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Chiral separation of dioxopromethazine in eye drops by CZE with charged cyclodextrin

Peter Mikuš *, Iva Valášková, Emil Havránek

Department of Pharmaceutical Analysis and Nuclear Pharmacy, Faculty of Pharmacy, Comenius University, Odbojárov 10, SK-832 32 Bratislava, Slovakia

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Abstract

Capillary zone electrophoresis (CZE) with carboxyethyl-β-cyclodextrin (CE-β-CD) dissolved in the operating buffer was used for the separation and determination of enantiomers of phenothiazine antihistaminic, dioxopromethazine, in commercial pharmaceutical preparation, eye drops. This chiral selector, negatively charged under given separating conditions (20 mmol/l ε-aminocaproic acid, acetic acid, pH 4.5), was effective in enantioresolution of the antihistamine even at its low concentrations (3–6 mg/ml) in the buffer solution. CZE identification and quantitation of the relevant constituents present in the preparation (dioxopromethazine enantiomers, phenylephrine) were based on the response of photometric absorbency detector, operating at a 275 nm detection wavelength. Changes in pH, type and concentration of chiral selector were studied in relation to chiral resolution. Acceptable validation criteria for sensitivity, precision, linearity and repeatability are included.

Keywords: Antihistamines; Capillary zone electrophoresis; Carboxyethyl-β-cyclodextrin; Charged cyclodextrin; Dioxopromethazine; Drugs; Enantioseparation; Pharmaceutical preparation

1. Introduction

Dioxopromethazine (DPZ) is histamine H1-receptor antagonist that decreases elevated permeability of capillaries and their dilatation induced by histamine released upon allergic reaction. The chemical structure of DPZ is shown in Fig. 1. Several methods have been reported for the quantitative determination of DPZ including spectrophotometry [1], HPLC [2,3] and capillary electrophoresis (CE) [4–7]. DPZ was determinated in various matrices including dusting powder, urine and eye drops. Some of the CE studies have been dealt with structurally related phenothiazines, see e.g. [8]. Here, the concentrations of thioridazine and promethazine in pharmaceutical preparations were determinated by capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC).

Enantiomeric separations of various drugs, among them antihistamines, by CE have been widely studied and the results have been reviewed

* Corresponding author. Tel.: +421-2-50259-350; fax: +421-2-55572-065.
E-mail address: mikus@fpharm.uniba.sk (P. Mikuš).

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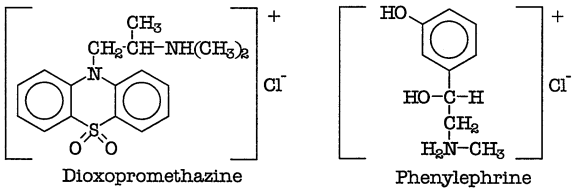

Fig. 1. Chemical structures of the studied drugs.

by several authors, see e.g. [9–16]. Phenothiazines including promethazine, ethopropazine, trimeprazine, methotrimeprazine, and thioridazine were separated by chiral CZE employing neutral both native and derivatized cyclodextrins (CDs), namely β-CD, γ-CD and hydroxypropyl-β-CD [17]. Enantioseparation of many chiral phenothiazine derivatives in CE using native and charged CDs has been published recently [18]. CE enantioseparations of the investigated drug, DPZ, have been examined using native β-CD as a chiral selector [4–7]. It was shown that β-CD was efficient chiral selector for the phenothiazine derivatives.

Given the simplicity and robustnes of cyclodextrin-based chiral separations [19] it was decided to focus on this chiral selectivity mechanism. CDs and their derivatives differ significantly in their selectivities in CE separations of various groups of compounds [20]. These differences provide a frame for a wide variety of the electrolyte systems suitable to the CE enantioseparations of ionogenic and nonionic pharmaceuticals [12]. Charged CD derivatives were used as chiral selectors in CE for the first time by Terabe [21] and the effects of opposite analyte-selector migration were illustrated by Chankvetadze [22]. Charged CDs migrating in opposite direction to the analytes were shown in many cases to be more effective chiral selectors in comparison with their native forms used in CE [23,24]. Some aspects of the use of charged cyclodextrins for CE enantioseparations of basic racemic drugs were discussed in [22]. Negatively charged CD derivatives were used in CE enantioseparations of antihistamines several times [18,25–27]. For example, carbinoxamine and doxylamine were separated to their enantiomers using sulfated β-CD [25] and chlorpheniramine by carboxymethyl-β-CD [26,27].

The aim of the present work was to develop simple, sensitive and selective method for determination of enantiomeric ratio of phenothiazine antihistaminic, dioxopromethazine, in commercial pharmaceutical preparation (eye drops). CZE with a negatively charged carboxyethyl-β-cyclodextrin (CE-β-CD) was expected to be more effective alternative to the CZE with a native β-CD. Moreover, the proposed method should be capable of quantifying enantiomers of DPZ in presence of phenylephrine (EPH) that is used in ophthalmology, in its lower concentration, as vasoconstrictor. The chemical structure of EPH is shown in Fig. 1. The use of CZE in a hydrodynamically (membrane) closed separation system, employing the capillary of a larger I.D., was expected to enhance some performance parameters (sensitivity, sample loadability, repeatability), see [28] and references cited inside, in comparison with commonly used hydrodynamically opened electrophoretic separation system. On the other hand, using this analytical approach the separation efficiency might be slightly decreased. Validation details of the proposed method include sensitivity, precision, linearity and repeatability.

2. Experimental

2.1. Instrumentation

A CS Isotachophoretic Analyzer (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 240 and 275 nm detection wavelengths. The signals from the detectors were led to a PC via a Unilab data acquisition unit (Fitek, Slovak Republic). ITP PRO32 WIN software (version 1.0) obtained from KasComp (Bratislava, Slovak Republic) was used for data acquisition and processing.

Prior to the use, the capillary was not particularly treated to suppress an electroosmotic flow (EOF). A dynamic coating of the capillary wall by means of a 0.2% m-HEC in background electrolyte solutions served for this purpose [29]. CZE analyses were carried out in cationic regime of the separation with direct injections of the samples. The experiments were performed in constant current mode [28]. The driving current was 100 μA. The temperature was 20 °C.

2.2. Chemicals and samples

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, USA). Methylhydroxyethylcellulose 30 000 (m-HEC), obtained from Serva (Heidelberg, Germany), served as an EOF suppressor in the carrier electrolyte solutions [29]. 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).

Native CDs were purchased from Aldrich. Carboxyethyl-β-cyclodextrin (DS 3, CE purity) is a commercial product of Cyclolab (Budapest, Hungary). Dioxopromethazine hydrochloride (DPZ) was obtained as a racemic mixture from Intermo (Netherlands). Phenylephrine hydrochloride (EPH) was obtained from Dolder (Switzerland). Commercial pharmaceutical preparation, eye drops Promefrin®, containing 2.5 mg/ml of DPZ and 1.0 mg/ml of EPH, was obtained from Unimed-Pharma (Bratislava, Slovak Republic). The drug samples were appropriately diluted with demineralized water prior to the analyses. Standard solutions of DPZ and EPH were prepared dissolving them in demineralized water.

3. Results and discussion

3.1. Method optimization

The principal operating parameters optimized in the present chiral CZE separation were cyclodextrin type and concentration, electrolyte composition and pH. Electrolyte systems (ES) used are ordered in Table 1.

3.1.1. Cyclodextrins

Native α- and β-CDs and derivatized CE-β-CD were used in our experiments aimed at finding a chiral selector suitable for the separation of enantiomers of dioxopromethazine (En1), (En2). Our CZE experiments with α-CD revealed that this host influenced the effective mobility of DPZ. However, it failed to resolve the enantiomers of DPZ. On the other hand, β-CD and its derivative were effective in this respect (see Fig. 2). The concentration of the native CD was varied over the range 2–10 mg/ml (ES 3 in Table 1). The charged CD was used at 2–6 mg/ml concentrations (ES 4 in Table 1). Regarding an appropriate conductivity of the electrolyte system lower concentrations of this selector were preferred. As shown in Fig. 3a, resolution of DPZ enantiomers increased with increasing CD concentration. It was found out that CE-β-CD was much more effective in resolution of the enantiomers than native β-CD due to the presence of its charge. It is obvious comparing both, functionalities c versus R in Fig. 3a and electropherograms (b) and (c) in Fig. 2. Trace (c) illustrates that a 3 mg/ml concentration of CE-β-CD was sufficient yet to obtain relatively high enantioresolution of DPZ. A complete enantioresolution of DPZ was possible to obtain at a 5.5 mg/ml concentration of charged CE-β-CD (see below). Based on recent ^{1}H-NMR spectroscopy findings [30], it seems appropriate to ascribe this remarkable chiral recognition capability of CE-β-CD to a cooperative action of the inclusion effect of its cavity with Coulomb interactions of oppositely charged complex forming partners. This

Table 1
Electrolyte systems

Parameter	ES 1	ES 2	ES 3	ES 4
Solvent	Water	Water	Water	Water
Carrier cation	ε-ACA	Gly	ε-ACA	ε-ACA
Concentration (mmol/l)	20	24	20	20
Counter ion 1	AcH	CitH	AcH	AcH
Concentration (mmol/l)	–	1.6	–	–
Counter ion 2	–	AcH	–	–
Concentration (mmol/l)	–	84	–	–
PH	4.5	3.2	3–5	3–5
EOF suppressor	m-HEC	m-HEC	m-HEC	m-HEC
Concentration (%, w/v)	0.2	0.2	0.2	0.2
Complexing agent	–	–	β-CD	CE-β-CD
Concentration (mg/ml)	–	–	2–10	2–6

ε-ACA, ε-aminocaproic acid; Gly, glycine; AcH, acetic acid; CitH, citric acid; m-HEC, methylhydroxyethylcellulose; β-CD, β-cyclodextrin; CE-β-CD, carboxyethyl-β-cyclodextrin.

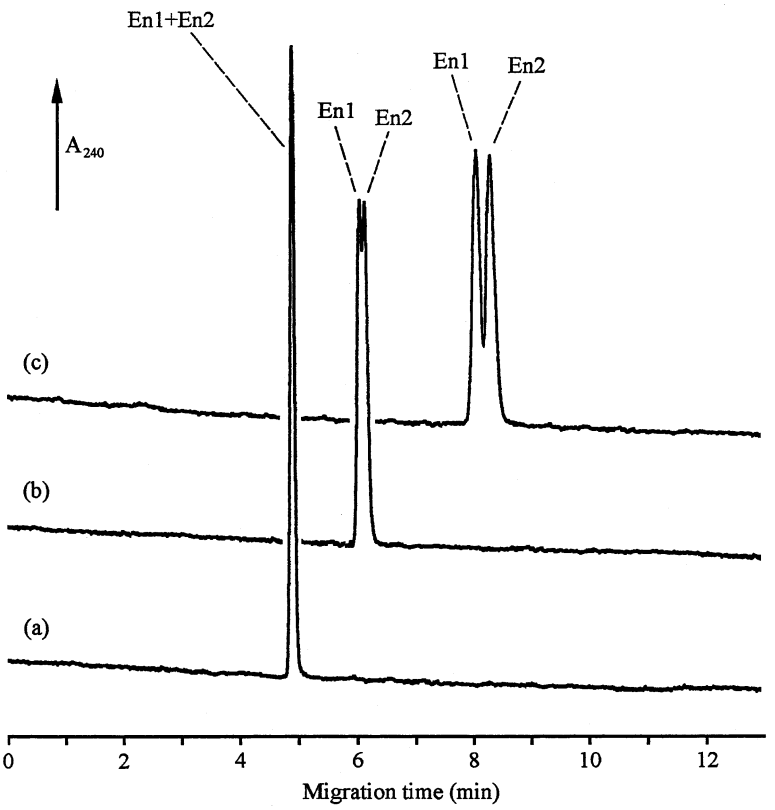

Fig. 2. CZE separations of dioxopromethazine enantiomers in the electrolyte systems containing different chiral selectors (Table 1). The concentrations of the analytes in the injected samples were 50 μmol/l. (a) The separation in ES 1, serving as a reference; (b) the separation in ES 3, containing native β-CD at a 10 mg/ml concentration; (c) the separation in ES 4 (pH 4.5), containing CE-β-CD at a 3 mg/ml concentration. Peak assignments: En1, En2, dioxopromethazine enantiomers. The driving current was stabilized at 100 μA [the corresponding driving voltage was 5.8 kV (a, b) and 5.3 kV (c). The detection wavelength was 240 nm.

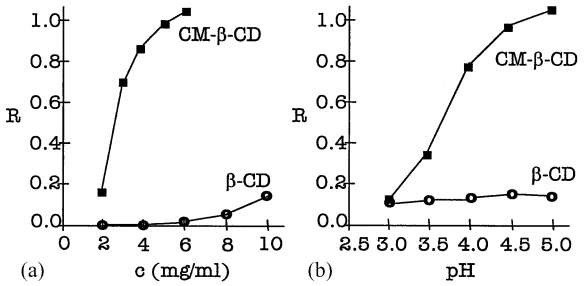

Fig. 3. Effect of pH and concentration of the chiral selector on resolution of dioxopromethazine enantiomers. (a) The concentration dependences of native β-CD and CE-β-CD were obtained at pH 4.5; (b) the effect of pH was examinated at 10 and 5 mg/ml concentrations of the native and derivatized CD, respectively. In the investigations the ES 3 and 4 (Table 1) were used. Concentrations of dioxopromethazine enantiomers in the analyzed sample were 50 μmol/l. The detection wavelength was 240 nm.

attribute (complex stability) as well as the countercurrent migration of the analyte and the chiral selector in CZE are expressed by the enantiomeric effective mobility difference equation proposed by Wren and Rowe [31] that supports/explains the results obtained.

3.1.2. Electrolyte composition and pH

Selectivity of achiral CZE method was examined in two buffers, ES 1 and 2, given in Table 1. It was found out that it was not princpally affected neither by different carrier cations nor pH of the buffer. The electrolyte system with ε-ACA was chosen for further investigations because it provided any better peak symmetry of DPZ than the electrolyte with Gly (probably due to a better match of the ionic mobilities of ε-ACA and DPZ).

The results obtained at various pH of the buffers demonstrated a significant role of the charge of the chiral selector in the separation selectivity. While enantioresolutions of DPZ were not practically influenced by pH changes when separated in presence of native β-CD, the strong dependence was observed with CE-β-CD (see Fig. 3b). Szemán et al. [32] demonstrated that various carboxymethyl-β-cyclodextrins (DS 2–8) were in their protonated forms at pH below 4–4.5. Our results were in accordance with those findings. The resolution of the enantiomers was increased with increasing pH, as can be seen in Fig. 3b. pH tested ranged from 3 to 5 and the enantioresolution was significantly increased at pH about 4 within this interval. Here, a residual charge of CE-β-CD could be responsible for the enantioresolution obtained within pH 3–4 (compare with [33] dealing with CE enantioseparation at a low pH using carboxymethyl-β-CD as a chiral selector). With respect to both, a sufficient dissociation of the carboxy group(s) of CE-β-CD and the effective mobility (migration time) of the analyte, pH 4.5 was chosen as the optimal buffer pH for the enantioseparations. At this pH baseline separation of En1 and En2 was obtained within 10 min using appropriate concentration of charged CE-β-CD (see above).

3.1.3. Wavelength optimization

Wavelength between 230 and 280 nm were assessed for maximum response. Use of 240 nm gave the highest response for DPZ. However, when separated DPZ and EPH simultaneously, 275 nm was chosen with respect to the fact that response of EPH decreased as wavelengths decreased.

3.2. Validation

The validation aspects assessed included performance parameters such as sensitivity, precision, linearity and repeatability. A lack of pure enantiomers of DPZ excluded to be evaluated migration order of the enantiomers. ES No. 4 (Table 1) was used to obtain validation data. Here, optimized concentration of CE–CD was 5.5 mg/ml and optimized acidobasic conditions were at pH 4.5.

Limit of detection (estimated as 3σ) of DPZ at 240 nm was 0.79 μmol/l when separated standard sample. When determined DPZ and EPH in the pharmaceutical preparation simultaneously (at a 275 nm of detection wavelength) limits of detection were 1.34 and 4.12 μmol/l, respectively.

Precision was assessed with or without internal standard at a 50 μmol/l concentration of each compound tested. In our study, EPH served as the internal reference. Twelve repeated injections of DPZ and EPH at different wavelengths gave the data presented in Table 2. As for the long-time repeatability, the separation and quantitation of

Table 2
Precision data for repeat injections of dioxopromethazine enantiomers and phenylephrine[a]

Factor	R.S.D. (%)									
	λ (240 nm)						λ (275 nm)			
	En1[b]	En1[c]	En2[b]	En2[c]	En1[b]	En1[c]	En2[b]	En2[c]	EPH[b]	EPH[c]
Migration time	0.22	0.29	0.21	0.31	0.34	0.42	0.36	0.41	0.31	0.40
Relative migration time	0.20	0.23	0.20	0.24	0.27	0.31	0.28	0.31	–	–
Peak area	0.56	0.75	0.58	0.77	0.66	0.84	0.70	0.87	0.75	0.86
Peak–area ratio	0.39	0.44	0.40	0.46	0.47	0.51	0.50	0.52	–	–

En1, En2, dioxopromethazine enantiomers; EPH, phenylephrine.
[a] The separations were carried out in the optimized ES 4 (see Table 1 and Section 3.2). The R.S.D. values of the migration parameters of the peaks were obtained from 12 parallel CZE runs repeated.
[b] On the same day.
[c] On different days (2 weeks between the series).

the compounds studied were repeated under the same conditions on different days and the results obtained are also in Table 2. These results clearly indicated that CZE separations in hydrodynamically (membrane) closed separation system provided highly reproducible migration data so that the internal standard was not necessary to use in order to obtain acceptable validation criteria. This is in the contrary to CE separations in hydrodynamically opened separation systems where the use of internal standards can more significantly both eliminate the imprecision due to injection volume (vacuum or pressure injection) and improve reproducibility of migration times [34].

The linearity of detector response (peak area) for DPZ and EPH was assessed over the range 10–100 μmol/l [limit of quantification (10σ) is involved]. This represents interval suitable for evaluation of these drugs in common preparations of pharmaceutical interest. The correlation coefficients obtained for En1, En2 and EPH were 0.9997, 0.9996 and 0.9994, respectively, when detected at 275 nm while it was 0.9998 for the enantiomers at 240 nm. Corresponding straight line equations were $y = -0.0335 + 45.5373x$, $y = -0.0286 + 43.8020x$ and $y = -0.0208 + 203{,}0450x$, respectively, at 275 nm, and $y = -0.0092 + 19.0531x$, $y = -0.0076 + 18.1373x$, respectively, at 240 nm. Use of the internal standard slightly improved correlation coefficients ($\sim 0.01\%$) reducing scatter of points due to random error. In all cases the peak areas of the peaks were normalized to their migration times to compensate for their differential detector residence times.

3.3. Analysis of pharmaceutical sample

In our research eye drops with 2.5 mg/ml (7.12 mmol/l) and 1.0 mg/ml (4.95 mmol/l) declared values of DPZ and EPH, respectively, was examined. The sample of an appropriately diluted preparation was separated and the electropherogram from the chiral separation is in Fig. 4. The baseline separation, short analysis time and low consumption of the charged chiral selector are merits of the proposed method. The results obtained using chiral and achiral CZE method are given in Table 3 and corresponding separation conditions include ES 1 and optimized ES 4, respectively (see Table 1 and Section 3.2). The contents of DPZ and EPH obtained using the proposed methods were in a good agreement with those declared. Moreover, the chiral method facilitated to determine the enantiomeric ratio of DPZ in the preparation (see Table 3 and Fig. 4). No detection interference was occurred separating the preparation at a 275 nm detection wavelength.

4. Conclusion

This work outlined the significant potential of CZE working in hydrodynamically (membrane) closed separation system, employing charged

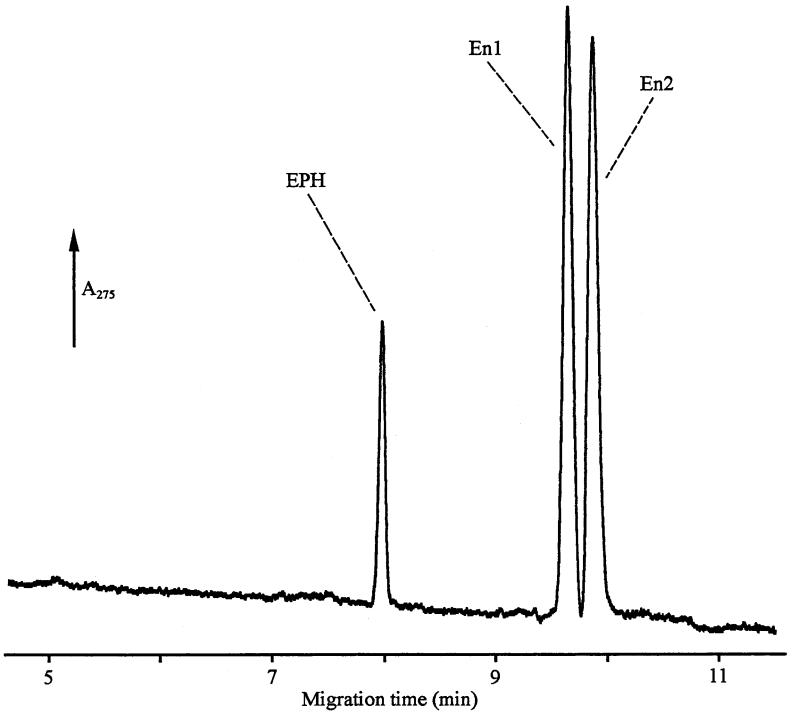

Fig. 4. Electropherogram from the determination of enantiomeric composition of dioxopromethazine in commercial pharmaceutical preparation (eye drops). The separation was carried out in the optimized ES 4 with CE-β-CD at a 5.5 mg/ml concentration and pH 4.5 (Table 1). The driving current was stabilized at 100 μA and the corresponding driving voltage was 4.9 kV. Peak assignments: En1, En2, dioxopromethazine enantiomers; EPH, phenylephrine. The preparation (see Section 2) was diluted 1:100 (v/v) with demineralized water before an injection. The electropherogram was obtained at a 275 nm detection wavelength.

chiral selector for providing highly effective chiral separations of pharmaceutical preparations. Baseline separations of dioxopromethazine enantiomers with high reproducibility and low consumption of the chiral selector are advantages of the proposed method. A notable performance

Table 3
Determination of dioxopromethazine and phenylephrine in eye drops[a]

Parameter	Method					
	Achiral			Chiral		
	DPZ	EPH	En1	En2	ΣEn1, En2	EPH
Average content[b] (mg/ml)	2.466	0.979	1.222	1.256	2.478	0.983
R.S.D.[c] (%), $n = 12$	0.69	0.77	0.75	0.78	–	0.80
Enantiomeric ratio (%)	–	–	49.31	50.69	–	–

DPZ, En1, En2, dioxopromethazine and its enantiomers; EPH, phenylephrine.

[a] The separations were carried out in the ES 1 and optimized ES 4 (see Table 1 and Section 3.2); the preparation analyzed (see Section 2) was diluted 1:100 (v/v) with water.

[b] For calculations, mean values of the corrected peak areas (an actual peak area of the analyte was divided by its migration time) of the analytes were used.

[c] The R.S.D. values of the corrected peak areas were obtained from 12 parallel CZE runs

was obtained using charged CE-β-CD comparing with native β-CD. The optimized conditions consisted of 5.5 mg/ml CE-β-CD dissolved in a 20 mmol/l ε-ACA-AcH–0.2% (w/v) m-HEC electrolyte, pH 4.5. The capillary of a larger I.D. (300 μm) employed provided favorable conditions in term of sensitivity of the photometric absorbance detection.

Successful validation was achieved including suitable assessments of sensitivity, linearity, precision and repeatability. It is concluded that the reported operating conditions are suitable for the routine assay and enantiopurity determination of dioxopromethazine.

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