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Research Article

Chiral separation of cetirizine by capillary electrophoresis

Chiral separation of cetirizine, a second-generation H_1-antagonist, was studied by CD-mediated CE. Several parameters, including pH, CD type, buffer concentration, type of co-ion, applied voltage and temperature, were investigated. The best conditions for chiral separation were obtained using a 75 mM triethanolamine-phosphate buffer (pH 2.5) containing 0.4 mg/mL heptakis(2,3-diacetyl-6-sulfato)-β-CD and 10% ACN. Online UV detection was performed at 214 nm, a voltage of 20 kV was applied and the capillary was temperature controlled at 25°C by liquid cooling. Hydrodynamic injection was performed for 1 s. The method was validated for the quantification of levocetirizine in tablets and for enantiomeric purity testing of the drug substance. Selectivity, linearity, LOD and LOQ, precision and accuracy were evaluated for both methods. The amount of levocetirizine dihydrochloride in the commercially available tablets was quantified and was found to be within the specification limits of the claimed amount (5 mg). The amount of distomer in levocetirizine drug substance was found to be 0.87 ± 0.09% w/w, which is in agreement with the certificate of analysis supplied by the company.

Keywords: Capillary electrophoresis / Cetirizine / Chiral separation / Heptakis(2,3-diacetyl-6-sulfato)-β-CD / Levocetirizine DOI 10.1002/elps.200500844

1 Introduction

Many drugs are marketed as racemic mixtures, although the individual enantiomers frequently differ in both their pharmacodynamic and pharmacokinetic profiles. The importance of stereochemistry and therefore also the need for enantioselective analysis methods has become well recognised [1–3]. For this purpose chromatographic techniques such as LC and GC were first developed [4, 5]. At present, LC still dominates chromatographic enantiomeric analysis in industry [6]. Over the last two decades, CE has proved to be a powerful alternative to LC for enantioselective analysis [4–11]. CE offers a tremendous flexibility for enantiomeric separations, because a wide variety of chiral additives are available. Compared to other analytical techniques like LC, CE offers several advantages including simplicity, short analysis times, high efficiencies, different separation mechanisms, small volumes and low running costs. The most frequently used chiral selectors in CE are CDs. The mechanism of chiral discrimination using CDs is the inclusion of the analytes into the cavity of the chiral selector. Additionally, secondary bonds between the rim of the CD and the analyte stabilize the complex. Enantioseparation is achieved if the binding constants of both enantiomers for the CD are different and/or if the mobilities of both enantiomer–CD complexes differ [12, 13]. In addition, the free and complexed form of the solute should have different mobilities.

Cetirizine is a potent, long-acting, second-generation histamine H_1 receptor antagonist used for the treatment of urticaria and allergic rhinitis [14–17]. Cetirizine is the acid metabolite of hydroxyzine and the structure of this zwitterionic drug is shown in Fig. 1. Three ionizable functions are present in the structure, namely a strong acid group (pK_a = 2.9), a strong basic group (pK_a = 8.0) and a weak basic group (pK_a = 2.2) [16–19]. Its H_1-antagonist activity is primarily due to levocetirizine, the *R*-enantiomer of cetirizine. Furthermore, the pharmacokinetic behavior of levocetirizine appears more favorable due to a lower volume of distribution and a slower renal clearance [16]. The eutomer is now also commercially available.

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Abbreviations: CM-β-CD, carboxymethyl-β-CD; **HDAS-β-CD**, heptakis(2,3-diacetyl-6-sulfato)-β-CD; **HDMS-β-CD**, heptakis(2,3-dimethyl-6-sulfato)-β-CD; **HP-β-CD**, hydroxypropyl-β-CD; **IS**, internal standard; **TM-β-CD**, hepatakis(2,3,6-tri-*O*-methyl)-β-CD

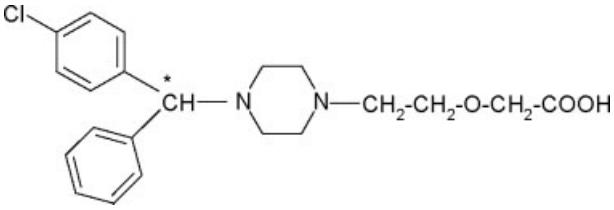

Figure 1. Structure of cetirizine.

Several assays have been described for the determination of cetirizine racemate in pharmaceutical preparations and biological fluids. LC [20–30] is the most widely used technique, in some cases coupled to MS/MS [31–36]. However, also TLC [37, 38], electrooxidation [39] and spectrophotometric [22] approaches have been described.

Only a few authors have investigated the chiral separation of cetirizine. The basis of most enantioselective assays described in literature is the separation of this antihistaminic drug on a chiral stationary phase by LC [15, 40–44]. Different stationary phases were used, namely α_1-acid glycoprotein [15, 40, 41, 44], cellulose tris(3,5-dimethylphenylcarbamate) [42, 43] and (*S*,*S*)-Whelk-01 [43].

The purpose of this work was to develop a CE method for the chiral separation of cetirizine. During method development, no literature data on enantioseparation of cetirizine with CE was available. It was only very recently that Mikuš *et al.* [45] have described the chiral separation of cetirizine using CD mediated CE. Optimized conditions consisted of 25 mmol/L morpholinoethanesulfonic acid (MES) buffer pH 5.2 containing 5 mg/mL randomly substituted sulfated-β-CD. After validation, the method was applied for the analysis of cetirizine enantiomers in various pharmaceutical preparations, all containing *rac.*-cetirizine.

In our study, several parameters that influence the chiral separation were first investigated, including pH, CD type, buffer concentration, type of co-ion, applied voltage and temperature. The optimized method was then validated for the enantioselective determination of levocetirizine in tablets and for the enantiomeric purity testing of the drug substance.

2 Materials and methods

2.1 Chemicals

Rac.-cetirizine dihydrochloride (purity >99%), levocetirizine dihydrochloride (purity >99%; enantiomeric impurity: 0.81%), 2-(4-((2-chlorophenyl)-phenylmethyl)-1-piperazinyl)ethoxy)-acetic acid, 2-(*p*-chlorobenzhydryl-piperazine)-2-ethoxyacetamide and UCB 30462 were a kind gift from UCB (Braine l'Alleud, Belgium). (1*R*,2*S*)-Ephedrine hydrochloride was purchased from Sigma (St. Louis, MO, USA). All CDs are of CE grade (purity >95%). Carboxymethyl-β-CD (CM-β-CD) (degree of substitution (DS) = 3) was purchased from Cyclolab (Budapest, Hungary). Hydroxypropyl-β-CD (HP-β-CD) (molar substitution = 0.6) was obtained from Aldrich (Gillingham, UK), heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) from Fluka (Buchs, Switzerland). Heptakis(2,3-diacetyl-6-sulfato)-β-CD (HDAS-β-CD) and heptakis(2,3-dimethyl-6-sulfato)-β-CD (HDMS-β-CD) were obtained from Antek Instruments (Houston, USA). *Ortho*-phosphoric acid (85%), sodium tetraborate, tris(hydroxymethyl)aminoethane (Tris), potassium hydroxide, ammonium hydroxide (37%) and triethanolamine were purchased from Merck (Darmstadt, Germany). Sodium hydroxide (1 M) was obtained from Carlo Erba (Milan, Italy). ACN was obtained from Acros Organics (Geel, Belgium). All reagents were of analytical or LC grade. The water used for preparing solutions was obtained from a Seralpur Pro 90 CN purification system (Seral, Germany).

2.2 CE equipment and conditions

A Beckman (Palo Alto, CA, USA) P/ACE 2100 System equipped with a UV detector and a temperature control system was used. All separations were performed in an uncoated fused-silica capillary (Beckman) with a total length of 37 cm (30 cm to the detector) × 75 μm internal diameter. Instrument control and data acquisition were performed by the chromatography software System Gold 7.11 (Beckman). Online UV detection was performed at 214 nm. Unless stated otherwise, a voltage of 20 kV was applied and the capillary was temperature controlled at 25°C by liquid cooling. Sample solutions were introduced by pressure (0.5 psi) for 1 s. Between runs, the capillary was flushed for 2 min with water and for 3 min with run buffer (20 psi). The separation buffer consisted of 0.075 M *ortho*-phosphoric acid, adjusted to pH 2.5 with 1 M triethanolamine. The buffer was filtered through a 0.2 μm membrane (Machery-Nagel, Düren, Germany). A stock solution of HDAS-β-CD was prepared at a concentration of 1 or 4 mg/mL in the phosphate buffer. Every day, this stock solution was diluted to the desired concentration with the separation buffer. If appropriate, organic modifier was added. Standard stock solutions of *rac.*-cetirizine dihydrochloride, levocetirizine dihydrochloride and (1*R*,2*S*)-ephedrine hydrochloride were prepared in water at a concentration of 1 mg/mL. All stock solutions were stored at 4°C. The solutions are stable during at least 2 wk.

2.2.1 Determination of levocetirizine in tablets

Before injection, the stock solution of levocetirizine dihydrochloride was diluted with water to the concentration needed (50–250 μg/mL) and (1*R*,2*S*)-ephedrine hydrochloride was added as internal standard (IS) in a concentration of 0.1 mg/mL. The sample was prepared as follows: 20 tablets were weighed and finely powdered. An accurately weighed aliquot of the powder equivalent to 6 mg levocetirizine dihydrochloride was dissolved in 20.0 mL water. After sonification (15 min), the sample was centrifuged for 15 min at 3000 rpm. The clear solution was diluted by a factor 2 in water and (1*R*,2*S*)-ephedrine hydrochloride was added.

2.2.2 Enantiomeric purity testing

The stock solution of *rac.*-cetirizine dihydrochloride was diluted with water to the concentration needed (10–100 μg/mL) and (1*R*,2*S*)-ephedrine hydrochloride was added as IS in a concentration of 0.1 mg/mL. Sample solutions were prepared at a concentration of 0.5 mg/mL levocetirizine dihydrochloride.

2.3 Data treatment

Resolution values were obtained from the System Gold software according to the following equation:

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

where t_1 and t_2 are the migration times (min) of the two enantiomers and w_1 and w_2 are the corresponding peak widths, measured at baseline as the distance between the inflection tangents.

Calculations were performed using relative corrected peak areas (rA_c). Corrected peak areas (A_c) are obtained by dividing the peak area by the corresponding migration time. Dividing these corrected peak areas by the A_c of the IS results in rA_c values.

Microsoft Excel 2000 and SPSS 12.0 were used for data analysis.

3 Results and discussion

3.1 Method development

3.1.1 Choice of pH and CD

Due to its zwitterionic nature, cetirizine can be considered as a cation at pH 3 and at pH 9 as an anion. Three different CD derivatives, TM-β-CD, HP-β-CD and CM-β-CD, were investigated at low and high pH. For these experiments two different BGE were used, namely 50 mM triethanolamine-phosphate buffer pH 3 and 20 mM sodium tetraborate buffer pH 9. No chiral separation could be observed under all tested circumstances.

The migration time of cetirizine increased largely when CM-β-CD was added at pH 3, although no chiral separation could be observed (without CM-β-CD: 9.6 min; +0.1 mM: 12.8 min; +0.5 mM: >30 min). To eliminate all negative charges and therefore possible repulsive forces, pH 2.5 was tested. A beginning of chiral separation could be observed using 1 mM CM-β-CD, at cost of long migration times (25 min). This strong retardation of the migration times prevents the use of higher CM-β-CD concentrations to obtain baseline separation. This was also observed by Mikuš *et al.* [45] when using either carboxyethyl-β-CD or sulfated-β-CD as chiral selector at low pH.

In following experiments, the ability of HDMS-β-CD and HDAS-β-CD to separate the cetirizine enantiomers at pH 2.5 was investigated. Baseline chiral separation of the antihistaminic drug was obtained with both single-isomer, sulphated CD derivates. Lower concentrations of HDAS-β-CD were necessary to obtain even higher resolution values as compared to HDMS-β-CD. Therefore, further experiments were performed with HDAS-β-CD as chiral selector.

Increasing the pH of the BGE containing HDAS-β-CD leads to a decrease in resolution and even a complete loss of resolution at pH 4. The separation of the enantiomers is based on inclusion complexation and is extra stabilised with electrostatic interaction between the positively charged nitrogens of the analyte and the negatively charge sulphate group of the CD. At pH 3 and higher the carboxylic function on cetirizine will be negatively charged. Therefore repulsion between the analyte and the CD can occur, leading to a decrease in resolution.

Further investigations were therefore performed using a 50 mM phosphate buffer pH 2.5 with 0.1 mg/mL HDAS-β-CD. However, a triangular shaped peak was observed, especially for the second migrating peak.

3.1.2 Concentration of the buffer

Peak triangulation due to electrokinetic dispersion is a peak deformation mechanism that occurs when the sample solution has a higher conductivity than the BGE and during separations where the migration velocity of the sample and buffer ions of the same charge are different [46]. Increasing the ionic strength of the BGE can reduce electromigration dispersion [46, 47].

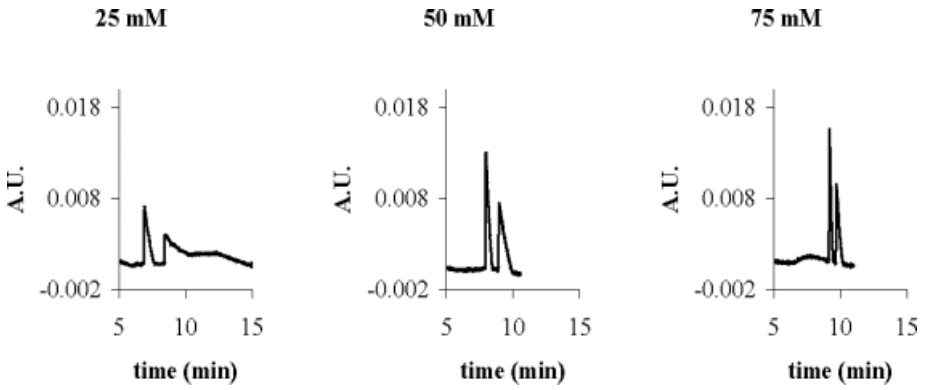

Figure 2. Influence of the buffer concentration on the chiral separation of cetirizine. Experimental conditions: capillary: 37 cm (30 cm effective length) × 75 μm id; detection: 214 nm; injection: 5 s (pressure); temperature: 25°C; applied voltage: 20 kV; separation solution: triethanolamine-phosphate buffer (pH 2.5) + 0.1 mg/mL HDAS-β-CD.

In Fig. 2 the separation of cetirizine is shown varying the concentration of the BGE. Using a 25 mM phosphate buffer, clearly peak dispersion occurred. More efficient peaks were observed with a buffer concentration of 50 and 75 mM. The best results were obtained with 75 mM. Higher concentrations could not be used due to the generation of high current values. The resolution between both cetirizine enantiomers in a 75 mM phosphate buffer pH 2.5 + 0.1 mg/mL HDAS-β-CD amounts to 1.7. In order to increase the resolution between both enantiomers other experimental parameters were optimised.

3.1.3 Influence of the co-ion

Peak dispersion is minimized when (i) the concentration of the BGE is much higher than that of the analyte and (ii) the mobilities of the analyte and the BGE co-ion are nearly identical [46, 48]. In the previous experiment the concentration of the BGE was augmented. To match the mobilities of the analyte and the BGE co-ion, five different co-ions were investigated, namely sodium, potassium, ammonium, Tris and triethanolamine. The results are shown in Fig. 3. The nature of the co-ion influenced the chiral separation and also the developed current. Due to the generation of high currents with sodium, potassium and ammonium, the experiments could only be performed at 15 kV. The peak shape and peak area could be improved by selecting potassium or ammonium as co-ion. However, in these cases the current was not stable and therefore these co-ions were not used in further experiments. Only the use of triethanolamine or Tris as co-ion resulted in stable currents and therefore triethanolamine was used in further experiments.

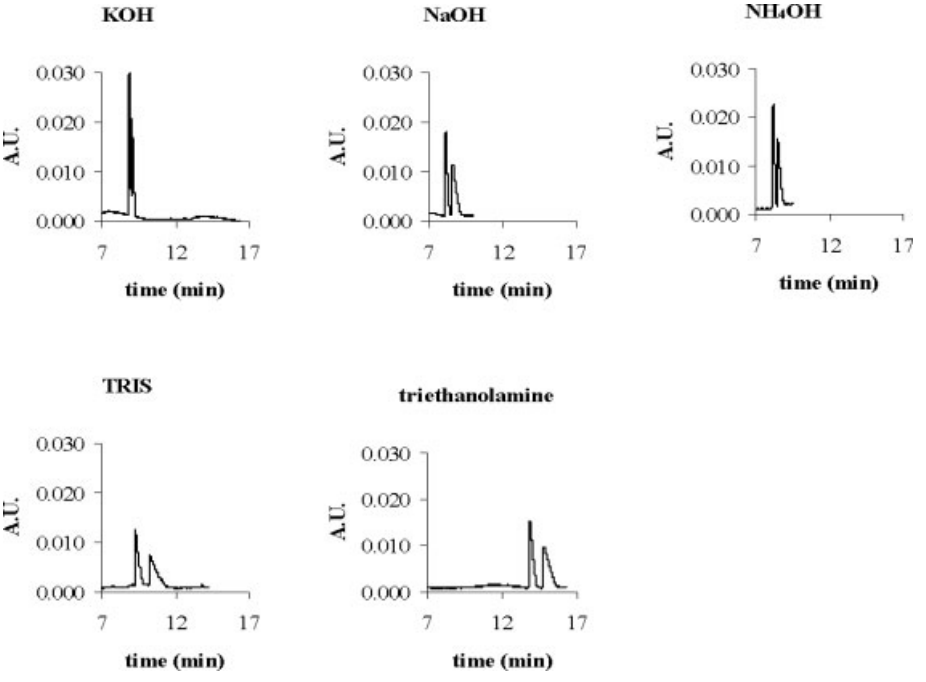

Figure 3. Influence of the co-ion on the chiral separation of cetirizine. Experimental conditions: see Fig. 2; applied voltage: 15 kV; separation solution: 75 mM phosphate buffer (pH 2.5) + 0.1 mg/mL HDAS-β-CD.

3.1.4 Applied voltage

In this experiment the voltage was varied between 10 and 25 kV. The migration times of the cetirizine enantiomers were shortened with increased voltage. In addition, the efficiency of the peaks was also increased. Higher voltages than 20 kV could not be used due to the generation of high currents. Therefore, 20 kV was selected for further experiments (current ±100 μA).

3.1.5 Temperature

The capillary temperature influences the mobility of the analytes, the kinetics and thermodynamics of the inclusion complexation process with CDs [47]. An increase in temperature causes a decrease in BGE viscosity and thus an increase in mobility of the analytes. Furthermore, an increase in temperature usually causes a decrease in the binding constants [49, 50]. The temperature was varied between 16 and 30°C. Lowering the temperature results in increased migration times and resolution.

The optimal conditions obtained until now are: 75 mM triethanolamine-phosphate buffer pH 2.5 + 0.1 mg/mL HDAS-β-CD; 20 kV; 16°C. Under these conditions however, triangular peaks are still obtained, especially for the second migrating peak (see Fig. 4a). Further optimization of the method is therefore still necessary.

3.1.6 Further optimization of the method

Higher concentrations of the chiral selector (0.4 mg/mL HDAS-β-CD) were combined with the addition of ACN as organic modifier (10% v/v). This resulted in increased peak efficiencies. In addition, higher concentrations of the standards were injected but over a shorter period of time (1 s instead of 5 s). The temperature was set at 25°C, which shortened the analysis period to 10 min. Figure 4b shows an electropherogram of the chiral separation of cetirizine under these optimised conditions.

3.1.7 Migration order

Rac.-cetirizine standard was spiked with levocetirizine to determine the migration order. The eutomer was migrating as the second peak, which is advantageous for this method, especially when testing the enantiomeric purity. Indeed, the resolution between both enantiomers is not high (2.0–2.5) and when injecting high sample concentrations peak tailing was observed. Under these circumstances it is more feasible to determine a minor impurity in front of the large tailing peak of the main component.

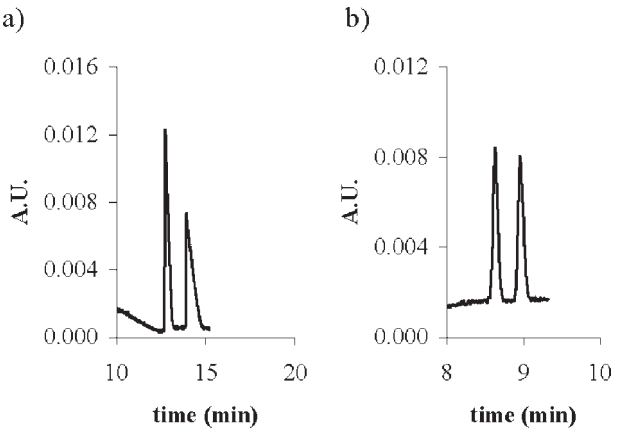

Figure 4. Optimization of separation conditions for the chiral separation of cetirizine. (a) Without organic modifier and 5 s injection; (b) with organic modifier and 1 s injection. Experimental conditions: see Fig. 2; (a) separation solution: 75 mM triethanolamine-phosphate buffer (pH 2.5) + 0.1 mg/mL HDAS-β-CD; temperature: 16°C; injection: 5 s (pressure); (b) separation solution: 75 mM triethanolamine-phosphate buffer pH 2.5 + 0.4 mg/mL HDAS-β-CD + 10% ACN; temperature: 25°C; injection: 1 s (pressure); current: ±90 μA.

3.1.8 System suitability test

During method development it was observed that the resolution between both enantiomers decreased upon using the same HDAS-β-CD stock solution for several days. For quantitative analysis the resolution should exceed 2.0. Therefore, the resolution between both enantiomers was tested every day with a 0.1 mg/mL rac.-cetirizine solution. If the resolution was lower than 2.0, a new stock solution of HDAS-β-CD was made. It was found that the stock solution could be used for maximal five consecutive days.

3.1.9 Selectivity

Selectivity of the method was evaluated by injecting three related substances of cetirizine, as described in the certificate of quality of levocetirizine obtained from UCB. 2-(4-((2-Chlorophenyl)phenylmethyl)-1-piperazinyl)ethoxy)-acetic acid (1) corresponds to related substance C described in the European Pharmacopea, 5th edition [51]. 2-(*p*-Chlorobenzhydryl-piperazine)-2-ethoxyacetamide (2) and UCB 30462 (4) are related substances described in the certificate of quality of levocetirizine supplied by the company. Stock solutions were prepared in ACN at a concentration of 1 mg/mL. Before injection, the stock solutions were diluted ten times with water. All three related substances were separated from both cetirizine enantio-

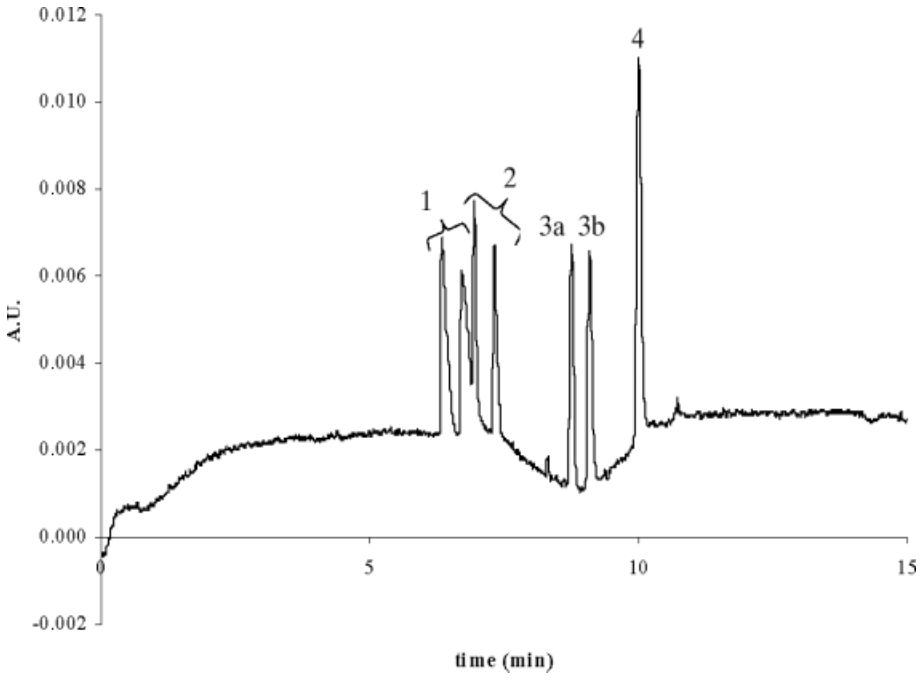

Figure 5. Selectivity of the method. (1) = 2-(4-((2-chlorophenyl)phenylmethyl)-1-piperazinyl)ethoxy)-acetic acid; (2) = 2-(*p*-chlorobenzhydryl-piperazine)-2-ethoxyacetamide; (3a) = *S*-(+)-cetirizine; (3b) = *R*-(−)-cetirizine; (4) UCB 30462; Experimental conditions: see Fig. 4b.

mers (Fig. 5) and also from the IS (1*R*,2*S*)-ephedrine hydrochloride, which is used in the following sections for quantification purposes. The enantiomers of both related substances (1) and (2) were also baseline-resolved with resolution values of, respectively, 1.8 and 2.6.

3.2 Determination of levocetirizine in tablets

The developed method was validated for the quantification of levocetirizine dihydrochloride (5 mg/tablet) in commercially available tablets (Xyzall®). (1*R*,2*S*)-Ephedrine hydrochloride was chosen as IS to correct for injection variability.

3.2.1 Linearity of the calibration line

The calibration curve was constructed with five levels of levocetirizine dihydrochloride distributed over the 50–250 μg/mL range. Calibration linearity was confirmed by absence of lack of fit. The following linear regression equation was obtained: $y = 0.0133 \pm 0.0007x - 0.0468 \pm 0.0293$; $r = 0.9990 \pm 0.0009$ ($n = 6$).

3.2.2 Analytical precision

Intraday precision was determined by comparing the relative corrected peak areas for six repeated injections of three standard concentrations (50, 150 and 250 μg/mL).

Table 1. Analytical precision – content assay

Concentration (μg/mL)	rA_c (mean ± SD)	RSD (%)
Intraday precision (n = 6)		
50	0.6435 ± 0.0201	3.1
150	1.9967 ± 0.0265	1.3
250	3.3288 ± 0.0707	2.1
Interday precision (n = 6)		
50	0.6272 ± 0.0289	4.6
100	1.2981 ± 0.0565	4.4
150	1.9243 ± 0.1117	5.8
200	2.5980 ± 0.1353	5.2
250	3.3066 ± 0.1401	4.2

Experimental conditions: see Fig. 4b

The interday precision of standard curves in the range 50–250 μg/mL was determined on six different days. The RSD values obtained for intra- and interday precision are lower than 3.1 and 6.0%, respectively. These results are summarised in Table 1.

3.2.3 Method precision

Six replicate assays on 1 day were performed to assess repeatability of the method. Interday method precision was assessed by injecting newly prepared samples on six

Table 2. Accuracy – content assay

Amount added (mg)	Amount found (mg)	Recovery (%)
4.50	4.37	97.1
4.50	4.40	97.7
4.50	4.56	101.4
6.00	5.94	99.0
6.00	5.67	94.6
6.00	5.78	96.4
7.50	7.45	99.4
7.50	7.47	99.6
7.50	7.44	99.1
Average ± SD		98.2 ± 2.0

Experimental conditions: see Fig. 4b.

different days in a time period of 1 month. The RSD values obtained for method repeatability and interday method precision were 3.3 and 6.6%, respectively.

3.2.4 Accuracy

Accuracy was assessed through a standard addition method. An accurately weighed aliquot of the tablet powder equivalent to 6 mg levocetirizine dihydrochloride was spiked with, respectively, 4.5, 6 and 7.5 mg of the eutomer. The samples were further prepared as described in Section 2.2.1. All samples were made in triplicate. Results are shown in Table 2. An average recovery of 98.2 ± 2.0% was obtained. A linear relationship was found between the added and calculated concentrations ($y = 1.0031x - 0.1215$; r = 0.9962). The 95% confidence intervals were determined for the slope (0.9251–1.0812) and the intercept (−0.5992−0.3562). These results show that the developed method is accurate for this application.

3.2.5 Assay

The amount of levocetirizine dihydrochloride in the commercially available tablets was quantified and was found to be 97.4 ± 3.3% (n = 6) of the claimed amount (5 mg). A typical electropherogram is shown in Fig. 6.

3.3 Enantiomeric purity testing of the drug substance levocetirizine

In a second study, the enantioselective method was validated and applied for the enantiomeric purity testing of levocetirizine dihydrochloride. In these experiments (1*R*,2*S*)-ephedrine hydrochloride was also chosen as IS to correct for injection variability.

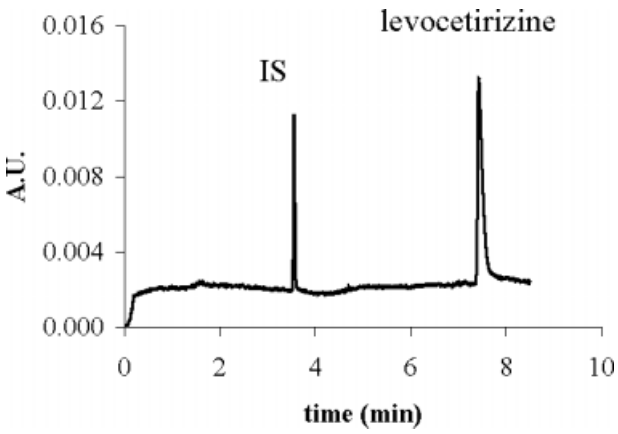

Figure 6. Typical electropherogram of the determination of levocetirizine dihydrochloride in tablets. Experimental conditions: see Fig. 4b.

3.3.1 Linearity of the calibration line

The calibration curve was constructed with five levels of *rac.*-cetirizine dihydrochloride distributed over the 10–100 μg/mL range, corresponding to 5–50 μg/mL *S*-(+)-cetirizine. The following linear regression equation was obtained: $y = 0.0119 \pm 0.0010x - 0.0008 \pm 0.0059$; $r = 0.9992 \pm 0.0008$ (n = 6). Calibration linearity was confirmed by absence of lack of fit.

3.3.2 LOD and LOQ

The LOD and LOQ, calculated as 3.3 and ten times the SD of the intercept (0.0059), divided by the slope of the calibration line (0.0119) (ICH Harmonised Tripartite Guideline, Q2B, Validation of analytical procedures: Methodology, CPMP/ICH281/95), amounts to 1.6 and 5.0 μg/mL, respectively. These limits are approximately three times better as those obtained by Mikuš *et al.* [45].

Due to peak overloading and the fact that the resolution between both enantiomers was not high, only samples of approximately 0.5 mg/mL of levocetirizine could be injected. Therefore, this method allows the detection of 0.3% m/m (1.6 μg/0.5 mg) and the precise and accurate quantification of approximately 1.0% m/m (5.0 μg/0.5 mg) enantiomeric impurity in samples of levocetirizine.

3.3.3 Analytical precision

Intraday precision was determined by comparing the relative corrected peak areas for six repeated injections of three standard concentrations of *rac.*-cetirizine (corresponding to 5, 25 and 50 μg/mL *S*-(+)-cetirizine). The

Table 3. Analytical precision – enantiomeric purity testing

Concentration (μg/mL)	rA_c (mean ± SD)	RSD (%)
Intraday precision (n = 6)		
5	0.0755 ± 0.0068	9.1
25	0.3538 ± 0.0118	3.3
50	0.7375 ± 0.0208	2.8
Interday precision (n = 6)		
5	0.0570 ± 0.0042	7.4
12.5	0.1539 ± 0.0133	8.6
25	0.3164 ± 0.0235	7.4
37.5	0.4664 ± 0.0232	5.0
50	0.6125 ± 0.0539	8.8

Experimental conditions: see Fig. 4b.

interday precision of standard curves in the range 5–50 μg/mL was determined on six different days. The results of intra- and interday precision are summarised in Table 3.

3.3.4 Method precision

Six replicate assays were performed to assess the method precision. Interday precision was obtained through injection of newly prepared samples of levocetirizine on six different days over a time period of 5 wk. The RSD values for method repeatability and interday precision were 10.8 and 12.2%, respectively. Considering the fact that the amount of *S*-(+)-cetirizine determined in these samples is near the LOQ of the method, method precision is acceptable.

3.3.5 Accuracy

Accuracy was assessed through a standard addition method. 0.5 mg/mL of levocetirizine dihydrochloride was spiked with, respectively, 10, 25 and 50 μg/mL of *rac.*-cetirizine dihydrochloride. This corresponds to 5, 12.5 and 25 μg/mL of the distomer or 1.0, 2.5 and 5.0% w/w of impurity. All samples were made in triplicate. Results are shown in Table 4. An average recovery of 95.8 ± 7.2% was obtained. A linear relationship was found between the added and calculated concentrations ($y = 1.0515x - 0.0904$; r = 0.9983). The 95% confidence intervals were determined for the slope (0.9966–1.1064) and the intercept (−0.1822−0.0014). These results show that the developed method is accurate for this application.

3.3.6 Assay

The amount of *S*-(+)-cetirizine dihydrochloride in the drug substance was quantified and was found to be 0.87 ± 0.09% w/w (n = 6) as compared to the amount of

Table 4. Accuracy – enantiomeric purity testing

Amount added (μg/mL)	Amount found (μg/mL)	Recovery (%)
5.10	4.85	95.1
5.10	4.24	83.2
5.10	4.42	86.6
12.75	11.95	93.7
12.75	12.87	101.0
12.75	12.47	97.8
25.50	26.99	105.8
25.50	25.20	98.8
25.50	25.62	100.5
Average ± SD		95.8 ± 7.2

Forexperimental conditions: see Fig. 4b.

levocetirizine dihydrochloride. A typical electropherogram is shown in Fig. 7. The result obtained with this new CE method was compared to the amount notified in the certificate of analysis (0.81%), using a one-sample t-test. There is no significant difference between the mean obtained with the CE method and the amount given in the certificate of analysis ($p > 0.05$). In addition, the intra- and interday method precision is 10.8 and 12.2%, which is acceptable when measuring at these low levels. Although the obtained result (0.87% w/w) is slightly lower than the calculated LOQ (1.0% w/w), this concentration could still be determined with acceptable precision and accuracy.

4 Concluding remarks

In this paper a CE method was developed for the chiral separation of cetirizine using a single-isomer, sulfated CD derivative as chiral selector. Several experimental parameters that influence chiral separation were investigated. The best conditions were found to be 75 mM triethanolamine-phosphate buffer (pH 2.5) containing 0.4 mg/mL HDAS-β-CD and 10% ACN. Online UV detection was performed at 214 nm. A voltage of 20 kV was applied and the capillary was temperature controlled at 25°C by liquid cooling. A1-s hydrodynamic injection further improved peak efficiency. Separation of cetirizine enantiomers was obtained in less than 10 min with a resolution of more than 2. The method was validated and applied for the quantification of levocetirizine in tablets and for enantiomeric purity testing of the drug substance. This work shows the potential of CE for chiral separations and for quantitative analysis of chiral compounds in pharmaceutical applications.

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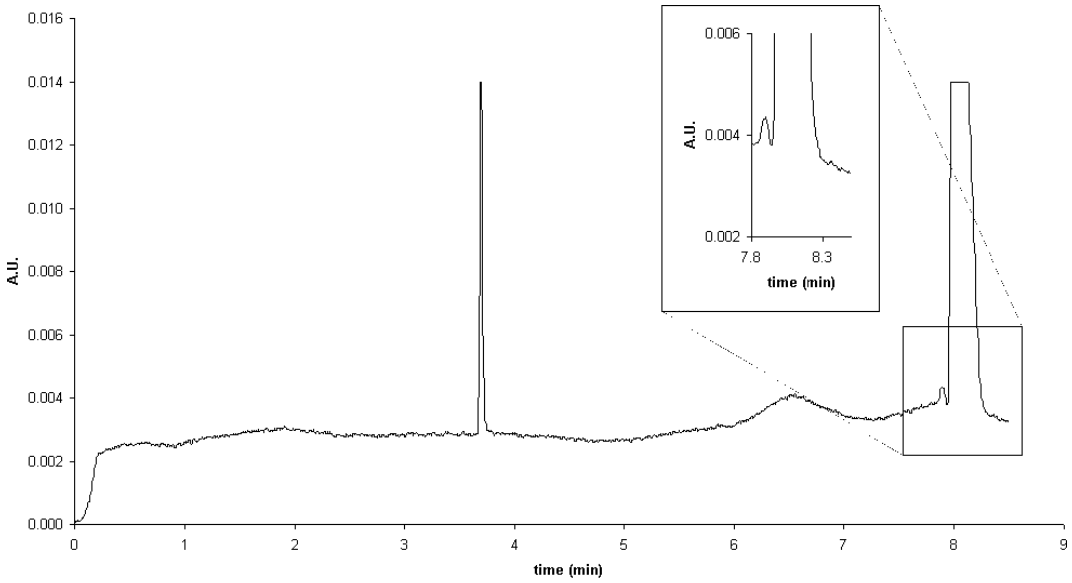

Figure 7. Typical electropherogram of bulk levocetirizine containing 0.9% w/w *S*-(+)-cetirizine. Experimental conditions: see Fig. 4b.

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