-packed with 5- μ m packing materials, ODS-Hypersil and SAS-Hypersil (Shandon Southern, Runcorn, Great Britain), C₂₂-Magnusil (Magnus Scientific, Sandbach, Great Britain) or Spherisorb-Phenyl (Phase Separations, Queensferry, Great Britain).

Solvents were made up using HPLC grade methanol (Fisons, Loughborough, Great Britain) and distilled water and were pumped at 1 ml min^{-1} using a Pye-Unicam XPS Pump or LDC DK Pump. The samples (usually $10 \mu\text{l}$) were injected using a Rheodyne 7125 syringe valve and detected with an Altex 153 absorbance detector at 254 nm and sensitivity 0.08 a.u.f.s.

Standard test mixtures

Retention index standards were made up using 25 or $50 \mu\text{l}$ of alkylarylketone in 10 ml of methanol, which was diluted 100-fold in methanol-water (50:50) before use.

Aliphatic ketones were examined at a concentration of $100 \mu\text{l}$ in 10 ml of methanol, and test compounds at 1 – $10 \mu\text{l}$ in 10 ml of methanol.

Retention index (I) determinations

Reported capacity factors are the mean values of repeated determinations. Using a least-squares correlation, $\log k'$ values for the standards were compared with the number of carbon atoms $\times 100$ (*i.e.* acetophenone $I = 800$). The retention index values of test compounds were determined from the equation $\log k' = aI + b$ using the values of a and b derived from the standard correlation curve. The values could also have been obtained from a semi-log plot of k' and $C_n \times 100$ (Fig. 1).

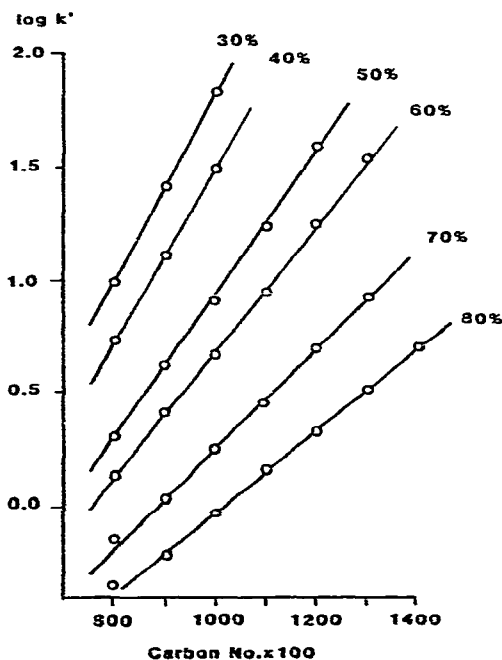


Fig. 1. Capacity factors for alkylarylketones compared with retention index (carbon number $\times 100$) using different percentages of methanol as eluent.

TABLE I
CAPACITY FACTORS AND RETENTION INDEX VALUES FOR ALKYLARYLKETONES AND TEST COMPOUNDS ON ODS-HYPERSIL WITH DIFFERENT SOLVENTS

Compound	Capacity factors, k' Solvent (methanol, %)					Retention index Solvent (methanol, %)					Nominal		
	30	40	50	60	70	80	30	40	50	60		70	80
<i>Retention index standards</i>													
Acetophenone	9.96	5.54	2.00	1.35	0.72	0.45	800	800	804	803	816	816	800
Propiophenone	25.9	13.1	4.04	2.58	1.07	0.61	900	899	900	904	894	891	900
Butyrophenone	67.9	31.3	8.00	4.59	1.74	0.93	1000	1000	993	993	991	995	1000
Valerophenone			17.3	8.79	2.88	1.42			1098	1094	1091	1099	1100
Hexanophenone			38.0	17.5	4.91	2.09			1205	1202	1198	1194	1200
Heptanophenone				33.6	8.32	3.25			1303	1302	1302	1302	1300
Octanophenone					14.1	4.97					1407	1407	1400
<i>Samples</i>													
Acetone	0.53	0.50	0.27	0.26			494	522	531	546			
Butan-2-one	1.19	0.96	0.50	0.51			579	600	614	651			
Pentan-2-one	3.09	2.13	0.95	0.78	0.55		678	690	703	718	761		
Hexan-2-one	8.78	5.53	2.00	1.47	0.77		787	800	804	816	829		
Octan-2-one			10.1	5.73	2.11	1.16			1025	1028	1029	1049	
Undecan-2-one					10.3	3.84					1344	1343	
Benzyl alcohol	3.84	2.57	1.08	0.83	0.48		701	711	720	727	735		
Benzaldehyde	6.67	4.16	1.58	1.14			758	767	772	776			
2-Phenylethanol	7.61	4.65	1.75	1.21	0.65		772	780	786	786	795		
<i>p</i> -Cresol	9.78	5.81	2.04	1.41	0.73	0.50	798	806	807	810	818	842	
Nitrobenzene	11.5	7.38	2.67	1.82	0.94	0.64	815	834	843	845	869	903	
Methyl benzoate	26.2	15.1	4.33	2.66	1.26	0.82	901	916	909	909	926	963	
Phenetole	42.9	26.1	7.08	4.75	2.03	1.18	952	979	976	999	1022	1053	
Toluene		30.6	9.08	5.63	2.31	1.39		998	1010	1025	1047	1094	

RESULTS AND DISCUSSION

Selection of retention index standards

For a series of compounds to be widely applicable as retention index standards, they should if possible satisfy the following criteria:

- (1) they should have a strong chromophore at 254 nm so that they can be added to unknown samples in small amounts to act as internal standards;
- (2) they should not be readily ionised to avoid changes in retention because of pH variations or the presence of ion-pairing reagents;
- (3) a range of members of the series should be readily available at reasonable cost;
- (4) the most polar member of the series should be eluted with a similar retention to water-soluble pharmaceuticals;
- (5) the standard compounds must be unreactive and stable in common LC solvents.

Although in GLC a homologous series of compounds is used as retention standards, in LC, which is based on polarity rather than volatility, either a homologous series or a series of compounds with repeating functional groups can be considered as possible standards. The latter could be based on systems such as the carbohydrates $H(CH_2OH)_nOH$ or poly-substituted aromatic systems, *i.e.* nitrobenzenes $Ar(NO_2)_n$. However, in neither case is a sufficiently wide range of compounds available, and the directive effect of aryl substituents means that the polarity effects are not cumulative. The carbohydrates would also require the use of a refractive index detector.

On the other hand the capacity factors of members of a homologous series have previously been shown to possess a simple relationship to carbon number, $\log k' = aC_n + b$, in which the values of a and b vary with the solvent¹¹⁻¹³. However, most simple monofunctional aliphatic compounds, including the alkan-2-ones used by Baker and Ma⁷, have only a weak chromophore (acetone¹⁴: λ_{max} , 265 nm; ϵ , $17\ 1\ mol^{-1}\ cm^{-1}$) and in the present study a concentration of 1% of the alkanones had to be used to obtain reasonable signals. Conjugated aliphatic compounds such as the 2-alkenals might be suitable, but series of homologues are not readily available.

Aromatic compounds, particularly those with conjugated systems, have medium-to-strong chromophores and would be easy to detect. The need for a fairly polar smallest homologue, in order to cover the wide range of retentions of samples of interest in HPLC, excludes the use of the relatively non-polar simple aryl hydrocarbons, ethers, halogen or nitro compounds, based on a comparison of their capacity factors^{15,16}. More polar systems such as carboxylic and sulphonic acids, amines and phenols are also excluded because of their sensitivity to pH changes. The remaining groups include alcohols, benzoate and phenyl alkanoate esters, amides and alkylarylketones. Of these only the phthalate esters and ketones are readily available although if needed the benzoates should be easy to synthesise. The phthalates, unfortunately, increase by two methylene groups between members and rapidly become very non-polar. It was therefore decided to examine the methyl to heptylphenylketones as the basis of a possible retention index system. Previously these compounds, which were all readily available, have been suggested as generally applicable relative retention standards although their use to form a scale was not examined¹⁷.

Alkylarylketones as a retention index scale

Using an ODS-Hypersil column and varying compositions of methanol-water (from 30% to 80%), the k' values of the alkylarylketones were compared with those of the alkan-2-ones and a number of test compounds (Table I). Using the k' values for the alkylarylketones, a retention index scale was derived based on number of carbon atoms $\times 100$ (*i.e.* acetophenone = 800) using a least-squares correlation. As expected from previous work the homologues showed a close correlation between $\log k'$ and $C_n \times 100$ (Fig. 1), and the slope and intercept of the curve changed with the composition of the solvent (Table II and Fig. 1). Using these calibrations retention index values were determined for all the compounds examined (Table I). Interestingly, benzaldehyde clearly does not behave as the zero member of the ketone series. The small deviations in the index values of the alkylarylketones from their nominal values are a guide to the precision of the results. The alkan-2-ones also followed a linear relationship between $\log k'$ and C_n but the slope was different from that determined for the aromatic ketones. The retention index values of the test compounds showed only a small but general increase with increasing proportion of methanol in the solvent, (Fig. 2), in contrast to a marked change of up to 300 units found by Baker and Ma⁷ for selected drugs when the alkan-2-one scale was used. Small variations in solvent composition would therefore have little effect on index values.

TABLE II

CORRELATION FACTORS FOR ALKYLARYLKETONE REFERENCE STANDARDS UNDER DIFFERENT CHROMATOGRAPHIC CONDITIONS AND COLUMNS*

Solvent (methanol, %)	Slope $\times 10^3$	Intercept	Correlation
<i>ODS-Hypersil</i>			
30	4.165	-2.33	0.9999
40	3.76	-2.27	0.9999
50	3.19	-2.27	0.9995
60	2.79	-2.11	0.9997
70	2.18	-1.92	0.9991
80	1.77	-1.79	0.9992
<i>SAS-Hypersil</i>			
30	3.35	-2.041	0.9997
<i>C₂₂-Hypersil</i>			
30	2.921	-1.8857	0.9986
<i>Spherisorb-phenyl</i>			
30	2.379	-1.4532	0.9987

* For the equation $\log k' = a(n \times 100) + b$.

Comparison of the alkylarylketones and alkan-2-ones shows that the lower members of the aliphatic series were more polar, the index value for acetone (*ca.* 500) requiring an extrapolation of the aromatic scale. Although this might suggest a limitation of the aromatic ketone scale when water-soluble drugs are being examined, in the previous study of barbiturates and propranolol analogues most of the compounds had retention index values on the aliphatic scale greater than 500, acetophenone having values of *ca.* 600⁸. Thus the alkylarylketone scale would largely cover these relatively polar compounds.

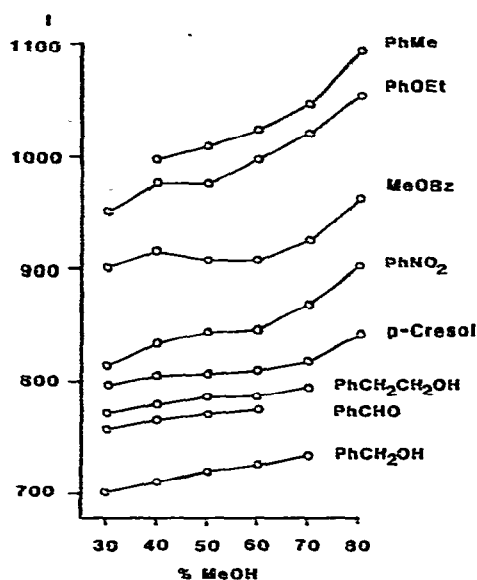


Fig. 2. Variation of retention index values of test compounds with eluent composition. Me = Methyl; Et = ethyl; Ph = phenyl; Bz = PhCO.

TABLE III

CAPACITY FACTORS AND RETENTION INDEX VALUES OF ALKYLARYLKETONES AND TEST COMPOUNDS ON SAS-HYPERSIL, C₂₂-MAGNUSIL AND SPHERISORB-PHENYL

Compound	SAS-Hypersil 30% methanol-water		C ₂₂ -Magnusil 30% methanol-water		Spherisorb-phenyl 30% methanol-water	
	k'	I	k'	I	k'	I
<i>Retention index standards</i>						
Acetophenone	4.40	801	2.92	805	2.96	809
Propiophenone	9.24	897	5.43	897	4.76	895
Butyrophenone	20.6	1001	10.3	992	8.00	990
Valerophenone			22.2	1106	14.4	1098
Hexanophenone					26.3	1208
Heptanophenone						
Octanophenone						
<i>Test compounds</i>						
Acetone	0.36	477	0.33	481	0.38	436
Butan-2-one	0.71	565	0.57	562	0.61	521
Pentan-2-one	1.56	667	1.03	650	1.02	614
Hexan-2-one	3.57	774	2.13	758	1.73	711
Octan-2-one			11.47	1008	4.88	900
Benzyl alcohol	1.71	677	1.08	657	0.69	543
Benzaldehyde	3.07	755	1.97	746	2.11	747
2-Phenylethanol	2.90	747	1.80	733	1.08	625
p-Cresol	3.69	779	1.90	741	1.04	618
Nitrobenzene	4.93	816	2.70	793	2.85	802
Methyl benzoate	9.06	895	5.13	889	3.68	848
Phenetole	10.46	914	5.36	895	2.96	809
Toluene	8.88	892	4.43	867	2.08	745

The aromatic system is considerably more sensitive to absorbance detection (acetophenone¹⁴: λ_{\max} , 199, 240, 278 nm; ϵ , 19,953, 12,589, 1071 l mol⁻¹ cm⁻¹), only 0.0025% (v/v) solutions being needed compared to 1% solutions of the aliphatic compounds. This is particularly useful for the longer chain compounds, which have a low solubility in methanol-water mixtures. Thus a set of standards could be added to an analyte sample in low concentration, without significantly altering the test solution.

Using only a single solvent system in each case all the compounds were also examined using SAS-Hypersil (Short Alkyl Silyl), C₂₂-Magnusil and Spherisorb-phenyl columns (Table III). In each case the calibration curve was again linear (Table II), but the values of the retention indexes of the test compounds differed between the columns.

Further studies making use of these differences as a method of characterising column materials in a similar way to the Rohrschneider constants in GLC, will be reported in the following paper¹⁸.

As the majority of LC analyses are carried out at ambient temperature, the effect of different column temperatures has not yet been examined but it would not be expected to have a dramatic effect over small temperature ranges.

CONCLUSION

The alkylarylketones represent an easy way to determine a retention index value for test compounds, which is relatively insensitive to changes in solvent composition. The standard ketones show a linear relationship between log k' and carbon number, and are readily detected using an absorbance detector. They cover the expected retention range of all but the most polar compounds, and have the potential to be used as the basis of tests of column polarity.

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