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Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 603–609
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Narrow-bore high performance liquid chromatographic method for the determination of cetirizine in human plasma using column switching

Chong-Kook Kim[a,*], Kyu Jeong Yeon[a], Eunmi Ban[a], Myung-Ja Hyun[a], Jin-Ki Kim[a], Mee-Kyung Kim[a], Su-Eon Jin[a], Jeong-Sook Park[b]

[a] *Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, San 56-1, Shillim-dong Kwanak-gu, Seoul 151-742, South Korea*
[b] *College of Pharmacy, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, South Korea*

Received 28 February 2004; received in revised form 13 November 2004; accepted 14 November 2004
Available online 19 December 2004

Abstract

An improved column switching high performance liquid chromatographic (HPLC) method was developed for determination of cetirizine in human plasma. Plasma samples were prepared by liquid–liquid extraction using methylene chloride. The samples extracted were initially injected into a clean-up Capcell Pak MF C_8 column and the peaks of cetirizine and internal standard were separated to an analytical C_{18} micro-column via column switching device. This analysis showed highly sensitive and selective results. Also, it was successfully applied to evaluate the pharmacokinetics of cetirizine in human volunteers after single oral administration.

Keywords: Cetirizine; Column switching; High performance liquid chromatography (HPLC); Pharmacokinetics

1. Introduction

Cetirizine (2-[2-[4[(4-chlorophenol)phenylmethyl]-1-piperazinyl]ethyloxy] acetic acid dihydrochloride) (Fig. 1) is an H_1-receptor antagonist in a group of the cyclizine class of compounds [1]. It is an active metabolite of hydroxyzine, a first generation H_1-receptor antagonist. Marked affinity of cetirizine for peripheral histamine H_1 receptors results in anti-allergic properties, but has the advantage that it lacks the CNS depressant effects often encountered in anti-histamines [2]. Cetirizine is a potent and well tolerated nonsedating anti-histamine drug for the treatment of seasonal and prennial allergic rhinitis and chronic urticaria [1–4].

Despite its widely use, only a few methods for the determination of cetirizine in biological fluids can be found in the literatures. Previously published assays include a gas chromatography (GC) [1], TLC with radio-labeled methods [4,10,11] and a high performance liquid chromatography (HPLC) with ultraviolet (UV) [3,5–8] or mass spectrometric detection [9]. Most investigators have used HPLC with UV and recently, two HPLC methods [7,8] have been reported for determination of cetirizine in plasma. However, these methods could not be reproduced under our laboratory conditions because of interfering peaks in some cases. Therefore, there was a need to develop and validate more specific method for pharmacokinetic studies of cetirizine in plasma than the previous papers'.

Column switching HPLC method has major advantages in terms of the increasing the sensitivity and specificity [12]. Also, this system is a simple composition of adding pump and columns to currently available HPLC and it can be operated by less trained user than mass spectroscopy. Therefore, column switching HPLC can be an alternative method for the determination of cetirizine in human plasma.

The aim of this study was to develop a specific and sensitive method using a narrow-bore HPLC with column

* Corresponding author. Tel.: +82 2 880 7867; fax: +82 2 873 7482.
E-mail address: ckkim@plaza.snu.ac.kr (C.-K. Kim).

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doi:10.1016/j.jpba.2004.11.022

switching for the determination of cetirizine in human plasma. Moreover, this method was used successfully to apply the pharmacokinetic studies of cetirizine in healthy volunteers.

2. Experimental

2.1. Materials

Cetirizine dihydrochloride (CTZ) (Fig. 1) was obtained from Hanmi Pharm. (Seoul, Korea). As an internal standard (IS), methyl paraben (Fig. 1) was purchased from Sigma (St. Louis, MO, USA). All solvents were HPLC grade from Burdick & Jackson (Muskegon, MI, USA) and water was purified by a Milli-Q system (Millipore Corp., Bedford, MA, USA). All other reagents were analytical reagent grade and used without further purification.

2.2. Preparation of standard solutions

Stock solutions of CTZ or IS were prepared by accurately weighing 10 mg of the compound into 1 ml of methanol. The CTZ standard was serially diluted with mobile phase 1 [15% (v/v) acetonitrile in 20 mM phosphate buffer (pH 2.8)] to yield 1, 2, 4, 10, 20, 40 and 80 μg/ml working standard solutions for the preparation of calibration curves. Also, the stock solution of IS was diluted with mobile phase 1 to yield 10 μg/ml internal standard working solution. The stock solutions were stored at −20 °C.

Plasma standards were prepared by spiking 5 μl of each CTZ working standard solution and 5 μl of IS working standard solution to 1 ml blank plasma (drug-free) in 10 ml glass tube. Quality control samples were prepared by spiking CTZ standards to produce the concentration pools of 10, 200 and 400 ng/ml. These samples were stored at −20 °C.

2.3. Preparation of plasma samples

The 1 ml of plasma samples was mixed with 6.0 ml methylene chloride and vortexed thoroughly for 30 s. After a centrifugation for 25 min at 2000 × *g*, the organic portion in each tube was carefully transferred to a 10 ml glass tube and evaporated to dryness under nitrogen gas at 30 °C. The residue in each tube was reconstructed by 500 μl of the mobile phase 1 and 210 μl was finally injected directly into the HPLC system.

2.4. Semi-microbore column switching HPLC

CTZ was determined by column switching HPLC with UV detection. All experiments were performed using an automated semi-microbore HPLC Nanospace SI-1 series (Shiseido, Tokyo, Japan) equipped with two 2001 pumps, a 2002 UV–vis detector, a 2003 autosampler, a 2004 column oven, a 2012 high-pressure switching valve and a 2009 degassing unit as schematically described in Fig. 2.

A Capcell Pak MF C_8 SG 80 column (50 mm × 4.6 mm i.d., 5 μm particles, Shiseido, Japan) was used for selective adsorption of CTZ in plasma. A Capcell Pak C_{18} UG 120V column (35 mm × 2.0 mm i.d., 5 μm particles, Shiseido) was used for concentration of CTZ as an intermediate column. A Capcell Pak C_{18} UG 120V column (250 mm × 2.0 mm i.d., 5 μm particles, Shiseido) was used as a separation column. The operation temperature was maintained at 35 °C during analysis. However, pretreatment and concentration column were operated at room temperature. Pump 1 and 2 were used

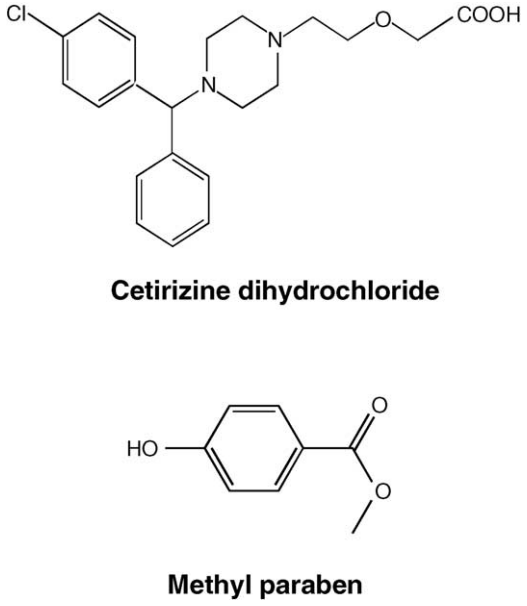

Fig. 1. Chemical structures of cetirizine dihydrochloride (CTZ) and methyl paraben as an internal standard (IS).

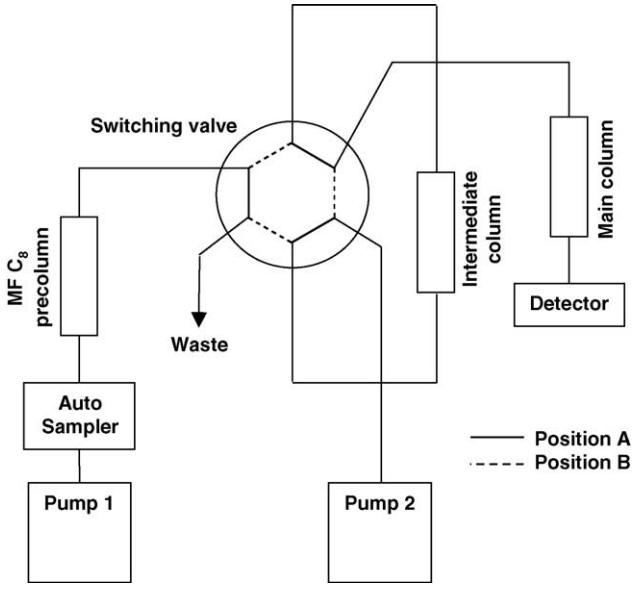

Fig. 2. Schematic diagram of a column swtitching system. (A) Sample loading, precolumn wash and re-equilibration position and (B) elution, separation.

Table 1
Time schedule of column switching HPLC for the analysis of cetirizine (CTZ) and methyl paraben (IS)

Time after injection (min)	Switching valve position	Comments
0.0–7.7	Loading (A)	Sample loading (210 μl) by mobile phase 1 at 0.5 ml/min
7.7–9.8	Concentration (B)	Transfer of CTZ and IS from pre-column to intermediate column by mobile phase 1 at 0.5 ml/min
9.8–23	Separation (A)	Intermediate column back-flush onto the analytical column followed by mobile phase 2 at 0.1 ml/min

Mobile phase 1: 15% (v/v) acetonitrile in 20 mM phosphate buffer (pH 2.8).
Mobile phase 2: 35% (v/v) acetonitrile in 20 mM phosphate buffer (pH 2.8).

to deliver mobile phase 1 at a flow rate of 0.5 ml/min and mobile phase 2 [35% (v/v) acetonitrile in 20 mM phosphate buffer (pH 2.8)] at a flow rate of 0.1 ml/min, respectively. UV detection was performed at 230 nm.

2.5. Analytical procedure

A schematic diagram of the HPLC system is showed in Fig. 2. Time program and switching valve position are showed in Table 1. Sample loading and chromatographic separation were performed as follows.

Step 1: Plasma sample solution (210 μl) was introduced to the pretreatment column via the autosampler using mobile phase 1 at a flow rate of 0.5 ml/min. At the time of sample injection, the column-switching valve was placed in position A. The pretreatment column kept CTZ. Residual proteins in plasma were eluted in void volume and wasted without passing through the analytical column and the detector.
Step 2: The switching valve was shifted to the position B at 7.7 min, thus CTZ is eluted from the pretreatment column between 7.7 and 9.8 min and introduced to the concentration column by isocratic elution using mobile phase 1 at a flow rate of 0.5 ml/min.
Step 3: Finally, CTZ adsorbed to the concentration column is introduced to the separation column by switching valve with back-flush technique using mobile phase 2 and a flow rate was reduced to 0.1 ml/min at this step. During the analysis, the pretreatment column was washed for the next analysis. It took 23 min to complete the above-mentioned analytical procedure for one sample.

2.6. Assay validation

2.6.1. Specificity and sensitivity

Specificity in relation to the endogenous components was demonstrated by analyzing standard CTZ, drug-free plasma, plasma spiked with CTZ and plasma obtained from subjects. The limit of detection (LOD) was based on S/N (signal-noise ratio) 10.

2.6.2. Linearity

Linearity was demonstrated by running plasma standards at six concentrations over the range of 10–400 ng/ml for five consecutive days. Standard samples were prepared by adding CTZ to blank plasma at concentrations of 10, 20, 50, 100, 200 and 400 ng/ml with 50 ng/ml IS and these were extracted and analyzed as described above. Peak area ratios of each CTZ to IS were measured and the calibration curve was obtained from least-square linear regression (no weighing factor). The suitability of the standard curve was confirmed by back-calculating the concentrations of the standard curve.

2.6.3. Precision and accuracy

Precision and accuracy were assessed in conjunction with the linearity studies using spiked plasma samples at each of six concentrations (nominally, 10, 20, 50, 100, 200 and 400 ng/ml) on four different days. The coefficient of variation (CV) was calculated from the ratio of the standard deviation (S.D.) to the mean. Accuracy was comparing the differences between the spiked value and the real concentrations and determined from the bias calculations.

2.6.4. Recovery

Recovery of CTZ from plasma was assessed using spiked blank plasma at three different concentrations (10, 100 and 400 ng/ml) and was estimated by comparing the mean peak ratios of the extracted spiked plasma to the mean peak area ratios of equivalent aqueous standard solutions.

2.6.5. Stability

Stability of CTZ in plasma under conditions of storage and handling relevant to conduct of pharmacokinetic studies (12 h at 4 °C and 21 days at −20 °C) was also investigated.

2.7. Pharmacokinetics of cetirizine in human

2.7.1. Subjects

The protocol of pharmacokinetic study was approved by the Korean Food and Drug Administration. A total of 10 healthy male subjects participated in this study after signing a consent form. The subjects had an age of 24 ± 5 years (22–32 years), body weight of 66 ± 7 kg (58–81 kg) and height of 171 ± 3 cm (164–178 cm). Subjects with a history of drug allergies or idiosyncrasies, renal or hepatic impairment or drug or alcohol abuse were excluded. Subjects who used medications of any kind within 2 weeks of the start or during the study were also excluded.

2.7.2. Drug administration

Subjects were advised not to take any medication for 2 weeks before the study and were requested to fast for at 10 h overnight the day before each treatment. A single oral

dose of a drug containing 5 mg CTZ was administered to 10 healthy volunteers after fasting. The drug was administered with 200 ml of water. Subjects were provided a standard meal at 4 h (lunch) and 8 h (supper) after drug administration in each treatment.

2.7.3. Blood sampling

Blood samples were drawn at 0.0 (pre-dose), and at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h (post-dose). Blood (8 ml) was collected by a syringe into a 15 ml centrifuge tube and immediately separated by centrifugation at 2000–3000 × *g*. Plasma from each sample was removed to clean-labeled vial and stored at −70 °C before analysis.

2.7.4. Pharmacokinetic data and analysis

The following parameters were assessed for the period of 0–24 h: the area under the plasma concentration–time curves from time zero to the last measurable cetirizine sample time (AUC_{0-24h}); maximum plasma concentration (C_{max}); time to reach the C_{max} (T_{max}) were obtained directly from the concentration–time plots of cetirizine.

3. Results and discussion

3.1. Column switching system of cetirizine

To determine the elution profile for the clean-up procedures of plasma, CTZ and IS of 100 ng/ml in extracted blank plasma were analyzed in the pretreatment column using a mobile phase 1. As shown in Fig. 3(A), CTZ and IS were observed around 7 and 8 min, respectively but the separation was not completely obtained. From these results, we devised a program for the analysis of these compounds. In the first step, the interfering endogenous compounds were wasted, then CTZ and IS were introduced to the concentration column from 7.7 to 9.8 min in intermediate column. Finally, CTZ and IS retained on the concentration column were eluted and

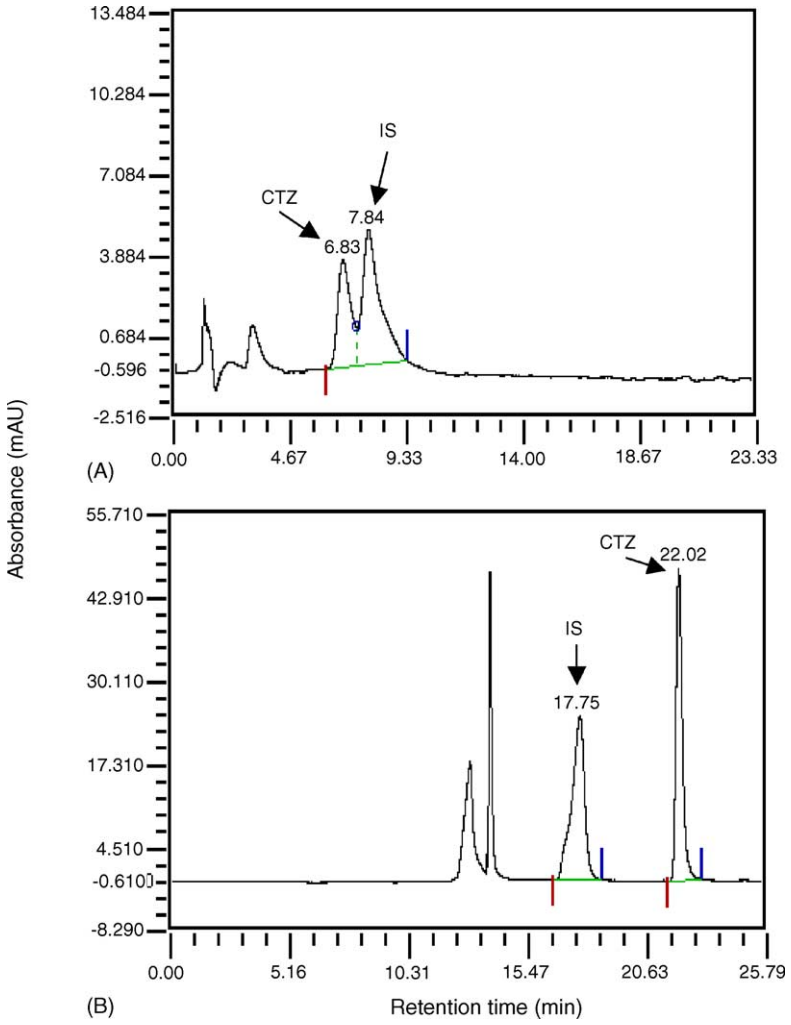

Fig. 3. Chromatograms of cetirizine (CTZ) and methyl paraben as an internal standard (IS); (A) without column switching method and (B) with column switching method.

introduced to the analytical column. Then CTZ and IS in plasma were analyzed using mobile phase 2. Fig. 3(B) shows the chromatogram of CTZ and IS using column switching method.

3.2. Specificity and sensitivity

Using the above determined column switching HPLC, typical chromatograms of CTZ and IS analyzed in human plasma

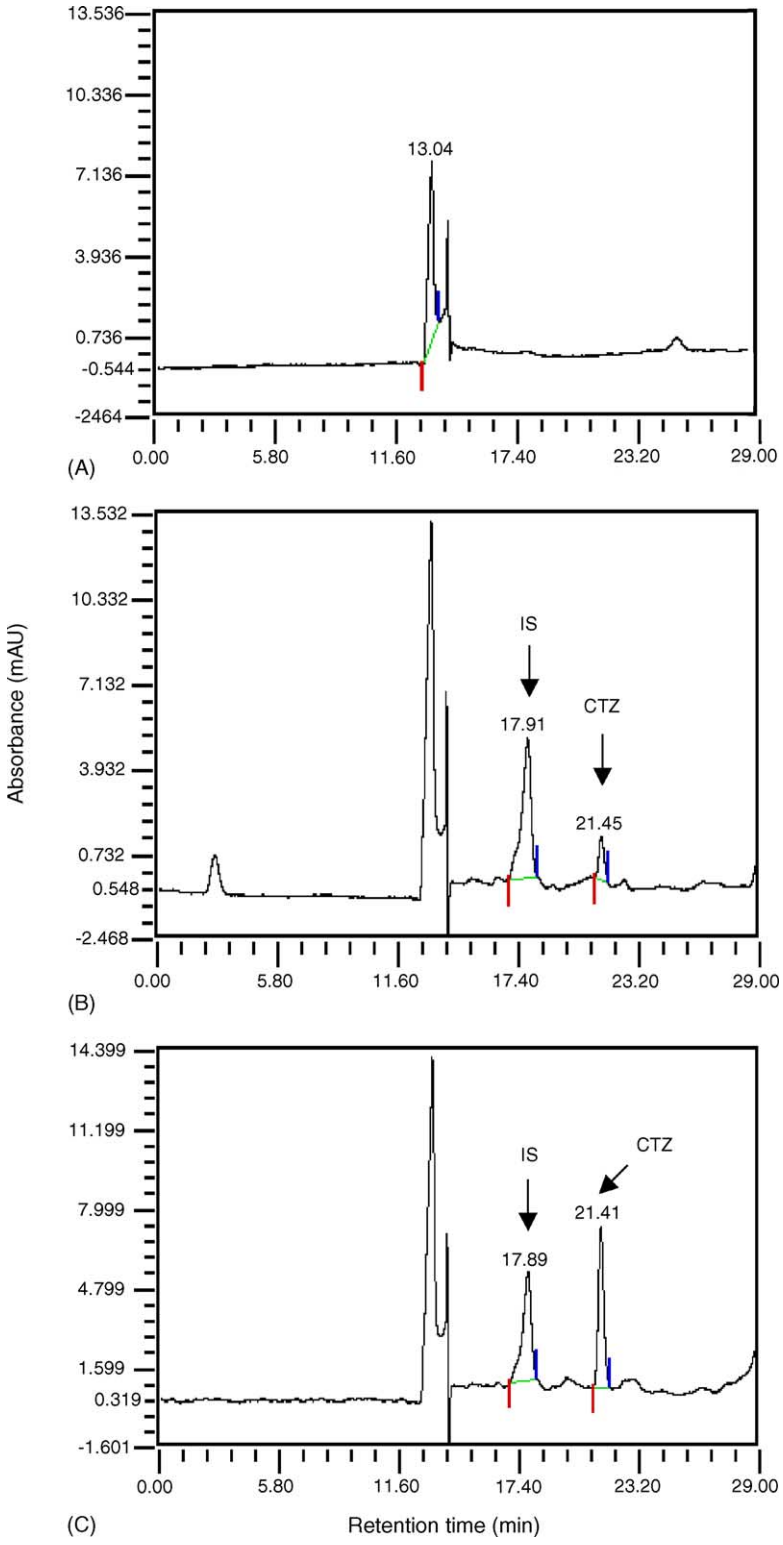

Fig. 4. Chromatograms of (A) blank plasma, plasma sample spiked at (B) 2 ng/ml (LOD) of cetirizine and (C) 50 ng/ml of cetirizine.

were presented in Fig. 4. Under this condition, the retention times for CTZ and IS were 17.9 and 21.4 min, respectively, with complete baseline resolution between peaks of CTZ and IS. Also, no interfering endogenous peaks were detected in chromatogram.

Using this method, the sensitivity of CTZ was good with the limit of determination (LOD) of 2 ng/ml as shown in Fig. 4(B). The LOD had a 2–10 times lower value than that obtained by Baltes et al. [1] including for GC-NPD method and by Moncrieff [3] applying for HPLC with UV detection. This enhancement of detection sensitivity may be obtained by increasing the volume of the plasma sample extracted introduced into precolumn without any additional concentration step.

3.3. Linearity

The assay was linear over the range of 10–400 ng/ml with typical calibration curve of $y = 0.0300(\pm 0.00163)x + 0.8170(\pm 0.187)$ (where y is the cetirizine concentration and x is ratio of peak area) with the correlation coefficient of 0.998(±0.001).

3.4. Precision and accuracy

The intra- and inter-day precision and the accuracy were determined by analyzing plasma samples spiked at 10, 20, 50, 100, 200 and 400 ng/ml. The intra-day precision was determined by analyzing four replicates in the same day. Precision of CTZ calculated as a coefficient of variation (CV) was always below 15% even at LOQ. Accuracy of CTZ expressed as a percentage of the measured concentration to the theoretical concentration ranged from 88.8 to 108.8%. The results of intra- and inter-day precision and accuracy for CTZ in human plasma are provided in Table 2.

3.5. Recovery

Several sample preparation and extraction methods for the LLE of CTZ in biological fluid have been reported. A rapid and efficient LLE of CTZ from plasma was achieved by using methylene chloride as an extraction solvent according to Zaater et al. [7].

Table 2
Precision and accuracy of cetirizine in human plasma ($n = 4$)

Concentration (ng/ml)	Precision (CV, %)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
10	14.5	14.8	88.8 ± 12.2	108.8 ± 4.6
20	10.9	14.9	103.5 ± 11.0	106.0 ± 7.4
50	8.2	7.2	101.8 ± 8.5	99.1 ± 5.2
100	4.2	10.2	101.0 ± 3.8	95.2 ± 5.8
200	10.9	9.0	98.8 ± 3.6	102.0 ± 3.3
400	8.2	5.8	100.2 ± 0.9	99.8 ± 0.6

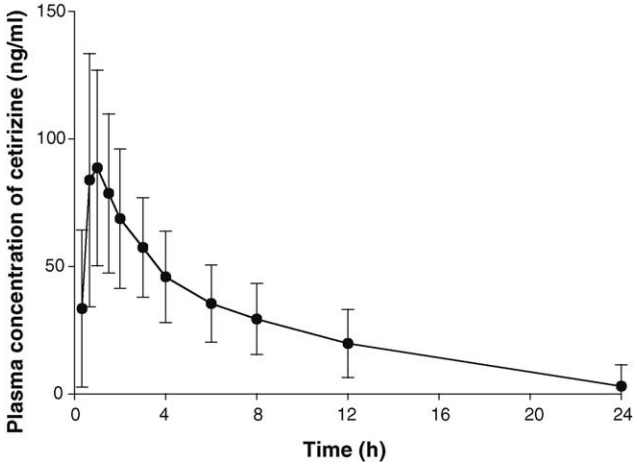

Fig. 5. Mean plasma concentration–time profile of cetirizine in healthy subjects after a single oral administration of 5 mg cetirizine ($n = 10$).

The extraction efficiency of CTZ was evaluated in blank plasma samples and mobile phase spiked with known amounts of CTZ and IS. Plasma samples were extracted as described above and the recovery was calculated by comparing the peak areas ratio of CTZ to IS obtained from the extracted working standard solutions in plasma and those resulting from the direct injection of the working standard solution of CTZ prepared in the mobile phase having the same concentrations. The extraction efficiency of CTZ measured was higher than 80% and it was almost quantitative.

3.6. Stability

CTZ and IS in plasma showed no tendency of degradation at −20 °C for 1 month. Plasma samples spiked with CTZ and IS showed no loss of analytes at 4 °C for 12 h and −20 °C for 21 days.

3.7. Pharmacokinetic application

The present study was applied to the analysis of CTZ in plasma of volunteers after an oral administration of CTZ tablet. Ten healthy volunteers were administered a single oral dose of cetirizine tablet (5 mg). Plasma samples were obtained during 24 h after CTZ administration. Fig. 5 shows the mean (±S.D.) of CTZ plasma concentration–time profiles for 10 volunteers. The area under the curve (AUC_{0-24h}) was 655 ng/ml min. The C_{max} of CTZ was reached at 0.96 h as 106 ng/ml. The pharmacokinetic parameters of CTZ are showed in Table 3. From the results, the analysis of this

Table 3
Pharmacokinetic parameters from 10 healthy subjects after the oral administration of 5 mg cetirizine ($n = 10$)

Parameters	Mean ± S.D.
$AUC_{0-24\,h}$ (ng h/ml)	655.24 ± 261.71
C_{max} (ng/ml)	106.08 ± 42.26
T_{max} (h)	0.96 ± 0.62

column switching HPLC method can be routinely applied to monitoring CTZ in human plasma.

4. Conclusion

We have established a specific and sensitive analytical method for the determination of CTZ in human plasma using column switching HPLC with UV detection. This method has the major advantages of eliminating the interfering compounds in plasma and concentrating analyte. Moreover, this method shows the excellent sensitive and specific results. Therefore, this method has been successfully used to provide the pharmacokinetic study of CTZ in human plasma and can monitor the concentration of CTZ routinely.

Acknowledgement

This work was supported in part by Research Institute of Pharmaceutical Sciences, Seoul National University.

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