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ORIGINAL RESEARCH

Comparison of HPLC and CE methods for the determination of cetirizine dihydrochloride in human plasma samples

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ABSTRACT: Two methods, capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC), for analysis of cetirizine dihydrochloride in small sample volumes of human plasma were compared. The CE and HPLC assays were developed and validated by analyzing a series of plasma samples containing cetirizine dihydrochloride in different concentrations using these two methods. The extraction procedure is simple and no complicated purification steps or derivatization are required. The analysis time in the HPLC method was shorter than that in the CE method, but solvent consumption was considerably lower in the CE method. The calibration curve was linear to at least 10–1000 ng/mL both for CE and HPLC with $r^2 = 0.9993$ and $r^2 = 0.9994$, respectively. The detection limits for cetirizine dihydrochloride were 3 and 5 ng/mL with CE and HPLC (a UV detector was applied in the both cases), respectively. Both methods were selective, robust and specific, allowing reliable quantification of cetirizine dihydrochloride, and could be useful for clinical and biomedical investigations. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: cetirizine dihydrochloride; human plasma; HPLC; CE; comparative study

INTRODUCTION

Cetirizine, a carboxylated metabolite of hydroxyzine, belongs to the piperazine class of second-generation of antihistamines. Owing to its structure, cetirizine negligibly penetrates the central nervous system and has fewer anticholinergic side effects compared with other antihistamines. This drug is well tolerated and is used for the treatment of seasonal and perennial allergic rhinitis and chronic idiopathic urticaria (Golightly and Greos, 2005; Du Buske, 1996; Campoli-Richards *et al.*, 1990). Furthermore, it has also be found to be well tolerated by patients with asthma (Mircarini *et al.*, 2001; Grant *et al.*, 1995) and is minimally metabolized (Nicolas *et al.*, 1999). Cetirizine is a racemate which consists equal amounts of (*R*)-levocetirizine and (*S*)-dextrocetirizine. However, (*R*)-levocetirizine is the most active enantiomer with 2-fold higher affinity for the H_1-receptor compared with racemic cetirizine (Gillard *et al.*, 2002). (*S*)-dextrocetirizine is more useful for urticaria treatment, whereas (*R*)-levocetirizine is used for the treatment of allergic disorders (Tillement *et al.*, 2003; Benedetti *et al.*, 2001).

A number of methods for quantification of cetirizine can be found in the literature. A high-performance liquid chromatography (HPLC) with ultraviolet detection (UV) is the most widely used technique (Macek *et al.*, 1999; Choi *et al.*, 2000a, b; Kim *et al.*, 2005; Zaater *et al.*, 2000; Moncrieff, 1992; Jelińska *et al.*, 2000, 2001; Paw *et al.*, 2002; Arayne *et al.*, 2005; Bajerski *et al.*, 2005; El Walily *et al.*, 1998; Jaber *et al.*, 2004; Nagaralli *et al.*, 2003; Likar *et al.*, 2005; Rosseel and Lefebvre, 1991) or liquid chromatography with spectrometric detection (LC-MS/MS) (Gergov *et al.*, 2001; Gupta *et al.*, 2005; de Jager *et al.*, 2002; Rudaz *et al.*, 2003; Song *et al.*, 2005; Tan *et al.*, 2006). Previously published methods included also gas chromatography (GC) (Baltes *et al.*, 1988), high-performance thin-layer chromatography (HPTLC) (Makhija and Vavia, 2001; Pandya *et al.*, 1996), electrooxidation (Güngör, 2004), spectrophotometric (El Walily *et al.*, 1998; Mahgoub *et al.*, 2003; Gowda *et al.*, 2001; Basavaiah and Charan, 2002; Basavaiah *et al.*, 1999; Gazy *et al.*, 2002), titrimetric technique (Basavaiah and Charan, 2002) for cetirizine determination and assay based on cetirizine–tetraphenylborate ion-pair analysis (Shoukry *et al.*, 1999). However, most of these procedures are time-consuming, not specific or selective enough. A literature survey reveals that the majority of the papers describe the measurement in pharmaceutical formulations (Jelińska *et al.*, 2000, 2001; Paw *et al.*, 2002; Arayne *et al.*, 2005; Bajerski *et al.*, 2005; El Walily *et al.*, 1998; Jaber *et al.*, 2004; Nagaralli *et al.*, 2003; Likar *et al.*, 2005; Rudaz *et al.*, 2003; Makhija *et al.*, 2001; Güngör, 2004; Mahgoub *et al.*, 2003; Gowda *et al.*, 2001; Basavaiah and Charan, 2002; Basavaiah *et al.*, 1999; Gazy *et al.*,

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2002; Shoukry *et al.*, 1999). There are only a few reports on the cetirizine analysis in human (Macek *et al.*, 1999; Kim *et al.*, 2005; Nagaralli *et al.*, 2003; de Jager *et al.*, 2002; Song *et al.*, 2005; Tan *et al.*, 2006; Baltes *et al.*, 1988; Pandya *et al.*, 1996) or animal plasma samples (Choi *et al.*, 2000a; Gupta *et al.*, 2005), which have been based on HPLC following some form of liquid–liquid extraction (LLE) (Macek *et al.*, 1999; Choi *et al.*, 2000a; Kim *et al.*, 2005; Nagaralli *et al.*, 2003; Gupta *et al.*, 2005; de Jager *et al.*, 2002; Tan *et al.*, 2006) or solid-phase extraction (SPE) (Song *et al.*, 2005). Some methods have been described for quantitation of cetirizine also in serum (Zaater *et al.*, 2000; Moncrieff, 1992), blood (Gergov *et al.*, 2001), urine (Choi *et al.*, 2000b; Rosseel and Lefebvre, 1991) or brain tissues (Gupta *et al.*, 2005). It is possible to find in the literature several articles on stereoselective investigation of cetirizine in biological material (Choi *et al.*, 2000a, b; Gupta *et al.*, 2005). Recently, in the literature only three electrophoretic methods for cetirizine determination are performed. One of them, reported by Van Eeckhaut and Michotte (2006), described separation of cetirizine enantiomers using a single-isomer, sulfated cyclodextrin derivative as chiral selector and with triethanoloamine-phosphate buffer at pH 2.5. Moreover, Mikuš *et al.* (2005) have also performed the chiral separation of racemic drug using sulfated-β-cyclodextrin (CD) mediated CE, but morpholinoethanesulfonic acid as buffer solution (at pH 5.2) was used. In a recently published paper, Uysal and Tuncel (2006) proposed a validated method, employing a buffer solution at pH 8.5 and UV detection at 200 nm. Unfortunately, all the electrophoretic methods allow the determination of cetirizine only in commercial pharmaceutical preparations, like tablets, drops or syrups. Although some of the aforementioned methods are precise and sensitive, there is no electrophoretic method for separation of racemic cetirizine in human plasma.

About 25% of all drugs used as therapeutic agent are chiral compounds administered to humans as racemates, and cetirizine is also marketed as a racemic mixture. In many pharmacokinetic investigations we need to determine the whole concentration of the drug and it is a essential to find the analytical conditions in which the signal for an enantiomeric drug can be separated from the biological matrix as only one peak. Measurement of the concentration of cetirizine enantiomers in plasma is required only for detailed pharmacokinetic studies.

In this paper are presented two analytical methods for the determination of racemic cetirizine in human plasma using capillary electrophoresis and high-performance liquid chromatography. The main goal of our study was to compare the performance and suitability of HPLC and CE techniques for the determination of cetirizine dihydrochloride in human plasma. The chief benefit of the presented methods is the minimal sample preparation involved, and that the procedure is a simple protein precipitation by acetonitrile in CE, and LLE by dichloromethane in HPLC. Both proposed methods are simple, inexpensive and efficient without complicated clean-up and derivatization procedures and can be successfully used in pharmacokinetic investigations.

EXPERIMENTAL

Reagents

Racemic cetirizine dihydrochloride and indometacin (used as an internal standard in HPLC) were kindly supplied by Biovena Pharma (Warsaw, Poland). Ephedrine hydrochloride (internal standard in CE) was provided by Fram-Impex (Gliwice, Poland). The organic solvents and reagents used for extraction and in the mobile phase were of analytical grade and used without further purification. *Ortho*-phosphoric acid 85%, sodium tetraborate decahydrate (pH 9.3; 20 mM) and sodium dihydrogenophosphate (pH 5.7; 10 mM) were obtained from POCh (Gliwice, Poland). The buffer solutions were prepared using triple-distilled water. Dichloromethane and acetonitrile, all of HPLC grade, were supplied by Merck (Darmstadt, Germany). Water was purified by triple distillation.

Instrumental parameters and conditions

CE technique. CE separation was performed with a P/ACE 2100 system equipped with a UV detector (Beckman Instrumental, Fullerton, CA). Samples were introduced from the anodic end of the fused-silica capillary (57 cm length and 75 μm i.d.) by 7 s vacuum injection. Detection at 50 cm from the injection end was by an on-line UV detector at 200 nm. The voltage applied was 25 kV and the temperature was set at 25°C. At the beginning of each day, the capillary was washed for 2 min with 0.1 M sodium hydroxide, 2 min with deionized water and finally with running buffer (5 min). The capillary was flushed between runs with 0.1 M hydrochloric acid (0.2 min), then 0.1 M sodium hydroxide (1 min) and finally with triple-distilled water (1 min). Before injection the capillary was rinsed with the running buffer for 1 min. Under these conditions, the migration times for cetirizine and the internal standard were 7.1 and 4.4 min, respectively. The total run time for each sample analysis was 9 min.

HPLC technique. The high-performance liquid chromatography system was purchased from Knauer (Berlin, Germany), and was equipped with a solvent pump (Mini-Star K-500), a K-2500 UV detector and a computer system for data acquisition (Eurochrom 2000). A Nucleosil-100 C_{18}, analytical column (5 μm, 125 × 4 mm i.d.) from Dr Ing. Herbert Knauer GmbH (Berlin, Germany) was used. As mobile phase a binary mixture of acetonitrile–water (60:40, v/v) adjusted to pH 2.7 with 85% *ortho*-phosphoric acid was applied. The flow-rate of 1 mL/min was found to be adequate for sample analysis. The chromatographic separation was performed at room temperature. The analytes were monitored at 227 nm.

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Under these conditions, the retention times for cetirizine and its internal standard were 4.2 and 3.3 min, respectively. The total run for each sample analysis including regeneration of the column was 6 min.

Standard solutions and sample preparation

Stock solutions of cetirizine and internal standards were made by accurately weighing 10.0 mg of the compound into 10 mL of triple distilled water. Further solutions were obtained by serial dilution of stock solution with water both for HPLC and CE analysis. Indometacin (internal standard, IS, for HPLC) was dissolved in acetonitrile, while ephedrine hydrochloride (IS for CE) was dissolved in triple-distilled water. Also, the stock solution of ISs was diluted to yield 100 and 10 μg/mL for CE and HPLC method, respectively. All solutions were stored in the refrigerator and protected from light.

Drug-free human plasma used for the preparation of calibration standards and quality control (QC) samples was purchased from University Hospital (Gdańsk, Poland) and stored at –20°C prior to use. Analytical research has been carried according to the recommendations of the local ethics committee. The plasma samples to be analyzed were removed from the freezer and thawed. QC samples, the calibration curve and blank plasma samples were extracted using a liquid–liquid extraction technique. Standard samples of calibration curves and QC samples were prepared by spiking different samples of 0.5 mL plasma each with known amounts of cetirizine dihydrochloride and internal standard solution (75 μL indometacin and 25 μL of ephedrine hydrochloride for HPLC and CE analysis, respectively). QC samples were prepared by spiking cetirizine and IS to produce the concentration pools of 50, 200 and 750 ng/mL.

For HPLC analysis, 4 mL of dichloromethane were added to each plasma sample. The resulting mixture was shaken mechanically for 10 min and centrifuged for 15 min at 3500 rpm/min. The organic layer was transferred into a clean test tube and evaporated to dryness in a water bath at 45°C with the aid of a gentle stream of air. The residue was reconstituted with 100 μL of acetonitrile–water (60:40, v/v). After shaking for 0.5 min and centrifugation for 8 min at 10,000 rpm/min, 20 μL was injected into the HPLC system for quantitation.

For CE analysis, 2 mL of acetonitrile was mixed with each plasma sample and vortexed thoroughly for 5 min. After a centrifugation for 5 min at 8000 rpm/min, the acetonitrile portion was transferred to a glass tube and evaporated to dryness at 45°C. The residue was dissolved in 200 μL of 2 mM borax solution, finally centrifuged and injected directly into the capillary.

Validation of analytical methods

Both CE and HPLC methods for determination of cetirizine in human plasma were validated for selectivity, accuracy, precision, linearity, recovery and stability. This assay has been carried out on the basis the replicate analysis of samples containing known amounts of cetirizine. Selectivity was determined by analysing drug-free blank human plasma samples which were tested for interference using the proposed methods, and these results were compared with those obtained from human plasma samples containing cetirizine and internal standard. The linearity of both methods was tested for the range of drug concentrations 10–1000 ng/mL. This assay was evaluated on the basis of the analysis of calibration curves constructed from samples of eight concentrations: 10, 20, 50, 100, 200, 500, 750 and 1000 ng/mL for cetirizine. The calibration curves were obtained by linear regression; the ratio of cetirizine peak height to internal standard peak height was plotted vs cetirizine concentration in ng/mL. The limit of detection (LOD) was determined as the lowest measurable sample concentration which was distinguishable from zero, defined as the peak being three times the baseline noise. The limit of quantification (LOQ) was defined as the lowest concentration at which the precision expressed by RSD% was lower than 15%, the accuracy expressed by a percentage of the nominal concentrations was within 80–120% and the ratio of signal to noise was better than 10. Within-day precision and accuracy were determined by the analysis of six replicates of calibration control samples at each concentration level of the calibration curves on the same day. Between-day precision and accuracy were assessed by analyzing the quality control samples (50, 200 and 750 ng/mL), which were tested on 10 different occasions. Within-day and between-day precision and accuracy were evaluated using back-calculated concentrations. Within-day precision was determined by calculating the relative standard deviation (RSD%). Assay accuracy was assessed by calculating the estimated concentrations as a percentage of the nominal concentrations.

The absolute recoveries of cetirizine and internal standards were determined by direct comparison of peak heights from extracted vs non-extracted plasma samples.

As part of the validation, the freeze–thaw stability of cetirizine was evaluated by comparing the stability of samples containing analyzed substance that had been frozen and thawed three times, with plasma samples that were thawed only once. This evaluation was based on back-calculated concentrations.

Selectivity

The selectivity of both methods was confirmed on the basis of analysis of different drug-free human plasma samples and extracts from plasma containing cetirizine and internal standard ($n = 6$). Typical electropherograms (CE) and chromatograms (HPLC) obtained with a drug-free plasma and a plasma sample containing 1000 ng/mL of cetirizine and internal standard are illustrated in Figs 1(A) and 2(A) and Figs 1(B) and 2(B), respectively. No interference was observed during the electrophoretic and chromatographic run of the plasma sample in the area where cetirizine or the internal standard peak appeared. Additionally, the specificity of both methods was confirmed by the identification of peaks of cetirizine and the internal standard in terms of the migration or retention times and UV spectrum. The analysis time was shorter with HPLC than with CE, 6 vs 9 min, respectively.

Linearity

The linearity of both methods was tested for the range of concentrations 10–1000 ng/mL using a 0.5 mL plasma sample.

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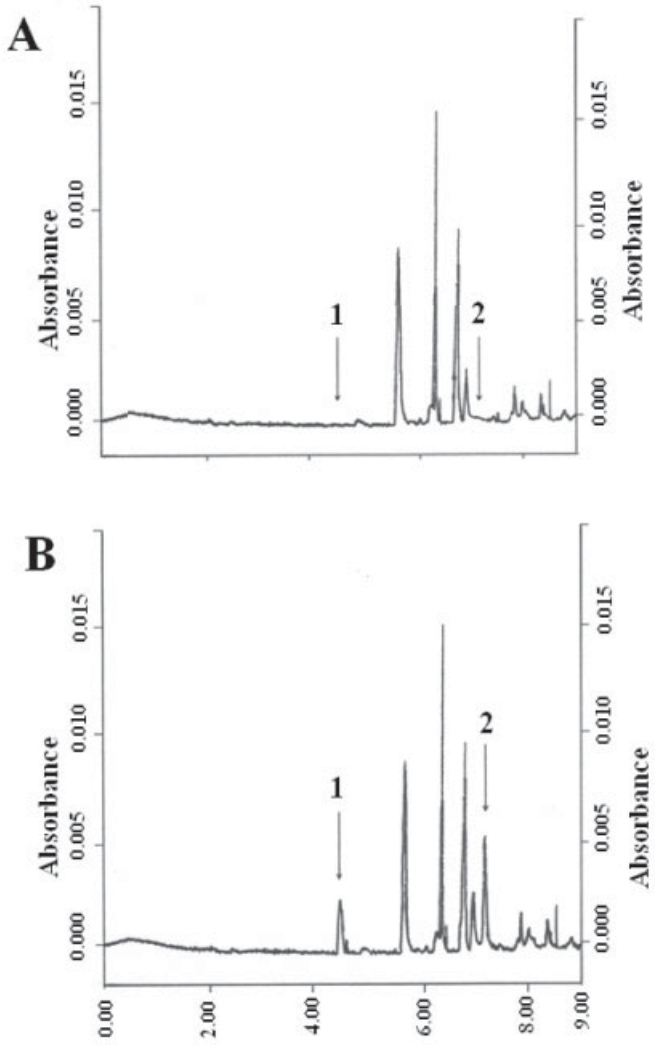

Figure 1. Typical electropherograms of blank human plasma extract (**A**) and plasma spiked with ephedrine hydrochloride (**1**) (internal standard – 50 μg/mL) and cetirizine dihydrochloride (**2**) (1000 ng/mL) (**B**).

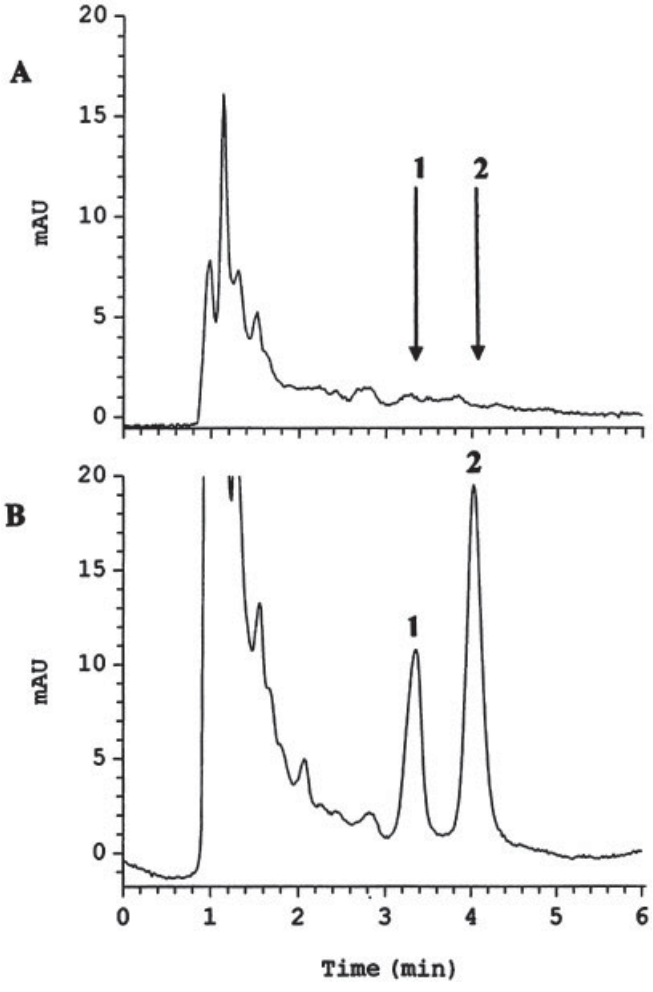

Figure 2. Typical chromatograms of blank human plasma extract (**A**) and plasma spiked with indometacin (**1**) (internal standard – 1500 ng/mL) and cetirizine dihydrochloride (**2**) (1000 ng/mL) (**B**).

Both methods exhibited a reliable linear response for the validated range of concentrations of cetirizine. The mean equations of the calibration curve including eight points for CE and HPLC were $H/H_{IS} = 0.0013\ (\pm\ 0.00002)\ C + 0.0154\ (\pm\ 0.01)$ and $H/H_{IS} = 0.0011\ (\pm\ 0.00001)\ C + 0.0058\ (\pm\ 0.0054)$ with correlation coefficients $r^2 = 0.9993$ and $r^2 = 0.9994$, respectively, where H/H_{IS} represents the ratio of cetirizine peak height and internal standard one and C represents the cetirizine concentration in ng/mL (Table 1). Standard errors are given in parentheses. The low value of the intercept and its relatively large standard errors confirm the specificity of the presented methods.

Limit of detection and quantitation

For sensitivity determination, the limits of detection (LOD) for cetirizine were calculated form six independent replications for both techniques and determined to be 3 and 5 ng/mL for CE and HPLC, respectively. The lowest standard concentration in the calibration curves was considered as the lower limit of quantitation for the presented methods. The limit of quantitation for cetirizine was proved to be 10 ng/mL, with precision 10.2 and 9.6% and accuracy 97 and 94% for CE and HPLC, respectively (Table 2). These methods were sufficiently sensitive, with a comparable quantification limit (Macek *et al.*, 1999; Kim *et al.*, 2005) or lower than for the earlier published HPLC methods with spectrophotometric detection (Choi *et al.*, 2000a; Zaater *et al.*, 2000; Moncrieff, 1992; Nagaralli *et al.*, 2003).

Precision and accuracy

The within-day and between-day precision and accuracy results for both presented methods are shown in Tables 2 and 3, respectively. The within-day precisions of the CE and HPLC method for cetirizine, expressed as RSD%, ranged from 10.2 to 2.3% and from 9.6 to 1.1%, respectively. The within-day accuracies for analysed substance were better than 94.7 and 94% for CE and HPLC techniques, respectively (Table 2). For the quality control samples at concentrations

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Table 1. Summary of precision and validation data for cetirizine dihydrochloride, obtained with CE and HPLC calibrations

	CE	HPLC
Linearity range (ng/mL)	10–1000	10–1000
Sample linearity		
Slope	0.0013 (± 0.0002)	0.0011 (± 0.00001)
Intercept	0.0154 (± 0.01)	0.0058 (± 0.0054)
Correlation coefficient (r^2)	0.9993	0.9994
Standard error	0.02	0.01
n	8	8
LOD (ng/mL)	3	5
LOQ (ng/mL)	10	10
Separation time (min)	9	6
Sample pretreatment	Protein precipitation (acetonitrile)	Liquid–liquid extraction (dichloromethane)

Table 2. Assay validation results obtained from within-run experiments for cetirizine dihydrochloride

	Within–run					
	CE method			HPLC method		
Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Precision RSD (%)	Accuracy (%)	Measured concentration (ng/mL)	Precision RSD (%)	Accuracy (%)
10	9.7 ± 1.0	10.2	97.0	9.4 ± 0.9	9.6	94.0
20	20.9 ± 1.7	8.0	104.5	21.2 ± 1.8	8.5	106.0
50	53.0 ± 4.0	7.6	106.0	48.8 ± 3.5	7.2	97.6
100	106.3 ± 6.4	6.1	106.3	90.2 ± 5.0	5.5	90.2
200	211.2 ± 9.7	4.6	105.6	209.9 ± 9.4	4.5	104.9
500	473.4 ± 21.2	4.5	94.7	503.8 ± 12.8	2.5	100.8
750	744.0 ± 23.1	3.1	99.2	732.7 ± 13.4	1.8	97.7
1000	1019.3 ± 23.1	2.3	101.9	997.8 ± 11.0	1.1	99.8

Mean concentration ± SD from six human plasma samples (n = 6).

Table 3. Assay validation results obtained from between-run experiments for cetirizine dihydrochloride analysed by CE and HPLC

	Between-run					
	CE method			HPLC method		
Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Precision RSD (%)	Accuracy (%)	Measured concentration (ng/mL)	Precision RSD (%)	Accuracy (%)
50	53.9 ± 5.6	10.4	107.8	51.4 ± 6.4	12.4	102.8
200	193.9 ± 8.6	4.5	97.0	197.0 ± 12.9	6.5	98.5
750	743.5 ± 25.2	3.4	99.1	747.0 ± 17.9	2.4	99.6

Mean concentration ± SD from 10 human plasma samples (n = 10).

of 50, 200 and 750 ng/mL of cetirizine, the between-day precisions were 10.4–3.4 and 12.4–2.2% and accuracies were 99.1–107.8 and 98.5–102.8% for CE and HPLC method, respectively (Table 3).

Recovery

The absolute recovery of cetirizine in plasma was determined at two different concentration levels (low and high) with six replicates for each concentration. Mean recoveries for cetirizine were 84.8 ± 1.5 and 88.1 ± 2.3% for CE, whereas for HPLC they were 94 ± 4.5 and 95.1 ± 2.3% at the 100 and 1000 ng/mL concentrations, respectively (Table 4). The absolute recoveries of internal standards (ephedrine hydrochloride in CE and indometacin in HPLC) were 88.0 ± 1.8 and 81.3 ± 4.3%, respectively. These data confirmed that the extraction provided adequate sensitivity to process the samples for both methods.

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Table 4. Absolute recovery of cetirizine dihydrochloride from human plasma ($n = 6$)

Concentration of cetirizine dihydrochloride (ng/mL)	Mean recovery (%)	RSD (%)	Range	n
CE method				
100	84.8 ± 1.5	1.8	82.9–87.0	6
1000	88.1 ± 2.3	2.6	84.9–91.8	6
Internal standard (ephedrine hydrochloride, 50 μg/mL)	88.0 ± 1.8	2.0	84.5–90.8	12
HPLC method				
100	94 ± 4.5	4.8	88.9–100.0	6
1000	95 ± 2.3	2.4	92.9–99.3	6
Internal standard (indometacin, 1500 ng/mL)	81.3 ± 4.3	5.2	77.0–86.8	12

Table 5. Results for processed plasma sample freeze–thaw cycles during 2 months

Plasma concentration of cetirizine dihydrochloride (ng/mL)				
Spiked	Initial	After first freeze–thaw cycle	After second freeze–thaw cycle	After third freeze–thaw cycle
CE method				
50	51.4 ± 1.4	49.8 ± 1.4	51.3 ± 1.7	50.7 ± 1.4
200	200.7 ± 1.6	198.4 ± 3.1	196.6 ± 2.4	195.4 ± 4.3
750	749.9 ± 11.1	745.9 ± 10.3	749.8 ± 9.6	749.1 ± 6.8
HPLC method				
50	50.2 ± 1.9	50.9 ± 2.0	51.0 ± 1.7	49.9 ± 1.2
200	201.9 ± 5.3	199.1 ± 5.1	199.7 ± 5.7	202.8 ± 5.7
750	756.7 ± 19.5	755.1 ± 16.2	754.1 ± 14.7	758.3 ± 18.3

Mean concentration ± SD from three human plasma samples ($n = 3$).

Freeze–thaw stability. In both methods, the freeze–thaw stability was evaluated using plasma samples containing 50, 200 and 750 ng/mL of cetirizine dihydrochloride. These tests were performed by measuring three replicates at each concentration over two months and the data obtained given in Table 5. The stability data were used to support repeat analyses. These results confirm that the analyte is stable in human plasma for three cycles of freeze and thaw, when stored at –20°C and thawed to room temperature, and can be handled under normal laboratory conditions without significant loss.

RESULTS

In this study, optimized and validated assays of both HPLC and CE methods for direct determination of racemic cetirizine in human plasma with UV detection have been developed and compared. The UV detection has been the most common detector used for its simplicity and low cost, while the mass spectrometry has been less frequently applied due to the high instrumentation cost. The sample preparation procedure developed in this work, including single liquid–liquid extraction, was optimized to eliminate time-consuming purification steps (Choi *et al.*, 2000a; Kim *et al.*, 2005; Pandya *et al.*, 1996), entailing a derivatization procedure (Baltes *et al.*, 1988) and solid-phase extraction (Song *et al.*, 2005), as used in previous work.

Several earlier published works have described the assay of racemic cetirizine in biological samples. Some of the methods are not suitable for routine analysis in clinical laboratories, because they require long pretreatment of biological samples, expensive chiral selectors or organic solvents, which are not readily available in many control laboratories. Moreover, determination of cetirizine is generally carried out with UV absorbance detection at around 220–230 nm. Unfortunately, this range of detection usually suffers from limited sensitivity and biological sample interference in some cases. Recently, one paper has described a validated CE study at 200 nm UV, but investigation was performed in pharmaceutical forms (Uysal and Tuncel, 2006). Therefore, in this work we proposed the development of a rapid analysis procedure for determination of racemic cetirizine in human plasma with only minor sample pre-treatment required at low wavelength UV detection (200 nm).

Likewise in this paper, the optimal electrophoretic and chromatographic parameters for the cetirizine separation were studied. The influence of the changes of pH buffer solution, as well as the changes of voltage and temperature and the electrophoretical migration

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of the analyzed drug were tested. One of the most important parameters for CE is the background electrolyte, especially its pH value. The best separation results were achieved with buffer solution composed of NaH_2PO_4 (pH 5.7; 10 mM) and $Na_2B_4O_7$ (pH 9.3; 20 mM). Lowering the pH of the electrolyte gives longer migration times for the analyzed drug and lower resolution of separation. Moreover, the electrophoretic condition gave good reproducibility of corrected peak signals and of the migration time for analytes (data not shown).

Furthermore, different parameters for HPLC analysis were varied, such as temperature, flow-rate and sample solvent. The effects of different organic solvents and acid solutions used to prepare the mobile phase were also investigated. The selectivity of the proposed method depends strongly on the percentage of acetonitrile, and the addition of 85% *ortho*-phosphoric acid to the mobile phase. Acetonitrile was chosen as a component of the mobile phase because a better peak symmetry was observed than after using methanol. *Ortho*-phosphoric acid added to the mobile phase shortened the retention times of cetirizine and internal standard and, additionally, the retention times of endogenous plasma peaks were modified to obtain clear peaks of analysed substances. Other experimental parameters such as the flow-rate of mobile phase and the temperature of analytical column had a minor influence on separation. The assay was performed at room temperature whereas the flow-rate of mobile phase was 1 mL/min.

Table 1 summarizes precision and validation data for cetirizine obtained with both techniques. These results show that all investigated parameters were similar, except for the analysis time, as well as the LOD of analyte. In terms of analysis time, under the HPLC conditions, the analytes were fully separated in a shorter time than for the CE method. Although the total separation times were 6 and 9 min for HPLC and CE, respectively, method development was faster with CE since it did not require extensive equilibration times compared with HPLC columns. Concerning analysis time, both methods offer substantial advantages in comparison with earlier investigations that analyzed cetirizine in plasma samples (Benedetti *et al.*, 2001; Macek *et al.*, 1999; Choi *et al.*, 2000b; Basavaiah and Charan, 2002).

Taking into account the sensitivity, the LOD value of cetirizine was lower in the CE technique. It is known that, when optical detectors are used for drug determination, the concentration sensitivity for HPLC tends to be better than that of CE. Notwithstanding, cetirizine is a relatively weak UV absorbing compound, particularly when it is dissolved in conventional organic solvent. Therefore the direct UV absorbance measurement of this drug, particularly at low concentrations, is poor. This is a problem, because most organic solvents (like acetonitrile) also strongly absorb radiation themselves at low-wavelength UV detection. Fortunately, in CE separation systems based on water buffer solutions, we can observe lower LOD of cetirizine for CE than for HPLC (Table 1). Moreover, the high transparency of the silica capillary wall allowed the use of low UV wavelengths, which successfully compensate for the poor sensitivity of the CE technique.

The within-day (as repeatability) and between-day laboratory investigations (as reproducibility) were also performed, and the resulting mean concentrations with standard deviations and relative standard deviation were determined at all the assayed levels (Tables 2 and 3). Plasma samples determined by the CE method gave worse within-laboratory repeatability than HPLC, whereas between-run accuracy was better for HPLC assay. The results showed that the HPLC method is slightly more accurate and repeatable than CE; on the other hand the CE method is more precise. The analysis of validation data leads to the conclusion that the results are similar, whereas the observed differences are insignificant.

Both proposed techniques may be applied as routine methods in bioavailability or bioequivalence studies and drug monitoring after enteric administration of a cetirizine therapeutic dose. These methods are sensitive enough for pharmacokinetic study at all intervals after dosing of drug and can be routinely applied to drug monitoring in humans.

CONCLUSION

The determined HPLC and CE methods with UV detection are specific, precise, accurate and can be used for pharmacokinetic and bioavailability studies of racemic cetirizine in therapeutic doses. There were no significant differences between the two techniques. Comparison of the HPLC and CE methods has shown that the first is more accurate and reproducible, while the latter is more sensitive and selective. Moreover, the many potential advantages, such as significantly reduced analysis costs, small injection volume and simplicity, should be taken into account.

Both methods have simple extraction procedures of cetirizine from human plasma, can be performed at room temperature and are based on readily available and inexpensive chemicals and conventional instruments. The low consumption of solvents (milliliters per day for CE vs centiliters per day for HPLC) and buffer additives is also a major advantage in CE. The results obtained in the validation process and in drug analysis for both techniques are encouraging and indicate suitability for routine analysis in any clinical laboratory.

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