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Simultaneous separation and enantioseparation of thalidomide and its hydroxylated metabolites using high-performance liquid chromatography in common-size columns, capillary liquid chromatography and nonaqueous capillary electrochromatography

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Abstract

The separation of thalidomide (TD) and its hydroxylated metabolites including their simultaneous enantioseparation was studied using three different polysaccharide-type chiral stationary phases (CSPs) in combination with polar organic mobile phases. Three different techniques, high-performance liquid chromatography in common-size columns, capillary LC and nonaqueous capillary electrochromatography were compared in terms of separation. As this study illustrates, polar organic mobile phases represent a valuable extension for less polar and polar aqueous–organic mobile phases in combination with polysaccharide CSPs. Chiralpak AD consisting of 25% of amylose-tris(3,5-dimethylphenylcarbamate) coated on wide-pore aminopropylsilanized silica gel exhibited higher resolving ability compared to the similar cellulose derivative (Chiralcel OD) as well as to cellulose-tris(4-methylbenzoate) (Chiralcel OJ) CSPs for this particular set of chiral analytes. Baseline separation and simultaneous enantioseparation of all three compounds could be achieved under optimized separation conditions. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Enantiomer separation; Thalidomide

1. Introduction

Thalidomide (1,3-dioxo-2[2′,6′-dioxopiperidine-3′-yl]-isoindole, TD) (Fig. 1) was introduced in 1956 under the trade name Contergan® [1]. The drug was withdrawn from the clinical practice due to severe teratogenic effects, which are mainly manifested in malformations of the limbs and defects of the ears, eyes and internal organs [2]. However, TD is reviving again [3,4] caused by its anti-inflammatory activity for the treatment of leprosy [5] and the recently discovered inhibition of the HIV-1 virus [6]. Furthermore, TD was found to suppress the release of tumor necrosis factor-α (TNF-α) [7,8]. The anti-inflammatory and immunmodulating properties of TD are used in the treatment of graft-versus-host disease following bone marrow transplantation, rheumatoid arthritis, Behçet's Syndrome, cachexia in AIDS and several dermatological diseases [3,9].

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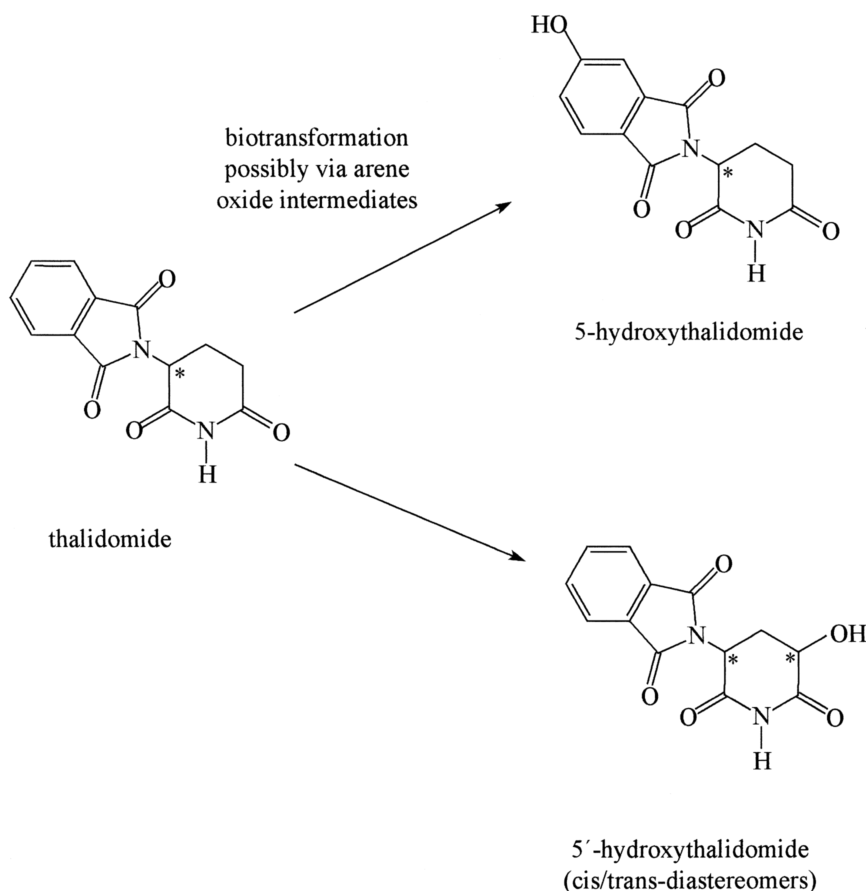

Fig. 1. Structure of thalidomide (TD) and its hydroxylated metabolites 5-hydroxythalidomide (5-OH-TD) and diastereomers of 5′-hydroxythalidomide (5′-OH-TD).

Recently, the United States Food and Drug Administration (FDA) gave marketing approval to TD for the treatment of erythema nodosum leprosum [4].

TD possesses a chiral carbon atom in position 1′ of the 2′,6′-dioxopiperidine-3′-yl moiety (Fig. 1). The teratogenicity of TD after intraperitoneal application to rats and mice was ascribed to its *S*-(−)-enantiomer [10]. However, these pharmacological experiments performed by Fickentscher and Köhler have to be re-evaluated because TD undergoes facile pH-dependent chiral inversion in aqueous media in the presence of endogenous substances like serum albumin [13]. In vitro *S*-(−)-TD is metabolised preferentially by hydroxylation of the phthalimide ring apparently via formation of arene oxide intermediates [11,12]. The stable metabolite of this pathway is 5-hydroxythalidomide (5-OH-TD) [11]. In contrast to *S*-(−)-TD, *R*-(+)-TD undergoes metabolic hydroxylation mainly in the 2′,6′-dioxopiperidine-3′-yl-ring by formation of diastereomeric 5′-hydroxythalidomide pairs [11–14]. Parallel to the different metabolic routes in vitro, fast enantiomerization of pure enantiomers of TD catalysed by (human) serum albumin was established in vivo [1115]. The question whether free radical-mediated oxidative damages to embryonic macromolecules [16] or differences in the metabolic pathways of the enantiomers of TD observed in in vitro experiments are responsible for the enantioselective teratogenicity remains unanswered.

Recently, it was reported that the incubation of racemic TD with fraction S9 from human liver contained both 5-OH-TD and one of the diastereomers of 5′-OH-TD, whereas only 5′-OH-TD was

detected in low concentrations in plasma samples from eight healthy male volunteers who had received racemic TD orally [17].

Despite intensive studies [11,15] many questions about the stereoselective metabolism of TD remain unanswered. The enantiomers of TD were previously resolved by high-performance liquid chromatography (HPLC) on several chiral stationary phases (CSPs) [10,11,13,15,18,19] as well as by capillary electrophoresis (CE) [12,20–23]. Pharmacokinetic *c–t* curves of the single enantiomers of TD were recorded under different conditions [11,13,15]. The enantioseparation of one of the metabolites, 5-OH-TD was also published [12]. However, until our recent CE study [24] no method was described which allowed the simultaneous separation and enantioseparation of native TD and its two biomedically relevant metabolites, 5-OH-TD and the *cis* isomer of 5′-OH-TD. This method is required in order to follow the enantioselectivity of the in vitro and the in vivo biotransformation in detail.

Although CE offers important advantages from the viewpoint of miniaturized scale, costs and flexibility, this technique still suffers somewhat of disadvantages such as reproducibility, sensitivity, etc. In addition, not all chiral selectors can be adapted to the CE mode because some of them are insoluble in CE buffers, possess a high detector response, lower chiral recognition ability in the mediums used in CE, etc. Moreover, a miniaturized technique with a stationary bed may be easier for on-line coupling with mass spectrometry (MS).

In this study an attempt was made to develop a method for the simultaneous separation and enantioseparation of TD and its biomedically relevant metabolites using common-size columns and furthermore to miniaturize this method using capillary LC and capillary electrochromatography (CEC) in nonaqueous buffers.

2. Experimental

2.1. Chemicals

Racemic TD was kindly provided by Grünenthal (Stolberg, Germany). The enantiomers of TD were obtained in our laboratory by preparative low-pressure liquid chromatography on a poly[*S*]-*N*-(1-cyclohexylethyl)-methacrylamide stationary phase [10]. 5-OH-TD was synthesized as described previously [25]. The *cis* isomer of 5′-OH-TD was a gift from Professor K. Eger (University of Leipzig, Germany) [26]. Microcrystalline cellulose (Avicel), wide-pore silica gel (LiChrospher 1000, 5 μm) and glacial acetic acid were from E. Merck (Darmstadt, Germany). Amylose B (M_r=16 000) was purchased from Nacalai Tesque (Kyoto, Japan). Ammonium acetate was purchased from Riedel-de Haën (Seelze–Hannover, Germany). Methanol and ethanol of HPLC quality, tetrahydrofuran, *n*-hexane, 2-propanol, acetonitrile, pyridine and benzene were from J.T. Baker (Deventer, The Netherlands). 4-Methylbenzoylchloride and 3,5-dimethylphenylisocyanate were supplied by Aldrich (Daisenhofen, Germany), (3-aminopropyl)-triethoxysilane was purchased from Fluka (Buchs, Switzerland).

The polysaccharide derivatives as cellulose-tris(3,5-dimethylphenylcarbamate), amylose-tris(3,5-dimethylphenylcarbamate) and cellulose-tris(4-methylbenzoate) (Fig. 2) were prepared as described previously [27–30]. All polysaccharide derivatives were isolated as methanol insoluble fractions. Before coating with the polysaccharide derivatives, wide-pore silica gel (LiChrospher 1000, 5 μm) was silanized using (3-aminopropyl)-triethoxysilane in benzene in the presence of a catalytic amount of dry pyridine at 80°C. The polysaccharide derivatives were dissolved in tetrahydrofuran and coated using different concentrations on previously aminopropyl-silanized wide-pore silica gel by a static technique.

2.2. Common-size and capillary columns

Common-size columns were purchased from Daicel (Tokyo, Japan) under the commercial names Chiralcel OJ, Chiralcel OD and Chiralpak AD (250× 4.6 mm). Capillary columns were packed as described previously [31–33]. Fused-silica capillaries of 100 μm I.D. from Polymicro Technologies (Phoenix, AZ, USA) were used. The inlet-end of the capillary was connected to a HPLC precolumn (50× 4.6 mm) which served as the reservoir for the slurry of the packing material in *n*-hexane–2-propanol (90:10, v/v). A commercially available HPLC column frit was connected to the outlet-end of the

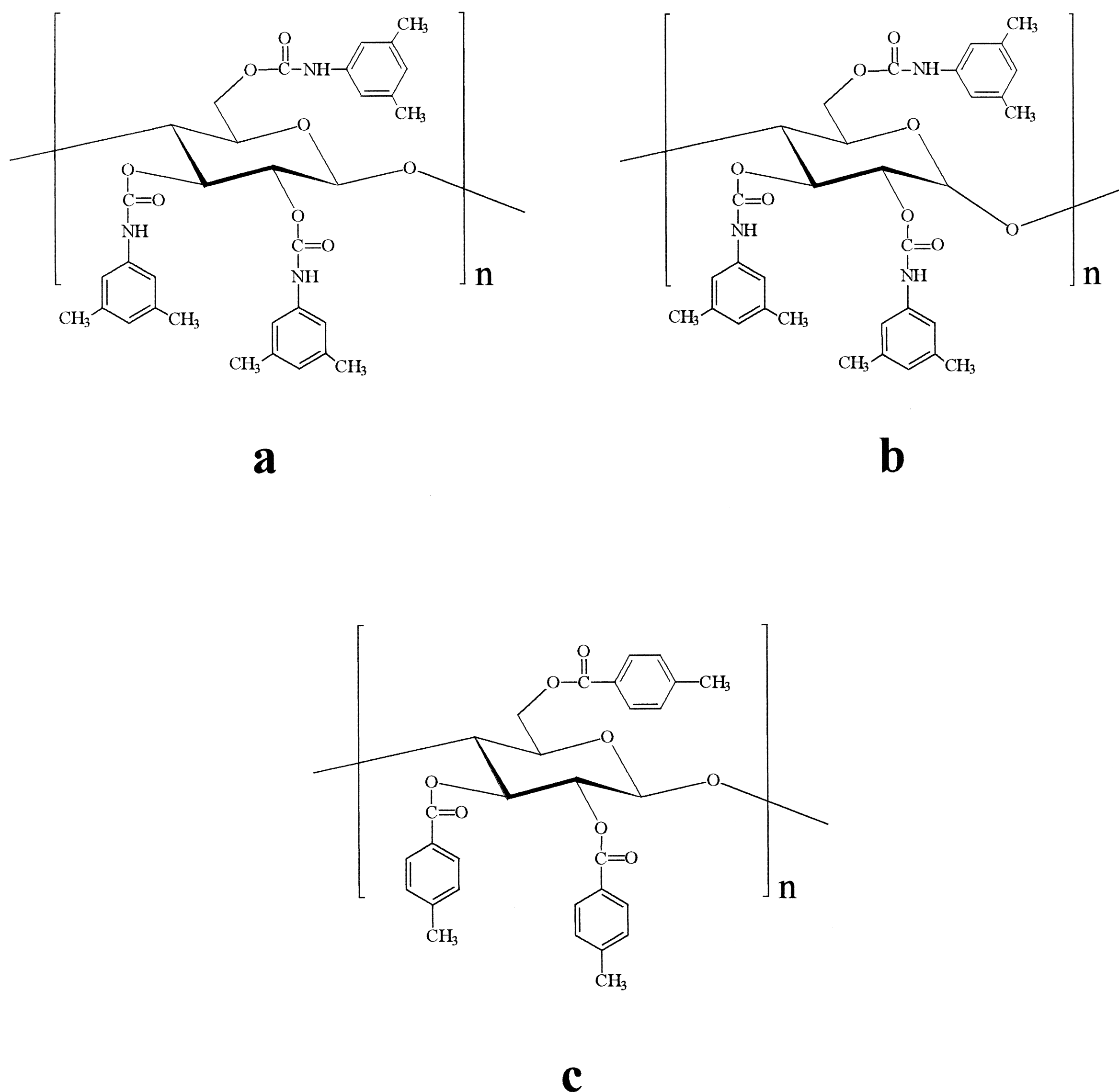

Fig. 2. Structure of the chiral stationary phases: (a) cellulose-tris(3,5-dimethylphenylcarbamate) (Chiralcel OD); (b) amylose-tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) and (c) cellulose-tris(4-methylbenzoate) (Chiralcel OJ).

capillary in order to retain the packing material. The slurry of the packing material was ultrasonicated in a water-bath (15 min) and transferred into the reservoir. The system was closed tightly, pressure up to 400 bar was applied using a Knauer pneumatic pump (Knauer, Berlin, Germany) and maintained for 1 h. After the complete reduction of the residual pressure (3–4 h), bidistilled water was pumped through the packed bed for 30 min. The outlet and inlet frits were sintered by local heating of the packed bed for approximately 10 s using a heating coil (700–800°C). The packed capillaries prepared according to this technique were used for capillary LC and CEC separations.

2.3. HPLC enantioseparations in common-size columns

HPLC enantioseparations in common-size columns were performed using a Merck–Hitachi L-6200A pump, a Merck–Hitachi 655A UV detector (E. Merck) and a Shimadzu C-R3A Chromatopac integrator (Shimadzu Europa, Düsseldorf, Germany).

2.4. Capillary LC and CEC

Capillary LC and CEC were performed with identical experimental set-ups using a HP ^{3D}CE (Agilent Technologies, Waldbronn, Germany) capillary electrophoresis instrument. Another experimental set-up for the capillary LC separations was a laboratory-made sample interface consisting of a stainless steel tee piece and a restrictor in analogy to that recently described by Taylor and Teale [34]. The stream of the mobile phase, generated using a commercial HPLC pump (Merck–Hitachi L-6200A), flows coaxially passing the capillary column inlet through a peek lead tube and leaves the tee piece through a restriction capillary with 50 μm I.D. The sample was introduced into the mobile phase stream with a Rheodyne sample injector equipped with a 20 μl loop (Rheodyne, Cotati, CA, USA) and further loaded onto the capillary by pressure. UV detection was carried out at 230 nm using a Grom capillary electrophoresis system 100 with a HP 3396A integrator (Agilent Technologies).

The apparent pH of ammonium acetate solutions in alcohols was adjusted with glacial acetic acid and measured with the pH meter pH522 (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) without any corrections.

3. Results and discussion

3.1. HPLC separations in common-size columns

Polysaccharide derivatives have been originally developed and basically used for enantioseparations in the normal-phase mode [27–30]. Alcohols, such as isopropanol (2-propanol) and ethanol in amounts of 1–10% (in rare cases up to 50%, v/v) are the most widely applied polar modifiers used in combination with apolar organic solvents such as *n*-hexane and *n*-heptane [35]. However, the enantioseparations in polar nonaqueous organic solvents have been scarcely studied especially with polysaccharide phenylcarbamates [33,36,37].

The separation of TD, 5-OH-TD and *cis*-5′-OH-TD on different polysaccharide-type columns using a single mobile phase (100% pure methanol) is shown in Fig. 3a and 3b. Chiralcel OD material exhibits chemoselectivity towards TD and its 5- and *cis*-5′-hydroxy metabolites in methanol but lacks enantioselectivity for all of these compounds (Fig. 3a). Chiralcel OJ material possesses enantioselectivity towards all three compounds in the same mobile phase but in contrast to Chiralcel OD, this material suffers from insufficient chemoselectivity (chromatogram not shown). The best combination of chemo- and enantioselectivity among these three materials tested towards the particular analytes of this study was found for Chiralpak AD (Fig. 3b). However, even with this CSP one of the enantiomers of *cis*-5′-OH-TD and *S*-(−)-TD partially overlapped. This did not allow a baseline enantioseparation of all six peaks in a single run.

Based on a previous experiment [33] ethanol has been used in order to modify the elution times and selectivity of a separation with Chiralpak AD. The separation pattern is not comparable to methanol as mobile phase because the elution order of TD and its metabolites is not the same (Fig. 4a). In addition, the peaks were wide and the analysis times impractically long. A mixture of ethanol–methanol (85:15, v/v) allowed one to combine the selectivity observed for both mobile phases in the separate runs (Fig. 4b). However, the disadvantages related to the long analysis time and wide peaks could not be avoided by the modification of the mobile phase. Further selectivity optimization was performed using a triple mobile phase system. The mobile phase combination methanol–ethanol–acetonitrile (80:14:6, v/v/v) allowed almost a baseline separation of all components (Fig. 4c).

Thus, polysaccharide derivatives may be very effectively used for the simultaneous separation and enantioseparation of chiral compounds in the presence of nonaqueous polar organic mobile phases.

It was not possible to determine the enantiomer migration order for all analytes because the pure

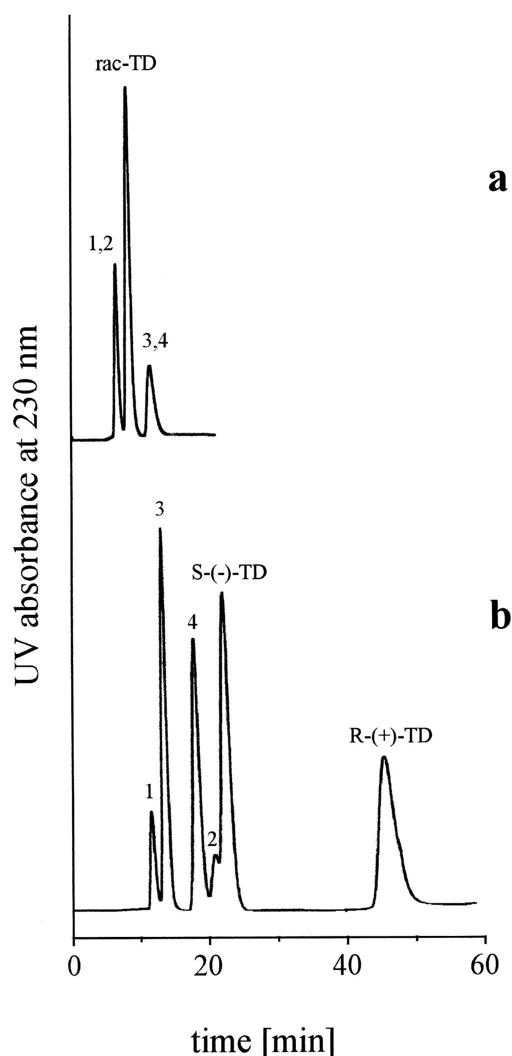

Fig. 3. HPLC separation of TD, 5-OH-TD and *cis*-5′-OH-TD on Chiralcel OD (a) and Chiralpak AD (b) columns (250×4.6 mm) using methanol as the mobile phase. Flow-rate: 0.5 ml/min; UV detection at 230 nm; 1, 2: enantiomers of *cis*-5′-OH-TD; 3, 4: enantiomers of 5-OH-TD.

enantiomers of 5-OH-TD and of the *cis* isomer of 5′-OH-TD were not available for us. Interestingly, the enantiomer migration order of TD was opposite with Chiralcel OJ compared to Chiralpak AD CSPs using ethanol or methanol as mobile phase (chromatogram not shown).

As one of the main goals of this study was to transfer the HPLC separation to CEC, the effect of ammonium acetate on the separation was studied. Ionizable compounds soluble in the separation medium are required in order to provide conductivity and to support the significant electroosmotic flow (EOF), but in this case ammonium acetate may exhibit some adverse effects and impairs the separation. In particular, the peaks corresponding to 5- and *cis*-5′-OH-TD coeluted when 20 m*M* ammonium acetate was added to the methanol–ethanol–acetonitrile mixture.

3.2. Enantioseparation of TD, 5-OH-TD and cis-5′-OH-TD in capillary LC

The separations in the capillary format were initially optimized with cellulose-tris(4-methylbenzoate) (Chiralcel OJ) using the HP ^{3D}CE system. Only partial separation was observed for six peaks with a methanol–ethanol mixture as mobile phase (Fig. 5). The elution times increased significantly with an increasing amount of ethanol but the selectivity of the separation could not be markedly improved.

A baseline resolution of all six peaks could not be achieved by variation of the content of the coated polysaccharide derivative. Therefore, the length of the packed bed of the capillary was increased, but the applied pressure limit of 12 bar of the HP ^{3D}CE equipment did not allow the use of packed beds longer than 25 cm. For this reason, another experimental set-up equipped with a laboratory-made injection unit and a conventional HPLC pump as described previously [34,38] was required to improve the separation on Chiralcel OJ and Chiralpak AD. The best results were obtained using capillaries packed with aminopropylsilanized silica previously coated with 20% amylose-tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) of 30 cm effective length. With this experimental set-up it was possible to detect five baseline resolved peaks. In particular, one of the enantiomers of 5-OH-TD and *cis*-5′-OH-TD overlapped (Fig. 6a). Optimization of double (methanol–ethanol or acetonitrile) or triple mobile phase (the same components) composition did not allow a baseline resolution of all six peaks.

Considering that Chiralpak AD exhibited excellent enantioselectivity but insufficient chemoselectivity in this particular separation, while Chiralcel OD com-

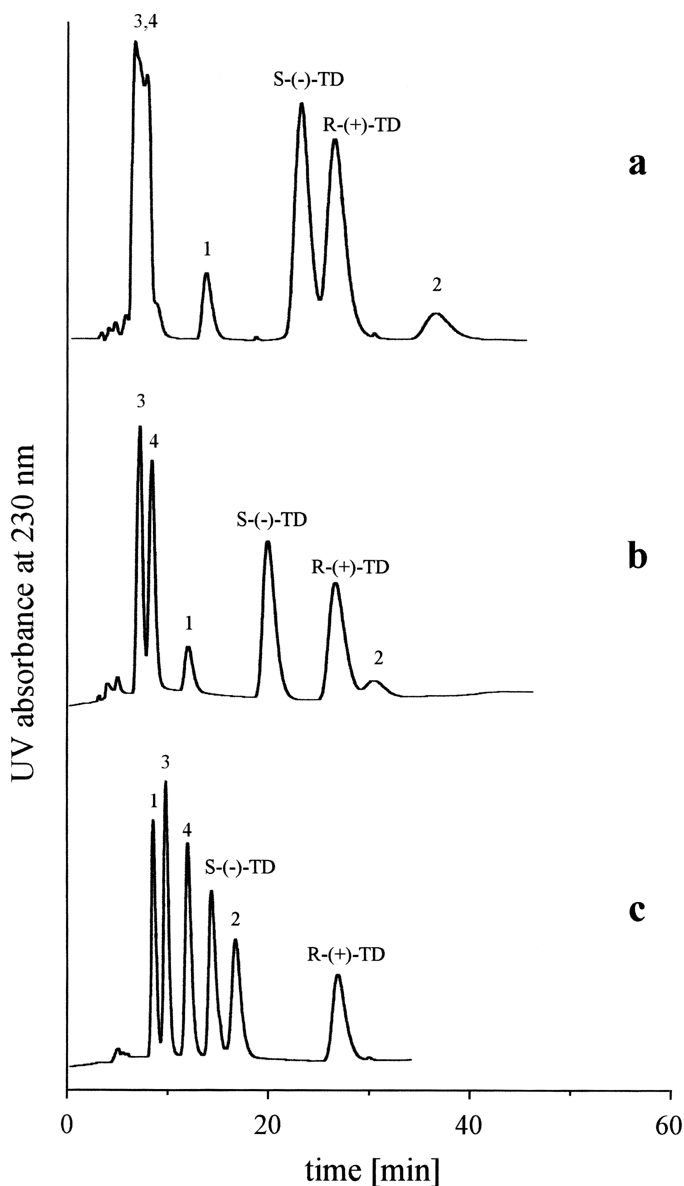

Fig. 4. HPLC separation of TD, 5-OH-TD and *cis*-5′-OH-TD on the Chiralpak AD column (250×4.6 mm) using 100% ethanol (a) ethanol–methanol (85:15, v/v) (b) and methanol–ethanol–acetonitrile (80:14:6, v/v/v) (c) as the mobile phase. Flow-rate: 1.0 ml/min (a and b), 0.7 ml/min (c); UV detection at 230 nm; 1, 2: enantiomers of *cis*-5′-OH-TD; 3, 4: enantiomers of 5-OH-TD.

pletely lacks enantioselectivity towards the same set of analytes but provides sufficient chemoselectivity, a combination (16% AD+4% OD) of these two polysaccharides were coated onto aminopropylsilica. A similar approach has been previously reported for HPLC by Zhang and Francotte [39]. In contrast to the pure AD material (Fig. 6b), it was possible to combine in this way the chemo- and enantioselectivity of both polysaccharide derivatives and to achieve almost a baseline resolution of all six components of the mixture with an optimized composition of the mobile phase (Fig. 6c).

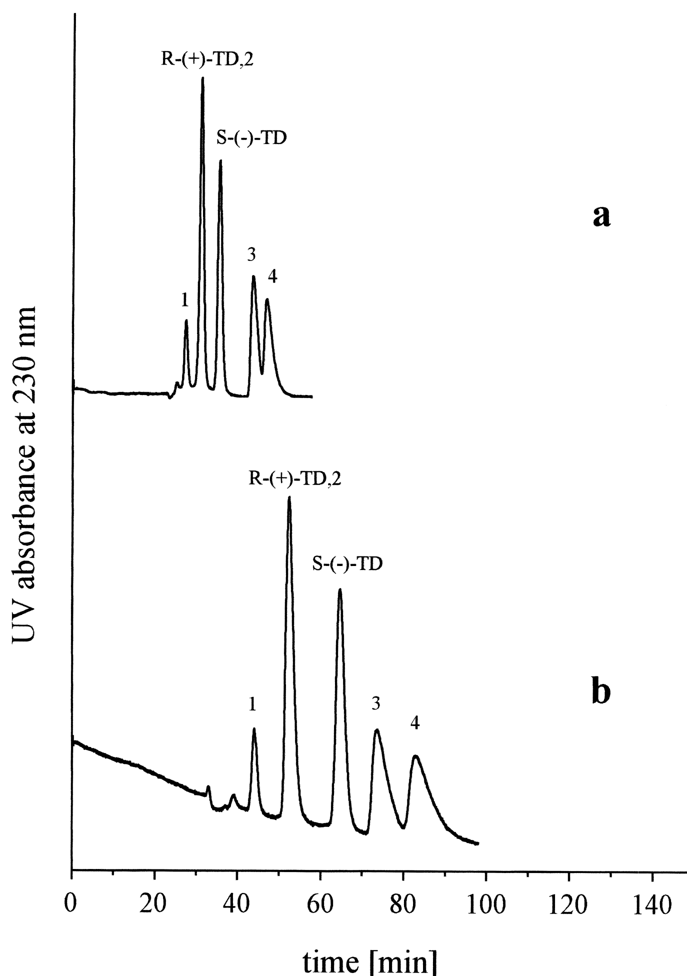

Fig. 5. Capillary LC separation of TD, 5-OH-TD and *cis*-5′-OH-TD on Chiralcel OJ using methanol–ethanol (80:20, v/v) (a) and (20:80, v/v) (b) as the mobile phase. Capillary: 20% cellulose-tris(4-methylbenzoate) coated on aminopropylsilica (5 μm); packed length: 25 cm; total length: 33.5 cm; I.D. 100 μm; applied pressure difference: 12 bar; UV detection at 230 nm; 1, 2: enantiomers of *cis*-5′-OH-TD; 3, 4: enantiomers of 5-OH-TD.

3.3. Enantioseparation of TD, 5-OH-TD and cis-5′-OH-TD in nonaqueous CEC

CEC offers several potential advantages compared to capillary LC such as higher peak efficiency, the potential to use smaller particles, etc. especially in separation of complex mixtures. However, CEC implies several requirements to the separation system. These include electric conductivity of the mobile phase, generation of the EOF, etc. From the viewpoint of the latter requirements, special difficulties may be encountered when nonaqueous mobile phases (background electrolytes) are used but as recent studies showed it is possible to perform CEC also in nonaqueous solvents [32,33,40–42].

Nonaqueous CEC separations of TD, 5-OH-TD and *cis*-5′-OH-TD in capillaries packed with aminopropylsilica coated with 16% AD+4% OD (a) and 20% AD (b) materials are shown in Fig. 7. The baseline separation of all six components could not be achieved but further optimization of the AD/OD ratio may allow to achieve this goal similar to

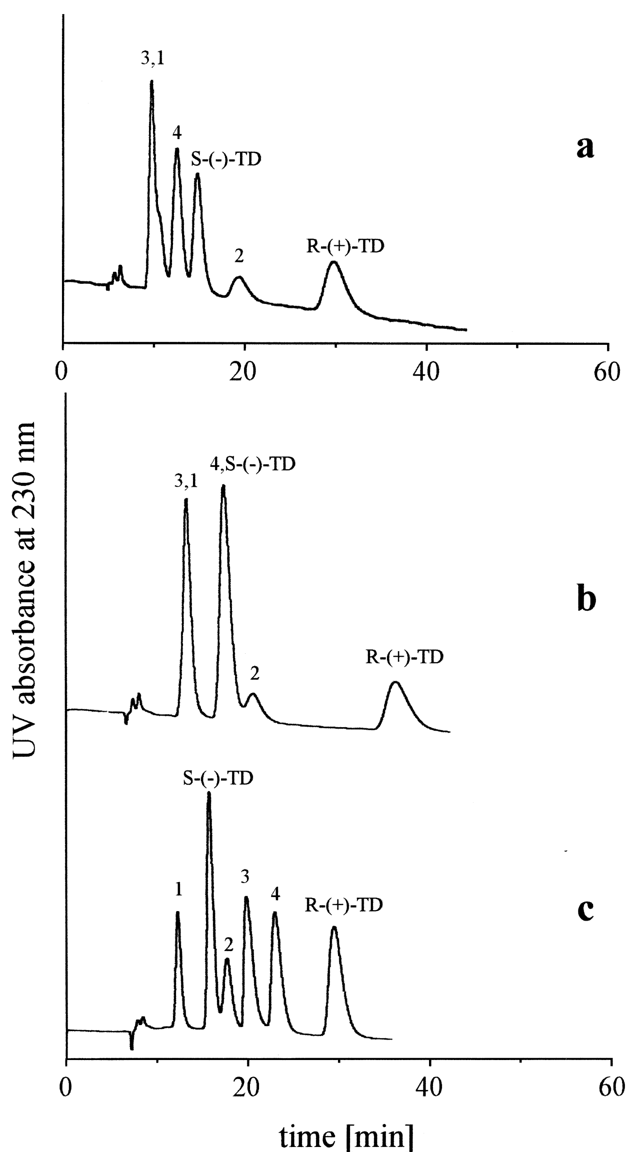

Fig. 6. Capillary LC separation of TD, 5-OH-TD and *cis*-5′-OH-TD in the capillaries packed with aminopropylsilica which was coated with 20% AD (a, b) and 16% AD+4% OD (c). Packed length: 30 cm; total length: 38.5 cm; I.D. 100 μm; mobile phase: methanol–ethanol (75:25, v/v) (a), methanol–acetonitrile (99:1, v/v) (b, c); flow-rate: 0.3 ml/min (a), 0.2 ml/min (b, c); UV detection at 230 nm; 1, 2: enantiomers of *cis*-5′-OH-TD; 3, 4: enantiomers of 5-OH-TD.

capillary LC also in the nonaqueous CEC mode. The improvement of the CEC separation system from this and several other viewpoints (particle size, composition and the pH of the separation buffer, etc.) are in progress at present.

4. Conclusion

The separation with nonaqueous polar organic mobile phases appears to be an alternative to aqueous and less polar organic mobile phases for enantio-

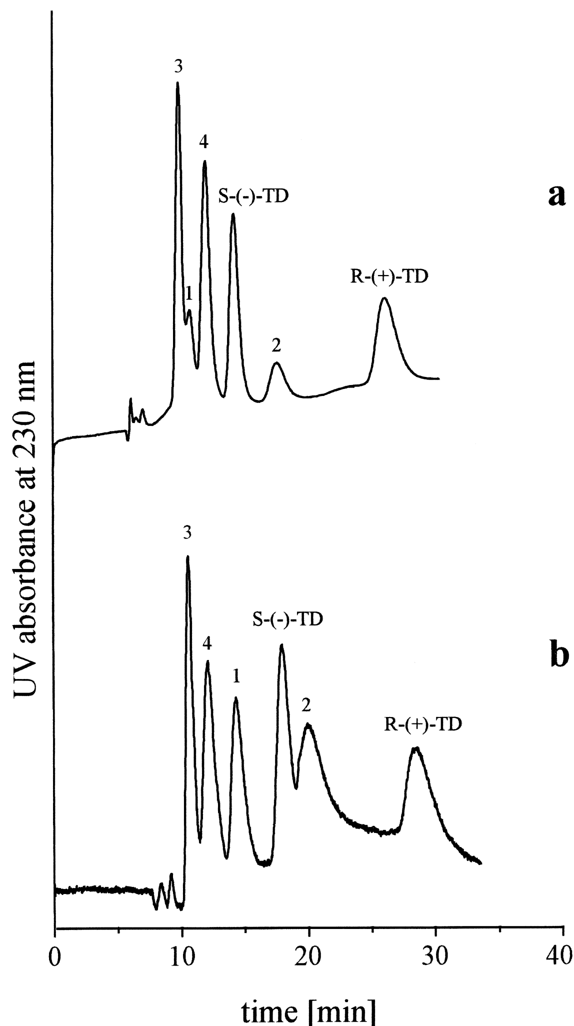

Fig. 7. Nonaqueous CEC separation of TD, 5-OH-TD and *cis*-5′-OH-TD in the capillaries packed with aminopropylsilica which was coated with 16% AD+4% OD (a) and 20% AD (b). Packed length: 25 cm; total length: 33.5 cm; I.D. 100 μm; separation medium: methanol–ethanol 75:25 (v/v) containing 2.5 m*M* ammonium acetate; applied voltage: −25 kV (current: 2.7 μA); UV detection at 230 nm; 1, 2: enantiomers of *cis*-5′-OH-TD; 3, 4: enantiomers of 5-OH-TD.

separations using polysaccharide-type CSPs. It is possible to transfer these separations to the capillary format (capillary LC and CEC) which offers several important advantages of the miniaturized techniques. In this study it was possible to achieve a baseline resolution of TD and its pharmacologically relevant metabolites, 5-OH-TD and *cis*-5′-OH-TD and to separate the enantiomers of all three compounds simultaneously.

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References

[1] K.S. Bauer, S.C. Figg, W.D. Figg, Biochem. Pharmacol. 55 (1998) 1827.
[2] W. Lenz, K. Knapp, Dtsch. Med. Wochenschr. 87 (1962) 1232.
[3] D. Stirling, Annu. Rep. Med. Chem. 30 (1995) 319.
[4] C. Marwick, J. Am. Med. Assoc. 278 (1997) 1135.
[5] R.L. Barnhill, A.C. Mc Dougall, J. Am. Dermatol. 7 (1982) 317.
[6] G. Kaplan, A.L. Moreira, Res. Immunol. 145 (1994) 685.
[7] H. Koch, Prog. Med. Chem. 22 (1985) 166.
[8] J.M. Gardner-Medwin, Exp. Opin. Invest. Drug. 5 (1996) 829.
[9] J.S. Ochonsky, J. Revuz, Eur. J. Dermatol. 3 (1994) 9.
[10] G. Blaschke, H.P. Kraft, K. Fickentscher, F. Köhler, Arzneim.-Forsch. 29 (1979) 1640.
[11] B. Knoche, G. Blaschke, Chirality 6 (1994) 221.
[12] C. Weinz, G. Blaschke, J. Chromatogr. B 674 (1995) 287.
[13] B. Knoche, G. Blaschke, J. Chromatogr. A 666 (1994) 235.
[14] M. Meyring, D. Strickmann, B. Chankvetadze, G. Blaschke, C. Desiderio, S. Fanali, J. Chromatogr. B 723 (1999) 255.
[15] T. Eriksson, S. Björkmann, B. Roth, A. Fyge, Chirality 7 (1995) 44.
[16] T. Parman, M.J. Wiley, P.G. Wells, Nat. Med. 5 (1999) 582.
[17] T. Eriksson, S. Björkmann, B. Roth, H. Björk, P. Höglund, J. Pharm. Pharmacol. 50 (1998) 1409.
[18] K. Nishimura, Y. Hashimoto, S. Iwasaki, Chem. Pharm. Bull. 42 (1994) 1157.
[19] H.Y. Aboul-Enein, M.R. Islam, J. Liq. Chromatogr. 14 (1991) 667.
[20] B. Chankvetadze, G. Endresz, G. Blaschke, Electrophoresis 15 (1994) 804.
[21] B. Chankvetadze, G. Endresz, G. Blaschke, J. Cap. Electrophoresis 2 (1995) 235.
[22] A. Aumatell, R.J. Wells, D.K.Y. Wong, J. Chromatogr. A 686 (1994) 293.
[23] G. Schulte, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 771 (1997) 259.
[24] M. Meyring, B. Chankvetadze, G. Blaschke, Electrophoresis 20 (1999) 2425.

[25] H.R. Hess, Ph.D. Thesis, University of Münster, Münster, 1989.
[26] U. Teubert, K. Zwingenberger, S. Wnendt, K. Eger, Arch. Pharm. 331 (1998) 7.
[27] Y. Okamoto, M. Kawashima, K. Hatada, J. Am. Chem. Soc. 106 (1984) 5357.
[28] Y. Okamoto, R. Aburatani, K. Hatada, J. Chromatogr. 389 (1987) 95.
[29] Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr. 363 (1986) 173.
[30] Y. Okamoto, R. Aburatani, T. Fukumoto, K. Hatada, Chem. Lett. 9 (1987) 1857.
[31] K. Krause, M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 837 (1999) 51.
[32] K. Krause, B. Chankvetadze, Y. Okamoto, G. Blaschke, Electrophoresis 20 (1999) 2772.
[33] M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A (2000) in press.
[34] M.R. Taylor, P. Teale, J. Chromatogr. A 768 (1997) 89.
[35] E. Yashima, Y. Okamoto, Angew. Chem. Int. Edn Eng. 37 (1998) 1021.
[36] J. Dingenen, B. Subramanian, in: A Practical Approach to Chiral Separations by Liquid Chromatography, VCH, Weinheim, 1994, pp. 115–181, Chapter 6.
[37] E. Francotte, J. Chromatogr. A 666 (1994) 565.
[38] D. Strickmann, B. Chankvetadze, G. Blaschke, C. Desiderio, S. Fanali, J. Chromatogr. A (2000) in press.
[39] T. Zhang, E. Francotte, Chirality 7 (1995) 425.
[40] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 839 (1999) 167.
[41] K.M. Whitaker, M.J. Sepaniak, Electrophoresis 15 (1999) 1341.
[42] A. Maruska, U. Pyell, J. Chromatogr. A 782 (1997) 167.