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On the capillary gas chromatographic separation of enantiomers of *N*-trifluoroacetyl-*O*-alkyl esters of selected amino acids on 2,3-di-*O*-pentyl-6-*O*-acyl cyclodextrins

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Abstract

In this work, the separation of enantiomers of *N*-TFA-*O*-alkyl amino acids on the 2,3-di-*O*-pentyl-6-*O*-acyl α-, β- and γ-cyclodextrin stationary phases has been studied. The influence of structure differences in the alkyl substituents bonded to the stereogenic carbon atom (R_1), as well as in the ester group (R_2) of the selected amino acid derivatives, and the selectivity of modified α-, β- and γ-cyclodextrin phases in gas chromatographic separation of derivatized amino acid enantiomers was studied in detail. A model set of *N*-TFA-alkyl esters of four amino acids was separated on five columns. The separation of enantiomers was evaluated in terms of the interactions of the alkyl substituents bonded to the stereogenic carbon (R_1) and/or the ester group (R_2) of the *N*-TFA-*O*-alkyl amino acid derivatives as well as the nature of the 3-*O*-acyl group in the 2,6-di-*O*-pentyl-3-*O*-acyl α-, β- and γ-cyclodextrins. It was shown that the variation in the enantiomeric separation with temperature and the retention order of enantiomers on a given cyclodextrin capillary column depends both on the nature of the bonded R_1 and R_2 alkyl groups. It was found that the temperature dependencies of selectivity factors, $\ln \alpha$ on $1/T$, were mostly non-linear. The thermodynamic data $\{\Delta(\Delta S)$ and $\{\Delta(\Delta H)\}$ which characterize the chiral recognition were used to gain more insight into the mechanistic aspects of enantio separation of the *N*-TFA-*O*-alkyl amino acid derivatives on 2,6-di-*O*-pentyl-3-*O*-acyl-α-, β- and γ-cyclodextrins.

Keywords: 2,6-Di-*O*-pentyl-3-*O*-acyl α- β- and γ-cyclodextrins; Enantiomer separation; *N*-Trifluoroacetyl-*O*-alkyl amino acid derivatives

1. Introduction

The gas chromatographic separation of biogenic amino acid enantiomers has been achieved quite successfully on several types of chiral stationary phases (CSPs) [1–9]. A uniform retention order for derivatized amino acids has been reported within sets of derivatives of the same type [2,3,5]. The analysis of *N*-trifluoroacetyl-*O*-methyl (*N*-TFA-*O*-methyl) derivatives on octakis(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin produces a uniform retention order, where the D-enantiomers were eluted prior to the L-enantiomers, except for proline [2]. *N*-TFA-*O*-methyl and isopropyl esters of 15 amino acids were analyzed on 6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-acetyl-β- and γ-cyclodextrins and 6-*O*-*tert*-butydimethylsilyl-2,3-di-*O*-*n*-butyryl-β- and γ-cyclodextrins [5]. The best separation for methyl esters was achieved on the γ-cyclodextrin CSP with the 2,3-di-*O*-*n*-butyryl substituents. The best separation for isopropyl esters was obtained on a β-cyclodextrin CSP with the 2,3-di-*O*-acetyl substituents, where the retention order was found to be D before L with the exception of proline [5].

Besides the CGC analyses of amino acids in analytical praxis, the amino acids are often used to study the retention mechanism in chiral CGC separations as their structures are relatively simple, easy to modify, and their enantiomers are accessible [10].

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It has been found that the retention of amino acid derivatives depends on the properties of the substituents bound to their amine and carboxylic acid groups [10,11]. The retention behavior of *N*-trifluoroacetyl-*O*-alkyl esters of selected amino acids with various types of alkyl ester groups was studied by capillary gas chromatography with modified cyclodextrin stationary phases. A model set of six different esters of five amino acids was analyzed on two columns coated with permethyl-β-cyclodextrin bonded to polysiloxane elastomer (ChirasilDex), and heptakis (6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-acetyl)-β-cyclodextrin dissolved in OV 1701 in a 1:1 ratio. It was found that the variation in the enantiomeric separation with temperature and the elution sequence of enantiomers depend on the nature of the alkyl groups bonded both to the stereogenic carbon atom and the ester group. A temperature dependent inversion of the elution sequence was observed for *N*-TFA-*O*-*n*-butyl valine derivative on a ChirasilDex column [10].

CGC separation of *N*-TFA-derivatives of amino acid isopropyl esters were studied on four types of cyclodextrin derivatives of 6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-acetyl or *n*-butyryl-β- and γ-cyclodextrins. All enantiomeric pairs of the studied amino acids were separated except tryptophane. Methyl esters showed the best separation on octakis(2,3-di-*O*-*n*-butyryl-6-*O*-*tert*.-butyldimethylsilyl)-γ-cyclodextrin, while the isopropyl esters were best separated on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin. Proline showed complete separation of its enantiomers as its *N*-TFA-*O*-isopropyl derivative, and the same derivative of alanine showed a very high separation factor ($\alpha = 1.81$) at 100 °C [5]. 2,6-Di-*O*-pentyl-3-*O*-propionyl-γ-cyclodextrin has been used for enantiomer CGC separation of amino acids, amines, alcohols, diols, epoxides, and lactones [12].

Studying the retention behaviour of enantiomers of *N*-TFA-*O*-*n*-propyl esters of several amino acids separated by gas chromatography on capillary columns coated with permethylated-β-CD, anchored to polysiloxane elastomer (ChirasilDex CB), and heptakis(6-*O*-*tert*-butyl dimethylsilyl-2,3-di-*O*-acetyl)-β-CD (TBDMSDA-β-CD) dissolved in OV 1701; it was found that the retention order and separation of enantiomers of amino acids depends on the polar and non-polar interaction contributions that were possible with the different chiral stationary phases. The separation of enantiomers of alanine *N*-TFA-*O*-*n*-propyl ester was better on TBDMSDA-β-CD (with a retention order of L, D) than on a ChirasilDex column (with a retention order of D, L). The separation of enantiomers of the *N*-TFA-*O*-propylester of proline was better on the ChirasilDex column than on TBDMSDA-β-CD, with both having a D, L retention order [10].

In our previous study [13], we evaluated the gas chromatographic separation of enantiomers of seven amino acid *N*-TFA-*O*-alkyl derivatives on four capillary columns coated with 2,3,6-tri-*O*-methyl-, and 2,6-di-*O*-methyl-3-*O*-pentyl-β- and γ-CD stationary phases. The separation of enantiomers was evaluated in terms of the non-polar interactions of the alkyl substituents bonded to the stereogenic carbon (R_1) and/or the ester group (R_2) of the *N*-TFA-*O*-alkyl amino acid derivatives as well as the 3-*O*-alkyl chain length of 2,6-di-*O*-methyl-3-*O*-alkyl-β- and γ-cyclodextrins [13]. In the current work, we have studied the gas chromatographic separation of enantiomers of *N*-TFA-*O*-alkyl amino acid esters on fused silica capillary columns coated with five 2,6-di-*O*-pentyl-3-*O*-acyl (trifluoroacetyl, propionyl and butyryl) derivatives of α-, β-and γ-cyclodextrins.

2. Experimental

2.1. Instruments

Capillary GC was performed using a HP 5890 gas chromatograph equipped with 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 signal of the FID was monitored by a HP 3396 integrator and via Peak 96 software transmitted into PC where it was evaluated by HP 3365 series II Chemstation software. One microliter of samples was injected into the column by split injection with split ratio of 100:1. The measurements were performed at isothermal conditions in the temperature range 60–160 °C with 10 °C increments.

2.2. Columns

2.2.1. Column A

Twenty-five milliliters capillary column with 0.25 mm i.d. coated with a 0.25 μm film thickness of 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-α-cyclodextrin (ATA).

2.2.2. Column B

Twenty-five meter capillary column with 0.25 mm i.d. coated with a 0.25 μm film thickness of a 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-β-cyclodextrin (BTA).

2.2.3. Column C

Twenty-five meter capillary column with 0.25 mm i.d. coated with a 0.25 μm film thickness of 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-γ-cyclodextrin (GTA).

2.2.4. Column D

Twenty-five meter capillary column with 0.25 mm i.d. coated with a 0.25 μm film thickness of a 2,6-di-*O*-pentyl-3-*O*-propionyl-γ-cyclodextrin (GProp).

2.2.5. Column E

Twenty-five meter capillary column with 0.25 mm i.d. coated with a 0.25 μm film thickness of a 2,6-di-*O*-pentyl-3-*O*-butyryl-γ-cyclodextrin (GBut).

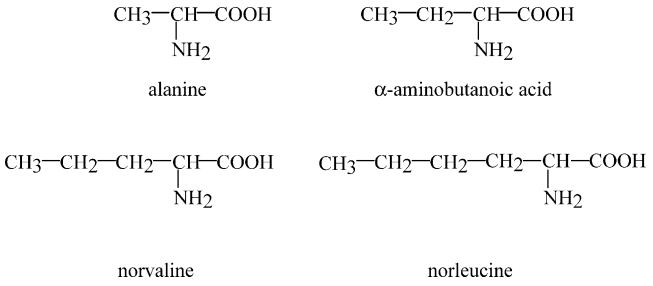
All columns were obtained from Advanced Separation Technologies Inc. (Whippany, NJ, USA) [14].

2.3. Analytes

The racemic mixtures as well as the pure L- or D-enantiomers of the amino acids were obtained from Sigma (Sigma–Aldrich Chemie GmbH). The methyl esters of all used amino acids, as well as, ethyl, *n*-propyl and *n*-butyl esters of *N*-TFA derivatives of alanine were prepared according to the following procedures:

Esterification: A 20% (v/v) solution of acetylchloride in the corresponding alcohol was added to the sample of amino acid and kept for 30 min at elevated temperature (from 90 to 120 °C according to the type of alcohol used) in a teflon cap closed vial. After reaction the excess reagent was removed with a stream of nitrogen.
Acylation: Fifty microliters of trifluoroacetic anhydride was added to the sample and the mixture was heated at 150 °C for 15 min. After removal of reagent the derivatives were dissolved in *n*-hexane, remaining acidic compounds extracted by water, followed by drying of the solution by anhydrous sodium sulphate.

The enantiomers of *N*-TFA-*O*-alkyl derivatives of following amino acids were used in this study:

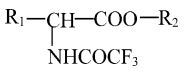


2.4. Chemicals

n-Hexane, *n*-pentane, methanol, ethanol, *n*-propanol, *n*-butanol and trifluoroacetic anhydride were purchased from Merck (Darmstadt, Germany).

3. Results and discussion

N-TFA-*O*-alkyl amino acids used in this study have the following general formula:



where R_1 and R_2 are the alkyl substituents bonded to the stereogenic carbon or to the carboxylate group, respectively. These derivatives were divided into two groups according to the nature of the R_1 and R_2 alkyl substituents:

(a) The first group contains the *N*-TFA-*O*-methyl esters of amino acids with a different linear alkyl chain bonded to the stereogenic carbon center (R_1): *methyl*- in alanine, *ethyl*- in 2-aminobutanoic acid, *n-propyl*- in norvaline and *n-butyl*- in norleucine. Using the members of this group1 the dependence of the GC enantiomer separation on the R_1 alkyl chain length may be studied.
(b) The second group contains the alanine-*N*-TFA-*O-methyl*, *ethyl*, *n-propyl* and *n-butyl* ester, respectively. Using the members of this group1 the dependence of enantioselectivity on the linear alkyl chain length of the bonded ester group (R_2) may be studied.

The retention sequence of the racemates was determined by a subsequent separation of the pure L-enantiomer standard. Enantiomers of D,L-*N*-TFA-*O*-alkyl amino acid esters were separated on five modified cyclodextrin capillary columns coated with:

(1) hexakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-α-CD,
(2) heptakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-β-CD,
(3) octakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-γ-CD,

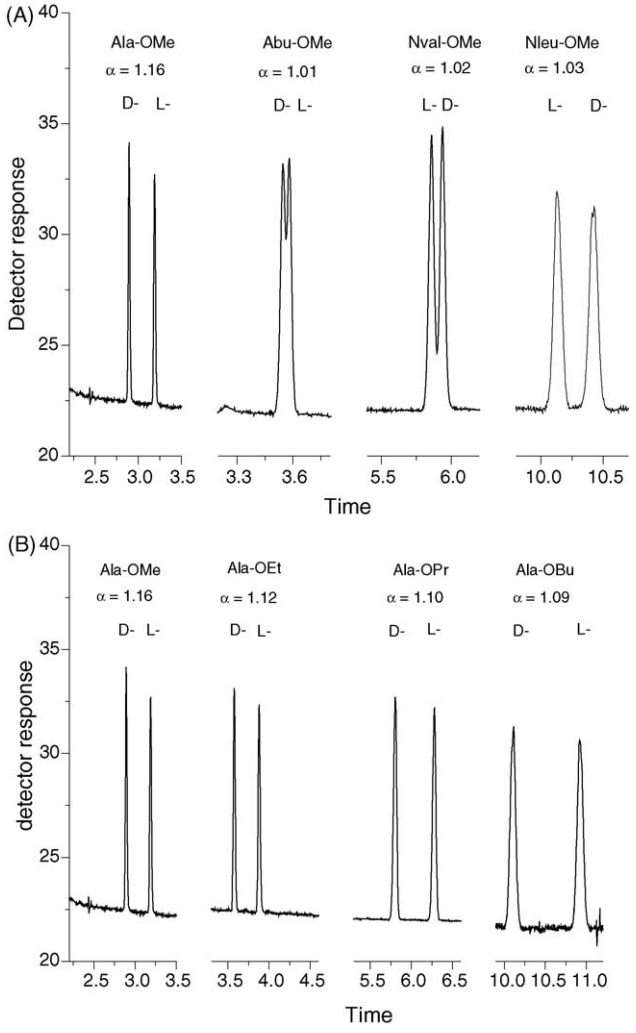


Fig. 1. Separation of enantiomers of *N*-TFA-*O*-alkyl amino acid derivatives obtained on 2,6-di-*O*-Pe-3-*O*-TFA-α-CD stationary phase at 100 °C. (A) Derivatives differing in R_1 substituent, (B) derivatives differing in R_2 substituent.

(4) octakis(2,6-di-*O*-pentyl-3-*O*-propionyl)-γ-CD,
(5) octakis(2,6-di-*O*-pentyl-3-*O*-butyryl)-γ-CD,

where the separation of enantiomers was correlated with the cavity size of the cyclodextrin (columns 1–3) and with the acidity of the acyl group bonded to -3-*O*-position of the γ-cyclodextrin. Dipole–dipole interactions are commonly identified in the mechanism of separation for 2,6-di-*O*-pentyl-3-*O*-TFA-α-, β-, and γ-CD stationary phases [11]. Similar interactions can be expected for the 2,6-di-*O*-pentyl-3-*O*-propionyl-γ-CD and 2,6-di-*O*-pentyl-3-*O*-butyryl-γ-CD chiral stationary phase. However, dispersion interactions and steric/repulsive interactions also may be important [15].

3.1. Separations on the 2,6-di-O-Pe-3-O-TFA-α-CD column

Fig. 1 shows the separation of enantiomers of the *N*-TFA-*O*-alkyl derivatized amino acids on a capillary column coated with 2,6-di-*O*-Pe-3-*O*-TFA-α-CD stationary phase at 100 °C. It follows from Fig. 1A that the retention order of the enantiomers changes with the length of the R_1 alkyl chain. While the enantiomers of *N*-TFA-derivatives of Ala-*O*-Me and Abu-*O*-Me elute with the same D,L retention order, the enantiomers of Nval-*O*-Me and Nleu-*O*-Me elute in the opposite order (L,D). The highest selectivity factor in Fig. 1A ($\alpha = 1.16$) belongs to the Ala-*O*-Me enantiomers. Fig. 1B shows that the retention order (D,L) of *N*-TFA-*O*-alkyl esters of alanine enantiomers is constant and does not depend on the R_2 alkyl chain length. The selectivity factors listed in Fig. 1B, however, decrease with the R_2 alkyl chain length. Comparison of Fig. 1A and B shows that the chiral recognition of the *N*-TFA-*O*-alkyl esters of the amino acid enantiomers is dependent on the length of alkyl chain R_1.

Fig. 2A shows the dependence of $\ln\alpha$ on the inverse of temperature ($1/T$) for enantiomers of *N*-TFA-*O*-alkyl derivatives for all studied amino acids on a capil-

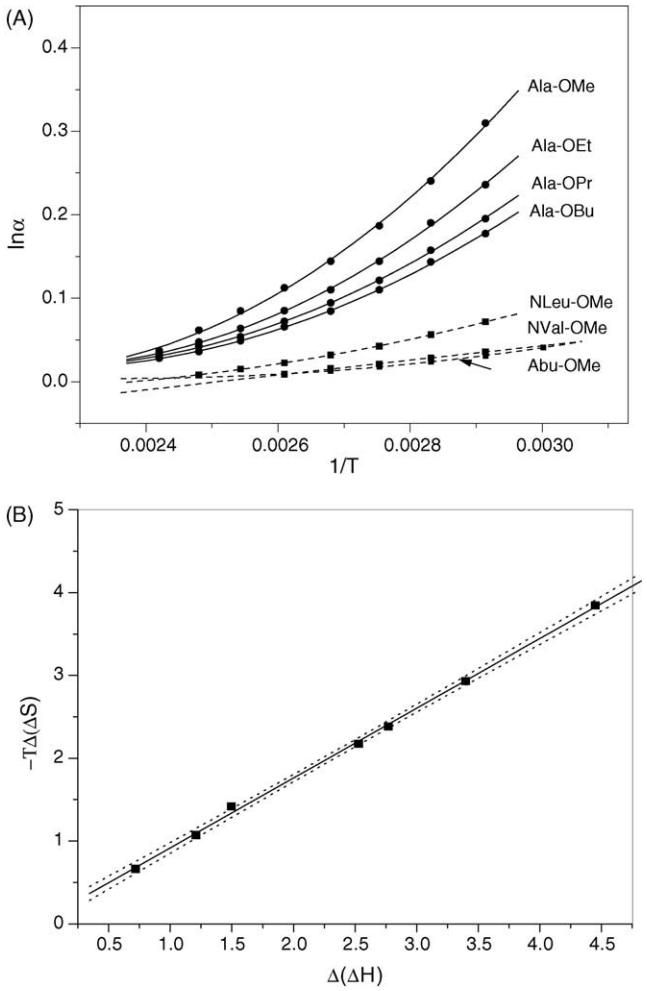


Fig. 2. Dependence of $\ln\alpha$ on reversed temperature ($1/T$) (A) and $T\Delta(\Delta S)$ [kJ/mol] on $\Delta(\Delta H)$ [kJ/mol] at 90 °C (B) obtained for enantiomers of *N*-TFA-*O*-alkyl amino acid derivatives on 2,6-di-*O*-Pe-3-*O*-TFA-α-CD stationary phase. Dotted lines in (B) shows 95% confidence interval.

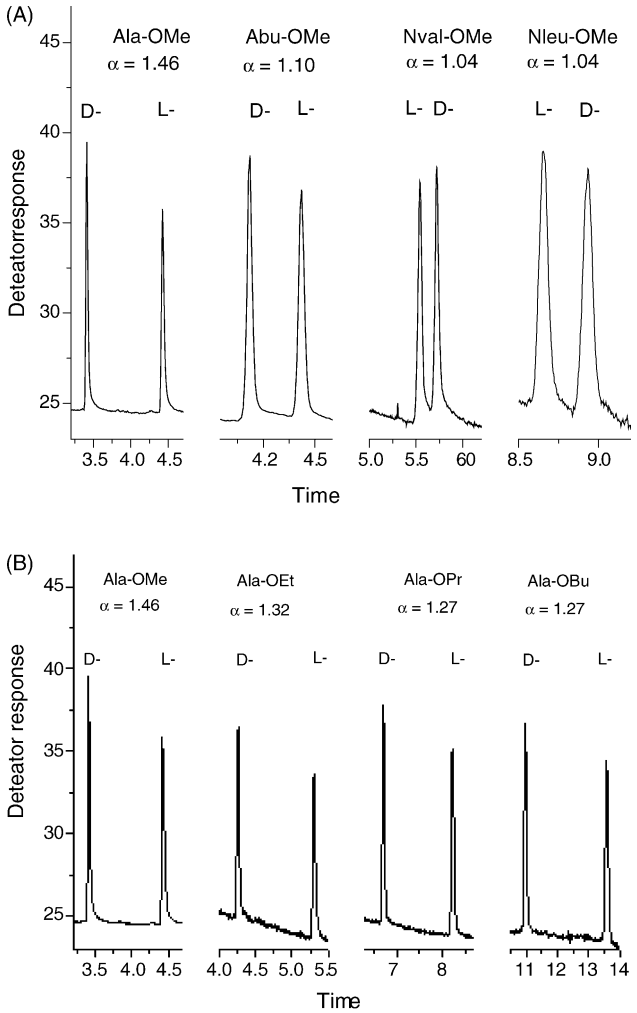


Fig. 3. Separation of enantiomers of *N*-TFA-*O*-alkyl amino acid derivatives obtained on 2,6-di-*O*-Pe-3-*O*-TFA-β-CD stationary phase at 100 °C. (A) Derivatives differing in R_1 substituent, (B) derivatives differing in R_2 substituent.

lary column coated with 2,6-di-*O*-Pe-3-*O*-TFA-α-CD. All dependencies are non-linear. The more non-linear curves in 1st group belong to enantiomers eluting with the L-, D-order. This indicates that intermolecular interactions of the enantiomers with the stationary phase chiral selector could be responsible for reversal in retention order are not additive.

Fig. 2B shows the dependence of $\Delta(\Delta S)$ on $\Delta(\Delta H)$ values obtained for enantiomers of the same amino acid derivatives on the same column at 90 °C. It follows from the data listed in Table 1 that the line found by regression analysis of the experimental data is linear (with a correlation coefficient 0.99934). Monotonous increase of $\Delta(\Delta S)$ values on $\Delta(\Delta H)$ shows no occurrence of steric interactions of enantiomers of the *N*-TFA-*O*-alkyl amino acids derivatives differing with the length of R_1 and R_2 alkyl chains with 2,6-di-*O*-Pe-3-*O*-TFA-α -CD chiral selector.

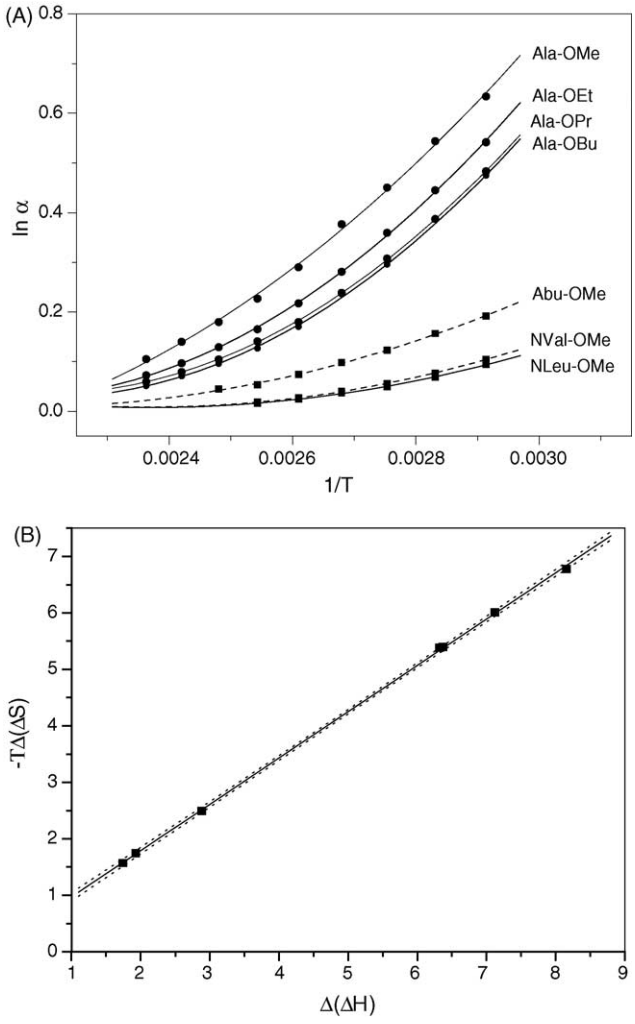


Fig. 4. Dependence of $\ln\alpha$ on the reverse of temperature ($1/T$) (A) and $T\Delta(\Delta S)$ [kJ/mol] on $\Delta(\Delta H)$ [kJ/mol] at 90 °C (B) obtained for enantiomers of *N*-TFA-*O*-alkyl amino acid derivatives on 2,6-di-*O*-Pe-3-*O*-TFA-β-CD stationary phase. The sense of dotted lines in (B) is given in the legend of Fig. 2A.

Table 1
The parameters of the dependence of $\Delta(\Delta S)$ on $\Delta(\Delta H)$ for enantiomers of *N*-TFA-*O*-alkyl derivatives of studied amino acid on 2,6-di-*O*-Pe-3-*O*-acyl-α-, β- and γ-CDs obtained at 90 °C

Column	Intercept	Slope	Correlation coefficient
2,6-Di-*O*-Pe-3-*O*-TFA-α-CD	0.0754	0.84244	0.99934
2,6-Di-*O*-Pe-3-*O*-TFA-β-CD	0.15309	0.81986	0.99986
2,6-Di-*O*-Pe-3-*O*-TFA-γ-CD	0.02035	0.83807	0.99999
2,6-Di-*O*-Pe-3-*O*-propionyl–CD	−0.14151	0.87399	0.99949
2,6-Di-*O*-Pe-3-*O*-butyryl–CD	−0.13169	0.88149	0.99987

3.2. Separations on the 2,6-di-O-Pe-3-O-TFA-β-CD column

Fig. 3 shows the separation of enantiomers of the *N*-TFA-*O*-alkyl derivatized amino acids on a capillary column coated with a 2,6-di-*O*-Pe-3-*O*-TFA-β-CD stationary phases at 100 °C. Comparison of Figs. 1 and 3 leads to analo-

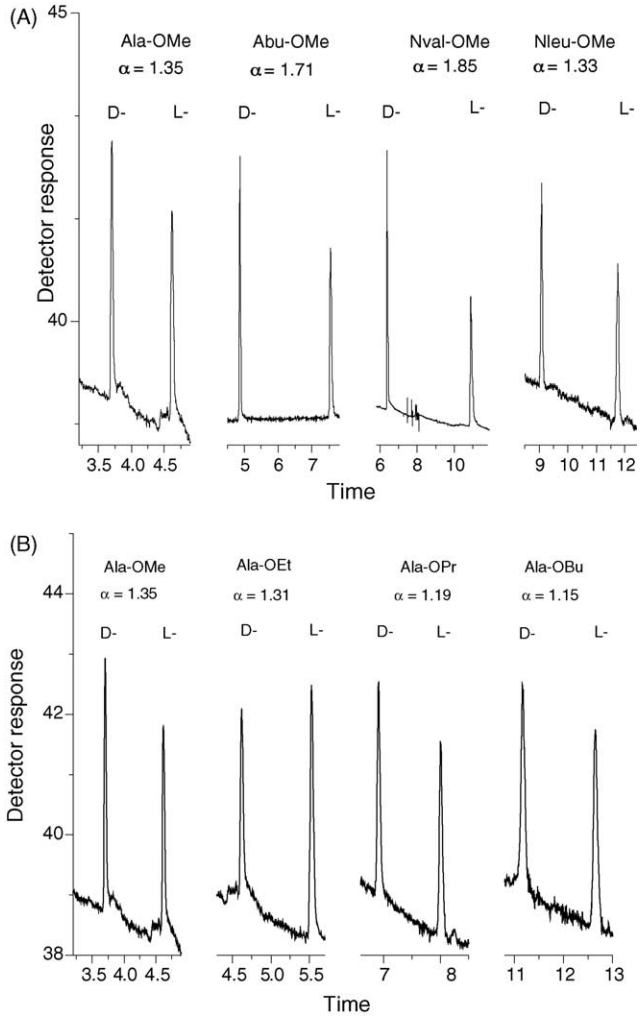


Fig. 5. Separation of enantiomers of *N*-TFA-*O*-alkyl amino acid derivatives obtained on 2,6-di-*O*-Pe-3-*O*-TFA-γ-CD stationary phase at 100 °C. (A) Derivatives differing in R_1 substituent, (B) derivatives differing in R_2 substituent.

gous conclusions on the retention order of enantiomers of these amino acids. However, the selectivity factors listed in Figs. 1 and 3 show higher values on the 2,6-di-*O*-Pe-3-*O*-TFA-β-CD column. Similar behaviors of the 2,6-di-*O*-Pe-3-*O*-TFA-α-CD and 2,6-di-*O*-Pe-3-*O*-TFA-β-CD columns can also be found by comparison of Figs. 2A and 4A. Comparison of Figs. 2B and 4B and the data listed in Table 1 shows, however, different slopes for the dependencies of $\Delta(\Delta S)$ on $\Delta(\Delta H)$ on the 2,6-di-*O*-Pe-3-TFA-α-CD and 2,6-di-*O*-Pe-3-*O*-TFA-β-CD columns. The higher slope of this dependence is connected with the higher contribution of the entropic term to overall interaction of enantiomers with the 2,6-di-*O*-Pe-3-*O*-TFA-β-CD column.

3.3. Separations on the 2,6-di-O-Pe-3-O-TFA-γ-CD column

The separation of enantiomers of the same series of derivatized amino acids was studied at 100 °C on a capillary column coated with a 2,6-di-*O*-Pe-3-*O*-TFA-γ-CD stationary phase. It is clear from the chromatogram in Fig. 5 that these separations are different from those in Fig. 1 (on the 2,6-di-*O*-Pe-3-*O*-TFA-α-CD column) and in Fig. 3 (on the 2,6-di-*O*-Pe-3-*O*-TFA-β-CD column). All enantiomers of the *N*-TFA-*O*-alkyl derivatives of the studied amino acid series elute in the same D-, L- order independently on the length of the R_1 and R_2 alkyl substituents.

The highest selectivity factor ($\alpha = 1.85$) is found for Nval-*O*-Me enantiomers. The selectivity factors of enantiomers listed in Fig. 5 increase with the length of the amino acid's R_1 alkyl chain, and for the *N*-TFA-*O*-alkyl alanine enantiomers, they decrease with the length of the R_2 alkyl chain. Comparison of Fig. 5A and B shows that the chiral recognition of the *N*-TFA-*O*-alkyl esters amino acid enantiomers depends mainly on the length of alkyl chain R_1.

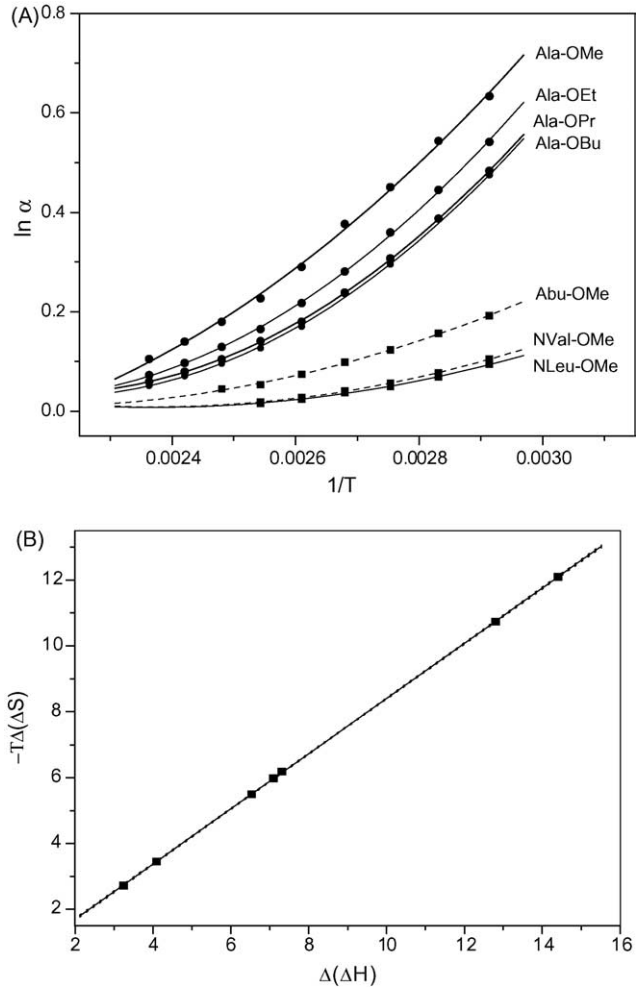


Fig. 6. Dependence of $\ln\alpha$ on reversed temperature ($1/T$) (A) and $T\Delta(\Delta S)$ [kJ/mol] on $\Delta(\Delta H)$ [kJ/mol] at 90 °C (B) obtained for enantiomers of *N*-TFA-*O*-alkyl amino acid derivatives on 2,6-di-*O*-Pe-3-*O*-TFA-γ-CD stationary phase. The sense of dotted lines in (B) is given in the legend of Fig. 2A.

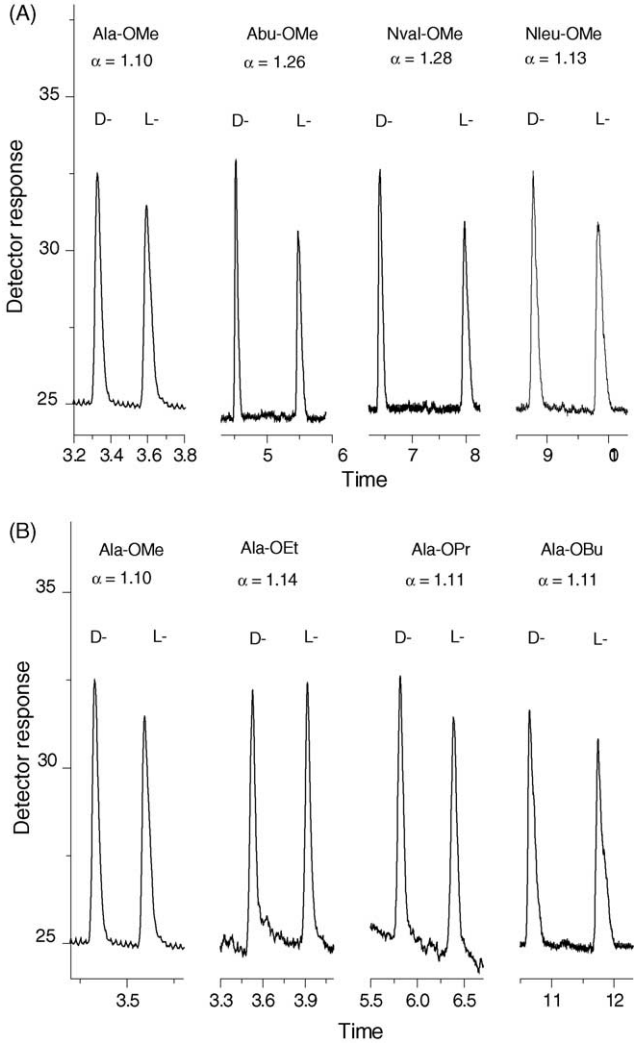


Fig. 7. Chromatograms of *N*-TFA-*O*-alkyl amino acid derivatives obtained on 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD stationary phase at 100 °C. (A) Derivatives differing in R_1 substituent, (B) derivatives differing in R_2 substituent.

From Figs. 1, 3 and 5 is obvious that gamma TFA cyclodextrin exhibits better enantioselectivity for studied amino acid derivatives than corresponding α and β analogs, which is in agreement with already published data [15].

Fig. 6A shows the non-linear dependence of $\ln \alpha$ on the inverse of temperature (1/*T*) for this series of derivatized amino acids on the capillary column coated with 2,6-di-*O*-Pe-3-*O*-TFA-γ-CD.

Fig. 6B shows the dependence of the $\Delta(\Delta S)$ on $\Delta(\Delta H)$ values obtained for the same series of amino acid enantiomers on the 2,6-di-*O*-Pe-3-*O*-TFA-γ-CD column at 90 °C. The slopes listed in Table 1 obtained on the 2,6-di-*O*-Pe-3-*O*-TFA-α-CD and 2,6-di-*O*-Pe-3-*O*-TFA-γ-CD columns are very similar but the correlation coefficient obtained on the last column is much higher. This looks like the R-groups are providing dispersion interactions and/or steric repulsive interactions.

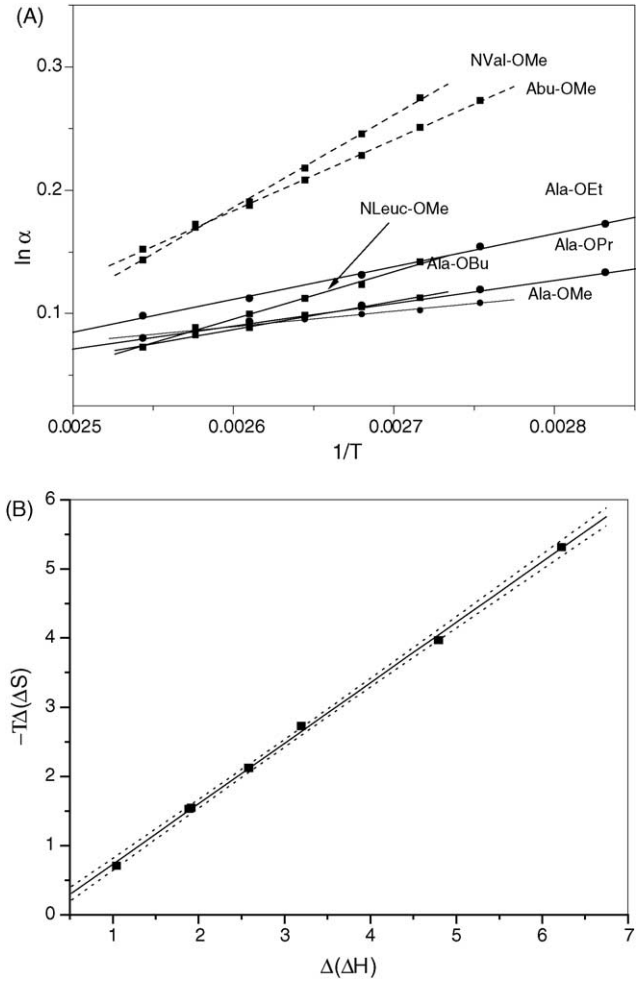


Fig. 8. Dependence of $\ln \alpha$ on reversed temperature (1/*T*) (A) and $T\Delta(\Delta S)$ [kJ/mol] on $\Delta(\Delta H)$ [kJ/mol] at 90 °C (B) obtained for enantiomers of *N*-TFA-*O*-alkyl amino acid derivatives on 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD stationary phase. The sense of dotted lines in (B) is given in the legend of Fig. 2A.

3.4. Separations on the 2,6-di-O-Pe-3-O-propionyl-γ-CD column

Fig. 7 shows the separation of enantiomers of the *N*-TFA-*O*-alkyl derivatives of the studied amino acids on a capillary column coated with 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD stationary phase at 100 °C. From this figure it follows that all these derivatized amino acid enantiomers elute in the D-, L-order.

A comparison of data listed in Figs. 5 and 7 show that the increase in the R_1 alkyl chain improves the separation of enantiomers on the 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD column to a lesser extent than on the 2,6-di-*O*-Pe-3-*O*-TFA-γ-CD column. The length of the R_2 alkyl chain has almost no influence on the chiral selectivity factors of the enantiomers of this amino acid series on the 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD column.

Fig. 8A shows the linear dependence of $\ln \alpha$ on the inverse of temperature (1/*T*), for enantiomers of the *N*-TFA-*O*-alkyl derivatives of all studied amino acids on a capillary col-

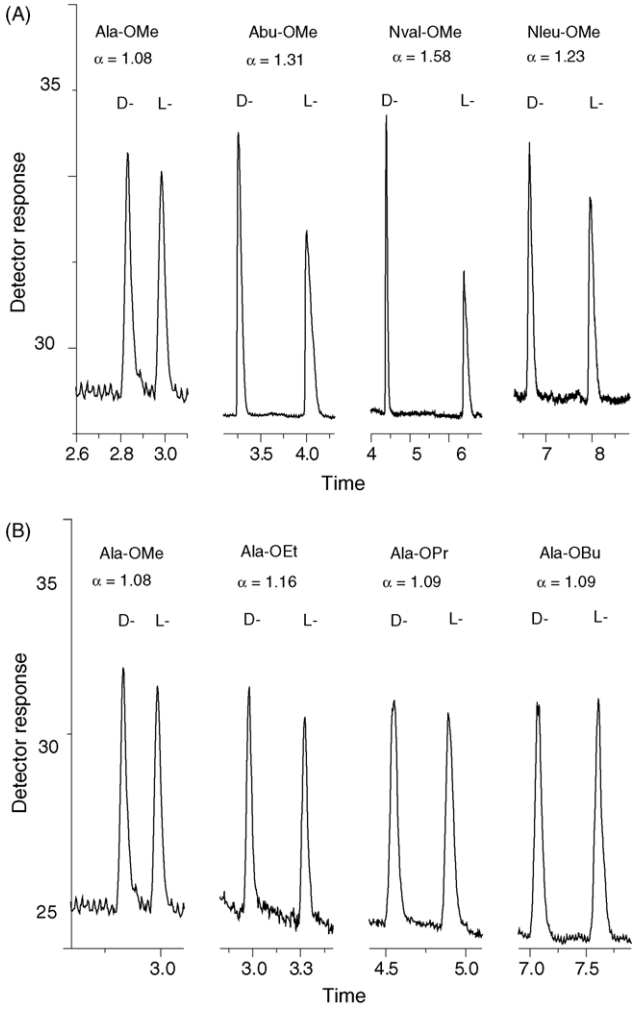


Fig. 9. Chromatograms of *N*-TFA-*O*-alkyl amino acid derivatives obtained on 2,6-di-*O*-Pe-3-*O*-butyryl-γ-CD stationary phase at 100 °C. A. Derivatives differing in R_1 substituent, (B) derivatives differing in R_2 substituent.

umn coated with 2,6-di-*O*-Pe-3-*O*-TFA-γ-CD. Fig. 8B shows the dependence of $\Delta(\Delta S)$ on $\Delta(\Delta H)$ values obtained for enantiomers of the same series of amino acid derivatives on the 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD column at 90 °C. The slope listed in Table 1 for 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD is slightly higher than was found for the 2,6-di-*O*-Pe-3-*O*-TFA-γ-CD column, but with a negative intercept on the *Y*-axis.

3.5. Separations on the 2,6-di-O-Pe-3-O-butyryl-γ-CD column

The separation of the enantiomers of *N*-TFA-*O*-alkyl derivatized amino acids on the 2,6-di-*O*-Pe-3-*O*-butyryl-γ-CD coated capillary column at 100 °C is shown in Fig. 9. From this figure it follows that the enantiomers of all studied amino acid derivatives elute with D-, L- retention order. The separation of these enantiomers improves with the length of the R_1 alkyl chain ($\alpha = 1.08$ for Ala-*O*-Me, $\alpha = 1.31$ for Abu-*O*-Me and $\alpha = 1.58$ for Nval-*O*-Me). The separation of these enantiomers depends on the alkyl chain length (R_1) to a greater extent than on the 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD column, which can be explained by the lower polarity of the 2,6-di-*O*-Pe-3-*O*-butyryl-γ-CD column. The dependence of the separation of enantiomers on the R_2 alkyl chain is not regular.

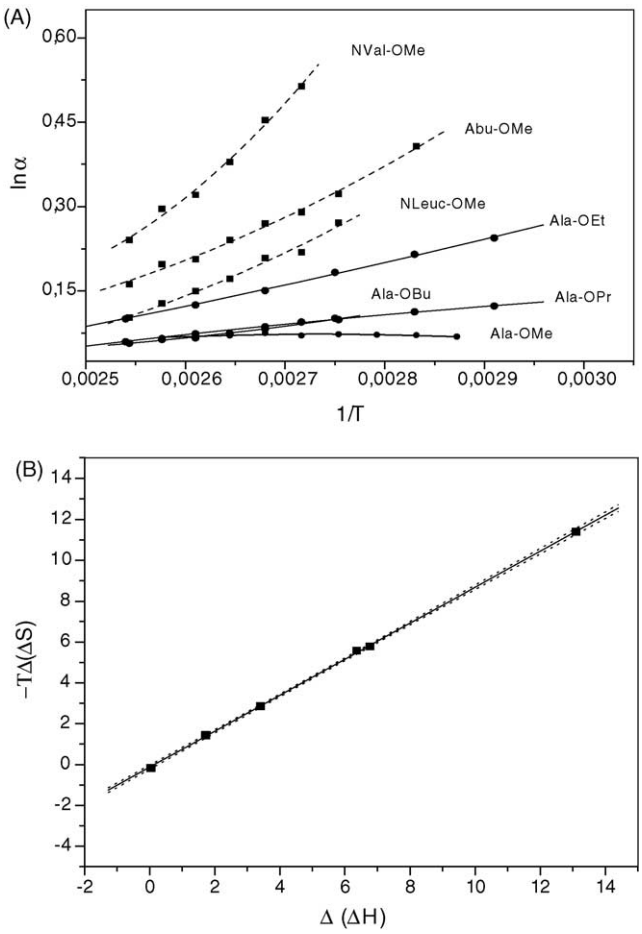


Fig. 10. Dependence of $\ln \alpha$ on reversed temperature ($1/T$) (A) and $T\Delta(\Delta S)$ [kJ/mol] on $\Delta(\Delta H)$ [kJ/mol] at 90 °C (B) obtained on 2,6-di-*O*-Pe-3-*O*-butyryl-γ-CD stationary phase at 100 °C. The sense of dotted lines in (B) is given in the legend of Fig. 2A.

Fig. 10A shows the linear dependence of $\ln \alpha$ on the inverse of temperature ($1/T$), for enantiomers of *N*-TFA-*O*-alkyl esters of alanine on a capillary column coated with 2,6-di-*O*-Pe-3-*O*-butyryl-γ-CD. These plots for enantiomers of all other *N*-TFA-*O*-methyl amino acid derivatives are nonlinear.

Fig. 10B shows the dependence of $\Delta(\Delta S)$ on $\Delta(\Delta H)$ values obtained for amino acid derivatives on the 2,6-di-*O*-Pe-3-*O*-butyryl-γ-CD column at 90 °C. The slope and the intercept on the Y-axis listed in Table 1 for 2,6-di-*O*-Pe-3-*O*-butyryl-γ-CD and 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD column are practically the same.

4. Conclusion

A comparison of chromatograms obtained for the separation of enantiomers of *N*-TFA-*O*-alkyl derivatives of some amino acids on the 2,6-di-*O*-Pe-3-*O*-butyryl-γ-CD, 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD and 2,6-di-*O*-Pe-3-TFA-α-, β- and γ-CD columns show that the separation is substantially affected by the nature of 3-*O*-substituent. It has been found that the elution order of enantiomers of the *N*-TFA-*O*-alkyl derivatives of the studied amino acids on 2,6-di-*O*-Pe-3-*O*-butyryl-γ-CD, 2,6-di-*O*-Pe-3-*O*-propionyl-γ-CD and 2,6-di-*O*-Pe-3-TFA-γ-CD was the same (D,L). The enantiomers of *N*-TFA-derivatives of Ala-*O*-Me and Abu-*O*-Me elute in the opposite order (L,D) on the 2,6-di-*O*-Pe-3-TFA-α- and β-CD columns.

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