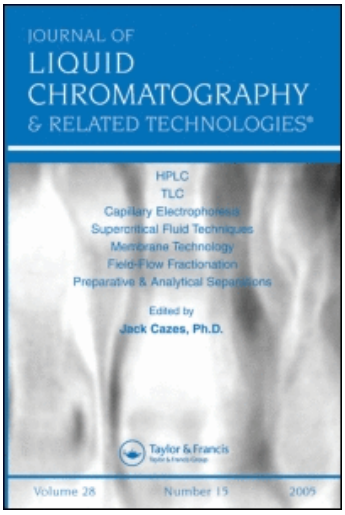

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Using Teicoplanin Based Chiral Stationary Phase to Explore Temperature Effects on Enantioseparation and Determination of Chiral Sulfoxides in Rat Serum

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Abstract: The methylated-teicoplanin aglycone chiral stationary phase (CSP) was used for the study of the enantioseparation and temperature behaviour of a set of chiral sulfoxides (nine different aryl methyl sulfoxides, 1-(methylsulfinyl) naphthalene and [(1,1-dimethyl-2-phenylethyl)sulfinyl]benzene) in polar organic mode. The effect of temperature on the HPLC separation of aromatic sulfoxides was studied between 10 and 50°C in methanol mobile phases with different concentration of organic modifier in mobile phase composition. The van't Hoff plots were constructed and thermodynamic data were determined from the slope and the intercept of linear van't Hoff plots for all 12 racemic compounds under the study. The van't Hoff plots ($ln\ k$ versus $1/T$ and $ln\ \alpha$ versus $1/T$) were linear for all enantiomers. The elution order (S (+) enantiomeric form eluted first) did not reverse in the temperature range of this study. Enthalpy-entropy compensation plots of ΔH_ivs.ΔS_i showed no significant linear correlation in methanol mobile phases used in this study. According to the enthalpy-entropy compensation plots of enantioselectivity, there is some significant linear correlation if the set of studied compounds is divided into two major groups. In order to observe the influence of temperature on retention and separation in biological matrix (rat serum), the solid phase extraction step was performed. The limit of determination (LOQ) was calculated for all studied compounds in the methanol

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mobile phase containing acetic acid (17.48 mmol/L) and diethylamine (4,79 mmol/L). The LOQ values were in the range of 7.0–24.2 ng/mL for racemic mixtures taking into consideration the recovery (82–96%) of the extraction procedure.

Keywords: Chiral separation, Enthalpy-entropy compensation, HPLC, Methylated-teicoplanin CSP, SPE, Sulfoxides

INTRODUCTION

Thus far, macrocyclic antibiotics, especially teicoplanin glycopeptides, have become suitable chiral selectors in many cases.[1–4] Their excellent chiral recognition capabilities is attributed to their ability of forming simultaneous stereospecific polar and ionic interactions with multiple chiral centres and binding sites, located in the cavities of the glycopeptide's basket like structure.[3,5] In general, the glycopeptide chiral selectors possess many functional groups (for example, hydroxyl, amine, amide linkages, carboxylic acid, aromatic moieties, and hydrophobic pockets) that offer different molecular interactions, including hydrophobic, ionic, hydrogen bonding, dipole–dipole, π-π and steric interactions.[6] Moreover they can operate in reversed phase, normal phase, and polar organic mode conditions.[7] Considerable work has been done in order to modify teicoplanin based chiral stationary phases, or in preparing the new ones with similar properties but potential higher resolving power. Despite the fact that the aglycone peptide "basket" is in general regarded as hydrophobic, the separation efficiency could be improved by methylation of teicoplanin aglycone by blocking the hydrogen bonding groups. Therefore, in the case of the methylated-teicoplanin aglycon (MTAG), the strong hydrogen bonding interactions can be additionally reduced.[6,8]

Chiral sulfoxides have become widely used as intermediates in synthetic reactions,[9,10] as important bioactive compounds[11,12] in asymmetric synthesis[13,14] and valuable reagents for drug synthesis.[15] The importance of efficient separations including chiral sulfoxides could not be underestimated, moreover in the case of determination of such compounds in biological matrix. Thermodynamic studies and the evaluation of temperature effects during the separation of chiral sulfoxides, can serve as a suitable approach to acquire some insight into the enantioseparation process.

Enthalpy-entropy compensation (EEC) is a term used to describe a compensation temperature, which is system independent for a class of similar experimental systems.[16,17] Melander et al.[18] have used the enthalpy-entropy compensation method in studies of hydrophobic interactions

and separation mechanism in reversed phase HPLC. Mathematically, enthalpy-entropy compensation can be expressed by the formula (1):

$$\Delta H_i = \beta \Delta S_i + \Delta G_\beta \tag{1}$$

where ΔG_β is the Gibbs free energy of the enantiomeric interactions (physicochemical interaction) in the chromatographic system at the compensation temperature (β), (β and ΔG_β are constants). The corresponding change in transfer enthalpy (ΔH_i) and transfer entropy (ΔS_i) is possible to obtain using van´t Hoff equation (2):

$$\ln k_i = \frac{-\Delta H_i}{RT} + \frac{\Delta S_i}{R} + \ln \phi \tag{2}$$

where k, ΔH_i, ΔS_i, R, T, and ϕ are the retention factor for the solute, partial molar enthalpy of transfer, partial molar entropy of transfer, the gas constant, the absolute temperature, and the phase ratio (that, is the volume of the stationary phase, (V_s), divided by the volume of the mobile phase, (V_m), respectively. The procedure involves plotting *ln k* against $1/T$, then setting the slope equal to - $\Delta H_i/R$ and solving for ΔH_i, and enable determining ΔS_i, from the intercept ($\Delta S_i + ln\ \phi$) of the plot. According to equation (1), when enthalpy-entropy compensation (EEC) is observed with a group of compounds in a particular chemical transformation (or interaction in the case of chromatographic retention), all of the compounds have the same ΔG_β at the compensation temperature β.

EEC can be also observed on the enantioselectivity simply plotting $\Delta(\Delta H_{2,1})$ versus $\Delta(\Delta S_{2,1})$. To calculate the thermodynamics parameters, we need to plot expression of ln α as a function of $1/T$ as it is in equation (3),

$$\ln \alpha = -\frac{\Delta(\Delta H)}{RT} + \frac{\Delta(\Delta S)}{R} \tag{3}$$

Using the linear regression model on this kind of dependence, $\Delta(\Delta H_{2,1})$, from the slope of the line, and $\Delta(\Delta H_{2,1})$, from the intercept, can be determined. The value of $\Delta H_{2,1}$ represents the difference in change of enthalpy between the couple of enantiomers and $\Delta S_{2,1}$ and in turn, the difference in change of entropy for the couple of enantiomers, for more and less retained ones.

The aim of this work was to explore the effect of the mobile phase composition on enantioseparation from the thermodynamic point of view. In addition, the aim was to illustrate the efficiency of separation of studied sulfoxides using MTAG CSPs even at higher temperatures,

without coelutions with matrix components of the biological sample. For this purpose, the solid phase extraction (SPE) procedure was developed and used before a chromatographic step.

EXPERIMENTAL

Materials

The names and structures of the chiral sulfoxides used in this study are given in Figure 1. All sulfoxide compounds were prepared at the Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, according to a method previously described in the literature.[19,20] HPLC grade solvents (methanol, acetic acid, diethylamine, water) were obtained from Merck (Germany).

Equipment

The HPLC chromatographic system (Hewlett Packard series 1100) consisted of a quaternary pump, an injection valve (Rheodyne 7724i) with a 20 μL sample loop, a switching valve (Valco), and a photodiode array detector and polarimetric detector (Chiralyzer, Ibz Messtechnik, Germany) connected in series. The column temperature was controlled with a column temperature box (LCT 5100, Ingos, Czech Republic).

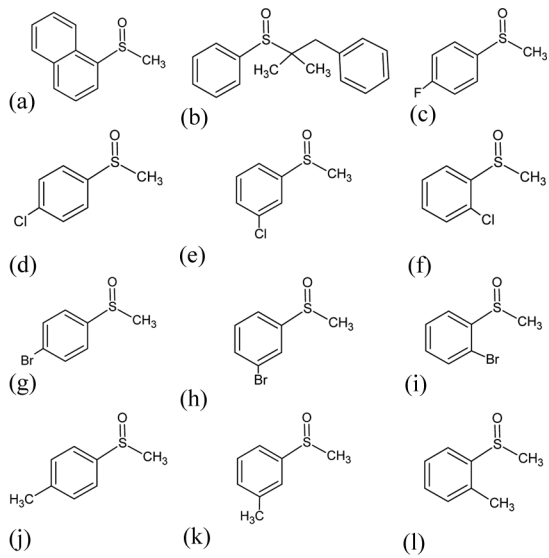

Figure 1. The structures of chiral sulfoxides used in this study.

Methods

The methylated-teicoplanin aglycone (MTAG) column (150 × 4.6 mm I.D.) (Astec, USA) was used for the study. Mobile phases consisted of pure methanol (MP1) and methanol containing 17.48 mmol/L acetic acid (Hac) and with different concentrations of diethylamine (Dea). The concentrations of Dea were as follows: zero concentration (MP2), 2.39 mmol/L (MP3), 4.79 mmol/L (MP4), 9.57 mmol/L (MP5), 14.36 mmol/L (MP6). Thermodynamic data were measured under isothermal conditions over a temperature range of 10–50°C at 10°C intervals. The precision of the controlled temperature was ±0.1°C. The analytes used for the thermodynamic study were dissolved in methanol (concentration 1 mg/mL). Standards solutions were diluted to final concentrations in the range of 2.5–100 μg/mL (5 concentrations) for each racemic mixture to obtain calibration curves. For the SPE procedure, serum samples were spiked with studied sulfoxides to give a concentration of 5 μg/mL. The cartridge was conditioned with 1 mL of methanol and 1 mL of purified water. Rat serum of 0.5 mL spiked with the studied analytes was injected into OASIS HLB (30 mg, 1 mL) (Waters, Ireland) cartridge. The sample was passed through the sorbent layer and washed with 1 mL of water/methanol (95/5 v/v). Analytes retained by the sorbent was eluted with 0.5 mL of the methanol containing 17.48 mmol/L acetic acid and 4.79 mmol/L diethylamine (MP4). Eluate (20 μL) was injected into the chiral column. The same procedure was applied for blank serum samples. UV absorption at a wavelength of 254 nm was used for detection. The elution order was confirmed with pure standards and polarimetric detection and for all the chiral sulfoxides separated using MTAG column the (S) (+)-enantiomer eluted first.

RESULTS AND DISCUSSION

The influence of mobile phase composition on retention for the first and the second eluted enantiomer is depicted in Figure 2. Retention factors slightly decrease with increasing concentration of Dea in mobile phase composition. In the case of the mobile phase consisting of pure methanol (MP1) the retention factors are bigger in comparison with all mobile phases containing some portion of organic modifier in mobile phase composition. It seems that not just the acidity and/or basicity but also ionic strength (mobile phases with and without organic modifiers in mobile composition were used) plays some kind of role in retention mechanisms of the studied compounds. This trend was clearly observed for all studied compounds. Studied sulfoxides were most retained in MP1. On the other hand, enantioselectivity factors showed a little increase in

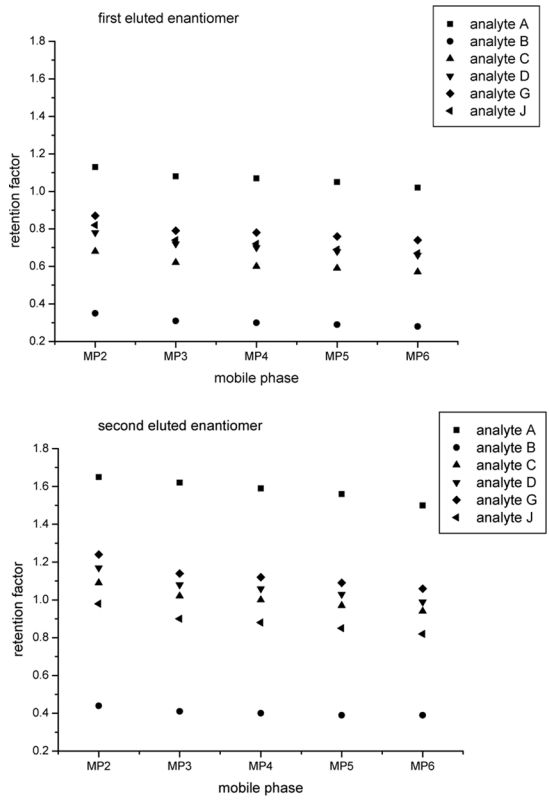

Figure 2. The influence of mobile phase composition on retention for the first and second eluted enantiomers using MTAG. (See Experimental for details.)

enantioselectivity factor values with increasing basicity of the mobile phase up to certain point (Figure 3). It means that not just the presence of the organic modifier but also the concentration ratio between acidic/basic modifiers has an influence on enantioseparation.

The Tables 1–6 summarize thermodynamic parameters obtained using equations 2 and 3. Linearity of these plots is not just the necessity for correct determination of thermodynamic parameters but also reveals the intensiveness of the correlation between variables. For all studied sulfoxides the correlation coefficient values of van′t Hoff plots (lnk_i vs $1/T$) were not less than 0.9916. In the case of vaṅt Hoff plots showing the influence of temperature on enantioselectivity (Figure 4), the correlation coefficient values were in the range of 0.9813–0.9999. According to obtained results, there is no change in retention and enantioseparation mechanisms within the temperature range under the study. In addition no coelutions or even changing of the elution order (S (+) of enantiomeric form

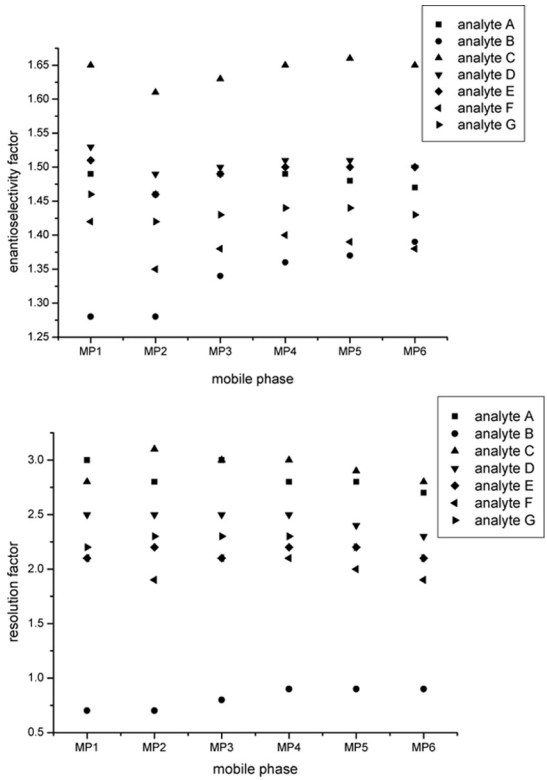

Figure 3. The influence of mobile phase composition on enantioselectivity factor for the first and second eluted enantiomers using MTAG. (See Experimental for details.)

eluted as the first) were observed for studied sulfoxides regardless of the mobile phase composition. The influence of temperature on retention just slightly varies with changing the mobile phase composition (Tables 1–6).

EEC plots of ΔH_i vs. ΔS_i did not show significant linear correlation ($r < 0.7$) for the set of studied compounds. Probably there are some significant differences in retention mechanism even within the same mobile phase, despite the similarities in values of ΔH_i. Even though the retention is enthalpy driven for all studied enantiomers, the influence of entropic contribution to the retention makes their individual retention mechanisms not similar. In other words, there is not any compensation temperature (β) for the set of studied compounds at which compounds have the same ΔG_β. This also reveals the different influence of stereogenic centre environment on retention.

Table 1. Thermodynamic parameters obtained from the van´ t Hoff dependence (lnk_i vs. 1/T) for the first and second eluted enantiomers and from the enantiosectivity van´ t Hoff plot in mobile phase MP1 (See Experimental for details.)

MP1	ΔH_1 ($kJ \cdot mol^{-1}$)	ΔS_1 ($J \cdot mol^{-1} \cdot K^{-1}$)	ΔH_2 ($kJ \cdot mol^{-1}$)	ΔS_2 ($J \cdot mol^{-1} \cdot K^{-1}$)	$\Delta(\Delta H_{2,1})$ ($kJ \cdot mol^{-1}$)	$\Delta(\Delta S_{2,1})$ ($J \cdot mol^{-1} \cdot K^{-1}$)	T_{iso}(°C)
Analyte							
A	−8,107	−7,42	−10,079	−11,08	−1,973	−3,66	265
B	−7,890	−16,59	−9,067	−18,67	−1,177	−2,08	293
C	−9,425	−16,28	−12,224	−21,92	−2,436	−4,41	279
D	−9,438	−15,11	−11,711	−19,58	−2,307	−4,60	229
E	−9,565	−17,38	−10,838	−18,57	−1,899	−3,29	303
F	−9,368	−14,41	−11,987	−20,71	−2,435	−5,66	157
G	−9,324	−13,90	−11,205	−17,44	−2,079	−4,20	222
H	−8,520	−13,09	−10,614	−16,82	−2,373	−4,68	234
I	−9,361	−13,21	−12,041	−19,51	−2,709	−6,37	152
J	−10,423	−18,29	−11,419	−19,99	−1,433	−3,14	183
K	−8,995	−15,72	−9,603	−15,03	−1,144	−1,05	811
L	−9,741	−15,39	−11,278	−18,92	−1,442	−3,23	174

The subscripts 1 and 2 refer to the first and second eluting enantiomer, respectively. Phase ratio used for the calculation of transfer entrophy: $\phi = 0{,}09$.
Tiso, theoretical temperature for elution order reversal.

Table 2. Thermodynamic parameters obtained from the van′ t Hoff dependence (lnk_i vs. 1/T) for the first and second eluted enantiomers and from the enantiosectivity van′ t Hoff plot in mobile phase MP2.(See Experimental for details.)

MP2	ΔH_1 ($kJ \cdot mol^{-1}$)	ΔS_1 ($J \cdot mol^{-1} \cdot K^{-1}$)	ΔH_2 ($kJ \cdot mol^{-1}$)	ΔS_2 ($J \cdot mol^{-1} \cdot K^{-1}$)	$\Delta(\Delta H_{2,1})$ ($kJ \cdot mol^{-1}$)	$\Delta(\Delta S_{2,1})$ ($J \cdot mol^{-1} \cdot K^{-1}$)	T_{iso}(°C)
Analyte							
A	−7,969	−6,95	−9,637	−9,63	−1,668	−2,68	350
B	−8,195	−17,62	−9,431	−19,90	−1,236	−2,29	267
C	−8,405	−12,81	−10,803	−17,34	−2,398	−4,53	257
D	−8,335	−11,41	−10,349	−15,21	−2,014	−3,80	257
E	−8,137	−12,64	−9,969	−15,98	−1,832	−3,34	275
F	−8,565	−11,79	−10,820	−17,25	−2,254	−5,46	139
G	−8,334	−10,56	−10,221	−14,29	−1,887	−3,73	232
H	−7,881	−10,99	−9,804	−14,44	−1,923	−3,44	285
I	−8,448	−10,21	−10,877	−16,01	−2,429	−5,81	145
J	−8,761	−12,58	−9,820	−14,77	−1,059	−2,20	208
K	−8,134	−12,78	−9,148	−13,68	−1,014	−0,90	854
L	−8,571	−11,41	−9,836	−14,26	−1,266	−2,85	170

The subscripts 1 and 2 refer to the first and second eluting enantiomer, respectively. Phase ratio used for the calculation of transfer entrophy: $\phi = 0{,}09$. *Tiso*, theoretical temperature for elution order reversal. ($\phi = 0{,}09$)

Table 3. Thermodynamic parameters obtained from the van´ t Hoff dependence (lnk_i vs. 1/T) for the first and second eluted enantiomers and from the enantiosectivity van´ t Hoff plot in mobile phase MP3 (See Experimental for details.)

MP3	ΔH_1 (kJ.mol^{-1})	ΔS_1 (J.mol^{-1}.K^{-1})	ΔH_2 (kJ.mol^{-1})	ΔS_2 (J.mol^{-1}.K^{-1})	$\Delta(\Delta H_{2,1})$ (kJ.mol^{-1})	$\Delta(\Delta S_{2,1})$ (J.mol^{-1}.K^{-1})	T_{iso}(°C)
Analyte							
A	−7,630	−6,15	−9,318	−8,76	−1,688	−2,61	373
B	−8,192	−18,59	−9,080	−19,28	−0,888	−0,70	1003
C	−8,300	−13,12	−10,530	−16,90	−2,230	−3,78	317
D	−7,985	−10,83	−9,914	−14,24	−1,929	−3,41	292
E	−7,633	−11,57	−9,408	−14,51	−1,775	−2,95	329
F	−8,114	−10,84	−10,222	−15,56	−2,108	−4,72	174
G	−7,864	−9,55	−9,664	−12,91	−1,800	−3,36	262
H	−7,367	−9,84	−9,249	−12,97	−1,882	−3,14	327
I	−8,017	−9,30	−10,319	−14,45	−2,303	−5,15	174
J	−8,581	−12,68	−9,585	−14,58	−1,004	−1,90	256
K	−8,045	−13,26	−9,020	−13,81	−0,975	−0,55	1509
L	−8,489	−11,76	−9,810	−14,67	−1,321	−2,92	180

The subscripts 1 and 2 refer to the first and second eluting enantiomer, respectively. Phase ratio used for the calculation of transfer entrophy: $\phi = 0{,}09$. *Tiso*, theoretical temperature for elution order reversal.*(* $\phi = 0{,}09$)

Table 4. Thermodynamic parameters obtained from the van´t Hoff dependence (lnk_i vs. 1/T) for the first and second eluted enantiomers and from the enantiosectivity van´t Hoff plot in mobile phase MP4.(See Experimental for details.)

MP4	ΔH_1 ($kJ \cdot mol^{-1}$)	ΔS_1 ($J \cdot mol^{-1}.K^{-1}$)	ΔH_2 ($kJ \cdot mol^{-1}$)	ΔS_2 ($J \cdot mol^{-1} \cdot K^{-1}$)	$\Delta(\Delta H_{2,1})$ ($kJ \cdot mol^{-1}$)	$\Delta(\Delta S_{2,1})$ ($J \cdot mol^{-1} \cdot K^{-1}$)	T_{iso}(°C)
Analyte							
A	−8,018	−7,69	−9,625	−10,04	−1,608	−2,35	411
B	−8,811	−21,11	−9,729	−21,78	−0,919	−0,68	1087
C	−8,600	−14,47	−10,798	−18,06	−2,198	−3,59	338
D	−8,258	−12,05	−10,139	−15,27	−1,881	−3,21	313
E	−8,079	−13,36	−9,682	−15,66	−1,603	−2,30	423
F	−8,430	−12,17	−10,472	−16,60	−2,042	−4,42	188
G	−8,160	−10,83	−9,958	−14,14	−1,798	−3,31	270
H	−7,755	−11,43	−9,517	−14,11	−1,762	−2,67	386
I	−8,277	−10,42	−10,616	−15,63	−2,339	−5,21	176
J	−8,931	−14,21	−9,898	−15,94	−0,967	−1,72	288
K	−8,504	−15,19	−9,314	−15,09	−0,809	0,10	(−8259)
L	−8,751	−12,98	−10,095	−15,95	−1,344	−2,97	179

The subscripts 1 and 2 refer to the first and second eluting enantiomer, respectively. Phase ratio used for the calculation of transfer entrophy: $\phi = 0{,}09$. *Tiso*, theoretical temperature for elution order reversal. Values in parenthesis do not make sense (lower than the absolute 0 K).

Table 5. Thermodynamic parameters obtained from the van´ t Hoff dependence (lnk_i vs. 1/T) for the first and second eluted enantiomers and from the enantiosectivity van´ t Hoff plot in mobile phase MP5.(See Experimental for details.)

MP5	ΔH_1 ($kJ \cdot mol^{-1}$)	ΔS_1 ($J \cdot mol^{-1} \cdot K^{-1}$)	ΔH_2 ($kJ \cdot mol^{-1}$)	ΔS_2 ($J \cdot mol^{-1} \cdot K^{-1}$)	$\Delta(\Delta H_{2,1})$ ($kJ \cdot mol^{-1}$)	$\Delta(\Delta S_{2,1})$ ($J \cdot mol^{-1} \cdot K^{-1}$)	T_{iso}(°C)
Analyte							
A	−8,112	−8,16	−9,641	−10,29	−1,529	−2,13	445
B	−8,989	−21,99	−9,933	−22,68	−0,944	−0,69	1100
C	−8,680	−14,98	−10,868	−18,53	−2,188	−3,55	344
D	−8,222	−12,16	−10,090	−15,34	−1,868	−3,18	314
E	−8,038	−13,45	−9,705	−15,96	−1,667	−2,51	392
F	−8,513	−12,64	−10,430	−16,67	−1,917	−4,02	203
G	−8,106	−10,86	−9,875	−14,10	−1,769	−3,24	274
H	−7,734	−11,56	−9,519	−14,31	−1,785	−2,75	376
I	−8,321	−10,75	−10,580	−15,71	−2,259	−4,95	183
J	−8,952	−14,56	−9,943	−16,35	−0,991	−1,80	279
K	−8,594	−15,77	−9,343	−15,44	−0,750	0,33	(−2547)
L	−8,835	−13,49	−10,176	−16,48	−1,342	−2,98	177

The subscripts 1 and 2 refer to the first and second eluting enantiomer, respectively. Phase ratio used for the calculation of transfer entrophy: $\phi = 0{,}09$. *Tiso*, theoretical temperature for elution order reversal. Values in parenthesis do not make sense (lower than the absolute 0 K).

Table 6. Thermodynamic parameters obtained from the van′ t Hoff dependence (lnk_i vs. 1/T) for the first and second eluted enantiomers and from the enantiosectivity van′ t Hoff plot in mobile phase MP6.(See Experimental for details.)

MP6	ΔH_1 (kJ.mol^{-1})	ΔS_1 (J.mol^{-1}.K^{-1})	ΔH_2 (kJ.mol^{-1})	ΔS_2 (J.mol^{-1}.K^{-1})	$\Delta(\Delta H_{2,1})$ (kJ.mol^{-1})	$\Delta(\Delta S_{2,1})$ (J.mol^{-1}.K^{-1})	T_{iso}(°C)
Analyte							
A	−8,066	−8,21	−9,561	−10,29	−1,495	−2,08	445
B	−8,951	−22,05	−9,963	−22,94	−1,012	−0,89	869
C	−8,728	−15,39	−10,970	−19,14	−2,241	−3,75	324
D	−8,317	−12,70	−10,214	−16,01	−1,897	−3,31	299
E	−8,172	−14,11	−9,792	−16,47	−1,620	−2,36	412
F	−8,544	−12,94	−10,543	−17,27	−1,998	−4,33	188
G	−8,112	−11,09	−9,900	−14,41	−1,788	−3,32	265
H	−7,796	−11,98	−9,566	−14,69	−1,770	−2,71	380
I	−8,412	−11,24	−10,601	−16,01	−2,189	−4,77	186
J	−9,034	−15,10	−9,992	−16,81	−0,958	−1,71	288
K	−8,686	−16,34	−9,453	−16,09	−0,766	0,25	(−3371)
L	−8,843	−13,77	−10,169	−16,75	−1,326	−2,98	172

The subscripts 1 and 2 refer to the first and second eluting enantiomer, respectively. Phase ratio used for the calculation of transfer entrophy: $\phi = 0{,}09$. *Tiso*, theoretical temperature for elution order reversal. Values in parenthesis do not make sense (lower than the absolute 0 K). ($\phi = 0{,}09$)

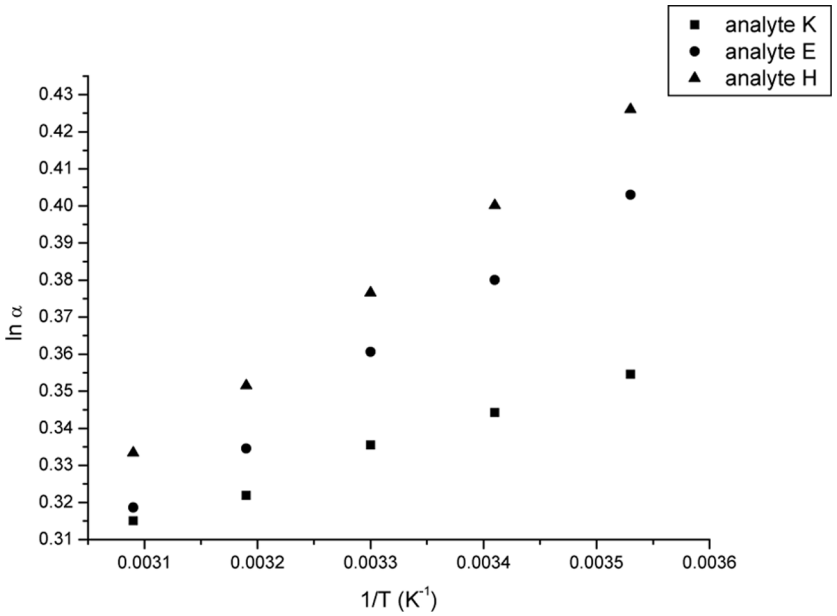

Figure 4. The dependencies of ln α vs. 1/t for chosen analytes for mobile phase MP6. (See Experimental for details.)

On the other hand, using EEC plots on enantioselectivity showed significant correlation only if the set of compounds was divided into two groups. Excluding 4-fluoro phenyl methyl sulfoxide (analyte C) from the first group A (analytes A, D, E, F, G, H, I, K), it gave correlation coefficients not less than 0.971 (Figure 5). The highest correlation was observed for the mobile phase containing pure methanol (MP1) in mobile phase composition. No significant difference in EEC plots was observed regardless of the mobile phase composition used in this study (Figure 6). This reveals some similarities in the enantioseparation mechanism for this set of compounds regardless of the basicity of the mobile phase. The rest of the analytes (second group B obtaining analytes B, J, and L) gave correlation equal to 0.9235, taking into correlation the values of $\Delta H_{2,1}$ versus $\Delta(S_{2,1})$ for all mobile phases used in this study (Figure 6). The values of enantioselectivity temperature T_{iso} at which the enantioselectivity factor is equal to 1 are given in Tables 1–6. The change of the transfer entalphy is always bigger for the second eluted enantiomer. In the case of 3- toluyl methyl sulfoxide (analyte K), the differences in transfer enthalpies for both enantiomers are smaller in comparison with other enantiomeric pairs. In addition, the enantioselectivity varies very slightly with temperature resulting in a positive intercept of the vańt Hoff dependence.

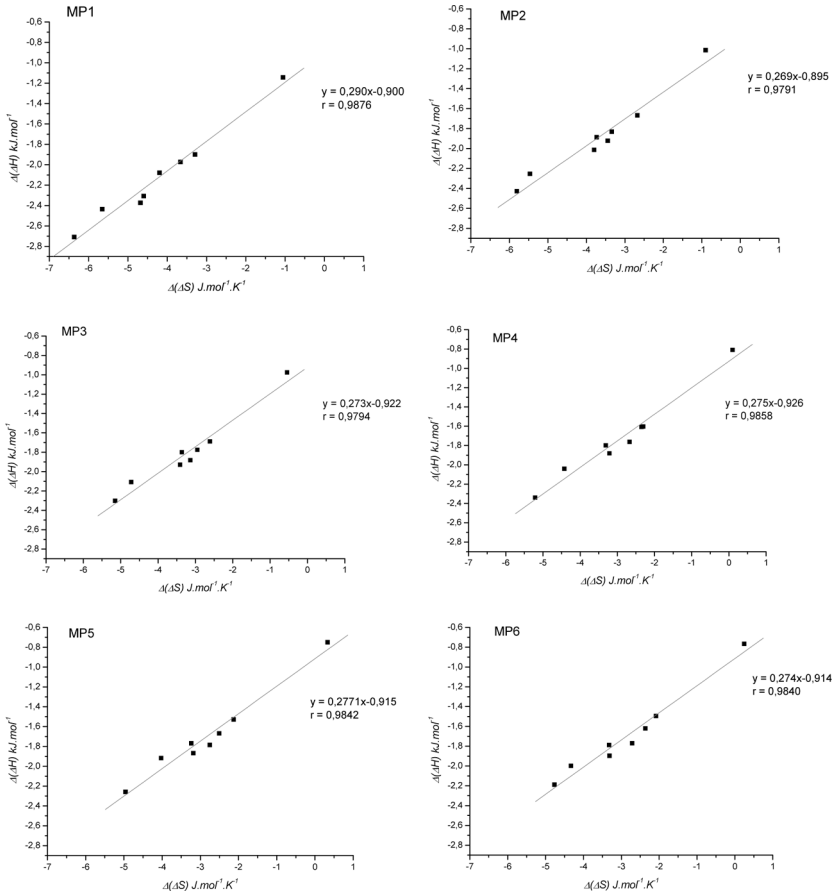

Figure 5. Enantioselectivity EEC plots of some sulfoxides selected from the set of the sulfoxides for each mobile phase. (See Experimental for details.)

As a consequence of such behaviour, the T_{iso}, theoretical temperature of coelution calculated for these enantiomers makes no sense in some cases (Tables 4–6). In most cases, the values of $\Delta(H_{2,1})$ are the biggest in mobile phase MP1. It means that in the case of mobile phases (MP2-MP6) the enantioselectivity factors change less with varing temperature as it does in the case of (MP1). Naturally, this is reflected also in values of $\Delta(S_{2,1})$ The F-test was used for the evaluation of results (level of probability $\alpha = 0.05$).

In order to find out, if MTAG CSP is suitable for separation and determination of chiral sulfoxides in real biological matrix, it was necessary to involve the extraction step before the chromatographic measurement. SPE procedure was performed as was described in the

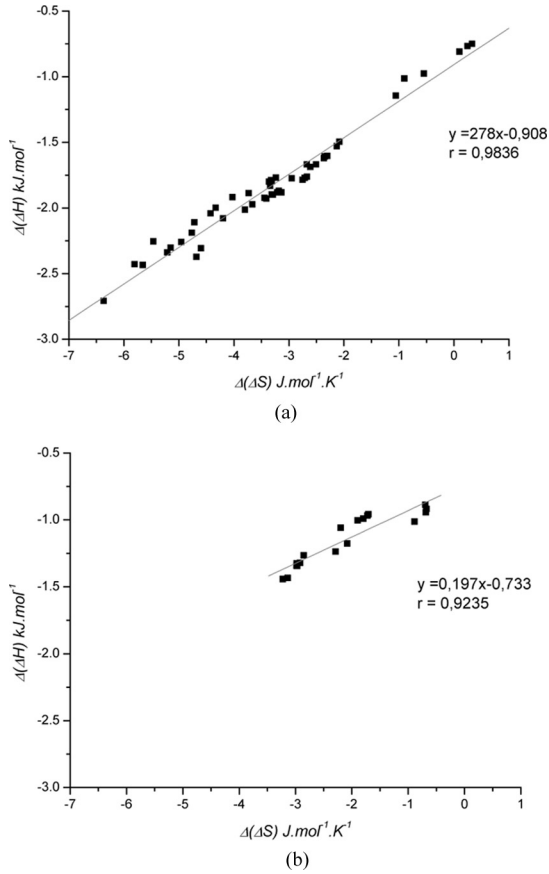

Figure 6. Enantioselectivity EEC plots showing correlation of two groups (Group A and B) of chiral sulfoxides for all mobile phases together. 4-Fluoro phenyl methyl sulfoxide is excluding from both groups.

Experimental part. The recovery of the SPE treatment is summarized in Table 7 together with LOD values for each of the seven racemic mixtures chosen for this purpose. In Figure 7 can be clearly seen the influence of temperature on retention and separation of studied sulfoxides in rat serum. It is evident that even at higher temperatures sufficient separation can be still observed without interference with the matrix of rat serum. Moreover, this showed how important it is to control temperature during the measurements and how significantly it is possible to control retention and separation with varying temperature.

Table 7. Slope and the intercept of the calibration lines ($y = a^*x + b$) with the correlation coefficients of these lines, LOQ and the recovery of the extraction step for studied sulfoxides.(See experimental for details.)

Analyte	Slope (a)	Intercept (b)	Correlation coefficient (r)	LOQ (ng/mL)	Recovery (%)
A	876,06	0,42	0,999	24,2	94
B	3865,99	0,60	0,999	6,3	82
C	1146,30	0,26	0,999	18,1	96
D	2927,69	−0,47	0,999	7,5	92
E	1868,29	−0,73	0,999	11,1	96
F	1575,42	0,81	0,999	13,9	91
G	3267,84	1,10	0,999	7,0	87

RSD of the recovery were in the range of 2–5% (n = 3)

CONCLUSION

There were not any significant changes observed in retention and enantioseparation mechanisms with increasing the concentration of diethylamine in mobile phase composition. Despite this, there are some differences regarding the retention of the studied compounds in pure methanol mobile phase with other polar-organic mode mobile phases. The position of the substituent on the aromatic ring seems to be significant. This was evident clearly in the case of 3-toluyl methyl sulfoxide using EEC. EEC also revealed some differences in the enantioseparation mechanism for 4-Fluro phenyl methyl sulfoxide in comparison with other sulfoxides. No coelution or changing of the elution order was observed within the temperature range. There were also no changes observed in

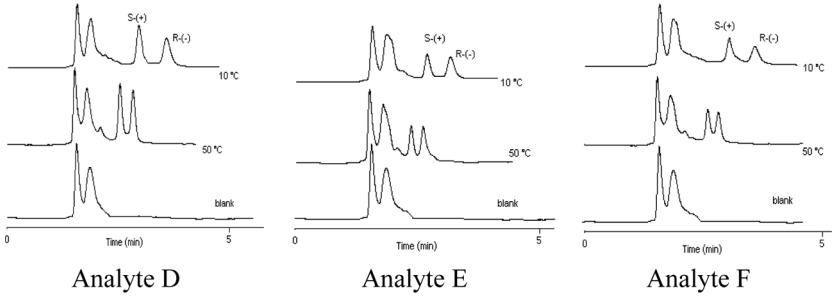

Figure 7. The influence of temperature on enatioseparation of chiral sulfoxides in rat serum. (See Experimental for details.)

retention or enantioseparation mechanisms within the temperatures under study. In addition, the study of the temperature effects on enantioseparation of studied sulfoxides in rat serum confirmed the necessity of proper control of column temperature. Moreover, the study showed how simple it is to control selectivity of the system with varying the chromatographic temperature. Eventually, according to the results, the MTAG column seems to be a suitable tool for the separation of chiral sulfoxides in biological matrix using the polar organic phase mode.

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