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# Bioequivalence Evaluation of Two Brands of Lisinopril Tablets (Lisotec and Zestril) in Healthy Human Volunteers

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**ABSTRACT:** The bioequivalence of two brands of lisinopril 20 mg tablets was demonstrated in 28 healthy human volunteers after a single oral dose in a randomized cross-over study, conducted at ACDIMA Center for Bioequivalence and Pharmaceutical Studies, Amman, Jordan. Reference (Zestril, AstraZeneca, UK) and test (Lisotec, Julphar, UAE) products were administered to fasting volunteers on 2 treatment days separated by a 2-week washout period; blood samples were collected at specified time intervals, and the plasma was separated and analysed for lisinopril using a validated LC-MS/MS method at ACDIMA Laboratory. The pharmacokinetic parameters $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{MAX}$, $T_{MAX}$, $T_{1/2}$ and the elimination rate constant were determined from the plasma concentration-time profiles for both formulations and were compared statistically to evaluate bioequivalence between the two brands, using the statistical modules recommended by the FDA. The analysis of variance (ANOVA) did not show any significant difference between the two formulations and 90% confidence intervals fell within the acceptable range for bioequivalence. Based on these statistical inferences it was concluded that the two brands exhibited comparable pharmacokinetic profiles and that Julphar's Lisotec is bioequivalent to Zestril of AstraZeneca, UK. Copyright © 2005 John Wiley & Sons, Ltd.

**Key words:** lisinopril; bioequivalence; pharmacokinetics; HPLC; Julphar

## Introduction

Lisinopril, a synthetic peptide derivative, is a long-acting angiotensin converting enzyme inhibitor, which has been shown to reduce blood pressure in patients with mild to moderate hypertension [1,2]. Lisinopril is chemically described as (S)-1-[$N_2$-(1-carboxy-3-phenypropyl)-L-lysyl]-L-proline dihydrate [3,4].

Lisinopril produces greater systolic and diastolic blood pressure reductions compared with hydrochlorothiazide and does not produce hypokalemia, hyperglycemia, hyperuricemia or hypercholestrolemia [5,6]. It is indicated for the treatment of hypertension either alone as initial therapy or concomitantly with other classes of antihypertensive agents [3,6].

Following oral administration of lisinopril, peak serum concentrations of lisinopril occur within about 7 h [6]. Its bioavailability ($\cong$25%) is not significantly affected by food, age or the co-administration of hydrochlorothiazide [5]. It is slightly bound to plasma proteins ($<$10%) and the volume of distribution is 124 l, and it does not undergo metabolism and is excreted unchanged entirely in the urine [6,7]. Based on urinary recovery, the mean extent of absorption is approximately 29% [8]. Upon multiple dosing, it exhibits an effective half-life of accumulation of 12 h [3,6]. Though many pharmacokinetics studies for lisinopril have been reported [2,5,7,8,9] very few [10] of them have focused

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on bioequivalence between two market brands. The present study demonstrated the bioequivalence of two market brands available in the Middle-East region.

The purpose of this study was to determine the bioequivalence of a new tablet formulation of lisinopril (Zestril 20 mg tablets) produced in United Arab Emirates by Gulf Pharmaceutical Industries-Julphar, in comparison with Zestril from AstraZeneca, UK.

## Materials and Methods

### *Study products*

| | |
|---|---|
| **Test Product**: | Lisotec – lisinopril 20 mg tablets |
| Batch No.: | 0008 Manufacturing date: 09/2003; Expiry date: 09/2005 |
| Manufacturer: | Gulf Pharmaceutical Industries-Julphar, UAE |
| **Reference Product**: | Zestril - lisinopril 20 mg tablets |
| Batch No.: | BR 067 Manufacturing date: 10/2002; Expiry date: 10/2006 |
| Manufacturer: | AstraZeneca, UK |

### *Study design*

Twenty-eight healthy adult volunteers participated in this comparative study at Ibn Al-Haytham Hospital, Amman, Jordan, as a joint venture with ACDIMA. Their mean age was $27.9 \pm 6.1$ years with a range of 18–42 years; the mean body weight was $73.4 \pm 6.4$ kg with a range of 61–87 kg and the mean height was $171.6 \pm 5.2$ cm with a range of 163–182 cm. The volunteers did not have any significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal or hematological diseases, as determined by their medical history, physical examination and routine laboratory tests (hematology, blood biochemistry and urine analysis) and were negative for hepatitis B antigen. Subjects were instructed to abstain from taking any drug including over-the-counter (OTC) for 2 weeks prior to and during the study period. They were informed about the aim and risks of the study by the clinical investigator, based on which they signed a written informed consent statement before entering the study. The study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of Good Clinical Practices. Before the start of the study the protocol was approved by the Institutional Review Board (IRB) of Ibn Al-Haytham Hospital.

### *Drug administration and sample collection*

The study was designed as a single dose, randomized, two treatment, two period crossover. In the morning of phase I, after an overnight fast (12 h) volunteers were given a single dose of either formulation (reference or test) of lisinopril 20 mg with 240 ml of water. No food was allowed until 4 h after dose administration. Water intake was allowed after 2 h of dose; water, lunch and dinner were given to all volunteers according to a time schedule. The volunteers were continuously monitored by Ibn Al-Haytham Hospital staff throughout the confinement period of the study. They were not permitted to lie down or sleep for the first 4 h after the dose. Approximately 7 ml blood samples were drawn into heparinized tubes through an indwelling cannula before (0 h) and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 24, 36, 48, 60, 72, 96, 120, 144 and 168 h after dosing. Blood samples were centrifuged at 4000 rpm for 5 min, plasma was separated and kept frozen at $-70^{\circ}$C until assayed. After a washout period of 14 days the study was repeated in the same manner to complete the crossover design.

### *Sample preparation for HPLC injection*

Extraction from plasma was accomplished by solid phase extraction (SPE); 25 µl of internal standard (enalapril 6 µg/ml) was added to the 1 ml plasma. 1.6 ml of hydrochloric acid was added to the plasma sample and the mixture was dispensed slowly into the SPE cartridge (the column was previously conditioned with 2 ml methanol and then twice with 2 ml water) by blowing the column with nitrogen. The SPE columns were washed five times with 2 ml of 0.037% hydrochloric acid. The extracts were

eluted into clean test tubes with 1 ml methanol followed by another 0.5 ml of methanol, then evaporated to dryness under nitrogen at 40°C. The residue was reconstituted with 100 µl of pure deionized water and 25 µl was injected into the HPLC column, where lisinopril and internal standards were separated from endogenous plasma substances.

### *Chromatographic conditions*

The plasma samples were analysed for lisinopril by a validated LC-MS-MS method. All solvents used were of HPLC grade and were purchased from Acros, Belgium; while other chemicals and reagents were of analytical grade. Lisinopril and internal standard were obtained from Julphar, UAE.

The LC-MS-MS consisted of a Waters 600 quaternary pump (Milford, USA), a Waters 717 autosampler (Milford, USA), Applied Biosystems API 3000 mass spectrometer detector (MDS Sciex, Canada); integration was done using Applied Biosystems Analyst® 1.4 software (MDS Sciex, Canada). Chromatographic separation was performed using Cyano Zorbax (250 × 4.6 mm, 5 µm) column from Waters, USA. The mobile phase consisted of 0.4% trifluoroacetic acid and acetonitrile (43:57 v/v) and eluted at a flow rate of 1.0 ml/min (a splitter was used after the column resulting in a final flow rate of 0.7 ml/min to the detector); the oven temperature was set at 50°C. Lisinopril was detected at *m/z* of 406.3 and MS/MS daughter at *m/z* of 84.1, while an internal standard (enalapril) was detected at *m/z* of 377.0 and MS/MS daughter at *m/z* of 234.4. The peak area was measured, and the peak area ratio of the drug to the internal standard and the concentration were calculated by the software. The method was validated following international guidelines [11].

### *Pharmacokinetic analysis*

Pharmacokinetic analysis was performed by means of model independent method using KineticaTM 2000 computer program [12]. The elimination rate constant ($\lambda_Z$) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life ($T_{1/2}$) was calculated as $0.693/\lambda_Z$. The area under the curve to the last measurable concentration ($AUC_{0-t}$) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t}+C_t/\lambda_Z$, where $C_t$ is the last measurable concentration.

### *Statistical analysis*

For the purpose of bioequivalence analysis $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ were considered as primary variables. Two-way analysis of variance (ANOVA GLM procedure; KineticaTM 2000 Computer program [12]) for crossover design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. The difference between two related parameters was considered to be statistically significant values of $p \leqslant 0.05$. Parametric 90% confidence intervals [13] based on the ANOVA of the mean test/reference (T/R) ratios of *AUCs* and $C_{max}$ were computed.

## Results and Discussion

Lisinopril was well tolerated by all volunteers; 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.

Under the described conditions, the lower limit of quantitation from 1 ml plasma was 1.0 ng/ml for lisinopril. The relationship between the concentration and the peak area ratio was found to be linear within the range 1.0–300 ng/ml. The intra-day accuracy of the method for lisinopril ranged from 100.3% to 106.4%, while the intra-day precision ranged from 6.65% to 10.90%. The inter-day accuracy ranged from 100.28% to 103.24%, while the inter-day precision ranged from 5.74% to 9.21%. The absolute recovery was 102.0% while the relative recovery ranged from 91.89% to 110.67%. The stability study showed that lisinopril was stable in plasma for 3 months when stored at −70°C. The method used in this study was found to be reliable, accurate, sensitive and rapid for detecting plasma levels of lisinopril.

Both formulations were readily absorbed from the gastrointestinal tract and lisinopril was measurable at the first sampling time (1.0 h) in all volunteers. The mean concentration-time profile of lisinopril for the two formulations is shown in Figure 1. The figure indicates that the mean plasma concentration profiles of the two brands were closely similar and superimposable. Peak concentrations of 79.8 ng/ml and 78.5 ng/ml were attained at 6.5 and 7.0 h, respectively, after drug administration and then declined rapidly and was detectable up to 72 h only.

Table 1 shows the pharmacokinetic parameters of lisinopril for two brands. The extent of absorption is a key characteristic of drug formulation and, therefore *AUC* is an important parameter for comparative bioavailability studies [14]. However, the other two parameters, $C_{max}$ and $T_{max}$, are also important features and could affect the therapeutic behavior of a drug [15] and hence were also considered in the study. The relative bioavailability of Lisotec was 105.0% for $AUC_{0-t}$, 104.0% for $AUC_{0-\infty}$ and 102.0% for $C_{max}$.

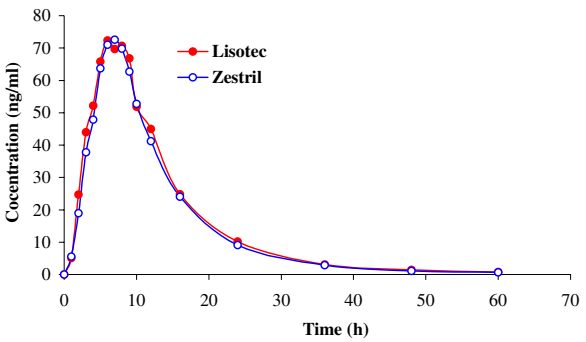


Figure 1. Mean plasma concentration of lisinopril after oral administration of single dose of two brands to 28 healthy human volunteers

The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent [16]. To demonstrate bioequivalence certain limits should be set depending on the nature of the drug, the patient population and the clinical end points. It is generally accepted that for basic pharmacokinetic characteristics, such as $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ the standard equivalence range is 0.8–1.25 [13]. The results of statistical analysis are shown in Table 1.

The mean and standard deviation of $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ of the two products did not differ significantly, suggesting that the blood profiles generated by Lisotec are comparable to those produced by Zestril. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations, with a value of $p>0.05$. 90% confidence intervals also demonstrated that the ratios of $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ of the two formulations lie within the FDA acceptable range of 80%–125%. The results are shown in Table 1.

For $T_{max}$ the parametric point estimate of difference (test−reference) was −0.50 h, and found to be within the acceptance limits ± 20% of the reference mean.

Plasma levels may be used as surrogate parameters for clinical activity; therefore the results of this study suggest comparable clinical efficacy of the two brands of lisinopril.

Table 1. Pharmacokinetic parameters of lisinopril for two brands (mean ± standard deviation, $n = 28$) and statistical analysis of ln-transformed data of lisinopril

| Pharmacokinetic parameter | Lisotec (test) | Zestril (reference) | Statistical analysis | |
|---|---|---|---|---|
| | | | ANOVA | 90% CI |
| $AUC_{0-t}$ (μg/ml.h) | 992.8 ± 520.5 | 948.3 ± 531.8 | 0.6108 (0.3812) | 88.9%–124.5% |
| $AUC_{0-\infty}$ (μg/ml.h) | 1016.3 ± 520.0 | 974.4 ± 532.7 | 0.6365 (0.3749) | 88.9%–123.4% |
| $C_{max}$ (μg/ml) | 79.8 ± 39.4 | 78.5 ± 45.1 | 0.5204 (0.4195) | 90.7%–124.3% |
| $T_{max}$ (h) | 6.5 ± 1.7 | 7.0 ± 1.39 | | |
| $T_{1/2}$ (h) | 10.1 ± 4.5 | 10.6 ± 6.4 | | |

Parenthesis values indicate analysis for periods.

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