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Enantioselective determination of pheniramine in pharmaceuticals by capillary electrophoresis with charged cyclodextrin

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Abstract

Cyclodextrin (CD)-mediated capillary zone electrophoresis (CZE) in hydrodynamically closed separation system was developed for the separation and quantitation of pheniramine (PHM) enantiomers. Several parameters affecting the separation were studied, including the type and concentration of chiral selector, carrier cation and counterion, and the pH of the buffer. A high effectivity of oppositely migrating carboxyethyl-β-cyclodextrin (CE-β-CD) to separate the PHM enantiomers was demonstrated in detail. The optimized chiral analysis of the antihistamine drug was performed in a buffer consisted of 20 mmol/l ε-aminocaproic acid adjusted to pH 4.5 with acetic acid, containing negatively charged CE-β-CD (2.5 mg/ml) as chiral selector and 0.2% (w/v) methylhydroxyethylcellulose (m-HEC) as an electro-osmotic flow (EOF) suppressor. Acceptable validation criteria for sensitivity, linearity, precision, accuracy/recovery were included. The proposed CZE method was successfully applied to the assay of PHM in pharmaceutical formulations using dioxopromethazine as an internal standard.

Keywords: Anti-histamine; Capillary zone electrophoresis; Chiral separation; Cyclodextrins; Drugs; Enantiomers; Pharmaceuticals; Pheniramine

1. Introduction

Pheniramine (PHM), *N*-[3-phenyl-3-(2-pyridyl)propyl]-*N*,*N*-dimethylamine (Fig. 1), is histamine H1-receptor antagonist used in preparations to treat allergies and respiratory infections, rhinitis, skin rashes and pruritus. Several analytical methods have been reported for the quantitative determination of PHM and halogen-substituted 3-phenyl-3-(2-pyridyl)propylamines including spectrophotometry [1], HPLC [2] and GC [3]. The drugs were determined in various matrices including pharmaceutical preparations (tablets) and biological fluids (human serum, plasma). Capillary electrophoresis (CE) has recently emerged as a promising analytical technique that consumes an extremely small amount of sample and that is capable of rapid, high-resolution separation, and reproducible quantitation of analytes. As an example, the determination of antihistamines in pharmaceuticals by a CE method is given [4].

Chiral CE separations of PHM and its halogen-substituted analogues have been accomplished using native and derivatized cyclodextrins (CDs) [5–9] and heparin [10] as chiral selectors. Given the simplicity and robustness of cyclodextrin-based chiral separations [11] 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. 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 have been used as chiral selectors in CE for the first time by Terabe [13]. A high effectivity of this type of CDs in CE enantioseparations can be explained at least by two phenomena: (i) an effect of opposite analyte-selector migration; and (ii) a higher stability of inclusion CD associates due to additional electrostatic

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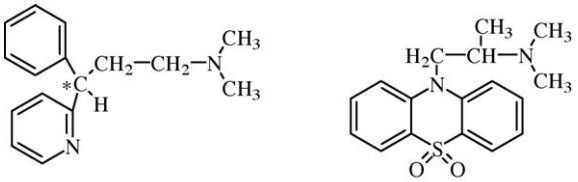

Fig. 1. Chemical structures of the drugs used.

interaction between the ionic substituents on the CD ring and the ionic guest compound [14,15]. Charged CDs were shown in many cases to be more effective chiral selectors in comparison with their native forms used in CE [16–18]. Sulfated-β-CD (7 mmol/l) and carboxyethyl-β-cyclodextrin (14 mmol/l) proved to give better resolution to the cationic racemic compounds, namely pheniramine, brompheniramine and chlorpheniramine, compared with CD with neutral substituents [9]. A baseline resolution of PHM enantiomers was achieved with carboxymethyl-β-CD (1.5 mmol/l) [6] and with carboxymethyl-β-CD polymer (4 mg/ml) or its mixture with native β-CD [8].

Although a number of CE methods have been reported for the qualitative chiral analysis of PHM (as cited above), only one paper [8] reported the validated quantitative chiral CE analysis of this drug. Performing the separations in hydrodynamically open CE system, using 50 μm I.D. capillary, the precision of the method (R.S.D. of peak areas) was 1.23–4.47% while the accuracy (absolute relative error) was 0.42–3.40% for 40–140 μmol/l PHM and $n = 5$. The detection limits of PHM enantiomers, based on a signal-to-noise ratio of 3, were 6 μmol/l.

The aim of the present work was to develop simple, sensitive and selective method for determination of enantiomeric ratio of PHM in commercial pharmaceutical preparations. By now, no attention has been paid to use CE in a hydrodynamically closed separation system for the analysis of PHM. Although used in practice rather rarely, this CE technology, minimizing impacts of non-separative transport processes on the migration velocities of the separated constituents, has inherent analytical advantages in situations when highly reproducible migration velocities are desired (e.g., peak area based quantitation of the analytes). The use of capillary of a larger I.D. was preferred in our work, as it should enable to enhance some performance parameters (sample loadability, separation capacity, sensitivity), see Ref. [19] and references given therein. The analytical advantages of using columns of larger I.D. include significantly reduced contributions of electromigration dispersion. At higher conductivities of the carrier electrolytes this gain, however, can be partially lost due to increased thermal dispersive effects. Considering these facts, parameters affecting the separation (enantioresolution, efficiency) were studied in detail, including the type and concentration of chiral selector, carrier cation and counterion, and the pH of the buffer. Validation details of the proposed method include sensitivity, linearity, precision, accuracy/recovery.

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 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 the use, the capillary was not particularly treated to suppress an electro-osmotic flow (EOF). A dynamic coating of the capillary wall by means of a 0.2% methylhydroxyethylcellulose (m-HEC 30000; Serva, Heidelberg, Germany) in background electrolyte solutions served for this purpose [20]. 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 [19] at 20 °C. The driving current applied was 100–120 μA.

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 de-mineralized 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).

PHM (as maleate salt) and dioxopromethazine (DPZ, as hydrochloride), Fig. 1, were obtained as working standards from ICN Biomedicals (Eschwege, Germany) and Unimed Pharma (Bratislava, Slovak Republic). PHM was used as a racemate while DPZ as a pure enantiomeric form. Commercial pharmaceutical preparation, granuled powder Fervex®, declared to contain 25 mg of PHM maleate per sack dose, was obtained from UPSA (Agen, France). Native CDs were purchased from Aldrich (Steinheim, Germany) and sulfated-β-

CD (*S*-β-CD) from Fluka. Carboxyethyl-β-cyclodextrin (DS 3, CE purity), CE-β-CD, is a commercial product of Cyclolab (Budapest, Hungary) while 6^{I}-deoxy-6^{I}-monomethylamino-β-CD (1MA-β-CD) was prepared in the laboratory using the procedure described elsewhere [21].

2.3. Procedures for sample and standard solution preparations

2.3.1. Standard solutions

Pure standard stock solutions of PHM and DPZ (serving as internal standard) were prepared in de-mineralized water at a 10^{-2} mol/l concentration and stored at −8 °C in the fridge. Working solutions were made by appropriate dilution of the stock solutions with de-mineralized water and directly injected into the CE equipment. The concentration of PHM in the injected model sample was 25 μmol/l (12.5 μmol/l for each enantiomer).

2.3.2. Pharmaceutical sample

A total of 8 sack doses were mixed and finely powdered. A portion of the powder equivalent to 10 mg PHM was weighed accurately, transferred to a 10 ml volumetric flask and suspended in 5 ml of de-mineralized water and 0.5 ml of 1 mol/l acetic acid. An appropriate amount of the internal standard was added. The flask was placed in ultrasonic water bath for 5 min before completion to volume with the water. The mixture was centrifuged (5000 rpm) for 5 min. The resulting solution was appropriately diluted with de-mineralized water prior to the analysis and filtered (a 1.2 μm pore size (Sigma)) before the injection into the CE equipment.

2.3.3. Calibration graph

Appropriate amounts of the PHM standard stock solution were diluted with de-mineralized water yielding concentrations of 5, 10, 15, 20, 25 and 30 μmol/l for each enantiomer. Ten replicate injections of each were made.

2.3.4. Recovery test

A portion of the powder (Fervex) equivalent to 5 mg PHM was weighed accurately into a 10 ml volumetric flask, dissolved in 5 ml of de-mineralized water and appropriate amount of PHM standard stock solution was added. Further procedure was the same as in the Section 2.3.2. After an appropriate dilution of resulting solution the concentration of added PHM standard varied as follows, 5, 10, 15, 20 and 25 μmol/l. Ten replicate injections of each sample were made.

2.4. Calculation

The sample resolution in the capillary was calculated by Eqs. (1) and (2),

$$R_s = \frac{2(t_2 - t_1)}{w_1 + w_2} \quad (1)$$

$$R_h = \frac{H_i - H_m}{H_i} \quad (2)$$

where R_s is resolution based on peak-width, t_1, t_2 are migration times of racemates/enantiomers, w_1, w_2 are baseline peak widths of racemates/enantiomers. R_h is resolution based on peak-height. If the baseline separation of two peaks cannot be achieved, R_h is applied. H_i is peak height of the lower peak of the two peaks and H_m is the height of the valley between the two peaks.

The separation efficiency (N) of the CE system was estimated using the following equation:

$$N = 5.54\left(\frac{t}{w_{0.5}}\right)^2 \quad (3)$$

where t is the migration time of the maximum of the peak and $w_{0.5}$ the width of the peak at 50% peak height.

3. Results and discussion

3.1. Method optimization

PHM represents a molecule which migration velocity is influenced by a protonation of two basic groups so that a cationic regime of separation is convenient for its analysis. 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 (in the first step) and to achieve a sufficient enantioresolution of PHM (in the second step). Electrolyte systems (Table 1) ES 1 and 2 were chosen for the free solution CE analyses while ES 3 and 4 were used for the CD-modified CE.

3.1.1. Separation in free solution without selector

PHM exhibited good migration properties in a relatively wide interval of pH of separation electrolyte as indicated by electropherograms (a) in Figs. 2 and 3. A buffer with ε-aminocaproic acid (ε-ACA) as a carrier cation adjusted to pH 5.2 with 4-morpholineethanesulfonic acid (MES) provided a

Table 1
Electrolyte systems

Parameter	ES 1	ES 2	ES 3	ES 4
Solvent	Water	Water	Water	Water
Carrier cation	Glycine	ε-ACA	Glycine	ε-ACA
Concentration (mmol/l)	25	20	25	20
Counter ion	Acetic acid	Acetic acid	Acetic acid	Acetic acid
pH	3.2	4.5	3.2	4.5
EOF suppressor	m-HEC	m-HEC	m-HEC	m-HEC
Concentration (%, w/v)	0.2	0.2	0.2	0.2
Complexing agent[a]	–	–	CDs	CDs
Concentration (mg/ml)[a]	–	–	0.5–20	0.5–20

[a] CD type (concentration range used, mg/ml): α-CD (1–20); β-CD (1–10); *S*-β-CD (0.5–5.0); CE-β-CD (0.5–15.0); 1 MA-β-CD (1–10).

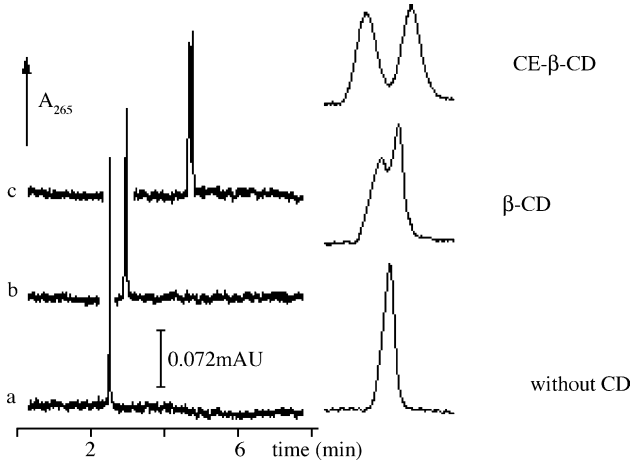

Fig. 2. CZE separations of PHM enantiomers under different complexing conditions at pH 3.2. (a) The separation in ES 1, serving as a reference; (b,c) the separations in ES 3, containing chiral selectors at 10 mg/ml concentrations. When *S*-β-CD (ES 3) used, no peak was obtained within 20 min. The driving current was stabilized at 100 μA. For other conditions see Section 2.

reasonable migration of PHM too, however, the intensity of detector response for the analyte was a bit lower so that this separation system was not considered further.

The slowly migrating organic molecules, as buffer constituents, were preferred to the fast migrating inorganic ions with respect to the minimization of thermal dispersion due to a Joule heat generated during the separation as well as the minimization of electromigration dispersion due to differences in the conductivity within the analyte zone and the zone of background electrolyte. Although *ε*-ACA buffer and higher pH (ES 2) produced more symmetric peak of PHM (compare detail peaks in Figs. 2a and 3a), the glycine buffer and lower pH (ES 1) were preferred in achiral analysis of PHM for the sake of shorter analysis time and higher separation efficiency.

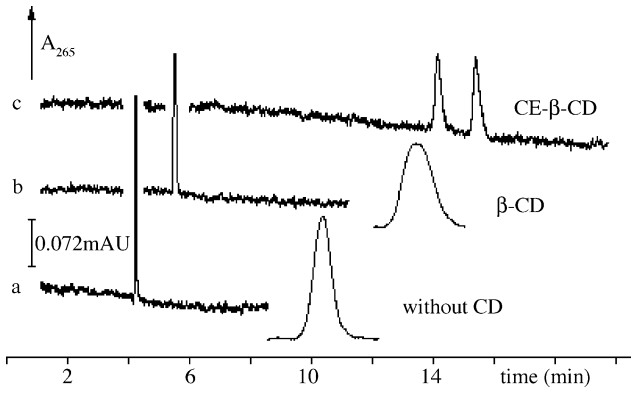

Fig. 3. CZE separations of PHM enantiomers under different complexing conditions at pH 4.5. (a) The separation in ES 2, serving as a reference; (b,c) the separations in ES 4, containing chiral selectors at 5 mg/ml concentrations. When *S*-β-CD (ES 4) used, no peak was obtained within 20 min. The driving current was stabilized at 120 μA. For other conditions see Section 2.

3.1.2. Separation in presence of CD additives

Native α- and β-CDs and various charged CD derivatives, namely CE-β-CD, *S*-β-CD and 1MA-β-CD were used in our experiments aimed at finding a chiral selector suitable for the separation of PHM enantiomers (PHM(1), PHM(2)). For the concentration ranges of CDs used see the legend to Table 1.

CE separations with native CDs revealed that β-CD had stronger interactions with the guest molecule studied than α-CD. In the contrary to α-CD, the interactions of PHM with β-CD were enantioselective and led to an enantioresolution of the drug (Fig. 2b). However, only partial separation of PHM enantiomers was reached.

Positively as well as negatively charged β-CDs were intended to enhance enantioresolution of PHM. CE experiments revealed that highly substituted *S*-β-CD and positively charged 1MA-β-CD were not suitable for this purpose. A highly charged *S*-β-CD, even if present at very low concentrations, caused extremely strong retardation of the analyte so that it could not migrate properly towards the cathode/detector (see the legends to Figs. 2 and 3). The lack of enantioselectivity of the cationic CD derivative can be explained in terms of the charge repulsion preventing stereoselective inclusion of PHM into the CD cavity. On the other hand, CE-β-CD was found out to be a highly effective chiral selector for CE separation of PHM enantiomers (Fig. 2c). A baseline enantioresolution was achieved at a 15 mg/ml concentration of CE-β-CD (ES 3) (Optimized chiral method 1). Advantages of this method included a high separation efficiency ($N_{PHM(1)}$ = 58,380, $N_{PHM(2)}$ = 60,195 theoretical plates) and short analysis time (ca. 7 min). An addition of CE-β-CD into the separation system reduced electromigration dispersion of PHM zones in the capillary (compare detail peaks (b) and (c) in Fig. 2).

The chiral separation of PHM with native β-CD and CE-β-CD was influenced by pH of buffer via changes in the stability of the associates and the differences in migration velocity between the free and associated form of the analyte. As expected, the effects were much more significant for the ionizable CE-β-CD and the enantioselectivity strongly enhanced with its ionization (compare Figs. 2c and 3c). On the other hand, the enantioresolution of PHM with native β-CD decreased at higher pH (compare Figs. 2b and 3b), probably, as a result of lower separation efficiency (higher molecular diffusion of less protoned PHM is expected). The concentration and pH dependences of enantioresolutions in Fig. 4 (for the dependence b, ES 3 with pH 3.8 was introduced additionally to the systems given in Table 1) clearly illustrate differences between native β-CD and CE-β-CD as chiral complexing agents in CE and implied their utilization in enantioseparations of PHE.

Optimized chiral method 2, validated and applied for enantioselective determination of PHM in pharmaceutical preparation was based on ES 4 and CE-β-CD (2.5 mg/ml) as a chiral buffer additive. A baseline resolution of the enantiomers with the use of small amount of chiral selector was a great advantage of this method. The separation efficiency

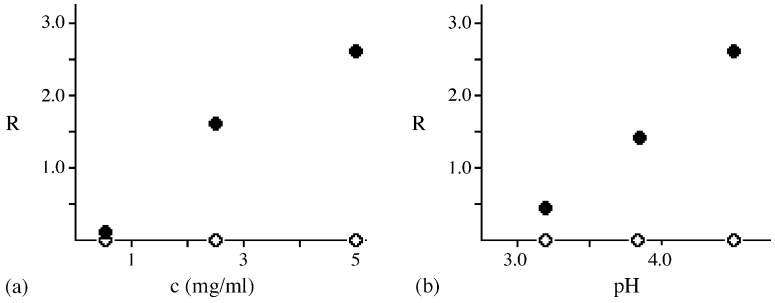

Fig. 4. Effect of pH and concentration of chiral selector on the resolution of PHM enantiomers. (a) The concentration dependences at 0.5, 2.5 and 5.0 mg/ml concentrations of CE-β-CD (●) and native β-CD (○) were obtained at pH 4.5 (ES 4); (b) the pH dependences were obtained at 5 mg/ml concentrations of the CDs and the ES 3 (pH 3.2 and 3.8) and ES 4 (pH 4.5) were used. For other conditions see Section 2.

($N_{PHM(1)}$ = 30,986, $N_{PHM(2)}$ = 27,757 theoretical plates) was sufficient for highly effective CE analyses of pharmaceuticals.

3.2. Validation

After optimization of the separation conditions, some analytical characteristics of the developed CZE method (Optimized chiral method 2) were investigated using standard solutions (validation samples), as given in the Section 2. The parameters involved were sensitivity, linearity, precision (run-to-run, day-to-day), accuracy/recovery. In all cases the peak areas of the peaks were corrected to their migration times to compensate for their differential detector residence times. A lack of pure enantiomers of PHM excluded to be evaluated migration order of the enantiomers.

The detection limits (estimated as 3σ) of PHM enantiomers (1,2) were 1.27 and 1.34 μmol/l, respectively, while the quantitation limits (estimated as 10σ) were 4.23 and 4.47 μmol/l, respectively. The concentration of the analyte in validation sample, corresponding to the limit of quantitation, was determined with acceptable precision (average R.S.D. = 0.43%, n = 10) and accuracy (absolute relative error 1.13%, mean recovery 98.87%, n = 10) under the stated conditions.

The linearity of detector response (peak area) for PHM was assessed over the range of 5.0–30.0 μmol/l for each enantiomer. This represents an interval suitable for evaluation of the drug in commercial pharmaceutical preparations. The straight line equations for PHM(1) and PHM(2) were $y = (0.1895 \pm 0.2898) + (9.2901 \pm 0.0114)x$ and $y = (0.1911 \pm 0.2937) + (9.2837 \pm 0.0106)x$, respectively, and corresponding determination coefficient was R^2 = 0.9996 in both cases. Use of the internal standard (DPZ) slightly improved the determination coefficient (~0.02%) reducing scatter of points due to random error.

Table 3
Recovery of drug from samples with known concentrations[a]

Amount of PHM standard (μmol/l)		Recovery (%)	
Added	Found (achiral/chiral method)[b,c]	Achiral method[b]	Chiral method[c]
5.00	4.99/4.97	99.80	99.40
10.00	10.01/10.03	100.10	100.30
15.00	15.11/14.94	100.73	99.60
20.00	20.04/19.96	100.20	99.80
25.00	24.97/24.88	99.88	99.52

[a] For the recovery test see Section 2.3.4.
[b] The separations were carried out in ES 1.
[c] The separations were carried out using Optimized chiral method 2.

The method was validated by evaluation of run-to-run and day-to-day precision. The results given in Table 2 clearly indicated that the CE separations in the hydrodynamically closed system provided highly reproducible migration and response data.

The results from the recovery test are presented in Table 3. The mean absolute recoveries using achiral and chiral CE method, determined by adding known amounts of PHM reference substance to the sample at the beginning of the process, were found to be 100.14 and 99.72% while corresponding accuracies (absolute relative errors) were 0.10–0.73 and 0.20–0.60%, respectively.

The obtained results clearly showed that the hydrodynamically closed CE separation system provided analyses of PHM with high precision and accuracy (compare with the data

Table 2
Precision data for repeat injections of PHM enantiomers[a]

Factor	RSD (%), n = 10			
	Run-to-run		Day-to-day	
	Enantiomer 1	Enantiomer 2	Enantiomer 1	Enantiomer 2
Migration time	0.22	0.22	0.28	0.29
Peak area	0.39	0.37	0.48	0.51

[a] The chiral analyses of model sample (25 μmol/l PHM) were carried out using Optimized chiral method 2.

Table 4
Determination of PHM and its enantiomeric ratio in granuled powder Fervex[a]

Parameter	Achiral method[b]	Chiral method[c]		
	PHM	PHM 1	PHM 2	ΣPHM 1,2
Average content (mg per dose)[d]	24.93	12.52	12.46	24.98
R.S.D. (%), $n = 10$	0.32	0.35	0.38	–
Enantiomeric ratio (%)	–	50.12	49.88	–

[a] For the sample preparation see Section 2.3.2.
[b] The separations were carried out in ES 1.
[c] The separations were carried out using Optimized chiral method 2.
[d] Relative response data were obtained using DPZ as an internal standard.

obtained by hydrodynamically open CE mode given in the Introduction section). Sensitivity of absorption photometric detection, depending on the optical pathway, was increased more than four times (ratio of I.D. was 1:6). Here, a higher thermal dispersion due to lower dissipation of Joule heat partially reduced this effect.

3.3. Application

The validated method was applied for the determination of PHM and its enantiomeric ratio in commercial pharmaceutical preparation, granuled powder Fervex. Results from the determinations are in Table 4. The content of PHM obtained by the proposed methods was in a good agreement with that declared. Quantitative estimations of the drug using the chiral and achiral methods brought consistent results and the absolute differences between determined and declared values were 0.08 and 0.28%, respectively. The chiral method facilitated to determine enantiomeric ratio of PHM in the preparation. The results in Table 4 indicated that the drug was present as a racemate. The electropherogram from the chiral analysis of the pharmaceutical sample is in Fig. 5. No detection interference was occurred separating PHM enantiomers and DPZ in the sample at a 265 nm detection wavelength.

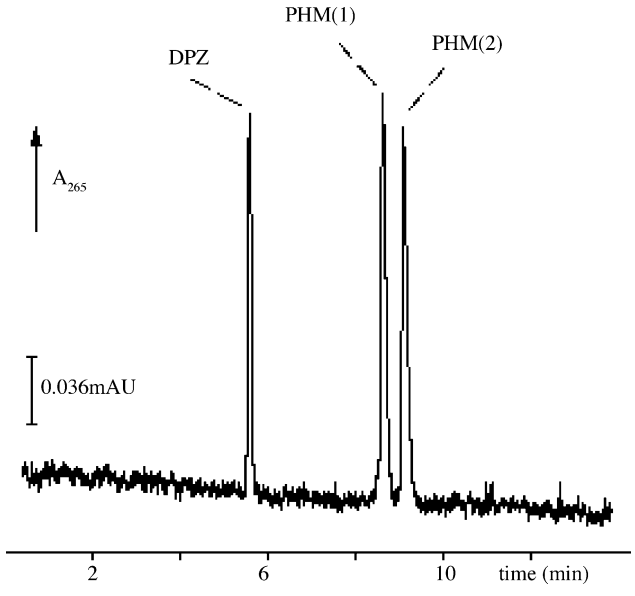

Fig. 5. Electropherogram from the determination of enantiomeric composition of PHM in commercial pharmaceutical preparation (granuled powder Fervex). The separation was carried out in the optimized ES 4 (2.5 mg/ml CE-β-CD) and the driving current was stabilized at 120 μA. The concentration of PHM in the sample was 50 μmol/l. Peak assignments: PHM(1), PHM(2) = pheniramine enantiomers; DPZ = dioxopromethazine. For the sample preparation and other conditions see Section 2.

4. Conclusion

This work outlined the significant potential of CE working in a hydrodynamically closed separation system, employing charged chiral selector, CE-β-CD, for providing highly effective chiral separations of PHM. It was demonstrated that CE-β-CD could be effectively applied in CE enantioseparations of the antihistamine when present in (at least partially) ionized form. On the other hand, native and highly charged forms of β-CD or the form with the same charge as analyte have not been effective for this purpose.

The baseline enantioseparation, short analysis time and low consumption of the chiral selector are merits of the proposed method, successfully validated and applied for the enantioselective determination of PHM in commercial pharmaceutical preparation. In addition, some performance parameters, namely precision, accuracy and sensitivity, were improved comparing to the hydrodynamically open CE system [8]. It is concluded that the reported operating conditions are suitable for the routine assay of PHM enantiomers. A modified chiral CE method employing considerably higher concentrations of CE-β-CD (>10 mg/ml) and lower pH (pH < 3.5) should be applied when a higher separation efficiency, shorter analysis time and a selectivity change required.

The proposed CE methods may be easily modified to enantiopurity determination of PHM using proper amount of CE-β-CD in the separation systems. Moreover, there are potentialities to adapt them to analyses of complex biological samples, as the CZE separation mode could be easily on-line combinable with the pre-concentration and purification CE modes (e.g. isotachophoresis) [22,23].

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References

[1] F. Onur, C. Yücesoy, S. Dermis, M. Kartal, G. Kökdil, Talanta 51 (2000) 269–279.

[2] K.M. Fried, A.E. Young, S.U. Yasuda, I.W. Wainer, J. Pharm. Biomed. Anal. 27 (2002) 479–488.
[3] J. Martens, J. Chromatogr. B 673 (1995) 183–188.
[4] C.P. Ong, C.L. Ng, H.K. Lee, S.F.Y. Li, J. Chromatogr. 588 (1991) 335–339.
[5] B. Chankvetadze, N. Burjanadze, G. Pintore, D. Bergenthal, K. Bergander, C. Mühlenbrock, J. Breitkreuz, G. Blaschke, J. Chromatogr. A 875 (2000) 471–484.
[6] A. Van Eeckhaut, M.R. Detaevernier, Y. Michotte, J. Chromatogr. A 958 (2002) 291–297.
[7] A. Schuster, C. Gotte, G. Bernhardt, A. Buschauer, Chirality 13 (2001) 285–293.
[8] H.L. Wu, C.H. Huang, S.H. Chen, S.M. Wu, J. Chromatogr. Sci. 37 (1999) 24–30.
[9] L.J. Jin, S.F.Y. Li, J. Chromatogr. B 708 (1998) 257–266.
[10] Y.K. Jin, A.M. Stalcup, Electrophoresis 19 (1998) 2119–2123.
[11] E. Schneiderman, A.M. Stalcup, J. Chromatogr. B 745 (2000) 83–102.
[12] S. Fanali, J. Chromatogr. A 792 (1997) 227–267.
[13] S. Terabe, Trends Anal. Chem. 8 (1989) 129–134.
[14] B. Chankvetadze, G. Endresz, G. Blaschke, Electrophoresis 15 (1994) 804–807.
[15] P. Mikuš, D. Kaniansky, R. Šebesta, M. Sališová, Chem. Listy 96 (2002) 693–697.
[16] P. Mikuš, D. Kaniansky, S. Fanali, Electrophoresis 22 (2001) 470–477.
[17] P. Mikuš, D. Kaniansky, J. Sep. Sci. 25 (2002) 872–876.
[18] P. Mikuš, I. Valášková, E. Havránek, J. Pharm. Biomed. Anal. 33 (2003) 157–164.
[19] D. Kaniansky, J. Marák, M. Masár, F. Iványi, V. Madajová, E. Šimuničová, V. Zelenská, J. Chromatogr. A 772 (1997) 103–114.
[20] D. Kaniansky, M. Masár, J. Bielčíková, J. Chromatogr. A 792 (1997) 483–494.
[21] R. Šebesta, M. Sališová, Enantiomer 4 (1999) 271–277.
[22] D. Kaniansky, Capillary isotachophoresis, capillary zone electrophoresis and their on-line coupling. D.Sc. Thesis, Comenius University, Bratislava, 2002, pp. 5–44.
[23] P. Mikuš, P. Kubačák, I. Valášková, E. Havránek, Pharmazie 58 (2003) 111–113.