Institute of Analytical Chemistry[1], Faculty of Chemical and Food Technology, Slovak Technical University, Department of Pharmaceutical Chemistry[2], Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic

Enantioseparation of chiral sulfoxides using teicoplanine chiral stationary phases and kinetic study of decomposition in human plasma

D. Meričko[1], J. Lehotay[1], J. Čižmárik[2]

Received July 30, 2008, accepted August 5, 2008

Professor Jozef Lehotay, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic
jozef.lehotay@stuba.sk

Pharmazie 63: 854–859 (2008) *doi: 10.1691/ph.2008.8219*

Teicoplanin chiral stationary phases (CHIROBIOTIC TAG and CHIROBIOTIC T) used in this study are suitable for enantioseparation of chiral sulfoxides in polar-organic phase mode. The method involves determination of chiral sulfoxides in human plasma on teicoplanin chiral stationary phase after the off-line SPE pre-treatment using OASIS HLB cartridges. The limit of determination was in the range of 0.004–0.026 μg/ml for individual racemic mixtures. The S(+) enantiomeric form eluted always as the first, except the 4-(methyl sulfinyl) biphenyl with less retained R(−) enantiomer. It was found that the rate constants of individual chiral sulfoxides depend on the type of halogen substituent. There was no significant difference in rate constants considering the position of Cl-substitution on aromatic ring of sulfoxides. Moreover, the rate constants of R(−) and S(+) forms of enantiomers are significantly different just in the case of 4-fluoro phenyl sulfoxide.

1. Introduction

Chiral sulfoxides are widely used as chiral controlers for asymmetric C−C bond formation processes (Capozzi et al. 2001) and as ligands in catalytic asymmetric synthesis (Owens et al. 2001). Moreover, some of the sulfoxides are useful binding blocks in the synthesis of natural and biologically active compounds presenting a variety of structures (Carreño 1995). Since stereogenic sulfinyl sulphur induces a strongly asymmetric environment, many synthetic groups were engaged in the design and development of new synthetic methods for generation of enantiopure sulfoxides. It is clear from the literature, that sulfoxide functionality plays a very important role in a variety of medical targets (Cotton et al. 2000). Together with increased use of these compounds in organic synthesis, the effective separation of the chiral sulfoxides is of analytical and preparative interest as well. Since 1959 (Farina et al. 1959), several approaches to enantiosepation of chiral sulfoxides occurred using protein (Allenmark and Bomgren 1982; Balmer et al. 1994), polysaccharides (Tanaka et al.1995; Donnoli et al. 2000) and cyclodextrin based chiral stationary phases (CSPs) (Küsters and Gerber 1997). The macrocyclic glycopeptides CSP's are very useful for the separation of enantiomers, including chiral sulfoxide molecules (Meričko et al. 2006). They possess several characteristics that allow them to interact with a variety of analytes and allow them to serve as chiral selectors. They have numerous stereogenic centres and a variety of functional groups, allowing them to have multiple interactions such as: hydrophobic, dipole-dipole and π-π interactions, as well as hydrogen bonding, steric repulsion and ionic or charge-to- charge interactions (Armstrong and Nair 1997; Gasper et al. 1996; Ward and Oswald 1997, Ward et al. 1995).

This paper shows both, versatility of off-line solid phase extraction (SPE) and effectivity of using glycopeptide chiral stationary phases (CHIROBIOTIC TAG and CHIROBIOTIC T) in polar-organic mode for determination of chiral sulfoxides and for kinetic study of their decomposition in human plasma.

2. Investigations, results and discussion

2.1. Chiral separation and mobile phase composition

The resolution factors and retention factors behaviour of the studied enantiomers (Fig. 1), as a function of the mobile phase composition (the amount of base in the mobile phase) in polar-organic mode for CHIROBIOTIC T and CHIROBIOTIC TAG columns, are documented in Tables 1, 2 and Fig. 2. As it is evident, the polar-organic mode is suitable for the separation of the sulfoxide enantiomers using teicoplanin columns. Despite the fact that there is no significant change in retention factors (k_i) with varying mobile phase composition, the enantioselectivity slightly increases with increasing diethylamide concentration in mobile phase using CHIROBIOTIC T column. The resolution factors were the highest in the case of methanol mobile phase containing 17.48 mmol/l acetic acid and 4.79 mmol/l diethylamine (MP3) (Fig. 2). Under such chromatographic conditions, the resolution factors ($R_{1,2}$) were in the range of 1.1–3.9. In the case of CHIROBIOTIC TAG higher retention factors and higher enantioselectivity factors (α) were observed in comparison with CHIROBIOTIC T. Unlike the CHIROBIOTIC T, no significant

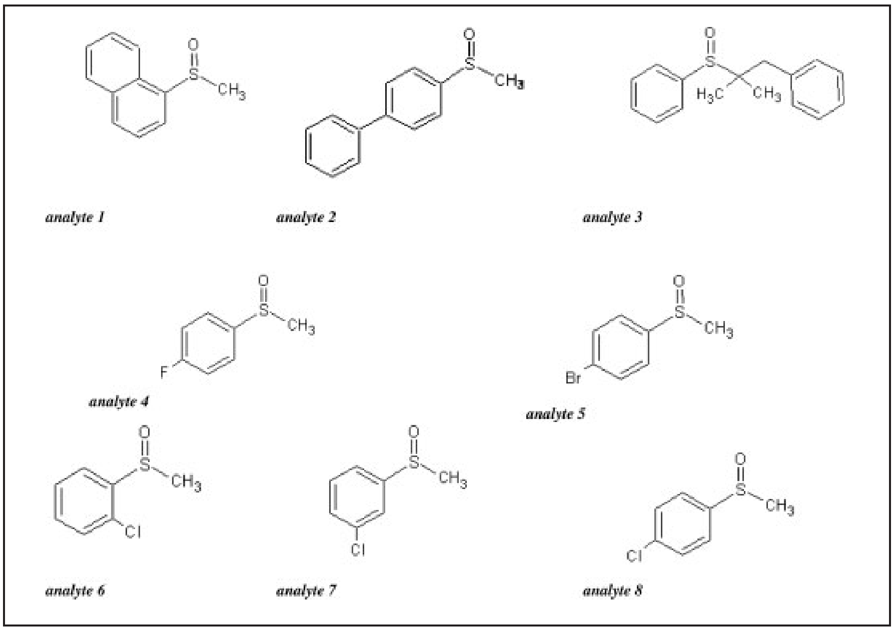

Fig. 1:
Chiral sulfoxide used for enantioseparation

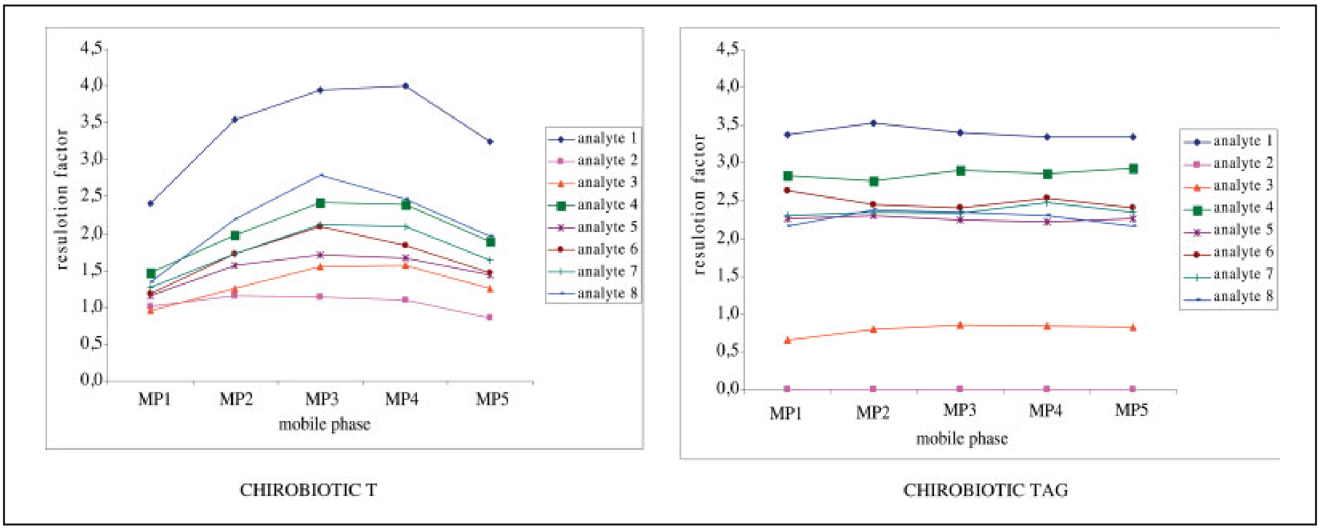

Fig. 2: Influence of mobile phase composition on values of resolution factors for both chiral stationary phase

Table 1: Chromatographic data of enantioseparation of chiral sulfoxides using CHIROBIOTIC T column

CHIROBIOTIC T										
Analyte	MP1		MP2		MP3		MP4		MP5	
	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α
1	0.44	1.41	0.41	1.50	0.45	1.51	0.43	1.54	0.40	1.56
2	0.36	1.19	0.34	1.20	0.37	1.18	0.36	1.18	0.33	1.18
3	0.11	1.51	0.09	1.78	0.11	1.68	0.10	1.74	0.08	1.92
4	0.28	1.34	0.25	1.43	0.28	1.42	0.26	1.43	0.24	1.46
5	0.32	1.23	0.29	1.29	0.33	1.28	0.31	1.29	0.28	1.30
6	0.30	1.27	0.27	1.34	0.30	1.33	0.29	1.34	0.26	1.36
7	0.24	1.34	0.21	1.43	0.24	1.41	0.23	1.43	0.21	1.45
8	0.31	1.30	0.28	1.42	0.31	1.43	0.30	1.44	0.27	1.46

k1 ± 0.09 (n = 3). α ± 0.06 (n = 3)
MP1-MeOH-Hac (17.48 mmol/l). MP 2-MeOH-Hac (17.48 mmol/l)- Dea (2.39 mmol/l). MP 3-MeOH-Hac (17.48 mmol/l)- Dea (4.79 mmol/l). MP 4-MeOH-Hac (17.48 mmol/l)- Dea (9.57 mmol/l). MP 5-MeOH-Hac (17.48 mmol/l)- Dea (14.36 mmol/l)

Table 2: Chromatographic data of enantioseparation of chiral sulfoxides using CHIROBIOTIC TAG column

CHIROBIOTIC TAG										
Analyte	MP1		MP2		MP3		MP4		MP5	
	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α
1	0.87	1.51	0.71	1.50	0.70	1.50	0.69	1.49	0.68	1.48
2	0.86	1.00	0.71	1.00	0.69	1.00	0.68	1.00	0.67	1.00
3	0.23	1.30	0.18	1.36	0.17	1.38	0.16	1.38	0.16	1.39
4	0.51	1.57	0.40	1.59	0.38	1.61	0.37	1.62	0.37	1.58
5	0.65	1.41	0.52	1.40	0.50	1.41	0.49	1.40	0.48	1.39
6	0.59	1.48	0.47	1.48	0.45	1.48	0.44	1.49	0.43	1.48
7	0.46	1.48	0.37	1.51	0.35	1.52	0.35	1.52	0.34	1.51
8	0.61	1.38	0.49	1.41	0.48	1.42	0.47	1.42	0.46	1.40

k1 ± 0.06 (n = 3). α ± 0.10 (n = 3)
MP1-MeOH-Hac (17.48 mmol/l). MP 2-MeOH-Hac (17.48 mmol/l)- Dea (2.39 mmol/l). MP 3-MeOH-Hac (17.48 mmol/l)- Dea (4.79 mmol/l). MP 4-MeOH-Hac (17.48 mmol/l)- Dea (9.57 mmol/l). MP 5-MeOH-Hac (17.48 mmol/l)- Dea (14.36 mmol/l)

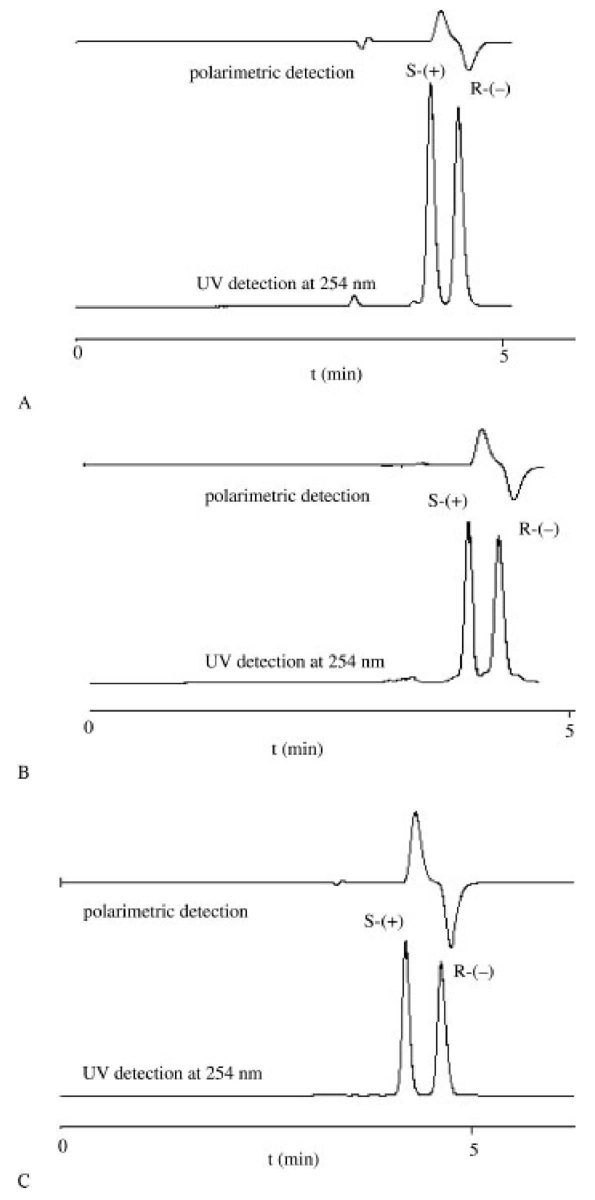

Fig. 3: Chromatograms of chiral sulfoxides separated on CHIROBIOTIC T in methanol mobile phase MP3 (See experimental for details.): A 4-chloro phenyl sulfoxide, B 3-chloro phenyl sulfoxide, C 2-chloro phenyl sulfoxide

change in resolution factors was observed with changing the mobile phase composition. The lowest resolution factors were documented for 4-(methyl sulfinyl) biphenyl regardless the mobile phase composition for both chiral columns. The influence of the position of the halogen substituent on separation can be demonstrated in the case of aryl-methyl sulfoxides with chloro substituent in 2-, 3- and 4-position (Fig. 3). The elution order of all enantiomers (S(+) enantiomer eluted first) were the same for all mobile phases. The exception was 4-(methyl sulfinyl) biphenyl with R(−) enantiomer eluted first. The teicoplanin aglycone (CHIROBIOTIC TAG) chiral stationary phase is considered more suitable for the separation of aryl-methyl sulfoxides in the polar organic mode (Meričko et al. 2007). This fact is also well documented in Fig. 2. In the case of [(1,1-dimethyl-2 phenylethyl) sulfinyl] benzene, separation is better using CHIROBIOTIC T in comparison with CHIROBIOTIC TAG for all mobile phase under the study. In addition, in the case of 4-(methyl sulfinyl) biphenyl, no separation was observed using CHIROBIOTIC TAG column (Fig. 4) with UV detection. Due to this fact, the determinations of all 8 chiral sulfoxides in human plasma after solid phase extraction (SPE) were performed using CHIROBIOTIC T column in methanol mobile phase MP3.

2.2. *Off-line solid phase extraction*

The performance of the off-line SPE was investigated with racemic standard solutions. The calibration with standard

Table 3: Limit of determination LOQ, correlation coefficients for calibration line and recovery of the SPE procedure for determination of chiral sulfoxides in human plasma

CHIROBIOTIC T			
analyte	LOQ (μg/ml)	r	Recovery (%)
1	0.026	0.9995	82
2	0.004	0.9990	64
3	0.009	0.9993	86
4	0.017	0.9995	88
5	0.007	0.9995	91
6	0.009	0.9991	85
7	0.016	0.9994	90
8	0.017	0.9994	93

r-correlation coefficient for calibration line. $y = a + bx$. (5 points in the range of 2.5–100 μg/mL measured for racemic mixture)
RSDs for recovery were 3–5 (%). (n = 3)

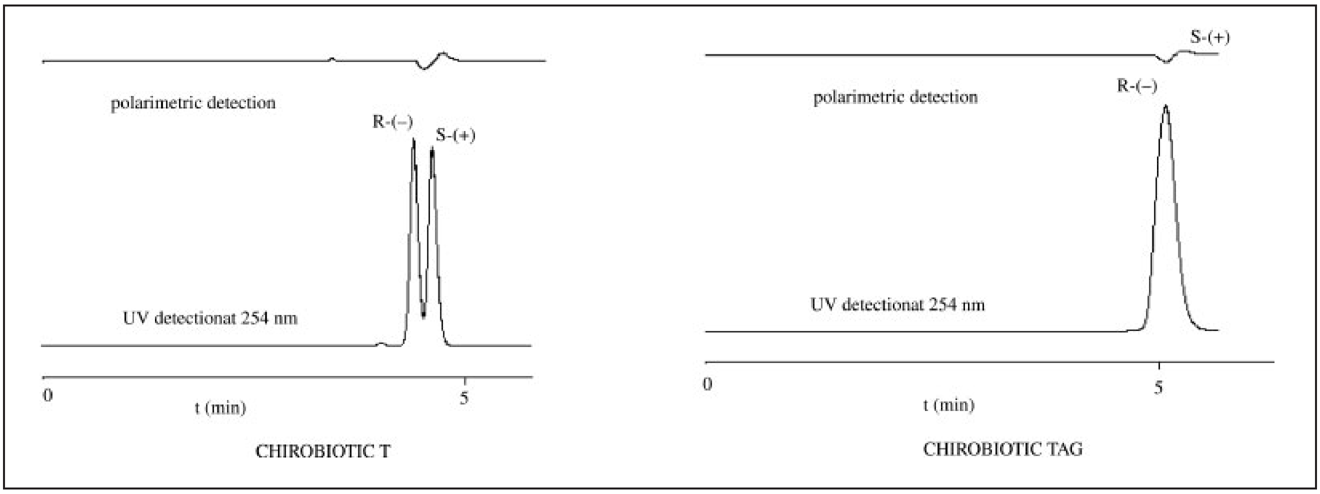

Fig. 4: Separation of 4-(methylsulfinyl) biphenyl using chiral stationary phase in methanol mobile phase MP3 (See experimental for details)

solutions was in the range of 2.5–100 μg/ml (5 points) and for all 8 sulfoxides the correlation coefficients were not less than 0.9990. The limit of determination (LOD) in plasma samples was in the range of 0.004–0.026 μg/ml for individual racemic mixtures. The recovery of the off-line clean up of analytes from spiked plasma at a concentration level 5 μg/ml was found to be 64–93 % with RSD of 3–5 % (n = 3) (Table 3).

2.3. In-vitro kinetic study

The developed HPLC method was applied to investigate the *in vitro* kinetics of decomposition of aryl methyl sulfoxides. Determination of chiral sulfoxides in human plasma after SPE procedure was performed using CHIROBIOTIC T column in methanol mobile phase MP3. The standard solution of racemic analytes was added to human plasma (t = 0) and the biological sample was incubated at 37 °C (time interval from 0 to 24 h). The same procedure was used for analysis of blank plasma solutions. In Fig. 5 there three chromatograms of blank samples are shown after different incubation times at 37 °C. Degradation of 4-bromo phenyl methyl sulfoxide is shown in Fig. 6. The time curve of the *in vitro* degradation of all enantiomers of analytes in human plasma is given in Fig. 7. The rate constants were determined using the linear dependences: ln (c/c_0) = f (t) (first order) and 1/c = f (t) (second order). The experimental rate constants (k) on the assumption that the reaction of degradation is of the first or the second order are summarised in Table 4. Curves depicted in Fig. 7 demonstrate the difference in the concentrations of the enantiomers after the treatment. It is evident, that degradation slightly decreases with the time of incubation. In the case of S(+) enantiomers of analytes 1, 3 and 5 the degradation strongly decreases after 12 h. This has similar trend

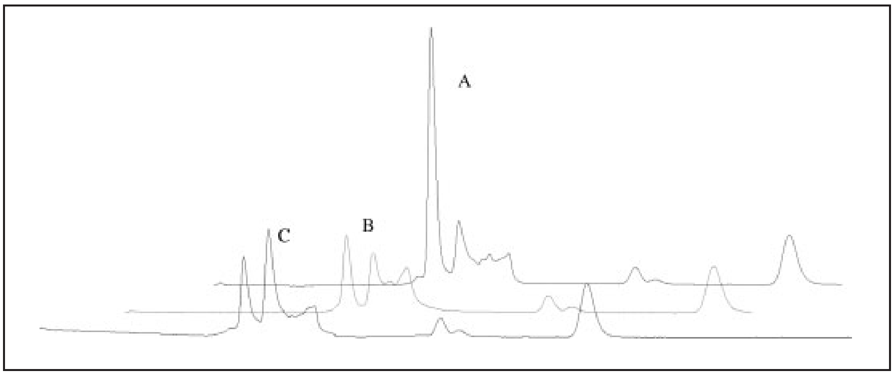

Fig. 5:
Chromatograms of the blank analyzed using CHIROBIOTIC T in MF3 after A 0(h), B 6(h) and C 12(h) incubation at 37 °C. (See experimental for details)

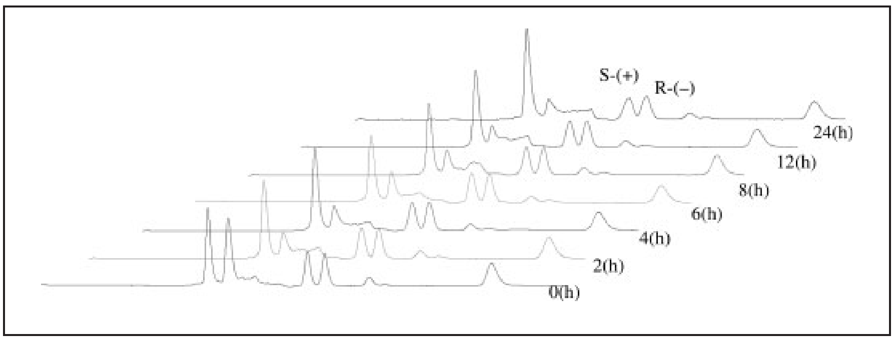

Fig. 6:
Degradation of S-(+) and R-(-) enantiomeric form of 4-bromo phenyl methyl sulfoxide in human plasma

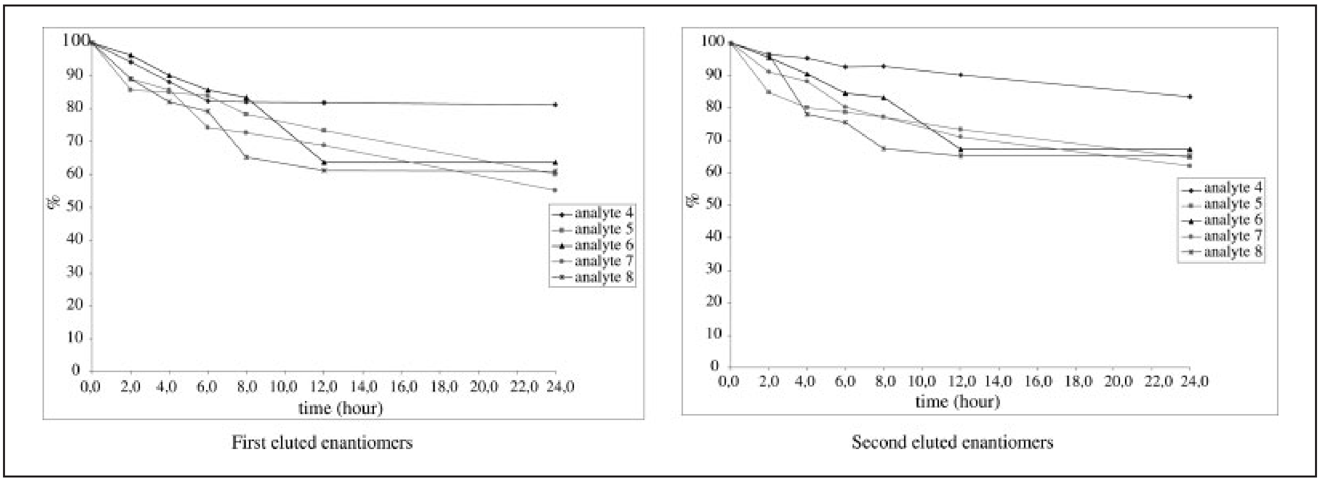

Fig. 7: Time curve of degradation for the first eluted analytes and second eluted analytes

Table 4: Rate constants of decomposition of selected chiral sulfoxides in human plasma

Analyte	First order section		Second order section	
	$k \times 10^5$ (s^{-1})	r (correlation coefficient)	k ($dm^3 \cdot mol^{-1} \cdot s^{-1}$)	r (correlation coefficient)
4-S-(+)	0.48	−0.89	0.39	0.90
4-R(−)	0.22	−0.96	0.17	0.97
5-S-(+)	0.63	−0.94	0.71	0.95
5-R(−)	0.62	−0.88	0.71	0.90
6-S-(+)	0.99	−0.96	1.01	0.94
6-R(−)	0.88	−0.98	0.88	0.97
7-S-(+)	0.88	−0.95	0.84	0.96
7-R(−)	0.78	−0.99	0.73	0.99
8-S-(+)	1.20	−0.98	1.16	0.98
8-R(−)	1.10	−0.94	1.02	0.95

Fisrt order section: k (rate constant), $k \pm 0.06 \times 10^5$ (s^{-1}). (n = 3)
Second order section: k (rate constant), $k \pm 0.04$ ($dm^3 \cdot mol^{-1} \cdot s^{-1}$) · (n = 3)

in the case of second eluted enantiomers R(−) of analytes 3 and 5. In other cases, the degradation in human plasma slightly continues even after 12 h of incubation. There is no significant difference in degradation of R(−) enantiomeric forms in comparison with the S(+) enantiomeric forms except the 4-fluoro phenyl methyl sulfoxide. In this case, there was even observed some difference in degradation in comparison with other analytes under study. This shows the significant influence of the halogen substituent on degradation. On the other hand, no significant difference was observed considering the position of 2,- 3,- and 4,-chloro substituent on degradation in human plasma. The degradation of studied compounds does not correspond significantly to any order with regard to the change in the concentration of the racemates during study (the correlation coefficients are close to 1 in both cases). It can be assumed that the mechanism of decomposition is very complicated and there is no possibility to describe it according to the first and/or second order model.

3. Experimental

3.1. Chemicals

Racemic aromatic sulfoxides were prepared at Iowa State Univerzity, Gilman Hall, USA and their structure is given in Fig. 1. Solvents (methanol) of HPLC grade and other chemicals (acetic acid, diethylamine, water, acetonitrile) of analytical grade were supplied by Merck (Germany).

3.2. Equipment

The HPLC chromatographic system Hewlett Packard (series 1100) consisted of a quaternary pump, autosampler, a switching valve Valco, a photodiode array detector and polarimetric detector (Chiralyzer, IBZ MESSTECHNIK, Germany) connected in series.

3.3. Chromatography

The chiral stationary phases used for separations of racemic sulfoxides were teicoplanin (CHIROBIOTIC T, 250 × 4.6 I.D., 10 μm) (Astec, USA) and teicoplanin aglycone chiral stationary phase (CHIROBIOTIC TAG, 250 × 4.6 I.D., 5 μm) (Astec, USA). There was a guard achiral column (SEPARON SGX C18, 10 × 4 I.D., 7 μm) (Watrex, Slovakia) connected before chiral column during the analysis of biological samples. Mobile phases consisted of methanol containing 17.48 mmol/l acetic acid (Hac) and with different concentration of diethylamine (Dea). The concentrations od Dea were as follows: zero concentration (MP1), 2.39 mmol/l (MP2), 4.79 mmol/l (MP3), 9.57 mmol/l (MP4), 14.36 mmol/l (MP5). The flow rate for the achiral and chiral columns was set at 1 ml/min. The temperature of the chromatographic columns was controlled at 20 °C. Injection volume was 20 μl. The wavelength of 254 nm was used for UV detection.

3.4. Sample preparation

The cartridge was conditioned with 1 ml of methanol, 1 ml of purified water. 0.5 ml of blood plasma spiked with studied analytes (concentration 5 μg/ml) was injected into OASIS HLB (30 mg, 1 ml) (Waters, Ireland) cartridge. Then 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 (MP3). 20 μl of eluate was injected into the chiral column. In order to avoid interferences in the case of 1-(methyl sulfinyl) naphthalene, the dilution of plasma sample with acetonitrile (5/1 v/v) and centrifugation (MPW-300 Mechanika precyzyjna, Poland) for 5 min was used before SPE procedure. The columns were conditioned with the mobile phase (MF3) before injection of plasma extracts or standard solutions.

Acknowledgements: Support from the Grant agency Slovak Republic (grants 1/0058/08), and APVV project No. 20-035-205 is gratefully acknowledged. The authors are thanks to the D.W. Armstrong for the donation of macrocyclic antibiotics chiral stationary phases and some standards of sulfoxide.

References

Allenmark S, Bomgren B (1982) Direct liquid chromatographic separation of enantiomers on immobilized protein stationary phases II. Optical resolution of a sulphoxide, a sulphoximine and a benzoylamino acid. J Chromatogr A 252: 297–300.
Armstrong DW, Nair UB (1997) Capillary electrophoretic enantioseparations using macrocyclic antibiotics as chiral selectors. Electrophoresis 18: 2331–2342.
Balmer K, Persson BA, Lagerström PO (1994) Stereoselective effects in the separation of enantiomers of omeprazole and other substituted benzimidazoles on different chiral stationary phases. J Chromatogr A660: 269–273.
Capozzi MAM, Cardellicchio C, Naso F, Spina G, Tortorella P (2001) Highly stereoselective route to dialkyl sulfoxides based upon the sequential displacement of oxygen and carbon leaving groups by Grignard reagents on sulfinyl compounds. J Org Chem 66: 5933–5936.
Carreño MC (1995) Applications of sulfoxides to asymmetric synthesis of biologically active compounds. Chem Rev 95: 1717–1760.
Cotton H, Elebring T, Larsson M, Li L, Sorensen H, Von Unge S (2000) Asymmetric synthesis of esomeprazole. Asymmetric synthesis of esomeprazole. Tetrahedron: Asymmetry 11: 3819–3825.
Donnoli MI, Superchi S, Rosini C (2000) Chromatographic resolution and elution order of alkyl aryl and aryl benzyl sulfoxides on cellulose-based chiral stationary phases. Enantiomers 5: 181–188.
Farina G, Montanari F, Negrini A (1959) Ricerche sull'etilenazione – Nota XVII. La stereochimica degli 1–2-diaril-vinilen-disolfossidi. Gazz Chim Ital 89: 1548–1563.
Gasper MP, Berthod A, Nair UB, Armstrong DW (1996) Comparison and modeling study of vancomycin, ristocetin A, and teicoplanin for CE enantioseparations. Anal Chem 68: 2501–2514.
Küsters E, Gerber G (1997) Enantiomeric separation of racemic sulphoxides on chiral stationary phases by gas and liquid chromatography. Chromatographia 44: 91–96.
Meričko D, Lehotay J, Skačáni I., Armstrong DW (2007) Separation and thermodynamic studies of chiral sulfoxides on teicoplanin-based stationary phase. J Liq Chrom Rel Technol 30: 1401–1420.
Meričko D, Lehotay J, Skačáni I, Armstrong DW (2006) Effect of temperature on retention and enantiomeric separation of chiral sulfoxides using teicoplanin aglycone chiral stationary phase. J Liq Chrom Rel Technol 29: 623–538.
Owens DT, Hollander JF, Oliver AG, Ellman JA (2001) Synthesis, utility, and structure of novel bis(sulfinyl)imidoamidine ligands for asymmetric Lewis acid catalysis [21]. J Am Chem Soc 123: 1539–1540.
Tanaka M, Yamazaki H, Hakusui H (1995) Direct HPLC separation of enantiomers of pantoprazole and other benzimidazole sulfoxides using cellulose-based chiral stationary phases in reversed-phase mode. Chirality 7: 612–615.
Ward TJ, Dann III C, Blaylock A (1995) Enantiomeric resolution using the macrocyclic antibiotics rifamycin B and rifamycin SV as chiral selectors for capillary electrophoresis. J Chromatogr A 715: 337–344.
Ward TJ, Oswald TM (1997) Enantioselectivity in capillary electrophoresis using the macrocyclic antibiotics. J Chromatogr A 792: 309–325.