ELSEVIER

Journal of Chromatography A, 844 (1999) 137–147

JOURNAL OF CHROMATOGRAPHY A

Enantioseparation of semisynthetic ergot alkaloids on vancomycin and teicoplanin stationary phases

Eva Tesařová[a,*], Kamil Záruba[b], Miroslav Flieger[c]

[a] *Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, 12800 Prague 2, Czech Republic*
[b] *Department of Analytical Chemistry, Institute of Chemical Technology, 16628 Prague 6, Czech Republic*
[c] *Institute of Microbiology, Academy of Sciences of the Czech Republic, 14220 Prague 4, Czech Republic*

Received 26 June 1998; received in revised form 2 March 1999; accepted 4 March 1999

Abstract

The macrocyclic antibiotics, vancomycin and teicoplanin, were used as chiral stationary phase selectors for the enantioselective separation of semisynthetic ergot alkaloids in reversed-phase high-performance liquid chromatography (RP-HPLC). The chromatographic behavior of the ergot preparations was investigated in order to obtain a deeper insight into the enantiodiscriminative process. A variety of factors, including mobile phase parameters such as the nature and concentration of the organic modifier, buffer concentration and pH, were examined. Conditions for the enantioseparation of real pharmaceutical preparations, i.e. lisuride, terguride and nicergoline, were found. Differences in the chiral stationary phases are presented and the interaction mechanism is discussed.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Antibiotics; Ergot alkaloids; Vancomycin; Teicoplanin; Lisuride; Terguride; Nicergoline

1. Introduction

Among numerous structurally different groups of pharmacologically active compounds, ergot alkaloids (EAs) [1,2] have an important place. The wide range of biological activities of EAs was explained on the basis of their structural similarity with mediators of neurotransmission [3]. Their actions are mediated by adrenergic [4], dopaminergic [5] or serotonergic receptors [6]. Among the numerous semisynthetic ergot preparations, nicergoline (α-adrenergic blocking agent [7]), lisuride (serotonin antagonist) and terguride (mixed D_2 agonist/antagonist of the pituitary [8] and central nervous system [9]) show considerable activity. Lisuride was introduced for the treatment of migraines and Parkinson's disease [10].

Considering the fact that the biological and pharmacological activities of drug components are strongly related to their molecular configuration [11], there is considerable interest in analytical methods that allow the determination of the enantiomeric composition of drugs and/or their metabolites.

So far, only a few reports have dealt with the enantioseparation of ergot alkaloids. One of them is a capillary electrophoretic method using native or modified β- and γ-cyclodextrins as chiral selectors (CSs) added to the background electrolyte [12]. A high-performance liquid chromatography (HPLC)

*Corresponding author. Tel.: +420-2-21952606; fax: +420-2-291958.
E-mail address: tesarove@natur.cuni.cz (E. Tesařová)

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00339-8

method on a ''tailor-made'' chiral column with 1-(3-amino propyl)-(5*R*,8*S*,10*R*)-terguride [13] and 1-allyl-(5*R*,8*S*,10*R*)-terguride [14] as chiral selectors was also successfully used for the separation of the previously mentioned semisynthetic ergot preparations.

Macrocyclic antibiotics (MAs) represent a recent class of powerful chiral selectors [15,16]. They contain numerous functional groups and many stereogenic centers. These sites offer the possibility of different interactions, which allow the separation of a wide variety of racemic compounds. Different MAs were successfully used for both chromatographic and electrophoretic separations of various types of enantiomers [15–27]. Chiral stationary phases (CSPs) based on the MA chiral selectors, vancomycin and teicoplanin, operate in all chromatographic separation modes, i.e. normal-phase and reversed-phase mode, and with polar–organic mobile phase [16–18].

Vancomycin- and teicoplanin-based CSPs (Chirobiotic V and Chirobiotic T, respectively) were utilized in our previous work for the chiral separation of some ergot alkaloids and blocked amino acids [22–24]. In this study, these MA-based CSPs were used for the enantioseparation of semisynthetic ergot alkaloids in reversed-phase mode. The influence of mobile phase parameters, such as buffer concentration, and the type and amount of organic modifier, on retention and enantioselectivity was studied. The possible mechanism of enantiodiscrimination is discussed.

2. Experimental

2.1. Materials

Some of the pure enantiomers of EAs used in this study were a kind gift from Galena (Opava, Czech Republic), others were prepared from their racemates using a previously described method in a semi-preparative mode [13].

Solvents and chemicals were of the following purity and origin: methanol and 2-propanol (p.a. grade; Penta, Chrudim, Czech Republic); acetonitrile (HPLC purity; Merck, Darmstadt, Germany); triethylamine (purity>99%; Sigma, St. Louis, MO, USA) and glacial acetic acid (p.a. grade; Lachema, Brno, Czech Republic).

2.2. Instrumentation

The HPLC system consisted of a Waters 501 pump (Millipore, Milford, MA, USA), an Ecom injection valve (Ecom, Prague, Czech Republic) and a variable wavelength UV detector (Knauer, Berlin, Germany). Data were processed on a PC Watrex 286 (Watrex, Prague, Czech Republic) using APEX 3.1. integration software (APEX, Prague, Czech Republic). The chiral columns used were Chirobiotic V and Chirobiotic T (Astec, Whippany, NY, USA), 250× 4.6 mm I.D., particle size 5 μm.

2.3. HPLC procedure

Sample solutions (0.5 mg/ml) were prepared by dissolving the individual enantiomers in methanol. Volumes (2–5 μl) of these solutions or their mixtures were injected into a chromatographic column.

Mobile phases were prepared by mixing a 0.1 or 1.0% solution of triethylamine in water (pH was adjusted to 4.0 with acetic acid) with appropriate volumes of organic modifiers (methanol, 2-propanol, acetonitrile). Buffer solutions were prepared in deionized water and filtered through 0.45 μm filters before use. Mobile phases were degassed by sonication.

The flow-rate of the mobile phases was 0.5 ml/min unless otherwise indicated. Detection was performed at 250 nm. Experiments were carried out at 24°C.

The void retention times were determined with KI solution (1 mg/ml). They were 4.20 and 4.05 min for the Chirobiotic V and Chirobiotic T columns, respectively (both measured at a flow-rate of 0.5 ml/min).

3. Results and discussion

3.1. Influence of mobile phase composition on retention and enantioresolution

Enantiomers of the pharmaceutical preparations of the ergot alkaloids nicergoline, lisuride and terguride,

and of one of the semisynthetic precursors, meluol (Fig. 1) were separated on two chiral stationary phases based on the macrocyclic antibiotics teicoplanin and vancomycin (Fig. 2), in reversed-phase separation mode.

One of the factors influencing enantioseparation is the pH of the mobile phase. It affects protonation of the basic nitrogen of the ergoline skeleton. It also affects the protonation and conformation of the chiral selectors used [18,21,23]. To keep these parameters

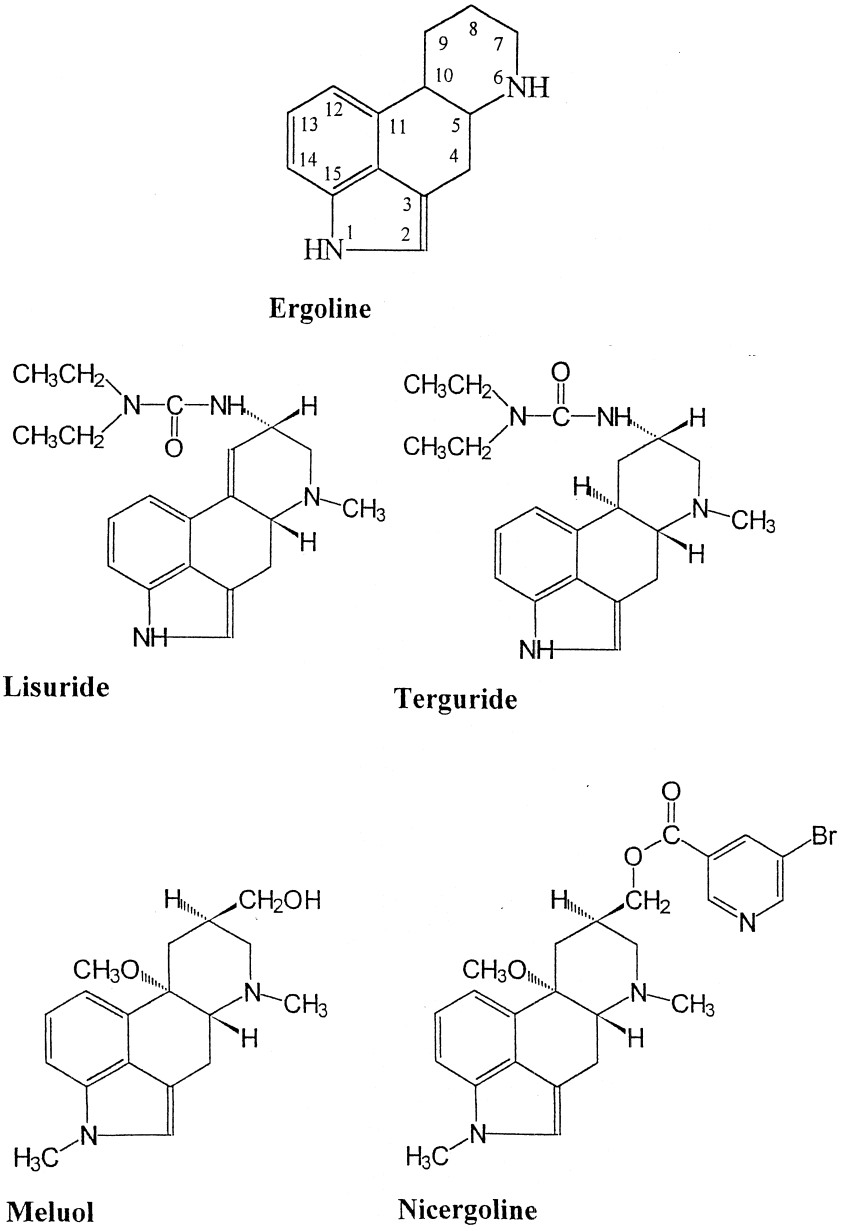

Fig. 1. Structures of the ergoline skeleton and ergot drugs used in this study.

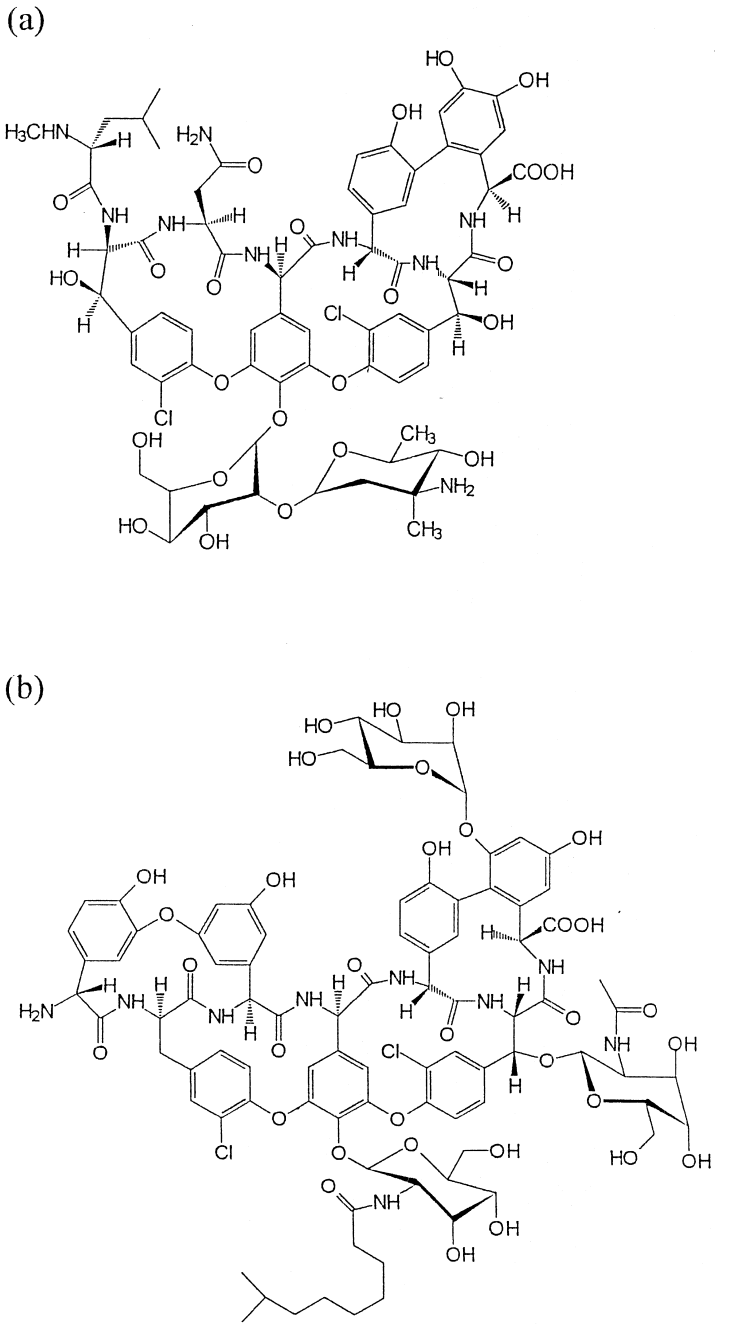

Fig. 2. Structures of the macrocyclic antibiotics vancomycin (a) and teicoplanin (b).

constant, we worked at a constant pH value of 4.0. (Our preliminary results have shown that enantioseparation at pH 6.0 was less efficient.)

Enantioseparation is markedly influenced by the type and concentration of the organic modifier (OM). Three organic modifiers, i.e. methanol (MeOH), acetonitrile (ACN) and 2-propanol (IPA), were used to study their influence on enantioseparation.

The chromatographic data (capacity factor of the first eluted enantiomer, k_1; separation factor, α; resolution, R) obtained on a vancomycin-based CSP are summarized in Table 1. Mobile phases consisted of 0.1% triethylamine acetate buffer (TEAA) and different portions of methanol and acetonitrile. The lowest retention of lisuride, terguride and meluol (but not for nicergoline) was obtained at a buffer–MeOH ratio of around 1:1. This indicates that these three compounds have similar mechanism(s) of interaction with the CSP, in contrast to nicergoline.

The addition of triethylamine acetate buffer was associated with lower enantioresolution and enantioselectivity. Replacement of methanol with acetonitrile led to peak shape improvement, a reduction of capacity factors, but also to deterioration of chiral resolution, as illustrated for lisuride in Fig. 3.

Conditions for the baseline separation of all of the substances under study were found. The best separations of lisuride, terguride and meluol enantiomers on vancomycin CSP were achieved in a mobile phase consisting of pure methanol. The possibility of obtaining enantioseparation with this easy-to-prepare mobile phase is advantageous for practical applications.

A more detailed study was performed on a teicoplanin-based CSP (Table 2). The plot of capacity factors versus methanol concentration for lisuride, terguride and meluol (Fig. 4) gave the same type of profiles (U-shaped curves). Nicergoline again demonstrated an exceptional behavior due to its different structure. Meluol, the least basic, with the best accessible stereogenic center, yielded the best enantiomeric resolution from the studied set of EAs on the teicoplanin CSP.

The influence of buffer concentration (1 and 0.1% TEAA) in the mobile phase containing MeOH as an organic modifier was determined for all EAs under study. As a consequence of increasing the buffer concentration, shorter retention times (lower capacity factors) were observed for all of these basic compounds. The influence on resolution of individual

Table 1
Retention data of ergot alkaloids on Chirobiotic V CSP[a]

Solvent	Conc. of solvent (%)	Retention parameter	Compound			
			Lisuride	Terguride	Meluol	Nicergoline
MeOH	100	k_1	9.11	11.55	4.10	0.64
		α	1.24	1.30	1.83	1.00
		R	2.42	2.38	2.29	0.00
	60	k_1	6.49	7.00	3.43	3.49
		α	1.26	1.21	1.10	1.11
		R	2.15	1.68	1.24	1.19
	40	k_1	12.80	5.46	5.32	5.28
		α	1.26	1.08	1.12	1.11
		R	1.77	0.48	1.37	1.23
	20	k_1	16.80	28.12	12.50	11.04
		α	1.24	1.16	1.10	1.09
		R	1.93	1.19	0.92	0.62
ACN	70	k_1	5.00	6.80	5.83	6.17
		α	1.06	1.05	1.07	1.00
		R	0.77	0.72	0.76	0.00
	60	k_1	3.37	4.43	3.46	3.61
		α	1.06	1.03	1.04	1.00
		R	0.59	0.37	0.54	0.00

[a] Mobile phase, organic modifier/buffer–0.1% TEAA, pH = 4.0.

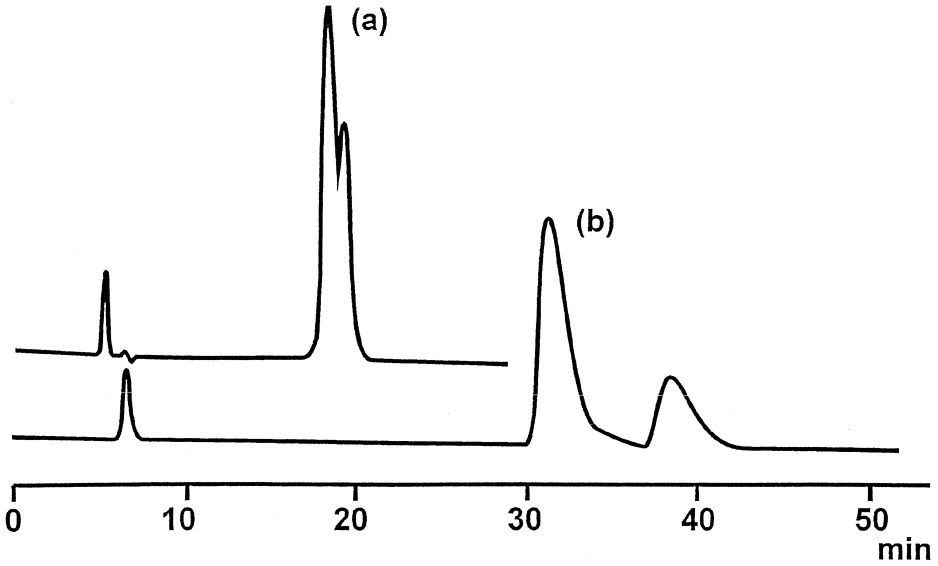

Fig. 3. Enantioseparation of lisuride on a vancomycin-based CSP. Influence of the type of organic modifier on the chiral separation. Conditions: stationary phase, Chirobiotic V; mobile phase, (a) 60% ACN in 0.1% TEAA, pH 4.0, (b) 60% MeOH in 0.1% TEAA, pH 4.0.

enantiomers differs from alkaloid to alkaloid and is strongly dependent on its structure. As an example, the enantioseparation of meluol, is shown in Fig. 5. The presence of triethylamine in the mobile phase reduces the solute–CSP interactions (both the chiral and achiral ones) but to unequal extents. The interaction of ergot alkaloids with CSP in the mobile phase without buffer is so strong that EAs are not eluted from the column even after 5-h runs. This was proved for lisuride in a methanol–water (60:40, v/v) mobile phase (flow-rate, 0.8 ml/min).

The effect of protonation of the studied compounds and of the chiral selector was also examined in a non-aqueous mobile phase consisting of pure methanol and methanol with 1% TEAA, respectively (Table 3). The addition of TEAA caused a significant reduction in retention for all of the compounds and a reduction of the enantioselectivity for lisuride and terguride.

Similar to the results obtained on the vancomycin CSP, the teicoplanin CSP also gave worse enantioresolution when mobile phases containing acetonitrile as an organic modifier (OM) were used (see Table 2). ACN, which is known from RP-HPLC to be a stronger elution agent than methanol, confirmed this behavior only if TEAA buffer was present in the mobile phase. Ergot alkaloids (except for nicergoline) were not eluted from the column with 100% ACN.

The influence of different OMs, i.e., MeOH, ACN and IPA (40% containing 1.0% TEAA), on the retention and enantiomeric resolution of meluol on the teicoplanin CSP is shown in Fig. 6. Most important is the change in the elution order of enantiomers (L-isomer is eluted first) using IPA. This less polar alcohol has a higher affinity for the teicoplanin-based CSP. It changes the accessibility of the interaction sites of teicoplanin (probably due to a change of CS conformation).

Comparison of the retention and enantioseparation of EAs on macrocyclic antibiotic-based CSPs at the same mobile phase composition shows higher affinity of the studied compounds to teicoplanin-bonded CSP. This is, however, not accompanied (except for meluol) by improved enantioresolution on this CSP.

D-Enantiomer was always eluted first on both CSPs. There were only two exceptions, both for meluol: on vancomycin-based CSP with pure methanolic mobile phase, and on teicoplanin CSP if IPA was used as an organic modifier.

Table 2
Retention data of ergot alkaloids on Chirobiotic T CSP[a]

Solvent	Conc.of solvent (%)	Conc. of TEA in buffer (%)	Retention parameter	Compound			
				Lisuride	Terguride	Meluol	Nicergoline
MeOH	100	–	k_1	20.94	24.96	n	0.69
			α	1.10	1.06	–	1.00
			R	1.37	0.93	–	0.00
	60	1.0	k_1	3.99	4.55	2.90	1.07
			α	1.07	1.00	1.19	1.00
			R	1.05	0.00	2.63	0.00
		0.1	k_1	10.57	19.30	11.87	1.11
			α	1.00	1.00	1.18	1.00
			R	0.00	0.00	3.63	0.00
	40	1.0	k_1	5.04	6.90	4.29	2.03
			α	1.08	1.00	1.25	1.00
			R	1.16	0.00	3.00	0.00
		0.1	k_1	7.62	19.47	15.92	2.32
			α	1.53	1.09	1.23	1.00
			R	1.56	0.58	3.76	0.00
	20	1.0	k_1	5.24	11.50	5.66	3.70
			α	1.08	1.03	1.28	1.00
			R	0.79	0.40	3.30	0.00
		0.1	k_1	38.33	45.28	16.65	4.08
			α	1.07	1.02	1.32	1.00
			R	0.49	0.41	4.15	0.00
ACN	100	–	k_1	n	n	n	0.90
			α	–	–	–	1.00
			R	–	–	–	0.00
	70	1.0	k_1	2.66	3.26	2.93	0.48
			α	1.00	1.00	1.09	1.00
			R	0.00	0.00	1.85	0.00
	60	1.0	k_1	2.46	2.83	2.26	0.59
			α	1.00	1.00	1.11	1.00
			R	0.00	0.00	1.96	0.00
	40	1.0	k_1	2.64	2.71	1.87	0.48
			α	1.00	1.00	1.09	1.00
			R	0.00	0.00	1.25	0.00
IPA	60	1.0	k_1	3.96	4.20	3.53	1.17
			α	1.07	1.00	1.07	1.00
			R	0.86	0.00	0.74	0.00

Note: n, not eluted during the measured time (2.5 h).
[a] Mobile phase: organic modifier/TEAA buffer.

3.2. Interaction mechanism

Several simultaneous interactions between the chiral selector and analyte are responsible for the retention and enantioseparation in HPLC [25]. Interactions that only increase the retention but do not enhance the enantiomeric separation can also participate in the interaction mechanism. There is no real correlation between the retention and resolution of EA enantiomers (viz. Tables 1 and 2). This fact indicates that enantioresolution is not dependent on the absolute value of the CS–analyte interaction energies (or association constants) but on their difference for individual enantiomers.

The main factors influencing the chromatographic behavior of the studied ergot alkaloids are: interac-

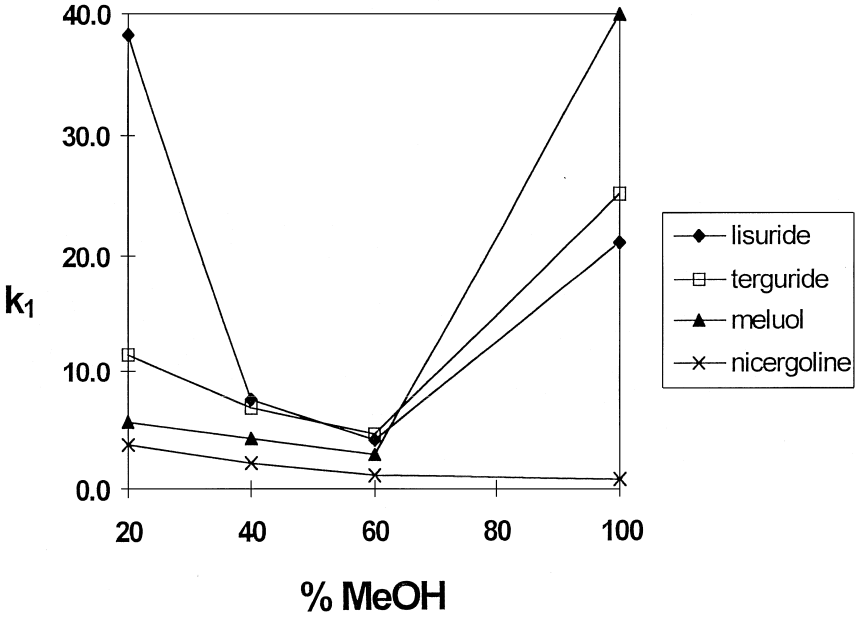

Fig. 4. Dependence of capacity factors on the methanol content in the mobile phase (MeOH and 0.1% TEAA buffer, pH 4.0) on Chirobiotic T CSP.

tions of basic nitrogen of the ergoline skeleton with acidic groups of the chiral selectors, the size and polarity of the substituent in position C(8), and π–π interactions.

On comparing the terguride and lisuride structures, which both have the diethyl–urea substituent in position C(8), it is obvious that the introduction of a double bond to the ergoline skeleton (in close proximity to the asymmetric carbon) enhanced enantioselectivity. It was confirmed by molecular modeling that the stereogenic site C(8) of lisuride is more accessible and the spatial arrangement of the sur-

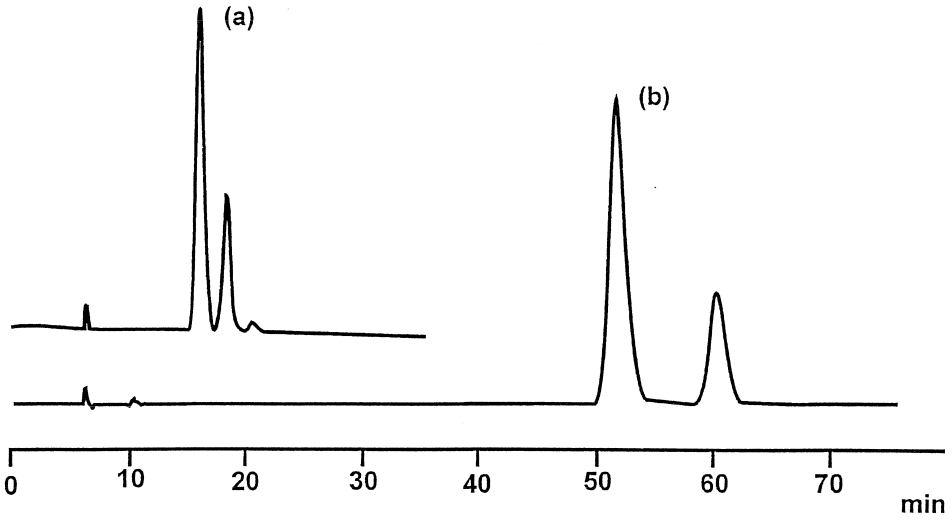

Fig. 5. Effect of buffer concentration on the enantioseparation of meluol. Conditions: stationary phase, Chirobiotic T; mobile phase, (a) 60% MeOH in 1.0% TEAA, pH 4.0 and (b) 60% MeOH in 0.1% TEAA, pH 4.0.

Table 3
Retention data for ergot alkaloids on Chirobiotic T CSP[a]

Mobile phase	Retention parameter	Compound			
		Lisuride	Terguride	Meluol	Nicergoline
100% MeOH	k_1	20.94	24.96	n	0.69
	α	1.10	1.06	–	1.00
	R	1.37	0.93	–	0.00
100% MeOH	k_1	1.74	2.47	2.24	0.09
with	α	1.00	1.00	1.13	1.00
1% TEAA	R	0.00	0.00	2.21	0.00

Note: n, not eluted during the measured time (2.5 h).
[a] Mobile phases: methanol, and methanol with 1% TEAA.

rounding groups is better suited for enantioselective interactions with the CS than in the case of terguride. This is, together with its higher conformational rigidity, the reason lisuride has always been better enantioresolved than terguride on both of the CSPs used.

The influence of the size and polarity of the substituent attached to the stereogenic center of the analyte is obvious from the rather different chromatographic behaviors of meluol and nicergoline. Better accessibility of the stereogenic center of meluol (due to the small hydroxymethyl substituent) makes possible suitable enantioselective interactions (mainly with the teicoplanin selector). Br–nicotinic acid, a substituent of nicergoline, is responsible for repulsive interactions with anionic sites of the chiral selector. This may be the reason for the very low capacity factors of this derivative in almost all of the separation systems studied.

The complementary effect of the enantioseparation abilities of vancomycin and teicoplanin CSPs is shown for examples of enantioresolution of lisuride

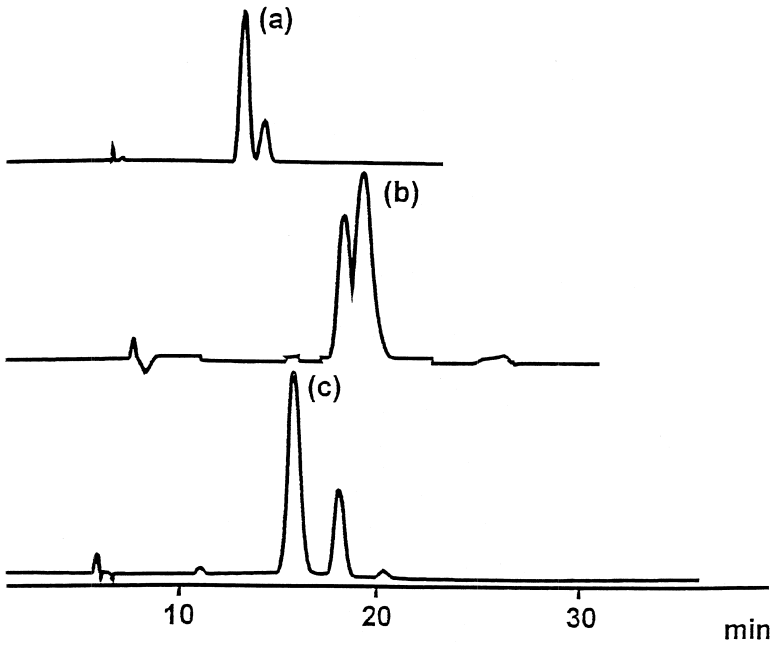

Fig. 6. Effect of different organic modifiers in the mobile phase on the retention and enantioresolution of meluol. Conditions: stationary phase, Chirobiotic T; mobile phase, 1.0% TEAA (pH 4.0): OM = 40:60; OM, (a) acetonitrile, (b) 2-propanol and (c) methanol.

and meluol in Fig. 7. (Composition of the mobile phase was the same in all cases). The primary MA–analyte interactions are similar. They seem to take place on the aglycone part of the CSs, which is similar for both glycopeptides [21]. The substitution and spatial arrangement of sugar moieties and other groups attached on the peptide backbone are different for vancomycin and teicoplanin. This results in their somewhat different enantioselectivities. Moreover, the geometrical arrangement of the whole CS molecule is an important factor [19]. Lisuride, which has a bigger substituent in position C(8), creates much tighter enantioselective interactions in the more opened vancomycin pocket. The closer (C-shaped) structure of teicoplanin allows a better inclusion fit of the small molecule of meluol. These complementary separations on the Chirobiotic V and T columns were observed previously [17,18].

4. Summary

Vancomycin- and teicoplanin-based chiral stationary phases were reported to show high enantioselectivity mainly for acidic and anionic compounds [18,20,21,26]. In this work, these CSPs were also proved to be suitable for the enantioseparation of basic drugs–semisynthetic ergot alkaloids (lisuride, terguride, meluol and nicergoline). An easy-to-prepare separation system, i.e., vancomycin-bonded CSP/methanol, convenient for pharmaceutical applications, was found.

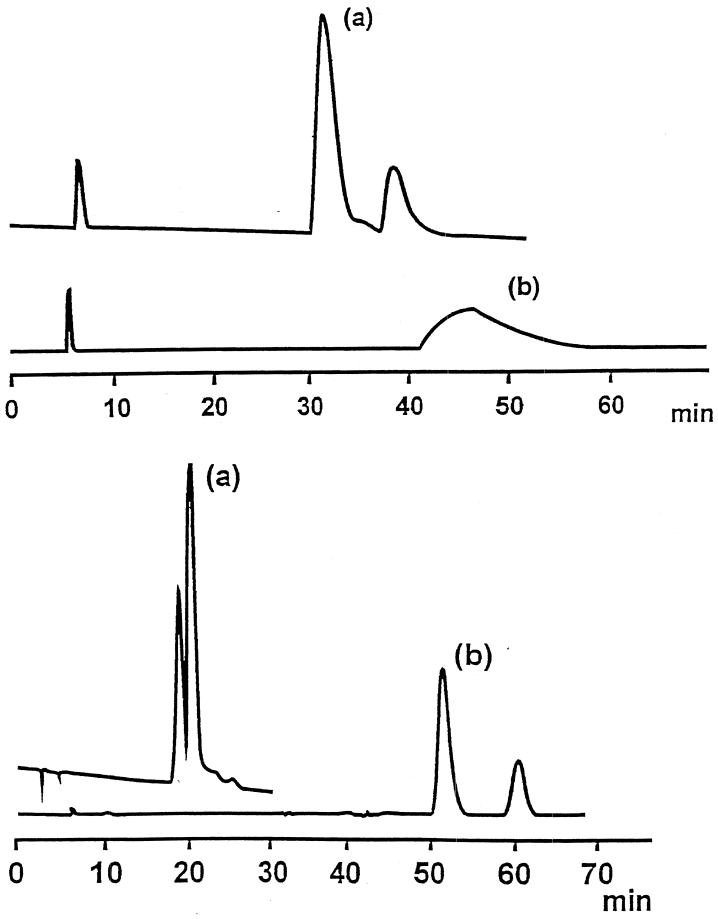

Fig. 7. Complementary separations on vancomycin- and teicoplanin-bonded chiral stationary phases for lisuride (top) and meluol (bottom). Columns: (a) Chirobiotic V and (b) Chirobiotic T. Mobile phase composition, 60% MeOH in 0.1% TEAA, pH 4.0, in all cases.

Despite the similarity among the studied derivatives and the quite similar structures of both of the chiral selectors, different influences of mobile phase composition (the quality and quantity of the organic modifier and the buffer concentration) on the enantioselectivity of individual EAs was observed. These results confirm the complexity of the interaction mechanism that is responsible for the enantioresolution.

Acknowledgements

The authors are grateful to Prof. D.W. Armstrong from the University of Missouri-Rolla who kindly supplied the CSPs, Chirobiotic V and Chirobiotic T. Financial support for this work by the Grant Agency of the Charles University is gratefully acknowledged.

References

[1] Z. Řeháček, P. Sajdl, Ergot Alkaloids, Academia, Prague, 1990.
[2] M. Flieger, M. Wurst, R. Shelby, Folia Microbiol. 42 (1997) 3.
[3] A. Fišerová, H. Kovářů, Z. Hajduová, V. Mareš, M. Starec, V. Křen, M. Flieger, M. Pospíšil, Physiol. Res. 46 (1997) 119.
[4] R.D. Aarons, A.S. Nies, J.G. Gerber, P.B. Molinoff, J. Pharmacol. Exp. Ther. 224 (1983) 1.
[5] B. Deleplaque, S. Vitielo, M. Le-Moal, P.J. Neven, Neurosci. Lett. 166 (1994) 216.
[6] K. Hellstrand, S. Hermodsson, Cell. Immunol. 127 (1990) 199.
[7] G. Arcari, L. Bernardi, G. Bosisio, S. Coda, G.B. Fragman, H.A. Glasser, Experientia 28 (1972) 819.
[8] T. Mizokawa, T. Akai, Y. Nakada, M. Yamaguchi, H. Nakagawa, S. Hasan, K.J. Rattig, H. Wachtel, Jpn. J. Pharmacol. 63 (1993) 269.
[9] Y. Ikoma, T. Akai, Y. Nakada, K. Hara, H. Wachtel, M. Yamaguchi, Folia Pharmacol. Jpn. 102 (1993) 113.
[10] M. Schulzer, E. Mak, D.B. Calne, Ann. Neurol. 32(6) (1992) Dec.
[11] J. Caldwell, J. Chromatogr. A 716 (1996) 3.
[12] S. Fanali, M. Flieger, N. Steinerová, A. Nardi, Electrophoresis 13 (1992) 39.
[13] M. Flieger, M. Sinibaldi, L. Cvak, L. Castellani, Chirality 6 (1994) 549.
[14] A. Messina, A. Girelli, M. Flieger, M. Sinibaldi, P. Sedmera, L. Cvak, Anal. Chem. 68 (1996) 1191.
[15] T.J. Ward, LC·GC Int. 6 (1996) 428.
[16] D.W. Armstrong, Y. Tang, S. Chen, Y. Zhou, Ch. Bagwill, J.-R. Chen, Anal. Chem. 66 (1994) 1473.
[17] D.W. Armstrong, Y. Liu, K.H. Ekborg-Ott, Chirality 7 (1995) 474.
[18] Chirobiotic Handbook, Advanced Separation Technologies, Whippany, NY, 1996.
[19] M.P. Gasper, A. Berthold, U.B. Nair, D.W. Armstrong, Anal. Chem. 68 (1996) 2501.
[20] A. Berthold, Y. Liu, Ch. Bagwill, D.W. Armstrong, J. Chromatogr. A 731 (1996) 123.
[21] D.W. Armstrong, U.B. Nair, Electrophoresis 18 (1997) 2331.
[22] E. Tesařová, K. Záruba, M. Flieger, Chem. Listy 90 (1997) 969.
[23] E. Tesařová, K. Záruba, V. Pacáková, J. Chromatogr. A, in press.
[24] E. Tesařová, part of oral presentation at 22nd International Symposium HPLC'98, St. Louis, MO, USA, May 2–8, 1998, Book of Abstracts, L-0504, p. 10.
[25] E. Tesařová, D.W. Armstrong, in: Z. Deyl, I. Mikšík, F. Tagliaro, E. Tesařová (Eds.), Advanced Chromatographic and Electromigration Methods in Biosciences, Elsevier, Amsterdam, 1998, p. 197, Ch. 5.
[26] A. Péter, G. Török, D.W. Armstrong, J. Chromatogr. A 793 (1998) 283.
[27] C. Desiderio, S. Fanali, J. Chromatogr. A 807 (1998) 37.