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Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 161–166

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Simultaneous enantioseparation of *cis*-diltiazem hydrochloride and its metabolite *cis*-desacetyldiltiazem using high-performance liquid chromatography and capillary electrophoresis

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Received 23 November 2000; received in revised form 1 June 2001; accepted 11 June 2001

Abstract

Two alternative methods were developed using high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) for a simultaneous enantioselective determination of Ca^{2+} blocking chiral drug *cis*-diltiazem hydrochloride (DLT) and its degradation product and metabolite *cis*-desacetyldiltiazem (Desac-DLT). The methods were compared from the viewpoint of analytical performance. The identification of HPLC peaks were performed using off-line electrospray ionization mass spectrometry. In addition, CE method was evaluated for quantitative determination of minor enantiomeric impurities in pharmacologically active (+)-*cis*-DLT.

Keywords: Capillary electrophoresis; Enantioseparation; HPLC; Chiral selector; Cyclodextrin; *cis*-Diltiazem hydrochloride; *cis*-Desacetyldiltiazem

1. Introduction

(+)-*cis*-Diltiazem hydrochloride (DLT) [(2S-*cis*)-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride] (Fig. 1a) is one of the widely used Ca^{2+} blocking drugs. DLT contains two centers of chirality and exists in the form of four stereoisomers. From these four stereoisomers (+)-*cis*-DLT is the pharmacologically active form which is in clinical use [1–4]. *cis*-Desacetyldiltiazem (*cis*-Desac-DLT) (Fig. 1b) is one of the degradation products and metabolites of DLT which has been reported to have 25–50% of the activity of *cis*-DLT in humans. Thus, simultaneous separation and enantioseparation of *cis*-DLT and *cis*-Desac-DLT represents certain interests from the viewpoint of purity test of

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0731-7085/02/$ - see front matter
PII: S0731-7085(01)00553-2

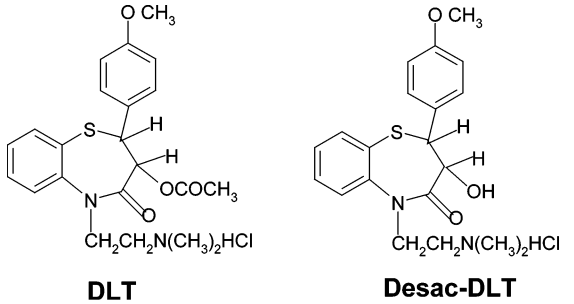

Fig. 1. Structure of diltiazem hydrochloride (DLT) and desacetyldiltiazem (Desac-DLT).

generic drug, drug forms, as well as for investigation of the metabolic profile of *cis*-DLT.

Several papers describe simultaneous separation and enantioseparation of DLT, its analogs, possible degradation products and metabolites using high-performance liquid chromatography (HPLC) [1–11] and capillary electrophoresis (CE) [12–21]. However, only few of them describe the simultaneous enantioseparation of *cis*-DLT and *cis*-Desac-DLT [16].

The goal of this study was to develop two alternative methods using HPLC and CE for a simultaneous separation and enantioseparation of *cis*-DLT and *cis*-Desac-DLT. In addition, the CE method was applied to a determination of the minor enantiomeric impurities in pharmacologically active (+)-*cis*-DLT.

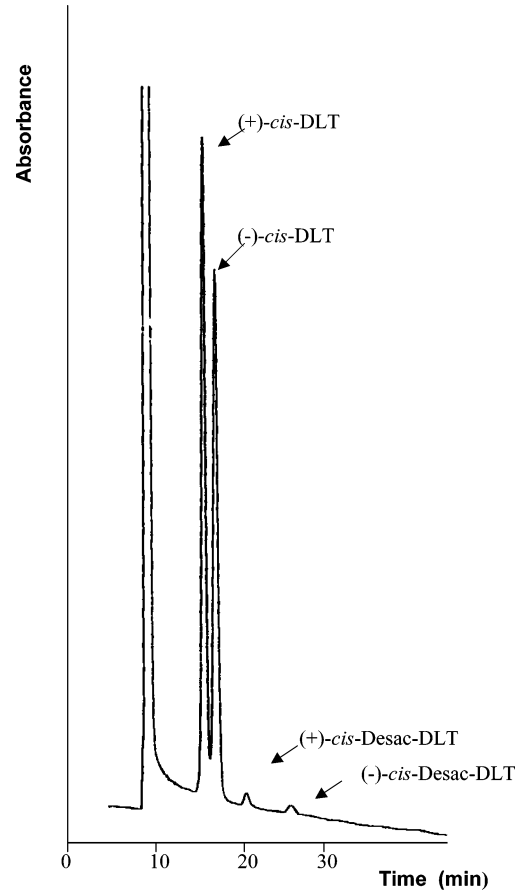

Fig. 2. HPLC enantioseparation of *cis*-DLT hydrochloride and *cis*-Desac-DLT on cellulose tris (3,5- dichlorophenylcarbamate) (CDCPC) using 2-propanol as a mobile phase. Mobile phase flow-rate 0.5 ml/min.

2. Experimental

2.1. Chemicals

cis-(±)-DLT was a gift from Tanabe Seiyaku (Osaka, Japan), *cis*-(+)-DLT was supplied from Sigma (Sigma-Aldrich, Deisenhofen, Germany), *cis*-(±)-Desac-DLT was obtained by desacetylation of *cis*-(±)-DLT at room temperature for several days. Sulfated cyclodextrin (SU-β-CD) with the average degree of substitution (D.S.) 14 was provided by Aldrich (Steinheim, Germany). Analytical and HPLC grade solvents were obtained from Merck (Darmstadt, Germany).

2.2. Chromatographic conditions

HPLC separations were performed on the HPLC system consisted of a Knauer isocratic Pump (Knauer, Berlin, Germany), a Rheodyne sample injector with a 20 μl loop (Rheodyne, Cotati, CA, USA), a variable-wavelength UV-detector 655A and a D-2500 chromato-integrator Merck-Hitachi (Merck, Darmstadt, Germany).

Chiral stationary phase (CSP) for HPLC enantioseparation was home-made cellulose tris (3,5-dichlorophenylcarbamate) (CDCPC) [22–26] coa-

ted on the wide-pore silica gel Daisogel-2000 (Daiso, Osaka, Japan). CDCPC was obtained by reacting of 3,5-dichlorophenylisocyanate (Aldrich, Steinheim, Germany) with vacuum-dried microcrystalline cellulose (Merck, Darmstadt, Germany) in dry pyridine at 90 °C overnight. The product was precipitated by dropwise addition to 200 ml methanol, separated by filtration and washed with methanol. Vacuum-dried (at 60 °C) CDCPC (0.75 g) was dissolved in 5 ml tetrahydrofuran (THF) and coated on the Daisogel-2000 which was silanized in advance using γ-glycidylaminopropyltriethoxysilane as described previously [22]. The CSP, which was prepared by this way was packed by slurry-packing technique into the stainless-steel column of 4.6 × 250 mm size. Mobile phase was 2-propanol with a flow-rate 0.5 ml/min.

2.3. Capillary electrophoretic conditions

CE enantioseparations were carried out on four different CE systems: a Grom modular capillary electrophoresis system 100 (Grom, Herrenberg, Germany) equipped with variable wavelength UV detector from Linear Instruments (Reno, USA), Beckman P/ACE 5010 capillary electrophoresis system and Beckman MDQ capillary electrophoresis system both equipped with variable wavelength UV detector (Beckman Instruments, Fullerton, CA, USA) and HP^{3D} capillary electrophoresis system equipped with the diode array UV detector from Agillent Technologies (Waldbronn, Germany). The samples were injected by pressure, 0.5 p.s.i. for 10 s. (1 p.s.i. = 6.8 kpa) or hydrodynamically with $\Delta h =$ 10 cm for different times.

Separation capillary was of either 40/47 or 50/60 cm effective/total length fused-silica (Polymicro Technologies, Phoenix, AZ, USA) with 50 μm I.D. Detection was performed at 214 nm.

2.4. Electrospray ionization mass spectrometry

Electrospray ionization mass spectra (ESI MS) were obtained using a quadrupole mass spectrometer Quatro LC-Z (Micromass) equipped with an electrospray interface. The solution of 1 mg/ml of (±)-*cis*-DLT in methanol and fraction of *cis*-Desac-DLT collected from the HPLC run were introduced into the ion source of the MS with a flow rate 5 μl/min using a syringe pump. The temperature of the source block was

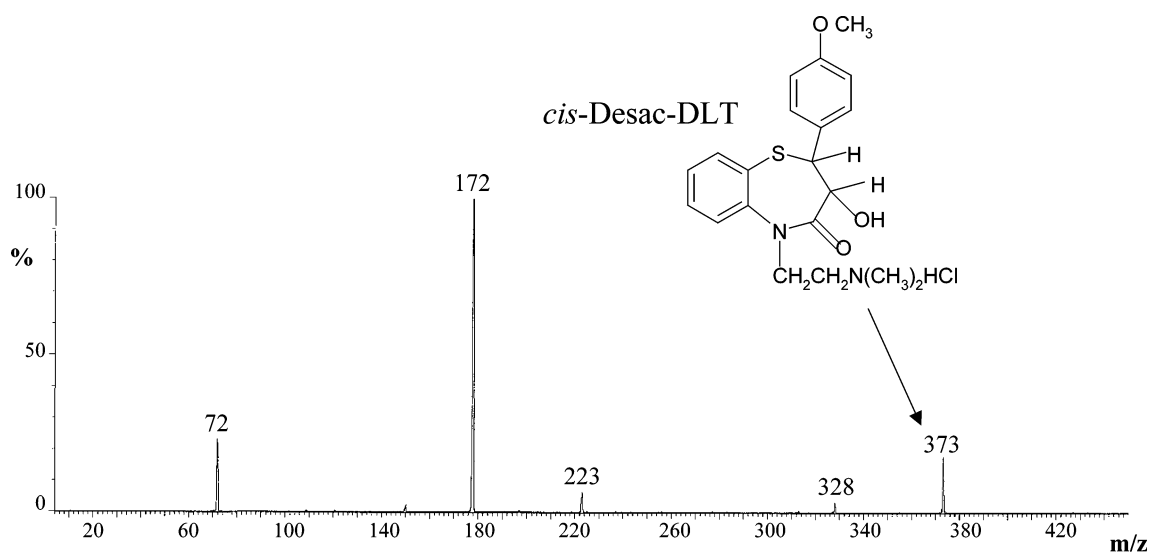

Fig. 3. Electrospray ionization mass spectrum of the compound contained in the peak N3 in the chromatographic run shown in Fig. 2.

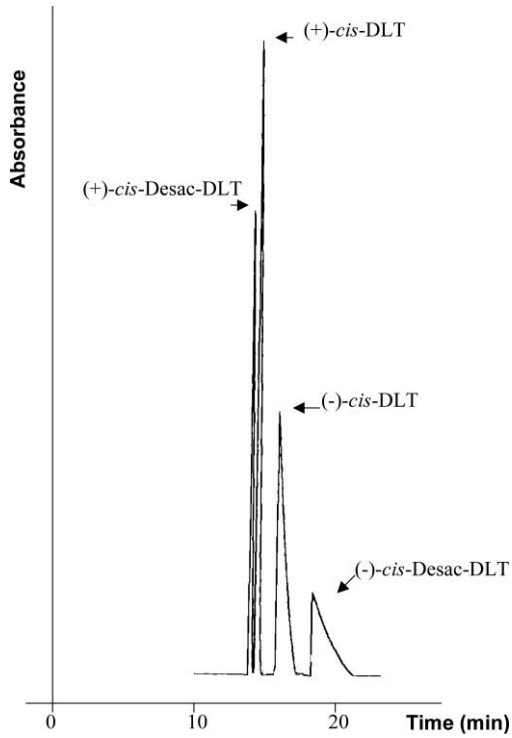

Fig. 4. CE enantioseparation of *cis*-DLT hydrochloride and *cis*-Desac-DLT using 1 mg/ml sulfated-β-CD as a chiral selector. Separation capillary: 40/47 cm total/effective length and 50 μm. I.D. Background electrolyte was 100 mM triethanolamine phosphate at pH 3.0.

60 °C and desolvation temperature 60 °C. The detection was performed in the positive ion mode. The applied voltage was 1.05 kV.

3. Results and discussions

3.1. Simultaneous HPLC enantioseparation of cis-DLT and cis-Desac-DLT

Several papers describe simultaneous enantioseparation of *cis*-DLT, its several analogs and possible metabolites using HPLC on chiral stationary phases (CSP) [1–11]. However, to the best of our knowledge, no study has been published on the simultaneous HPLC separation and enantioseparation of (±)-*cis*-DLT and (±)-*cis*-Desac-DLT, although the latter represents one of the major degradation product and metabolite of *cis*-DLT.

Various commercially available polysaccharide-type CSPs were examined in the normal and reversed-phase mode. None of these CSPs allowed the separation of all four components. Then, same CSPs were studied in polar organic mode with different alcohols as mobile phases. The best separation of all four components was observed on cellulose tris (3,5-dichlorophenylcarbamate) (CD-CPC) using 2-propanol as a mobile phase (Fig. 2) [25].

No reference standard was available for (±)-*cis*-Desac-DLT. Therefore, the fractions corresponding to each peaks were collected and analyzed using ESI-MS. The mass spectra obtained (Fig. 3) clearly confirmed that the peaks N3 and N4 were two enantiomers of (±)-*cis*-Desac-DLT.

3.2. CE enantioseparation of cis-DLT and cis-Desac-DLT

Several cyclodextrin-type chiral selectors were examined in order to achieve simultaneous enantioseparation of (±)-*cis*-DLT and (±)-*cis*-Desac-DLT. The complete separation of four components was achieved using commercially available randomly substituted sulfated β-CD (SU-β-CD) (Fig. 4). The CE method was used in order to follow the kinetics of desacetylation of *cis*-DLT in aqueous methanolic and methanolic solutions (1/1, v/v). The reaction proceeds rather intensively in 0.1% aqueous methanolic solution and the degree of desacetylation exceeds 21% in 24 h at a room temperature (Fig. 5a). The degree of deacetylation in pure methanolic solution was approximately 2% at a room temperature in 24 h (Fig. 5b).

Rather high separation factor of (+)-*cis* and (−)-*cis*-DLT using the CE technique described in this study allowed to use the method for determination of small enantiomeric impurities of (−)-*cis* DLT in (+)-*cis*-DLT. The spiked samples with the content of 0.1–2% of *cis*-(−)-DLT were analyzed using four different CE-sys-

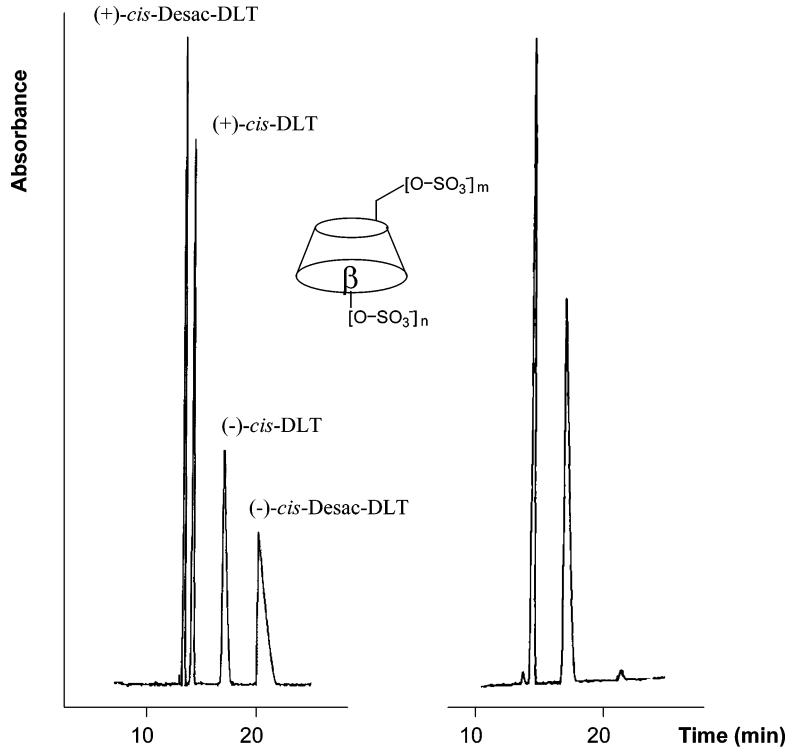

Fig. 5. Electropherograms of hydrolysis products of *cis*-DLT after 24 h at a room temperature in aqueous methanolic (1/1, v/v) (a) and pure methanolic solutions (b). The separation conditions were as indicated in the legend to Fig. 4. The separation was performed using Beckman P/ACE 5010 instrument.

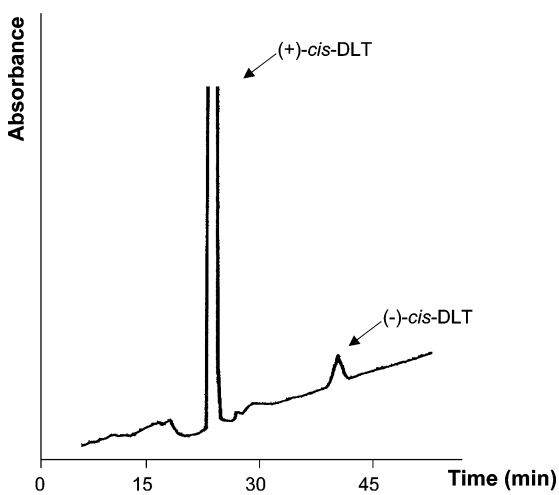

Fig. 6. Electropherogram of (+)-*cis*-DLT containing 0.2% (m/m) of (−)-*cis*-DLT. Separation capillary: 50/60 cm total/effective length and 50 μm. I.D. Background electrolyte was 100 mM triethanolamine phosphate at pH 3.0. The separation was performed using Beckman MDQ instrument.

tems: Grom 100 CE, Beckman P/ACE 5010 CE, Beckman P/ACE MDQ CE and HP^{3D} CE. The calibration curves were linear in the concentration range studied with the correlation coefficients between 0.988 and 0.990 depending on the instrument. The method allowed a clear detection and quantification of the enantiomeric impurity on the level of 0.2% (Fig. 6). It was possible to correctly evaluate the content of the minor enantiomer using the major one as the internal standard. The method could be transferred from equipment to equipment and from laboratory to laboratory.

Acknowledgements

The authors thank the 'Deutsche Forschungsgemeinschaft', the 'Fonds der Chemischen Industrie and the' Ministerium für Frauen, Jugend, Familie und Gesundheit des Landes Nordrhein–Westfalen' for financial support.

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