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COMPARISON OF REVERSED-PHASE LIQUID CHROMATOGRAPHY COLUMNS USING "ROHRSCHNEIDER" TYPE CONSTANTS

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SUMMARY

Using a set of aromatic reference compounds, the retention characteristics of reversed-phase high-performance liquid chromatographic columns can be expressed in terms of "Rohrschneider" type constants. These are based on the differences in retention indices of the reference compounds using the alkylarylketone scale, between the test column and solvent conditions and an ideal hexane–water partition system. The system can be used to quantitatively differentiate between phases coated with different alkyl chains and phenyl groups, and could be used to define the retention specifications of a column.

INTRODUCTION

It is often difficult to compare the results of reversed-phase liquid chromatography obtained in different laboratories, because of differences in columns coated with nominally identical bonded phases, particularly octadecylsilyl (ODS silica, and in variations in the ways results are expressed either as capacity factor, k', which is very susceptible to solvent composition, relative retention times to standards, or retention times at a particular flow-rate. Part of this problem could be largely solved by the use of a retention index scale based on a widely accepted homologous series. Two alternative systems using alkylarylketones[1] or alkan-2-ones[2] have been proposed and in both cases index values have been found to vary only slightly with solvent composition but to be dependent on column bonded phase.

It is harder to compensate for differences between columns because these often result from the different methods used to bond the stationary phase to the silica and the extent to which unreacted silica hydroxyl groups are capped[3]. It is therefore very difficult to define columns for use in a particular application such as an official method without specifying a particular make or brand or to select alternatives to a column reported in the literature. It would be highly desirable if the polarity and absorption of a column material could be determined in some way similar to the Rohrschneider[4] or McReynolds[5] constants used in gas–liquid chromatography (GLC), which could then be used to define the retention performance required to

carry out an assay and to compare columns of different makes or coated with different phases.

The present paper proposes a method for defining retention characteristics based on the use of the alkylarylketone-retention index-scale and a set of reference compounds the index values of which are compared to a standard system. The outline of a similar proposal based on the alkan-2-one scale and a standard ODS-column was mentioned by Baker but has not been examined further[2].

EXPERIMENTAL

Retention index values

Index values for reference compounds were determined as described previously[1].

Partition coefficients

Hexane–water partition coefficients were measured at $25 \pm 1°C$ using HPLC-grade hexane (Fisons Scientific, Loughborough, Great Britain) and distilled water. The concentration of the test compound in the hexane phase was determined by UV spectroscopy.

RESULTS AND DISCUSSION

In order to be able to produce a set of factors characteristic of the retention behaviour of a chromatography column, two components are needed. Firstly in order to eliminate differences in retention due to flow-rate, column length, and if possible solvent composition, a set of retention index standards are needed to provide a scale independent of absolute k' values. Secondly, a set of retention-test reference compounds are required, chosen so that they are characteristic of a range of structural types to test different possible interactions with the column. The index values of the test compounds could in themselves serve as retention factors but as with the Rohrschneider constants in GLC[4] the difference in index values compared to a standard column of optimum low polarity would be a more useful guide.

As reported in the previous paper, the alkylarylketones, which can be readily detected by UV detectors, can be used to provide an index scale which is largely independent of solvent compositions[1] and this scale was adopted as the basis of the present work.

Set of test compounds

In their choice of test compounds for use in GLC, Rohrschneider[4] and McReynolds[5] selected compounds, which were characteristic of typical sample groups. The final set of test substances, benzene, ethanol, methyl *n*-propyl ketone, nitropropane, pyridine, and in some cases 2-methyl-2-pentanol and iodobutane, would not be suitable for HPLC as most have only weak chromophores and could not easily be detected at 254 nm.

Initially therefore a number of aromatic compounds, benzaldehyde, benzyl alcohol, 2-phenylethanol, *p*-cresol, methyl benzoate, phenetole, nitrobenzene, and toluene were examined, the choice of compounds, *i.e.* methyl rather than ethyl ester,

being chosen so that 2 or 3 compounds could be examined in the same run. An attempt to include as a basic compound either ethylpyridine or β-phenylethylamine in the set was unsuccessful as without specially adjusting the solvent conditions only broad and distorted peaks were obtained. Because of their sensitivity to pH changes carboxylic acids were also not included. The capacity factors and retention index values for the test compounds on four reversed-phase columns using methanol–water (30:70) as eluent were determined earlier (Table I)[1].

TABLE I

RETENTION INDEX VALUES OF TEST COMPOUNDS*

From ref. 1, solvent methanol–water (30:70).

Test compound	Column			
	ODS-Hypersil	SAS-Hypersil	C_{22}-Magnusil	Spherisorb-phenyl
Benzaldehyde	758	755	746	747
Phenetole	952	914	895	809
Toluene	998*	892	867	745
Methyl benzoate	901	895	889	848
Nitrobenzene	815	816	793	802
Acetophenone	800	800	800	800
Benzylalcohol	701	677	657	543
2-Phenylethanol	772	745	733	625
p-Cresol	798	779	741	618

* Solvent methanol–water (40:60).

2-Phenylethanol and benzyl alcohol behaved in a very similar way, the difference between their index values remaining nearly constant on all four columns, the former was therefore not studied further. The index value of benzaldehyde remained virtually constant relative to the alkylarylketone scale suggesting that similar factors effected the aldehyde and ketone groups. The remaining six compounds, toluene, phenetole, methyl benzoate, nitrobenzene, benzyl alcohol and p-cresol, could be classified using the differences between their partition coefficients (log P) in octanol–water and hexane–water (Table II). This is a guide to their polarities and roughly follows the elution order (Table I). The set of test compounds thus includes compounds both more and less polar than the ketone index scale. The differences between the log P values of the test compounds in the two systems emphasises the effect of sample polarity on the distribution into the non-aqueous phase.

Standard reference-partition system

Although the index values of the test compounds could themselves be used to characterise the retention properties of different columns or packing materials, they cannot be related directly to the properties of the column. This is because the index value reflects both the polar interaction of the functional group of the compounds with the stationary phase and the lipholicity of the hydrocarbon grouping which depends on the size and shape of the molecule. As it is the former interaction which is

TABLE II

PARTITION COEFFICIENTS FOR TEST COMPOUNDS AND ALKYLARYLKETONES IN
OCTANOL–WATER AND HEXANE–WATER

Compound	Log P*		$Log\ P_{octanol-water}\ -$ $Log\ P_{hexane-water}$
	Octanol–water	Hexane–water	
Phenetole	2.51	3.05**	−0.54
Toluene	2.58	2.87	−0.29
Methyl benzoate	2.17	2.19**	−0.02
Nitrobenzene	1.84	1.47	0.37
Benzylalcohol	1.02	−0.76	1.78
p-Cresol	1.95	−0.35	2.30
Acetophenone	1.66	1.15	0.51
Propiophenone	2.20	1.97**	0.23
Butyrophenone	(2.70)***	2.74**	(−0.04)

  * From ref. 6 unless stated.
 ** Measured in this study.
*** Predicted value as addition of CH_2 usually increase log $P_{octanol-water}$ by 0.5.

primarily of interest it is necessary to compare the index values with a standard system in which the polar interaction with the stationary phase is minimised.

Any attempt to use as a standard an alkane-bonded silica column/mobile phase system, such as the ODS-silica/water system proposed by Baker and Ma[2], will be difficult to implement because of a lack of a guaranteed degree of coating or capping of the column. There will almost always be some residual polar interaction present.

The alternative is a reproducible two-phase liquid–liquid partition system, the distribution coefficients of which for the test compounds can be determined. These values can then serve as a standard against which the effective distribution coefficients of the column systems can be compared. Although a lot of work has been carried out using the octanol–water partition system because of its similarities to biological systems, octanol has considerable polarity and would interact with the polar test compounds. Test column systems could then have both greater and lesser interactions and interpretation of the results would be confused.

It would therefore be desirable to use an alkane–water reference partition system to represent an extreme non-polar–polar interaction system. In much the same way Rohrschneider and McReynolds in devising constants for GLC used squalane as their standard stationary phase, because it was the least polar stationary phase in common use.

Because the distributions coefficients for many of the test compounds are already available it is therefore proposed to use as a baseline standard the hexane–water partition system. In order to make comparisons easier to determine, rather than using distribution coefficients of the standard system and test columns, the theoretical retention index values for the test compounds will be calculated as if they had been measured on a hexane–water liquid–liquid partition column.

Since the capacity factor k' of a partition chromatography system is proportional to the distribution coefficient P (eqn. 1), the relationship eqn. 2 can be derived.

$$k' = P \frac{\text{(Volume of stationary phase)}}{\text{(Volume of mobile phase)}} \tag{1}$$

$$\log k' = \log P + \text{constant} \tag{2}$$

It has been shown for a homologous system that the capacity factor depends on the number of carbon atoms (eqn. 3)[7]. Thus for all compounds in the same system, k' must depend on their retention index (I) values (eqn. 4).

$$\log k' = a\, C_n + b \tag{3}$$

$$\log k' = a'\, I + b' \tag{4}$$

Combining eqns. 2 and 4 gives eqn. 5 and hence if two compounds are compared we obtain eqn. 6.

$$I = \alpha \log P + \text{constant} \tag{5}$$

$$\Delta I = \alpha\, \Delta \log P \tag{6}$$

The partition coefficients of homologous alkylarylketones in hexane–water (Table II) give a mean value for $\Delta \log P = 0.793$ and hence $\alpha = 126$. Although in octanol–water systems $\Delta \log P$ for different homologous series is approximately 0.5, the reported values for hexane–water partitions are very variable, e.g. n-alkanols 0.58, ω-arylalkanols ($Ph(CH_2)_n OH$) 0.43, alkylpyridines 0.66 and alkylamphetamines 0.91[6].

Using the experimental value for α and the partition coefficients of the test compounds (Table II) their theoretical retention indices in hexane–water can be calculated from eqn. 6 (Table III).

Although it might seem that compounds with the same carbon skeletons should have similar index values, on the assumption that hexane would show little or no polar interaction with the test compounds, different interactions will occur with the aqueous phase.

Retention constants of reversed-phase columns

Comparison of the hexane–water indices of the test compounds with their retention indices from Table I gives a set of constants which represent the relative interaction of the test compounds with the four columns in comparison with the interaction of the alkylarylketones (Table IV). Because of its long retention the index value for toluene on ODS-silica was not measured and the value for methanol–water (40:60) has been used. From the earlier study this is expected to be very similar[1].

As can be seen the different groups interact as expected, greater retentions being found for the more polar compounds, implying some residual polarity in the alkyl-bonded reversed-phase columns.

TABLE III

CALCULATION OF THEORETICAL INDEX VALUES FOR HEXANE–WATER SYSTEMS

Test compound	Log P	Δ Log P*	ΔI**	I
Phenetole	3.05	1.90	239	1039
Toluene	2.87	1.72	217	1017
Methyl benzoate	2.19	1.04	131	931
Nitrobenzene	1.47	0.32	40	840
Acetophenone	1.15	0.00	0	800
Benzylalcohol	−0.76	−1.91	−241	559
p-Cresol	−0.35	−1.50	−189	611

* $\log P - \log P$ (acetophenone).
** From $\Delta I = \alpha\Delta \log P$: $\alpha = 126$.

Each column gives a different set of constants characteristic of the retention properties of the column-eluent combination, variations between columns such as the change in relative interaction of toluene and methyl benzoate, very similar on ODS-Hypersil but markedly different on SAS-Hypersil, being clearly indicated.

The considerable differences observed for the phenyl-bonded phase have been examined further and will be discussed in more detail in a subsequent paper[8].

Using indices reported previously[1] the variations of ODS-Hypersil with solvent composition was calculated (Table V). There is a slow but steady change on moving to higher percentages of methanol presumably as a result of differences in the interactions with the mobile phase, a problem not faced with the GLC constants. Therefore comparisons between columns should always be made using the same eluent solvent in each case, ideally this should be water but for most columns this would be impractical as k' of the reference compounds would be too long.

TABLE IV

RETENTION CONSTANTS FOR TEST COMPOUNDS ON FOUR COLUMNS

Retention constant = retention index − retention index (hexane–water). Mobile phase methanol–water (30:70).

Test compound	Column			
	ODS-Hypersil	SAS-Hypersil	C_{22}-Magnusil	Spherisorb-phenyl
Phenetole	−87	−125	−144	−230
Toluene	−19*	−125	−150	−272
Methyl benzoate	−30	−36	−42	−83
Nitrobenzene	−25	−24	−47	−38
Acetophenone	0	0	0	0
Benzylalcohol	142	118	98	−16
p-Cresol	187	168	130	7
Σ_6^1 constant	168	−24	−155	−632
Σ_6^1 constant	490	596	611	646

* Mobile phase methanol–water (40:60).

TABLE V.

VARIATION IN RETENTION CONSTANTS WITH COMPOSITION OF MOBILE PHASE FOR ODS-HYPERSIL

Based on retention index values in ref. 1.

Test compound	Retention constants					
	Methanol (%)					
	30	40	50	60	70	80
Phenetole	−87	−60	−63	−40	−17	14
Toluene	−	−19	−7	8	30	77
Methyl benzoate	−30	−15	−22	−22	−5	32
Nitrobenzene	−25	−6	3	5	29	63
Acetophenone	0	0	0	0	0	0
Benzylalcohol	142	152	161	168	176	−
p-Cresol	187	195	196	199	207	231
Σ_6^1 constant	−	247	268	318	420	−
Σ_6^1 $\overline{constant}$	−	447	452	442	462	−

In order to be able to see if a simplification of the set of constants could be used as a rough guide to the overall retention ability of the column a number of ways were examined to relate the retention constants. The span of constants between phenetole and p-cresol varied from 274 to 224 on ODS-Hypersil but on the other three columns was 293, 274 and 237, and thus was little help. The summation of the constants, Σ_6^1 constant, was dependent on solvent (Table V) but varied markedly between stationary phases (Table IV) and therefore is a guide at constant solvent composition.

The one value, which was relatively intensitive to solvent composition was the summation of the absolute values of the constants, Σ_6^1 $\overline{constant}$, but the reason for this is not clear, although the other columns gave different values and it could be used as a guide.

It should be emphasised that these values and constants are relative retention values, the most retentive column (highest k' values) being the ODS-Hypersil. Similarly the change in the constant of toluene between ODS-Hypersil and C_{22}-Magnusil is more probably due to a change in the index scale, caused by a change in the RCOPh interaction, rather than a large difference in the toluene interaction.

The variations in retention indices of the test compounds on different stationary phases, emphasise a problem in the use of HPLC to directly determine log $P_{octanol-water}$ for structure activity studies, unless the column is impregnated with octanol[9]. Similarly attempts to predict retention indices on ODS-silica from log $P_{octanol-water}$ values or Hansch π additivity constants will be difficult if polar groups are present. If the change is in an alkyl chain prediction is easier and Baker obtained a good correlation for calculated relative k' values for the alkyl barbiturates[10] and N-substituted 3-propananilidonortropane analogues[11]. However attempts to predict the retentions of propranolol analogues[10] and of narcotics and related compounds[11] gave only poor correlations, in the latter case discrepancies between observed and calculated values varying from +200 to −422 index units. Polar and in particular

ionisable groups caused the largest difficulties. Equivalent changes to different molecules such as the metabolic formation of glucuronides have been shown to cause similar relative changes in retention index[12], even though the additional group is very polar.

As is clear from the present study log $P_{\text{octanol-water}}$ and log $P_{\text{hexane-water}}$ can differ greatly and both can differ from the apparent log $P_{\text{ODS-silica/methanol-water}}$.

CONCLUSIONS

Using the alkylarylketones as a reference scale the retentions of a set of test compounds can be compared with a theoretical hexane–water partition chromatograph to give a set of retention constants which are a guide to the retention properties of a column eluent combination. The retention constants differ between columns and change with eluent composition.

REFERENCES

1 R. M. Smith, *J. Chromatogr.*, 236 (1982) 313.
2 J. K. Baker and C.-Y. Ma, *J. Chromatogr.*, 169 (1979) 107.
3 K. K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979.
4 L. Rohrschneider, *J. Chromatogr.*, 17 (1965) 1.
5 W. O. McReynolds, *J. Chromatogr. Sci.*, 8 (1970) 685.
6 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biochemistry*, Wiley, New York, 1979.
7 H. Colin and G. Guiochon, *J. Chromatogr. Sci.*, 18 (1980) 54.
8 R. M. Smith, *J. Chromatogr.*, (1982) in press.
9 S. H. Unger, J. R. Cook and J. S. Hollenberg, *J. Pharm. Sci.*, 67 (1968) 1364.
10 J. K. Baker, *Anal. Chem.*, 51 (1979) 1693.
11 J. K. Baker, R. E. Skelton, T. N. Riley and J. R. Bagley, *J. Chromatogr. Sci.*, 18 (1980) 153.
12 J. K. Baker, *J. Liquid Chromatogr.*, 4 (1981) 271.