ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 467–478

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Comparative enantioseparation of selected chiral drugs on four different polysaccharide-type chiral stationary phases using polar organic mobile phases

Bezhan Chankvetadze [a,1], Irma Kartozia [b], Chiyo Yamamoto [c], Yoshio Okamoto [c,*]

[a] *Molecular Recognition and Separation Science Laboratory, School of Chemistry, Tbilisi State University, Chavchavadze Ave 1, 380028 Tbilisi, Georgia*

[b] *Institute of Pharmaceutical Chemistry, University of Münster, Hittorfstr. 58-62, 48149 Münster, Germany*

[c] *Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Chikusa-ku, 464-8603 Nagoya, Japan*

Received 24 July 2001; received in revised form 31 August 2001; accepted 10 September 2001

Dedicated to Professor Dr Gottfried Blaschke on the occasion of his 65th birthday.

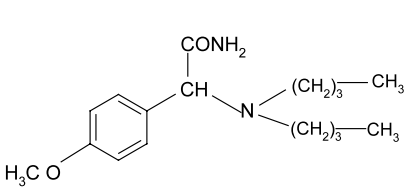
Abstract

The enantiomers of randomly selected chiral drugs and drug analogs of various structural and pharmacological groups were resolved on four different polysaccharide-type chiral stationary phases (CSP) using pure methanol and acetonitrile as mobile phases. Polysaccharide phenylester type CSP, Chiralcel-OJ although resolving the enantiomers of some chiral drugs was less universal in the combination with methanol and acetonitrile as mobile phases. Among polysaccharide phenylcarabamates amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak-AD) was superior over the corresponding cellulose derivative, cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel-OD). However, another derivative of cellulose, namely, cellulose tris(3,5-dichlorophenylcarbamate) (CDCPC) exhibited higher chiral recognition ability compared to Chiralpak-AD material. This study confirms previous findings about the applicability of polysaccharide type CSPs in so called polar organic mode as well as shows high potential of CDCPC as a practically useful CSP for High performance liquid chromatography (HPLC) enantioseparations.

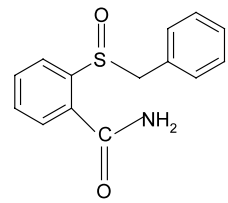
Keywords: Chiral drugs; Enantioseparations; HPLC; Polysaccharide type chiral stationary phases; Chiralcel-OD; Chiralcel-OJ; Chiralpak-AD; Cellulose tris(3,5-dichlorophenylcarbamate)

* Corresponding author. Tel.: +81-52-789-3146; fax: +81-52-789-3188.
E-mail address: okamoto@apchem.nagoya-u.ac.jp (Y. Okamoto).
[1] Present address: Institute of Pharmaceutical Chemistry, University of Münster, Hittorfstr. 58-62, 48149 Münster, Germany.

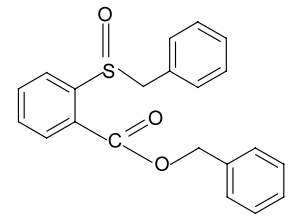
0731-7085/02/$ - see front matter
PII: S0731-7085(01)00648-3



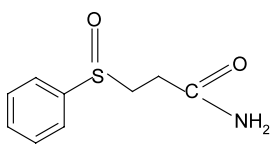
1. Ambucetamide



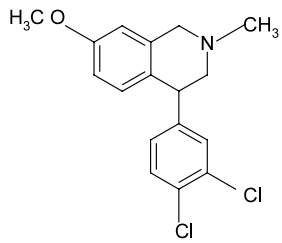
2. 2-(Benzylsulfinyl)benzamide



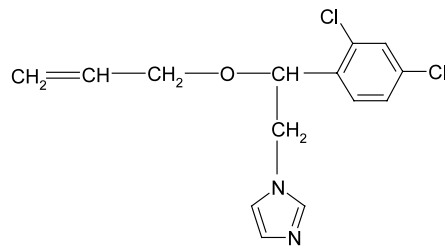
3. Benzyl 2-(benzylsulfinyl)benzoic acid benzyl ester



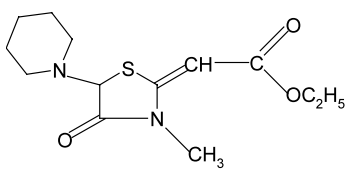
4. 2-(Phenylsulfinyl)ethylamide



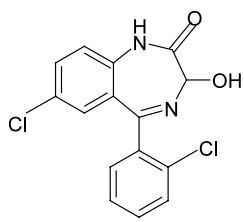
5. Diclofensine



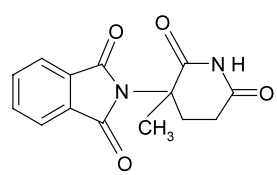
6. Enilconazole



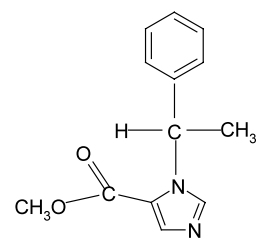
7. Etozolin



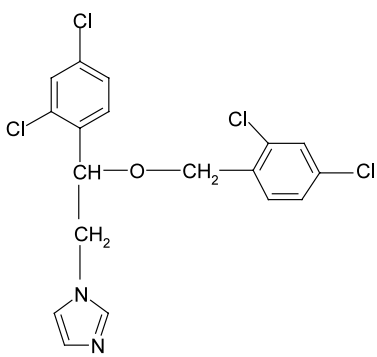
8. Lorazepam



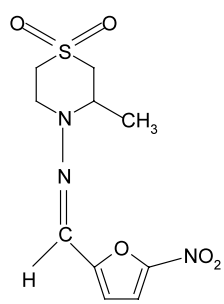
9. C-Methylthalidomide



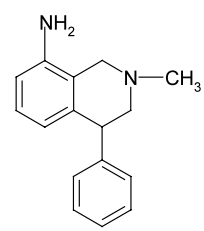
10. Metomidate



11. Miconazole

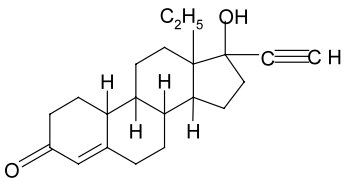


12. Nifurtimox

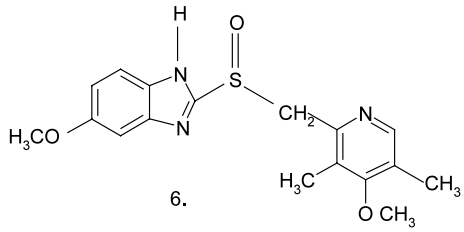


13. Nomifensine

Fig. 1. Structure of chiral analytes.

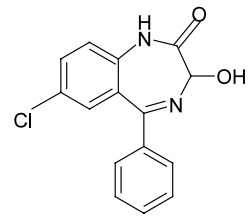


14. Norgestrel

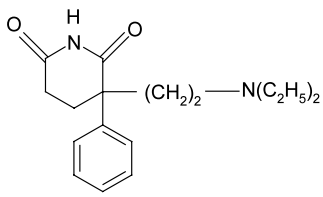


6.

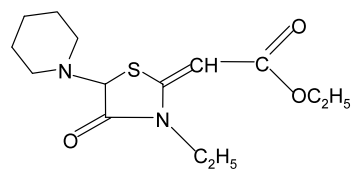
15. Omeprazole



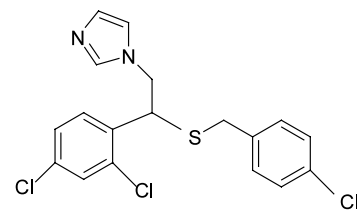
16. Oxazepam



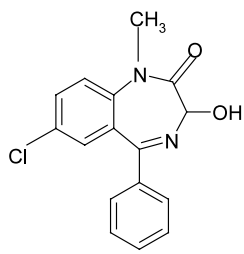
17. Phenylglutaramide



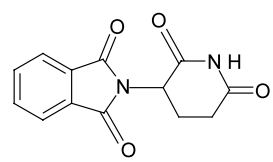
18. Piprozolin



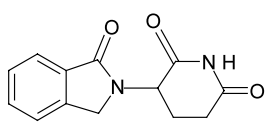
19. Sulconazole



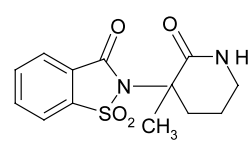
20. Temazepam



21. Thalidomide



22. Thalidomide analog (EM-12)



23. Thalidomide analog (EM-310)

Fig. 1. (*Continued*)

1. Introduction

Polysaccharide derivatives belong to the most widely used chiral stationary phases (CSP) for High performance liquid chromatography (HPLC) enantioseparations [1,2]. These materials are very useful for analytical [1–6] and preparative scale [1,2,7–10] enantioseparations. On the analytical scale polysaccharide type CSPs can be successfully used not only in the common size HPLC columns but

also in miniaturized techniques such as capillary liquid chromatography and capillary electrochromatography [11–16].

Polysaccharide phenylester and phenylcarbamate derivatives were originaly proposed as CSP for normal-phase HPLC [1–7]. Later, it was shown that these materials can also effectively be used in reversed-phase chromatographic mode with aqueous-organic mobile phases [17–19]. Although reverse and normal phase modes cover almost completely the potential applications of CSPs in HPLC, the polar organic mode becomes increasingly popular for various CSPs during last few years. Pure polar organic eluents may offer the advantages of alternative chiral recognition mechanisms, higher solubility of some analytes, be cheaper and easier to be removed from the analytes. These properties may appear especially useful in preparative scale applications of HPLC or simulating moving bed (SMB) chromatography.

Although, some previous studies indicate the applicability of polasaccharide type CSPs in combination with polar organic solvents [19–26], no systematic comparative study for the chiral analytes of various structural and pharmacological groups has been reported.

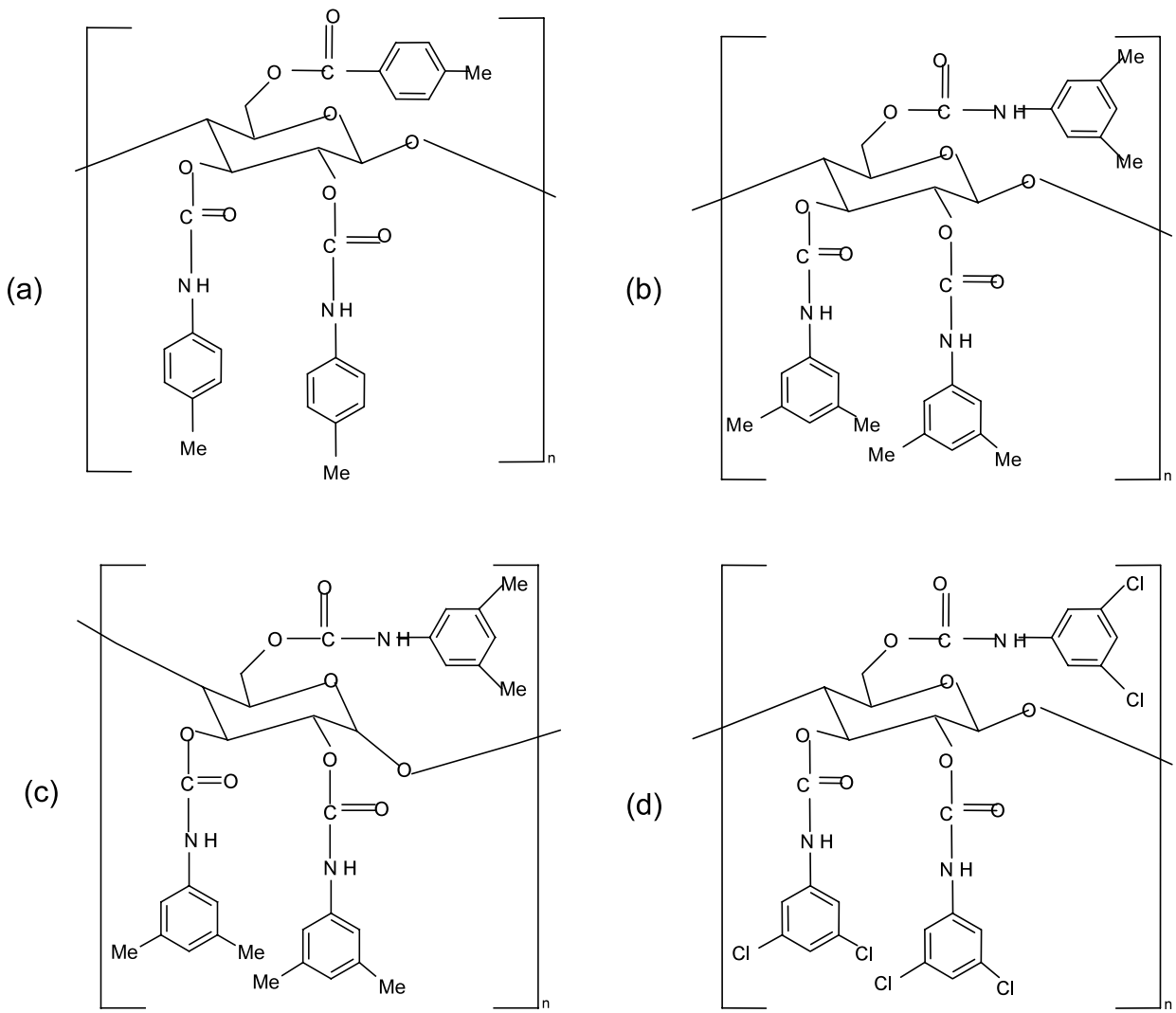


Fig. 2. Structure of cellulose (a, b, d) and amylose (c) derivatives used as chiral selectors in this study.

Table 1
Enantioseparation of selected chiral drugs and drug analogs on Chiralcel-OJ column using pure methanol as a mobile phase

Analyte	MEOH			
	k'_1	k'_2	α	R_s
Ambucetamide	0.10	0.41	4.10	–
2-Benzylsulfinylbenzoic acid benzyl ester	0.91	1.12	1.23	1.0
Enilconazole	1.17	1.17	1.00	–
Etozolin	0.98	7.40	7.55	4.0
Lorazepam	0.44	0.44	1.00	–
C-methylthalidomide	0.69	0.69	1.00	–
Metomidate	1.20	1.20	1.00	–
Miconazole	1.32	2.15	1.63	0.6
Nifurtimox	1.53	2.25	1.47	1.0
Nomifensin	0.19	0.60	3.00	1.2
Norgestrel	0.22	0.83	3.75	1.5
Omeprazole	0.26	0.26	1.00	–
Oxazepam	0.20	0.20	1.00	–
Piprozolin	0.69	4.93	7.14	4.0
Sulconazole	1.92	1.92	1.00	–
Temazepam	2.86	2.86	1.00	–
Thalidomide	0.65	0.98	1.50	0.5
Thalidomide analog, EM-310	0.57	0.73	1.28	–

Table 2
Enantioseparation of selected chiral drugs and drug analogs on Chiralcel-OD column using pure methanol or acetonitrile as a mobile phase

Analyte	MEOH				ACN			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
Ambucetamide	0.11	0.33	3.00	0.8	0.15	1.20	8.02	5.0
2-Benzylsulfinylbenzamide	0.81	1.62	2.00	2.2	0.82	1.55	1.89	2.5
2-Benzylsulfinylbenzoic acid benzyl ester	1.40	1.57	1.12	0.8	0.15	0.15	1.00	–
2-Phenylsulfinylethylamide					0.15	0.15	1.00	–
Enilconazole					0.68	0.68	1.00	–
Etozolin	0.76	1.05	1.38	1.2	0.12	0.23	1.88	0.6
Lorazepam	0.34	0.34	1.00	–	1.72	2.49	1.45	0.5
C-methylthalidomide	0.47	1.70	3.60	4.0	0.20	0.3	1.50	0.5
Metomidate	0.30	0.30	1.00	–	0.12	0.12	1.00	–
Miconazole	1.09	1.16	1.07	–				
Nifurtimox	0.68	0.74	1.08	–				
Norgestrel	0.41	0.41	1.00	–	2.47	2.47	1.00	–
Oxazepam	0.40	1.11	2.77	0.59				
Piprozolin	0.66	1.41	2.14	3.5				
Sulconazole	0.81	0.81	1.00	–	1.55	1.72	1.11	0.5
Temazepam	0.41	0.50	1.23	0.5	0.37	0.55	1.49	1.0
Thalidomide	0.65	0.73	1.13	0.4				
Thalidomide analog, EM-12	0.23	0.29	1.28	–				
Thalidomide analog, EM-310	1.00	1.00	1.00	–	0.30	0.46	1.56	1.1

The goal of the present study was to evaluate the potential of three most commonly used commercially available polysaccharide type CSPs such as Chiralcel-OJ, Chiralcel-OD and Chiralpak-AD, and one less studied cellulose derivative, namely cellulose tris(3,5-dichlorophenylcarbamate) (CD-CPC) in the enantioseparation of randomly selected chiral drugs using pure methanol and acetonitrile as mobile phases.

2. Experimental

2.1. Materials and reagents

Chiral drugs and drug analogs (Fig. 1) were from various commercial sources and used without any further purification. Microcrystalline cellulose (Avicel), HPLC grade methanol and acetonitrile were from Merck (Darmstadt, Germany). 3,5-Dichlorophenylisocyanate, pyridine and tetrahydrofuran were from Aldrich (Deisenhofen, Germany). Macroporous silica gel Daisogel SP-2000 with the pore size of 200 nm and particle diameter 7 μm was from Daiso (Osaka, Japan).

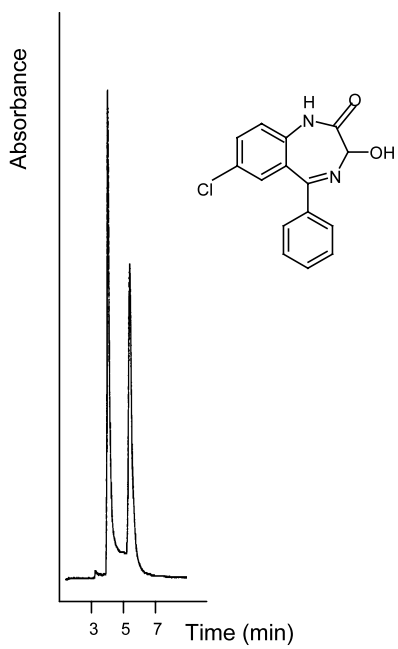


Fig. 3. Enantioseparation of oxazepam on Chiralcel-OD column using pure methanol as a mobile phase.

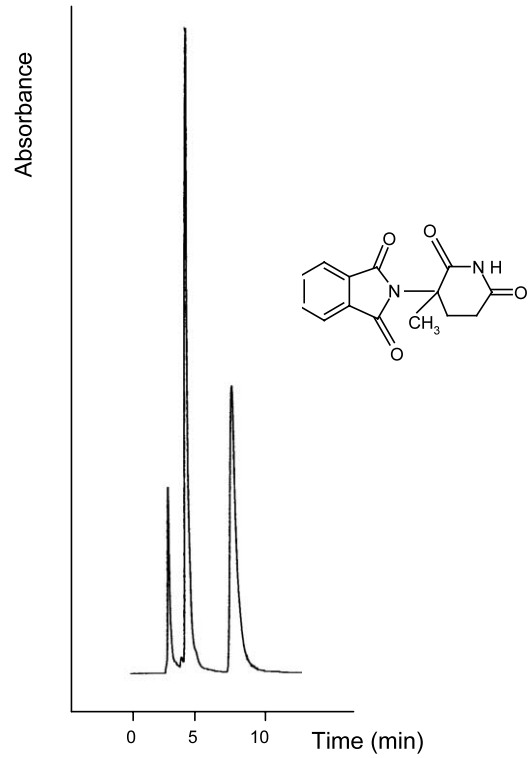


Fig. 4. Enantioseparation of C-methylthalidomide on Chiralcel-OD column using pure methanol as a mobile phase.

2.2. Preparation of HPLC column containing DCPC

CDCPC was synthesized as previously described [5,24–26] by the reaction of microcrystalline cellulose (Avicel) with an excess of 3,5-dichlorophenylisocyanate in dry pyridine at approximately 100 °C overnight and isolated as a methanol insoluble fraction. Elemental analysis, IR and ^{1}H-NMR spectra showed that hydroxyl groups of cellulose were almost completely converted into the carbamate moieties.

Column packing material was prepared as described previously [5,24–26] by dissolving CDCPC in tetrahydrofuran and coating on the macroporous silica gel Daisogel SP-2000. The packing

material was packed by conventional high-pressure slurry packing technique into the stainless-steel column of 250 × 4.6 mm size.

2.3. High performance liquid chromatography (HPLC)

HPLC enantioseparations were performed on the system consisted of isocratic Knauer HPLC pump 64 (Knauer, Berlin, Germany), Merck Hitachi 655 A variable wavelength UV monitor and Merck Hitachi D-2500 chromato-integrator (Merck, Darmstadt, Germany). The 250 × 4.6 mm stainless steel columns under the commercial names Chiralcel-OJ, Chiralcel-OD and Chiralpak-AD (Fig. 2) were from Daicel Chemical Ind. (Tokyo, Japan). The mobile phases were pure methanol or acetonitrile with a flow rate 1 ml/min. The detection was performed at 254 nm.

3. Results and discussion

Successful enantioseparations using Chiralcel-OJ column in combination with pure polar organic mobile phases have been reported previously [22,23]. However, as it has been prevailed in a recent study [26], this material is less universal CSP in combination with pure methanol, ethanol or 2-propanol as mobile phases compared to the phenylcarbamate derivatives of polysaccharides. In Ref. [26] only few members of chiral sulfoxides were studied with the sulfur atom as the centre of chirality. The results of the more extensive screening performed in this study are in good agreement with the previous results. Thus, from 18 analytes eight were not resolved at all and a baseline enantioseparation was observed only for four compounds (Table 1). For some compounds (thalidomide and analog, miconazole,

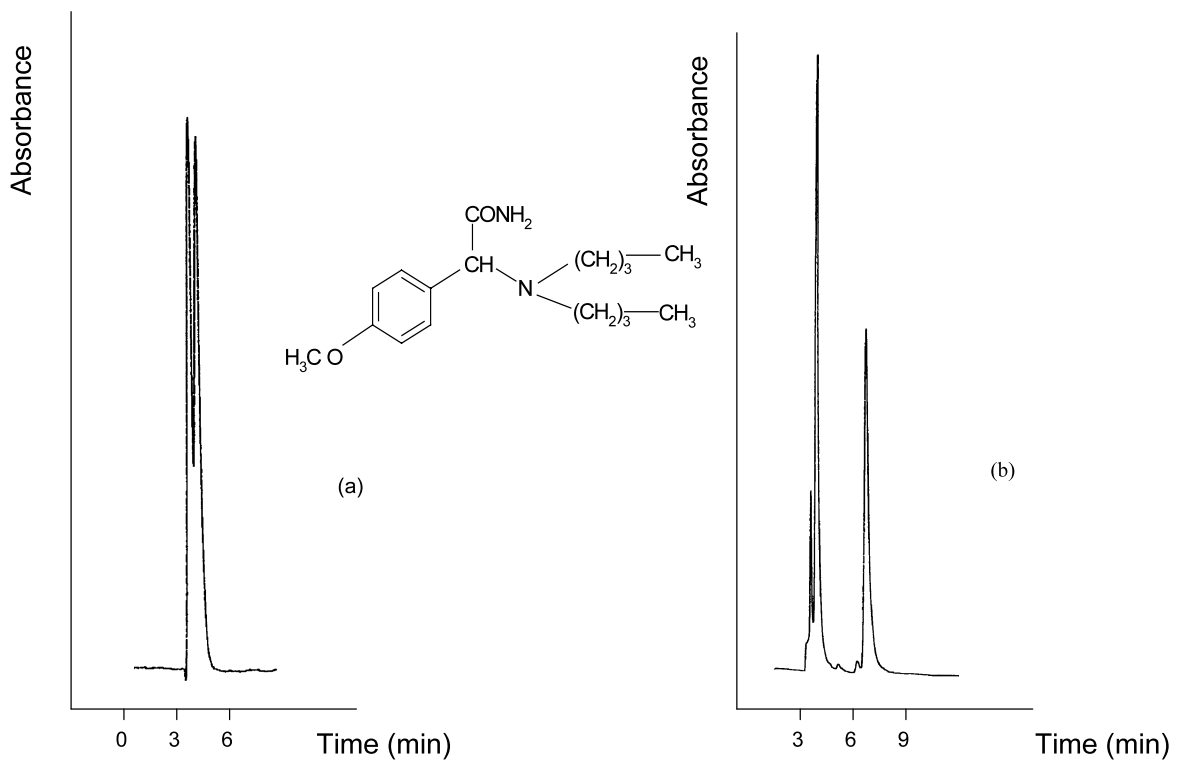


Fig. 5. Enantioseparation of ambucetamide on Chiralcel-OD column using methanol (a) and acetonitrile (b) as mobile phases.

Table 3
Enantioseparation of selected chiral drugs and drug analogs on Chiralpak-AD column using pure methanol or acetonitrile as a mobile phase

Analyte	MEOH				ACN			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
Ambucetamide	0.31	0.44	1.42	1.00	2.79	4.02	1.44	1.5
2-Benzylsulfinylbenzamide	0.53	0.61	1.15	0.40	0.82	3.93	4.80	3.5
2-Benzylsulfinylbenzoic acid benzyl ester	1.10	1.34	1.22	1.5	0.75	1.21	1.61	1.0
2-Phenylsulfinylethylamide	0.52	0.52	1.00	–	1.85	1.85	1.00	–
Enilconazole	0.49	0.49	1.00	–	0.76	0.76	1.00	–
Etozolin	1.60	5.56	3.47	7.9	0.32	0.45	1.40	1.2
Lorazepam	0.48	0.53	1.11	–	0.75	1.27	1.70	0.6
C-methylthalidomide	4.17	8.44	2.02	3.0	4.46	6.74	1.51	1.5
Metomidate	0.70	1.09	1.56	3.0	0.14	0.14	1.00	–
Miconazole	0.26	0.34	1.32	0.4	1.15	2.33	2.03	1.2
Nifurtimox	3.35	4.09	1.22	1.5	0.10	0.22	2.20	1.2
Norgestrel	0.23	0.23	1.00	–	0.82	0.82	1.00	–
Omeprazole	1.89	5.68	3.00	5.0	6.02	9.43	1.57	1.2
Oxazepam	0.61	1.15	1.88	2.0	1.40	1.40	1.00	–
Piprozolin	0.89	2.11	2.37	5.0	0.14	0.22	1.57	0.3
Sulconazole	1.06	1.20	1.14	0.6	1.67	1.67	1.00	–
Temazepam	1.11	1.11	1.00	–	0.86	1.17	1.36	1.0
Thalidomide	2.68	7.74	2.89	9.0	1.22	1.34	1.10	–
Thalidomide analog, EM-12	0.99	1.62	1.64	2.5	0.09	1.32	14.62	6.0
Thalidomide analog, EM-310	0.71	1.10	1.54	2.5	0.94	2.21	2.35	2.5

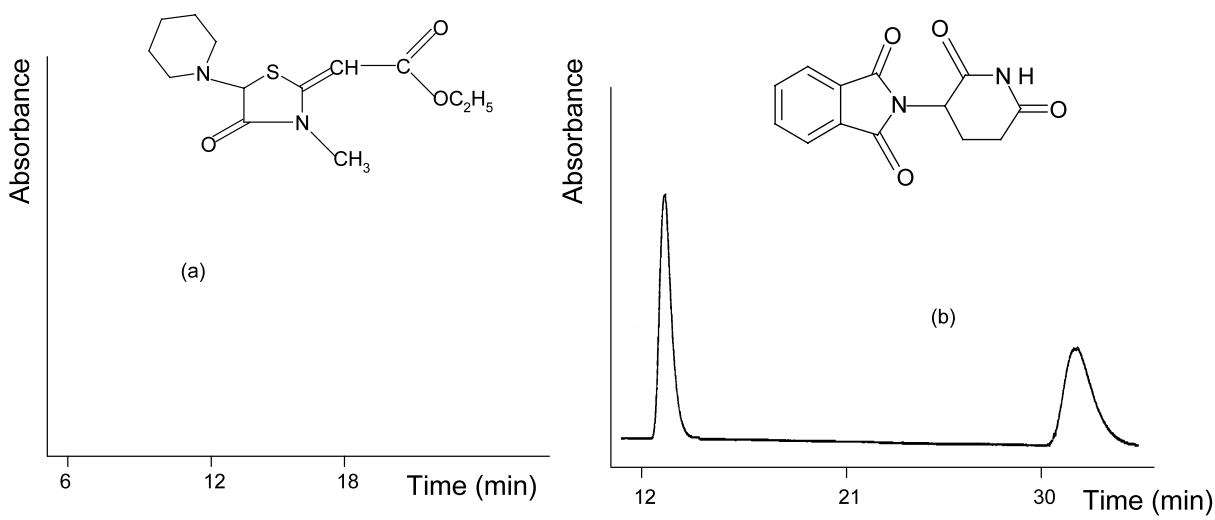


Fig. 6. Enantioseparation of etozolin (a) and thalidomide (b) on Chiralpak-AD column using methanol as a mobile phase.

nifurtimox) separation factors were rather high but low peak efficiency did not enable a baseline enantioseparation to be observed. Although the enantioseparation factor for ambucetamide was rather high on OJ column, this did not allow significant enantioseparation due to extremely low retention factor ($k' = 0.10$). The results of enantioseparation with pure acetonitrile as a mobile

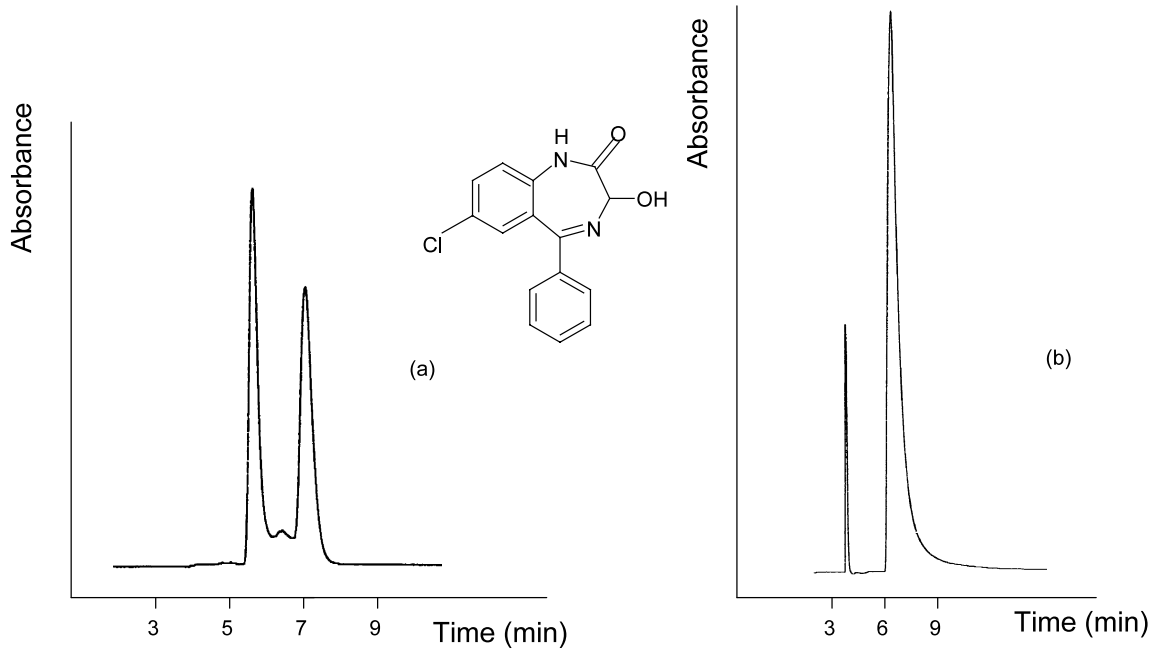


Fig. 7. Enantioseparation of oxazepam on Chiralpak-AD column using methanol (a) and acetonitrile (b) as mobile phases.

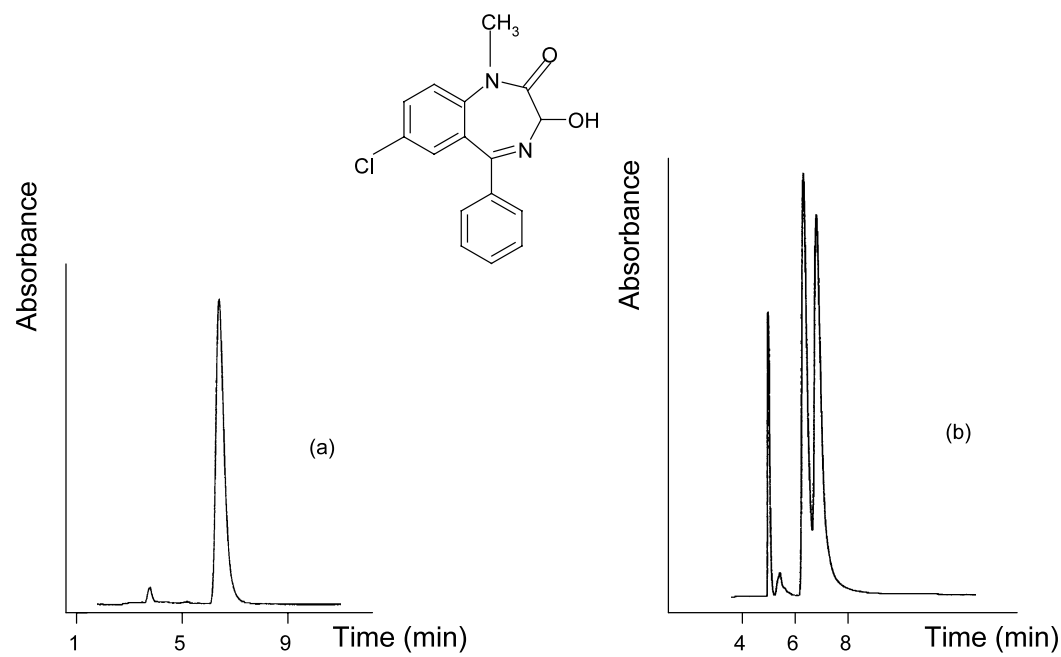


Fig. 8. Enantioseparation of temazepam on Chiralpak-AD column using methanol (a) and acetonitrile (b) as mobile phases.

Table 4
Enantioseparation of selected chiral drugs and drug analogs on CDCPC using methanol or acetonitrile as a mobile phase

Analyte	MEOH				ACN			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
Ambucetamide					1.43	8.26	5.78	5.4
2-Benzylsulfinylbenzamide	0.81	2.69	3.32	6.4	1.46	18.68	13.44	15
2-Benzylsulfinylbenzoic acid benzyl ester	0.33	0.66	2.00	2.0	0.98	1.58	1.61	2.0
2-Phenylsulfinylethylamide	0.33	0.42	1.28	0.9	2.23	3.25	1.46	1.2
Diclofensine					0.35	0.47	1.33	0.6
Enilconazole	0.63	0.63	1.00	–	3.32	3.97	1.19	0.7
Etozolin	0.90	2.13	2.36	5.0	0.42	0.59	1.41	1.1
Lorazepam					0.50	0.65	1.30	0.8
C-methylthalidomide	0.60	1.22	2.03	1.8	0.30	0.70	2.32	2.0
Metomidate	0.34	0.34	1.00	–	0.75	0.96	1.27	0.6
Miconazole	2.35	2.35	1.00	–	6.73	8.07	1.20	1.0
Nifurtimox	1.21	1.21	1.00	–				
Nomifensin	1.77	1.77	1.00	–	1.60	1.80	1.12	–
Norgestrel	1.27	1.70	1.34	1.7	1.71	2.08	1.22	1.1
Omeprazole	0.72	1.24	1.72	2.5	2.60	3.14	1.21	0.7
Oxazepam	0.32	0.49	1.54	1.0	0.63	1.16	1.84	1.1
Piprozolin	0.75	1.63	2.2	4.7	0.33	0.57	1.72	1.5
Phenylglutarimide					1.34	1.57	1.17	0.5
Sulconazole	0.63	0.63	1.00	–	4.40	5.14	1.17	0.8
Temazepam	1.38	1.63	1.18	1.0	1.16	1.94	1.68	2.5
Thalidomide	0.95	1.11	1.17	1.1	0.33	0.43	1.30	0.6
Thalidomide analog, EM-12	0.99	2.57	2.59	5.2	0.88	1.30	1.47	1.5
Thalidomide analog, EM-310	0.48	0.73	1.54	1.5	0.59	1.40	2.38	2.2

phase were even worse and low enantioselectivity with very low retention factors (0.15–0.20) were observed only in the case of etozolin and piprozolin (data not shown in the Table 1).

Chiralcel-OD column exhibited somewhat higher chiral recognition ability in both pure methanol and acetonitrile compared to Chiralcel-OJ column. Thus, from 17 chiral analytes examined using methanol as a mobile phase enantioselectivety was observed for 12 (Table 2). However, a baseline enantioseparation could be observed only for few analytes. Chiral diazepine derivative oxazepam was resolved with relatively high enantioseparation factor but the enantiomerization was observed on the column at the room temperature (Fig. 3). One of the thalidomide analogs, C-methylthalidomide could be resolved with relatively high resolution on Chiralcel-OD column using methanol as a mobile phase (Fig. 4).

Acetonitrile in general was less suitable compared to methanol as a mobile phase with Chiralcel-OD material. However, some complementary enantioseparations could be found with these two mobile phases. Thus, for instance, oxazepam was resolved when methanol was used, but not resolved when acetonitrile was used as a mobile phase. The opposite effect was observed for other compounds. Thus, some of the thalidomide analogs and sulconazole were not recognized enantioselectively in methanol but recognized in acetonitrile. This effect was very significant in the case of ambucetamide. The enantiomers of this analyte were hardly resolved with Chiralcel-OD column in methanol (Fig. 5a) but very good enantioseparation was observed in acetonitrile (Fig. 5b).

Chiralpak-AD column which contains the same phenylcarbamate moiety as Chiralcel-OD column but attached to the amylose backbone instead of cellulose, exhibited rather high enantiomer resolving ability compared to its cellulosic analog (Table 3). Thus, from 20 chiral analytes examined no enantioselectivity was observed only for four in

methanol (2-phenylsulfinylethylamide, enilconazole, norgestrel, temazepam) and for six (2-phenylsulfinylethylamide, enilconazole, metomidate, norgestrel, oxazepam, sulconazole) in acetonitrile (Table 3). High enantioselectivites and resolution factors were observed for several chiral analytes (etozolin, piprozolin, omeprazole, and especially, thalidomide) in methanol (Fig. 6). Methanol appeared to be more suitable mobile phase compared to acetonitrile. Thus, for example, the enantiomers of oxazepam were resolved on Chiralpak-AD column (again the enantiomerization was observed) in methanol (Fig. 7a) but no enantioseparation was observed in acetonitrile (Fig. 7b). In contrast to this, other diazepine derivative, temazepam was resolved into enantiomers in acetonitrile (Fig. 8b) but no enantioseparation at all was observed in methanol (Fig. 8a). From the CSPs tested, CDCPC exhibited comparable enantiomer resolving ability to Chiralpak-AD column in methanol and markedly better properties compared to all chiral columns studied with acetonitrile as a mobile phase. Thus, from 19 chiral analytes examined the enantiomers of 13 were resolved in methanol and from 22 analytes examined the enantiomers of all 22 were resolved in acetonitrile with CDCPC column (Table 4). At this stage it was difficult to find any clear structure-retention or -separation factor dependencies. On the same column, some of the analytes were longer retained in methanol and others in acetonitrile as a mobile phase. The same applied to the separation factor. This finding is in accordance with previous results [26] and indicate that the intermolecular forces involved in analyte retention and enantioseparation are multivariate. Thus, the behavior of the separation system depends not only on the mobile and stationary phase but also on the nature of the analyte.

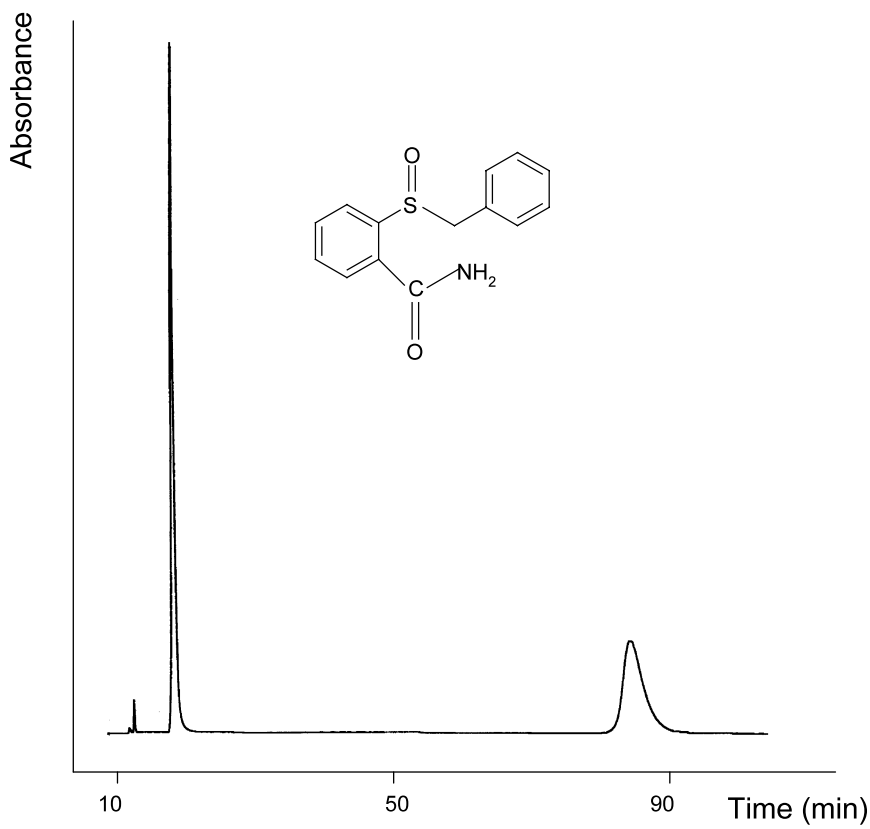


Fig. 9. Enantioseparation of (2-behzylsulfinyl)-benzamide on CDCPC using pure acetonitrile as a mobile phase.

The enantioseparation of 2-(benzylsulfinyl)benzamide is shown in Fig. 9 in order to illustrate the potential of CDCPC as a powerful CSP for HPLC enantioseparation in polar organic mode.

References

[1] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. 37 (1998) 1020–1043.
[2] E. Yashima, C. Yamamoto, Y. Okamoto, Synlett (1998) 344–360.
[3] Y. Okamoto, M. Kawashima, K. Yamamoto, K. Hatada, Chem. Lett. (1984) 739–742.
[4] A. Ichida, T. Shibata, I. Okamoto, Y. Yuki, H. Namikoshi, Y. Toda, Chromatographia 19 (1984) 280–284.
[5] Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr. 363 (1986) 173–186.
[6] Y. Okamoto, R. Aburatani, T. Fukumoto, K. Hatada, Chem. Lett. (1987) 1857–1860.
[7] E. Francotte, J. Chromatogr. A 576 (1992) 1–45.
[8] J. Dingenen, J.N. Kinkel, J. Chromatogr. 666 (1994) 627–650.
[9] E. Francotte, J. Chromatogr. A 906 (2001) 379–397.
[10] M. Schulte, J. Strube, J. Chromatogr. A 906 (2001) 399–416.
[11] K. Krause, M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 837 (1999) 51–63.
[12] S. Mayer, X. Briand, E. Francotte, J. Chromatogr. A 875 (2000) 331–339.
[13] M. Meyring, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 876 (2000) 157–167.
[14] M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 887 (2000) 439–455.
[15] K. Otsuka, C. Mikami, S. Terabe, J. Chromatogr. A 887 (2000) 457–463.
[16] M. Girod, B. Chankvetadze, Y. Okamoto, G. Blaschke, J. Sep. Sci. 24 (2001) 27–34.
[17] K. Ikeda, T. Hamasaki, H. Kohno, T. Ogawa, T. Matsumoto, J. Sakai, Chem. Lett. (1989) 1089–1090.
[18] A. Ishikawa, T. Shibata, J. Liq. Chromatogr. 16 (1993) 859–878.
[19] K. Tachibana, A. Ohnishi, J. Chromatogr. A 906 (2001) 127–154.
[20] A.M. Krstulovic, G. Rossey, J.P. Porziemsky, D. Long, I. Chekrum, J. Chromatogr. 411 (1987) 461–465.
[21] H.Y. Aboul-Enein, V. Serignese, J. Bojarski, J. Liq. Chromatogr. 16 (1993) 2741–2749.
[22] J. Dingenen, in: G. Subramanian (Ed.), A Practical Approach to Chiral Separation by Liquid Chromatography, VCH, New York, 1994, pp. 115–181 Chapter 6.
[23] C. Weinz, G. Blaschke, H.M. Schiebel, J. Chromatogr. B 690 (1997) 233–242.
[24] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Chem. Lett. (2000) 1776–1777.
[25] B. Chankvetadze, C. Yamamoto, Y. Okamoto, Comb. Chem. High Troughput Screening (CC&HTS) 3 (2000) 497–508.
[26] B. Chankvetadze, C. Yamamoto, Y. Okamoto, J. Chromatogr. A 922 (2001) 127–137.