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CORRELATION AND PREDICTION OF THE k' VALUES FOR MOBILE PHASE OPTIMIZATION IN HPLC

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ABSTRACT

The tripartite "PRISMA" optimization model is summarized, which includes all possible solvent combinations between 1-5 solvents. The solvent composition is characterized by the solvent strength (ST) and the selectivity points (PS).

The results show that a correlation between the selectivity points for equilibrated column systems at a constant solvent strength (horizontal function) can be described by the function $k' = a(PS)^2 + b(PS) + c$. In all cases in 12, 8, and 4 selectivity points, the k' values were measured along the edges of the triangle through two basic selectivity points (181-118, 118-811 or 811-181). The function obtained from the k' values measured at 12 or 8 points correlated with a high significance with the function obtained from only 4 points. The vertical correlation at constant selectivity points between various solvent strengths can be described by $ST = a \ln(k') + b$. Because the vertical correlation can be linearized, measurements on 3 solvent strengths levels are needed to calculate the k' values in all selectivity points in the spatial design. These correlations are also relevant when modifiers are used in constant amounts, using various substance classes of naturally-occurring compounds of differing polarity, both correlations were shown to be valid. From the presented

correlations of the k' values and the selectivity points, the chromatographic behavior of substances to be separated can be predicted at all selectivity points within the "PRISMA" model for isocratic separation. Based on these relationships a mobile phase optimization strategy is suggested.

INTRODUCTION

With the introduction of sophisticated automated HPLC instruments, many mobile phase optimization procedures and criteria have been described for HPLC and are summarized extensively by Berridge [1,2] and Schoenmakers [3]. Kirkland und Glajch [4] suggested a solvent strength prism for the optimization of multisolvent gradient elution. Based on this three-dimensional representation and the solvent classification of Snyder [5], as well as the seven point optimization method from Glajch, Kirkland and Snyder [6], the "PRISMA" optimization system was developed in our laboratory for optimization of the mobile phase and transfer between various chromatographic methods. This model is not only applicable to HPLC [7,8] and various preparative column chromatographic methods, but also for mobile phase optimization in planar chromatography [e.g.,9-13] for analytical and preparative purposes.

During our previous work with the "PRISMA" model, we achieved suitable HPLC separations of different substance classes using a simple method [14,15]. By first testing the various four solvent combinations determined by the basic selectivity points in the geometrical design and according to the results obtained by testing other solvent combinations in the region of the one giving the best separation, many separation problems could be solved. The efficiency of this process also relied on the experience of the chromatographer, although selection of the suitable mobile phase was more systematic and faster than by trial and error requiring no sophisticated instrumentation (calculator and HPLC system with 1 pump). Working with this system for many separation problems, we observed some trends between the k' values and the selectivity points. Therefore, the horizontal and vertical correlation between the selectivity points and k' values in the regular part of the

model was studied in RP modus on various substance classes [16] in isocratic mode from which the flavonoid glycosides from *Betula* species and the furocoumarin isomers from *Heracleum sphondylium* are chosen as typical examples for apolar and polar compounds, respectively. A strategy to elaborate the optimal mobile phase composition with these correlations is proposed here, which could be a part of an automated method development system, which also would consider e.g., the column type, ion strength, pH, temperature.

THEORETICAL

The "PRISMA" Model

The "PRISMA" model consists of three parts (Fig.1a): the base, symbolizing the modifier (dark grey in Fig.1); the regular part of the prism (white in Fig.1) with congruent base and top surfaces; and the irregular truncated top prism (light grey in Fig.1a).

The solvent strength values of the modifier(s) are treated by the "PRISMA" model as an additive term. For the sake of simplicity, the solvent strength values of the modifiers are neglected, since they are usually present in low, constant concentrations (generally between 0.1-3%, e.g., acids, ion pairs). The "PRISMA" model itself can be visualized as a graphic spatial representation of the solvent strength and the proportions of the components which determine selectivity. If the solvent strengths are plotted vertically and if the two dimensional representation of the solvent concentrations, which primarily influences the selectivity, is plotted on the horizontal plane, a prism is obtained with an equilateral triangle as its base (Fig.1). The lengths of the edges of the prism (S_A , S_B , S_C) correspond to the solvent strengths of the neat solvents (A, B, C) in question. As different solvents have differing solvent strengths, the length of the edges of the prism are generally unequal, so that as Fig. 1a shows, the top plane of the prism will not be parallel and congruous with its base. If the prism is cut parallel to the base at the height of the lowest edge -determined by the solvent with the lowest solvent strength in the system- the lower part gives a regular prism (Fig.1b) where the top and bottom planes are parallel equilateral triangles. The top part of the system is an irregular prism.

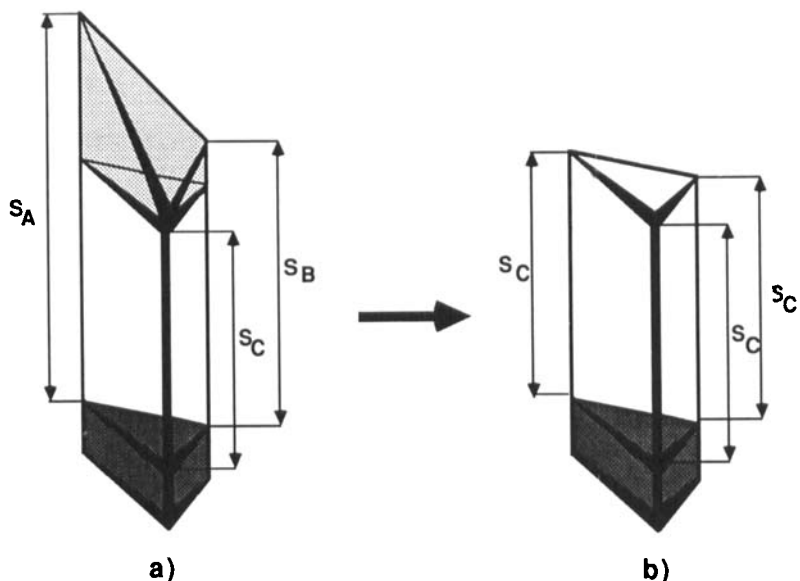


Figure 1 The "PRISMA" mobile phase optimization model.

- a) The complete model, consisting of the base symbolizing modifier(s) (dark grey), the regular part (white) and the irregular top part (light grey).
 b) The regular part and the base of the model (generally used for mobile phase optimization in reversed phase chromatography).

In normal phase (NP) chromatography, the upper irregular part is used for characterization of eluents for the separation of polar compounds, while the regular part characterizes eluents for the separation of nonpolar substances. In typical reversed phase (RP) chromatography, the regular part is used for the separation, independently of the polarity of the compounds to be separated.

The irregular part of the model

The three corners of the top cover plate (which is an irregular triangle) of the prism, represent the three undiluted neat solvents (Fig.2a). The corner corresponding to the longest edge of the prism is equivalent to solvent A (the solvent with the highest solvent strength), while solvent C (the solvent with the lowest solvent strength) corresponds to the corner of

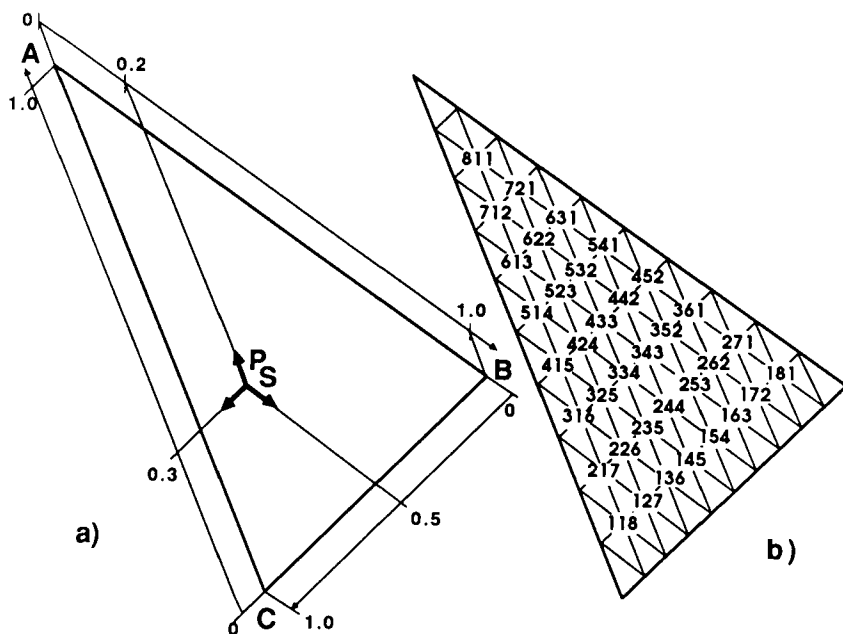


Figure 2 Combination of three neat solvents (A, B, C) on the irregular top triangle of the "PRISMA" model.

a) The volume fractions of the three solvents A,B and C in point P_S .

b) The selectivity points in the top triangle representing the combinations of the three solvents with three-digit numbers.

the shortest edge. In the triangle shown in Fig.2a, a certain solvent composition (P_S) can be characterized by the volume fractions of the corners. Here the volume fraction of solvent A is 0.3; that of solvent B is 0.2, while the volume fraction of solvent C is 0.5 (this means that in the eluent characterized by point P_S , the v/v % concentration of solvent A is 30 %, that of solvent B is 20 %, while that of solvent C is 50 %).

This point of the triangle, where the ten-fold values (P_A, P_B, P_C) of all three characteristic volume fractions are integers, can be defined by a three-digit number. This number - where the sum of the digits is 10- can be obtained by multiplying the volume fractions by 10 and arranging them in order of diminishing solvent strength. So that the solvent composition shown in Fig.2a can be defined by point 325 (which means

a mixture of 30% solvent A, 20% solvent B, and 50% solvent C). Fig.2b shows all four solvent compositions on the cover plate of the prism. They are characterized by integer three-digit numbers and defined as selectivity points (P_S). (As the center of the triangle cannot be described using the system defined previously, it is characterized per definition as $P_S=333$, referring to the fact that this composition is obtained if equal amounts are taken from all three solvents). Because the three solvents selected generally differ in solvent strength, all selectivity points on the surface of the cover plate of the irregular part represent different solvent strengths.

The points along the edges of the cover plate represent mixtures of two solvents (A and B, B and C, A and C). Inside the irregular triangle, the selectivity points represent mixtures of the three solvents (A and B and C). Dilution of an eluent mixture with a solvent having zero solvent strength gives mixtures characterized by the same selectivity point but having lower solvent strengths. These solvents are represented by the inside points of the upper irregular part of the prism. Of course, eluent mixtures with a solvent strength lower than solvent C are excluded.

Because in the irregular top triangle the solvent strength differs in each selectivity point, which greatly influences the separation, the steps between two selectivity points are often too large. The solvent mixtures between these selectivity points can then be described by three two-digit numbers (e.g., $P_S=20-58-22$). Even finer adjustments can be made (e.g., $P_S=19.7-58.3-22$), but usually a 1% accuracy is sufficient

The regular part of the model

The base and top plane of the regular part of the prism are congruent equilateral triangles. The height of this part of the prism corresponds to the solvent strength value of the weakest solvent. Due to the original selection of a decreasing order of solvent strength for solvent A, B, and C, this is solvent C. This means, that corner C of the regular prism represents undiluted solvent C. The solvent mixtures represented by corners AD and BD (see Fig.3) can be obtained by diluting solvents A and B to the solvent

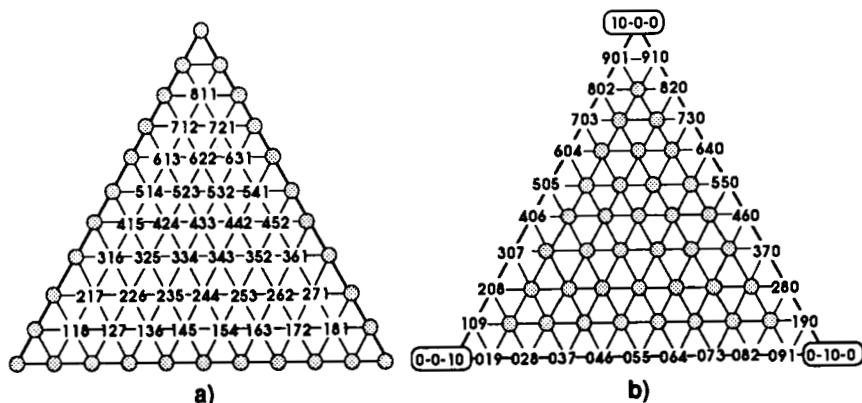


Figure 3 The selectivity points in the regular part of the "PRISMA" modell.

a) Selectivity points in the triangle representing four solvent combinations (including the solvent strength regulator).

b) Selectivity points along the edges of the triangle representing three solvent combinations; the corners representing two solvent combinations.

strength of C, with a solvent of zero solvent strength. Eluents characterized by other points on the top cover plate can be obtained by mixing the solvents represented by the corners of the top cover plate in the volume proportions corresponding to the point in question. The points symbolizing four solvent compositions frequently used in optimization (the selectivity points) are characterized -similarly to the points on the top irregular part- by three-digit numbers (see Fig.3a). In Fig.3b, the selectivity points representing the two- and three-solvent compositions are given.

The selectivity points on the vertical planes of the regular part of the prism can be obtained by diluting the solvent mixtures with a zero strength solvent. The solvent strength values decrease from top to bottom; at the base of the prism, the solvent strength value is zero. Hexane is used to reduce the solvent strength in NP chromatography and water is used in RP chromatography. If sections are prepared from the regular prism parallel to the base, triangles with different solvent strengths are obtained. Obviously, all points on one of these triangles represent the same solvent strength, while all points on a vertical straight line correspond to the same selectivity points.

Calculation of the mobile phase composition

Construction of the irregular and regular parts of the prism and of the solvent composition corresponding to individual points is demonstrated in Fig.4 with THF ($S_A=4.5$), ACN ($S_B=3.2$) and MeOH ($S_C=2.6$) in RP chromatography; water ($S_D=0$) is used to adjust solvent strength. Point C on the top of the regular prism corresponds to undiluted MeOH, while the other two corners (A_D and B_D) are obtained by diluting THF and ACN with the zero solvent strength water to a solvent strength of 2.6. This corresponds to a mixture of 57.78% THF and 42.22% water (corner A_D) and a mixture of 81.25% ACN and 18.75% water (B_D), respectively.

The total solvent strength of a four solvent eluent is the sum of the solvent strengths of the volume fractions of the single solvents [17]: $S_T = \psi_A \times S_A + \psi_B \times S_B + \psi_C \times S_C$, which gives a solvent strength of 3.92 in the case of $P_S=631$ on the top irregular triangle ($S_T = 0.6 \times 4.5 + 0.3 \times 3.2 + 0.1 \times 2.6 = 3.92$). The same relationship between the concentrations of A, B and C is valid by dilution of this solvent mixture to $S_T = 2.6$.

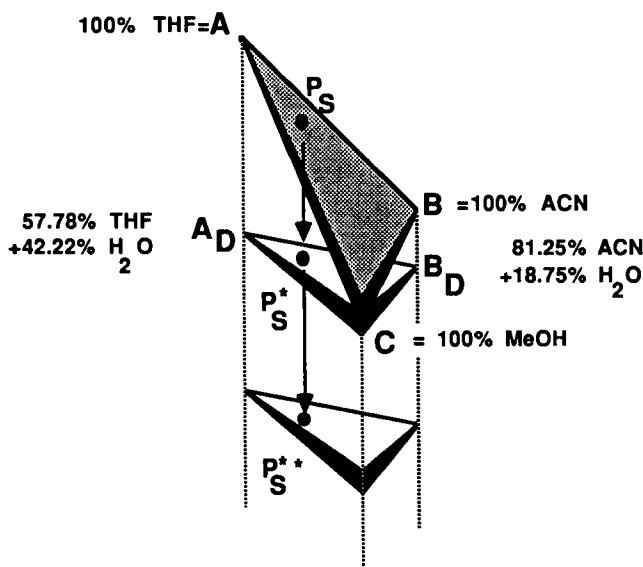


Figure 4 Example for the calculation of the mobile phase composition with the "PRISMA" model in RP chromatography.

For the calculation of the mobile phase composition it has to be considered that in the regular part of the prism minimum two corners of the triangles represent solvents containing the solvent strength regulator. In the case of the solvent strength 2.6 and $P_S^*=631$ (Fig. 4), the ψ of the solvent mixture represented by corner A_D (57.78% THF and 42.22 % water) is 0.6, the volume fraction of the solvent mixture representing corner B_D (81.25 % ACN and 18.75 % water) is 0.3, while that of corner C (100% MeOH and 0% water) is 0.1. Accordingly, the concentration of THF in the final mixture (in $P_S^*=631$ at $S_T=2.6$) is 34.67% (0.6×57.78), that of ACN is 24.38% (0.3×81.25), MeOH is 10% (0.1×100), and water is 30.95% ($100 - 34.67 - 24.38 - 10$).

Dilution of the solvent characterized by the point P_S^* ($S_T=2.6$; $P_S=631$) with water in different proportions, gives mixtures with the same selectivity point ($P_S^*=P_S^{**}$) but with lower solvent strengths [e.g., $S_T=1.3$; $P_S=631$ {17.33%(34.67:2) THF + 12.19%(24.38:2) ACN + 5% (10:2) MeOH + 65.48% (30.95:2+50) water}]. Obviously the same solvent composition can also be obtained by first calculating the compositions at the edges of the prism, corresponding to the solvent strength desired, and subsequently mixing these in the proportions given by the volume fractions (selectivity point).

Relationship between the irregular and regular parts of the model

As may be derived from the calculation example mentioned above, the relationship between volume fractions of the top irregular and the regular part of the model are not identical, because as may be seen in Fig.4, the regular triangle with the highest solvent strength is geometrically a projection of the top irregular triangle. Only point C is identical in both triangles, all other points characterize other solvent compositions.

The selectivity points in the top irregular triangle can be described as $P_S = P_A; P_B; P_C$. In the highest regular triangle, point C also represents a neat solvent ($P_C = P_{CD}$), where the corners consist of P_{AD} and P_{BD} instead of P_A and P_B . So the selectivity points on the top regular triangle can be described as $P_S^* = P_{AD}; P_{BD}; P_{CD}$, where

$$PAD = (SA \times PA) / ST; PBD = (SB \times PB) / ST; \text{ and } PCD = (SC \times PC) / ST = PC$$

The conversion of the selectivity points between the irregular and regular triangle can be made as follows:

$$PS^* = (SA \times PA) / ST ; (SB \times PB) / ST ; (SC \times PC) / ST ;$$

The projection of THF, ACN, and MeOH on the top irregular triangle ($ST=3.92$) in $PS=631$, will be at

$$ST=2.6, PS^* = (4.5 \times 60) / 3.92 ; (3.2 \times 30) / 3.92 ; (2.6 \times 10) / 3.92 \text{ which gives the selectivity point } PS^* = 68.88- 24.49- 6.63 \text{ rounded off as } PS^* = 68.9- 24.5- 6.6.$$

That means the relation between the different solvents ($c_A : c_B : c_C$) is constant within the top irregular triangle for the different solvent strengths (in sections parallel to the top irregular triangle) and are also constant within the regular part at horizontal sections. So, if PS is defined as a selectivity point in the top irregular triangle and PS^* and PS^{**} in the regular part, $PS \neq PS^* = PS^{**}$, therefore, the eluent always has to be characterized with the solvent strength and the selectivity point.

EXPERIMENTAL

Furocoumarin isomers from *Heracleum sphondylium* (for structures see Table 1) and the flavonoid glycosides from *Betula* species (see Table 2) were isolated and identified in the Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, Switzerland.

Table 1 Structures of the flavonoid glycosides investigated.

R ₁	R ₂	Symbol	Structure
-Galactose	-OH	BF-1	
-Rutinose	-H	BF-2	
-Galactose	-H	BF-3	
-Glucuronic acid	-H	BF-4	
-Arabinopyranose	-H	BF-5	
-Arabinofuranose	-H	BF-6	
-Rhamnose	-H	BF-7	

Table 2 Structures of the furocoumarin isomers investigated

Name	R ₁	R ₂	Symbol	Structure
Sphondin	-H	-OCH ₃	FC-1	
Pimpinellin	-OCH ₃	-OCH ₃	FC-4	
Iso-Bergaptene	-OCH ₃	-H	FC-5	
Iso-Pimpinellin	-OCH ₃	-OCH ₃	FC-2	
Bergaptene	-OCH ₃	-H	FC-3	

Separation of all compounds was performed on a Spherisorb ODS II (3 μ m) 100 x 4 mm I.D. cartridge (Knauer, Berlin, FRG). All organic solvents [tetrahydrofuran (THF), acetonitrile (ACN) and methanol (MeOH)] were of HPLC quality (Romil Chemical, Shepshed, Leics, England); acetic acid pro analysis was obtained from Merck (Darmstadt, FRG). Water was freshly distilled and filtered through a 0.45 μ m membrane filter (Millipore, Bedford, MA, USA). For all experiments, a flow rate of 1ml/min was used. Between the single chromatographic experiments, the column was always equilibrated for 20 min. The temperature of the column was kept at 25^o C for all analyses.

A LC 41 four solvent HPLC with autosampler (both from Bruker-Franzen, Bremen, FRG) coupled with an Epson QX-16 computer and a FX-80 printer (Seiko Epson, Nagano, Japan) were used in these experiments. A Gilson model 116 UV detector (Gilson Medical Electronics, Middleton, WI, USA) was operated at a wavelength of 313 nm for furocoumarins and at 254 nm for the flavonoid glycosides.

The correlation between the k' values and the selectivity points were calculated with a Macintosh II computer (Apple Computer, Cupertino, CA, USA) using Statview 512+ (Brain Power, Calabas, CA, USA) and Cricket Graph (Cricket Software, Malvern, PA, USA) programs.

RESULTS AND DISCUSSION

Correlation between the selectivity points

In our experiments, the eluent combinations were systematically changed including the three basic selectivity points, where the mobile phase was described by three-digit numbers (see Fig.3a) at a constant solvent strength. The k' values of the separated compounds were displayed versus the selectivity points which symbolizes the composition of the eluent. The linear, quadratic, and cubic correlations were studied with the raw data as well as with the transformed data ($\ln k'$ and $1/k'$) keeping in mind, that the optimization strategy requires an exact correlation based on the measured data, if possible without transformation. For the various solvent combinations tested, the correlation was excellent using quadratic functions of the raw data and the transformed data ($\ln k'$ or $1/k'$). The correlations were tested in all three directions, along all lines (see in Fig.3, 118-181, 217-271, 316-361, 415-451, 514-541 and 181-811, 172-712, 163-613, 154-514, 145-415, etc.), and including all points on the selectivity triangle. Different solvent strength levels and several substance classes were examined. The best and simplest correlation was found with the quadratic function using the measured k' values without transformation. In Fig.5 the quadratic correlation between the measured k' values of the flavonoid glycosides tested as an example for highly polar compounds in twelve selectivity points ($PS=190$; 10-85-05; 181; 172; 163; 154; 145; 136; 127; 118; 10-05-85; 109), is shown and the corresponding r^2 values are given. Since the selectivity point is a symbol in the geometrical model, the actual calculation was made with the proportion of one of the two solvents which are varied; the amount of the other was determined by the first, while the portion of the third was always constant. Similar functions were obtained ($r^2 > 0.989$) with all substance classes tested; therefore, the correlation between the k' values and the selectivity points at a constant solvent strength level can be generalized and expressed by the function:

$$k' = a (PS)^2 + b (PS) + c$$

Since for a quadratic mathematical function requires a minimum of four data points two other points were selected between the basic selectivity points (between 811 and 181 these were 631 and 361; between 181

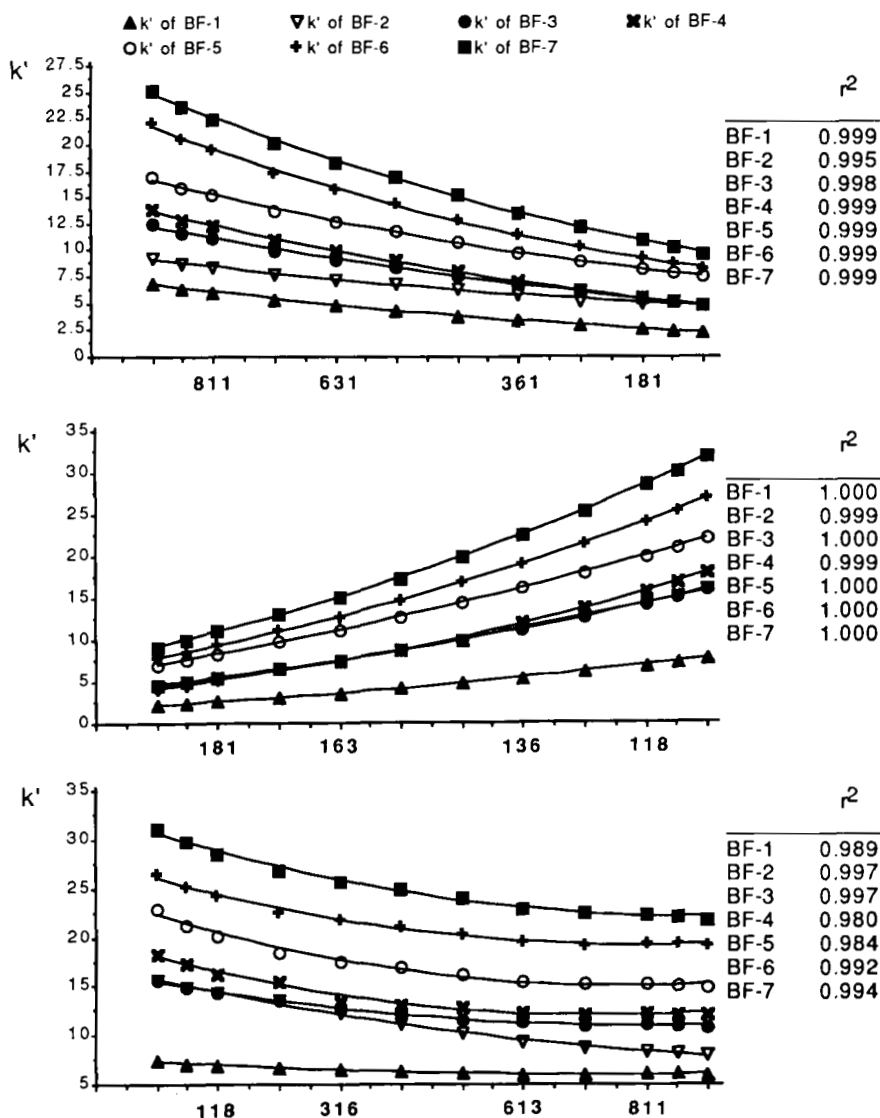


Figure 5 Correlations between the k' values of the flavonoid glycosides and the 12 selectivity points at a constant solvent strength. Mobile phase: $S_T=0.44$, combinations of THF, ACN, MeOH, water determined by the selectivity points, + 5% acetic acid as modifier, flow rate: 1 ml/min.

and 118 these were 163 and 136, between 118 and 811, 316, 613). From the data obtained with the solvent combinations described by these selectivity points, the k' values of the points in between (721; 541; 451; 271; 172; 154; 145; 127; 217; 415; 514; 712) could be calculated. No significant difference was found between the functions calculated from 12, 8, or 4 points. To demonstrate the negligible difference between the measured and calculated k' values, some data from the flavonoid glycosides are given in Table 3. Generally the difference between the measured and calculated k' values was less than 0.1. Larger differences were obtained when two or three compounds eluted together (see in Table 3, e.g., BF-3 and BF-4 in $PS=190$, BF-2, BF-3 and BF-4 in $PS=172$), so recognition of the peak maxima was not correct enough with the integrator.

Similarly good correlations were obtained for apolar compounds. The results of the separations of five furocoumarin isomers tested are shown in Fig.6. The k' values of the furocoumarins are plotted versus all selectivity points with three-digit numbers around the selectivity triangle (181-118-811) at a constant solvent strength. The validity of the quadratic function at the horizontal plane (see Fig.6) is also recognized by the depicted regression coefficients which varied between 0.99 and 1.0.

After the horizontal function we tested also the vertical relationship between the k' values and the solvent strength in defined selectivity points. Using four solvent systems, we found that the correlation can be described by the function:

$$\ln k' = m(PS) + n$$

Because a linear mathematical function requires a minimum of three measured data points, five different solvent strengths were tested in the three basic selectivity points. The results may be seen in Fig.7 for the five furocoumarins tested in a solvent strength range of 1-12 %.

When changing the solvent strength in a four solvent system, the relationship of the three organic solvents is constant ($CA:CB:CC = \text{const}$); the system may be considered a pseudo binary eluent system.

Table 3 Difference between the measured and calculated k' values of the flavonoid glycosides investigated.

		Selectivity points													
		190	10-85-05	181	172	163	154	145	136	127	118	10-05-80	109		
BF-1	k' measured	2.11	2.34	2.57	3.07	3.52	4.06	4.68	5.31	6.03	6.80	7.21	7.68		
	k' calculated	2.15	2.34	-	2.99	-	4.04	4.65	-	6.03	-	7.26	7.62		
	difference	0.04	0	-	0.08	-	0.02	0.03	-	0	-	0.05	0.06		
BF-2	k' measured	4.08	4.58	5.11	6.53	7.43	8.60	9.88	11.15	12.60	14.14	14.95	15.83		
	k' calculated	4.15	4.65	-	6.24	-	8.60	9.88	-	12.63	-	14.41	15.66		
	difference	0.07	0.07	-	0.29	-	0	0	-	0.03	-	0.54	0.17		
BF-3	k' measured	4.53	5.02	5.50	6.53	7.43	8.60	9.88	11.15	12.60	14.14	14.95	15.83		
	k' calculated	4.70	5.09	-	6.43	-	8.58	9.81	-	12.59	-	14.96	15.80		
	difference	0.17	0.07	-	0.01	-	0.02	0.07	-	0.01	-	0.01	0.03		
BF-4	k' measured	4.53	5.02	5.50	6.53	7.43	8.60	9.88	11.85	13.67	15.64	16.66	17.73		
	k' calculated	4.77	5.34	-	6.38	-	8.75	10.18	-	13.65	-	16.72	17.85		
	difference	0.24	0.32	-	0.15	-	0.15	0.3	-	0.02	-	0.06	0.12		
BF-5	k' measured	6.94	7.63	8.33	9.82	11.08	12.59	14.34	16.03	17.92	19.87	20.89	21.96		
	k' calculated	7.11	7.70	-	9.65	-	12.65	14.27	-	17.89	-	20.9	21.96		
	difference	0.17	0.07	-	0.18	-	0.04	0.07	-	0.03	-	0.01	0		
BF-6	k' measured	7.72	8.54	9.36	11.14	12.67	14.54	16.75	18.89	21.32	23.89	25.26	26.72		
	k' calculated	7.92	8.60	-	10.90	-	14.55	16.63	-	21.30	-	25.27	26.64		
	difference	0.20	0.06	-	0.24	-	0.01	0.12	-	0.02	-	0.01	0.08		
BF-7	k' measured	9.10	10.06	11.02	13.13	14.98	17.22	19.84	22.40	25.32	28.38	30.00	31.74		
	k' calculated	9.25	10.07	-	12.83	-	17.21	19.7	-	25.28	-	29.99	31.66		
	difference	0.15	0.01	-	0.30	-	0.01	0.14	-	0.04	-	0.01	0.08		

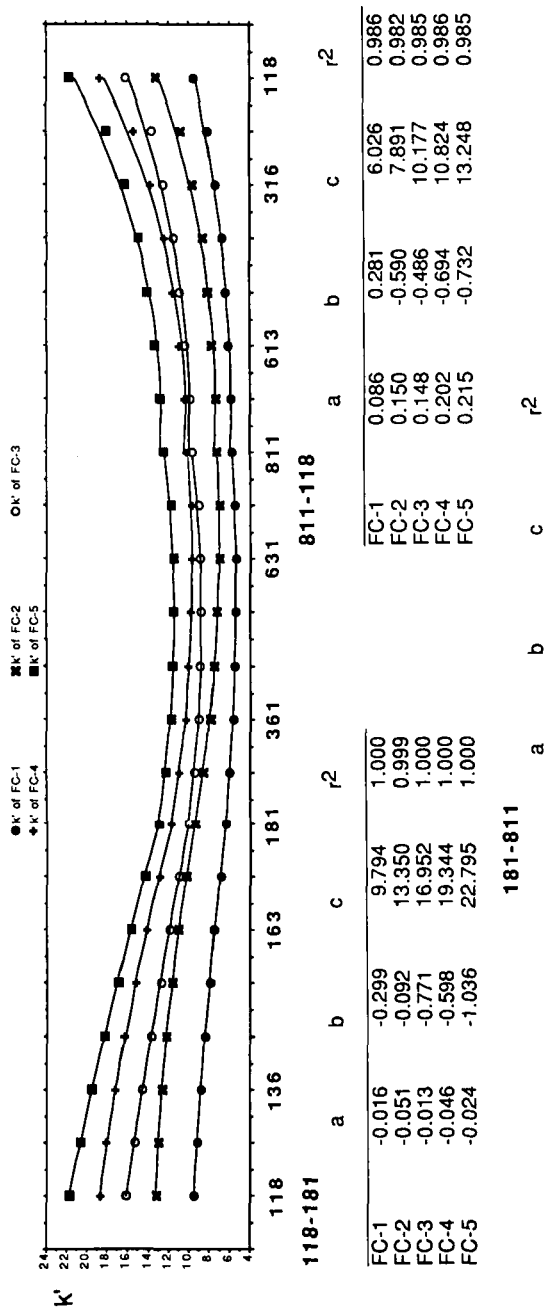


Figure 6 Correlations and functions between the k' values of the furcoumarin isomers and the 8 selectivity points at a constant solvent strength. Mobile phase: $ST=1.0$ combinations of THF, ACN, MeOH, water determined by the selectivity points; flow rate 1.0 ml/min.

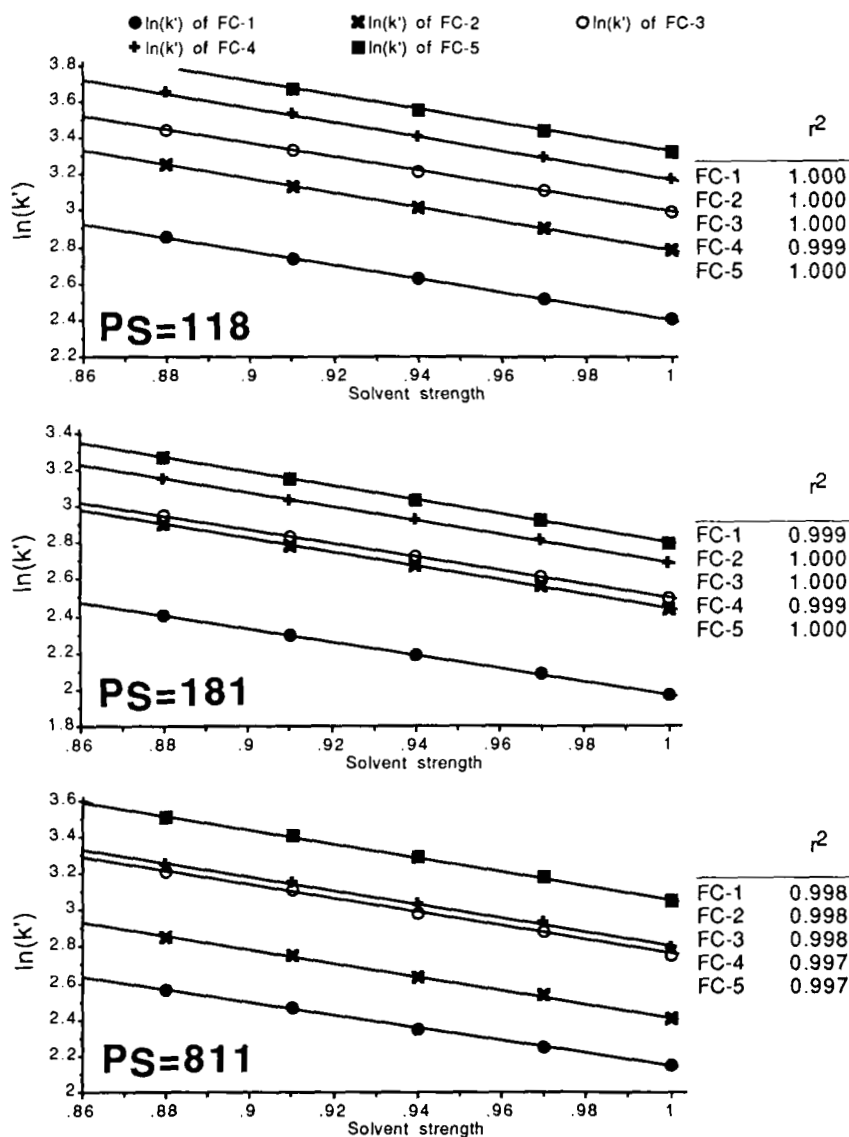


Figure 7 Correlations between the k' values of the furocoumarin isomers and the solvent strength in the three basic selectivity points. Mobile phase: combinations of THF, ACN, MeOH, water determined by the selectivity points at $S_T=1.0, 0.97, 0.94, 0.91$ and 0.88 ; flow rate 1.0 ml/min.

These results show that the correlation known already for binary eluent systems [see in ref. 3] is also valid in the "PRISMA" model.

Proposed optimization strategy with correlation of the selectivity points

The following strategy is based on the vertical and horizontal correlation between the selectivity points and the k' values, where the number of compounds to be separated is known. The evaluation of peak recognition and separation criteria based on this optimization strategy is in preparation [18].

So the proposed strategy is as follows: After selection of the three suitable solvents according to the Snyder classification [5], optimization of the mobile phase composition is started.

I. Measurement of k' values on level ST_1 .

Considering the number of separated substances and the separation time, the solvent strength is first adjusted stepwise in selectivity point 333 with the solvent strength regulator. At the determined solvent strength (ST_1), the k' values of selectivity points representing four solvent combinations along the edges of the triangle between the basic selectivity points (811-181-118) and the selectivity points around the center of the triangle (433-343-334) are measured (see points marked black in Fig.8a).

II. Calculation and prediction of k' values on level ST_1 .

From each of the four measured selectivity points along a line, the mathematical function is calculated as $k' = a (PS)^2 + b (PS) + c$ for the substances to be analyzed. This is followed by calculation of the k' values of the selectivity points between the measured points from the obtained function (see points marked grey in Fig.8b). The k' values of the remaining selectivity points are predicted with the function obtained with the help of the calculated selectivity points (see remaining points in Fig.8b) including all combinations of four and three solvents. Some data from furcoumarin isomers are given in Table 4 to demonstrate the good correlations between the measured and predicted k' values

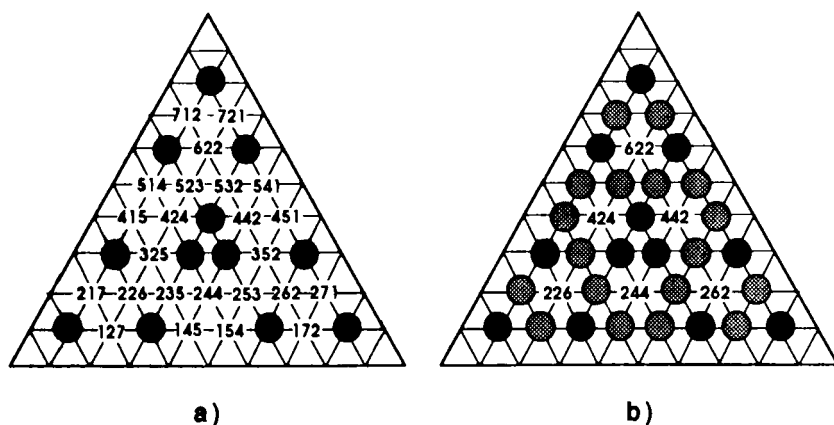


Figure 8 Strategy for mobile phase optimization using the "PRISMA" model.

a) Measurement of the k' values in 12 selectivity points at a selected solvent strength level. Black points in triangle indicate the selectivity points to be measured.

b) Calculation and prediction of the k' values of the remaining selectivity points at this solvent strength level. Grey points indicate the selectivity points at which the k' values can be calculated with functions obtained from measured values. Prediction of the k' values in the remaining points with functions obtained from the measured and calculated data.

III. Determination of k' values on two other solvent strength levels

Depending on the results and the aim of the optimization, the strategy described in I. and II. has to be repeated on two other solvent strength levels. Whether the solvent strength has to be reduced, increased or both depends on the data obtained with the first experiments. As a general guideline, the difference between the three solvent strength levels should not exceed 10-15%.

IV. Calculation and prediction of the optimum separation

After calculation and prediction of the k' values on the three selected solvent strength levels, the retention surfaces in the regular part of the model can be calculated for each compound in the spatial design using the vertical and horizontal functions. Between the consecutive retention surfaces the minimal resolution values can be calculated [18].

Table 4 Difference between the measured and predicted k' values of the furocoumarin isomers investigated.

		Selectivity points					
		226	262	622	442	424	244
FC-1	measured	7.65	6.32	5.88	5.79	6.37	6.96
	predicted	7.66	6.27	5.67	5.73	6.38	6.96
	difference	0.01	0.05	0.21	0.06	0.01	0
FC-2	measured	10.42	9.00	7.52	7.78	8.34	9.77
	predicted	10.40	8.94	7.29	7.72	8.33	9.67
	difference	0.02	0.06	0.26	0.06	0.01	0.1
FC-3	measured	12.79	10.05	9.65	9.37	10.64	11.35
	predicted	12.83	9.99	9.40	9.15	10.66	11.41
	difference	0.04	0.06	0.25	0.22	0.02	0.06
FC-4	measured	14.62	11.70	10.38	10.54	11.70	13.21
	predicted	14.57	11.63	10.17	10.48	11.78	13.10
	difference	0.05	0.07	0.21	0.06	0.08	0.11
FC-5	measured	17.04	13.21	12.55	12.33	13.99	15.10
	predicted	17.06	13.16	12.32	12.24	14.02	15.11
	difference	0.02	0.05	0.09	0.09	0.03	0.01

CONCLUSION

Correlations between the selectivity points and k' values on the horizontal plane and in the vertical plane of the "PRISMA" model expressed as functions are highly significant considering the measured, calculated, and predicted data for isocratic runs at a constant solvent strength level. The horizontal quadratic correlation presented here allows the calculation and prediction of the k' values in all selectivity points with the help of the 12 defined selectivity points. (A simple linear approach is also sufficient in some cases, but testing various substance classes a quadratic function gave the best correlation in all cases.) For the quadratic correlation essentially less data points would be needed, because of three unknown coefficients in the equation. With more data,

like the 12 measurements proposed, departure of the quadratic model assumption can be tested, so more experimental runs are required than by using other recently published optimization methods [1,3]. To achieve the optimum separation with a two, three, or four solvent system, the third dimension is needed; therefore, the vertical correlation can be employed. Because this can be linearized, three solvent strengths levels are sufficient. The presented optimization strategy can be employed in all cases where the number of compounds to be separated is known. To obtain a suitable resolution between the compounds to be separated, the first two steps described are generally sufficient. The correlations are also relevant when modifiers are used in a constant amount, which was the case for the flavonoid glycosides. The proposed mobile phase optimization strategy is the base for an automated mobile phase optimization procedure for Bruker-Franzen four solvent HPLC system, which is in preparation [18].

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REFERENCES

- [1] Berridge, J.C., Techniques for the automated optimisation of HPLC separation, Wiley, Chichester, 1985.
- [2] Berridge, J.C., Chemistry in Britain, 1063 (1987).
- [3] Schoenmakers, P.J., Optimization of chromatographic selectivity, Elsevier, Amsterdam, Oxford, New York, Tokyo, 1986.
- [4] Kirkland, J.J., Glajch, J.L., J.Chromatogr. 255, 27 (1983).
- [5] Snyder, L.R., J.Chromatogr. Sci., 16, 223 (1978).
- [6] Glajch, J.L., Kirkland, J.J., Snyder, L.R., J. Chromatogr. 238, 269 (1982).
- [7] Nyiredy, Sz., Meier, B., Erdelmeier, C.A.J., Sticher, O., HRC & CC, 8, 186 (1985).
- [8] Nyiredy, Sz., Sticher, O., HRC & CC, 10, 208 (1987).
- [9] Nyiredy, Sz., Erdelmeier, C.A.J., Meier, B., Sticher, O., Planta med., 241 (1985).
- [10] Nyiredy, Sz., Erdelmeier, C.A.J., Meier, B., Sticher, O., GIT Suppl. Chromatogr. , 4/85, 24 (1986).

- [11] Dallenbach-Toelke, K., Nyiredy, Sz., Meier, B., Sticher, O., *J.Chromatogr.*, **365**, 63 (1986).
- [12] Nyiredy, Sz., Application of the "PRISMA" model for the selection of eluent-systems in Overpressure Layer Chromatography (OPLC), Labor MIM, Hungary, 1987.
- [13] Nyiredy, Sz., Dallenbach-Toelke, K., Sticher, O., in Proceedings of the Fourth International Symposium on Instrumental High Performance Thin-Layer Chromatography (Ed. Traitler, H., Studer, H., Kaiser R.E.) Institute for Chromatography, 1987, pp.289-300.
- [14] Dallenbach-Toelke, K., Nyiredy, Sz., Meier, B., Sticher, O., *Planta med.*, 189 (1987).
- [15] Vuorela, H., Dallenbach-Toelke, K., Nyiredy, Sz., Hiltunen, R., Sticher, O., *Planta med.*, in press.
- [16] Nyiredy, Sz., Meier, B., Dallenbach-Toelke, K., Sticher, O., Eleventh International Symposium on Column Liquid Chromatography, Amsterdam, 1987, Abstr. No. Th-P-18.
- [17] Lehrer, R., *Int. Laboratory* **11**, 76 (1981).
- [18] Nyiredy, Sz. et al. in preparation.