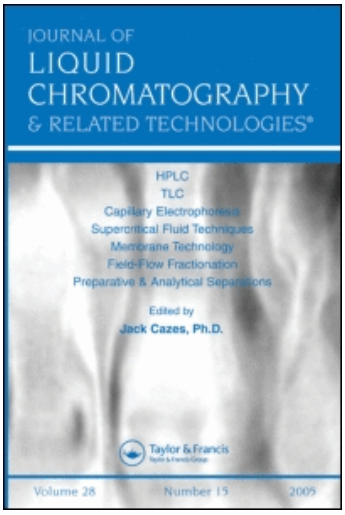

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Selectivity Tuning in an HPLC Multicomponent Separation

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Selectivity Tuning in an HPLC Multicomponent Separation

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Abstract: The optimisation of the selectivity of two columns coupled in series has been investigated in HPLC. Two columns with different polarities (PEG and CN) were coupled in series via a T connector and the retention of analytes chromatographed on the column system was tuned by varying the individual mobile phase flows in the coupled columns. The flow rate ratio necessary for receiving an optimum selectivity was calculated on the basis of the measured retention factors on the individual columns. The performance of this method has been studied by the separation of a model mixture of 17 analytes with various functionalities.

Keywords: Two columns coupled in series, Selectivity tuning in HPLC

INTRODUCTION

Selectivity is a powerful tool to improve the resolution of chromatographically separated sample constituents. Analysis of complex samples by HPLC requires relatively high selectivity in comparison to capillary GC since the HPLC columns commonly exhibit substantially lower efficiencies than capillary columns in GC.[1,2]

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Selectivity relates to the difference in physicochemical interactions between the solutes and the chromatographic system. In chromatography, selectivity is commonly expressed as the relative retention α of a critical pair of sample components:[1]

$$\alpha = \frac{k_j}{k_i} \tag{1}$$

where k are retention factors of adjacent compounds i and j and $k_j > k_i$.

It is obvious that if $\alpha = 1$, the components i and j are not resolved. In such cases the interactions between a chromatographic system and compounds i and j do not differ, and the separation system is considered non-selective.

Because of limited separation efficiency, selectivity is the most important tool in HPLC for achieving the required resolution R_{ji} as denoted in Eq. (2).[1]

$$R_{ji} = \frac{\sqrt{n_i}}{4}\frac{k_i}{k_i + 1}(\alpha - 1) \tag{2}$$

where n_i is the number of theoretical plates and k_i – is the retention factor of the less retained peak i.

For a given pair of compounds and separation mechanisms, the selectivity of the HPLC system depends on the nature of both mobile, as well as stationary phases.[1–5] Interactions of the solute with mobile and stationary phases may include a mixture of non-polar dispersive and specific polar forces. The terms "polar" and "non-polar" have been commonly used to describe a property of both the solute, as well as mobile and stationary phases. These terms/concepts should not be confused with selectivity. Selectivity is the result of the sum of the total complex interactions of two solutes with the mobile and the stationary phases.[3–5].

The selectivity of a stationary phase can be changed discontinuously by selection of a proper column packing, (by its polarity or other parameters), or continuously tuned by: using tailored-made stationary phases; using mixed stationary phases; by coupling different polarity columns in series (e.g. dual column chromatography or two-dimensional chromatography).

The selectivity is usually "optimized" on a stationary phase chosen by the chromatographer using a trial and error approach. The optimization of selectivity of the separation system is then simplified to the tuning of the mobile phase composition in the isocratic and/or gradient mode.

After selecting the stationary phase and the mobile-phase components, several isocratic experiments are required to build a retention model. Multivariate optimization, however, allows finding the best combination of the parameters. One of the more common goals of any chromatographic analysis is to achieve the desired separation in the shortest possible time. The criterion describing the optimum selectivity of a chromatographic system to achieve this goal for multicomponent samples is not well defined.

Different computer-assisted chromatographic optimization methods have been developed. DryLab[6] uses retention data from scouting runs for

subsequent retention and resolution prediction via simulation. ChromSword[7,8] exploits structure fragments and dipole–dipole interactions to predict retention behavior. EluEx[9–11] helps to plan initial experimental conditions from chemical structures of separated solutes. The more recently introduced LabExpert software can plan experiments, collect and evaluate results, and adjust chromatographic conditions in real time, according to predefined decision schemes, until a satisfactory separation is achieved.[12]

It should be pointed out that most of the method development strategies, as well as many types of chromatography software have been focused on achieving the optimized separation of a complex mixture. This is an important milestone in the development of an HPLC method, but in addition to separation there are many other method parameters that also need to be optimized.[13,14]

In our previous paper, we have described the computer assisted procedure for the selectivity tuning of two reversal enantioselectivity HPLC columns {(R,R) Whelk-O 1 and (S,S) Whelk-O 1} coupled in series.[15] The performance of this method for adjusting the required selectivity was studied using the separation of enantiomers of alkoxysubstituted esters of phenylcarbamic acid. It was demonstrated that by adjusting the mobile phase flows in the individual columns, the elution order of enantiomers could be controlled.

The aim of this paper is to optimize the selectivity of a HPLC dual-column system for a multicomponent sample separation, evaluate mathematical models for the description of system performance, use the derived models for prediction and optimization of a chromatographic separation, and to prepare an off-line optimization procedure with new selectivity criterion for multicomponent samples.

THEORETICAL BACKGROUND ON THE RETENTION IN TWO COLUMNS COUPLED IN SERIES

The retention time of any compound in a column series ($t_{R,AB}$) is the sum of the retention times of the compound in the individual columns ($t_{R,A}$ and $t_{R,B}$):

$$t_{R,AB} = t_{R,A} + t_{R,B} \tag{3}$$

The retention time can be calculated from the basic chromatographic equation:

$$t_R = t_M(1 + k) \tag{4}$$

where t_M is mobile phase hold-up time and k is the retention factor calculated from Eq. (5):

$$k = \frac{t_R - t_M}{t_M} \tag{5}$$

The retention factor of any compound in two columns coupled in series can be found by combination of Eqs. (3)–(5):

$$k_{AB} = \frac{t_{M,A}}{t_{M,AB}} k_A + \frac{t_{M,B}}{t_{M,AB}} k_B \tag{6}$$

since the mobile phase hold-up time in two columns coupled in series ($t_{M,AB}$) can be found from the mobile phase hold-up times of the individual columns ($t_{M\ A}$ and $t_{M,B}$):

$$t_{M,AB} = t_{MA} + t_{MB} \tag{7}$$

The mobile phase hold-up time can be calculated from the formula used for calculation of the mobile phase velocity rate ($\bar{u}$) in corresponding columns:

$$\bar{u} = \frac{F_m}{A_m} \tag{8}$$

where F_m is the mobile phase flow rate ($F_m = V_M/t_M$, where V_M is a volume of the column occupied by the mobile phase), A_m is the cross section of the column occupied by the mobile phase ($A_m = \varepsilon \pi r^2$, where ε is column porosity and r - is the column radius).

From Eq. (8), it follows that the mobile phase hold-up time can be calculated from the column porosity (ε), a total column volume ($V_c = \pi r_c^2 L$, where r_c is a column radius and L is its length), and a mobile phase flow rate (F_m):

$$t_M = \frac{\varepsilon V_c}{F_m} \tag{9}$$

Combination of Eqs. (6) and (9) leads, after rearrangement, to the following expression:

$$k_{AB} = \frac{\varepsilon_A V_{c,A} F_{m,B}}{\varepsilon_A V_{c,A} F_{m,B} + \varepsilon_B V_{c,B} F_{m,A}} \cdot k_A + \frac{\varepsilon_B V_{c,B} F_{m,A}}{\varepsilon_A V_{c,A} F_{m,B} + \varepsilon_B V_{c,B} F_{m,A}} \cdot k_B \tag{10}$$

From this equation, it follows that the retention factor in a column series (k_{AB}) depends on the physical column characteristics (porosity ε and total column volumes V_c), the mobile phase flow rates ($F_{m,\ A}$ and $F_{m,\ B}$) and the retention factors of a compound in the individual columns (k_A and k_B).

Equation 10 is usually written in the simplified form:

$$k_{AB} = x_A k_A + x_B k_B \tag{11}$$

where x_A and x_B are weight factors, which can be found from Eqs. (10–11):

$$x_A = \frac{\varepsilon_A V_{c,A} F_{m,B}}{\varepsilon_A V_{c,A} F_{m,B} + \varepsilon_B V_{c,B} F_{m,A}} \tag{12}$$

or

$$x_B = \frac{\varepsilon_B V_{c,B} F_{m,A}}{\varepsilon_A V_{c,A} F_{m,B} + \varepsilon_B V_{c,B} F_{m,A}} \tag{13}$$

From Eqs. (10)–(13), it follows that the retention factor of any compound separated in two columns coupled in series (k_{AB}) depends on the choice of individual column polarities (expressed by the retention factors k_A and k_B) and the contribution of these polarities to overall column series polarity (expressed by the weight factors x_A and x_B). Thus, the overall polarity of two columns coupled in series can be tuned by adjusting the mobile phase flow in the individual columns, since weight factors (x_A and x_B), often called relative retentivities (Φ_A and Φ_B), determine the contributions of the individual column polarities to the overall polarity of the column series.[2,15]

Equation 11 can be written in the form:

$$k_{AB} = k_A + x_B(k_B - k_A) \tag{14}$$

which is a linear dependence of k_{AB} on x_B.

EXPERIMENTAL

A Varian Vista 5500 liquid chromatograph and Varian LC-50 UV variable wavelength detector (Varian, Walnut Creek, USA) were used. The mid-point flow of mobile phase was delivered using a HP 1050 pump (Hewlett-Packard, Avondale, USA).

Two columns were coupled in series using a low dead volume T-piece, allowing the addition of the drainage of a second flow (see Fig. 1). Both column series CN–PEG and PEG–CN were evaluated in this study. The first series was used for experiments where $F_{m,CN} < F_{m,PEG}$. The second series was for experiments where $F_{m,PEG} < F_{m,CN}$. Flows of the mobile phase in the individual columns ($F_{m,CN}$ and $F_{m,PEG}$) were set according to a three level experimental design,[16] both in the CN–PEG and PEG–CN series.

Column A

Nucleosil 100/5 μm, CN bonded phase, 150 mm × 4.6 mm I.D. (Alltech[17]).

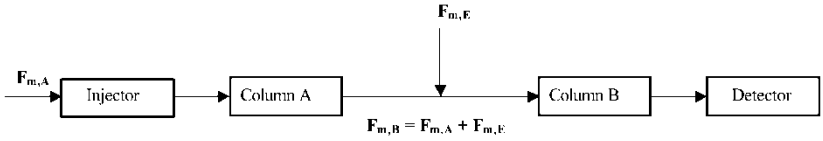

Figure 1. Schematic of the mobile phase flows in the dual HPLC column series. $F_{m,A}$ = flow of the mobile phase in the column A; $F_{m,E}$ = flow of the mobile phase into the T connector; $F_{m,B}$ = flow of the mobile phase in the column B ($F_{m,B} = F_{m,A} + F_{m,E}$).

Column B

Nucleosil 100/5 μm, PEG crosslinked phase, 150 mm × 4.6 mm I.D. (Column was prepared in Laboratory of Organic Chemistry, Gent University, Belgium).

Both columns were straight phase columns; the mobile phase composition hexane/dioxane 90/10% (vol/vol) was used throughout the experiment. Two sets of the seventeen substances listed in Table I was used in this study. Octyl benzene was injected for the determination of mobile phase hold-up time.

RESULTS AND DISCUSSION

An optimization procedure for selectivity tuning in HPLC is illustrated on a dual column system with a variation of mobile phase flow rates in the individual columns. The possibilities and limitations for variation of selectivity in a column system consisting of a bonded –CN phase column and a column containing PEG crosslinked stationary phase were stated according to experimental observations. The system was operated in the normal phase mode. This HPLC procedure evolved from previous studies involving gas chromatographic systems with flow variation. Since liquids can be considered

Table 1. Composition of test samples I and II

Functional group	Compound	Sample I	Sample II
–OH	Phenol	60 mg	60 mg
–OH	m-cresol	50 μl	50 μl
$-COOCH_3$	di-me-phthalate	25 μl	—
–OH	Benzhydrol	120 mg	120 mg
–OH	o-cresol	70 μl	70 μl
di $-COOC_2H_5$	di-et-phthalate	35 μl	35 μl
PAH	Coronene	30 mg	4.5 mg
di –C═O	9,10-antraquinone	1 mg	1.3 mg
PAH	benzo-a-pyrene	2.5 mg	1.6 mg
di $-COOCH_3$	di-me-terephthalate	9.4 mg	10.4 mg
PAH	Fluorene	2.5 mg	3.9 mg
$-COOCH_2C_6H_5$	benzoyl benzoate	35 μl	35 μl
–NH	Dibenzylamine	20 μl	20 μl
–C═O	Acetophenone	0.5 mg	1 μl
–OH	2,6-di-me-phenol	100 mg	92.8 mg
PAH	1,2,5,6-dibenzoantracene	13 mg	5.0 mg
PAH	Triphenylene	0.5 mg	0.1 mg

non-compressible, HPLC is less problematic than HRGC because of its flow additivity.[15,18] Also, it was observed that the system must be completely equilibrated, and the temperature controlled in order to obtain the best results. Connection of the columns via a simple metal T-piece provided an additional inlet stream for the mobile phase in the middle of the system. It did not cause additional broadening of peaks. A comparison of a number of theoretical plates for four test compounds (Fig. 2) shows very little difference between the directly coupled and the T-piece coupled system. The columns were connected in both orders. In the following text, A denotes the CN column and B denotes the PEG column.

Since HPLC retention data is not as reproducible as that in gas chromatography, both columns were carefully checked for their performance throughout the experiment. The temperature of the columns set was monitored and termostated at $22 \pm 2^{\circ}$C. Table 2 illustrates the retention data variation over one month with daily use of the system. It is obvious that especially for phenolic types of compounds on the PEG stationary phase and ester type compounds on cyano phase, the retention deviation ranges to a maximum of 10% of the original value.

Two models were used for optimization of the selectivity and for providing a description of the overall retention of compounds in CN–PEG or PEG–CN system as a function of flow rates $F_{m,CN}$, $F_{m,PEG}$: for the series CN–PEG flow rates $F_{m,CN} = F(pump1)$, $F_{m,PEG} = F_{m,\,CN} + F_{m,\,E}\,(pump2)$; and for the

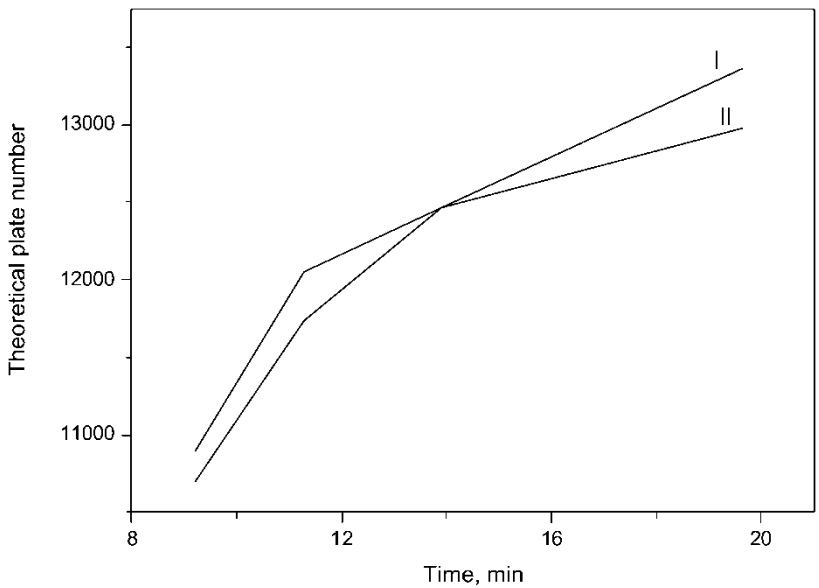

Figure 2. Comparison of the number of theoretical plates for a HPLC column system coupled directly (I), and via a T-piece (II).

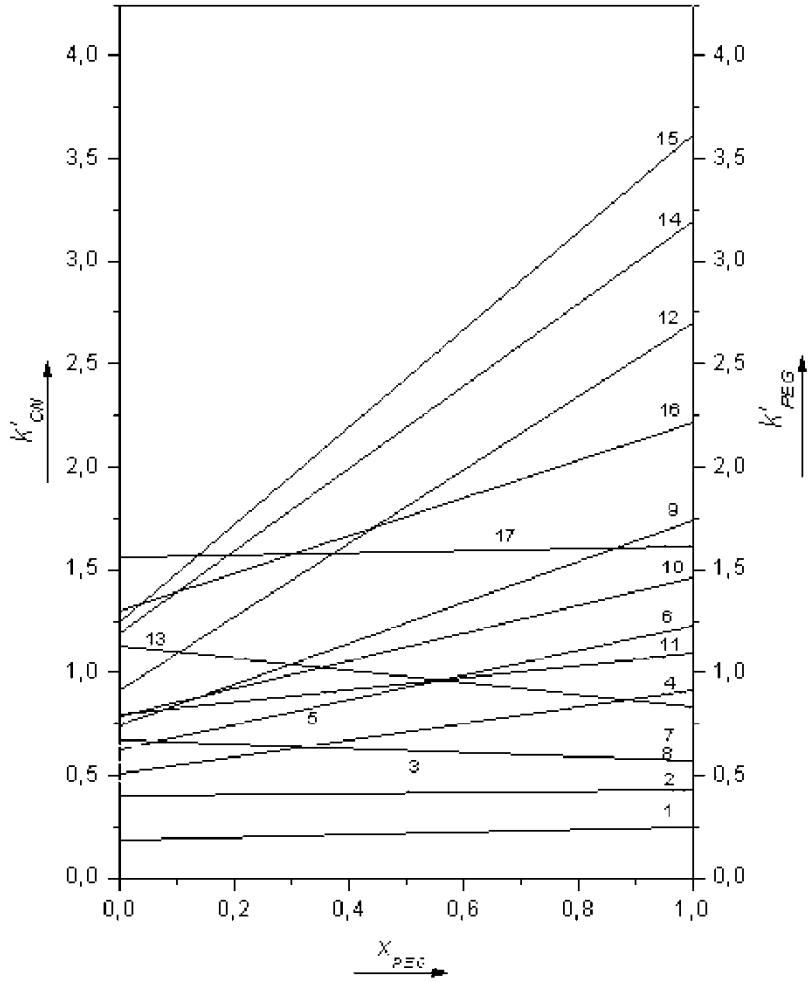

Figure 3. Dependence of the retention factors (k_i) of model sample constituents on the weight factor (x_{PEG}). For details see Experimental.

series *PEG–CN* flow rates $F_{m,PEG} = F(pump1)$, $F_{m,\ CN} = F_{m,\ PEG} + F_{m,\ E}$ (*pump2*).

For the calculation of data needed for optimization of the column series CN and PEG selectivity, the mobile phase hold-up times in the column series ($t_{M,CN-PEG} = t_{M,PEG-CN}$), retention factors of sample constituents in the column CN and PEG series ($k_{i,CN-PEG} = k_{i,PEG-CN}$), and peak widths at half peak heights ($w_{i,h/2,CN-PEG} = w_{i,h/2,PEG-CN}$) were approximated by mathematical models.

Table 2. HPLC retention factor reproducibility on obtained for model sample constituents on single PEG crosslinked and –CN bonded columns in duration of column system use

		CN			PEG		
		Retention factor, k			Retention factor, k		
No.	Compound	Sample 1	Sample 2	Δk, %	Sample 1	Sample 2	Δk %
1	Fluorene	0.186	0.186	0.00	0.252	0.260	2.98
2	Dibenzylamine	0.405	0.392	−3.32	0.430	0.435	1.16
3	Benzoylbenzoate	0.469	0.461	−1.74	0.513	0.513	−0.10
4	Triphenylene	0.509	0.497	−2.41	0.909	—	—
5	benzo(a)pyrene	0.566	0.555	−1.98	1.085	1.095	0.92
6	2,6-di-me-phenol	0.627	0.625	−0.32	1.227	1.272	3.63
7	di-me-terephthalate	0.642	0.629	−2.07	0.645	0.660	2.33
8	Acetophenone	0.668	0.647	−3.25	0.568	0.573	0.97
9	Coronene	0.739	0.714	−3.50	1.735	1.762	1.56
10	1,2,5,6-di-benzoanthracene	0.790	0.761	−3.81	1.461	1.479	1.20
11	9,10-anthraquinone	0.796	0.776	−2.58	1.092	—	
12	o-cresol	0.914	0.909	−0.55	2.700	2.703	0.11
13	di-et-phthalate	1.129	1.121	−0.71	0.836	—	—
14	m-cresol	1.191	1.180	−0.93	3.193	3.279	2.69
15	Phenol	1.249	1.275	2.04	3.620	4.096	13.15
16	Benzhydrol	1.298	1.296	−0.15	2.217	2.314	4.38
17	di-me-phthalate	1.559	1.514	−2.97	1.618	1.623	0.31

The dependence of the retention factors of all analytes in the column series ($k_{i,CN-PEG} = k_{i,PEG-CN}$) on the flows in the individual columns ($F_{m,CN}$ and $F_{m,PEG}$) can be described by the second order polynomial:

$$k_{i,CN-PEG} = k_{i,PEG-CN} = a_0 + a_1 \cdot F_{m,CN} + a_2 \cdot F_{m,PEG} + a_3 \cdot F_{m,CN}^2 + a_4 \cdot F_{m,PEG}^2 + a_5 \cdot F_{m,CN} \cdot F_{m,PEG} \quad (15)$$

where the various a's are coefficients, and F_m is the mobile phase flow in the CN and PEG column respectively.

The dependence of the mobile phase hold-up time in the column series on flows $F_{m,CN}$ and $F_{m,PEG}$ has been approximated by the following equation:

$$t_{M,CN-PEG} = t_{M,PEG-CN} = b_0 + \frac{b_1}{F_{m,CN}} + \frac{b_2}{F_{m,PEG}} \quad (16)$$

where the various b's are coefficients, and F_m is the mobile phase flow in the CN and PEG columns, respectively.

The dependence of the peak half width on retention time is given by the following linear equation:

$$w_{i,h/2,CN-PEG} = w_{i,h/2,PEG-CN} = c_0 + c_1 \cdot t_{R,i,CN-PEG} = c_0 + c_1 \cdot t_{R,i,PEG-CN} \quad (17)$$

where the c's – are coefficients and $t_{R,I}$'s – are retention times of the sample constituents obtained on the CN and PEG column series. The dependence of the peak half width, $w_{i,h/2}$, on retention time, $t_{R,i}$, for all fully separated peaks on the column series CN–PEG and PEG–CN at all experimental combinations of $F_{m,CN}$ and $F_{m,PEG}$ are shown in Fig. 4.

The coefficients for Eqs. (15)–(17) were found by regression analysis of the data obtained from the separation of the model sample constituents using 12 experiments, in the parameter space $F_{m,A}$, $F_{m,B}$ (0.5–1.5 mL min^{-1}). The separations at $F_{m,A} = F_{m,B}$ were checked on the both CN–PEG and PEG–CN column series. Figure 5 shows the chromatograms obtained for equal flows, i.e., $F_{m,CN} = F_{m,PE\ G} = 0.5$ mL/min for both the CN–PEG (A) and PEG–CN (B) series. Differences in retention times on both chromatograms resulted from the fact that the flow rates of different manufacturers of HPLC pumps were both inaccurate and unequal. Figures 6 and 7, however, shows that after the correction of the pre-set and measured flows, the retention on both column series, CN–PEG and PEG–CN, were almost the same for the flows $F_{m,CN} = F_{m,PEG} = 1.0$ mL/min and $F_{m,CN} = F_{m,PEG} = 1.5$ mL/min, respectively.

Figures 8–10 shows the separation of a mixture on the constituents in CN–PEG and PEG–CN column series at other $F_{m,CN}$, $F_{m,PEG}$ flows. Regression analyses were performed on a PC using Origin 4.1 software.[19] Coefficients for Eq. (15) were tested both by a residual analysis as well as by the F-test and the T-test. It has been found that the F values for a_0, a_1

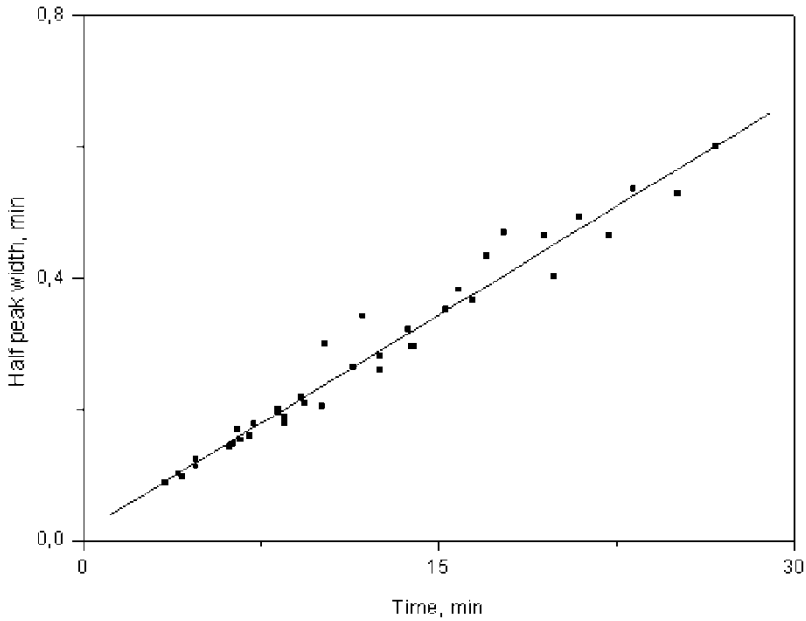

Figure 4. Dependence of the peak half widths, $w_{i,h/2}$, on retention time, $t_{R,i}$, for all fully separated peaks with the column series CN–PEG and PEG–CN at all experimental combinations of $F_{m,CN}$ and $F_{m,PEG}$.

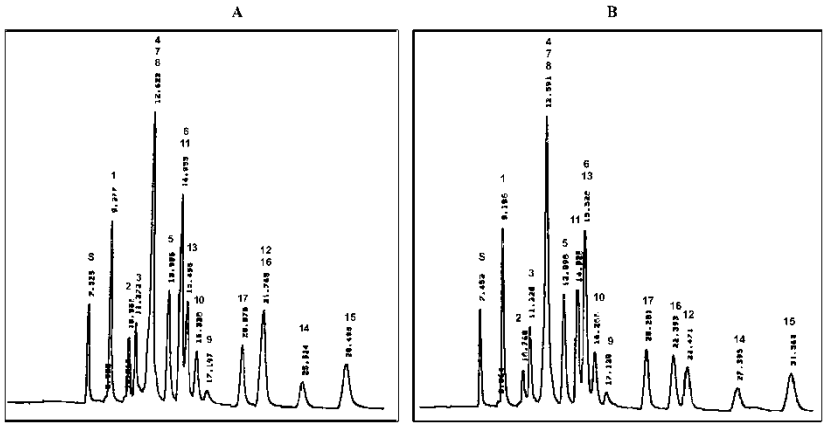

Figure 5. Chromatograms obtained by separation of the test mixture constituents in the column series CN–PEG (**A**) and PEG–CN (**B**) with equal mobile phase flows ($F_{m,CN} = F_{m,PEG} = 0.5\,\mathrm{mL/min}$).

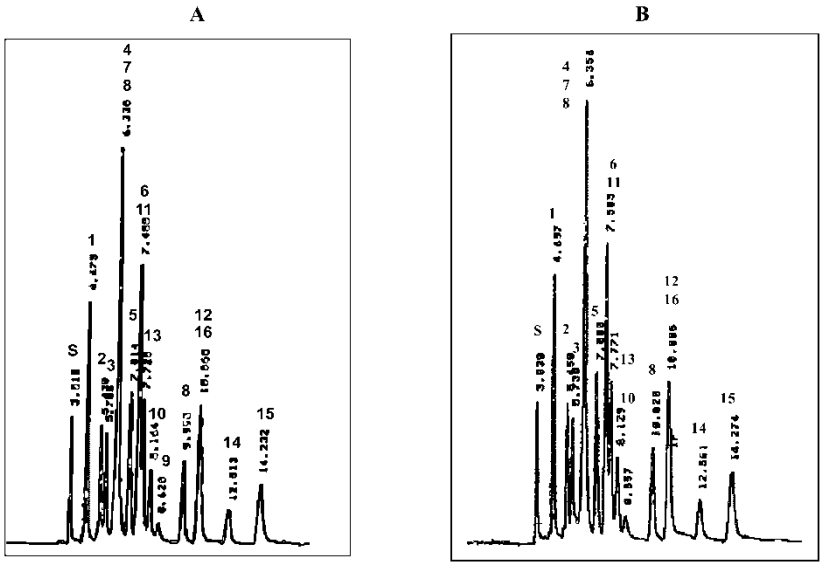

Figure 6. Chromatograms obtained by separation of the test mixture constituents in the column series CN–PEG (**A**) and PEG–CN (**B**) with equal mobile phase flows ($F_{m,CN} = F_{m,PEG} = 1.0\,\mathrm{mL/min}$).

and a_3 coefficients were higher for compounds, which did not differ dramatically in retention factors on A and B columns. The F values for the a_4 coefficients were always higher than the a_3 coefficients for compounds, which had substantially different retention factors on the A and B columns.

A computer program written for a GC dual column system was transformed for input of retention factors, mobile phase hold-up time, and last peak retention time.[18] The optimization procedure was based on a comparison of the predicted and required resolution factors. The predicted resolution factors were calculated for all adjacent peak pairs using the predicted retention factors (calculated from Eq. (15) for all combinations of $F_{m,CN}$ and $F_{m,PEG}$ with a 0.01 mL/min step), according to the modified Eq. (2):

$$R^{pr}_{ji,CN-PEG}(F_{m,CN}, F_{m,PEG}) = R^{pr}_{ji,PEG-CN}(F_{m,CN}, F_{m,PEG}) = \frac{\sqrt{n_{ji}}}{4} \cdot \frac{k_i}{1+k_i} \cdot \frac{k_j - k_i}{k_i} \tag{18}$$

where k_i and k_j are retention factors predicted for compounds i and j at the flows $F_{m,CN}$ and $F_{m,PEG}$.

The number of theoretical plates, n_i, was calculated for all peaks from the peak half widths (found from Eq. (15) for all combinations of $F_{m,CN}$ and

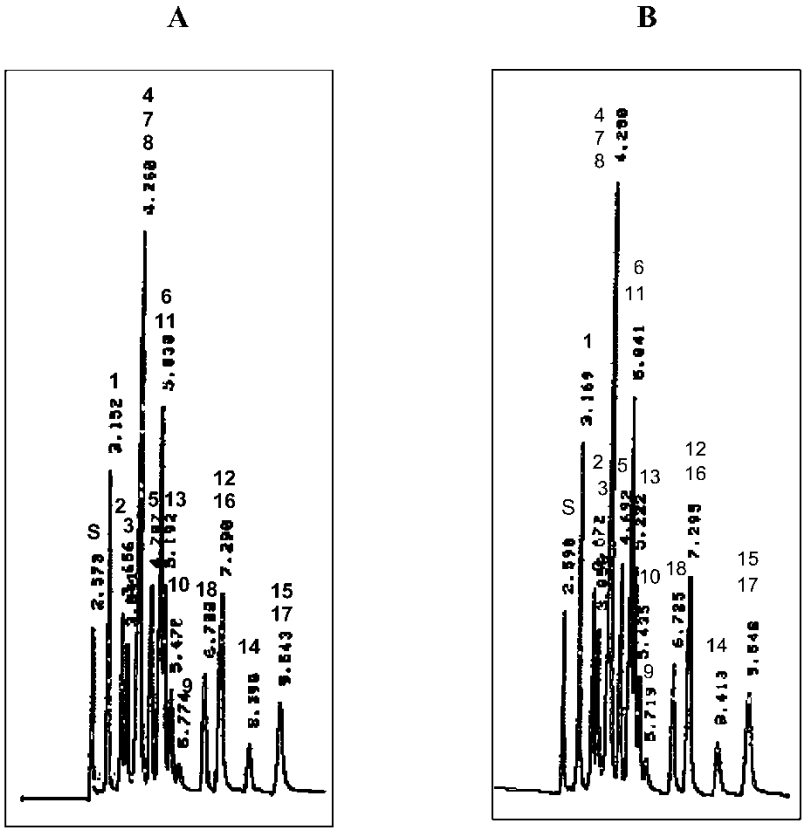

Figure 7. Chromatograms obtained by separation of the test mixture constituents in the column series CN–PEG (**A**) and PEG–CN (**B**) with equal mobile phase flows ($F_{m,CN} = F_{m,PEG} = 1.5\,\mathrm{mL/min}$).

$F_{m,PEG}$ with the 0.01 mL/min step), according to the equation:

$$n_i = 5.54\left(\frac{t_{R,i,CN-PEG}}{w_{h/2,CN-PEG}}\right)^2 = 5.54\left(\frac{t_{R,i,PEG-CN}}{w_{h/2,PEG-CN}}\right)^2 \tag{19}$$

The resolution factors predicted of all adjanced peak pairs at the given combination of flows ($F_{m,CN}$ and $F_{m,PEG}$ changed in the range of 0.5–1.5 mL/min with the 0.01 mL/min step) were used to calculate the selectivity criterion (C_p), which shows the maximum number of peaks resolved with a chosen resolution factor in the shortest time. C_p was calculated according to the formula:[18]

$$C_p(F_{m,A}, F_{m,B}) = \sum_{i=1}^{n} m_i - \frac{t_{\max}}{t_{\max} - t_{R,n}} \tag{20}$$

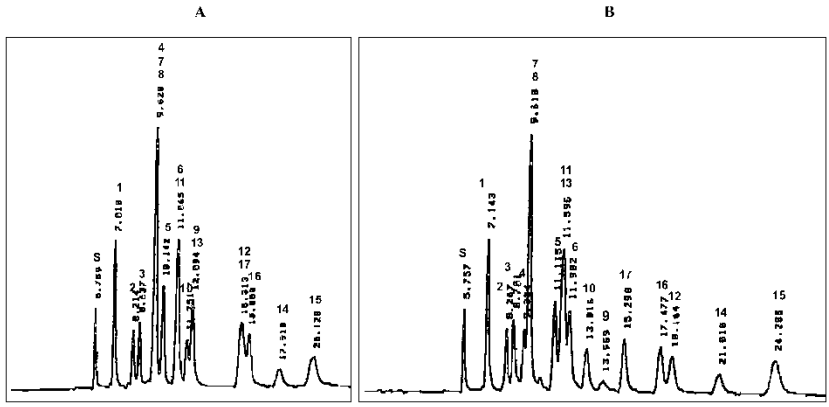

Figure 8. Chromatograms obtained by separation of the test mixture constituents in the column series: CN–PEG with the mobile phase flows: $F_{m,CN} = 0.5\,\mathrm{mL/min}$ and $F_{m,PEG} = 1.0\,\mathrm{mL/min}$, and PEG–CN with the mobile phase flows: $F_{m,PEG} = 0.5$ mL/min and $F_{m,CN} = 1.0\,\mathrm{mL/min}$.

where $m_i = 1$ if $R_{ji} \geq R_{req}$ else m = 0; the t_{max} is the selected maximum acceptable analysis time, and $t_{R,n}$ is the retention time of the last eluted peak. The value of maximum acceptable analysis time is arbitrarily chosen so that for any analysis $t_{max} > t_{R,n}$.

The first term of the right hand side in Eq. (20) gives the number of compounds resolved on a chromatogram equal to or better than the required resolution (threshold), and it is the primary part of the criterion, C_p. Since

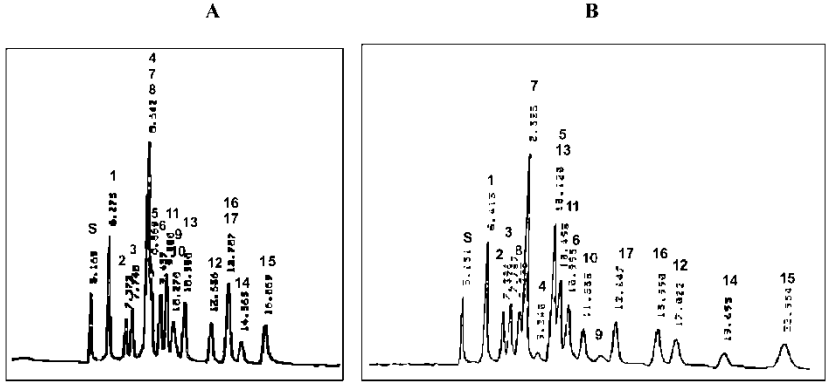

Figure 9. Chromatograms obtained by separation of the test mixture constituents in the column series: CN–PEG with the mobile phase flows: $F_{m,CN} = 0.5\,\mathrm{mL/min}$ and $F_{m,PEG} = 1.5\,\mathrm{mL/min}$, and PEG–CN with the mobile phase flows: $F_{m,PEG} = 0.5$ mL/min and $F_{m,CN} = 1.5\,\mathrm{mL/min}$.

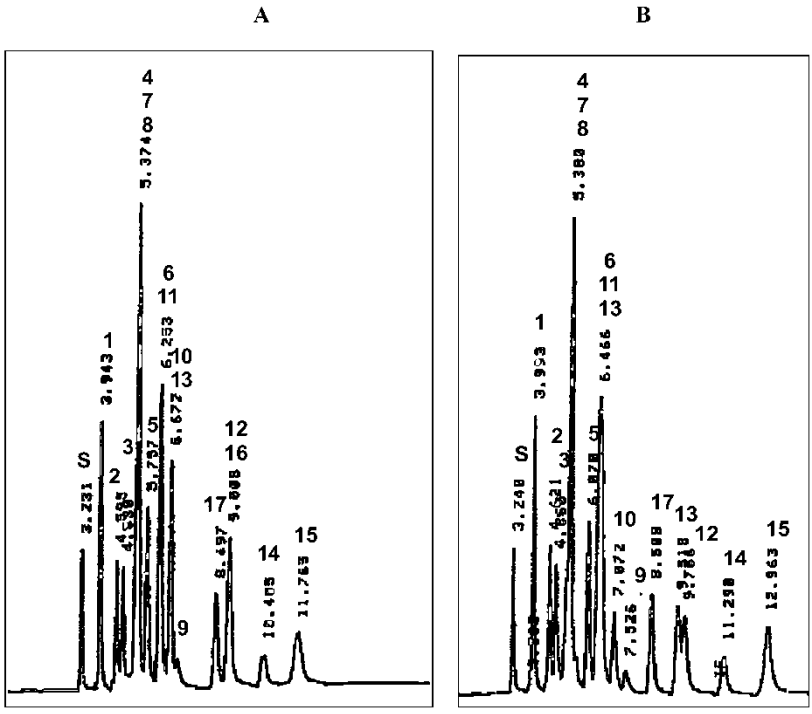

Figure 10. Chromatograms obtained by separation of the test mixture constituents in the column series: CN–PEG with the mobile phase flows: $F_{m,CN} = 1.0\,\text{mL/min}$ and $F_{m,PEG} = 1.5\,\text{mL/min}$, and PEG–CN with the mobile phase flows: $F_{m,PEG} = 1.0\,\text{mL/min}$ and $F_{m,CN} = 1.5\,\text{mL/min}$.

the C_p criterion is hierarchical, the second term is used to judge analyses with an equal number of resolved peaks.[20] From a set of all calculated $C_p(F_{m,CN}, F_{m,PEG})$ values, the maximum number corresponds to the optimum flows ($F_{m,CN,opt}$, $F_{m,PEG,opt}$) where the column series, CN–PEG and PEG–CN, exhibits optimum selectivity.

Tables 3 and 4 list results of the optimization for the selected resolution factors $R_{ji,req} = 0.6$, 0.8, and 1.0. Beside the values of the C_p criterion, the corresponding times of analysis and optimum flows are shown in Table 3. Figure 11 shows the separation of sample components at optimum flows for $R_{ji,req} = 0.6$ and $R_{ji,req} = 1.0$, respectively. Figure 12 shows good correlation between the real (A) and simulated (B) chromatograms at optimum flows for $R_{ji,req} = 0.8$.

Comparison of Figs. 11 and 12 shows that the optimum of column series selectivity depends *inter alia* on the selected resolution of the sample constituents.

Table 3. Comparison of HPLC optimization results

$R_{ji,reql}$	$F_{m,CN,opt}$	$F_{m,PEG,opt}$	C_p	$t_{R,n}$
1.00	1.34	0.72	11.5	17.54
0.80	1.44	0.52	14.36	22.26
0.60	1.50	0.64	14.45	19.32

The optimization procedure is based on the retention surfaces, described by Eq. (15), and was compared to a procedure based on Equation (14), where the corresponding x_{CN}, x_{PEG} values were calculated from the mobile phase hold-up values and the mobile phase flows in individual columns. Individual compound retention data on the CN and PEG columns were used for prediction of the retention factors on the CN–PEG and PEG–CN column series (see Fig. 3). It has been found that the optimization procedure based on the retention surfaces was more apropriate, as it is based on the statistical treatment of the needed data. The chromatographic model based on Eq. (14) is simplier, but it requires more precise data for construction of Fig. 3 and the "real" values of the mobile phase hold-up time in both columns.

Table 4. Comparison of predicted and Experimental retention factors ($k_{i,ic}$ and $k_{i,e}$, respectively) for required resolution factor ($R_{ji,req}$)

		$R_{ji,req} = 1.0$		$R_{ji,req} = 0.8$		$R_{ji,req} = 0.6$	
No.	Compound	$k_{i,c}$	$k_{i,e}$	$k_{i,c}$	$k_{i,e}$	$k_{i,c}$	$k_{i,ee}$
1	Fluorene	0.24	0.23	0.24	0.23	0.24	0.23
2	Dibenzylamine	0.43	0.41	0.44	0.41	0.43	0.41
3	Benzoylbenzoate	0.50	0.49	0.51	0.49	0.51	0.48
4	Triphenylene	0.73	0.74	0.80	0.76	0.76	0.74
5	Benzo(a)pyrene	0.91	0.87	0.97	0.91	0.94	0.88
6	2,6-Dimethyl phenol	1.06	1.05	1.12	1.10	1.09	1.06
7	Dimethyl terephthalate	0.66	0.64	0.67	0.64	0.66	0.63
8	Acetophenone	0.64	0.60	0.61	0.58	0.63	0.58
9	Coronene	1.40	1.33	1.48	1.38	1.44	1.35
10	1,2,5,6-Di-benzoanthracene	1.23	1.17	1.30	1.21	1.26	1.17
11	9,10-Anthraquinone	1.01	0.96	1.04	0.98	1.02	0.96
12	o-Cresol	2.12	2.09	2.27	2.24	2.20	2.14
13	Diethyl phthalate	0.99	0.96	0.96	0.91	0.98	0.92
14	m-Cresol	2.61	2.58	2.78	2.75	2.70	2.63
15	Phenol	3.17	3.12	3.40	3.35	3.29	3.19
16	Benzhydrol	2.00	1.94	2.09	1.99	2.05	1.94
17	Dimethyl phthalate	1.63	1.57	1.65	1.55	1.64	1.53

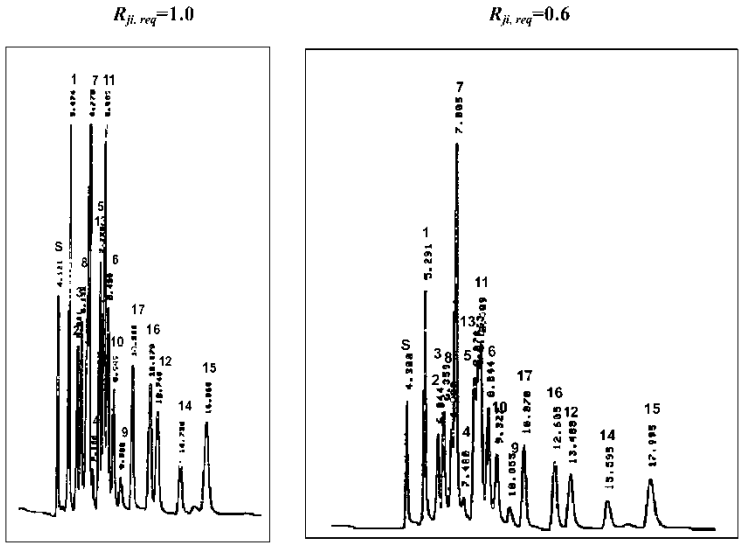

Figure 11. Chromatogram obtained by separation of the test mixture constituent in the column series PEG–CN at optimum selectivity for $R_{ij,req} = 1.00$ and $R_{ij,req} = 0.60$.

CONCLUSION

A procedure for the optimization of the selectivity of a multicomponent HPLC sample is described for a system that utilizes two columns of different polarity in series. It has been shown that the selectivity of serially coupled column does

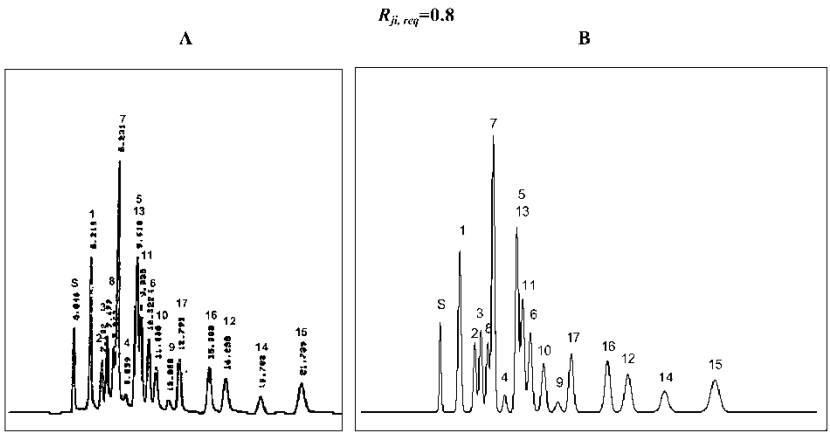

Figure 12. Comparison of the experimental (**A**) and simulated (**B**) chromatograms of the test mixture constituent in the column series PEG–CN at optimum selectivity for $R_{ij,re\ q} = 80$.

not depend on the columns order if the flows in the individual columns are equal. The selectivity of the column series was tuned by the mobile phase flows in the individual columns. The optimization algorithm was based on mathematical models.

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