SEPARATION OF METHIONINE ENANTIOMERS BY USING TEICOPLANIN AND CYCLOFRUCTAN COLUMNS

KATARÍNA HROBOŇOVÁ[1], ZUZANA DEÁKOVÁ[1,2], JAKUB MORAVČÍK[1], JOZEF LEHOTAY[1,3], DANIEL W. ARMSTRONG[4], ANNA LOMENOVÁ[1]

[1]*Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia (katarina.hrobonova@stuba.sk)*
[2]*Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Sasinkova 2, 811 08 Bratislava, Slovakia*
[3]*Department of Chemistry, Faculty of Natural Sciences, University of SS. Cyril and Methodius in Trnava, Nam. J. Herdu 2, Trnava, SK-917 01, Slovakia*
[4]*Department of Chemistry, University of Texas at Arlington, 700 Planetarium Place, TX, 76019 Arlington, USA*

Abstract: Methionine is a naturally occurring amino acid. Its enantiomeric separation by using high performance liquid chromatography on various types of chiral stationary phases was studied. The effect of mobile phase composition on enantioselectivity and retention was considered. The separation of the enantiomers was attained in different separation modes – reversed phase mode for the macrocyclic antibiotic chiral stationary phases (teicoplanin, teicoplanin aglycone), normal phase and polar organic phase modes for the isopropyl carbamate cyclofructan 6 chiral stationary phase. It was shown that the hydrogen bonding, dipole interactions, steric effects between methionine molecules and stationary phases play an important role in the separation of enantiomers.

Key words: methionine, enantiomers, cyclofructan 6-based column, teicoplanin-based column, mobile phase composition, HPLC

1. Introduction

Methionine (Fig. 1) is a sulfur-containing essential amino acid that participates in protein synthesis. It plays an important role as a precursor for the synthesis of other sulphur containing amino acids (cysteine and taurine) (HARDY, 1985). Some studies have indicated that D-methionine is poorly utilized and excreted in the urine (STEGING *et al.*, 1986).

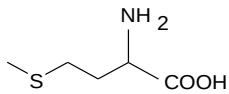

Fig. 1. The chemical structure of methionine.

Methionine is continuously converted to homocysteine and adenosine. Significant amounts of methionine are subsequently regenerated via the remethylation of

DOI 10.1515/nbec-2015-0009

homocysteine. This enzymatic reaction is catalyzed by methionine synthase (VISSER *et al*., 2011; KUO *et al*., 2005; MINER *et al*., 1997).

Many studies are focused on enantiomeric separations of amino acids by HPLC. Direct analysis of amino acids or their derivatives is by chiral ligand-exchange chromatography (ILISZ *et al*., 2006) or by a chiral stationary phase (CSP) based on cyclodextrins (WANG *et al*., 2010; REMSBURG *et al*., 2008), crown-ether (CHEN *et al*., 2006; LEE *et al*., 2010), macrocyclic glycopeptides (POPLEWSKA *et al*., 2007; GUILLEN-CASLA *et al*., 2010), polysaccharide derivatives (LEE *et al*., 2008), and proteins (WINKLER *et al*., 2009).

Macrocyclic glycopeptides are chiral selectors mainly used in separation techniques such as HPLC (XIAO *et al*., 2004; ILISZ *et al*., 2006) and capillary electrophoresis (ARMSTRONG *et al*., 1997). The most frequently utilized glycopeptide chiral selector for amino acids enantioseparation are teicoplanin and teicoplanin aglycone (POPLEWSKA *et al*., 2008; ILISZ *et al*., 2009). The variety of stereoselective interactions of the teicoplanin (also other macrocyclic glycopeptides) chiral selector is related to the presence of many stereogenic centers and the heterogeneity of its functional groups. The structure of these chiral selectors (Fig. 2) indicates that interactions including hydrogen bonding, π-π complexation, dipole stacking, steric interactions, hydrophobic interactions, electrostatic interactions, and inclusion are present. Macrocyclic antibiotic CSPs can be operated in all common separation modes, reversed phase (aqueous polar organic mobile phase; RP), normal phase (little polar alkane alcohol mobile phase; NP), and polar organic phase (non-aqueous organic mobile phase; PO) (ARMSTRONG *et al.*, 1995).

Cyclofructans (CFs) are macrocyclic oligosaccharides consisting of six or more β-(2→1) linked D-fructofuranose units (from six to eight; CF6, CF7, CF8). Each fructofuranose unit contains four stereogenic centers and three hydroxyl groups that can be derivatized in order to improve the enantioseparation performance of CF CSPs. Cyclofructan based CSPs are the newest approach used for chiral HPLC (SUN *et al*., 2009). The development of derivatized cyclofructan CSPs is based on aliphatic or aromatic functionalization of a native chiral selector. It was reported, that aliphatic-derivatized CF6 are suitable for separation of racemic primary amines. These CSPs can be operated in all separation modes (NP, RP, PO) (SUN *et al*., 2010; JANEČKOVÁ *et al*., 2011).

This work is focused on comparison of HPLC separation and interaction abilities of two different types of CSPs and three separation modes for the resolution of methionine enantiomers. These CSPs included derivatized cyclofructan 6 and macrocyclic antibiotic chiral selectors. The influence of the mobile phase composition on various chromatographic parameters (retention factor, resolution) was investigated.

2. Material and methods

2.1 Chemicals

L-Methionine and DL-Methionine were purchased from Sigma-Aldrich. Organic solvents of HPLC gradient grade, acetonitrile, methanol, ethanol, and n-hexane were a

product of Merck. Glacial acetic acid (100 % purity), trifluoroacetic acid, and triethylamine of analytical grade purity were purchased from Merck.

A stock solutions of racemic mixture and L-enantiomeric form were prepared in water (doubly deionized water, < 18 MΩ cm^{-1}) at concentration 1.0 mg mL^{-1}. Working solutions were obtained by appropriate dilutions and were prepared weekly. The solutions were filtered through a 0.45 μm nylon membrane filter before the HPLC analysis.

2.2 Instrumentation and chromatographic conditions

I) The HPLC system consisted of an isocratic pump (DeltaChrom SDS 030, Watrex), an injection valve (Rheodyne), and electrochemical detector (Coulochem II, ESA). Detector was composed of guard cell Model 5020 and analytical cell Model 5010A (ESA). Analytical cells had potential set to + 0.65 V (E1), + 0.9 V (E2) and + 1.4 V (E0) according to GARAIOVA *et al*. (2013). Enantiomeric separations were performed on teicoplanin (Chirobiotic T) or teicoplanin aglycone (Chirobiotic TAG) columns (4×250 mm I.D, 5 μm) (ASTEC). The mobile phases for consisted of methanol or acetonitrile and water in different volume ratios. The flow rate was 0.6 mL min^{-1}. A nylon filter (0.45 μm) was used for the mobile phases filtering. The columns were thermostated with a JET STREAM II Plus HPLC column thermostat (WO Industrial Electronics) at 25 °C. System peak obtained by injection of methanol or acetonitrile to the studied separation system served for determination of the dead time. The elution order of enantiomers was L-form, D-form in all cases.

II) The Agilent Technologies HPLC (series 1200) consisted from a binary pump, an injection valve (Rheodyne), a column thermostat, a diode array and polarimetric detector (Chiralizer, IBZ Messtechnik) were used for the assay. The chiral column IP-CF6 (4×250 mm I.D, 5 μm) (ASTEC) was used for separation (the chiral selector was isopropyl carbamate cyclofructan 6). The temperature was maintained at 23 or 0 °C. The mobile phases for polar organic mode consisted of methanol, acetonitrile, acetic acid, and triethylamine. In all experiments the acid and basic additive percentages in the mobile phase were fixed at 0.3/0.2 (v/v). The mobile phases for normal phase mode were composed of n-hexane and ethanol in different volume ratios, small addition of trifluoroacetic acid (0.1 %, v) was used. A nylon filter (0.45 μm) was used for the mobile phases filtering. The flow rate was 0.8 mL min^{-1} and the injection volume was 20 μL. UV detection of the analytes was performed at 210 nm. System peak obtained by injection of methanol (PO mode) or ethanol (NP mode) to the studied separation system served for determination of the dead time. The elution order of enantiomers was L-form, D-form in all cases.

The all measurements were done in three replicates. The retention factor (k) values of first and second eluted enantiomer were calculated as the ratio of differences between retention time of enantiomer (t_R) and dead time (t_M) to dead time. The resolution values (R_S) of L- and D- enantiomers were calculated by ratio of difference between retention times of enantiomers (t_{RD}, t_{RL2}) to sum of peak widths at half peak height (w_{05D}, w_{05L}) as follow: $R_S = 1.18 * (t_{RD}-t_{RL}) / (w_{05D} + w_{05L})$.

3. Results and discussion

The purpose of this study was to select suitable chromatographic conditions to directly separate methionine enantiomers (Fig. 1). Two types of chiral stationary phases were tested with the aim to obtain complementary HPLC conditions from the point of view of the separation mode used. The interaction abilities of tested CSPs were studied.

3.1 HPLC enantioseparation in reversed phase mode

Teicoplanin based CSPs (Fig. 2) were used for enantioseparations in the reversed phase separation mode. Enantiomeric recognition of the methionine was studied in a mobile phase composed of water and organic solvent (methanol or acetonitrile). Different organic modifier/water ratios were tested as shown in Table 1 and Fig. 3.

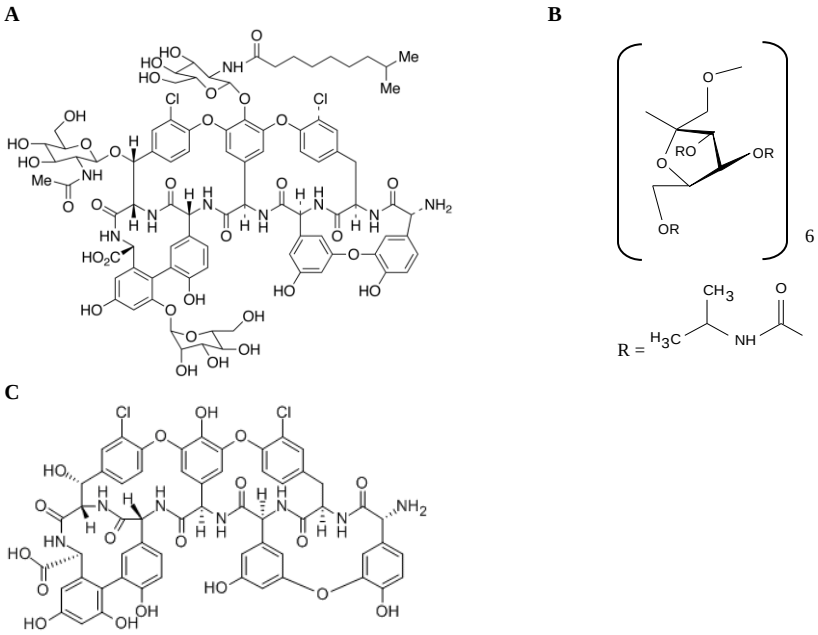

Fig. 2. Structure of tested chiral selectors. Teicoplanin (A), Isopropyl carbamate cyclofructan 6, IP-CF6 (B), Teicoplanin aglycone (C) (ARMSTRONG *et al.*, 1995; BERTHOD *et al.*, 2000; SUN *et al.*, 2009).

The retention factor vs. methanol/water or acetonitrile/water ratio showed a U-shaped retention curve, which indicates that different interactions take place at different solvent compositions. Increased retention and selectivities were obtained when the mobile phase contain more than 40 % methanol or more than 50 % acetonitrile. Higher retention (higher values of retention factors) is probably the result of higher solubility of the selected amino acid in water in comparison to organic

solvent. Table 1 data also indicates that the retention is higher in methanol containing mobile phase then in acetonitrile containing mobile phase. The same trend was observed for resolution values of enantiomeric forms (Fig. 3). Higher R_S values were obtained in the mobile phases containing methanol as an organic modifier. It can be supposed that the formation of hydrogen bonds supported in methanol may increase the enantioselectivity. The suitable mobile phase composition for separation of methionine enantiomers on teicoplanin chiral stationary phase was methanol/water (70/30 v/v).

The separation of methionine enantiomers was also studied on a column containing the teicoplanin aglycone (Fig. 2) as a chiral selector. This CSP is similar to teicoplanin based CSP, but without saccharide moieties (BERTHOD *et al*., 2000). The separations were accomplished with methanol/water or acetonitrile/water mobile phases. Among these two organic modifiers better enantioselectivity and resolution was observed using methanol as the modifier. Figure 3 and Table 1 show that the concentration of methanol has a significant effect on enantiomeric separation of methionine enantiomers.

Table 1. Effect of methanol and acetonitrile percentage on the values of retention factors (k) of L- and D-methionine in the reversed phase mode[a] by the teicoplanin and teicoplanin aglycone CSPs.

Teicoplanin CSP							
			Methanol (%)				
	5	**10**	**20**	**40**	**50**	**70**	**85**
k_1	2.4	2.3	2.1	2.1	2.2	2.6	3.5
k_2	2.9	2.9	2.8	3.2	3.5	4.5	7.1
			Acetonitrile (%)				
	5	**10**	**20**	**40**	**50**	**70**	**80**
k_1	2.1	1.7	1.6	1.4	1.7	3.1	6.5
k_2	2.4	1.9	1.8	1.6	2.0	3.9	8.6
Teicoplanin aglycone CSP							
			Methanol (%)				
	5	**10**	**20**	**40**	**50**	**70**	**80**
k_1	1.7	1.8	1.8	1.9	2.0	2.3	2.5
k_2	2.5	2.7	3.1	3.9	4.5	6.2	7.3

[a] mobile phase: methanol/water or acetonitrile/water (v/v); column temperature: 25 °C; flow rate: 0.6 mL min^{-1}; RSD ≤ 5 %

The comparison of chromatographic results obtained on the teicoplanin and teicoplanin aglycone CSPs may show the effect of saccharide moieties in enantiorecognition. The α values of methionine are 1.2-1.6 times higher on the aglycone CSP than on the native teicoplanin CSP. The higher resolutions (R_S > 3.9) were observed for aglycone CSP in comparison with teicoplanin CSP (R_S > 0.7) obtained in tested methanol percentage interval. These differences suggest that the saccharide units of the native teicoplanin molecule have a negative influence on chiral

recognition process of methionine enantiomers. This also indicates that the aglycone part of teicoplanin molecule is dominant in enantiorecognition. It is probably the effect of steric hindrance, where the sugar parts occupy the inside of "basket" and limits the accessibility of other molecules to binding sites, i.e., blocking the possible interaction on the aglycone surface (BERTHOD *et al.*, 2000) The retention factor vs. methanol/water ratio dependencies (Table 1 and Fig. 3) showed that the overall polarities of the stationary phases used in this study are similar.

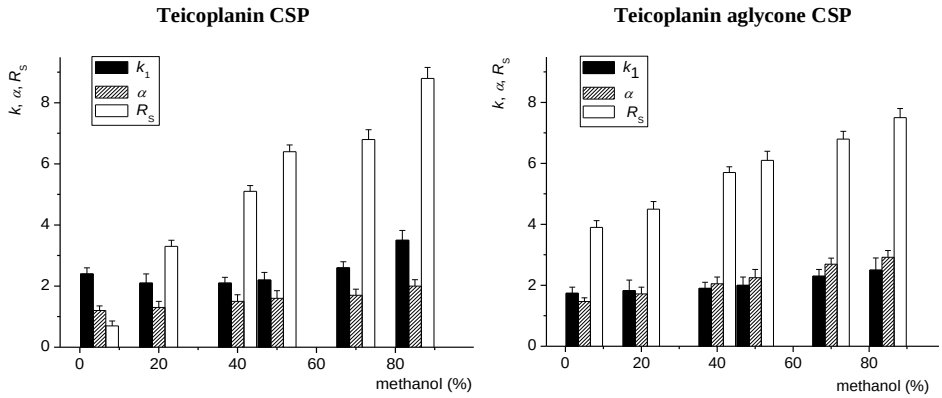

Fig. 3. Effect of methanol concentration on enantioseparation of methionine in reversed phase mode[a] by the teicoplanin and teicoplanin aglycone CSPs. [a] mobile phase: methanol/water (v/v); column temperature: 25 °C; flow rate: 0.6 mL min^{-1}.

The tested teicoplanin and teicoplanin aglycone CSPs are extremely selective for the enantiomers of methionine. Figures 4A and 4B show chromatograms of methionine on the two CSPs with the methanol/water 70/40 (v/v) mobile phase. The L-enantiomeric forms elute first and D-form elute second in all tested mobile phases.

3.2 HPLC enantioseparation in polar organic mode

In 2009, Sun et al. demonstrated that the polar organic separation mode was preferred for separation of enantiomers of primary amines on novel class of cyclofructan based CSPs. The derivatization on the chiral selector proved to be essential for enantioselectivity. Several different binding sites have been demonstrated on isopropyl carbamate cyclofructan 6 (IP-CF6) (Fig. 2) including hydroxyl and carbamate groups which may have interactions with analyte via hydrogen bonding or dipolar interactions. The possible existing interactions also include steric effects in the chiral groove. (SUN *et al.*, 2009) The combination of interactions may have a different effect on the stabilization of the complex CSP-enantiomer.

IP-CF6 CSP and mixture of methanol/acetonitrile/acetic acid/triethylamine as mobile phase were used for HPLC separation of methionine enantiomers. The influence of mobile phase composition on the retention and enantioresolution on IP-

CF6 CSP was first studied to obtain an effective enantioseparation ($R_S \geq 1.0$). The concentration of methanol in mobile phases increased from 10 % to 85 % and acidic and basic modifier concentrations were kept the same (0.3 % of acetic acid, 0.2 % of triethylamine). The results shown in Figure 5 documented that increasing the concentration of methanol decreases the resolution values from 2.6 to 0.3. The retention factor values plots of the first and the second eluted enantiomers exhibited an

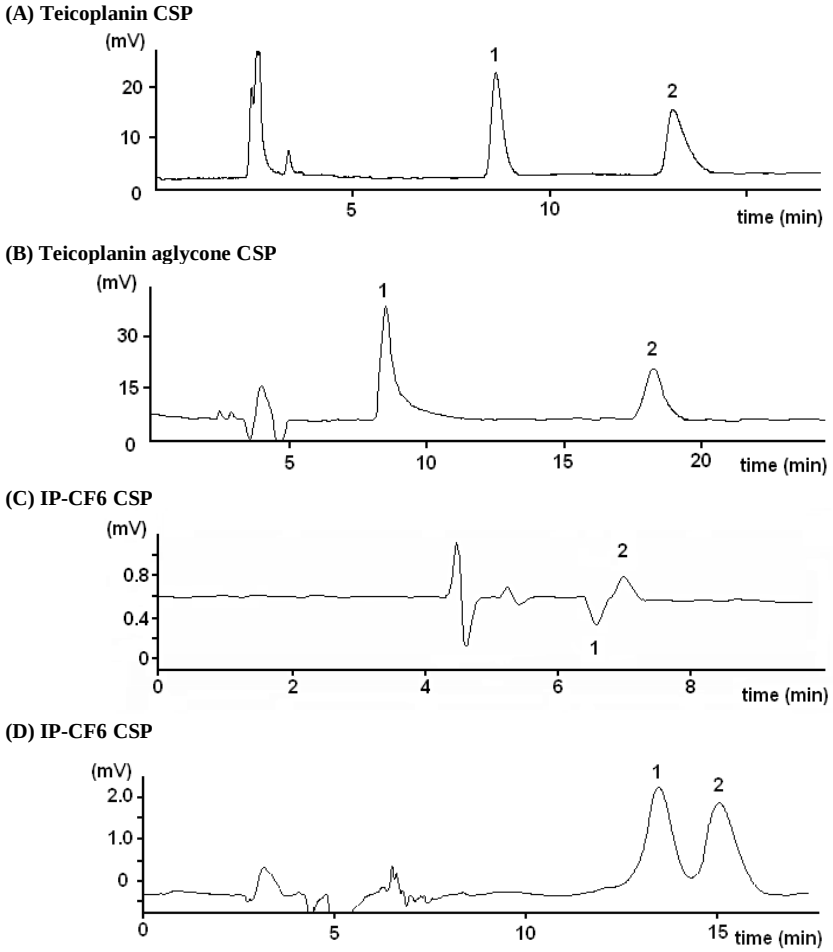

Fig. 4. Chromatograms of L- (1) and D- (2) methionine in RP separation mode by the teicoplanin (A) and teicopanin aglycone (B) CSPs, PO separation mode (C) and NP separation mode (D) by the IP-CF6 CSP. Conditions: (A) mobile phase: methanol/water (70/30 v/v), temperature: 25 °C, flow rate: 0.6 mL min^{-1}, electrochemical detection; (B) mobile phase: methanol/water (70/30 v/v), temperature: 25 °C, flow rate: 0.6 mL min^{-1}, electrochemical detection; (C) mobile phase: methanol/acetonitrile/acetic acid/triethylamine (75/25/0.3/0.2 v/v/v), temperature: 23 °C, flow rate: 0.8 mL min^{-1}, polarimetric detection; (D) mobile phase: ethanol/n-hexane/trifluoroacetic acid (50/50/01 v/v/v), temperature: 0 °C, flow rate: 0.8 mL min^{-1}, spectrofotometric detection at 210 nm.

observable dependence with varying methanol concentration. The differences in the retention factors with change methanol concentration could be explained by competition of methanol and methionine for hydrogen bonding sites on IP-CF6 CSP. The suitable mobile phase for separation of methionine enantiomers was mixture methanol/acetonitrile/acetic acid/triethylamine (75/25/0.3/0.2 v/v/v/v). The highest value of resolution was observed with 20 % methanol, resulting from longer retention. Figure 4C shows the chromatogram of methionine separation on IP-CF6 column in polar organic separation mode. The L-enantiomeric form elutes first and D-form elutes second. The previous results demonstrated that decreasing the column temperature can increase selectivity and resolution (MORAVČÍK *et al*., 2013).

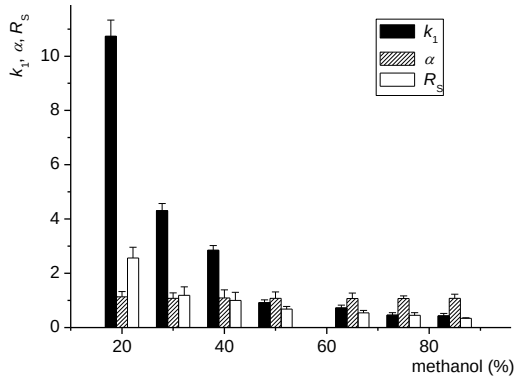

Fig. 5. Effect of methanol concentration on enantioseparation of methionine in polar organic mode[a] by the IP-CF6 CSP. [a]mobile phase: methanol/acetonitrile/0.3 % acetic acid/0.2 % triethylamine, column temperature: 23 °C, flow rate: 0.8 mL min^{-1}.

3.3 HPLC enantioseparation in the normal phase mode

Since the short retention of methionine enantiomers on IP-CF6 CSP in the polar organic separation mode was observed (retention times from 6 to 7 min), the normal phase separation mode was tested. The mobile phases consist of n-hexane, ethanol, and trifluoroacetic acid. The amount of acidic modifier was constant, 0.1 % (v) in all measurements. The different n-hexane/ethanol ratios (50-95 % of ethanol) were tested as it is shown in Figure 6. The increased retention and selectivity were obtained when the mobile phase contained less than 65 % of ethanol. Also the increasing of ethanol concentration caused decreasing of resolution values from 1.1 to 0.5. The retention factor and the resolution values vs. n-hexane/ethanol ratio plot indicate that in the interval 95-65 % of ethanol is favorable influence the hydrogen donation groups from mobile phase and therefore probably the hydrogen bonding interactions are contribute to the enantiomeric separation of methionine complexes with IP-CF6 CSP. In mobile phases containing lower than 65 % of ethanol decreases the portion of available hydrogen donation groups in the mobile phase, probably are preferred interactions in the stationary phase, which results increase in retention and also in enantioresolution. There is no significant influence of different mobile phase composition on the

selectivity factors (α = 1.42 – 1.48). Figure 4D shows the chromatogram of methionine on the IP-CF6 CSP with the n-hexane/ethanol/trifluoroacetic acid 50/50/0.1 (v/v/v) mobile phase. The L-enantiomeric form elutes first and D-form elutes second in all tested mobile phases.

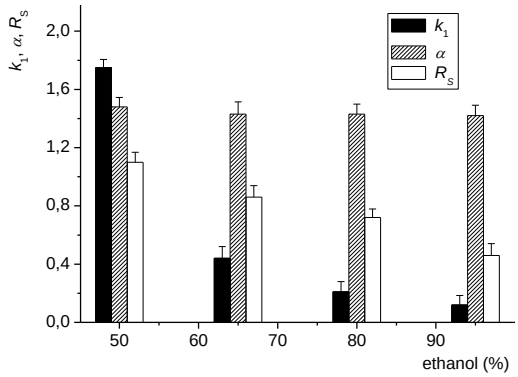

Fig. 6. Effect of ethanol concentration on enantioseparation of methionine in normal phase mode[a] by the IP-CF6 CSP. [a] mobile phase: n-hexane/ethanol/trifluoroacetic acid v/v/0.1, column temperature: 0 °C, flow rate: 0.8 mL min^{-1}.

The tested IP-CF6 CSP in NP separation mode is so more selective resolving enantiomers of methionine in comparison to PO mode.

4. Conclusions

The HPLC separation conditions (stationary phase, mobile phase composition) were proposed for separation of methionine enantiomers. The separation of enantiomers was achieved on macrocyclic antibiotic (teicoplanin, teicoplanin aglycone) CSPs in reversed phase separation mode and on newly developed cyclofructan-based (isopropyl carbamate cyclofructan 6) CSP in normal phase and polar organic separation modes. It appears that hydrogen bonding, steric and dipole interactions are of great importance for these separations. The proposed separation systems are complementary and may be used depending on the type of sample matrix.

Acknowledgements: This study was financially supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and of the Slovak Academy of Sciences (grant VEGA no. 1/0499/14).

References

ARMSTRONG, D.W., NAIR, U.B.: Capillary electrophoretic enantioseparations using macrocyclic antibiotics as chiral selectors. Electrophoresis, 18, 1997, 2331-2342.

ARMSTRONG, D.W., YOUBANG, L., EKBORGOTT, K.: A covalently bonded teicoplanin chiral stationary phase for HPLC enantioseparations. Chirality, 7, 1995, 474-497.

BERTHOD, A., CHEN, Z., KULLMAN, J.P., ARMSTRONG, D.W., GASPARRINI, F., D`ACQUARICA, I., VILLANI, C., CAROTTI, A.: Role of the carbohydrate moieties in chiral recognition on teicoplanin-based LC stationary phases. Anal. Chem., 72, 2000, 1767-1780.

CHEN, J.J., ZHANG, D.T., SHEN, B.C., ZHANG, X.J., XU, B.J., XU, X.Z.: Enantioseparation of D,L-α-amine acid on crown ether chiral stationary phases. Chin. J. Anal. Chem., 34, 2006, 1535-1540.

GARAIOVA, I., MUCHOVÁ, J., NAGYOVÁ, Z., MIŠĽANOVÁ, C., ORAVEC, S., DUKÁT, A., WANG, D., PLUMMER, S.F., ĎURAČKOVÁ, Z.: Effect of a plant sterol, fish oil and B vitamin combination on cardiovascular risk factors in hypercholesterolemic children and adolescents: a pilot study. Nutr. J., 12, 2013, 1-8.

GUILLEN-CASLA, V., LEON-GONZALEZ, M.E., PEREZ-ARRIBAS, L.V., POLO-DIEZ, L.M.: Direct chiral determination of free amino acid enantiomers by two-dimensional liquid chromatography: application to control transformations in E-beam irradiated foodstuffs. Anal. Bioanal. Chem., 397, 2010, 63-75.

HARDY, P.M.: In Chemistry and Biochemistry of Amino acids; G.C. BASRRETT, Ed., Chapmen and Hall: New York, 1985, p. 6.

ILISZ, I., BERKECZ, R., PETER, A.: HPLC separation of amino acid enantiomers and small peptides on macrocyclic antibiotic-based chiral stationary phases: A review. J. Sep. Sci., 29, 2006, 1305-1321.

ILISZ, I., BERKECZ, R., PETER, A.: Retention mechanism of high-performance liquid chromatographic enantioseparation on macrocyclic glycopeptide-based chiral stationary phases. J. Chromatogr. A, 1216, 2009, 1845-1860.

ILISZ, I., TOURWE, D., ARMSTRONG, D.W., PÉTER, A.: High-performance liquid chromatographic enantioseparation of unusual secondary amino acids on a D-penicillamine-based chiral ligand-exchange column. Chirality, 18, 2006, 539-543.

JANEČKOVÁ, L., KALÍKOVÁ, K., VOZKA, J., ARMSTRONG, D.W., BOSÁKOVÁ, Z., TESAŘOVÁ, E.: Characterization of cyclofructan-based chiral stationary phases by linear free energy relationship. J. Sep. Sci., 34, 2011, 2639-2644.

KUO, H.K., SOROND, F.A., JEN-HAU CHEN, J.H., HASHMI, A., MILBERG, W. P., LIPSITZ, L.A.: The role of homocysteine in multisystem age-related problems: A systematic review. J. Gerontology, 60, 2005, 1190-1201.

LEE, T., LEE, W., HYUN, M.H., PARK, J.H.: Enantioseparation of β-amino acids on an 18-crown-6-tetracarboxylic acid-bonded silica by capillary electro-chromatography. J. Chromatogr. A, 1217, 2010, 1425-1428.

LEE, K.A., YEO, S., KIM, K.H., LEE, W., KANG, J.S.: Enantioseparation of N-fluorenylmethoxycarbonyl α-amino acids on polysaccharide-derived chiral stationary phases by reverse mode liquid chromatography. J. Pharm. Biomed. Anal., 46, 2008, 914-919.

MINER, S.E.S., EVROVSKI, J., COLE, D.E.C.: Clinical chemistry and molecular biology of homocysteine metabolism: An update. Clin. Biochem., 30, 1997, 189-201.

MORAVČÍK, J., HROBOŇOVÁ, K.: High-performance liquid chromatographic method for enantioseparation of underivatized α-amino acids using cyclofructan-based chiral stationary phases. Nova Biotechnol. Chim., 12, 2013, 108-119.

POPLEWSKA, I., KRAMARZ, R., PIATKOWSKI, W., SEIDEL-MORGENSTERN, A., ANTOS, D.: Behavior of adsorbed and fluid phases versus retention properties of amino acids on the teicoplanin chiral selector. J. Chromatogr. A, 1192, 2008, 130-138.

POPLEWSKA, K.R., PITKOWSKI, W., SEIDEL-MORGENSTERN, A., ANTOS, D.: Influence of preferential adsorption of mobile phase on retention behavior of amino acids on teicoplanin chiral selector. J. Chromatogr. A, 1173, 2007, 58-70.

REMSBURG, J.W., ARMSTRONG, D.W., PÉTER, A., TÓTH, G.: LC enantiomeric separation of unusual amino acids using cyclodextrin-based stationary phases. J. Liq. Chromatogr. Rel. Technol., 31, 2008, 219-230.

STEGINK, L.D., BELL, E.F., FILER, L.J., ZIEGLER, E.E., ANDERSEN, D.W., SELIGSONÄŽ, F.H.: Effects of equimolar doses of L-methionine, D-methionine and L-methionine-dl-sulfoxide on plasma and urinary amino acid levels in normal adult humans. J. Nutr., 116, 1986, 1185-1192.

SUN, P., ARMSTRONG, D.W.: Effective enantiomeric separations of racemic primary amines by the isopropyl carbamate-cyclofructan6 chiral stationary phase. J. Chromatogr. A, 1217, 2010, 4904-4918.

SUN, P., WANG, C., BREITBACH, Z.S., ZHANG, Y., ARMSTRONG, D.W.: Development of new HPLC chiral stationary phases based on native and derivatized cyclofructans. Anal. Chem. 81, 2009, 10215-10226.

VISSER, W.F., VERHOEVEN-DUIF, N.M., OPHOFF, R., BAKKER, S., KLOMP, L. W., BERGER, R. DE KONING, T.J.: A sensitive and simple ultra-high-performance-liquid chromatography–tandem mass spectrometry based method for the quantification of D-amino acids in body fluids. J. Chromatogr. A, 1218, 2011, 7130-7136.

WANG, Y., YOUNG, D.J., TAN, T.T.Y., NG, S.C.: Click preparation of hindered cyclodextrin chiral stationary phases and their efficient resolution in high performance liquid chromatography. J. Chromatogr. A, 1217, 2010, 7878-7883.

WINKLER, M., KLEMPIER, N.: Enantioseparation of nonproteinogenic amino acids. Anal. Biomed. Chem., 393, 2009, 1789-1796.

XIAO, T.L., ARMSTRONG, D.W.: Enantiomeric separations by HPLC using macrocyclic glycopeptide-based chiral stationary phases. Chiral Separations Methods in Molecular Biology, 243, 2004, 113-171.