Selectivity Optimization of Stationary Phases

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A method for stationary-phase optimization in liquid chromatography has been developed that complements existing optimization procedures for mobile phases. Using a selectivity triangle (or a selectivity tetrahedron), chromatographers can systematically find the optimum stationary phase for their analyses. With this method it is suggested that the expensive testing of columns for particular separation problems is rendered unnecessary. For illustrative purposes, the selectivity optimization procedure is applied to a set of non-polar and medium-polarity reversed-phase columns. The subsequent mixed-packing ("target") column derived from this stationary phase mixed set provided optimum selectivities for high- and medium-polarity compounds.

Introduction

The selectivity (S)[1–3] for two compounds (1 and 2) in a chromatographic phase system consisting of a stationary phase (K) and a mobile phase (F) is given by the equation

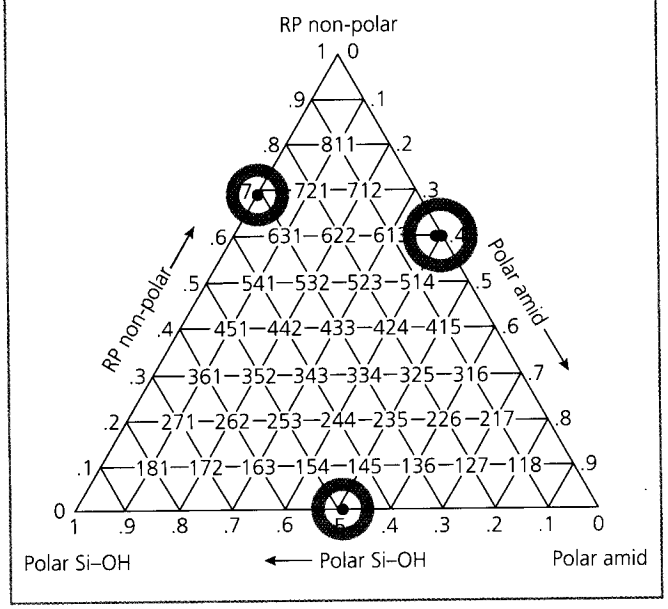
$$S_{21} = S_{K21} + S_{F21} = \ln\alpha_{21} = \ln\frac{t'_2}{t'_1} = \ln t'_2 - \ln t'_1 \qquad [1]$$

where α_{21} is the selectivity coefficient, and t'_1 and t'_2 are the respective net retention times for compounds 1 and 2. S can be considered as a direct measure of Gibbs free energy for the separation $\Delta\Delta G°_{21}$, and the selectivity contributions of the phase system are additive. It proves convenient to multiply the numerical value of S by 100 such that, generally, it lies between 10 (for just separated compounds) and 100 (for well-separated compounds). With no separation, S is equal to 0.

The optimization of S by systematic modifications of mobile-phase composition (F) is well known,[4,5] and mostly used in reversed-phase chromatography using water as the base solvent and acetonitrile or methanol as the organic tuning solvents. Optimum eluent composition can be established after two gradient runs and subsequent computer evaluation.

However, finding the optimum stationary phase can be a troublesome and expensive operation. Often many packings must be tested as there is no established protocol enabling chromatographers to select the most favourable column. Generally, columns with very similar selectivities are examined and the "right" column is often found by chance.

Figure 1: Selectivity triangle. Within the grey circles lie four points which characterize the calculated phase compositions according to Table 1. On the left side the point 69/31/00, on the basis side the point 00/49/51, on the right side the point 60/00/40 and within the triangle the point 60/01/39. For further comments see text.



Therefore, using modern, polar, reversed-phase materials, a selectivity set consisting of three or four columns with varying selectivities has been developed. Figure 1 shows a selectivity triangle, such as one used for solvents,[6] in which each corner represents a pure packing with a definite selectivity.

A non-polar, reversed-phase column (RP non-polar) is placed at the apex of the triangle. On the lower left corner a polar, reversed-phase packing (Polar Si-OH) is positioned. The polarity of this phase results from a large portion of surface silanol groups. Such phases possess an extended polar (matrix) selectivity,[3,7,8] an alternative to the so-called "embedded polar (amide) phases."[3,9] A phase of this latter type, with an embedded urea group, was placed at the lower right corner of the triangle. To produce a selectivity tetrahedron, a further phase can be included; in this instance a relatively polar phase with a secondary amino group embedded between the spacer and alkyl chain,[3,10] allowing additional pH tuning. A representation of this selectivity tetrahedron is shown in Figure 2.

Using these four phases, 11 optimization possibilities provide good conditions for solving difficult separation problems. The four phases correspond to the four corners of the tetrahedron. There are six combinations of any two phases with their concentrations in the mixtures along the six tetrahedron edges, and each allowing the application of a simple interpolation diagram (Figure 3). Additionally, there are four combinations of three-phase mixtures, with their concentrations defined on the tetrahedron faces. One four-phase combination is possible, with its mixture concentrations defined inside the three-dimensional tetrahedron space.

Our method, shown here for reversed-phase chromatography, should also be applicable to normal-phase chromatography. Investigations are currently in progress.

The computerized treatment of retention times yields the optimum composition of a hypothetical "target" column, as well as the gross or net retention times of components on the target column, the retention time difference Δt_{Rlk} of the critical pair l,k (Table 1) and, if required, the selectivities (S_{ji}) and resolutions (R_{Sji}) of neighbouring compounds on the target column (Table 3).

The program compares differences in the square roots of the retention times of neighbouring peaks (ΔSQR) for all possible mixed columns in 1% compositional increments. The optimum

Figure 2: Selectivity tetrahedron. The non-polar phase of Figure 1 lies at the apex of the tetrahedron. The base area represents a selectivity triangle with three polar phases. There exist three triangles, each consisting of a non-polar and two different polar phases. The triangle of Figure 1 forms the front of the tetrahedron. In total, there are 6 double, 4 triple and 1 quadruple possible phase combinations. For comments to the points 45/36/19 and 59/10/31 see text.

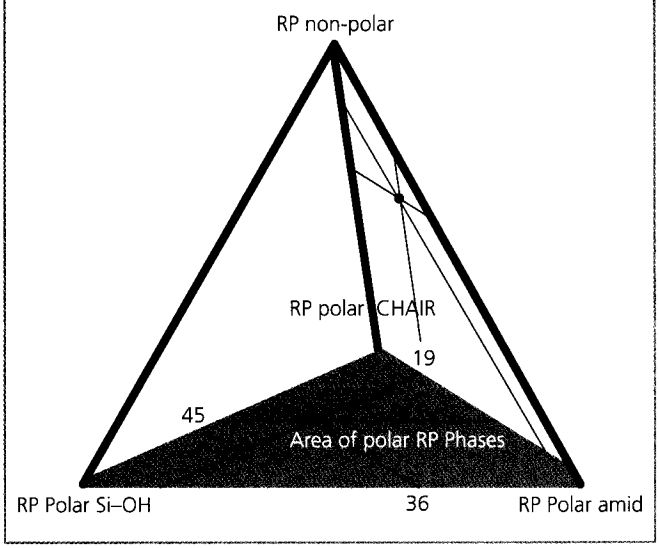


Figure 3: Graphical interpolation of the retention times for two phases mixed with each other. The retention times on the ordinates stem from Table 2. The interpolated target retention values at a mixing proportion of 60/40 (arrow) fully agree with the values of the target column, calculated by the computer program (Table 3, column 2). These values are compared with the experimentally found values in column 3 of the table. Compounds: 1 = Phenyl-2-propanol, 2 = Phenyl-1-propanol, 3 = Phenylpropionitrile, 4 = Cyanazin, 5 = Metoxuron.

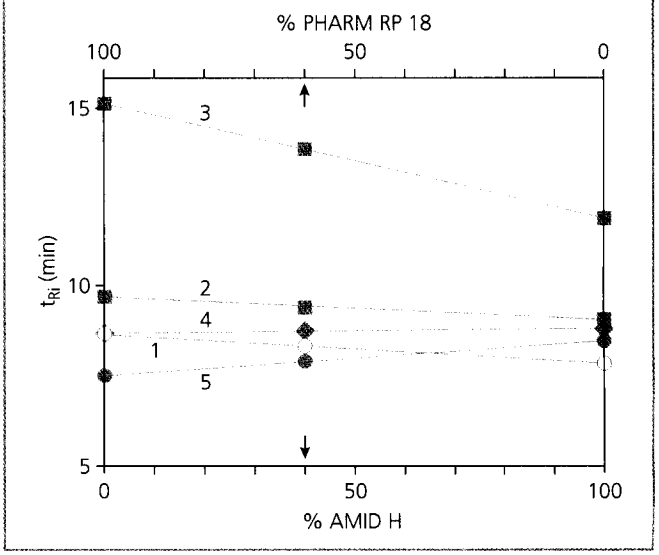


Table 1: Calculated target columns by means of the computer program.

	Parameters of the concentration spots according to Figure1			Critical pairs	
Combination	PHARM RP18	RP 18 M500	AMID H RP 18	Δt_{Rlk}*	Compounds
1	60	00	40	0.43	1/4
2	00	49	51	0.14	4/5
3	69	31	00	0.41	2/4
4	60	01	39	0.44	1/4

* Distance of the compounds with the most unfavourable retention times.

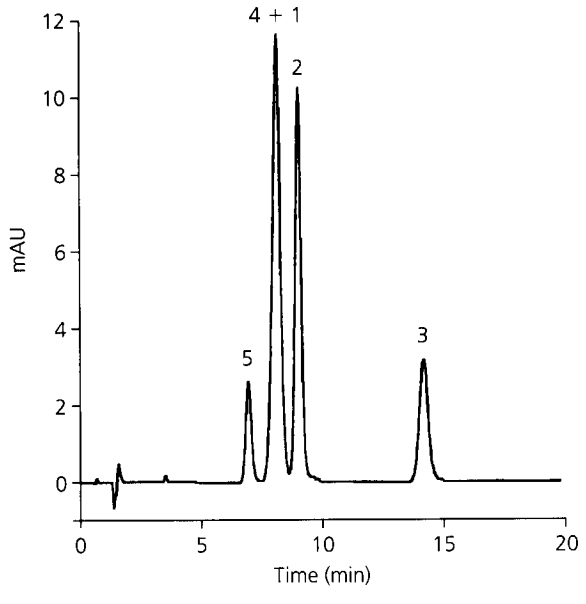
packing mixture is determined as that which generates the maximum ΔSQR value for the critical peak pair in the separation. All the above mentioned columns and materials are commercially available. The target column, however, must be produced.

Experimental

Equipment: The UV detector was acquired from Knauer (Berlin, Germany), the pumps from Bischoff (Leonberg, Germany), the column oven from SepServ (Berlin, Germany) and the injection port from Rheodyne (Bensheim, Germany). Various analytes were obtained from Sigma-Aldrich (Taufkirchen, Germany) and solvents from Mallinckrodt-Baker (Deventer, The Netherlands).

Columns: The following SepServ columns were used in this study: non-polar = UltraSep ES PHARM RP18 (150 × 3 mm), embedded polar = UltraSep ES AMID H RP18 P (150 × 3 mm), extended polar = UltraSep ES RP18 M500 (150 × 3 mm), embedded amino = UltraSep ES CHAIR (150 × 3 mm), target colums with mixed packings (150 × 3 mm).

Figure 4: Chromatogram on UltraSep ES PHARM RP18. For conditions see Experimental section. Compounds: 1 = Phenyl-2-propanol, 2 = Phenyl-1-propanol, 3 = Phenylpropionitrile, 4 = Cyanazin, 5 = Metoxuron.



Conditions: Eluent, acetonitrile/water (30/70 v/v); flow-rate, 0.4 mL/min; temperature, 25 °C; detection, 254 nm.

Results and Discussion

Figures 4, 5 and 6 show the separations of a five-component mixture on three standard columns of the described selectivity set. The mixture cannot be separated easily and each chromatogram shows one pair of unresolved peaks. Therefore, we calculated optimum "target" columns using the computer program. The results are summarized in Table 1. Corresponding to the four packing combinations, four spots are shown on the selectivity triangle (Figure 1). From the fifth

Figure 5: Chromatogram on UltraSep ES RP18 M500. For conditions see Experimental section. For peak designations see Figure 4.

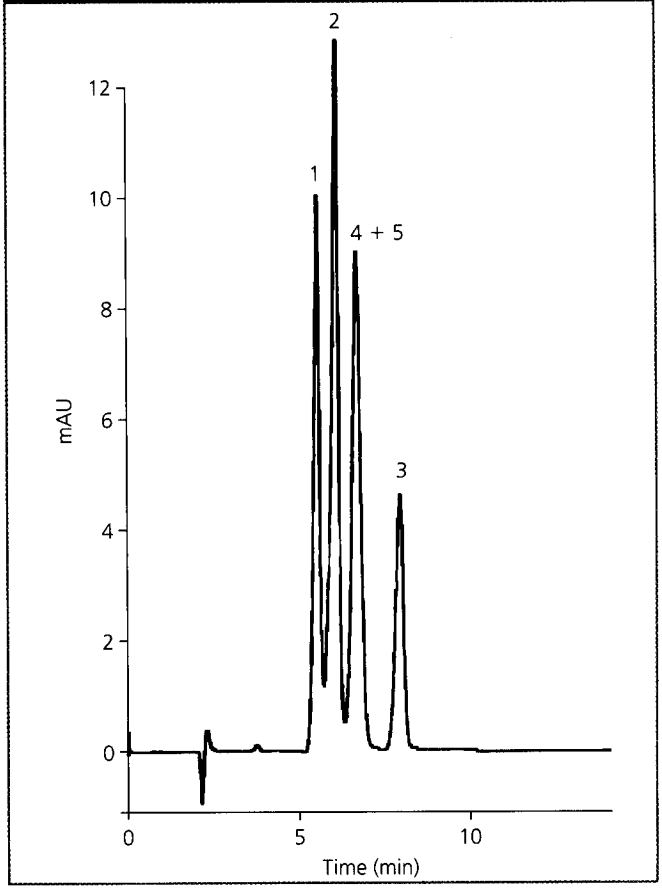


Table 2: Selected target column.

Compound	Gross retention times t_{Ri} (min)		t_{Ri} calculated for TARGET column (min)
No.	PHARM RP18	AMID H RP18P	
1	8.79, 8.81, 8.80 ($\bar{x}$ = 8.80)	7.85, 7.89, 7.86 ($\bar{x}$ = 7.87)	8.43
2	9.71, 9.75, 9.72 ($\bar{x}$ = 9.73)	9.07, 9.09, 9.0 ($\bar{x}$ = 9.08)	9.47
3	15.19, 15.20, 15.23 ($\bar{x}$ = 15.21)	12.09, 12.05, 12.04 ($\bar{x}$ = 12.06)	13.95
4	8.84, 8.80, 8.78 ($\bar{x}$ = 8.81)	8.94, 8.96, 8.93 ($\bar{x}$ = 8.94)	8.86
5	7.61, 7.63, 7.58 ($\bar{x}$ = 7.61)	8.59, 8.58, 8.59 ($\bar{x}$ = 8.59)	8.00

The standard deviation according to ASTM over all values amounts ± 0.02 min.

column of Table 1, we learn that only three spots and target columns, respectively, could be on the short list (combinations 1, 3 and 4). We selected combination 1. Its parameters 06/04/00 lie on the right side of the triangle, between the RP non-polar and polar amide corners. The value of 0.43 for Δt_{Rlk} suggests a separation just sufficient for all compounds. A target column based on this calculated mix was prepared and the compound mixture separated (Figure 7). As expected, all compounds were resolved.

Table 3: Separation parameters for the selected target column.

Compound No.	t_{Ri} (calc.)	t_{Ri} found	$S_{ji} \times 100$*	R_{Sji}*
5	8.00	8.01; 8.02		
1	8.43	8.34; 8.37	6	1.0
4	8.86	8.83; 8.86	6	1.0
2	9.47	9.49; 9.47	8	1.3
3	13.95	13.91; 13.93	45	7.6

* The values were calculated with t_M = 1.6 min (thiourea) and N = 6300 TP per column length

Figure 6: Chromatogram on UltraSep ES AMID H RP18 P. For conditions see Experimental section. For peak designations see Figure 4.

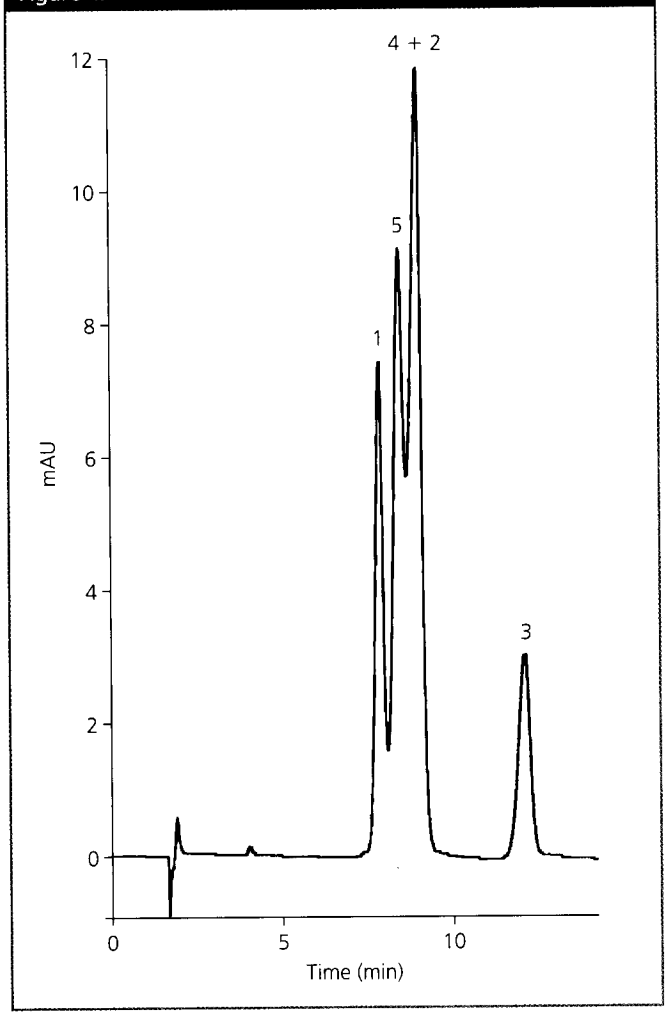
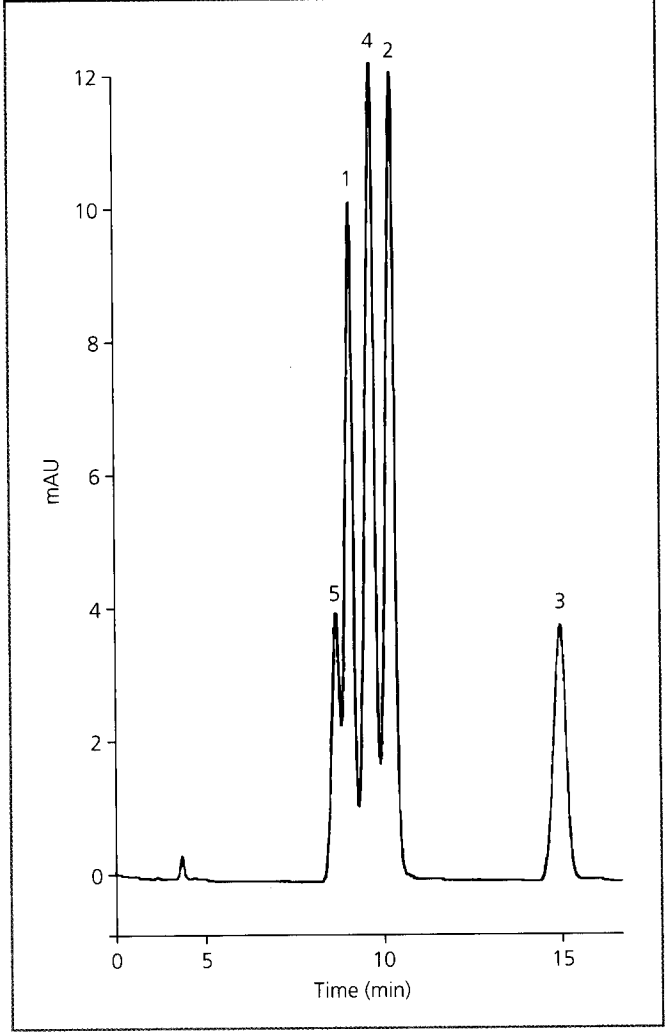


Table 2 compares the retention times observed on selectivity set columns with those calculated for the target column. Table 3 compares these calculated values with those found experimentally. Furthermore, Table 3 provides the calculated selectivities and resolutions of the target column.

By including the UltraSep ES CHAIR column in the optimization procedure, the area of the polar RP phases in the tetrahedron (Figure 2) revealed an optimum ternary mix composition at 45/36/19 (UltraSep ES CHAIR/ UltraSep ES RP18 M500/UltraSep ES AMID H RP18 P), for which a Δt_{Rlk} value of 0.50 was calculated. Resolutions of 1.3 and 4.8 were achieved for the first three and the last compound pairs, respectively (see Figure 7). A similarly good result gave the ternary phase combination shown in the right tetrahedron area of Figure 2, represented by point 59/10/31 (UltraSep ES PHARM RP18/ UltraSep ES CHAIR/ UltraSep ES AMID H RP18 P). On this basis, it was not difficult to achieve baseline separation for all compounds using a longer column.

In our example separation problem, the computer calculations showed that a mixing of all four phases gave no further advantages.

Figure 7: Chromatogram on a calculated and produced target column. For conditions see Experimental section. For peak designations see Figure 4.



Conclusions

Using phases with different selectivities, optimization of a given separation problem can be realized systematically by creating a calculable "target" column. Our procedure is similar to the optimization of mobile phases. One needs several chromatographic runs (as many as columns included) to evaluate the results either graphically or, more quickly and extensively, by means of a computer program.

Normally, chromatographers cannot pack "target" columns themselves and, with regard to the set columns, packing materials must be in stock. Therefore, commercial support is necessary. We believe that this paper will stimulate other manufacturers to offer column sets also, so that chromatographers can choose between several delivery sources. This guarantees a general application of the new method.

References

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