Selectivity Tuning in Chiral Dual Column Gas Chromatography

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Abstract

The enantioselective tuning of two columns coupled in series is investigated in chiral high-resolution gas chromatography. Two columns with opposite enantioselectivities (Chirasil-L-Val and Chirasil-D-Val) are coupled in series via a T connector, and the relative retention of enantiomers chromatographed on the system is changed by varying the individual carrier gas flow rates in the coupled columns. The flow-rate ratio necessary for the required selectivity is calculated on the basis of the measured retention factors on the individual columns. The performance of this method for adjusting selectivity is studied by the separation of enantiomers of the *N*-TFA-*O*-methyl esters of six amino acids. It is demonstrated that the change of the coupling point carrier gas pressure, at the constant inlet and outlet pressures, may change the enantioselectivity of the given column series to such an extent that the enantiomer elution order may be reversed.

Introduction

Effective multicomponent analysis in gas chromatography (GC) involves a combination of efficiency and optimal stationary-phase selectivity. Selectivity is a powerful tool to improve the resolution of a sample's constituents. In chromatography, the selectivity is commonly expressed as the relative retention (α) of a critical pair of sample components:

$$\alpha = k_j/k_i \qquad \text{Eq. 1}$$

where k is the retention factor of compounds i and j in which usually $k_j > k_i$ (1).

It is obvious that if $\alpha = 1$, then the separation system is nonselective for those components.

The enantioselectivity of a GC stationary phase can, in a manner analogous to achiral chromatography (2), be changed discontinuously by the selection of proper column packings or continuously by tailor-made stationary phases, mixed stationary phases, or coupling different polarity columns in series (e.g., dual column chromatography or two-dimensional chromatography).

The last method has not been fully explored for the chromatographic separation of enantiomers, even though this approach has been used successfully in achiral separations (3–6).

The aim of this study is to show a procedure for the selectivity tuning of two columns coated with different chiral selectors and coupled in series for the direct separation of enantiomers. This is done by changing individual column flow rates in serially coupled columns operated at equal column temperatures. The carrier gas flow rates in individual columns (u_A,u_B) were selected as variables for selectivity tuning. The separation of a mixture of D- and L-enantiomers of the *N*-trifluoroacetyl (TFA)-*O*-methyl esters of six amino acids was used for the study of the selectivity tuning procedure.

If two columns with different stationary-phase selectivities are coupled in series, the overall selectivity of the column series can be tuned by changing the contribution of individual column selectivities. In an analytical praxis it is convenient to keep constant all of the column parameters depicted in Figure 1 except the temperatures (T_A, T_B) and carrier gas flow rates (u_A,u_B) in the individual columns (6). The selectivity factor of compounds i and j in a column series can

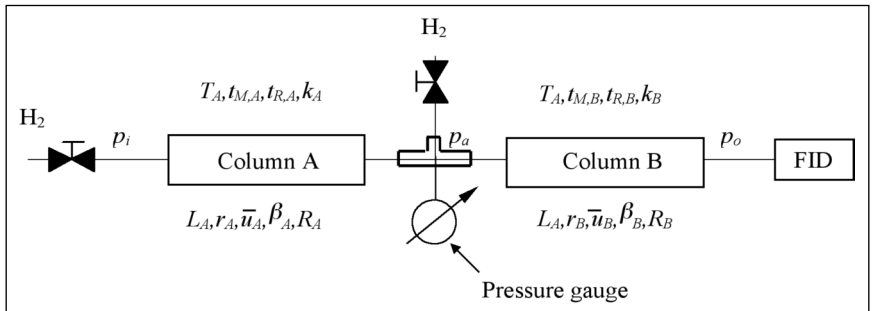

Figure 1. Schematic of two columns coupled in series via a low, dead-volume T junction: temperature, T; retention time, t_R; column inner diameter, r; column phase ratio, β; and column resistance, R.

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then be calculated from the formula:

$$\alpha = (k_{AB,j}(T_A,T_B,u_A,u_B)) / (k_{AB,i}(T_A,T_B,u_A,u_B)) \quad \text{Eq. 2}$$

where $k_{AB}(T_A,T_B,u_A,u_B)$ is the retention factor in the column *AB* series heated at the temperatures T_A and T_B and u is the average mobile phase flow rate ($u = F_m/A_m$, where F_m is the flow and A_m the column mobile phase cross-section). Index *A*, *B* stands for the first and second column in a series, respectively.

The retention factor of a solute in a coupled column series at equal temperatures ($T = T_A = T_B$) can, for isothermal conditions, be calculated from the following equation:

$$k_{AB}(u_A,u_B) = x_A \cdot k_A(u_A,u_B) + x_B \cdot k_B(u_A,u_B) \quad \text{Eq. 3}$$

where x is a weight (retentivity) factor, calculated at a given temperature from the following equation:

$$x_A = t_{M,A} / t_{M,AB} = L_A \cdot u_B / (L_A \cdot u_B + L_B \cdot u_A) \quad \text{Eq. 4}$$

or

$$x_B = t_{M,B} / t_{M,AB} = L_B \cdot u_A / (L_A \cdot u_B + L_B \cdot u_A) \quad \text{Eq. 5}$$

where t_M is the gas holdup time and L the column length.

From equations 2–5 it follows that the overall selectivity of a column series, at isothermal conditions, can be tuned by changing the mobile phase flow rate in individual columns (which, in turn, control the contributions of each column via the retentivity factors x_A and x_B).

Experimental

Equipment

Capillary GC using single and coupled columns in series was done on an HP 5890 GC equipped with a split–splitless injector and flame ionization detector (FID). Hydrogen with a flow rate of 40–50 cm/s was used as a carrier gas. The FID signal was monitored with an HP 3396 integrator using Peak 96 software transmitted into PC, in which it was evaluated by HP 3365 Series II ChemStation software (all products were purchased from Hewlett-Packard, Avondale, PA). The final chromatograms were adapted using Microcal Origin software (One Roundhouse Plaza, Northampton, MA).

Two capillary columns (Chirasil-L-Val and Chirasil-D-Val) were coupled in series in both sequences (*AB* or *BA*) using press fit connectors or according to the schematic shown in Figure 1. The inlet of the first column was in both cases coupled to the injection port. The outlet of the first column was coupled to the inlet of the second column directly through a press-fit connector or to the inlet of the T-piece (Figure 2). The second inlet of the T-piece was coupled to a manostat (Carlo Erba, Milan, Italy), allowing for the change of carrier gas pressure in it. The outlet of the T-piece was coupled to the inlet of the second column via a press fit connector (reduction from 0.50 to 0.30 mm). The outlet of the column B was inserted into the jet FID.

Separations on individual Chirasil-D-Val and Chirasil-L-Val columns were accomplished isothermally in the temperature range of 70°C to 140°C with 10°C steps. The hydrogen carrier gas inlet pressure was 150 kPa. Separations in both column series (Chirasil-D-Val + Chirasil-L-Val and Chirasil-L-Val + Chirasil-D-Val) also were done using the temperature interval 70°C to 140°C with 10°C steps. Hydrogen was used as a carrier gas. Its pressure at the joint point of columns coupled in series (p_a) was changed in the range of 30 to 180 kPa using the constant inlet (p_i = 180 kPa) and outlet (p_o = 101 kPa) pressures.

One microliter of the *N*-TFA-*O*-methyl esters of the amino acids dissolved in *n*-hexane (10 mg of each of the amino acid derivatives in 10 mL of *n*-hexane) were injected using a split–splitless injector with the 1:100 split ratio. The injector temperature was 250°C, and the FID temperature was 300°C.

Columns

Column A was a Chirasil-L-Val 25-m FS capillary column with a 0.25-mm i.d. coated with approximately a 0.25-µm film thickness of L-valine-*tert*-butylamide anchored to polysiloxane polymer. Column B was a Chirasil-D-Val 25-m FS capillary column with approximately a 0.25-mm i.d. coated with a 0.25-µm film thickness of L-valine-*tert*-butylamide anchored to polysiloxane polymer. Both columns were prepared at the Department of Organic Chemistry, University of Tuebingen (Tuebingen, Germany) according to the published procedures (8,9).

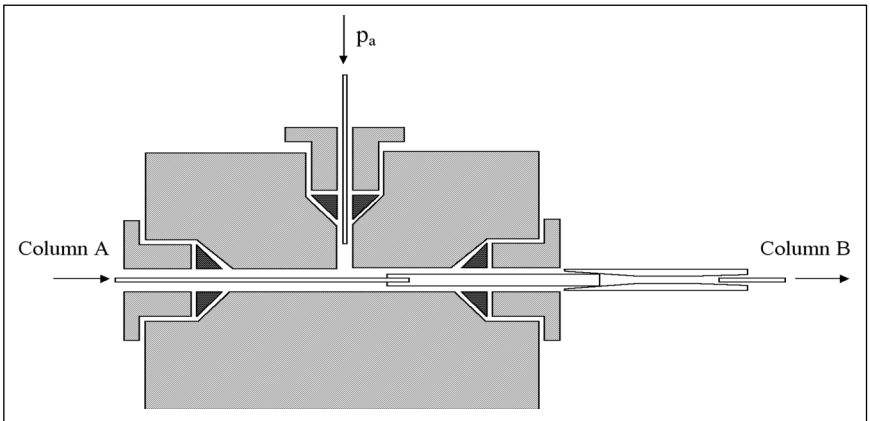

Figure 2. Schematic of the T connector.

Analytes

Racemic mixtures as well as the pure L- or D-enantiomers of amino acids were obtained from Sigma (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The methyl esters of the *N*-TFA derivatives of all of the amino acids were prepared according to the previously published procedure (10). The list of enantiomers of the *N*-TFA-*O*-methyl esters of the amino acid used in the GC separation studies is given in Table I.

Results and Discussion

Separation of enantiomers of the *N*-TFA-*O*-methyl esters of the amino acids on individual columns

The separations of enantiomers of the *N*-TFA-*O*-methyl esters of the amino acids by capillary gas–liquid chromatography on Chirasil-D-Val and Chirasil-L-Val columns at 100°C are shown in Figures 3 and 4, respectively. In both cases, the best separation was obtained for *N*-TFA-*O*-methyl leucine enantiomers (peaks 11 and 12) and the worst was for *N*-TFA-*O* methyl proline enantiomers (peaks 5 and 6). The elution order of all of the enantiomers of these analytes was the D- then L-enantiomer for the Chirasil-L-Val column and the L- then D-enantiomer from the Chirasil-D-Val column. A comparison of the chromatograms in Figures 3 and 4 shows that the Chirasil-D-Val column exhibited lower separation efficiency than the Chirasil-L-Val column.

Data determined for enantiomers of the *N*-TFA-methyl esters of amino acids on single Chirasil-D-Val and Chirasil-L-Val columns at 100°C are listed in Table II. The selectivity factors (α) in Table II were calculated according to equation 2 and therefore do not reflect the elution order of the enantiomers. A comparison of the retention factors listed in Table II shows that the Chirasil-D-Val column had a thicker film of stationary phase than the Chirasil-L-Val column. In spite of this difference the selectivity factors of the *N*-TFA-*O*-methyl ester enantiomers of all of the amino acids listed in Table II indicate the full enantioreversibility of both columns.

A comparison of the enantiomeric elution order and the configuration of the chiral selector shows that L-enantiomers were more retained on the Chirasil-L-Val column and D-enantiomers were more retained on the Chirasil-D-Val column. This indicates that the interaction of derivatized amino acids with the same configuration was stronger than that of those with different configurations. This conclusion, however, is not valid in general because on cyclodextrin-based stationary phases (with the *R*-configuration of glucopyranose units) enantiomers elute both in *S*,*R* and *R*,*S* orders, and the elution order can also be changed by separation temperature (10).

Table I. List of Enantiomers of *N*-TFA-*O*-Methyl Derivatives of Amino Acids Used in This Work

Label	Amino acid derivative
1	D-*N*-TFA-*O*-methyl ester of alanine
2	L-*N*-TFA-*O*-methyl ester of alanine
3	D-*N*-TFA-*O*-methyl ester of valine
4	L-*N*-TFA-*O*-methyl ester of valine
5	D-*N*-TFA-*O*-methyl ester of proline
6	L-*N*-TFA-*O*-methyl ester of proline
7	D-*N*-TFA-*O*-methyl ester of norvaline
8	L-*N*-TFA-*O*-methyl ester of norvaline
9	D-*N*-TFA-*O*-methyl ester of serine
10	L-*N*-TFA-*O*-methyl ester of serine
11	D-*N*-TFA-*O*-methyl ester of leucine
12	L-*N*-TFA-*O*-methyl ester of leucine

Separation of enantiomers of the *N*-TFA-*O*-methyl esters of the amino acids on columns coupled in series

Because the carrier gas was compressible, it was expected that the overall selectivity of the coupled columns would be affected by the column order.

Figure 5 shows the separation of enantiomers of the *N*-TFA-*O*-methyl esters of the amino acid analytes in the *AB* columns (Chirasil-L-Val (*A*) coupled to Chirasil-D-Val (*B*)) at 100°C. It might be expected that putting columns of opposite enantioselectivity and equal length would eliminate the enantiomeric separation. However, as can be seen from Figure 5, this was not always the case. Partial resolution of enantiomers of *N*-TFA-*O*-methyl norvaline (peaks 7 and 8), serine (peaks 9 and 10), and leucine (peaks 11 and 12) in Figure 5 can be explained both by gas compressibility and the thicker film of the chiral selector in the Chirasil-D-Val column. The thicker films asserted themselves in the coupled columns through both retention and retentivity factors as indicated in equations 3–5. The effect of

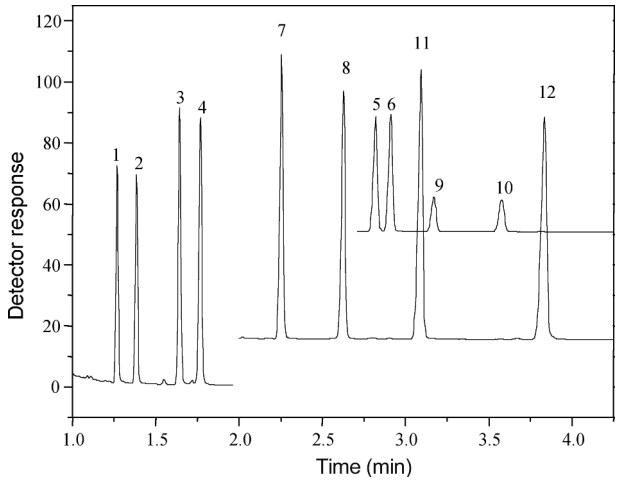

Figure 3. Separation of enantiomers of the *N*-TFA-*O*-methyl derivatives of the amino acids on the Chirasil-D-Val column at 100°C. The peak numbers correspond with the numbering in Table I.

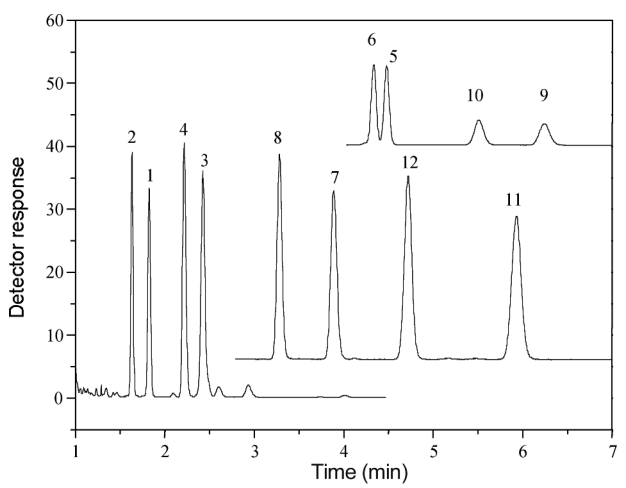

Figure 4. Separation of enantiomers of the *N*-TFA-*O*-methyl derivatives of the amino acids on the Chirasil-L-Val column at 100°C. The peak numbers correspond with the numbering in Table I.

the stationary-phase film thickness on the retention factor in this instance was, however, more significant than the effect from the compressibility of the carrier gas, which was asserted in the column series through the retentivity factors.

Figure 6 shows the separation of enantiomers of *N*-TFA-*O*-methyl amino acid derivatives using the column series BA (Chirasil-D-Val (B) coupled to Chirasil-L-Val (A)) at 100°C. The contribution of the B column in the column series BA was greater than that of the A column (both by the contribution of retention and retentivity factors). The separation enantiomers of the *N*-TFA-*O*-methyl ester of alanine (2,1), valine (4,3), norvaline (8,7), and proline (6,5) in this series was better than in the previous series. The impact of the Chirasil-D-Val column in this column series was apparent also from the elution order of the resolved enantiomers in Figures 5 and 6.

The dependence of the retention factors ($k_{i,\ L\text{-}Val}$) on the weight factor ($x_{D\text{-}Val}$) for the column series *AB* at 100°C is depicted in Figure 7. This dependence was constructed by substituting retention factors obtained for individual columns at 100°C (values listed in Table II) into equation 3. The perpendiculars in Figure 7 were constructed from the retention factors ($k_{i,AB}$) obtained for enantiomers of the derivatized amino acids in the column series *AB* (left perpendicular) and *BA* (right perpendicular). The perpendiculars were constructed for the average value of the weight factor ($x_{D\text{-}Val}$) calculated from the equation:

$$k_{i,AB} = k_{i,L-Val} + x_{D-Val}(k_{i,D-Val} - k_{i,L-Val}) \qquad \text{Eq. 6}$$

Table II. Retention Times, Retention Factors, and Selectivity Coefficients of Enantiomers of *N*-TFA-*O*-Methyl Esters of the Amino Acids Obtained for Single Chirasil-L-Val and Chirasil-D-Val columns at 100°C

	Chirasil-L-Val			Chirasil-D-Val		
Label	t_R	k	α	t_R	k	α
1	1.260	0.68	1.24	1.820	1.48	1.23
2	1.380	0.84		1.620	1.21	
3	1.640	1.19	1.15	2.421	1.69	1.14
4	1.770	1.36		2.213	1.48	
5	2.820	2.76	1.04	4.125	4.63	1.04
6	2.910	2.88		3.980	4.43	
7	2.250	2.00	1.25	3.884	4.30	1.24
8	2.620	2.49		3.277	3.47	
9	3.160	3.21	1.17	5.885	7.03	1.17
10	3.570	3.76		5.152	6.03	
11	3.090	3.12	1.32	5.925	7.08	1.30
12	3.830	4.11		4.715	5.43	

using retention factors obtained on individual columns and on both column series. Intersections of perpendiculars and straight lines for dependencies $k_{i,AB}$ on $x_{D\text{-}Val}$ shows the elution order of the enantiomers both for column series *AB* and *BA*.

Column series overall selectivity tuning

From the theoretical section, it can be seen that the overall selectivity of two columns coupled in series can be tuned by the change of separation temperatures, the carrier gas flow rates in individual columns, or both. It has already been shown that the last parameter can be easily tuned by the change of the column's coupling point carrier gas pressure at constant inlet and outlet pressures (11). The coupling point carrier gas pressure (p_a) was changed using an extra hydrogen stream in the T-connector (Figure 2).

A column's p_a was changed both for column series *AB* and *BA*. In both instances

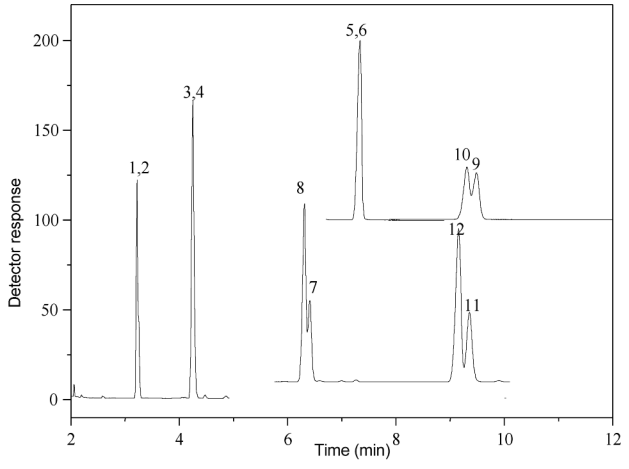

Figure 5. Separation of enantiomers of the *N*-TFA-*O*-methyl derivatives of the amino acids on the *AB* column series at 100°C. The peak numbers correspond with the numbering in Table I.

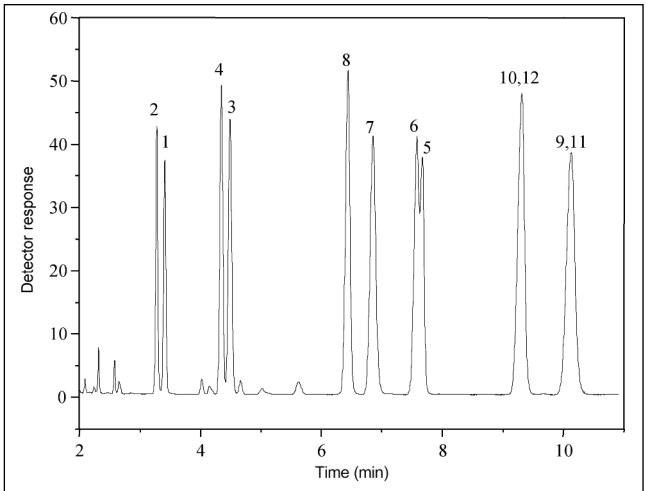

Figure 6. Separation of enantiomers of the *N*-TFA-*O*-methyl derivatives of the amino acids on the *BA* column series at 100°C. The peak numbers correspond with the numbering in Table I.

the experimental values of p_a were higher than the pressure (p_m) calculated for the columns directly coupled in series via press-fit connectors (12). Because the individual columns have different pneumatic resistances, the p_m values were different for the column series *AB* and *BA*. Experimental values of p_a were changed from 160 to 180 kPa for the column series *AB* and from 130 to 180 kPa for the column series BA, respectively.

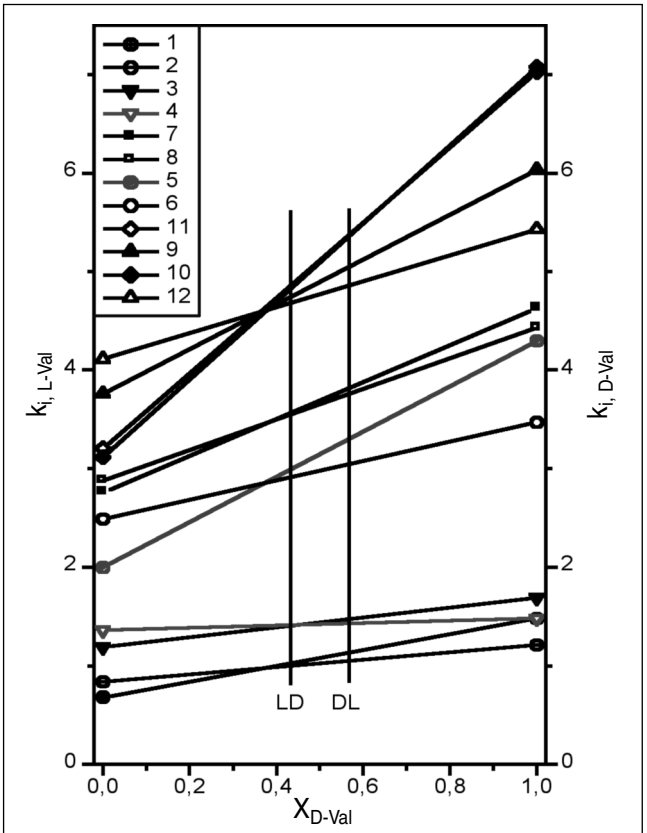

Figure 7. Dependence of the retention factors $k_{i,AB}$ of enantiomers of the *N*-TFA-*O*-methyl derivatives of the amino acids on the weight factor ($x_{D\text{-}Val}$) for the column series *AB* at 100°C. The peak numbers correspond with the numbering in Table I.

Figure 8 shows the separation of enantiomers of the *N*-TFA-*O*-methyl ester of the amino acids obtained on the coupled column series *AB* (Chirasil-L-Val + Chirasil-D-Val) at 100°C with p_a = 160 kPa and p_a = 180 kPa. Separation of the same enantiomers on the BA column series (Chirasil-D-Val [B] coupled to Chirasil-L-Val [A]) at 100°C with p_a = 130 kPa (A), p_a = 140 kPa (B), and p_a = 160 kPa (C) is shown in Figure 9.

Figure 9 shows that the change in the coupling pressures from p_a = 130 to p_a = 160 produced a change in the elution orders of the enantiomers with the exception of proline, whose enantiomers were not separated under these conditions. The retention order of the separated enantiomers was D- then L-enantiomer at p_a = 130 kPa. At p_a = 140 kPa enantiomers were not separated and at p_a = 160 kPa they eluted in the opposite order (L- then D-enantiomer). This example illustrates that the change of carrier gas flow rate (which influences the overall selectivity of a dual column series through the contributions of the individual column selectivities) can easily be performed by controlling the column's coupling point pressure while maintaining constant inlet and outlet pressures.

Conclusion

Enantiomeric selectivity tuning can be accomplished in isothermal GC by differentially controlling the flow rate in two coupled columns that have chiral stationary phases of opposite enantioselectivity. The carrier gas flow rates in the individual column can be controlled by altering the gas pressure at the coupling point of the two columns while maintaining constant inlet and outlet pressures. By adjusting this pressure between 130 and 160 kPa, the elution order of racemic *N*-TFA-*O*-methyl esters of amino acids can be changed from the D-enantiomer eluting before the L-enantiomer, the coelution of the D- and L-enantiomers, to the L-enantiomer eluting before D-enantiomers. Additional factors that affect this type of pressure-controlled selectivity tuning are the carrier gas compressibility, which can also cause different flow rates in the two

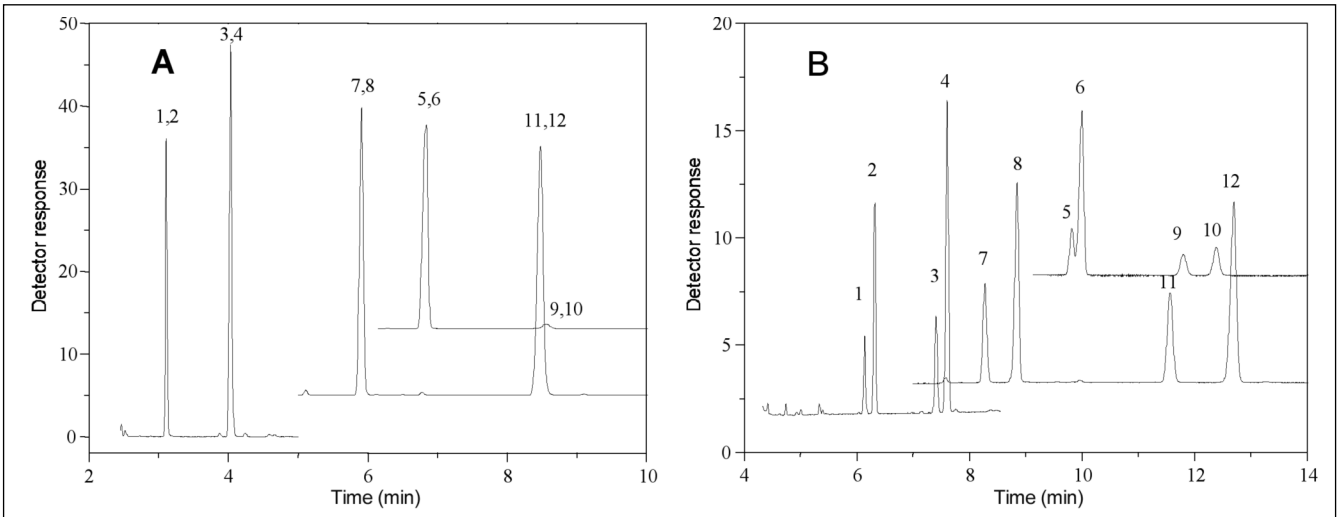

Figure 8. Separation of the enantiomers of *N*-TFA-*O*-methyl derivatives of the amino acids on the *AB* coupled column series at 100°C with (A) p_a = 160 kPa and (B) p_a = 180 kPa.

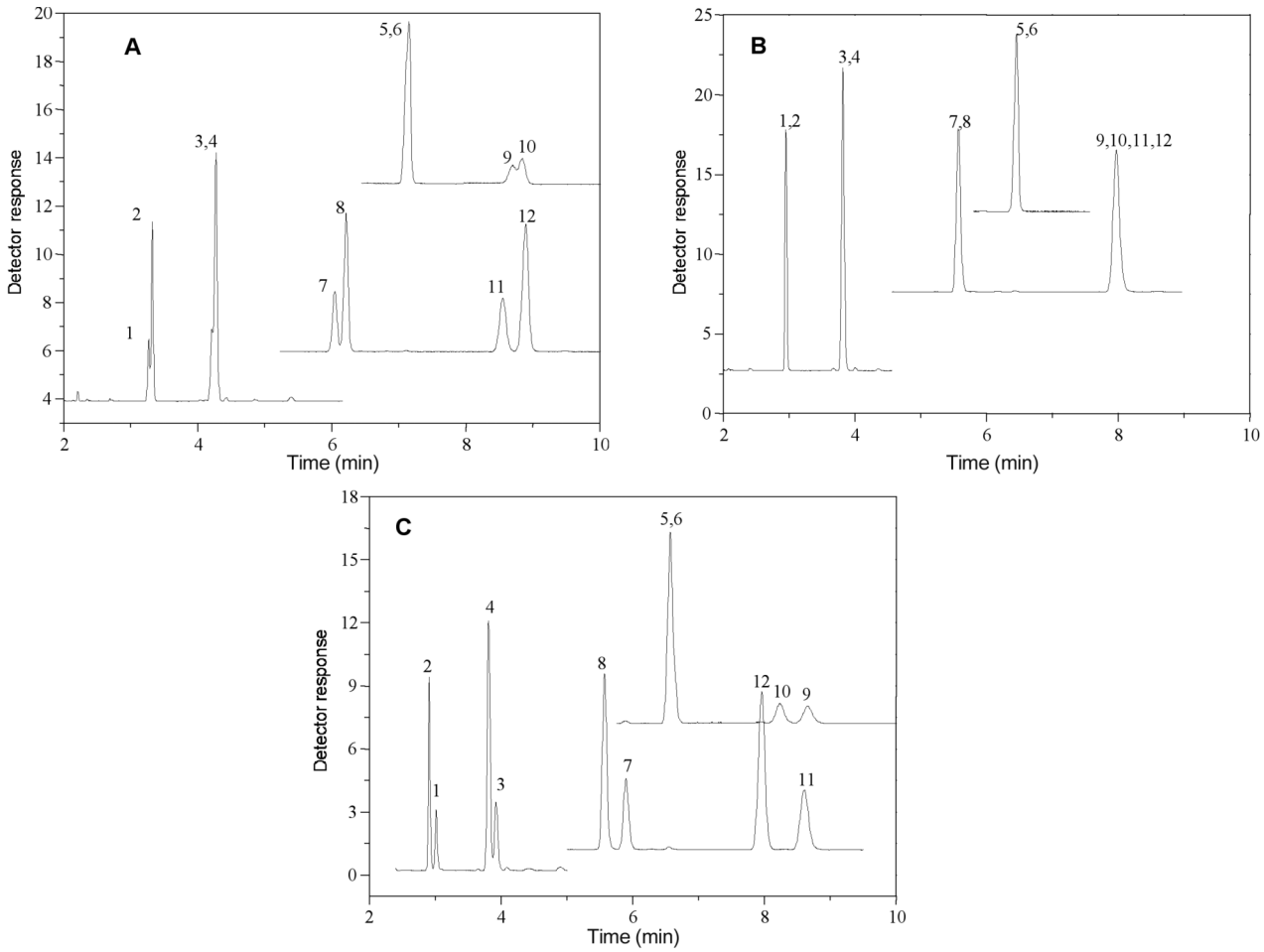

Figure 9. Separation of the enantiomers of *N*-TFA-*O*-methyl derivatives of amino acids on the *BA* coupled column series at 100°C with (A) p_a = 130 kPa, (B) p_a = 140 kPa, and (C) p_a = 160 kPa.

columns, and different chiral stationary-phase film thicknesses on the two columns. The second of these factors produced the more noticeable effect in this study.

Acknowledgments

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