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The Role of Stationary Phases in Reversed-Phase Liquid Chromatography in the Application of Solvent Optimization Procedures

The development of liquid chromatographic separation procedures, which meet high standards of reliability and ruggedness, is of increasing importance. A number of software programs are available to make method development in laboratory practice less time consuming and also less dependent on the degree of knowledge of the analyst. Method development, as well as the subsequent routine analysis, generally start from the assumption of a constant column quality (that is, selectivity). In this study the role of the column in method development in reversed-phase liquid chromatography was investigated. It is shown that the influence of nominally identical stationary phases from different manufacturers on the results of method development procedures is significant. This also implies that the transferability of chromatographic separation conditions between "identical" columns is poor.

Method development in high performance liquid chromatography (HPLC) is still not straightforward and suffers from non-systematic, time-consuming procedures in many laboratories. Many application procedures are determined using trial-and-error approaches. The resulting methods depend strongly on the knowledge and experience of the chromatographer. In a number of instances, this may lead to an uneconomic use of time and materials. In general, method development can be performed from one of three starting points.

- *Trial-and-error procedures.* The separation is performed using a number of often arbitrarily selected eluent mixtures. The eluent composition displaying the best results is adopted for use.
- *Sequential optimization procedures.* Each eluent composition is based on the results of previous experiments. By performing this step-by-step procedure, an optimum eluent composition may be achieved.
- *Modelling methods.* The systematic changes in the eluent composition are controlled and explained using a retention model. Subsequently, from a limited number of experiments, the optimal eluent composition is mathematically predicted.

Methods 1 and 2, and especially method 1, are very often applied in laboratory practice. The large number of time-consuming experiments to be performed make these methods economically unattractive. Moreover, a systematic knowledge base of all the considered separations is not compiled. With method 2, there is also a risk of finding a local optimum, and, therefore, missing a better optimum for the separation. In principle, method 3 does not suffer from the above-mentioned disadvantages, and, therefore, it may be an attractive alternative for laboratories in which applications are regularly developed.

In addition, by using method 3, the selectivity in reversed-phase (RP) HPLC separations can be greatly improved by making use of quaternary eluent mixtures of acetonitrile, methanol, tetrahydrofuran (THF), and water. These eluents cover a large selectivity area in RP chromatography (1,2). Snyder described a method allowing the optimal

TABLE I: List of the Columns Used, and Their Properties

Column	Particle Size (µm)	Column Length (cm)	Column Inner Diameter (mm)	Relative Acidity According to Ref. 19
Zorbax RX-ODS	5	15	4.6	⇓
Nova-Pak C-18	4	20	5	⇓
Lichrosorb RP-18	5	15	4.6	⇓
Zorbax ODS	5	15	4.6	⇓

TABLE II: List of the Test Mixtures Used for the Solvent Optimization Procedures

Test Mixtures			
1	2	3	4
p-hydroxybenzoic acid *n*-alkyl ester	*n*-alkylbenzenes	2-*n*-alkylpyridines	Practical sample
methyl ester	benzene	propylpyridine	methyl ester
ethyl ester	methylbenzene	hexylpyridine	ethyl ester
propyl ester	ethylbenzene	heptylpyridine	benzene
butyl ester	propylbenzene	octylpyridine	methylbenzene
	butylbenzene	nonylpyridine	hexylpyridine
			heptylpyridine

eluent composition using four solvents in the eluent to be derived (3). However, this method is time-consuming, and it is difficult to completely interpret the data to establish an optimal eluent composition.

To overcome these disadvantages, and to obtain objective and rapid optimal eluent compositions, several manufacturers have produced computer software programs, such as Diamond (Unicam, Cambridge, UK), ICOS (Hewlett-Packard, Waldbronn, FRG), and Drylab (LC Resources, California, USA). These methods are based on retention modelling.

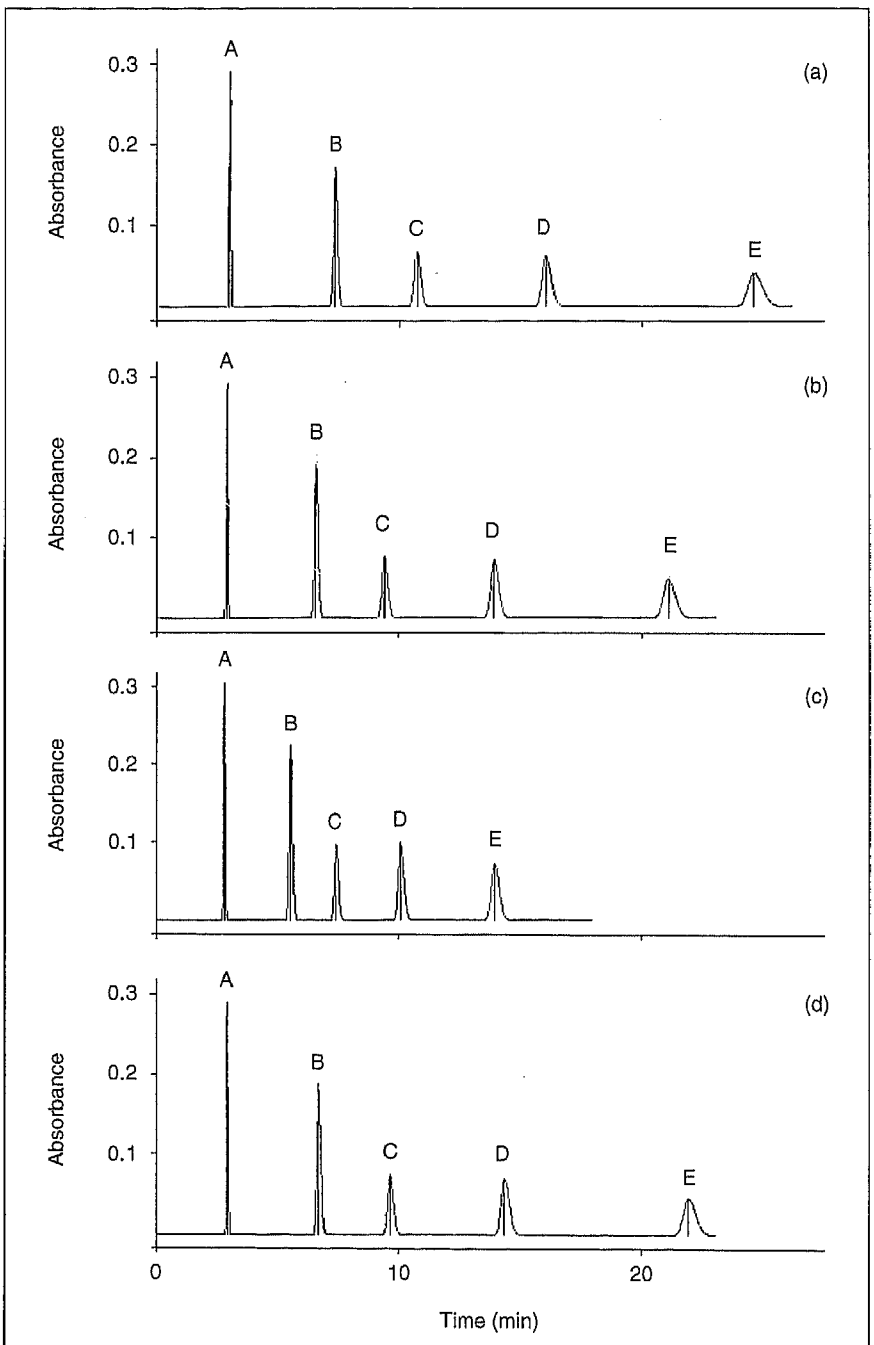

FIGURE 1: The effect of the selection of different optimization criteria on the optimal solvent composition. Column: Lichrosorb RP-18; test mixture: alkylpyridines; Optimization criteria: (a) SMIN function, eluent composition: MeOH–ACN–THF–H_2O (34.8:1.9:23.3:40.0 v/v); (b) TNE function, eluent composition: MeOH–ACN–THF–H_2O (42.5:11.2:13.3:33.0 v/v); (c) STMIN function, eluent composition: MeOH–ACN–THF–H_2O (0:35.4:23.3:41.3 v/v); (d) RSTAR function, eluent composition: MeOH–ACN–THF–H_2O (52.2:1.9:13.3:32.6 v/v). Peaks: A = 2-*n*-propylpyridine, B = hexylpyridine, C = heptylpyridine, D = octylpyridine, E = nonylpyridine.

With the Diamond system used in this study, retention times are measured using several solvents that all produce chromatograms with convenient peak retention factors, typically in the range 1–10. Models are then derived that predict the retention times for each solute. These predictions may then be used to suggest the optimum solvent composition. As users need to know when changes in peak order occur, peaks are deconvoluted, tracked, and assigned as the process continues. A full discussion of the different systems is, however, beyond the scope of this article. For more detailed information, see references 4 and 5.

In addition to a good optimization method used by the chromatographer, the applied RP columns must be of constant and of reproducible quality with all three methods to determine the optimal solvent composition. The chromatographic properties of the applied stationary phase should be constant during the application of solvent-optimization procedures. The same is true when the analyses need to be carried out routinely. This allows reliable qualitative and quantitative analysis results to be obtained, which is particularly relevant when the analytical and data-acquisition procedures are run in automatic modes. Also, given the finite lifetime of HPLC columns, from time to time these columns have to be replaced. It is important that the chromatographic properties of a column are similar to those of the one it

TABLE III: Optimal Eluent Composition Determined for Test Mixture 1

Column	Optimal Eluent Composition (%) H_2O	MeOH	ACN	THF
Zorbax RX-ODS	56.3	17.6	26.1	0.0
Nova-Pak C-18	46.3	52.7	1.0	0.0
Lichrosorb RP-18	45.8	49.2	5.0	0.0
Zorbax ODS	50.8	21.8	27.4	0.0

TABLE IV: Optimal Eluent Composition Determined for Test Mixture 2.

Column	Optimal Eluent Composition (%) H_2O	MeOH	ACN	THF
Zorbax RX-ODS	34.5	30.2	35.3	0.0
Nova-Pak C-18	31.2	55.6	10.3	2.9
Lichrosorb RP-18	32.8	49.6	17.6	0.0
Zorbax ODS	28.3	41.7	30.0	0.0

TABLE V: Optimal Eluent Composition Determined for Test Mixture 3.

Column	Optimal Eluent Composition (%) H_2O	MeOH	ACN	THF
Zorbax RX-ODS	43.4	0.0	43.8	12.8
Nova-Pak C-18	39.1	0.0	53.1	7.8
Lichrosorb RP-18	33.0	42.5	11.2	13.3
Zorbax ODS	44.3	0.0	36.7	19.0

TABLE VI: Optimal Eluent Composition Determined Test Mixture 4

Column	Optimal Eluent Composition (%) H_2O	MeOH	ACN	THF
Zorbax RX-ODS	47.1	4.9	46.2	1.8
Nova-Pak C-18	49.0	23.1	17.0	10.9
Lichrosorb RP-18	41.3	8.9	35.9	13.9
Zorbax ODS	40.9	7.0	48.6	3.5

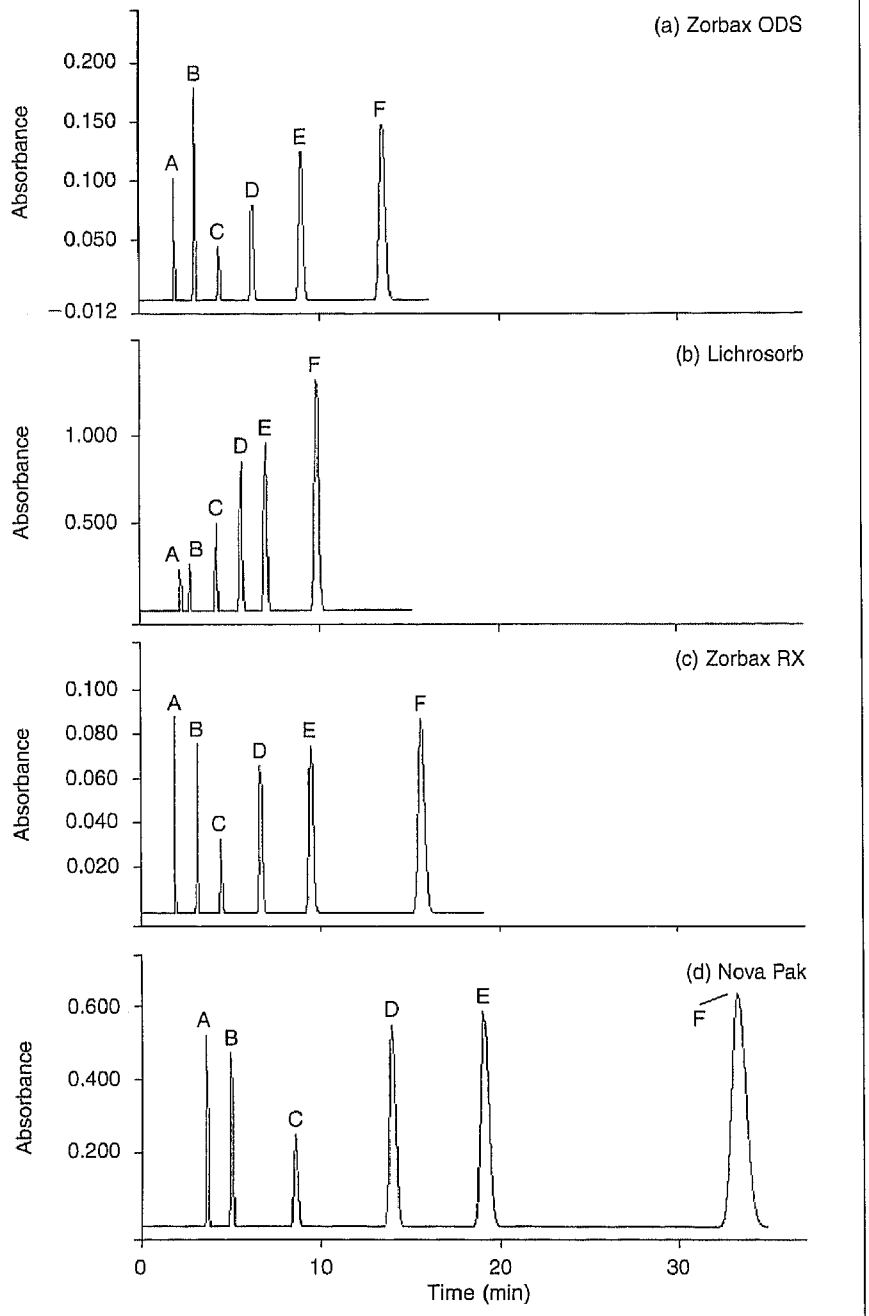

FIGURE 2: Chromatograms of the practical test mixture 4 on columns 1–4 (summarized in Table I), operating under their optimal eluent compositions (presented in Table VI). Flow rate: 1 mL/min; detection: UV. Peaks: A = *p*-hydroxybenzoic acid methyl ester, B = *p*-hydroxybenzoic acid ethyl ester, C = benzene, D = methylbenzene, E = hexylpyridine, and F = heptylpyridine.

replaced, to guarantee an uncomplicated continuation of the analysis.

It is recognized that large differences in chromatographic properties and chemical stability occur between nominally identical RP stationary phases (6–9). These differences can also sometimes be observed in different batches of a specific RP phase from the same manufacturer (10). Predominant factors in this respect are: the differences between the silica substrates used by the manufacturers, the way that silanes are bonded to the surface of the support, and the surface coverage of the attached ligands (11–13). These differences between "identical" RP phases considerably limit the benefit of optimization procedures and the transferability of chromatographic procedures from column-to-column.

A specific problem of silica-based RP phases is their chemical instability when they are used under practical conditions. This instability is accompanied by a change in the chromatographic properties of the stationary phase or even the deterioration of the packed bed in the column (8,11–15). In fact, the above-mentioned problems with RP phases are still the driving force behind the efforts of research groups involved in the area of HPLC stationary phases. Nevertheless, chromatographers are regularly confronted with the problem of selecting the proper stationary phase for a particular separation. This is complicated by the fact that a large variety of apparently identical RP phases (that is, C_{18} phases) are available. Until recently, little objective product information has been available to support the analyst — only details of some bulk properties have been on offer. In addition, practical and useful test procedures for judging columns on their potential applicability are not available. Test methods have been suggested by Jandera (16) and Smith (17). An evaluation of these characterization methods for RP phases revealed that applied extrapolation procedures, in particular, may provide erroneous results (18).

In this study a different approach was used for studying the differences between nominally identical RP phases, compared with the usual spectroscopic investigations. Instead of performing chromatographic standard test procedures, several nominally identical RP18 columns were subjected to a solvent-optimization procedure using a number of specific samples. In addition, to include in this study the possible influence of the silica substrate, RP phases were selected from the "less-acidic" versus the "more-acidic" ranking described by Leach et al. (19). To emphasize the possible influence of the differences between nominally identical RP columns on the optimal solvent composition, simple test substances were selected. Mixtures of neutral, acidic, and basic homologue series were used as the preliminary test samples. To mimic a more practical sample, another test mixture was prepared containing a number of these neutral, acidic, and basic homologues. It was shown that the

selection of an RP column to solve a specific application problem has a significant effect on the optimal eluent composition calculated by the computer software program.

This work confirms, through the approach of solvent-optimization procedures, that significant differences in selectivity and resolution may occur between "identical" RP columns, as was also indicated by spectroscopic and other studies. Finally, the strong limitations of the transferability of optimal solvent compositions between apparently identical RP columns in chromatographic practice are discussed.

EXPERIMENTAL

Chemicals: The applied solvents — methanol, acetonitrile, and unstabilized THF — were of HPLC-grade from FSA Laboratory Supplies (Loughborough, UK). To the THF, a small amount of 2-*tert*-butyl-4-methylphenol was added as a stabilizer. However, the use of unstabilized THF showed more stable baselines in the chromatograms. With unstabilized THF, the amount of peroxide was controlled by the Merckoquant peroxide-tester (Merck AG, Darmstadt, FRG). HPLC-grade water was prepared by filtering deionized water through a Milli-Q water-purification system (Millipore, Bedford, Massachusetts, USA). Before use, the solvents were filtered through appropriate 0.45-μm membrane filters (Millipore).

Columns: The columns investigated in this study were all nominally identical ODS columns.

- Zorbax RX-ODS (Rockland Technologies, West Chester, Pennsylvania, USA)
- Nova-Pak C-18 (Millipore)
- Lichrosorb RP-18 (Merck)
- Zorbax ODS (Rockland Technologies).

The column properties are summarized in Table I.

Samples: The compounds used in the test mixtures were of reference grade. Test mixture 1 consisted of a homologous series of *n*-methyl to *n*-butyl *p*-hydroxybenzoic acid esters (Sigma, St. Louis, Missouri, USA). Test mixture 2 was a homologous series of benzene to butylbenzene (Pierce, Rockford, Illinois, USA). Test mixture 3 was prepared from a series of alkylpyridines, which were synthesized in-house.

Of each of these three test mixtures, two compounds were selected to produce a practical test mixture (test mixture 4), to prepare a sample in which different classes of compounds were represented. The concentrations of test components in the mixtures injected were approximately 10^{-3}% w/w. The test mixtures are listed in Table II.

Equipment: The HPLC equipment consisted of a PU4100M liquid chromatograph with single to quaternary isocratic or gradient solvent control (ATI Unicam, Cambridge, UK). The eluent flow was 1.0 mL/min in all experiments, at ambient temperature. The column effluent was monitored using a PU4120 diode-array detector (ATI Unicam). The processing of the detector signals was performed on a personal computer equipped with PU6003 diode-array software, and solvent optimization was performed using the Diamond PU6100 solvent-optimization software (ATI Unicam).

RESULTS AND DISCUSSION

The four nominally identical ODS columns to be investigated were subjected to the solvent-optimization procedure, using the test mixtures 1–4 as the samples. The Diamond solvent-optimization system allows the user to select a number of different criteria to perform the optimization procedure. The major selection possibilities of this system are

- maximum resolution
- maximum resolution at minimum analysis time
- most even spacing of peaks.

In this study, the optimization criterion was chosen as the maximum separation of the two closest peaks and a minimum retention time of the last peak (20–22). As an example, Figure 1 shows the effect of selecting different optimization criteria for the Lichrosorb column using the pyridines as the test mixture. In Figure 1(a) the SMIN function has been chosen, which provides the maximum separation for the closest pair of peaks. The function TNE, which is used in Figure 1(b) is again providing a maximum separation but is biased towards producing a short chromatogram. Figure 1(c) (STMIN) has an even larger bias towards producing short elution times. RSTAR, which was used in Figure 1(d), produces an even separation of peaks, and RNT produces a combination of RSTAR and TNE. For the purposes of this investigation, a single criterion function, TNE, was used throughout. TNE = $SMIN^2/(1+k_w)$, where k_w is the retention factor of the last peak.

The results of the optimized eluent composition of the columns, for the four test samples, are presented in the Tables III–VI. With each specific sample, it is clear that there are large differences in the optimal eluent composition between the columns. This indicates that the different columns show different "surface activity" to the solutes. As an example, the chromatograms of test sample 1 on the columns operating under their specific optimal eluent composition are presented in Figure 2.

It is obvious that nominally identical RP stationary phases may show different optimal eluent compositions. This is a complicating factor for chromatographic practice. When a specific column has been adopted for an application, the chromatographic experimental conditions are not transferable to other apparently identical RP columns.

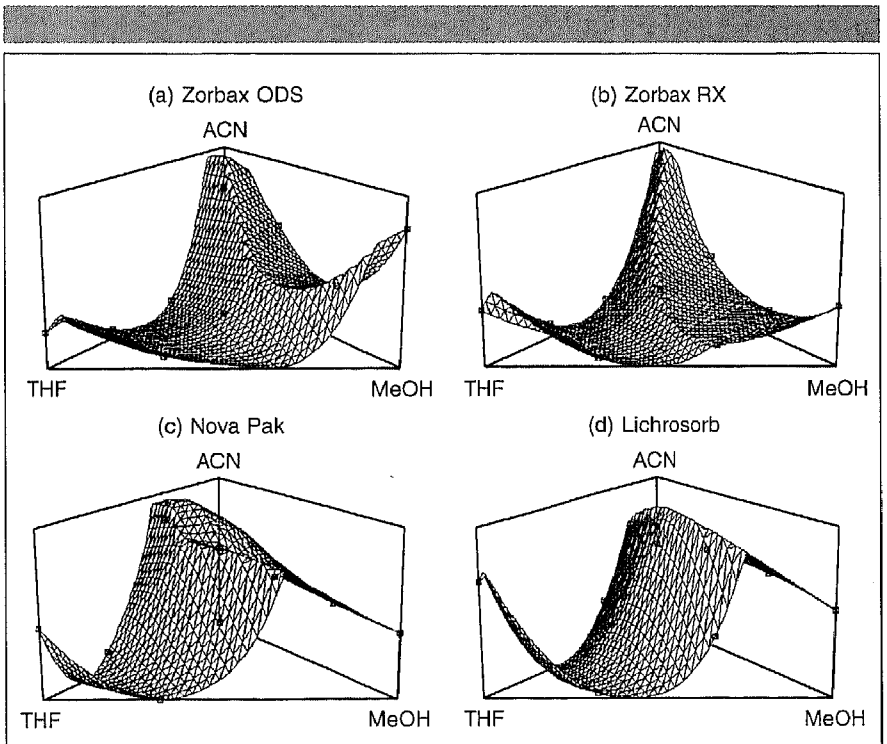

FIGURE 3: Three-dimensional plots of the TNE response functions for the optimization of the practical test mixture 4. The percentages of the organic modifiers in the binary eluent mixtures in the three corners of the isoeluotropic triangle are (a) column: Zorbax ODS; eluent composition: 1 = MeOH–H_2O (70.0:30.0 v/v), 2 = ACN–H_2O (60.7:39.3 v/v), 3 = THF–H_2O (35.1:64.9 v/v); (b) column: Zorbax RX, eluent composition: 1 = MeOH–H_2O (64.9:35.1 v/v), 2 = ACN–H_2O (52.8:47.2 v/v), 3 = THF–H_2O (36.3:63.7 v/v); (c) column: Nova Pak; eluent composition: 1 = MeOH–H_2O (66.1:33.9 v/v), 2 = ACN–H_2O (48.6:51.4 v/v) 3 = THF–H_2O (36.2:63.8 v/v); (d) column: Lichrosorb, eluent composition: 1 = MeOH–H_2O (71.5:28.5 v/v) 2 = ACN–H_2O (68.6:31.4 v/v), 3 = THF–H_2O (39.7:60.3 v/v).

TABLE VII: The Retention Factors k', the Separation Factors α, and the Retention/Separation Terms z of Test Samples 1–3 for the Columns Operating Under Their Optimal Solvent Compositions.

Alkylbenzenes												
Zorbax RX-ODS			Nova-Pak C-18			Lichrosorb RP-18			Zorbax ODS		
k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$
1.61			1.56			1.73			1.57		
	1.76	0.32		1.72	0.31		1.66	0.30		1.64	0.28
2.84			2.69			2.88			2.58		
	1.63	0.32		1.53	0.28		1.55	0.29		1.52	0.27
4.64			4.11			4.45			3.91		
	1.78	0.39		1.69	0.36		1.65	0.35		1.64	0.34
8.27			6.96			7.34			6.41		
	1.76	0.40		1.72	0.39		1.66	0.37		1.64	0.36
14.5			11.99			12.2			10.5		

Alkylesters												
Zorbax RX-ODS			Nova-Pak C-18			Lichrosorb RP-18			Zorbax ODS		
k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$
1.26			1.00			1.53			1.31		
	2.03	0.36		1.97	0.33		1.75	0.31		1.87	0.33
2.56			1.97			2.68			2.45		
	2.15	0.45		2.06	0.41		1.89	0.39		1.97	0.41
5.51			4.06			5.06			4.82		
	2.17	0.50		2.11	0.47		1.99	0.45		2.01	0.46
11.9			8.58			10.1			9.69		

Alkylpyridines												
Zorbax RX-ODS			Nova-Pak C-18			Lichrosorb RP-18			Zorbax ODS		
k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$
0.56			0.60			0.86			0.84		
	4.59	0.56		3.63	0.50		3.67	0.55		3.81	0.56
2.57			2.18			3.16			3.20		
	1.65	0.32		1.60	0.29		1.58	0.31		1.57	0.30
4.24			3.48			5.00			5.02		
	1.61	0.33		1.58	0.31		1.55	0.31		1.54	0.31
6.81			5.50			7.77			7.75		
	1.61	0.35		1.59	0.33		1.56	0.33		1.55	0.33
11.0			8.75			12.14			12.0		

Practical sample												
Zorbax RX-ODS			Nova-Pak C-18			Lichrosorb RP-18			Zorbax ODS		
k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$
0.42			0.86			1.15			0.49		
	2.95	0.37		1.77	0.26		1.39	0.17		2.62	0.35
1.24			1.52			1.59			1.29		
	1.76	0.30		2.16	0.41		1.82	0.33		1.76	0.30
2.19			3.27			2.90			2.28		
	1.72	0.33		1.81	0.38		1.44	0.25		1.61	0.30
3.76			5.92			4.19			3.66		
	1.52	0.29		1.44	0.27		1.30	0.19		1.54	0.30
5.73			8.51			5.43			5.65		
	1.76	0.39		1.84	0.43		1.48	0.29		1.59	0.33
10.07			15.67			8.04			8.96		

TABLE VIII: Calculated Values of the Resolutions of a Separation System for Several Values of α and k', Assuming a Plate Number of 10,000 for the Column.

	$k'/(1+k') = 0.75$		$k'/(1+k') = 0.5$		$k'/(1+k') = 0.99$	
α	z	R	z	R	z	R
1.02	0.014	0.35	0.010	0.25	0.020	0.49
1.05	0.036	0.90	0.024	0.60	0.048	1.19
1.10	0.068	1.70	0.045	1.13	0.090	2.27
1.15	0.098	2.45	0.065	1.63	0.130	3.26
1.20	0.125	3.13	0.083	2.08	0.167	4.17

The reasons for the different optima can be visualized in plots of the response surfaces. Figure 3 shows the 3-dimensional plots of the chromatogram quality response function TNE for the optimization of the practical sample number 4 on the four columns. The triangular base represents the isoeluotropic triangle, and the corners of this triangle are binary mixtures of water and one of the organic modifiers. Peak separation increases are indicated in the vertical direction. The view direction is towards the acetonitrile–water corner of the isoeluotropic triangle. For the Zorbax ODS column there are two optima on the methanol and acetonitrile corners. For the Lichrosorb and Nova-Pak columns, optima at the acetonitrile–tetrahydrofuran edge can be observed. Finally, the Zorbax RX column shows an optimum at the acetonitrile corner.

This six-peak separation is relatively simple, so it is not unexpected that the response surfaces show optima on the edge of the triangle. To examine the data in more detail, using the chromatograms of test samples 1–4, the retention factor k' and the separation factor, α, were calculated for each column operating under its optimal solvent composition. As the columns under investigation differed in plate number and length in some instances, the retention/separation

TABLE IX: The k', α, and z values of the Columns Operating Under Optimal Solvent Composition (OSC) of Two Specific Columns, Using Test Mixture Number 4.

OSC: Zorbax RX-ODS; eluent: MeOH–ACN–THF–H_2O (4.9: 46.2: 1.8: 47.1 v/v)											
Zorbax RX-ODS			Nova Pak C-18			Lichrosorb RP-18			Zorbax ODS		
k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$
0.43			0.42			0.59			0.30		
1.34	3.12	0.39	0.77	1.84	0.20	1.12	1.89	0.25	0.82	2.73	0.29
2.41	1.80	0.31	2.18	2.83	0.44	2.96	2.65	0.47	1.56	1.90	0.29
4.20	1.74	0.34	3.77	1.73	0.33	4.54	1.53	0.29	2.59	1.66	0.29
6.04	1.44	0.26	5.53	1.47	0.27	11.38	2.51	0.55	3.46	1.34	0.20
10.8	1.79	0.40	9.58	1.73	0.38	19.14	1.68	0.39	5.87	1.70	0.35

OSC: Zorbax ODS; eluent: MeOH–ACN–THF–H_2O (7.0: 48.6: 3.5: 40.9 v/v)											
Zorbax RX-ODS			Nova Pak C-18			Lichrosorb RP-18			Zorbax ODS		
k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$	k'_i	$\alpha_{j,i}$	$z_{j,i}$
0.70			0.26			0.55			0.42		
2.00	2.86	0.43	0.45	1.75	0.13	0.85	1.56	0.17	1.28	3.05	0.38
3.49	1.75	0.33	1.51	3.35	0.42	2.00	2.35	0.38	2.31	1.80	0.31
5.98	1.71	0.36	2.37	1.57	0.26	3.16	1.57	0.28	3.79	1.64	0.31
10.2	1.70	0.37	3.24	1.36	0.20	6.99	2.22	0.48	5.75	1.52	0.29
17.4	1.71	0.39	5.31	1.64	0.33	11.21	1.60	0.35	9.36	1.63	0.35

term, z, was calculated, and forms part of the chromatographic resolution equation:

$$z = \frac{\alpha_{j,i} - 1}{\alpha_{j,i}} \cdot \frac{k_j}{k_j + 1} \qquad [1]$$

where $\alpha_{j,i}$ is the separation factor of the components j and i, and k_j is the retention factor of compound j.

The results are summarized in Table VII. The data show that significant differences in the z-values of the columns were observed. These apparently small differences may strongly influence the resolution of complex samples. This is demonstrated in Table VIII, where the resolution is calculated for a selectivity range from 1.02 to 1.20 at three different $k'/(1+k')$ values.

From the data in Tables VII and VIII it can be concluded that relatively small differences in the z values of "identical" columns operating under their optimal solvent condition may result in different resolution patterns, particularly with samples containing many compounds. Finally, the transferability of the optimal solvent composition of a specific column to other "identical" columns was investigated. Therefore, each column was also tested under the optimal eluent conditions of the other columns. As an example, Table IX presents the resulting k', α, and z values of sample 4 on the four investigated columns operating under the optimal conditions of the Zorbax and Zorbax RX columns. From these data it can be concluded that the transferability of optimal solvent conditions between nominally identical columns is poor.

Currently, in laboratory practice, analysts need to be able to transfer eluent conditions, not only from one "identical" column to another, but also between different laboratories using nominally identical columns. This study shows that this requirement is difficult to meet.

CONCLUSIONS

In this study the differences in chromatographic behaviour of nominally identical RP stationary phases for HPLC were investigated with the intention of establishing optimal solvent conditions of these phases.

It was shown that even with the simple test mixtures used in this study, significant differences in the optimal solvent conditions between nominally identical RP phases were obtained, confirming that large differences between these columns may exist. Furthermore, it was shown that the retention/separation values of the investigated columns operating under their optimal eluent conditions differed, resulting in significant differences in the resolution obtainable on these columns.

Finally, it was also shown that the transferability of optimal eluent conditions between these nominally identical columns is poor, preventing the use of these columns in interlaboratory studies. From a practical point of view, currently, this problem can only be tackled by using the specific stationary phase on which an application has been developed.

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