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Research Article

CHIRAL SEPARATION OF CETIRIZINE ENANTIOMERS BY CYCLODEXTRIN MEDIATED CAPILLARY ELECTROPHORESIS

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ABSTRACT

Chiral separation cetirizine, a second generation H1 antagonist was studied by cyclodextrine (CD) mediated capillary electrophoresis. The influence on the separation of several parameters including pH and concentration of the background electrolyte (BGE), CD type and concentration, applied voltage and temperature were studied and the electrophoretic and analytic parameters were optimized. The best conditions for the chiral separation were obtained using 25mM disodium hydrogeno-phosphate – 25mM sodium didydrogeno-phosphate (1:1) as BGE, 5mM sulfobuthyl ether- β-CD as chiral selector, a voltage of + 20kV, temperature of 20°C, injection pressure/time of 50mbar/ 1sec, UV detection at 230nm. The analytical performance of the method was evaluated. The proposed method was successfully applied to the enantioselective assay of cetirizine in pharmaceutical formulations. CE proved to be a rapid, specific, reliable and cost-effective method for the chiral separation of cetirizine enantiomers and can be useful for laboratories performing routine analysis.

Key words: chiral separation, cetirizine, capillary electrophoresis

INTRODUCTION

Cetirizine (CET), (R,S)-[2-[4-[(4-chlorophenyl) phenylmethyl] - 1- piperazinyl] ethoxy] acetic acid, is a second generation selective H1 receptor antagonist used in the treatment of hay fever, allergies, angioedema and urticaria (Block and Beale, 2011).

CET is the major metabolite of hydroxyzine (a first generation H1 anti-histamine, used for its anxiolytic effects), resulting from complete oxidation of the primary alcohol moiety.

This compound is zwitterionic and relatively polar and thus does not penetrate or accumulate in the CNS. The advantages of this compound appear to be its long action (once-daily dosing), rapid onset of activity, minimal CNS effects and a lack of clinically significant effects on cardiac rhythm (Martindale, 2011) .

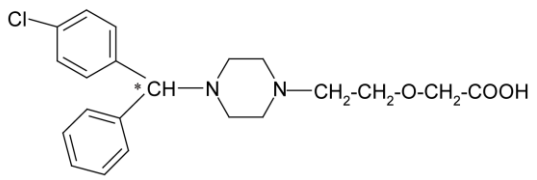

Figure 1. Chemical structure of cetirizine. The asterix denote the chiral center.

Structurally it is a piperazine derivative and posses in its structure an asymmetric carbon atom, resulting in the existence of a S- and R-enantiomer. The chemical structure of CET is presented in figure 1.

The levorotatory enantiomer (R-enantiomer) of CET, levocetirizine is the more active form; and is marketed also as pure enantiomer. The R-enantiomer is has a 30-fold higher affinity than the S-enantiomer, and dissociates more slowly from H1 receptors. Levocetirizine displays its action on the same receptor and CNS selectivity profile as the racemate, CET, and exhibits the same therapeutic advantages (Block and Beale, 2011).

It is obvious, from above mentioned facts regarding the stereochemistry of CET, that the control of enantiomeric composition of pharmaceuticals containing CET is a permanent necessity and also a challenge.

Capillary electrophoresis (CE) proved to be a powerful alternative in chiral analysis to the more frequently used chromatographic techniques. The advantages of using CE for the enantioseparation of chiral substances are being related to the: high resolution power, simple and rapid method development, low consumption of solvent, sample and chiral

selector and especially with the high selectivity in choosing and changing the chiral selector (Gubitz *et al*., 1997).

Usually in CE a direct method of separation is used, by simply adding the chiral selector to the background electrolyte (BGE). The chiral selector will interact with the two enantiomers during the electrophoretic process, forming labile diastereoisomeric complexes. The separation of the two enantiomers can take place only if the two diastereoisomers possess different stability constants, causing the two enantiomers to move with different velocities (Gubitz *et al*., 2008).

Cyclodextrines (CDs) are cyclic oligosaccharides composed of several glucopyranose units. CDs are chiral due to the presence of asymmetric carbons on the glucose units. CDs whether native or derivatized, neutral or ionic are by far the most common chiral selectors employed in CE for the enantiomeric separation of chiral substances. Using CDs as chiral selectors, analytes must fit the cavity either with the whole molecule or with their hydrophobic part on forming inclusion-complexes stabilized by secondary bonds between the rim of the chiral selector and the substituents of the analyte asymmetric center. The complex formed during the electrophoretic process, in equilibrium with the free analyte, possesses a different mass responsible for the change of the effective mobility (Fillet *et al*., 1998; Fanali, 2000).

Recently some CE methods have been used to investigate the enantioseparation of cetirizine; using quite different types of CDs and electrophoretic conditions (Mikus *et al*., 2005; Van Eeckhaut *et al*., 2006; Chou *et al*., 2008).

Chou *et al.* reported a CE method for enantioseparation of CTZ using a borate buffer (pH 8.7) with 1% w/v sulfated β-CD as the chiral selector; van Eeckhaut *et al.* reported a CE separation of CTZ enantiomers with the phosphate buffer (pH 2.5) using heptakis (2,3-diacetyl-6-sulfato)- β-CD and triethanolamine and acetonitril as the chiral selector, co-ion and organic modifier, respectively; Mikus *et al.* 2005 described a CE enantioseparation of CTZ, using dynamic coating of capillary with methylhydroxylethylcellulose, randomly substituted sulfated- β -CDs and 25mM morpholinoethanesulfonic acid (pH 5.2) as the chiral selector and buffer solution. As seen in these reports, the enantioseparation conditions were not very simple or reagents used were very expensive.

The aim of the present work was to develop an alternative simple, rapid an efficient method CD-mediated CE method for the enantioseparation of CTZ, the optimization of electrophoretic conditions and the determination of its isomers, in commercial pharmaceuticals.

MATERIALS AND METHODS

Apparatus

All experiments were performed on a Agilent 6100 CE system (Agilent, Germany) equipped with a diode array UV detector. The data was processed using Chemstation 7.01 (Agilent, Germany) software. Separation was performed in a 48 cm (40 cm effective length) x 50 μm I.D. uncoated fused silica-capillaries (Agilent, Germany). The pH of the buffer solutions was measured with the Terminal 740 pH–meter (Inolab, Germany). The UV spectrum of CET was recorded with Specord 210 spectrophotometer (Analytik Jena, Germany).

Chemicals and reagents

The racemic mixture R,S–cetirizine hydrochloride and the pure enantiomer levocetirizine (R-cetirizine) hydrochloride of pharmaceutical grade were obtained from RA Chem Pharma Limited (Balanagar, Hyderabad, India). As an internal standard (IS), we used another H1 antihistamine, loratadine obtained from Tonira Pharma Limited (Ankleshwar, Gujarat). The following reagents of analytical grade were used: phosphoric acid (Pernix Pharma, Hungary), methanol, sodium hydroxide (Lach Ner, Czech Republic), sodium tetraborate, disodium hydrogenophosphate, sodium didydrogenophosphate (Merck, Germany). Purified water was provided by a Milli-Q Plus water purification system (Millipore, USA).

As chiral selectors we used the following cyclodextrine (CD) derivatives of research grade: native neutral CD (α-CD, β-CD, γ-CD), derivatized neutral CD (hydroxypropyl-β-CD - HP-β-CD, randomly methylated β-CD – RAMEB), anionic substituted charged CD

(sulfobuthyl ether- β-CD – SBE-β-CD). All CDs were obtained from Cyclolab (Budapest, Hungary) with the exception of SBE-β-CD - Capsitol (Cydex, USA).

For the determination from commercial pharmaceutical preparations we used Zyrtec tablets (UCB Pharma, Germany) each tablet containing 10mg CET, obtained from a local pharmacy.

Sample preparation

CET sample stock solutions were prepared by dissolving the substance in water in a concentration of 100μg/mL and later diluted to the appropriate concentration. The samples were introduced in the system at the anodic end of the capillary by hydrodynamic injection. All samples and buffers were filtered through a 0.45μm syringe filter and degassed by ultrasound for 5min before use.

To determine CET from tablets, twenty tablets from the same batch product were weighed and pulverized, and the net weight of each tablet was calculated. An amount of powder equivalent to the average weight of a single tablet was accurately weighed and transferred to a 250mL volumetric flask. Water was added to the mark, the flask was sonicated for 15min to affect complete dissolution, and the solution was filtered through a 0.45μm syringe filter. The samples were centrifuged at 3500rpm for 10min, the supernatant was diluted; further the same procedures were followed as for the preparation of standard solutions for the CE separation.

Electrophoretic technique

Before use, the capillary was pretreated successively with 0.1 M NaOH, water, and running buffer. At the beginning of each workday, the capillary was rinsed with running buffer for 10 min; between each injection, the capillary was rinsed with buffer for 1 minute.

In the preliminary analysis we applied some "standard" electrophoretic conditions for a CE analysis: temperature 20°C, applied voltage + 25 kV, injection pressure/time 50 mbar/3 sec, sample concentration 10μg/mL.

Previously we recorded the UV spectra of CET and found absorption maximum at 231 nm. 210 and 230 nm were elected as appropriate wavelengths for the UV detection.

Enantioselectivity evaluation

The separation factors (α) were calculated as the ratio of the migration times of the optical isomers, and the resolution (R) was obtained by the $R=2(t_2-t_1)/(w_1+w_2)$ equation, where the migration times (t_1 and t_2) and the peak-widths (w_1 and w_2) were marked for the slow and fast migrating enantiomers, respectively.

RESULTS AND DISCUSSION

Preliminary analysis

In order to find the suitable conditions for the chiral separation of CET, a series of preliminary experiments were conducted in an achiral system with different buffer compositions at different pH values. In the preliminary analysis we used: 25mM phosphoric acid (pH-2.1), 25mM sodium didydrogeno-phosphate (pH-5.0), 25mM disodium hydrogenophosphate-sodium didydrogenophosphate (1:1) (pH-7.0) and 25 mM sodium tetraborate (pH-9.3) BGE respectively and we modified the pH of the buffer by adding a 0.1M sodium hydroxide solution. CET can be detected on the entire studied pH interval (2.5-11.00).

CET has three ionizable moieties resulting in pKa values of 2.2, 2.9 and 8.0, and depending on the pH it predominantly exists as a zwitterion. CET will be negatively or positively charged depending on the pH of the environment, as it posses two basic and one acidic functions, offering the possibility of using either an acidic or an alkaline running buffer for its determination (Nojavan *et al.*, 2011).

The type and concentration of CD added to BGE is of primary importance in achieving chiral resolution. Initial concentration of 10mM neutral CDs were dissolved in the BGE, while for charged CDs we added a concentration of 5mM in order to limit the increase of ionic strength which generated high currents.

No chiral separation was observed using native neutral CDs (α-CD, β-CD, γ-CD) or derivatized neutral CDs (HP-β-CD, RAMEB), as we observed only an increase in the migration times of the analyte. The only CD, which exhibited obvious chiral interactions, was the anionic ionized SBE- β-CD.

Consequently we can conclude that SBE-β-CD proved to be the optimum chiral selector for the separation of CET enantiomers.

Optimization of the analytical method

The influence of several parameters on enantiomeric resolution, such as the nature and concentration of CD used as chiral selector, buffer composition and pH, direction and intensity of the EOF, addition to the buffer of an organic solvent, applied voltage and capillary temperature, were studied in order to obtain an increased separation resolution and a short analysis time.

The charge of the CD can play an important role in the resolution mechanism; the electrostatic interactions with the analytes, the movement of the chiral selector in the opposite direction of the two enantiomers and the possibility of separating uncharged analytes being considered to be the main advantages in using this type of CD. SBE-β-CD contains four modified primary hydroxyl groups with a butyl chain and sulfonic groups; and its chemical features allow its use in a charged mode (negatively charged) over a wide pH range (2-11) (Ren *et al.*, 1999)

Compared with neutral selectors, the effect of the concentration of the charged chiral selectors on the selectivity of enantioseparation can be more pronounced. In this work the concentration of the chiral selector was investigated experimentally for SBE- β-CD concentrations from 1 to 10 mM. The increase of the concretion improve slightly the enantioresolution of the separation, but high concentration generates elevated currents and instability of the electrophoretic system. The optimum CD concentration was set at 5 mM SBE- β-CD.

At low acidic pH, SBE- β-CD is negatively charged and CET is positively charged, while the EOF was close to zero; under electric field SBE- β-CD will move towards anode and the enantiomers towards cathode, leading to very long migration times and only slight chiral interactions. SBE- β-CD proved to be efficient as chiral selector for the enantioseparation of CET in neutral and alkaline pH of the BGE (pH 6-10). We obtained the best chiral resolution at a pH of 7.0.

An increase of the BGE concentration improved the resolution of the enantiomers, increasing also the migration times of the analytes due to the reduction of EOF. The upper limit for buffer concentration is limited by the increase of the generated current and resulting Joule heating which reduces the efficiency of the separation. The optimum BGE concentration was set at 25mM disodium hydrogenophosphate - 25mM sodium didydrogenophosphate (1:1).

Addition of an organic modifier such as methanol or acetonitrile to the BGE resulted in longer migration times but there was no significant improvement in the chiral separation.

An increase of the temperature caused a decrease in buffer viscosity, and thus a decrease in migration time; but also a decrease in the binding constant of the inclusion complex between the analyte and the CD. The optimum capillary temperature was set at 20^0C.

Running voltage did not have a strong effect on the resolution; but application of a higher voltage led to a shorter migration time. The optimum voltage was set at + 20 kV.

A high injection pressure of 50 mbar and a rapid injection time of 1 second provided a reasonable sample load and maintained resolution.

The migration order of the two enantiomers was determined by injecting a solution of the racemate enriched with the pure enantiomer separately. The first peak to pass the detector window was determined to be R(+)-CET followed by S(-)-CET.

Taking in consideration the aspects presented above we can conclude that the optimum electrophoretic conditions for the CET enantioseparation are: BGE: 25mM disodium hydrogenophosphate – 25mM sodium didydrogenophosphate (1:1); chiral selector: 5mM SBE- β-CD; buffer pH: 7.0; applied voltage: + 20kV, temperature: 20°C, injection pressure/time: 50mbar/1 sec, UV detection at 230nm.

Applying the optimized conditions we obtained a chiral resolution of 2.54 and a separation factor of 1.08, with a migration time of 6.40 for the R-enantiomer and 7.0 for the S-enantiomer.

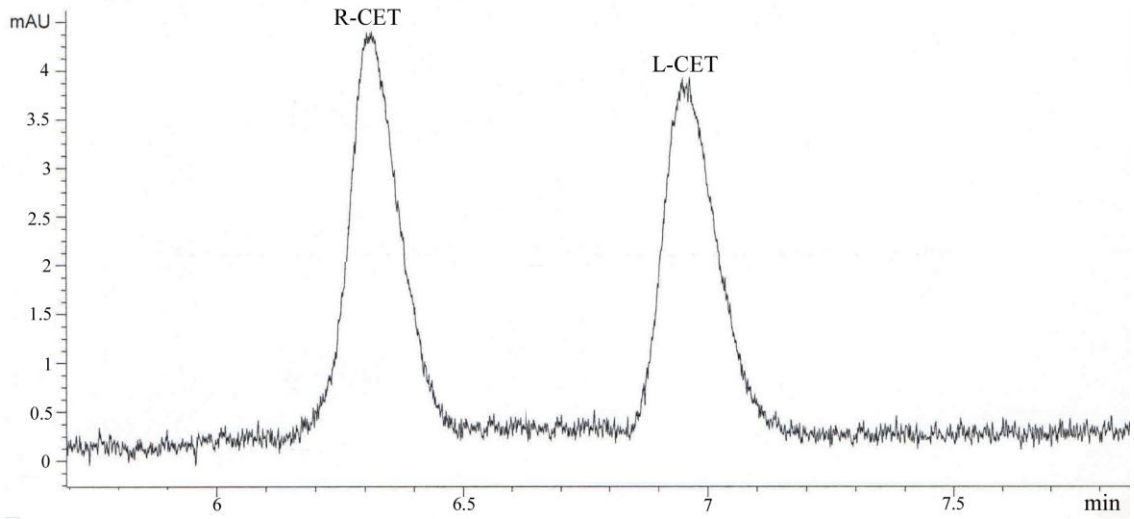

Figure 2. Capillary electrophoretic separation of CET enantiomers using SBE- β-CD as chiral selector (experimental conditions: BGE: 25 mM disodium hydrogenophosphate – 25mM sodium didydrogenophosphate (1:1), chiral selector: 5mM SBE- β-CD, pH–7.0, voltage + 20kV, temperature 20°C, hydrodynamic injection 50 mbar/1 sec., sample concentration 5μg/mL, UV detection 230nm)

Table I Calibration data for CET chiral separation (calibration range: 2.5 - 50 μg mL^{-1})

Enantiomer	Regression equation	Correlation coefficient	LOD (μg/mL^{-1})	LOQ (μg/mL^{-1})
R(+) CET	y = 0.4735x+0.8381	0.995	2.64	7.92
S(-) CET	y = 0.4576x+0.8066	0.998	2.90	8.20

The electropherograms of the enantioseparation of CET using SBE- β-CD as chiral selectors are presented in **figure 2.**

Analytical performance

The proposed separation method was evaluated on the basis of precision (migration time and peak area), linearity, limit of detection (LOD) and limit of quantification (LOQ). For all these measurements the optimized separation parameters were used.

Loratidine was used as an internal standard (IS); its migration time being lower than the one of the CET enantiomers. Quantification was accomplished on the basis of CET enantiomer to IS peak-area ratios (peak area of CET enantiomer/peak area of IS).

Calibration plots were constructed by preparing standard solutions (n=3) at six concentrations in a specific concentration range (concentration range: 2.5-50 μg/mL^{-1}), and showed good linearity (table I). High correlation coefficients were obtained and the intercepts of the plots were not significantly different from zero.

The limits of detection (LOD) and quantification (LOQ) were estimated as: standard deviation of regression equation/slope of the regression equation multiplied by 3.3 and 10, respectively (table I).

The precision of the method was determined by measurement of repeatability (intra-day) and intermediate precision (inter-day), expressed as RSD% for a series of measurements.

Standard solutions of CET and internal standard were prepared in methanol and injected on three consecutive days, six times a day (table 2).

Table II Intra and inter-day repeatability of carvedilol to SI peak-area ratio (n-number of experiments; k-day of experiment; RSD / % -relative standard deviation; sample concentration, IS concentration - 10μg/mL^{-1})

Enantiomer	Repeatability			Intermediate precission n=6, k=3
	Day 1 n=6	Day 2 n=6	Day 3 n=6	
		RSD / %		
R(+) - CET	0.75	1.08	1.45	1.80
S(-) -CET	0.78	1.15	1.58	1.90

In order to demonstrate that the developed method can be used in true samples, we applied the optimized conditions for the enantiomeric separation of CET from Zyrtec tablets, each tablet containing 10 mg CET. The peaks obtained from the tablets were similar to those from CET standard and there was no interference from the matrix. The content of a tablet was found to be 9.85 ± 0.20 mg (mean ± SD, n = 6). The content of CET, obtained by the proposed method, was in a good agreement with that declared by the manufacturers.

The chiral method permitted determination of the enantiomeric ratio of CET in the preparation; the results indicated that the drug was present as a racemate.

CONCLUSION

The selectivity of the enantiomeric separation for CET will obviously depend on the nature and concentration of the CD added to the BGE. Moreover, the pH of the buffer will influence the charge of the analyte and that of ionizable CD derivatives as well as the EOF. Further factors such as the nature and the concentration of BGE, the applied voltage, capillary temperature and injection parameters can also affect enantioseparation.

It was demonstrated that chargeable CDs (SBE- β-CD) offer much higher flexibility in optimizing separation problems of CET enantiomers. The faster migration of the R(+)-CET indicates that this enantiomer have weaker interaction with the selected CD, while the interaction of S(-)-CET has a stronger one.

This work demonstrates the potential of CE for chiral separations and for quantitative analysis of chiral compounds in pharmaceutical applications.

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