HIGH – PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ENANTIOSEPARATION OF UNDERIVATIZED α-AMINO ACIDS USING CYCLOFRUCTAN – BASED CHIRAL STATIONARY PHASES

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Abstract: Chromatographic columns with chiral stationary phases based on chemically – bonded derivatized cyclofructans were evaluated for HPLC enantioresolution of underivatized α-amino acids. The analytical study of chiral separation of these analytes was focused on the selection of suitable chiral stationary phase and mobile phase. Using isopropyl carbamate cyclofructan 6 as a chiral stationary phase, α-amino acid optical isomers were separated. The retention and enantioseparation of chiral amino acids were also influenced by a mobile phase composition. The mixture methanol/acetonitrile/acetic acid/triethylamine (75/25/0.3/0.2 v/v/v/v) was found to be the most effective mobile phase for HPLC separation of studied compounds. HPLC enantioresolution of chiral amino acids was thermodynamically studied. Based on the enthalpy and entropy contribution values calculated from the van´t Hoff equation, HPLC enantioseparation under chosen chromatographic conditions was found to be an enthalpically driven.

Key words: amino acids, cyclofructan – based chiral selectors, HPLC, thermodynamic study

1. Introduction

Chirality was discovered in 1848 by Louis Pasteur (FLACK, 2009). Since most biologically important compounds exist in two enantiomeric forms that can possess differences in their physiological activity, the impetus for the separation of optical isomers and the preparation of enantiomerically pure compounds in the field of biotechnology, chemistry and pharmacology is still increasing (ILISZ *et al.*, 2012). Chromatographic methods, i.e. capillary electrophoresis (CE), gas chromatography (GC), high – performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), thin layer chromatography (TLC), have been frequently used for the enantioresolution of chiral analytes. Among them, enantioselective HPLC method is one of the most powerful and widely used separation techniques at both analytical and preparative scales (DING *et al.*, 2004).

Amino acids play an important role in living organisms. They serve as the basic structural units of peptides and proteins. Most proteinogenic α-amino acids contain an asymmetric carbon atom. Their stereoisomers can differ markedly in physiological effects. Chiral separation of these substances is a great analytical challenge in several research areas, such as asymmetric syntheses in organic chemistry, biochemistry of amino acids, pharmaceutics, the dating of archaeological materials and the study of

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food processes in human body (SHPIGUN *et al.*, 2002). The recent direct HPLC separations of amino acid stereoisomers and related compounds are summarized in Table 1.

Table 1. Summary of chiral stationary phases for direct HPLC separations of amino acid stereoisomers.

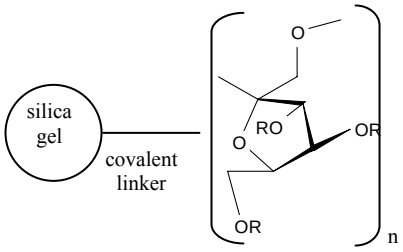
Separated stereoisomers	Chiral selectors	Mobile phases	References
	Cyclodextrins		
20 unusual amino acids	α-CD, β-CD, acetylated β-CD, 3,5-dimethylphenyl-carbamoylated β-CD	0.1% aqueous TFAA (pH 4-5)	REMSBURG *et al.*, 2008
	Crown-ether		
β-amino acids, proteinogenic D,L-amino acids, aliphatic and aromatic β^3-amino acids	(+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid	H_2O/MeOH + 10 mmo.L^{-1} AcOH, H_2O/MeOH (80/20-20/80 v/v) + 5-10 mmol.L^{-1} AcOH, H_2O/MeOH (50/50 v/v) + 0.02 mol.L^{-1} H_2SO_4	CHEN *et al.*, 2006; WANG *et al.*, 2010; BERKECZ *et al.*, 2006
β-amino acids	(3,3´-diphenyl-1,1´-binaphtyl)-20-crown-6	H_2O/MeCN (80/20 v/v) + 0.01 mol.L^{-1} H_2SO_4 + 1.0 mmol.L^{-1} $CH_3CO_2NH_4$	CHOI *et al.*, 2008
proteinogenic amino acids	pseudo-18-crown-6 having 1-phenyl-1,2-cyclohexanediol unit	hexane/EtOH/TFAA/H_2O (75/25/0.5/0.2 v/v/v/v)	HIROSE *et al.*, 2005
	Macrocyclic glycopeptide		
N-methyloxycar-bonyl-α-amino acids	ristocetin A, teicoplanin, vancomycin	15 mmol.L^{-1} NH_4OAc (pH 4.1)/MeOH (80/20 v/v), MeOH/MeCN/AcOH/TEA (25/75/0.25/ 0.25 v/v/v/v)	BOESTEN *et al.*, 2006
tryptophan, phenylalanin, leucin	teicoplanin, teicoplanin aglycone, methylated teicoplanin aglycone	1% TFAA/MeOH (60/40 v/v) MeOH/MeCN/AcOH/TEA (55/45/0.3/0.2 v/v/v/v)	XIAO *et al.*, 2006

AcOH – acetic acid, NH_4OAc – ammonium acetate, CD – cyclodextrin, CSP – chiral stationary phase, EtOH – ethanol, MeCN – acetonitrile, MeOH – methanol, TEA – triethylamine, TFAA – trifluoroacetic acid

Cyclofructans (CFs) belong to a relatively small group of macrocyclic oligosaccharides. They consist of six or more β-(2→1) linked D-fructofuranose units. Native cyclofructans have limited capabilities as chiral selectors (SUN *et al.*, 2009). Recently, it was found that aliphatic and aromatic functionalized CFs could be highly selective for chiral molecules containing a primary amine functional group (JIANG *et al.*, 2009).

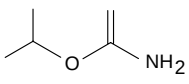
In the present work derivatized cyclofructans were tested as chiral selectors for the direct HPLC enantioseparation of underivatized α-amino acids. Isopropyl carbamate

cyclofructan 6 (IP-CF6), (R)-naphtylethyl carbamate cyclofructan 6 (RN-CF6) and dimethylphenyl carbamate cyclofructan 7 (DMP-CF7) (Fig. 1) chiral stationary phases (CSP) were selected. The influence of the mobile phase composition on the values of chromatographic parameters (retention factor, resolution) was investigated. The enantioseparation process was thermodynamically characterized by the values of enthalpy and entropy contributions calculated from the van´t Hoff equation.

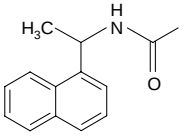


R = derivatization group
n = 6 (cyclofructan 6, CF6), 7 (cyclofructan 7, CF7)

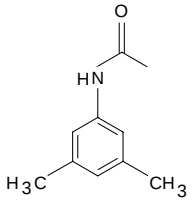
Derivatization groups



isopropyl carbamate (IP)

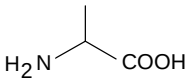


(R)-naphtylethyl carbamate (RN)

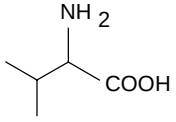


dimethylphenyl carbamate (DMP)

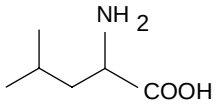
Fig. 1. Scheme of stationary phases based on chemically bonded derivatized cyclofructan and chemical structures of used derivatizing groups.



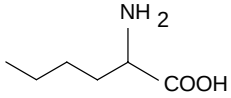
alanine (Ala)



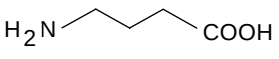
valine (Val)



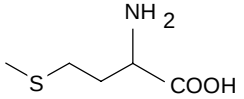
leucine (Leu)



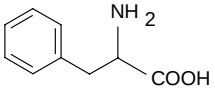
norleucine (Nle)



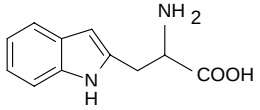
γ-aminobutyric acid (GABA)



methionine (Met)



phenylalanine (Phe)



tryptophan (Trp)

Fig. 2. Chemical structures of separated proteinogenic α-amino acids and γ-aminobutyric acid.

2. Materials and methods

2.1 Chemicals and samples

Racemic mixtures of certain proteinogenic amino acids (alanine, leucine, methionine, norleucine, phenylalanine, tryptophan, valine) and γ-aminobutyric acid (Fig. 2) were purchased from Sigma Aldrich. Acetonitrile, methanol, acetic acid and triethylamine were purchased from Merck. The solutions of racemic mixtures of each amino acid were prepared by dissolving in methanol (concentration 10 $mg.mL^{-1}$). The homogenization of prepared solutions was performed by an ultrasonic bath (Bandelin Sonorex RK 100H).

2.2 HPLC analysis

The HPLC separation of chiral α-amino acids were carried out on a Agilent (series 1100) HPLC system equipped with a binary pump, an injection valve (Rheodyne) with a 20 μL injection loop, column thermostat, spectrophotometric detector and a chromatographic datasystem. Direct separation of α-amino acid enantiomers were performed on chromatographic columns with chiral stationary phases based on chemically – bonded derivatized cyclofructans IP-CF6, RN-CF6 and DMP-CF7 (4×250 mm I.D, 5 μm). The temperature range of 0 – 35 °C was chosen for the thermodynamic study of the HPLC enantioseparation of selected analytes. The mobile phases that were tested consisted of methanol and acetonitrile as organic solvents and acetic acid and triethylamine as ionic modifiers. The different concentration of methanol (50, 65, 75 and 85 vol. %) was used to investigate the influence of the mobile phase composition on the retention and enantioresolution of α-amino acid racemic mixtures. The ratio of ionic modifiers was constant (0.3/0.2 v/v). The flow rate was 0.8 $mL.min^{-1}$. The chromatograms of eluted optical isomers were sxcanned at the wavelength of 210 nm.

3. Results and discussion

3.1 The influence of the chiral stationary phase composition on the retention and enantioseparation of chiral amino acids

Chiral columns based on chemically – bonded cyclofructans with aliphatic (IP-CF6) and aromatic moieties (RN-CF6, DMP-CF7) were tested in order to select the appropriate CSP for HPLC separation of studied chiral amino acids. The mobile phase methanol/acetonitrile/acetic acid/triethylamine (75/25/0.3/0.2 v/v/v/v) was used for all chromatographic separations.

Results given in Table 2 show that the RN-CF6 chiral selector exhibits no enantioselectivity for the amino acid enantiomers used in this study. All chiral amino acids eluted with retention factors from 0.09 to 0.25. The highest retention factor values were obtained for aromatic amino acids (phenylalanine and tryptophan). The

lower retention factor value was obtained for aliphatic amino acids. The enantioseparation of amino acid enantiomers was not achieved.

The DMP-CF7 chiral column was also evaluated. This chromatographic column is based on a chemically – bonded CF7 with an aromatic substituent which allows different types of interactions between separated enantiomers and the chiral selector. Among them, π-π interactions, dipole-dipole interactions and hydrogen bonds generally occur. Using this CSP, the HPLC enantioresolution of amino acid optical isomers was not achieved. Compared to the RN-CF6 chiral column, the retention factor values were higher (Table 2). Based on the relationship between a structure of analytes and their retention behavior the amino acids with more polar functional groups were retained in the DMP-CF7 CSP for a longer period of time.

Table 2. Chromatographic results for separation of studied chiral amino acids by cyclofructan – based chiral stationary phases.

CSP	**RN-CF6**	**DMP- CF7**	**IP- CF6**		
Amino acid	k	k	k_1	k_2	R_S
Leu		-	0.22 ± 0.01	0.25 ± 0.01	0.31 ± 0.01
Val	-	0.28 ± 0.01	0.26 ± 0.01	-	-
Trp	0.23 ± 0.01	0.30 ± 0.01	0.27 ± 0.01	0.32 ± 0.01	0.33 ± 0.01
Phe	0.25 ± 0.00	0.30 ± 0.01	0.29 ± 0.01	0.35 ± 0.01	0.36 ± 0.02
Nle	-	-	0.37 ± 0.02	0.40 ± 0.01	0.40 ± 0.02
Met	0.09 ± 0.00	0.36 ± 0.02	0.46 ± 0.02	0.50 ± 0.02	0.25 ± 0.01
GABA	0.10 ± 0.00	0.37 ± 0.02	0.47 ± 0.02	0.52 ± 0.02	0.44 ± 0.02
Ala	0.19 ± 0.01	0.39 ± 0.02	0.69 ± 0.03	0.76 ± 0.03	0.51 ± 0.02
t_M **[min]**	5.04	4.14	4.88		

The HPLC enantioseparation of proteinogenic amino acids was achieved by the IP-CF6 chiral column. Resolution values given in Table 2 show that enantiomers of some analytes were partially separated (R_S = 0.25 – 0.51). The highest enantioresolution was obtained for amino acids with aliphatic substituents. Amino acids with aromatic moieties were separated with lower resolution values. Based on these results, steric effects can be predicted to have a dominant role during the enantioseparation process under the mentioned conditions (IP-CF6 CSP, polar – organic separation mode).

3.2 The influence of the mobile phase composition on the retention and enantioseparation of chiral amino acids

The influence of mobile phase composition on the retention and enantioresolution of selected amino acids was observed. All chromatographic separations were performed in the polar – organic separation mode (methanol/acetonitrile/acetic acid/triethylamine). The values of methanol concentration in tested mobile phases were 50, 65, 75 and 85 vol. %. Small amounts of ionic modifiers (0.3 vol. % of acetic acid, 0.2 vol. % of triethylamine) were added to prepared mobile phases. The resolution values of chiral amino acids (Table 3) were calculated for tested mobile phases. Results show that the HPLC enantioseparation of studied analytes is markedly

influenced by the mobile phase composition. With an increasing value of methanol volume percent concentration the resolution values were decreasing. Based on these results, the mixture methanol/acetonitrile/acetic acid/triethylamine (75/25/0.3/0.2, v/v/v/v) was found to be the most effective mobile phase for the HPLC separation of all set of chiral α-amino acids. The resolution values obtained for this mobile phase composition were from the interval 0.25-0.51.

Table 3. The influence of methanol concentration in used mobile phases on the resolution values of chiral amino acids.

Amino acid	**methanol/acetonitrile/acetic acid/triethylamine (v/v/v/v)**			
	50/50/0.3/0.2	**65/35/0.3/0.2**	**75/25/0.3/0.2**	**85/15/0.3/0.2**
	R_S			
GABA	0.76 ± 0.03	0.67 ± 0.03	0.44 ± 0.02	0.35 ± 0.01
Ala	0.65 ± 0.03	0.59 ± 0.02	0.51 ± 0.02	0.40 ± 0.01
Leu	0.81 ± 0.03	0.44 ± 0.02	0.31 ± 0.01	0.26 ± 0.01
Met	0.58 ± 0.02	0.34 ± 0.01	0.25 ± 0.01	0.21 ± 0.01
Nle	0.55 ± 0.02	0.46 ± 0.02	0.40 ± 0.01	0.35 ± 0.01
Phe	0.54 ± 0.02	0.42 ± 0.02	0.36 ± 0.01	0.31 ± 0.01
Trp	0.56 ± 0.02	0.42 ± 0.02	0.33 ± 0.01	0.28 ± 0.01
t_M [min]	4.09	4.06	4.27	4.50

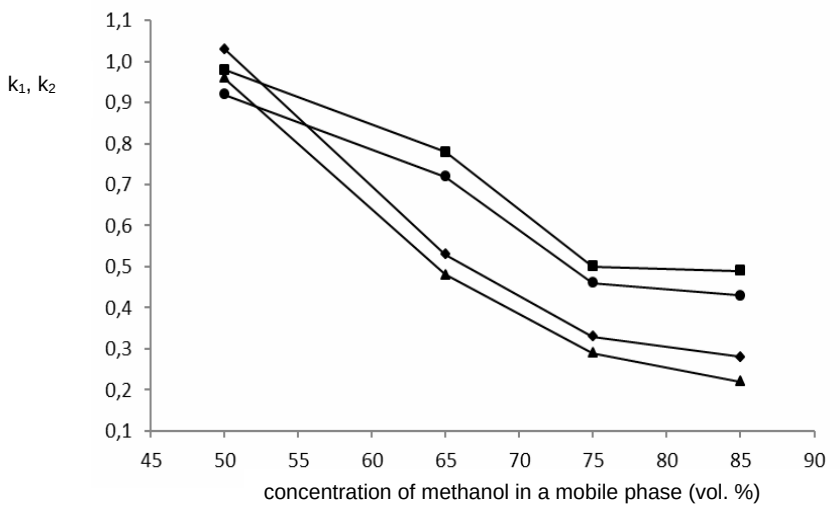


Fig. 3. The dependence of retention factors (k_1, k_2) of methionine (■,●) and phenylalanine (♦,▲) on the concentration of methanol in a mobile phase.

The retention factor values of eluted amino acid enantiomers exhibited an observable dependence on the change of methanol volume percent concentration. This dependence for selected analytes (methionine, phenylalanine) is graphically

represented in Fig. 3. The increase in methanol content in a mobile phase has the same effect on the retention of studied compounds as on the resolution.

3.3 The influence of the column temperature on the retention and enantioseparation of chiral amino acids

The column temperature is one of the factors that can affect elution parameters. Moreover, increasing the temperature usually causes the enantioresolution values to get lower. Thermodynamic study of chiral separation provides useful information about enantioseparation mechanism and the nature of the interactions between optically active analytes and chiral stationary phases. Based on the values of enthalpy and entropy contribution calculated from the van´t Hoff equation, separation of optical isomers can be characterized as an enthalpically or an entropically driven process. Thermodynamics is the effective tool for evaluating both enantioselective separation and chiral selectors (WENG *et al.*, 2004).

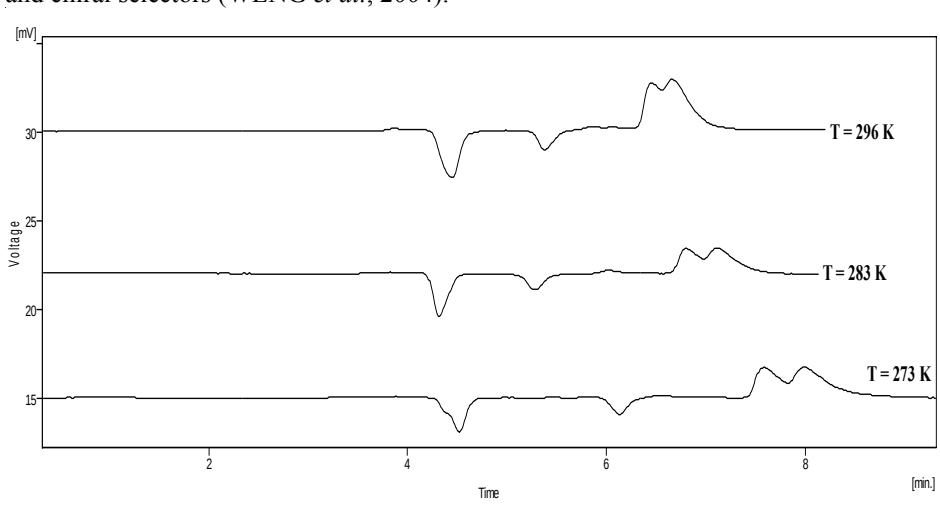


Fig. 4. HPLC chromatograms of methionine racemic mixture in the temperature interval 273 – 296 K. Chromatographic conditions: stationary phase: isopropyl carbamate cyclofructan 6, mobile phase: methanol/acetonitrile/acetic acid/ triethylamine (75/25/0.3/0.2 v/v/v/v), flow rate: 0.8 mL.min^{-1}, detection: spectrophotometric (λ = 210 nm), injected volume: 20 μL.

The effect of the column temperature on the retention and resolution of chiral amino acids was investigated. The HPLC enantioseparation of selected analytes was carried out at eight different temperature values (0 °C, 5 °C, 10 °C, 15 °C, 20 °C, 23 °C, 30 °C and 35 °C). When the column temperature was increasing, the retention factor values of eluted amino acid racemic mixtures were decreasing.

The HPLC chromatograms of methionine enantioseparation in the temperature interval 273 – 296 are shown in Fig. 4. The resolution values (Table 4) were also markedly influenced by the temperature at which enantiomeric separations were realized. The influence of the column temperature on the enantioseparation of selected amino acids exhibited the same trend as the temperature dependence of the retention of

separated amino acid enantiomers. The resolution of chosen chiral amino acids reached its maximum value at 0 ºC. With the increasing column temperature, the HPLC enantioseparation of studied compounds was getting worse (lower values of R_S).

For the thermodynamic characterization of the chromatographic enantioseparation of studied compounds, the van´t Hoff equation was used. This equation represents the dependence of the natural logarithm of the retention factor value (ln k) on reciprocal value of the column temperature ($1/T$). The van´t Hoff analysis is suitable for the determination of the fundamental thermodynamic parameters, i.e. the standard partial molar enthalpy of transfer (ΔH_i) and the standard partial molar entropy of transfer (ΔS_i). The values of these parameters were calculated according to the equation:

$$\ln k_i = -\Delta H_i/RT + \Delta S_i/R + \ln \varphi$$

where k_i is the retention factor, ΔH_i [Jmol^{-1}] is the standard partial molar enthalpy of transfer, ΔS_i [JK^{-1}mol^{-1}] is the standard partial molar entropy of transfer, R is the gas constant (8.314 JK^{-1}mol^{-1}), T [K] is the column temperature and φ is the phase ratio defined as the volume of the stationary phase (V_S) divided by the volume of the mobile phase (V_M). The value of the standard partial molar enthalpy of transfer (ΔH_i) was calculated from a slope of $-\Delta H_i/R$ and the value of the standard partial molar entropy of transfer (ΔS_i) was calculated from an intercept of $\Delta S_i/R$. The determination of these thermodynamic characteristics requires the knowledge of the phase ratio. It is usually difficult to calculate the phase ratio because the value of the stationary phase volume (V_S) is approximate in many cases. Its value is relatively easy to calculate only for liquid – liquid chromatography (CHESTER *et al.*, 2003).

The values of the standard partial molar enthalpy $\Delta(\Delta H_i)$ and entropy change $\Delta(\Delta S_i^*)$ of chosen amino acid racemic mixtures are given in Table 5. The values of the entropic contribution $\Delta(\Delta S_i^*)$ also included the value of the phase ratio. The values of the standard partial molar Gibbs energy (ΔG_i) of both amino acid enantiomers (Table 5) were calculated for two different temperatures (273 K and 308 K) according to the Gibbs – Hemholtz equation:

$$\Delta G_i = \Delta H_i - T\Delta S_i^*$$

The standard partial molar Gibbs energy change $\Delta(\Delta G_i)$ of chosen amino acid racemic mixtures (Table 5) was also determined for temperatures of 273 K and 308 K. The standard partial molar enthalpy of the enantiomer with a higher value of retention time (ΔH_2) reached higher values than the standard partial molar enthalpy of the first eluted enantiomer (ΔH_1). This fact was observed for all separated amino acid racemic mixtures. Enantioselective interactions between a chiral selector and the second eluted enantiomer are more preferred than enantioselective interactions between a chiral selector and the first eluted optical isomer. Higher values of the standard partial molar enthalpy change $\Delta(\Delta H_i)$ were obtained for racemic mixtures of α-amino acids with an aliphatic substitution (leucine, methionine, norleucine). The standard partial molar enthalpy change $\Delta(\Delta H_i)$ exhibited lower values for alanine, γ-aminobutyric acid and phenylalanine.

Table 4. The resolution values of the chosen chiral amino acids in the temperature interval 273 – 308 K.

T [K]	273	278	283	288	293	296	303	308
Amino acid	R_S							
Trp	0.47 ± 0.02	0.44 ± 0.02	0.41 ± 0.01	0.38 ± 0.01	0.35 ± 0.01	0.33 ± 0.01	0.30 ± 0.01	0.28 ± 0.01
Leu	0.66 ± 0.02	0.60 ± 0.02	0.48 ± 0.02	0.44 ± 0.02	0.36 ± 0.01	0.31 ± 0.01	0.29 ± 0.01	0
Phe	0.53 ± 0.02	0.50 ± 0.02	0.47 ± 0.02	0.42 ± 0.01	0.38 ± 0.01	0.35 ± 0.01	0.32 ± 0.01	0.29 ± 0.01
Nle	0.72 ± 0.03	0.62 ± 0.02	0.56 ± 0.02	0.49 ± 0.02	0.43 ± 0.02	0.40 ± 0.01	0.37 ± 0.01	0
Met	0.71 ± 0.03	0.68 ± 0.03	0.62 ± 0.02	0.54 ± 0.02	0.37 ± 0.01	0.25 ± 0.01	0.21 ± 0.01	0.19 ± 0.00
GABA	0.63 ± 0.02	0.61 ± 0.02	0.57 ± 0.02	0.52 ± 0.02	0.48 ± 0.02	0.44 ± 0.02	0.38 ± 0.01	0.35 ± 0.01
Ala	0.70 ± 0.03	0.67 ± 0.03	0.61 ± 0.02	0.55 ± 0.02	0.53 ± 0.02	0.51 ± 0.02	0.49 ± 0.02	0.46 ± 0.02

Table 5. The values of thermodynamic parameters of chosen chiral amino acids calculated from the van't Hoff equation.

Amino acid	$\Delta(\Delta H°)$ [Jmol^{-1}]	$\Delta(\Delta S°*)$ [JK^{-1}mol^{-1}]	$(\Delta G_1°)_{273\,K}$ [Jmol^{-1}]	$(\Delta G_2°)_{273\,K}$ [Jmol^{-1}]	$\Delta(\Delta G°)_{273\,K}$ [Jmol^{-1}]	$(\Delta G_1°)_{308\,K}$ [Jmol^{-1}]	$(\Delta G_2°)_{308\,K}$ [Jmol^{-1}]	$\Delta(\Delta G°)_{308\,K}$ [Jmol^{-1}]
Phe	-286 ± 15	0.35 ± 0.05	1953 ± 179	1528 ± 140	-425 ± 22	3544 ± 324	3151 ± 288	-393 ± 20
Met	-993 ± 91	-2.69 ± 0.32	871 ± 80	613 ± 57	-258 ± 14	2407 ± 220	2243 ± 205	-164 ± 15
Trp	-985 ± 90	-2.06 ± 0.25	2281 ± 209	1858 ± 170	-423 ± 22	3688 ± 337	3337 ± 305	-351 ± 18
GABA	-616 ± 57	-1.37 ± 0.17	838 ± 77	597 ± 56	-241 ± 13	2360 ± 216	2167 ± 198	-193 ± 10
Ala	-290 ± 15	-0.31 ± 0.04	-108 ± 10	-313 ± 16	-205 ± 11	1400 ± 128	1206 ± 110	-194 ± 10
Leu	-2130 ± 195	-6.10 ± 0.80	2069 ± 190	1605 ± 147	-464 ± 24	4663 ± 426	4413 ± 403	-250 ± 13
Nle	-1007 ± 92	-2.63 ± 0.31	1487 ± 136	1198 ± 110	-289 ± 15	2997 ± 274	2800 ± 256	-197 ± 10

The chromatographic enantioseparation is also influenced by steric effects. The diffusion of separated optical isomers into interaction parts of a chiral selector is dependent on the size of analyte molecules. The standard partial molar entropy of enantiomers (ΔS_1^*, ΔS_2^*) is connected with steric interactions and can be also used for the enantioselectivity evaluation. The entropic contribution of the second eluted enantiomer (ΔS_2^*) exhibited higher values than the entropic contribution of the enantiomer with a lower value of retention time (ΔS_1^*). This means that molecules of the second eluted enantiomer were retained in more interaction parts of a chiral selector than molecules of the first eluted enantiomer. Based on the values of the enthalpic and entropic contribution to the change of Gibbs energy, the HPLC enantioseparation of chosen amino acids under selected chromatographic conditions (IP-CF6 column, polar – organic separation mode) was found to be an enthapically driven process. It means that the second eluted enantiomer and a chiral selector form a diastereomeric complex with a higher stability constant value than the enantiomer with a shorter elution time. The van't Hoff plots of chosen chiral amino acids are graphically represented in Fig. 5. The coefficient of determination values (R^2~0.99) indicate the linear dependence of retention of studied compounds on the column temperature. The retention mechanism did not change with the temperature change.

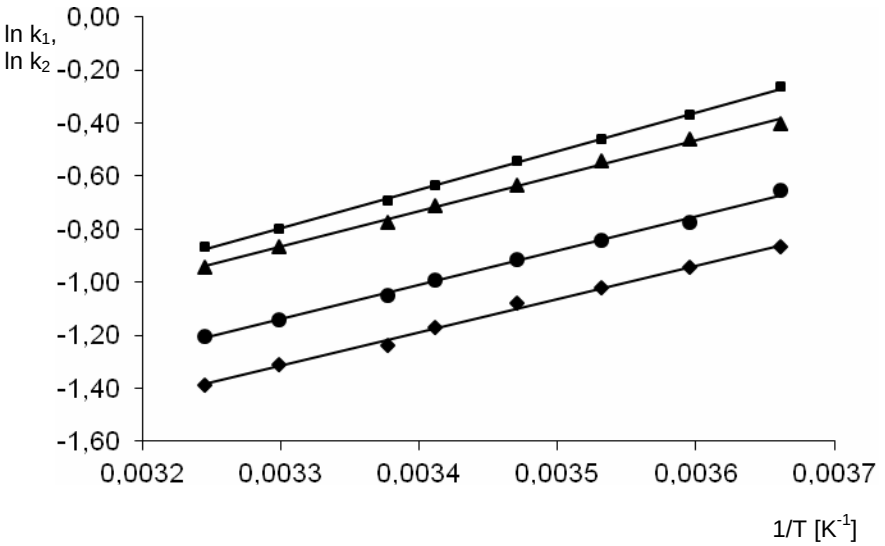


Fig. 5. The van't Hoff plots of chosen chiral amino acids. (■,▲) phenylalanine, (●,♦) methionine

4. Conclusions

Different types of cyclofructan – based chiral selectors were tested for HPLC separation of chiral amino acids. Among them, isopropyl carbamate cyclofructan 6 showed the ability to separate optical isomers in the polar – organic separation mode

(mobile phase: methanol/acetonitrile/acetic acid/triethylamine, 75/25/0.3/0.2 v/v/v/v). Based on the results of thermodynamic study in the temperature interval 0 ºC – 35 ºC, the HPLC enantioseparation of studied racemic mixtures can be characterized as an enthapically driven process.

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