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Bioequivalence Evaluation of Two Brands of Cetirizine HCl 10 mg Tablets (Zyrix and Zyrtec) in Healthy Male Volunteers

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ABSTRACT – The purpose of the present study was to evaluate the bioequivalence of two cetirizine HCl tablets, Zyrtec tablet (UCB Pharm. Co., Ltd. Korea, reference product) and Zyrix tablet (Kukje Pharm. Co., Ltd., Korea, test product), according to the guidelines of Korea Food and Drug Administration (KFDA). After adding an internal standard (diazepam), plasma samples were extracted using 1 mL of dichloromethane. Compounds extracted were analyzed by reverse-phase HPLC with ultra-violet detector. This method for determination cetirizine is proved accurate and reproducible with a limit of quantitation of 10 ng/mL in male plasma. Twenty-four healthy male Korean volunteers received each medicine at the cetirizine HCl dose of 10 mg in a 2 × 2 crossover study. There was a one-week wash out period between the doses. Plasma concentrations of cetirizine were monitored for over a period of 24 hr after the administration. AUC (the area under the plasma concentration-time curve) was calculated by the linear trapezoidal rule. C_{max} (maximum plasma drug concentration) and T_{max} (time to reach C_{max}) were compiled from the plasma concentration-time data. Analysis of variance was carried out using logarithmically transformed AUC and C_{max}. No significant sequence effect was found for all of the bioavailability parameters indicating that the crossover design was properly performed. The 90% confidence intervals for the log transformed data were acceptable range of log 0.8 to log 1.25 (e.g., log 0.93-log 1.08 for AUC_{0-t}, log 0.91-log 1.08 for $AUC_{0-\infty}$ and log 1.01-log 1.11 for C_{max}). The major parameters, AUC and C_{max} met the criteria of KFDA for bioequivalence indicating that Zyrix tablet is bioequivalent to Zyrtec tablet.

Key words – Cetirizine HCl, Bioequivalence, Pharmacokinetics, HPLC, Zyrtec, Zyrix

Cetirizine HCl, (2-[2-[4[(4-chlorophenol) phenylmethyl]-1-piperazinyl]ethyloxy] acetic acid HCl), a member of the cyclizine class of compounds is an H_1-receptor antagonist and is an active metabolite of hydroxyzine, a first generation H_1-receptor antagonist.[1] Cetirizine's marked affinity 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 antihistamines.[2] This is owing to the fact that it is highly selective and has less affinity for calcium channel receptor, adrenergic α_1-, dopamine d_2-, serotonin 5-HT_2 receptors and muscarinic receptors than do other common anti-allergic drugs. Cetirizine was characterized by two-compartmental kinetics with a rapid absorption phase (Ka=1.0-1.4 hr^{-1}), a rapid distribution phase (alpha=0.33-0.69 hr^{-1}) and a slower terminal half-life of 13.2-13.6 hr (beta=0.051-0.052 hr^{-1}) after a 10 mg oral administration.[3]

Several methods have been described in the literature for the determination of cetirizine in biological fluids, including high-performance liquid chromatography (HPLC) with UV spectrophotometry[4,5] and LC-MS/MS.[6] In the present paper, we describe a reliable method for quantifying nanograms of cetirizine in plasma using liquid extraction of cetirizine in dichloromethane. The chromatographic conditions were optimized and the results of the validation in terms of linearity, accuracy, precision, recovery, detection, quantitation limits and specificity are provided. The applicability of this method in pharmacokinetic studies is evaluated. Kukje Pharm. Co., Ltd. (Korea) has developed a new formulation of cetirizine tablet: Zyrix 10 mg this study assessed, hence the bioequivalence of this newerly developed formulation with a reference formulation, Zyrtec 10 mg (UCB Pharm. Co., Ltd. Korea) in twenty-four healthy Korean volunteers. Typical bioavailability, including AUC (the area under the plasma concentration-time curve) and C_{max} (the maximum drug concentration) parameters were compared.

Materials and Methods

Materials and reagents

Cetirizine HCl and diazepam (Figure 1) were provided from

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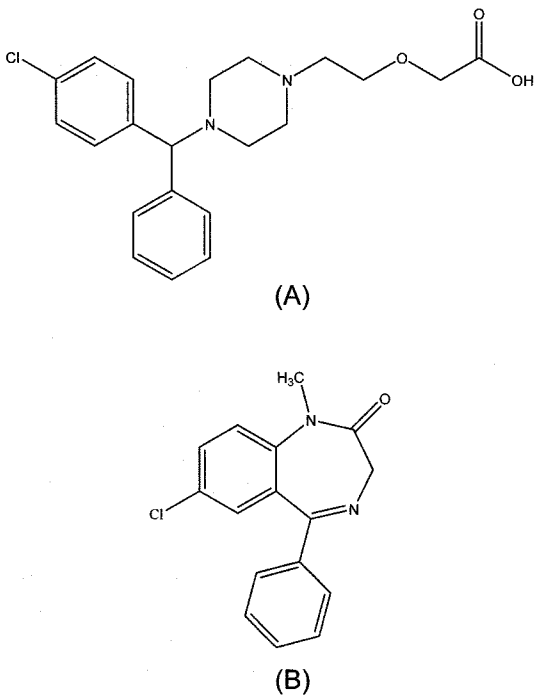

Figure 1–Chemical Structures of (A) cetirizine and (B) diazepam.

Kukje Pharm. Co., Ltd. The solvents, i.e., acetonitrile, dichloromethane and perchloric acid (all first grades) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). A Milli-Q® (Millipore Co., Milfore, MA, USA) water purification system was used to obtain the purified water used for the HPLC analysis. All other chemicals and solvents were of the highest analytical grade available. The test product, Zyrix (10 mg cetirizine HCl, Kukje Pharm. Co., Ltd., Korea) and the reference product, Zyrtec (10 mg cetirizine HCl, UCB Pharm. Co., Ltd. Korea) were supplied in the form of tablets.

Calibration standards

The primary stock solution of cetirizine were prepared up at 1000 μg/mL in water and stored at -70°C. The internal standard stock solution was in water producing a concentration of 10 μg/mL. Cetirizine stock solution was serially diluted with water to obtain concentration of 100, 200, 500, 1000, 2000 and 5000 ng/mL. These standard solutions were employed for the preparation of calibration graphs.

Selection of volunteers

The study population consisted of twenty-four healthy male Korean volunteers with an average age of 22.17 years and an average weight of 68.46 kg. The volunteers were selected after passing a clinical screening procedure including a physical examination and laboratory tests (blood analysis; hemoglobin, hematocrit, WBC, platelet, differential counting of WBC, blood urea nitrogen, total bilirubin, cholesterol, total protein, albumin, alkaline phosphatase, glucose fasting, sGOT, and sGPT, urine analysis; specific gravity, color, pH, sugar, albumin, bilirubin, RBC and cast). The volunteers were excluded if there was any possibility of their being sensitive to this type of medication, had a history of any illness of the hepatic, renal or cardiovascular systems, or a history of excessive alcohol intake or other medications. This was done to ensure that the existing degree of variation would not be due to an influence of illness or other medications.

Prescribe for volunteers and sample collection from volunteers

All of the volunteers avoided using other drugs for at least one week prior to the study and until its completion. They also refrained from consuming xanthine-containing foods, alcoholic beverages and other beverages for 48 hr prior to each dosing and until the collection of the last blood sample. Each volunteer received an oral dose of 10 mg of cetirizine HCl in a standard 2 × 2 cross-over model in a randomized order. Half life of cetirizine HCl dose 10 mg was reported 7.95 ± 0.45 hr,[4] and then we had a one week washout period between the doses. All of the participants signed a written consent form after they had been informed of the nature and details of the study in accordance with the Korean Guidelines for Bioequivalence Test.[7] The subjects were hospitalized (Kyung Hee Medical Center, Seoul, Korea) at 10:00 p.m. on the eve of the study and fasted overnight (10 hr) and 4 hr after each drug administration. At 7:00 a.m., their median cubital vein was cannulated and 7 mL blood samples were drawn into heparinized tubes. The doses were taken at 8:00 a.m. on each dosing day along with 240 mL of water. At 4 hr after the oral administration, all of the subjects were given standardized meals. The subjects were not allowed to remain in the supine position or to sleep until 8 hr after the oral administration. Approximately 7 mL blood samples were collected via the cannula at the following times; predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hr after the administration. On each occasion, the blood sample was centrifuged immediately, and this sample was frozen at -70°C until the HPLC analysis.

Sample preparation for HPLC injection

10 μL of internal standard working solution (diazepam 10 μg/mL) was added to 1 mL plasma sample. The samples were vortexed briefly for 2 min and 1 mL of dichloromethane was added. This mixture was shaken and centrifuged at 3,000 rpm for 10 min. The organic layer was separated and evaporated to

dryness at 40°C in Speed-Vac (Holbrook instruments INC., USA). The final residue was reconstituted into 200 μL of mobile phase, vortexed again for 10 min and then centrifuged at 15,000 rpm for 20 min. The resulting clear supernatant from each sample was transferred to an autosampler vial and a 100 μL aliquot was injected into the HPLC system.

HPLC Analysis and validation of method

All plasma samples were analyzed for cetirizine concentration according to a sensitive, selective and accurate high-performance liquid chromatography (HPLC) method, which was developed and validated.

The chromatographic system consisted of a Millipore Waters HPLC system: 515 Pump, 717 plus Autosampler, 486 Tunable Absorbance Detector, Temperature Control Module and controlled by Waters Empower Software. The separation was achieved on a Thermo Hypersil GOLD (250 × 4.5 mm i.d., 5 μm) reversed-phase column at 35°C. The mobile phase consisted of 13 mM dihydro-sodium phosphate (pH 2.8 with perchloric acid) and acetonitrile (61:39, v/v). The mobile phase was eluted at a flow rate of 1 mL/min. Ultra-violet (UV) detection of cetirizine and internal standard was performed at 230 nm. The autosampler was controlled at 4°C. The peak height was measured, and the peak height ratio of the drug to the internal standard and the concentration were calculated. All samples from each single volunteer were measured on the same day in order to avoid inter-assay variation.

The standard solutions over the range of 10-500 ng/mL using 1 mL plasma samples were employed for the preparation of calibration curves. In order to assess the intra-day coefficient of variation (CV) and accuracy of the method, five calibration curves were obtained in one day. The precision and accuracy for inter-day assay were assessed at the same concentration and repeated for five different days.

Statistical analysis of pharmacokinetic parameters

Non-compartmental analysis was performed to estimate pharmacokinetic parameters of cetirizine HCl. C_{max} (maximum plasma drug concentration) and T_{max} (time to reach C_{max}) were obtained directly from the data, without interpolation. AUC_{0-t} (area under the plasma concentration versus time curve from time zero-pre-dose-to time of last quantifiable concentration) was calculated using the linear and logarithmic trapezoidal rule. The terminal first order constant (Ke) was determined by a least squares fit of the terminal plasma concentrations. The constant Ke was used to extrapolate $AUC_{t-\infty}$ (area under the plasma concentration versus time curve from time of last quantifiable concentration to infinite). $AUC_{0-\infty}$ (area under the plasma concentration versus time curve from time zero-pre-dose-extrapolated to infinite time) is obtained from AUC_{0-t} plus $AUC_{t-\infty}$.[8]

Primary PK parameters required in assessing bioequivalence were evaluated by PK software WINNONLIN® standard version 3.1 software[9] and K-BE test for windows.[10] Bioequivalence between the products was determined by calculating 90% confidence intervals (90% C.I.) for the ratio of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values for the test and reference products, using logarithmic transformed data. Analysis of variance (ANOVA) was used to assess group and period effects.

Results and Discussion

Unexpected incidents that could have influenced the outcome of the study did not occur. There was no drop-out and all volunteers who started the study continued to the end and were discharged in good health. Both products were well tolerated, with no reported adverse events.

Cetirizine and diazepam (I.S.) were well separated from the biological background under the described chromatographic conditions at retention times of 9.5 and 14.8 min, respectively (Figure 2). The peaks were good shape, completely resolved one. No interference with constituents from plasma matrix was observed. The mobile phase used guaranteed good repeatability of retention times.

Under the validation conditions described, the lower limit of quantification from 1 mL plasma was 10 ng/mL for cetirizine. The relationship between the concentration and peak height ratio was found to be linear within the range 10-500 ng/mL for cetirizine. The intra-day accuracy of the method for cetirizine ranged from 97.3% to 112.0% while precision ranged from 10.6% to 13.6%. The inter-day accuracy of the method for cetirizine ranged from 98.0% to 104.0% while precision ranged from 5.7% to 11.0% (Table I). The mean recoveries of the 10, 100 and 500 ng/mL levels were 90.2, 89.7 and 98.1%, respectively. To evaluate cetirizine stability in human plasma, drug-free plasma samples were spiked at 10, 100 and 500 ng/mL. After extraction, samples were arranged in the autosampler and analyzed. In the short-term stability study, cetirizine was founded to be stable for 24 hr at 4°C and room temperature. In the long-term stability study, the plasma samples spiked with cetirizine also showed no loss of analyte when they were stored for two months at -70°C. The final stability test was demonstrated after three freeze-thaw cycles. No significant deterioration of the analyte was observed under any of these conditions. The proposed method used in this study was found to be reliable, accurate, sensitive and rapid for detecting

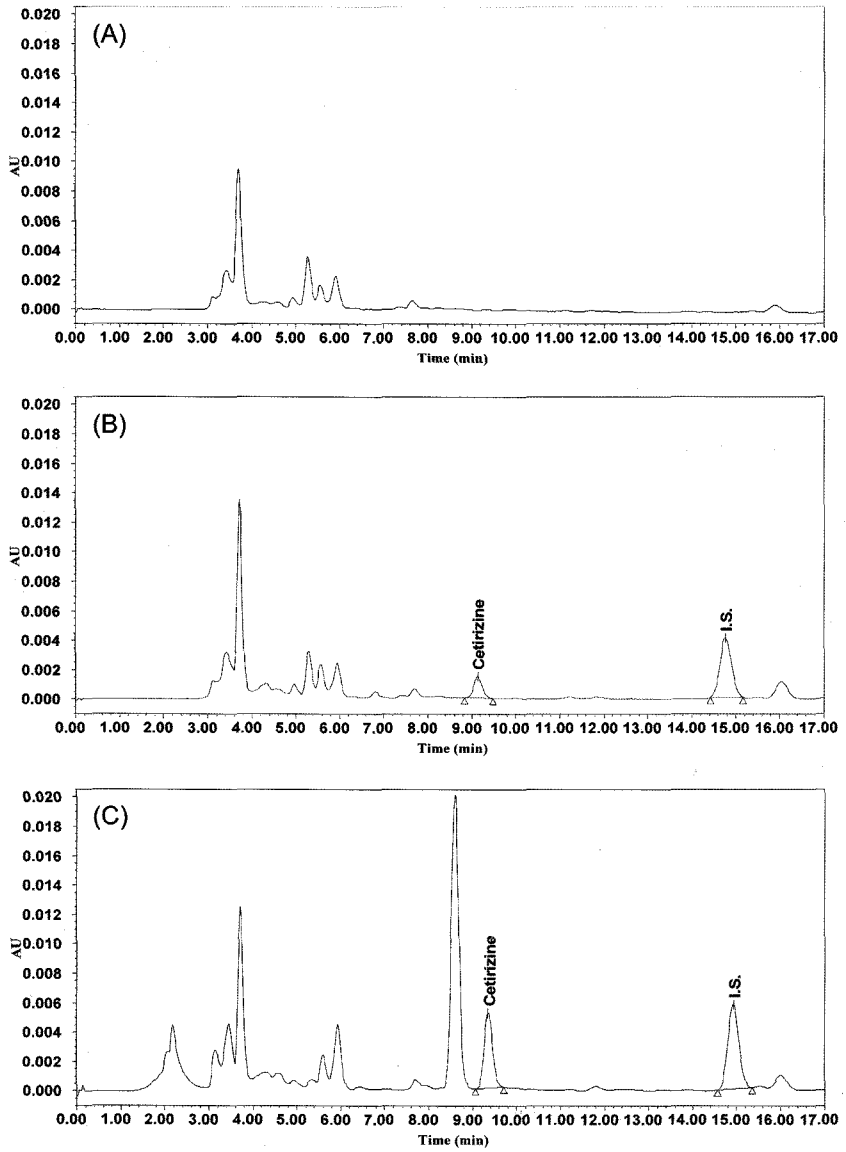

Figure 2–Chromatogram of (A) blank human plasma, (B) plasma spiked with cetirizine (100 ng/mL) and internal standard, and (C) plasma from a volunteer 3 hr after an oral administration of 10 mg cetirizine HCl.

Table I–*Precision and Accuracy for the Determination of Cetirizine in Human Plasma (n = 5)*

Normal Concentration (ng/mL)	Precision (%)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
10	10.6	11.0	112.0	104.0
20	12.6	9.2	100.0	98.3
50	10.7	8.1	97.3	98.0
100	13.6	7.9	98.6	102.0
500	11.5	5.7	99.3	100.4

plasma levels.

After validation, about 600 volunteer's plasma samples (twenty four individuals, twelve time intervals and two period) were processed with HPLC. The mean concentration-time profile of cetirizine for the two products is shown in Figure 3. The figures indicate that the mean plasma concentration profiles of the two cetirizine HCl products were closely similar and overlaped. Peak concentrations of 301.02 ng/mL (test) and 285.38 ng/mL (reference) for cetirizine were attained at 1.0 hr equally, after drug administration and then declined broadly and were detectable up to end time point (24 hr).

Table II shows the average values of pharmacokinetic parameters after administration of two brands of cetirizine HCl. The extent of absorption is a key characteristic of drug formulation and, therefore AUC is an important parameter for

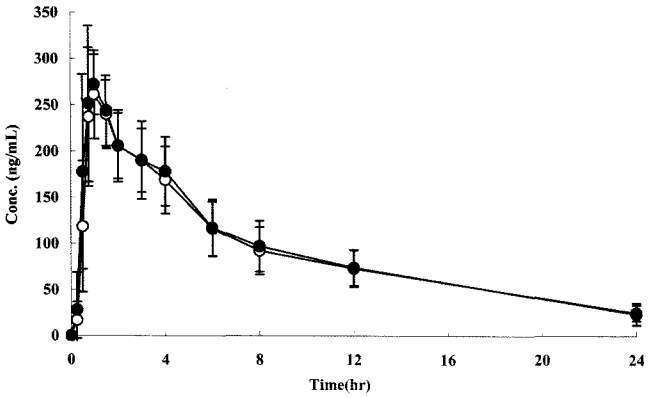

Figure 3–Mean (± S.D., n=24) plasma concentration-time curves of cetirizine following oral adminstration of Zyrtec (○) and Zyrix (●) tablet at the dose of 10 mg of cetirizine HCl.

comparative bioequivalence study.[11] However, the other two parameters, C_{max} and T_{max}, are also important features and could affect the therapeutic behavior of a drug[12] and hence were also considered in the study. The relative bioavailability on the basis of the cetirizine was 93.5% to 107.8% for AUC_{0-t}, 91.4% to 107.6% for $AUC_{0-\infty}$ and 100.9% to 110.7% for C_{max}. The estimate value of AUC_{0-t} being > 80% of the estimate value of $AUC_{0-\infty}$ implied that the sampling scheme was sufficiently long to ensure adequate description of the absorption phase.

Pharmacokinetic studies of cetirizine HCl have been

Table II–*Pharmacokinetic Parameters of Cetirizine HCl 10 mg Tablets (mean±S.D., n=24)*

Pharmacokinetic Parameters	Zyrix (test)	Zyrtec (reference)
AUC_{0-t} (ng·hr/mL)	2115.9 ± 523.5	2228.89 ± 740.3
$AUC_{0-\infty}$ (ng·hr/mL)	2400.8 ± 666.6	2368.6 ± 492.9
C_{max} (ng/mL)	301.0 ± 36.6	285.4 ± 39.1
T_{max} (hr)	1.0 ± 0.3	1.1 ± 0.5
$T_{1/2}$ (hr)	8.06 ± 1.3	7.82 ± 1.3
Ke (hr^{-1})	5.47 ± 4.3	6.35 ± 3.5

Table III–*Bioavailability Parameters in Normal and Logarithmic Scales for Each Subject Obtained after Oral Administration of Zyrtec and Zyrix Tablets at the Cetirizine HCl Dose of 10 mg*

Subjects	Zyrtec Tablet					Zyrix Tablet				
	AUC_{0-t} (ng·hr/mL)	Ln AUC_{0-t}	C_{max} (ng/mL)	Ln C_{max}	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	Ln AUC_{0-t}	C_{max} (ng/mL)	Ln C_{max}	T_{max} (hr)
A1	1780	3.25	264	2.42	1.00	1701	3.23	246	2.39	1.00
A2	1754	3.24	319	2.50	0.75	1884	3.28	313	2.50	0.75
A3	2176	3.34	374	2.57	0.75	1907	3.28	353	2.55	0.50
A4	2040	3.31	251	2.40	1.50	2245	3.35	280	2.45	1.00
A5	2520	3.40	306	2.49	1.50	2360	3.37	292	2.47	1.00
A6	1511	3.18	216	2.34	0.75	2703	3.43	349	2.54	0.75
A7	2066	3.32	231	2.37	1.00	1840	3.26	250	2.40	0.75
A8	2261	3.35	321	2.51	0.75	2170	3.34	327	2.52	0.75
A9	3053	3.48	324	2.51	3.00	3216	3.51	322	2.51	2.00
A10	2348	3.37	278	2.45	1.50	2370	3.37	279	2.45	0.75
A11	1853	3.27	262	2.42	0.75	1060	3.03	260	2.42	1.00
A12	2082	3.32	245	2.39	1.00	2206	3.34	243	2.39	1.50
B1	1993	3.30	321	2.51	0.50	2571	3.41	322	2.51	0.75
B2	2498	3.40	334	2.52	1.00	2641	3.42	334	2.52	1.50
B3	1432	3.16	278	2.45	1.00	1185	3.07	302	2.48	1.00
B4	2392	3.38	263	2.42	1.50	2399	3.38	281	2.45	1.00
B5	2524	3.40	314	2.50	0.75	2405	3.38	318	2.50	0.75
B6	1534	3.19	273	2.44	0.75	1342	3.13	271	2.43	1.00
B7	2019	3.31	318	2.50	1.00	2056	3.31	360	2.56	0.75
B8	1682	3.23	277	2.44	0.75	1706	3.23	257	2.41	1.50
B9	2188	3.34	290	2.46	2.00	2796	3.45	359	2.56	1.00
B10	1859	3.27	286	2.46	0.75	1688	3.23	315	2.50	0.75
B11	2591	3.41	278	2.44	1.00	2480	3.39	277	2.44	1.00
B12	1660	3.22	215	2.33	1.00	1842	3.27	303	2.48	0.50
Mean	2076.04	3.31	285.38	2.45	1.09	2115.91	3.31	301.02	2.48	0.97
(S.D.)	400.15	0.08	39.07	0.06	0.54	523.49	0.12	36.60	0.05	0.35

Table IV–*Statistical Results of Bioequivalence Evaluation between Two Cetirizine HCl Tablets*

	Parameters		
	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
Difference (%)	1.92	1.36	5.48
F value[a)]	0.15	0.42	0.68
Test/Ref estimate	1.00	0.99	1.06
90% C.I.	93.5%-107.8%	91.4%-107.6%	100.9%-110.7%

#The AUC and C_{max} values were calculated on the basis of log transformed data.
a)α=0.05 F(1,22)=4.301.

reported previously.[4-6] The one was assayed plasma cetirizine concentrations using a HPLC-UV method, and reported the following pharmacokinetic parameters: AUC_{0-t} 3382 ± 420 ng·hr/mL and C_{max} 286 ± 32 ng/mL after a single oral dose of 10 mg cetirizine HCl tablet.[4] The other was assayed plasma cetirizine concentrations using LC-MS/MS after protein precipitation and reported the following pharmacokinetic parameters: AUC_{0-t} 2714 ng·hr/mL and C_{max} 302 ng/mL after a single oral dose of 10 mg cetirizine HCl tablet.[6] In our study, AUC_{0-t}, and C_{max} for cetirizine were 2076.04 ± 400.15 ng·hr/mL (Zyrtec) and 2115.91 ± 523.49 ng·hr/mL (Zyrix), 285.38 ± 39.07 ng/mL (Zyrtec) and 301.02 ± 36.60 ng/mL (Zyrix), respectively (Table III), which were a little different to the results of other previous studies. These differences might be regarded as ethnic or factorial factor.

No significant sequence effect was found for all of the bioavailability parameters indicating that the cross-over design was properly performed. Geometric means of the parameters (Table IV) are given for the test and reference products separately for each period and as combined estimates. The difference of the test product/mean of the reference product for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were 1.92%, 1.36% and 5.48%, respectively. No significant period effect in AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} was detected in this study. Ninety percent confidence intervals also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two products lie within the KFDA acceptable range of 80%-125%.[13]

The results of this study suggest equivalent clinical efficacy of the two brands of cetirizine.

Conclusions

It was shown that this method is suitable for the analysis of cetirizine in human plasma samples collected for bioequivalence studies. Using this method, the bioequivalence of two different 10 mg cetirizine HCl tablet products was examined in twenty-four healthy normal male volunteers. The statistical analysis results based on comparisons of the two pivotal parameters (AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}) demonstrated the bioequivalence of these two tablet products of cetirizine HCl.

Acknowledgements

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