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Liquid chromatographic enantioseparation of β-blocking agents with (1*R*,2*R*)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate as chiral derivatizing agent

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Abstract

The applicability of (1*R*,2*R*)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate [(*R*,*R*)-DANI] as a recently developed chiral derivatizing agent for the enantioseparation of a series of β-blockers is described. The thiourea diastereomers formed were analyzed by reversed-phase high-performance liquid chromatography, mixtures of water and methanol or acetonitrile being used for elution. Conditions of derivatizations (temperature, reagent excess and reaction time) were optimized, and the effects of organic modifiers on the retention and separation were investigated; the diastereomers could readily be baseline separated with methanol-containing mobile phases with resolutions between 1.58 and 2.72. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Derivatization, LC; Enantiomer separation; β-Blockers; (1*R*,2*R*)-1,3-Diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate

1. Introduction

In recent decades, more than 40 β-blocking agents have been introduced into therapeutic practice, and made a major contribution to many aspects of medicine and clinical science. They were used initially in the treatment of angina and cardiac arrhythmias, but their application has been extended, for example, to the control of systemic blood pressure and the relief of intraocular pressure [1].

The pharmacophore of β-blockers is the aryloxy amino alcohol substructure (Fig. 1). The (*S*) enantiomers are known to be much more potent as β-adrenergic antagonists than the (*R*) enantiomers, e.g., the eudismic ratios for propranolol and metoprolol are 130 and 270, respectively [2]. However, most of them are marketed as racemates. Besides the different pharmacological effects of the enantiomers, their metabolic pathways, dispositions and pharmacokinetics may also differ. Accordingly, as a consequence of the increasing demand for the availability of enantiomerically pure pharmaceutical preparations, there is growing interest in the separation of the enantiomers and/or determination of their enantiomeric purity.

The stereoselectivity of the pharmacology of β-

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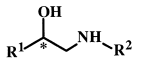

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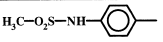
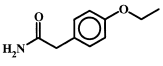
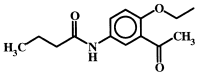
Sotalol		—$CH(CH_3)_2$
Atenolol		—$CH(CH_3)_2$
Acebutolol		—$CH(CH_3)_2$
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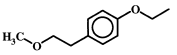
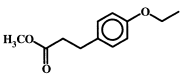
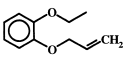
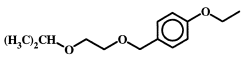
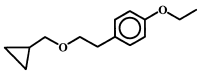
Metoprolol		—$CH(CH_3)_2$
Esmolol		—$CH(CH_3)_2$
Oxprenolol		—$CH(CH_3)_2$
Bisoprolol		—$CH(CH_3)_2$
Betaxolol		—$CH(CH_3)_2$
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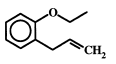
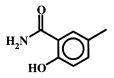
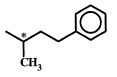
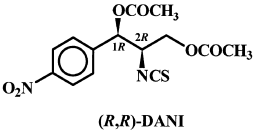
Alprenolol		—$CH(CH_3)_2$
Labetalol		

Fig. 1. Structures of β-blockers investigated.

blockers has given rise to a great deal of literature describing the resolution of enantiomers by high-performance liquid chromatography (HPLC). Besides the direct modes of enantioseparation, i.e., the application of chiral stationary phases [3–14] or chiral mobile phase additives [15], the use of chiral derivatizing agents (CDAs) offers an indirect mode [16–18]. Despite the fact that the latter approach is the oldest one and encounters some drawbacks, a number of publications have reported the application of CDAs for this purpose even in the last 10 years [3,19–27]. Some of these proved particularly useful for the resolution of β-blockers, e.g., (*R*)- and (*S*)-1-(1-naphthyl)ethyl isocyanate [28,29], (*S*)-menthyl chloroformate [9,30–32], (*S*)-α-methylbenzyl isocyanate [33], (*R*)- and (*S*)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(*N*,*N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazole [34], 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate [35,36] and (1*R*,2*R*)- and (1*S*,2*S*)-*N*-[(2-isothiocyanato)cyclohexyl]-3,5-dinitrobenzoylamide [37].

The purpose of the present study is to describe the applicability of a recently developed CDA, (1*R*,2*R*)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate [(*R*,*R*)-DANI, Fig. 2] [38–40], which broadens the spectrum of methods available for the enantioseparation of β-blockers. The advantages of this CDA are that it is accessible in the (*S*,*S*) enantiomeric form, which makes appropriate selection of the elution sequence possible, it can readily be synthesized in enantiomerically pure form [=99% enantiomeric excess (ee) after derivatization with (*S*)-Val of known enantiomeric purity; Aldrich 99% ee] [39], and it is stable both in the solid and in the solution phase. The thiourea diastereomers formed were analyzed on reversed-phase (C_{18}) columns, mixtures of water and methanol (MeOH) or acetonitrile (MeCN) being used for elution. The reaction conditions (reagent excess, temperature and reaction



(*R*,*R*)-DANI

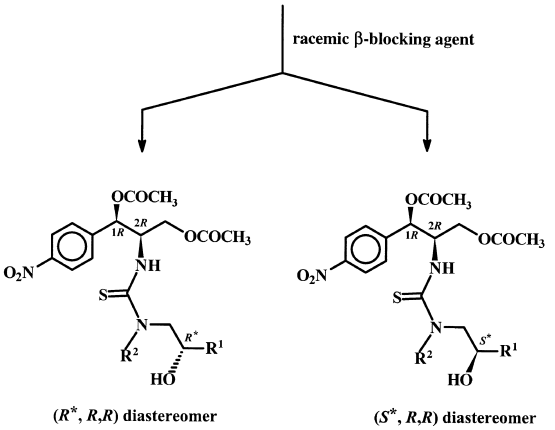


Fig. 2. Derivatization procedure with (*R*,*R*)-DANI.

time) were optimized. The effects of organic modifiers on the retention and separation were investigated.

2. Experimental

2.1. Chemicals and reagents

Trifluoroacetic acid (TFA) and triethylamine (TEA) of analytical-reagent grade, MeOH and MeCN of HPLC grade were obtained from Merck. 0.1% aqueous TFA was prepared with triply distilled water and purified by filtration through a 0.45-μm Millipore filter, type HV (Molsheim, France). (*R*,*R*)-DANI was synthesized according to Ref. [38]. Racemic atenolol and propranolol were purchased from Bioeel (Targu-Mures, Romania). Enantiomerically pure (*R*)- and (*S*)-alprenolol were from Sigma (St. Louis, MO, USA), (*R*)-atenolol was from Aldrich (Steinheim, Germany) and (*S*)-propranolol was from Fluka (Buchs, Switzerland). The other β-blockers studied were extracted from pharmaceutical formulations: acebutolol from Acebutolol 200 (ct-Arzneimittel Chemische Tempelhof, Germany), pindolol from Pindolol 15 (ct-Arzneimittel Chemische Tempelhof), metoprolol from Metoprolol 100 (ct-Arzneimittel Chemische Tempelhof), oxprenolol from Trasicor (Chinoin, Hungary), bisoprolol from Concor (Merck, Germany) and betaxolol from Lokren (Synthelabo, France); or directly diluted from aqueous injections: sotalol from Sotalex 1% (Bristol-Myers Squibb, Germany), esmolol from Brevibloc (Baxter Deutschland, Germany) and labetalol from Trandate (Glaxo Wellcome, UK); they were found to be chemically pure on the basis of the chromatograms obtained after derivatization.

2.2. Apparatus

The HPLC system consisted of an M-600 low-pressure gradient pump equipped with an M-486 tunable absorbance detector, and Millenium software version 2.1 (Waters Chromatography, Milford, MA, USA). The injector with a 20-μl loop was from Rheodyne (Cotati, CA, USA). The columns used for reversed-phase analyses were Nova-Pak C_{18}, 150×3.9 mm I.D., 4 μm particle size (Waters Chromatography), and APEX ODS, 250×4.6 mm I.D., 5 μm particle size (Jones Chromatography, Hengoed, UK).

2.3. Derivatization procedure

A 10-μl volume of a 33 m*M* aqueous solution of the amino alcohol was diluted with 56 μl water–MeCN (1:1) (if the analyte was in free base form) or 0.5% TEA in water–MeCN (1:1) (if the analyte was in salt form). To this solution, 66 μl of the CDA (10 m*M* in MeCN) was added; the molar ratio of the reagent to the β-blocker was 2:1 (in those cases where only pharmaceutical formulations were available, 100% extraction efficiency was suggested). The mixture was kept at room temperature for 3 h. The thiourea diastereomers produced were injected onto a reversed-phase column after a fivefold dilution with the eluent. The derivatives were detected at 245 nm.

3. Results and discussion

3.1. Derivatization kinetics

(±)-Atenolol in free base form was derivatized with (*R*,*R*)-DANI in the absence and also in the presence of TEA (0.5% or 1%) as base component. The derivatization yields of samples were found to be independent of the presence of TEA, and TEA was therefore not applied in further experiments with atenolol.

Study of the rate of derivatization at room temperature revealed that with a twofold molar excess of the CDA the reaction was complete within 3 h (Fig. 3). At a lower ratio (CDA–analyte, 1.1:1), more than 5 h was needed for completion, while at a higher excess (CDA–analyte, 4:1), the reaction time shortened to 1.5 h. The reaction rates for the individual enantiomers were similar; only a very slight kinetic resolution was detected before completion.

A temperature elevation had a favorable effect on the reaction time. At 50°C, only 1 h was required for plateau formation of the derivatives, and the higher temperature did not cause any degradation of the thioureas formed.

When these results are compared with those obtained for α-amino acids in a previous study [39], it can be concluded that the reactivity of the amino

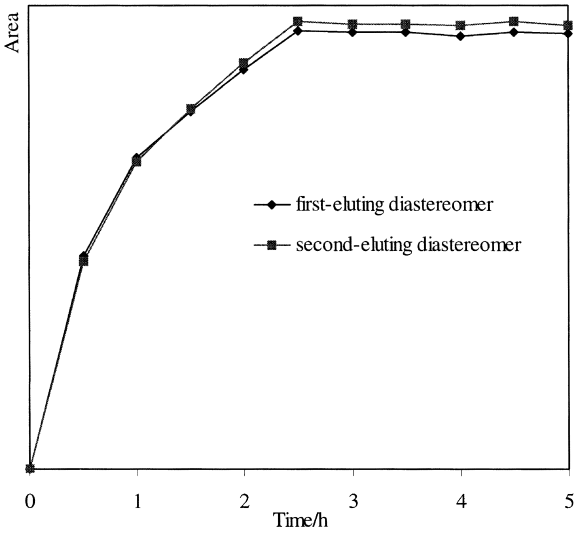


Fig. 3. Study of time course of yield of derivatization of (±)-atenolol with (*R*,*R*)-DANI. Conditions of derivatization: time, 0–5 h; temperature, ambient; molar ratio of CDA–analyte=2:1; without base catalyst. Chromatographic conditions: column, APEX ODS; flow-rate, 0.8 ml min^{-1}; detection, 245 nm.

function of the β-blockers towards isothiocyanate is significantly higher than that of the amino acids. This emphasizes the necessity to check the quantitativeness of derivatization for different groups of compounds.

No racemization of either the CDA or the amino alcohols was observed under any of the reaction conditions tested. The derivatives can be stored in a deep-freezer without decomposition for at least 1 week. The ratio of the peak areas of the first- and second-eluting diastereomers at 245 nm after completion was 1.00:1.03. Very similar results were obtained for propranolol in respect of the derivatization kinetics.

3.2. HPLC results

The thiourea derivatives were analyzed under reversed-phase conditions, with mixtures of water and MeOH or MeCN as eluents. Since the β-blockers examined do not contain other ionizable functionalities besides the secondary amino group to be derivatized, there was no necessity to add buffers to the mobile phase for ion suppression; additionally, the peak shapes were adequate when either pure water or 0.1% aqueous TFA (pH~2) was used as the aqueous part of the eluent.

MeOH as organic modifier proved much more effective than MeCN. The chromatographic data, the retention factors (k), separation factors (α) and resolutions (R_s) in Table 1, show that the diastereomers derived from several β-blocking agents were baseline-separated ($R_s>1.5$) with MeOH-containing eluents within reasonable retention times ($t_R<25$ min). In all cases, when the eluent strength, i.e., the MeOH content of the mobile phase, was decreased, the analysis time became longer and the R_s value became better, as expected. When MeCN was applied as organic modifier, complete coelution of the derivatives occurred, with even longer retention times than those observed for baseline-separated peaks with MeOH.

The aromatic group of the β-blockers gives these compounds their lipophilic character. Since a more apolar analyte is retained more strongly on an apolar stationary phase, it is necessary to increase the eluent strength to elute the more lipophilic compound with a similar retention time as that for the less apolar

Table 1
Chromatographic data on (*R*,*R*)-DANI-derivatized β-blockers with MeOH as organic modifier[a]

Compound	MeOH in eluent (%)	k_1	k_2	α	R_s
Sotalol	65	2.40	2.72	1.13	0.78
	60	4.13	4.85	1.17	1.39
	58	5.15	6.08	1.18	1.58
Atenolol	60	4.67	5.58	1.19	1.62
	58	5.54	6.69	1.21	1.79
Acebutolol	65	5.38	6.22	1.16	1.58
	60	10.94	13.08	1.20	2.42
Pindolol	65	5.61	6.87	1.22	2.63
Metoprolol	70	5.50	6.65	1.21	2.13
Esmolol	75	3.46	4.01	1.16	1.29
	70	5.55	6.69	1.21	2.32
Oxprenolol	75	3.67	4.28	1.17	1.47
	70	6.74	8.24	1.22	2.72
Bisoprolol	75	3.92	4.51	1.15	1.21
	70	7.59	9.11	1.20	2.23
Betaxolol	75	5.50	6.45	1.17	2.00
Propranolol	75	5.35	6.52	1.22	2.33
Alprenolol	75	5.80	7.04	1.21	2.24

[a] Column, APEX ODS 250×4.6 mm I.D. (5 μm); flow-rate, 0.8 ml min^{-1}; detection, 245 nm; eluent, water–MeOH (%, v/v). 1, First-eluting diastereomer; 2, second-eluting diastereomer; $k=(t_R-t_0)/t_0$; $\alpha=k_2/k_1$; $R_s=2(t_2-t_1)/(w_1+w_2)$.

one. Indeed, the wide-ranging polarity variation of the β-blockers investigated was reflected by the significantly different MeOH contents (from 60 to 75%) needed for elution of the different thioureas from the reversed-phase with very similar analysis times (Fig. 4).

In most cases, the excess reagent peak eluted before the derivatives. Exceptions were the two most hydrophilic, sotalol and atenolol; for these, the reagent peak eluted later than the thioureas, but this did not interfere with the diastereomers in any case.

The sequences of elution of the diastereomers were determined for alprenolol, atenolol and propranolol, which were available in enantiomerically pure form, and were in all cases found to be (*S**,*R*,*R*)<(*R**,*R*,*R*), where the asterisk refers to the configuration of the chiral center in the analyte. The environments of the chiral centers and the reaction centers in the β-blockers are practically the same, and it therefore seems safe to assume that the sequence of elution remains the same for the other amino alcohols (the only exception may be sotalol, in which the aryloxymethyl ligand is replaced by an aryl group, which according to the Cahn–Ingold–Prelog rule causes a different priority of the substituents connected to the chiral center).

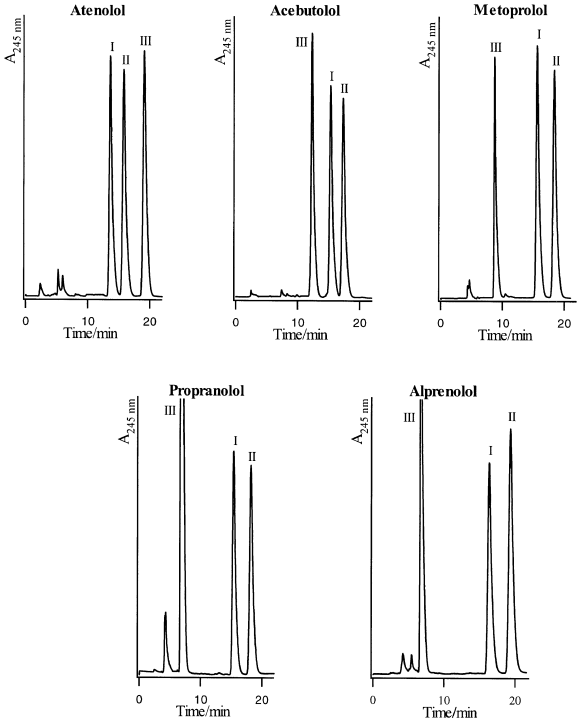


Fig. 4. Chromatograms of some β-blocker diastereomers formed with (*R*,*R*)-DANI. Column, APEX ODS; flow-rate, 0.8 ml min^{-1}; detection, 245 nm; eluent, atenolol water–MeOH (40:60, v/v), acebutolol water–MeOH (35:65, v/v), metoprolol water–MeOH (30:70, v/v), propranolol and alprenolol water–MeOH (25:75, v/v).

The limit of detection for atenolol and for propranolol at 245 nm at a signal-to-noise ratio of 3:1 was determined to be ca. 0.2 nmol ml^{-1}.

Of the examined β-blockers, labetalol contains two chiral centers and four stereoisomers therefore exist. Analyses were performed on two different C_{18} phases that displayed quite different selectivities; results are given in Table 2. On the APEX ODS column (Jones Chromatography), all four isomers were detected (water–MeOH, 25:75, v/v), but no baseline separation could be achieved by lowering the MeOH content. On the Nova-Pak C_{18} phase (Waters Chromatography) only three distinct peaks were detected in the chromatogram (water–MeOH, 40:60, v/v), two isomers in the middle were coeluted. When MeCN was applied as organic modifier, three peaks were again detected, but the selectivity was different, i.e., now the first two isomers were coeluted. Combination of the two organic modifiers, MeOH and MeCN, resulted in only a partial separation of the peaks, even after an analysis time of 60 min.

4. Conclusions

An indirect HPLC method was developed for the separation of enantiomers of a series of β-blockers with (*R*,*R*)-DANI as CDA. The derivatization could be carried out quantitatively under mild conditions and the thioureas obtained proved relatively stable. Extreme differences were found between the selectivities of the organic modifiers applied: with MeOH-containing eluents, good resolutions were readily achieved, whereas MeCN was not selective at all. The separability of the four stereoisomers of labetalol was investigated on two different C_{18} phases, but the complete resolution of all isomers was not successful on either column. However, it should be mentioned that only a small number of

Table 2
Chromatographic data on (*R*,*R*)-DANI-derivatized labetalol isomers[a]

Column	Organic component in eluent (%)	k_1	k_2	k_3	k_4	$\alpha_{1,2}$	$\alpha_{2,3}$	$\alpha_{3,4}$	$R_{s;1,2}$	$R_{s;2,3}$	$R_{s;3,4}$
APEX ODS	MeOH										
	75	2.63	3.01	3.26	3.49	1.14	1.08	1.07	0.91	0.56	0.47
	70	4.74	5.69	6.09	6.64	1.20	1.07	1.09	1.04	0.40	0.52
	65	10.14	13.24	13.24	14.70	1.31	1.00	1.11	1.06	0.00	0.43
Nova-Pak	MeOH										
	70	2.49	3.17	3.17	3.58	1.27	1.00	1.13	1.23	0.00*	0.60
	65	6.25	8.22	8.22	9.28	1.32	1.00	1.13	1.65	0.00*	0.91
	60	12.79	17.64	17.64	21.01	1.38	1.00	1.19	3.07	0.00	1.74
	MeCN										
	45	10.73	10.73	11.95	13.22	1.00	1.11	1.11	0.00	0.96	1.06
	42	19.22	19.22	21.62	24.11	1.00	1.12	1.12	0.00	1.18	1.11

[a] Columns, APEX ODS 250×4.6 mm I.D. (5 μm), Nova-Pak C_{18} 150×3.9 mm I.D. (4 μm); flow-rate, 0.8 ml min^{-1}; detection, 245 nm; eluent, water–MeOH or water–MeCN (%, v/v). 1, First-; 2, second-; 3, third-; 4, fourth-eluting isomer; $k=(t_R-t_0)/t_0$; $\alpha_{n,n+1}=k_{n+1}/k_n$; $R_{S;n,n+1}=2(t_{n+1}-t_n)/(w_n+w_{n+1})$; *a shoulder detectable on the second peak.

publications describe the baseline separation of all four stereoisomers of labetalol by HPLC using a CDA, e.g., Ref. [22].

In conclusion, the CDA used in this study offers a simple, effective and readily available method for the enantioseparation of several β-blockers.

Acknowledgements

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References

[1] C.L. Davies, J. Chromatogr. 531 (1990) 131.
[2] H.Y. Aboul-Enein, I.W. Wainer (Eds.), The Impact of Stereochemistry on Drug Development and Use, Wiley, New York, 1997.
[3] J. Bojarski, Chem. Anal. 42 (1997) 139.
[4] X.J. Yang, T. Fukushima, T. Santa, H. Homma, K. Imai, Analyst 122 (1997) 1365.
[5] A. Ceccato, P. Hubert, J. Crommen, J. Chromatogr. A 760 (1997) 193.
[6] P.V. Petersen, J. Ekelund, L. Olsen, S.V. Ovesen, J. Chromatogr. A 757 (1997) 65.
[7] H.Y. Aboul-Enein, L.I. Al-Duraibi, Enantiomer 4 (1999) 451.
[8] K. Fulde, A.W. Fraham, J. Chromatogr. A 858 (1999) 33.
[9] V.L. Lanchote, P.S. Bonato, P.M. Cerqueira, V.A. Pereira, E.J. Cesarino, J. Chromatogr. B 738 (2000) 27.
[10] H.Y. Aboul-Enein, I.A. Al-Duraibi, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 1817.
[11] I. Le Potier, S.L. Tamisier-Karolak, P. Morin, F. Megel, M. Taverna, J. Chromatogr. A 829 (1998) 341.
[12] J. Ekelund, A. van Arkerns, K. Bronnum-Hansen, K. Fich, L. Olsen, P.V. Petersen, J. Chromatogr. A 708 (1995) 253.
[13] C.J. Welch, S.R. Perrin, J. Chromatogr. A 690 (1995) 218.
[14] D.W. Armstrong, S. Chen, C. Chang, S. Chang, J. Liq. Chromatogr. Rel. Technol. 15 (1992) 545.
[15] G. Bazylak, H.Y. Aboul-Enein, J. Liq. Chromatogr. Rel. Technol. 22 (1999) 1171.
[16] M.W. Skidmore, in: K. Blau, J.N. Halket (Eds.), Handbook of Derivatives for Chromatography, Wiley, New York, 1993, p. 215.
[17] G. Subramanian (Ed.), A Practical Guide to Chiral Separations by Liquid Chromatography, VCH, New York, 1994.
[18] G. Lunn, L.C. Hellwig (Eds.), Handbook of Derivatization Reactions for HPLC, Wiley, New York, 1998.
[19] S. Görög, M. Gazdag, J. Chromatogr. B 659 (1994) 51.
[20] T. Toyo'oka, Biomed. Chromatogr. 10 (1996) 265.
[21] J.M. Dakers, D.W. Boulton, J.P. Fawcett, J. Chromatogr. B 704 (1997) 215.
[22] D.M. Desai, J. Gal, J. Chromatogr. 579 (1992) 165.
[23] R. Büschges, H. Linde, E. Mutschler, H. Spahn-Langguth, J. Chromatogr. A 725 (1996) 323.
[24] Y.H. Wen, S.S. Wu, H.L. Wu, J. Liq. Chromatogr. 18 (1995) 3329.
[25] O.P. Kleidernigg, W. Lindner, Chromatographia 44 (1997) 465.
[26] E. Frigerio, E. Pianezzola, S. Benedetti, J. Chromatogr. A 660 (1994) 351.
[27] T. Toyo'oka, Y.-M. Liu, Analyst 120 (1995) 385.
[28] J. Szymura-Oleksiak, M. Walczak, J. Bojarski, H.Y. Aboulenein, Chirality 11 (1999) 267.

[29] F.J. Belas, M.A. Phillips, N.R. Srinivas, R.H. Barbhaiya, I.A. Blair, Biomed. Chromatogr. 9 (1995) 140.
[30] K.H. Kim, P.W. Choi, S.P. Hong, H.J. Kim, Arch. Pharm. Res. 22 (1999) 608.
[31] R.B. Miller, Y. Guertin, J. Liq. Chromatogr. 15 (1992) 1289.
[32] F. Li, S.F. Cooper, M. Cote, J. Chromatogr. B 668 (1995) 67.
[33] J.L. Beal, S.E. Tett, J. Chromatogr. B 715 (1998) 409.
[34] T. Toyo'oka, M. Toriumi, Y. Ishii, J. Pharm. Biomed. Anal. 15 (1997) 1467.
[35] L. Olsen, K. Bronnum-Hansen, P. Helboe, G.H. Jorgensen, S. Kryger, J. Chromatogr. 636 (1993) 231.
[36] T. Shimizu, M. Hiraoka, H. Nakanomo, J. Chromatogr. B 674 (1995) 77.
[37] O.P. Kleidernigg, K. Posch, W. Lindner, J. Chromatogr. A 729 (1996) 33.
[38] M. Péter, A. Péter, F. Fülöp, Chromatographia 50 (1999) 373.
[39] M. Péter, A. Péter, F. Fülöp, J. Chromatogr. A 871 (2000) 115.
[40] A. Péter, M. Péter, F. Fülöp, G. Török, G. Tóth, D. Tourwé, J. Sápi, Chromatographia 51 (2000) 148.