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Computerized optimization of selectivity for direct capillary gas chromatographic multicomponent separations of enantiomers[1]

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Abstract

A computer-assisted procedure was elaborated for optimization of selectivity of two capillary columns coupled in series for direct gas chromatographic multicomponent separations of enantiomers. Two fused-silica capillary columns (column A coated with permethyl-β-cyclodextrin (ChirasilDex) and column B coated with a mixture of heptakis(6-*O*-*tert*.-butyldimethylsilyl-2,3-*O*-diacetyl)-β-cyclodextrin and polysiloxane OV-1701 in 1:1 ratio (TBDMSDAC)} were coupled in series via a T-piece. Each column was placed in an independently heated oven. Selectivity was tuned by variation of temperatures of the two columns (T_A,T_B) and carrier gas pressure in the column coupling point (p_m) under a constant inlet and outlet pressure. The chromatograms were recorded under working conditions (T_A,T_B and p_m) calculated using the Doehlert experimental design. Optimization procedure was based on mathematical models. A new threshold criterion for optimization of selectivity suitable for multicomponent sample separation was introduced. The elaborated optimization procedure was verified by separating D- and L-enantiomers of *N*-trifluoroacetyl/*O*-alkyl derivatives of alanine, valine, 2-aminobutanoic acid and proline. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Selectivity optimization; Alanine; Valine; 2-Aminobutanoic acid; Proline

1. Introduction

A combination of the necessary column efficiency and optimum stationary phase selectivity is the best approach to capillary gas chromatographic (GC) analysis of multicomponent samples [1]. Three methods are generally used for a change of stationary phase selectivity [2] that could be implemented in enantioselective separations: (i) Changing the nature of the stationary phase. The selectivity of the stationary phase (chiral selector) is usually adjusted stepwise by changing the chiral selector for direct GC separations of enantiomers [3–6]. Moreover, it has been observed that the overall selectivity of a chiral column can be influenced by the polarity of the polysiloxane polymer used for the preparation of capillary columns with diluted cyclodextrin selectors [5,7]. (ii) Packing the column with a mixture of

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stationary phases such as hexakis(6-*O*-*tert.*-butyldimethysilyl-2,3-*O*-dimethyl)-α-cyclodextrin and heptakis(6-*O*-*tert.*-butyldimethysilyl-2,3-*O*-dimethyl)-β-cyclodextrin [5]. (iii) Coupling two or more columns with different stationary phases in a series.

The last two methods have not yet been fully exploited for GC separation of enantiomers, even though this approach was successfully applied to achiral separations [8–10].

If the fixed-length columns with stationary phases with a different selectivity are coupled in series, the overall selectivity of the column series can be tuned by changing the contribution of individual column selectivities. The overall selectivity can be adjusted between the selectivities of the individual columns depending both on the carrier gas flow-rates and temperatures in the individual capillary columns [8–10].

The aim of this paper is to describe a computer-assisted procedure for the optimization of selectivity of two capillary columns coated with different chiral selectors coupled in series for the direct separation of a complex mixture of enantiomers. Two fused-silica capillary columns (column A coated with permethylated-β-cyclodextrin and column B coated with heptakis(6-*O*-*tert.*butyldimethylsilyl-2,3-*O*-diacetyl)-β-cyclodextrin dissolved in OV-1701 in a 1:1 ratio) were coupled in series via a T-piece and placed in independently heated ovens. The temperatures of both individual columns (T_A, T_B) and the carrier gas pressure in the column coupling point (p_m) were selected as variables. The optimization procedure was based on a mathematical model which was evaluated by a threshold criterion. The validity of the optimization procedure has been verified by the separation of a mixture of D- and L-enantiomers of *N*-trifluoroacetyl/*O*-alkyl esters of alanine, valine, 2-aminobutanoic acid and proline.

2. Experimental

2.1. Gas chromatography

The gas chromatographic system consisted of two independently controlled ovens (Fractovap 2350 and Fractovap 4180; Carlo Erba, Strumentazione, Milan, Italy) (Fig. 1) interfaced with a separately heated stainless-steel tube as shown in Fig. 2. The inlet of the first column (ChirasilDex placed in Fractovap 2350) was coupled to an all-glass inlet stream splitter injection port. The outlet of column A was led through the interface to the Fractovap 4180 GC where it was coupled to the first inlet of the T-piece (see Fig. 2). The second inlet of the T-piece was coupled to the Fractovap 4180 injection port allowing for a change of carrier gas pressure in the T-piece. The outlet of the T-piece was coupled to the inlet of the column B (TBDMSDAC placed in Fractovap 4180 GC) via a 50 mm×0.50 mm fused-silica capillary and press fit connector (reduction

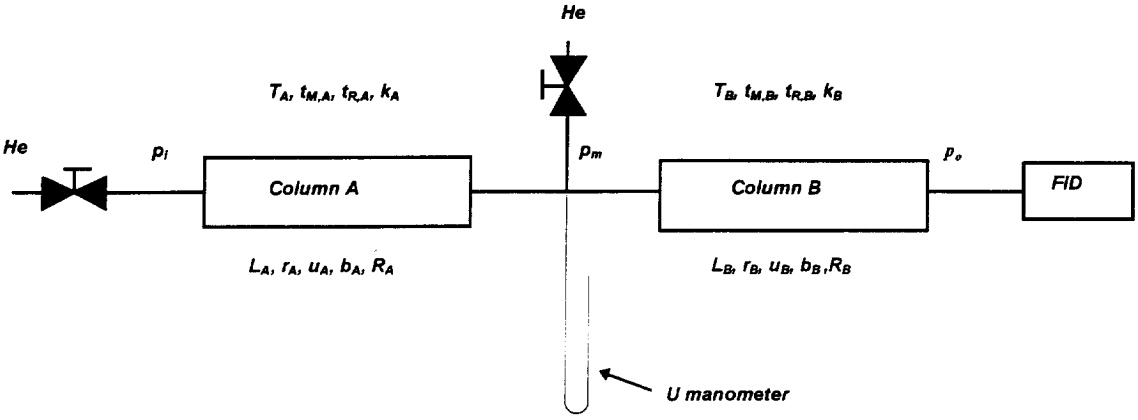

Fig. 1. Scheme of the GC instrument containing two capillary columns placed in two separately heated ovens. Column A (ChirasilDex) was placed in the Fractovap 2350 GC, and Column B (TBDMSDA) was placed in the Fractovap 4180 GC. For details see Section 2.

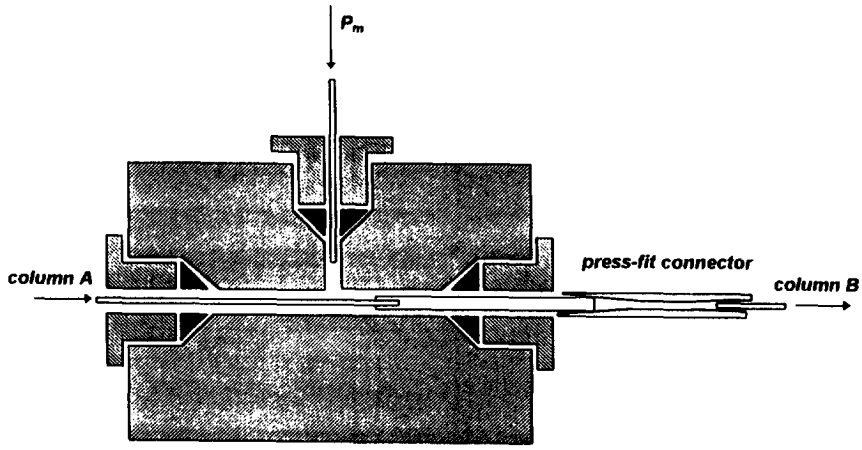

Fig. 2. Scheme of column coupling T-piece.

from 0.50 to 0.30 mm; see Fig. 2). The outlet of the column B was inserted into the jet of the Fractovap 4180 GC FID. The detector signal was monitored using an electrometer Model EL 480 (Carlo Erba) and an HP 3396 integrator. Peak 96 software was used for transmission of data from the integrator to an IBM compatible PC 386 where they were evaluated by an HP 3365 series II CHEMSTATION software (all purchased from Hewlett-Packard, Avondale, PA, USA).

2.2. Capillary columns

The following fused-silica capillary columns were used in this study: Column A, 25 m capillary column with 0.25 mm I.D. coated with permethyl-β-cyclodextrin anchored to a silicone polymer (ChirasilDex, CHROMPACK, Middelburg, The Netherlands) [11], and Column B, 30 m long capillary column with 0.32 mm (I.D. coated with a mixed stationary phase (heptakis(-6-*O*-*tert.*-butyldimethylsilyl-2,3-di-*O*-acetyl)-β-cyclodextrin diluted in OV 1701 in a 1:1 ratio, (TBDMSDA), prepared according to the procedure of Takeoka et al. [12,13]).

2.3. Operating conditions

Helium (99.996%, Linde, Bratislava, Slovakia) was used as a carrier gas with a constant inlet pressure $p_i = 130$ kPa (relative). The inlet stream splitter was operated with a split ratio of 1:100. The column outlet pressure of the carrier gas was atmospheric. The intermediate carrier gas pressure (p_m pressure in T-piece) was varied between 40 and 90 kPa (relative). The temperature of individual capillary columns was changed between 90°C and 140°C (T_A) and 120°C and 170°C (T_B), respectively. Thirteen chromatograms were recorded using the experimental parameters (p_m, T_A and T_B showed in Table 2 and Fig. 3) found in selected parameter space by the Doehlert method of experimental design.

2.4. Amino acid sample

A model sample consisted of D- and L-enantiomers of *N*-trifluoroacetyl/*O*-alkyl esters of alanine, valine, 2-aminobutanoic acid and proline (Table 1). *N*-TFA/*O*-alkyl derivatives were prepared from pure amino acids using trifluoroacetyl anhydride and the corresponding alcohol using the recently published procedures [14].

2.5. Computation

Curve fitting and optimization were performed with an IBM compatible 386 PC.

A program written in Pascal was developed for calculation of optimum experimental parameters ($p_{m,opt}$, $T_{A,opt}$ and $T_{B,opt}$) using a novel criterion for monitoring selectivity of two columns coupled in

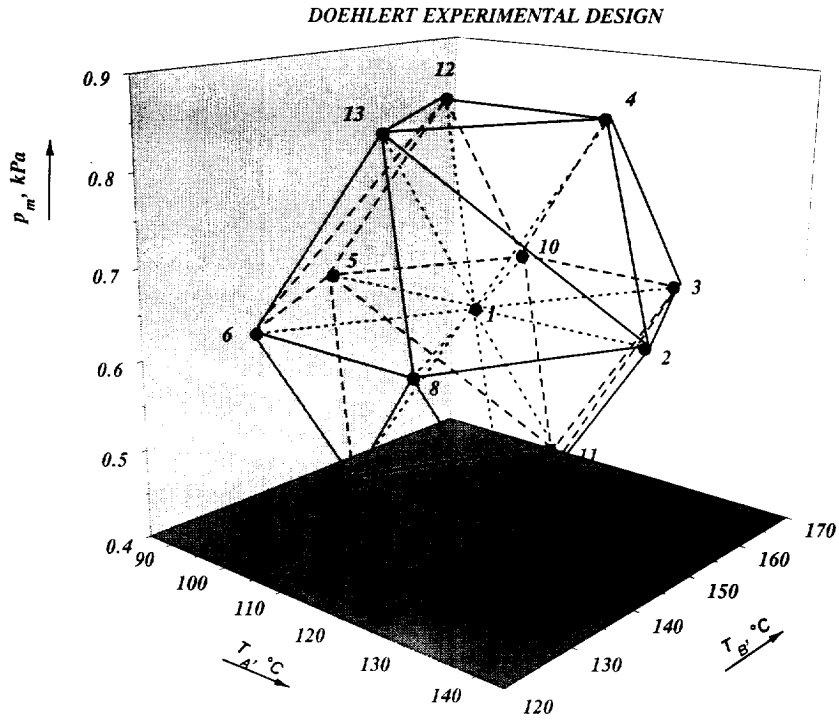

Fig. 3. Working parameters for column temperatures (T_A, T_B) and column coupling carrier gas pressure (p_m) found by the Doehlert experimental design.

Table 1
List of *N*-trifluoroacetyl/*O*-alkyl esters of amino acids in a model sample

Label	*N*-TFA-*O*-Alkyl amino acid derivative
1	*N*-trifluoroacetyl/*O*-methyl ester of D-alanine
2	*N*-trifluoroacetyl/*O*-methyl ester of L-alanine
3	*N*-trifluoroacetyl/*O*-methyl ester of D-valine
4	*N*-trifluoroacetyl/*O*-methyl ester of L-valine
5	*N*-trifluoroacetyl/*O*-ethyl ester of D-2-amino butanoic acid
6	*N*-trifluoroacetyl/*O*-ethyl ester of L-2-aminobutanoic acid
7	*N*-trifluoroacetyl/*O*-propyl ester of D-2-aminobutanoic acid
8	*N*-trifluoroacetyl/*O*-propyl ester of L-2-aminobutanoic acid
9	*N*-trifluoroacetyl/*O*-butyl ester of D-2-aminobutanoic acid
10	*N*-trifluoroacetyl/*O*-butyl ester of L-2-aminobutanoic acid
11	*N*-trifluoroacetyl/*O*-pentyl ester of D-2-aminobutanoic acid
12	*N*-trifluoroacetyl/*O*-pentyl ester of L-2-aminobutanoic acid
13	*N*-trifluoroacetyl/*O*-methyl ester of D-proline
14	*N*-trifluoroacetyl/*O*-methyl ester of L-proline

series during a multicomponent sample GC separation.

3. Results and discussion

3.1. Selectivity of a column series

Recently, we have shown that the retention order of D- and L-enantiomers of *N*-trifluoroacetyl/*O*-alkyl esters of alanine, valine, 2-aminobutanoic acid and proline on capillary columns coated with permethyl-β-cyclodextrin (ChirasilDex) and heptakis(6-*O*-*tert*.-butyldimethylsilyl-2,3-*O*-diacetyl)-β-cyclodextrin diluted in OV 1701 in a 1:1 ratio, (TBDMSDA) was reversed at certain temperatures [14]. The overall selectivity of this column series is between individual column selectivities and can be tuned inter alia by changing the column temperatures (T_A and T_B) and/or carrier gas velocity in individual columns (u_A and u_B), as described for the achiral column series [9,15]. The selectivity factor of compounds i and j in a column series can be calculated from the formula:

$$\alpha = \frac{k_{ab,j}(T_A, T_B, u_A, u_B)}{k_{ab,i}(T_A, T_B, u_A, u_B)} \quad (1)$$

where $k_{AB}(T_A, T_B, u_A, u_B)$ is the retention factor in a column series which can be expressed by the following equation [8–10]:

$$k_{AB}(T_A, T_B, u_A, u_B) = k_A(T_A) + X_B\{k_B(T_B) - k_A(T_A)\} \quad (2)$$

where X_B is a retentivity factor, calculated from:

$$X_B = \frac{t_{M,B}}{t_{M,AB}} = \frac{L_B.u_A}{L_A.u_B + L_B.u_A} \quad (3)$$

where t_M is the gas hold-up time and L, column length [8–10], index A, and B stands for the first and the second column in a series, respectively. The index AB denotes the column series.

From the Eqs. (1)–(3) it follows that the selectivity of a column series can be tuned both by the different temperature of the individual columns (T_A and T_B), as well as by the contribution of individual columns expressed by the retentivity factor X_B. From Eq. (2) it follows that the temperature of individual columns affects significantly the individual retention factors k_A and k_B (exponential decrease with increasing temperature) and, to a minor extent, the flow-rate of the carrier gas and hence the gas hold-up times (by influence on viscosity). The input, column coupling point, and outlet pressures determine the pressure conditions along the column system. In the case of the columns coupled directly at a constant inlet and outlet pressures, the columns' coupling point pressure is adjusted depending on the individual column resistances. If the column connection is coupled via a T-piece, the column coupling pressure can be adjusted around the default value. This way the pressure drops and the gas hold-up time in individual columns will be influenced in the opposite way, which significantly affects the retentivity in Eq. (3).

The tuning of the overall column selectivity is illustrated in Figs. 4 and 5 by a separation of D- and L-enantiomers of *N*-trifluoroacetyl/*O*-propyl ester of alanine. Fig. 4A shows a change of retention order of the L- and D-enantiomers of *N*-trifluoroacetyl/*O*-propyl ester of alanine with a change in temperature of the second column (from 110°C to 130°C) at a constant temperature of the first column (90°C). At the lowest temperature of the second column (110°C), the retention order of the enantiomers on a column series is equal to that observed on a single TBDMSDA column [14]. The contribution of the second column to the overall column selectivity decreases with increasing temperature. At T_B = 130°C the retention order is the same as that observed on a single ChirasilDex column [14].

Fig. 4B shows a change of retention order of the L- and D-enantiomers of *N*-trifluoroacetyl/*O*-propyl ester of alanine with the change of the first column temperature (from 110°C to 130°C) at a constant temperature of the second column (120°C). At the lowest temperature of the first column (80°C), the retention order on the column series is equal to that observed using a single ChirasilDex column [14]. Reversed retention order can be seen at the temperature T_A = 100°C where the contribution of the first column to the overall selectivity is minimized, and therefore the retention order is equal to that observed on a single TBDMSDA column [14].

Since all other separation conditions were constant (excluding small changes of carrier gas velocity in

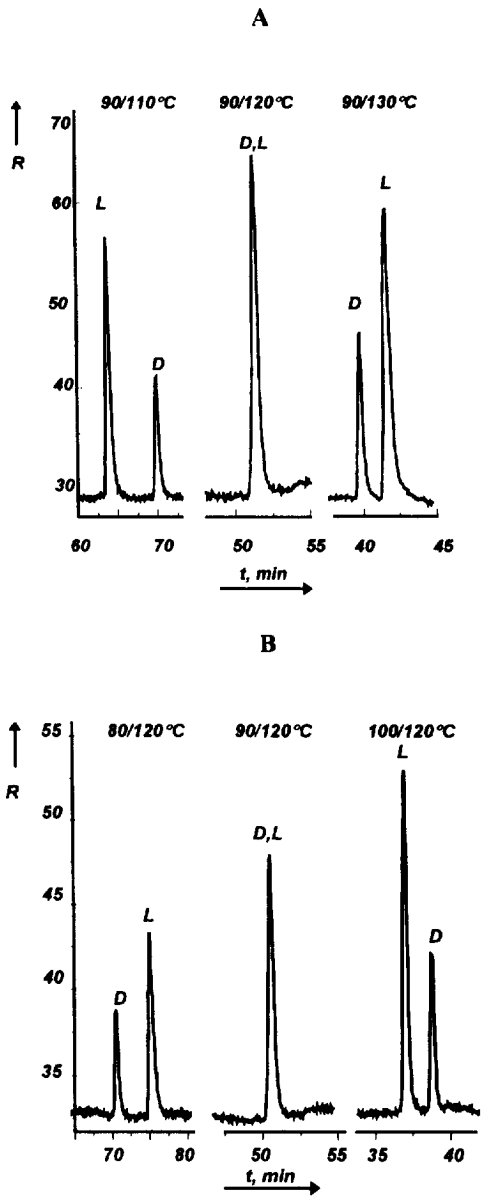

Fig. 4. Dependence of retention order of the L- and D- enntiomers of *N*- trifluoroacetyl/*O*-propyl ester alanine on temperature. A, Constant temperature of the first column ($T_A = 90°C$) and various temperatures of the second column ($T_B = 110–130°C$). B, Constant temperature of the second column ($T_B = 120°C$) and various temperatures of the first column ($T_A = 80–100°C$). For details see Section 2.

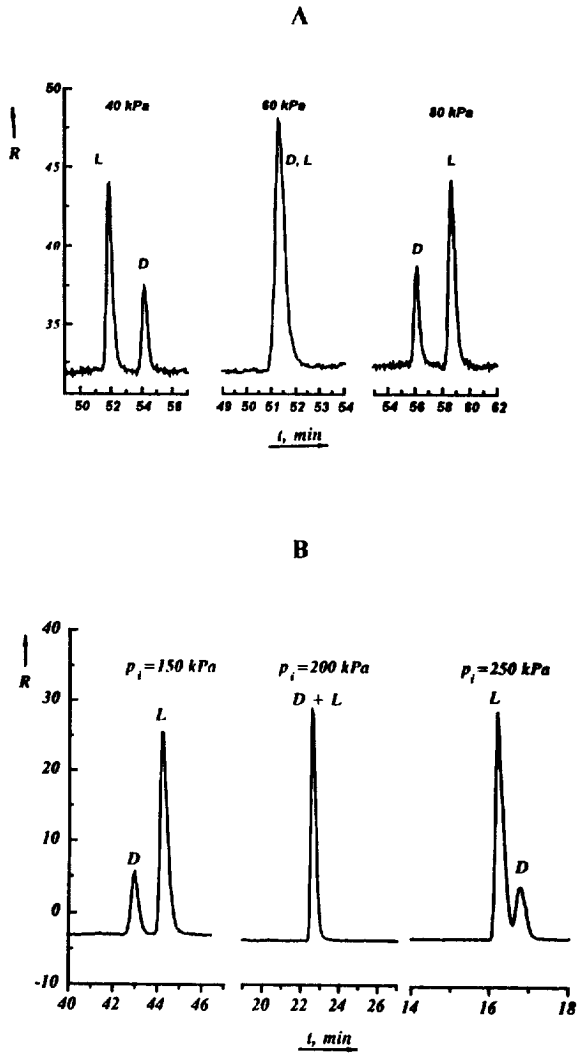

Fig. 5. Dependence of retention order of the L- and D- enntiomers of *N*-trifluoroacetyl/*O*-propyl ester alanine on carrier gas flow-rate. A, Constant temperatures of the first column ($T_A = 90°C$) and the second column ($T_B = 120°C$), as well as the carrier gas inlet pressure ($p_i = 130$ kPa) and different column coupling point pressures ($p_m = 40–80$ kPa). B, Constant temperatures of the first column ($T_A = 90°C$) and the second column ($T_B = 135°C$), as well as column coupling point pressure ($p_m = 110$ kPa) and different carrier gas inlet pressure ($p_i = 150–250$ kPa). For details see Section 2.

individual columns caused by column temperature variation), the inversion temperatures in Fig. 4A and Fig. 4B are the same.

A reverse of selectivity of a column a series for

enantiomers of a given amino acid derivative can also be obtained by tuning the carrier gas flow-rate in individual columns at constant column temperatures: for instance by changing the column coupling carrier gas pressure (p_m from 40 to 80 kPa) at constant inlet and outlet pressures (Fig. 5A), or changing the carrier gas inlet pressure (p_i from 150 to 250 kPa) at constant column coupling pressure ($p_m = 110$ kPa; Fig. 5B). In addition to the variation of the retention order illustrated above, the variation of temperature significantly affects the overall retention time. In contradiction, the variation of the columns' coupling point pressure affects the retention order with minor changes of the overall retention times.

3.2. Optimization of selectivity

From the above it follows that: temperatures (T_A, T_B) and carrier gas flow-rates (u_A, u_B) are the relevant factors which dramatically influence the overall selectivity of the two columns coupled in a series; the flow-rates in an individual column can easily be tuned by changing columns coupling pressure (p_m) at constant inlet and outlet pressures.

The parameters T_A, T_B, p_m were chosen as relevant in our optimization procedure. Input data (gas hold-up times, retention times of all sample species) were read from 13 chromatograms recorded under experimental conditions (T_A, T_B, p_m) for the separation of D- and L-enantiomers of *N*-trifluoroacetyl/*O*-alkyl esters of alanine, valine, 2-aminobutanoic acid and proline. The separation conditions were determined using the Doehlert experimental design (Fig. 3) and are listed in Table 2. The effect of the temperature of the first column on the overall column series selectivity is illustrated in Fig. 6A showing the chromatograms obtained at the lowest (bottom chromatogram) and the highest temperature (upper chromatogram) of the first column. Fig. 6B illustrates the dependence of column series selectivity on the temperature of the second column, where the upper chromatogram was recorded at the lowest, and the bottom chromatogram at the highest temperature. The influence of column series selectivity on the column coupling pressure can be seen in Fig. 6C; the upper chromatogram was recorded at the highest and the bottom chromatogram at the lowest column coupling pressure.

Table 2
Chromatographic working parameters of experiments found according to the Doehlert experimental design

Experiment number	T_A (°C)	T_B (°C)	p_m (kPa)
1	115	145	65
2	140	145	65
3	127.5	167	65
4	127.5	152	85
5	90	145	65
6	102.5	123	65
7	102.5	137	45
8	127.5	123	65
9	127.5	137	45
10	102.5	167	65
11	115	159	45
12	102.5	152	85
13	115	131	85

T_A, T_B are the temperatures of column A and column B, respectively, and p_m is the intermediate column pressure.

The following mathematical model was used to describe the dependence of the retention factors ($k_{AB,i}(T_A, T_B, p_m)$) on the working parameters T_A, T_B, p_m:

$$\ln k_{AB,i}(T_A, T_B, p_m) = b_1 + b_2\frac{1}{T_A} + b_3\frac{1}{T_B} + b_4.\ln p_m + b_5.(\ln p_m)^2 + b_6\frac{\ln p_m}{T_A}. + b_7\frac{\ln p_m}{T_B} + b_8\frac{(\ln p_m)^2}{T_A} + b_9\frac{(\ln p_m)^2}{T_B} \quad (4)$$

The coefficients b_1–b_9 were determined by multiple linear regression analysis (MLRA) of experimental values $k_{AB,i}(T_A, T_B, p_m)$ and the corresponding parameters T_A, T_B, p_m. With the known coefficients b_1–b_9 of Eq. (4), $k_{AB,i}(T_A, T_B, p_m)$ values of all sample constituents were calculated for all combinations of temperatures (T_A, T_B in a 1°C steps) and columns' coupling pressures (p_m in a 5 kPa steps) in the chosen parameter space. This choice of the increments was determined by the GC instruments used that allowed variations of the temperature in 1°C and pressure in 5 kPa steps. For each combination of parameters T_A, T_B, p_m, the selectivity factor

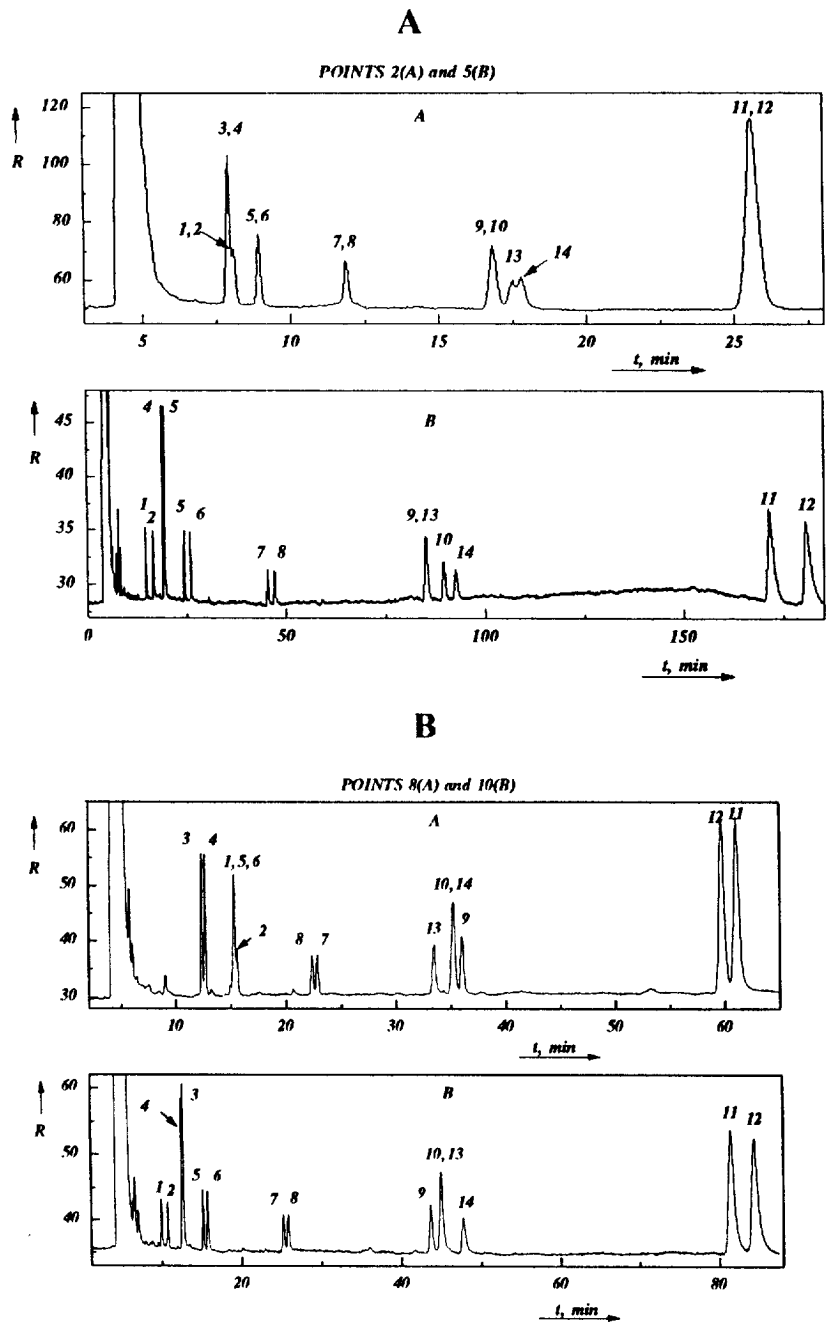

Fig. 6. Separation of D- and L-enantiomers of *N*-trifluoroacetyl/*O*-alkyl esters of alanine, valine, 2-aminobutanoic acid and proline in the column series at working parameters found by the Doehlert experimental design. (A) Experiments 2 (A) and 5 (B). (B) Experiments 8(A) and 10 (B). (C) Experiments 9(A) and 13(B). For details see Section 2.

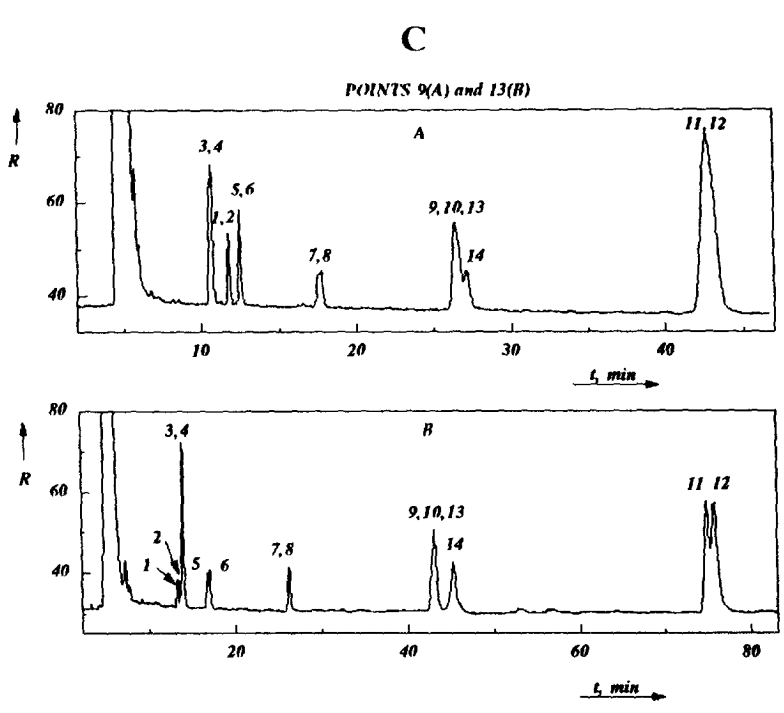

Fig. 6. (*continued*)

$\alpha(T_A, T_B, p_m)$ was calculated for all adjacent peak pairs:

$$\alpha(T_A, T_B, p_m) = \frac{k_j(T_A, T_B, p_m)}{k_i(T_A, T_B, p_m)} \quad (5)$$

where i and j denote the first and second eluted peaks, respectively.

The calculated $\alpha(T_A, T_B, p_m)$ values were compared with the critical value (α_{req}). Based on this comparison, a threshold criterion $C(T_A, T_B, p_m)$ was calculated for all combinations of T_A, T_B, p_m parameters. This criterion is suitable for monitoring the selectivity of a column series for a multicomponent sample, since it is based on the number of peaks when the selectivity factor is higher than α_{req} and can be found from the following equation:

$$C(T_A, T_B, p_m) = \sum_{1}^{n-1} m_i \quad (6)$$

where n is the number of solutes in a sample, $m_i = 1$ if $\alpha(T_A, T_B, p_m) \geq \alpha_{req}$, elsewhere $m_i = 0$. The optimum selectivity of the columns coupled in series was obtained under the separation conditions listed in Table 3, where the selectivity criterion reached a maximum value [12].

Fig. 7 shows a chromatogram obtained under optimum separation conditions where all species were resolved in 205 min, except for compounds 9 and 13 (*N*-trifluoroacetyl/*O*-butyl ester of D-2-aminobutanoic acid and *N*-trifluoroacetyl/*O*-metyl ester of L-proline).

In conclusion, it can be stated that the selectivity of two chiral columns coupled in a series can be tuned by changing individual column temperatures and carrier gas flow-rates similarly as with achiral columns. The proposed threshold selectivity criterion

Table 3
Chromatographic working parameters for optimum column series selectivity

Working parameters	Value
$T_{A,opt}$ (°C)	92
$T_{B,opt}$ (°C)	168
$p_{m,opt}$ (kPa) (rel)	80
$C(T_A, T_B, p_m)$ optimum conditions	12

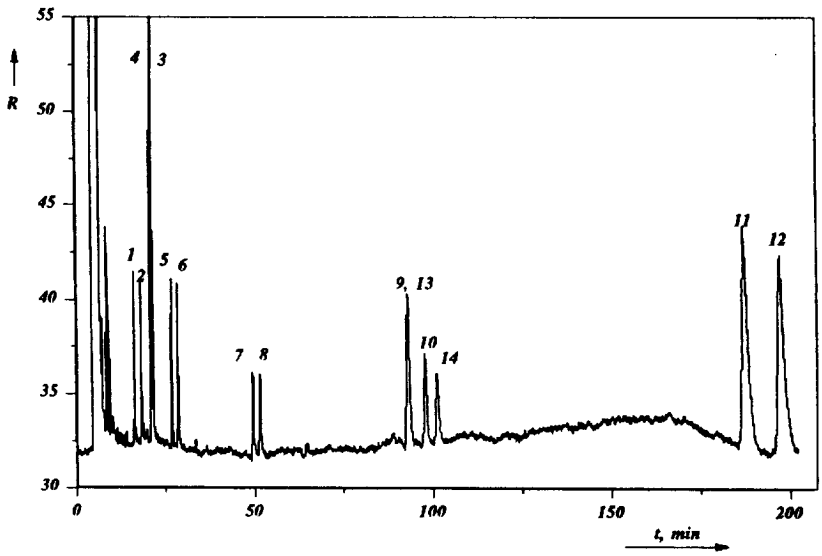

Fig. 7. Separation of D- and L-enantiomers of *N*-trifluoroacetyl/*O*-alkyl esters of alanine, valine, 2-aminobutanoic acid and proline in the column series under optimum working parameters ($T_{A,opt}$ = 92°C, $T_{B,opt}$ = 168°C and $p_{m,opt}$ = 80 kPa). For identification of peaks see Table 1.

can be used for monitoring selectivity of chromatographic columns for multicomponent sample analysis.

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