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THE USE OF RETENTION INDICES TO MEASURE THE SOLVENT SELECTIVITY OF TERNARY ELUENTS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

The retention indices, measured on the alkylarylketone scale, of a set of column test compounds (toluene, nitrobenzene, *p*-cresol, 2-phenylethanol, and *N*-methylaniline, are used to determine the changes in selectivity of a series of ternary eluents prepared from methanol–0.02 *M* phosphate buffer pH 7 (60:40), acetonitrile–0.02 *M* phosphate buffer, pH 7 (50:50) and tetrahydrofuran–0.02 *M* phosphate buffer, pH 7 (35:65). The analyses were carried out on a Spherisorb ODS reversed-phase column. The selectivity changes were often non-linear between the binary compositions.

INTRODUCTION

One of the principal advantages of reversed-phase high-performance liquid chromatography (HPLC) compared to other analytical techniques is the ability to examine a wide range of samples by using different combinations of columns and eluents. Part of this versatility is a result of the different bonded phases which are available but mainly because the overall polarity and selectivity of the eluent can be changed by the use of different organic modifiers, the addition of ion-pair reagents or the control of the pH.

The solvophobic theory of separation suggests that whereas the hydrocarbonaceous stationary phase is largely inert and has little effect on selectivity, the interaction of a sample with the mobile phase is mainly responsible for selectivity differences. However, the possible choice of organic modifiers is limited by their availability in a pure state, compatibility with detectors, cost, and viscosity. Glajch *et al.*¹ have proposed, on the basis of the sample–solvent interactions on a selectivity triangle^{2,3}, that the three solvents methanol, acetonitrile and tetrahydrofuran provide a range of different types of interactions which the analyst can exploit to give wide differences in selectivity.

Using this concept a number of groups have developed optimisation techniques for the separation of mixtures in reversed-phase chromatography based on mapping or simplex routines for ternary and quaternary eluent mixtures of these solvents with water as an overall polarity modifier^{4–9}.

A disadvantage of these optimisation techniques is that they either require a full knowledge of the experimental retentions over a range of eluent compositions for mapping methods or use a sequential multistep trial and error series of measurements to locate the optimum separation in the simplex method. It should be possible to relate the retentions in binary eluents to separations with ternary eluents and thus predict likely suitable combinations, which should reduce the number of steps needed to determine the optimum ternary mixtures.

However, because of the poor reproducibility of methods in HPLC between different columns or laboratories each of these studies will only be valid as the optimisation of that particular system of column and eluent. Whereas the techniques are valuable as general methods, the results are unique and the whole procedure has to be repeated for each new sample and chromatographic system. This poor transferability between systems is a consequence of differences in the methods used to report retentions, the different specific interactions of nominally identical columns, and small interlaboratory differences in eluent compositions, temperature and eluent pH.

In order to derive relationships and results which can be used in optimisation studies, it is therefore important to be able reproducibly to measure and report retentions and hence selectivity. Although capacity factors are widely used in liquid chromatography, the values are very dependent on the accurate measurement of the column void volume, whose value frequently differs depending on the measurement technique¹⁰. In earlier studies with binary eluents the retention index (RI) values of analytes, based on the alkylarylketone scale, were shown to provide an alternative more robust method for recording and reporting retentions. The retention indices are largely independent of the exact value of the void volume, eluent composition, and chromatographic conditions¹¹⁻¹³. This work led to a comparison of the selectivity of columns of different bonded phases using the retention indices of a series of column test compounds in a similar technique to the use of Rohrschneider constants in gas-liquid chromatography^{14,15}. The column test compounds were also used to distinguish between isoelutotropic binary eluents containing different organic modifiers and could quantify the small specific selectivity differences between nominally equivalent octadecylsilyl bonded column materials¹².

The present paper uses these concepts to determine the selectivities, using a single column, of a set of ternary eluents prepared by combining binary mixtures of methanol, acetonitrile and tetrahydrofuran. The method provides quantitative selectivity measurements and reproducible retention data that could form the basis of a more theoretical approach to optimisation and would enable the selectivity of different systems to be directly compared.

EXPERIMENTAL

Chemicals

Alkylarylketones, acetophenone, propiophenone, butyrophenone, and valero-phenone, and column test compounds, toluene, nitrobenzene, 2-phenylethanol, *p*-cresol, and *N*-methylaniline were laboratory grade from a number of sources. Methanol, acetonitrile and tetrahydrofuran (HPLC grade) and potassium dihydrogen phosphate (AR grade) were from Fisons Scientific Equipment, Loughborough (U.K.).

Equipment

HPLC was carried out on a 5- μ m Spherisorb ODS column (10 cm \times 5 mm) at ambient temperature using a Pye Unicam 4010 pump and an Altex 153 fixed wavelength detector at 254 nm. Samples (10 μ l) were injected using a Reodyne 7125 valve and the results were recorded using a Hewlett-Packard 3390 integrator. The eluents were prepared by volume from the organic modifiers (methanol, acetonitrile and tetrahydrofuran) and 0.02 M aqueous potassium dihydrogen phosphate buffer, which had been adjusted to pH 7.0. A solution of sodium nitrate was used to determine the column void volume.

Procedures

Triplicate samples of the retention index alkylarylketones and the five column test compounds were injected. The retention indices were calculated from the mean capacity factors as described earlier based on $RI_{\text{ketone}} = \text{carbon number} \times 100$ (ref. 11).

RESULTS AND DISCUSSION

It was proposed to measure the retention of the column test compounds in a range of eluent combinations based on three binary eluents in order to test the ability of the retention indices of the column test compounds to discriminate between the selectivities of the ternary eluents.

Snyder³ has proposed that there are three principal sample-solvent interactions (electron donating, electron withdrawing and dipole), and therefore a minimum of three test compounds with different interaction characteristics, together with retention index standards to test polarity effects, should be sufficient to characterise any separation system. In a previous study the selectivities of a series of binary eluent-column systems were compared, based on the retention indices of the four test compounds, toluene, nitrobenzene, *p*-cresol and 2-phenylethanol, calculated using the alkylarylketone scale¹². These compounds had been selected initially as representatives of different Snyder selectivity groups and their ability to discriminate between eluents and column materials was confirmed by a multivariate analysis.

However, because of residual uncapped silanol groups, most alkyl bonded silica columns also show some specific column interaction effects as a result of silanophilic ion-exchange activity. This can cause problems of poor peak shape and retention reproducibility particularly for amines and other basic compounds¹⁶. *N*-Methylaniline was therefore also examined in this study to test if it would provide any additional information. However, if it was not ionised it should not add to the overall selectivity assessment, as it should reflect interaction effects already characterised by the other neutral test compounds. Aniline, the only aromatic amine studied by Snyder³, had similar solvent interaction properties to nitrobenzene and non-ionised *N*-methylaniline might therefore be anticipated to behave in an analogous manner.

Based on the reported relative elution strengths of the organic modifiers (methanol, 2.6; acetonitrile, 3.1; tetrahydrofuran, 4.4; and water, 0.0)¹⁷, isoelutropic eluents of 60% methanol, 50% acetonitrile and 35% tetrahydrofuran with a phosphate buffer were selected as the binary starting points for the present study. Com-

TABLE I
CAPACITY FACTORS OF RETENTION INDEX STANDARDS AND COLUMN TEST COMPOUNDS IN TERNARY ELUENTS

Mobile phase, organic component-0.02 M phosphate buffer (pH 7). Key: PhMe, toluene; cresol, *p*-cresol; PhEtOH, 2-phenylethanol; PhNO₂, nitrobenzene; PhNHMe, N-methylaniline; PhCOMe, acetophenone, PhCOEt, propiophenone; PhCOPr, butyrophenone; PhCOBu, valerophenone.

Eluent (% composition)	Capacity factors										
	Aceto-nitrile	Tetrahydrofuran	PhMe	Cresol	PhEtOH	PhNO ₂	PhNHMe	PhCOMe	PhCOEt	PhCOPr	PhCOBu
60	0	0	3.05	0.82	0.78	1.42	1.02	1.10	1.82	2.99	5.22
48	10	0	3.05	0.94	0.89	1.50	1.18	1.18	1.90	3.00	5.06
36	20	0	3.25	1.02	0.92	1.65	1.34	1.25	2.05	3.25	5.52
24	30	0	2.93	0.85	0.76	1.51	1.24	1.10	1.84	2.92	4.86
12	40	0	3.42	1.01	0.84	1.88	1.55	1.33	2.17	3.37	5.42
0	50	0	3.51	1.09	0.91	2.02	1.76	1.43	2.24	3.31	4.97
0	40	7	3.94	1.30	0.95	2.13	1.85	1.41	2.27	3.46	5.35
0	30	14	4.37	1.52	1.02	2.29	2.01	1.42	2.34	3.62	5.67
0	20	21	5.71	2.04	1.21	2.87	2.49	1.64	2.82	4.49	7.62
0	10	28	8.99	3.12	1.49	4.26	3.46	1.99	3.88	6.74	11.98
0	0	35	9.98	3.79	1.61	4.94	4.11	2.12	4.19	7.31	12.96
12	0	28	7.82	2.90	1.34	3.86	3.07	1.69	3.22	5.63	10.22
24	0	21	6.18	2.11	1.18	2.95	2.24	1.35	2.49	4.28	7.73
36	0	14	5.41	1.77	1.09	2.48	1.83	1.26	2.26	3.89	7.07
48	0	7	3.94	1.17	0.86	1.73	1.27	1.01	1.76	2.94	5.27

binations of these eluents should then give ternary eluents with very similar elution strengths. To reduce any effects due to protonation of the N-methylaniline the aqueous component was buffered to pH 7.0. The same Spherisorb ODS column was used throughout the study to prevent any changes due to column variation.

The retention times of the four retention index standards (acetophenone to valerophenone) and the five column test compounds were measured in the three binary solvents and in three series of ternary mixtures prepared by combinations of the binary eluents. The column void volume was measured in each case with an aqueous solution of sodium nitrate and was found to vary from 1.1 min in methanol and acetonitrile–buffer eluents to 1.3 min in tetrahydrofuran–buffer eluent. As the same column was used throughout the study this variation illustrates the problems in determining an accurate value for the void volume. These changes could be caused by Donnan repulsion excluding the analyte from part of the column in polar eluents¹⁸. In addition, Jinno has found that the measured void volumes varied with the concentration of the sodium nitrate solution¹⁹.

Based on the measured retention times the capacity factors of the column test compounds were determined (Table I). Although the eluents were selected to be isoeluotropic, with the exception of *p*-cresol, the tetrahydrofuran–buffer combination is clearly a much weaker eluent. The changes in the capacity factors with eluent composition were irregular but there are clearly some differences in selectivity with eluent composition however these are not easy to quantify. In earlier studies Schoenmakers *et al.*²⁰ have found that the relationship between the capacity factors and ternary eluent composition was not linear but curved as the result of second order interaction effects. However, Colin *et al.*²¹ reported that if an alternative method for

TABLE II
RETENTION INDICES OF COLUMN TEST COMPOUNDS IN TERNARY ELUENTS
Conditions and samples as in Table I.

Eluent (% composition)			Retention indices					
Methanol	Acetonitrile	Tetrahydrofuran	Slope*	PhMe	<i>p</i> -Cresol	PhEtOH	PhNO ₂	PhNHMe
60	0	0	2.24	999	746	737	851	788
48	10	0	2.08	998	753	743	850	801
36	20	0	2.13	995	758	739	857	815
24	30	0	2.13	998	746	722	863	822
12	40	0	2.02	1001	739	701	872	831
0	50	0	1.79	1014	731	688	880	847
0	40	7	1.92	1029	779	707	891	858
0	30	14	1.99	1041	811	723	901	872
0	20	21	2.14	1048	840	734	909	881
0	10	28	2.58	1048	871	746	923	888
0	0	35	2.59	1053	891	747	935	905
12	0	28	2.57	1057	892	765	937	900
24	0	21	2.51	1061	875	775	933	886
36	0	14	2.48	1055	859	774	918	865
48	0	7	2.37	1049	828	771	898	842

* Slope of log k' against carbon number $\times 100$ for the alkylarylketones (PhCOMe to PhCOBu).

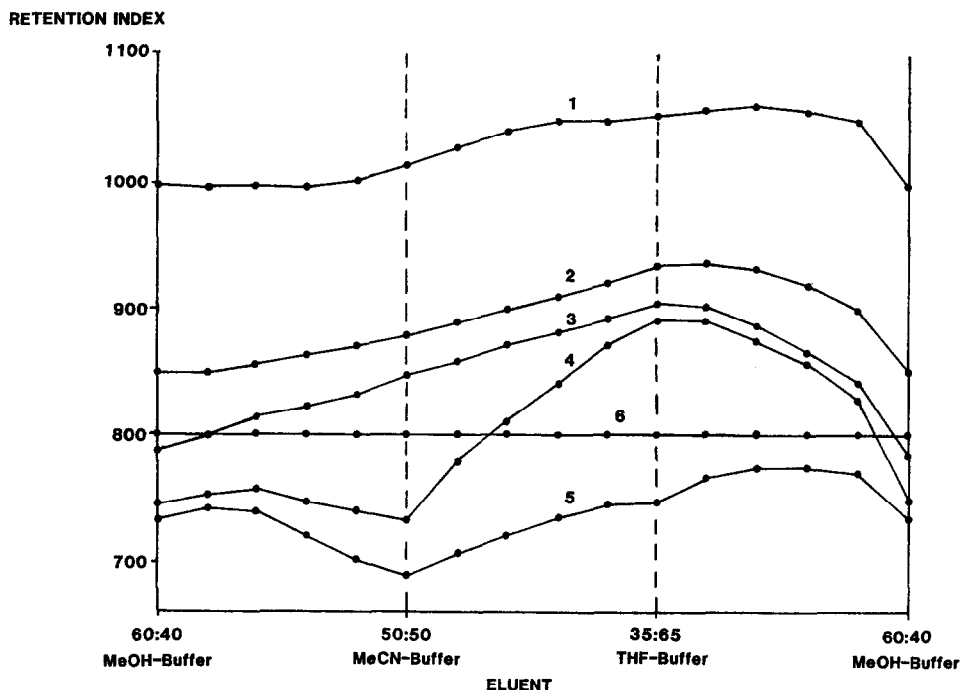


Fig. 1. Variation of retention indices of column test compounds with composition of ternary eluents. (1) Toluene; (2) nitrobenzene; (3) *N*-methylaniline; (4) *p*-cresol; (5) 2-phenylethanol; (6) acetophenone (standard RI = 800).

the determination of the void volume based on the linearisation of the retentions of the *n*-alkanols was used, there was a linear relationship between the capacity factors of analytes and the composition of ternary eluents.

For each eluent there was a linear relationship between the logarithm of the capacity factors and the carbon number of the alkylarylketones [$\log k' = a + b \times (\text{carbon number})$], correlations between 0.9996 and 0.9969]. Although the eluents should have been isoelutropic the slope (b) which represents the methylene selectivity of the system changed markedly with the eluent composition (Table II) and was much lower for acetonitrile than for methanol or tetrahydrofuran eluents.

Based on the capacity factors, the retention indices of the column test compounds were calculated (Table II). The values for the binary mixtures were very similar to the corresponding indices found previously for 70% methanol, 50% acetonitrile and 40% tetrahydrofuran–water eluents, respectively¹², confirming the insensitivity of retention indices to the proportion of organic component. Clear trends in selectivity are apparent, which are particularly obvious when the retention indices are plotted against eluent composition (Fig. 1). In contrast to the capacity factors the changes in the retention indices with eluent composition are regular. Almost all the observed relationships are curved, suggesting that the effect of the eluent on selectivity is dependent on secondary effects which differ with the analyte.

If the methanol–buffer and acetonitrile–buffer combinations are examined the

changes in the retention indices of the less polar test compounds are nearly linear with eluent composition but small deviations (± 10 units) occur to both higher and lower values. As predicted N-methylaniline behaved in a similar manner to nitrobenzene. The relatively small deviations are in accord with the work of Jandera *et al.*²² who concluded that the capacity factors of analytes in ternary acetonitrile-methanol eluents could be predicted with sufficient accuracy from a linear relationship between the binary retentions without using more complex relationships which would require additional test measurements at intermediate compositions. However, large deviations (up to 20 units) in retention indices were observed for the more polar samples, *p*-cresol and 2-phenylethanol. The ternary combinations of acetonitrile-buffer and tetrahydrofuran-buffer also show slightly curved relationships between retention indices and eluent composition.

The most noticeable deviations from linearity (up to 40 units) were observed for the tetrahydrofuran-buffer and methanol-buffer ternary eluents. In each case the curve was not symmetrical and the last few percent of THF seemed to cause the greatest change. A similar effect has been reported previously by Tanaka *et al.*²³, who found that the addition of small amounts of tetrahydrofuran to a methanol-water eluent had a much larger effect than subsequent larger additions. They attributed this effect to a displacement of methanol from the silanol groups on the silica surface by the less polar tetrahydrofuran and suggested that small amounts of tetrahydrofuran could therefore exert a major effect on selectivity without changing solvent strength.

As anticipated, in all the eluent combinations N-methylaniline and nitrobenzene behaved in a similar manner, confirming their similar interactions (Fig. 1). The amine is clearly not contributing any additional factors to the study of the overall eluent selectivity, which has been confirmed in a subsequent more detailed study of the effect of eluent composition, pH and temperature on the retention indices of homologous N-alkylanilines²⁴.

For each ternary system the relationship between the differences in the retention indices in the two binary eluents and the deviation from linearity in the intermediate ternary eluents was examined. However, there appeared to be no correlation between the two factors.

CONCLUSIONS

The retention of the test compounds can be used to distinguish between the differences in selectivity of the ternary eluents. In order to compare selectivities or optimise conditions it is much better to express the relative retentions as retention indices rather than as capacity factors as the former are more independent of the chromatographic conditions or the values adopted for the void volume. However, the retention indices cannot be derived by linear interpolation of the values in the corresponding binary eluents as in each case there was a non-linear relationship that was often unsymmetrical. In order to determine the full optimisation map it would therefore be necessary to make a number of measurements on intermediate ternary compositions.

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