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Determination of dimethindene enantiomers in pharmaceuticals by capillary electrophoresis with carboxyethyl-β-cyclodextrin

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Cyclodextrin-mediated capillary zone electrophoresis in a hydrodynamically closed separation system with suppressed electroosmotic flow and UV absorbance photometric detection was developed for a high effective separation and quantitation of dimethindene enantiomers in various pharmaceutical formulations (solution, gel, capsules). The running buffer consisted of ε-aminocaproic acid (20 mmol/l) adjusted to pH 4.5 with acetic acid, negatively charged carboxyethyl-β-cyclodextrin (2.5 mg/ml), serving as chiral selector, and 0.1% (w/v) methylhydroxyethylcellulose, serving as an electroosmotic flow suppressor. The proposed method was successfully validated appraising parameters of sensitivity, linearity, precision, accuracy/recovery and robustness and it is useful for routine use.

1. Introduction

Dimethindene maleate, *N*,*N*-dimethyl-3-[1-(2-pyridinyl)ethyl]-l-*H*-indene-2-ethanamine maleate (DIM), is a histamine H1-receptor antagonist used in preparations to treat allergies and respiratory infections, rhinitis, skin rashes and pruritus. Chiral capillary electrophoresis (CE) is currently intensively implemented in pharmaceutical and clinical analyses. CE has been reported for the quantitative determination of DIM enantiomers in model samples and body fluids using various buffer additives, such as native and derivatized cyclodextrins (CDs) (Chankvetadze et al. 1998; Heuermann and Blaschke 1994; Matsunaga et al. 2002; Prien and Blaschke 1997; Rudolf and Blaschke 1999) and sulfated galactosaminoglycans (Gotti et al. 1999), as chiral selectors. Charged CD derivatives (phosphated, carboxylated) appeared to be the most effective chiral selectors among all chiral buffer additives employed as they enabled to achieve baseline enantioseparation at low concentrations (< 5 mg/ml) in separation electrolytes.

The aim of the present work was to examine carboxyethyl-β-cyclodextrin (CE-β-CD) as chiral selector for DIM and to develop a simple, sensitive and selective capillary zone electrophoresis (CZE) method for determination of DIM enantiomers in commercial pharmaceutical formulations, suitable for routine use.

2. Investigations, results and discussion

The principal operating parameters optimized in the present CE separations were type and concentration of CD, carrier cation and counterion and pH of buffer. These parameters were optimized with respect to minimize electromigration and thermal dispersion effects (use of low-mobility buffer constituents) and to achieve a sufficient enantioresolution of DIM (charged CE-β-CD was found to be more effective than native β-CD). Sufficient ionization of both, analyte and chiral selector, was achieved at pH 4.5 resulting in a baseline enantioseparation of DIM with a low concentration of the oppositely migrating CE-β-CD and a short analysis time. Electroosmotic dispersion was successfully suppressed using methylhydroxyethylcellulose (m-HEC) as a capillary surface-coating additive. Optimized separating conditions consisted of 20 mmol/l ε-aminocaproic acid adjusted to pH 4.5 with acetic acid, 2.5 mg/ml CE-β-CD and 0.1% (w/v) m-HEC.

The developed CE method was validated and the resulting data are given in Table 1. In calculations the areas of the peaks were corrected to their migration times to compensate for their differential detector residence times. The data in Table 1 clearly indicate that parameters of the proposed method, i.e. sensitivity, linearity, precision (repeatability, intermediate precision, reproducibility), accuracy/recovery and robustness, are favorable for its routine use in pharmaceutical analysis.

In this work, DIM enantiomers were determined in various pharmaceutical formulations including solution, gel and capsules. Results from the determinations are given in Table 2. The contents of DIM, obtained by the proposed method, were in good agreement with those declared by the manufacturers. The absolute differences between determined and declared values did not exceed 1.3% with RSD less than 1.5%. The chiral method clearly confirmed racemic composition of the active compound in the preparations. The electropherogram from the chiral analysis of the pharmaceutical sample (here, the commercial peroral solution Fenistil® is given as an example) is shown in the Fig. No detection interferences from the sample solvent,

Table 1: Performance parameters of the enantioselective CZE method for dimethindene[a]

Parameter		Parameter	
Sensitivity[b]		Precision[e], RSD (%)	
LOD (μg/ml)	0.3	Repeatability	0.6
LOQ (μg/ml)	1.0	Intermediate precision	1.3
Linearity[c]		Reproducibility	2.1
Line equation (1)	$y = 0.0630 + 0.2736x$	Recovery[f] (%)	
r^2 (1)	0.9996	Peroral solution	100.1
Line equation (2)	$y = 0.0916 + 0.2647x$	Gel	99.2
r^2 (2)	0.9994	Capsules	98.8
Robustness test[d]	$< 3\%$	Accuracy[g], RE (%)	0.7

[a] For the optimized separating conditions see the section 2; other working conditions, the section 3. Concentration of DIM standard in the injected samples was 10 μg/ml, unless otherwise stated.
[b] The limit of detection (LOD) was estimated as 3σ while the limit of quantitation (LOQ) as 10σ (σ, average height of the baseline noise).
[c] Linearity of detector response (peak area) for DIM enantiomers [(1) and (2), enantiomers according to their migration order] was assessed over the concentration range 2.0–10.0 μg/ml with five replicate injections of each sample.
[d] Robustness test examined the effect that deliberate variations in operational parameters (concentration of chiral selector, 2.5–3.0 mg/ml, and carrier ion, 20–25 mmol/l) had on the analysis results (enantioresolution of DIM). Fluctuations of R are expressed in %.
[e] Precision is expressed as relative standard deviation (RSD) of peak areas obtained from five consecutive runs (repeatability), five runs, each on different day (intermediate precision) and five runs performed on another analyzer with the same parameters as described in the section 3.2 (reproducibility).
[f] For the recovery test DIM standard was added into different dosage forms (peroral solution, gel, capsules) and the detector responses were compared to those obtained from DIM samples prepared in pure solvent (demineralized water). Five replicate injections of each sample were done.
[g] Accuracy is expressed as average relative error, RE (%), obtained from the recovery test

Table 2: Enantioselective determination of dimethindene in pharmaceuticals[a]

Parameter	Oral solution	Gel	Capsules
Average content[b]	per 1 ml	per 1 g	per 1 capsule
declared (mg)	1	1	4
determined ± RSD (%)	100.4 ± 0.8	99.3 ± 1.1	98.7 ± 1.5
Enantiomeric ratio, (1)/(2)	50.3/49.7	49.5/50.5	50.8/49.2

[a] For the optimized separating conditions see the section 2 (other working conditions and sample preparation procedures, the section 3). Concentration of DIM in the injected samples was ~10 μg/ml.
[b] Average content of DIM in pharmaceuticals (Fenistil) was calculated (from five consecutive runs) as a sum of enantiomers (1) and (2) using their corrected peak areas.

impurities and dosage form excipients were seen separating DIM enantiomers in the samples at a 265 nm detection wavelength.

It is concluded that the proposed method, with CE-β-CD as a chiral selector, is suitable for highly effective (reliable, simple, fast and economic) enantioselective screening of pharmaceuticals containing DIM as an active ingredient.

3. Experimental

3.1. Samples and reagents

The carrier electrolyte solutions were prepared from chemicals obtained from Merck (Darmstadt, Germany), Aldrich (Steinheim, Germany), and Fluka (Buchs, Switzerland) in water demineralized by a Rowapure-Ultrapure water purification system (Premier, Phoenix, Arizona, U.S.A.). All chemicals used were of analytical grade or additionally purified by the usual methods. The solutions of the electrolytes were filtered before use through disposable membrane filters (a 1.2 μm pore size) purchased from Sigma (St. Louis, MO, USA). Racemic DIM (maleate salt) was obtained as working standard from USP (USP Convention, Rockville, MA, USA). Commercial pharmaceuticals, Fenistil® drm. gel, Fenistil® por. gtt. sol., Fenistil® 24 por. cps. pro., are products of Novartis (Nyon, Switzerland) and they were obtained commercially. Native β-CD was purchased from Aldrich (Steinheim, Germany). CE-β-CD (DS 3, CE purity) is a commercial product of Cyclolab (Budapest, Hungary).

3.2. Instrumentation and capillary zone electrophoresis conditions

A CS Isotachophoretic Analyzer EA 101 (Villa-Labeco, Spišská Nová Ves, Slovak Republic) was used in a single-column configuration of the separation unit. The separation unit consisted of the following modules: (i) a CZE injection valve with a 100 nl internal sample loop (Villa-Labeco); (ii) a column provided with a 300 μm I.D. (650 μm O.D.) capillary tube made of fluorinated ethylene-propylene copolymer (FEP) of 210 mm total length (160 mm to the photometric detector); (iii) a counter-electrode compartment with a hydrodynamically (membrane) closed connecting channel to the separation compartment (Villa-Labeco).

The CZE column was provided with an on-column conductivity detector (Villa-Labeco) and with a LCD 2083 on-column photometric detector with variable wavelengths, 190–600 nm (Ecom, Praha, Czech Republic). In this work the photometric detector was set at a 265 nm detection wavelength. The signals from the detectors were led to a PC via a Unilab data acquisition unit (Villa-Labeco). ITP Pro32 Win software (version 1.0) obtained from KasComp (Bratislava, Slovak Republic) was used for data acquisition and processing.

Prior to use, the capillary was not particularly treated to suppress an electroosmotic flow. A dynamic coating of the capillary wall by means of a 0.2% (w/v) m-HEC 30 000 (Serva, Heidelberg, Germany) in background electrolyte solutions served this purpose (Kaniansky et al. 1997). CZE analyses were carried out in the cationic regime of the separation with direct injections of the samples. The experiments were performed in constant current mode at 20 °C. The driving current applied was 100–120 μA.

3.3. Sample and standard solution preparations

A standard stock solution of DIM was prepared in diluted acetic acid at 1 mg/ml concentration and it was stored at −8 °C in the freezer. Working solutions were made by appropriate dilution of the stock solution with demineralized water so that the concentrations of the drug in the injected model samples were in the 0.1–10 μg/ml range.

Pharmaceuticals present in liquid or gel dosage forms were weighed accurately and then appropriately diluted with slightly acid demineralized water. Solid pharmaceuticals were prepared for analysis in the following way: ten capsules were finely powdered in the agate mortar. A portion of the powder equivalent to 5 mg of DIM was weighed accurately, transferred to a 10 ml volumetric flask and suspended in 5 ml of demineralized water and 0.5 ml of 1 mol/l acetic acid. The flask was placed in an ultrasonic water bath for 10 min. The mixture was kept for 12 h at a room temperature, shaken, completed to volume with water, ultrasonicated for 10 min and again kept for 12 h at a room temperature. The mixture was centrifuged (8000 rpm) for 10 min. The resulting solution was appropriately diluted with demineralized water prior to the analysis and filtered [at

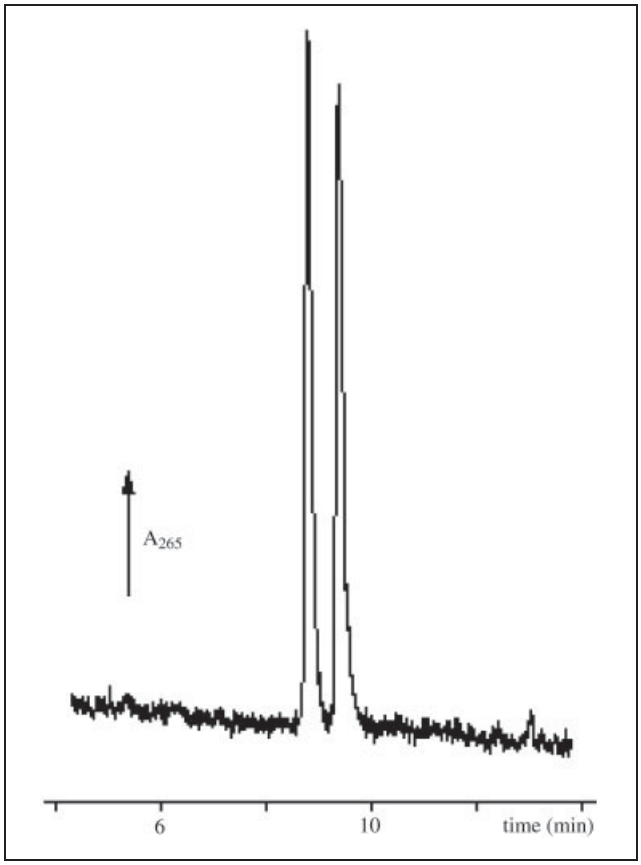

Fig.: Electropherogram from the enantioselective determination of DIM in commercial pharmaceutical preparation (peroral solution Fenistil). The separating conditions consisted of 20 mmol/l ε-aminocaproic acid buffer adjusted to pH 4.5 with acetic acid, 2.5 mg/ml CE-β-CD and 0.1% (w/v) m-HEC. The driving current was stabilized at 120 μA and detection wavelength was set at 265 nm. The concentration of DIM enantiomers in the sample was ~5 μg/ml. For the sample preparation and other conditions see the section 3

1.2 μm pore size (Sigma)] before injection into the CE equipment. The concentrations of the drug in the injected pharmaceutical samples were in all cases within the 1–10 μg/ml range.

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