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ORIGINAL ARTICLE

# BIOEQUIVALENCE STUDIES OF TWO IRANIAN GENERIC FORMULATIONS OF CAPTOPRIL IN HEALTHY VOLUNTEERS

Seyed-Mohsen Foroutan PhD*•, Afshin Zarghi PhD*, Ali-Reza Shafaati PhD*, Arash Khoddam PharmD**

**Department of Pharmaceutical Chemistry, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, **Noor Research and Educational Institute, Tehran, Iran*

**Background and Objective** – Captopril is a widely used antihypertensive drug and is formulated by several pharmaceutical companies in Iran. This study was conducted to compare the bioavailability of two captopril formulations with reference products of the same strength in healthy volunteers.

**Methods** – The relative bioavailability of captopril was determined in two single-blind, single dose, randomized, crossover, two-phase studies. The relative bioavailability of the test product (first study: generic captopril 25 mg tablets; second study: generic captopril 50 mg tablets) with respect to the reference product (first study: Capoten® 25 mg tablets; second study: Capoten® 50 mg tablets, Bristol-Myers Squibb) was determined. Twelve healthy volunteers in two groups took part in these studies and took either the test or reference tablets in the first phase and received the other tablet in the second phase of each study.

**Results** – In the first study, the mean values for the variable peak plasma concentration ($C_{max}$) were 459.8 ng/mL for the reference and 466.6 ng/mL for the test product. The mean values for the variable area under the curve ($AUC_{0-t}$) were 1392.5 ng.hr/mL and 1403.2 ng.hr/mL for the reference and test product, respectively. In the second study, the mean values for the variable $C_{max}$ were 535.5 ng/mL for the reference and 517.2 ng/mL for the test product. The mean values for the variable $AUC_{0-t}$ were 1518.8 ng.hr/mL and 1444.5 ng.hr/mL for the reference and test product, respectively. The 90% confidence intervals for the test/reference mean ratios of $C_{max}$ and $AUC_{0-t}$ were between 0.90 and 1.19, which is within the conventional bioequivalence range of 80 – 125%.

**Conclusion** – The test products were bioequivalent to the reference (Capoten®) in terms of the rate and extent of absorption of captopril in both 25 mg and 50 mg strengths.

**Keywords** bioequivalence captopril tablet HPLC human serum



## Introduction

Captopril is a widely used antihypertensive drug that inhibits angiotensin-converting enzyme (ACE, kinase II), the enzyme that converts angiotensin I to angiotensin II and may also reduce the degradation of bradykinin. It is used in the management of hypertension, in heart failure, following myocardial infarction, and in diabetic nephropathy.[1] About 60 – 75% of a dose of captopril is absorbed from the gastrointestinal tract and peak plasma concentrations are achieved within about one hour. Captopril is largely excreted in the urine, 40 to 50% as unchanged drug, the rest as disulphide and other metabolites. The elimination half-life has been reported to be 1 to 3 hours. Excellent reviews provide more details on the pharmacokinetics of captopril and bioavailability of its tablets[2 – 4] and the clinical pharmacokinetics of captopril and other ACE inhibitors.[5]

Bioequivalence studies on generic drug products manufactured in Iran have been conducted by the Ministry of Health and Medical Education to assure high quality of the drug products available in Iran. The aim of this work, as

• **Correspondence:** *S. M. Foroutan PhD, Department of Pharmaceutical Chemistry, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran. P.O. Box: 14155-6153.*

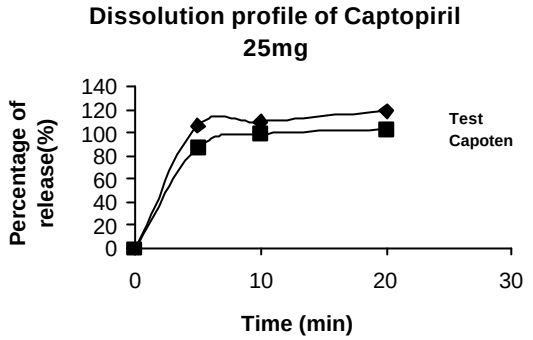


**Figure 1.** Dissolution behavior of Capoten tablets and captopril tablets at each 25 mg.

part of the overall studies on Iranian generic products, was to compare the bioavailability of two captopril formulations containing 25 and 50 mg captopril, respectively, with reference products of the same strength in healthy volunteers. Formulation parameters were accounted for by carrying out *in vitro* characterization of the dosage forms.

## Material and Methods

### *In vitro* characterization

Weight variation assessment and content uniformity and content assays were conducted on 20 tablets of each brand according to the United States Pharmacopeia[6] (USP) procedure for captopril tablets.[5] The dissolution profile of the captopril test and reference products were determined according to the USP 24 procedure, in which 5-mL samples must be removed at 0, 5, 10 and 20 minutes. Samples were diluted with dissolution medium and assayed spectrophotometrically at 212 nm. The amount of captopril dissolved at each time was calculated using a calibration curve prepared on the day of the study.

### *In vivo* studies

### Study protocol

Twelve healthy, nonsmoking, male volunteers (mean age ± SD, 26.7 ± 3.2 years; weight, 72.1 ± 4.20 kg; height, 174.6 ± 2.1 cm) in two groups took part in this study. All volunteers gave written informed consent after they had received detailed instructions about the study performance, restrictions and possible adverse events that may be experienced as a result of taking the drug. All volunteers were in good physical health according to physical examination and hematological and urinary laboratory tests. Subjects did not take any other medications for at least 2 weeks prior to and throughout the study. Each subject fasted overnight before the experiment, and food was withheld for 4 hours after dosing. Tablets were swallowed with 150 mL water. A light, normal lunch consisting of cheese, bread and water was given to all subjects 4 hours after dosing. A washout period of 1 week was included between the administration of each product. In the second phase, the test and reference tablets were crossed between the two groups of volunteers.

### Blood sampling

After predose blood samples had been drawn, volunteers ingested the appropriate study drug, according to the randomization schedule, with 200 mL tap water. Blood samples (5 mL) were taken via an indwelling venous cannula at the following times: before drug administration (0 hr), and 20, 40, 60, 90, 120, 150, 180, 240, 300 and 360 minutes after drug administration. Within 10 minutes of collection, blood samples were centrifuged at 3000 rpm for 9 – 11 min and after clot retraction from each sample, two aliquots of plasma were transferred to labeled tubes. All samples were handled at room temperature before storage at –20ºC pending captopril assays.

### Assay

Analysis was performed using a normal phase high-performance liquid chromatographic (HPLC) method described previously.[7] To 1 mL plasma in a 5-mL glass tube was added 50 ìL water containing lorazepam (10 ìg/mL) as internal

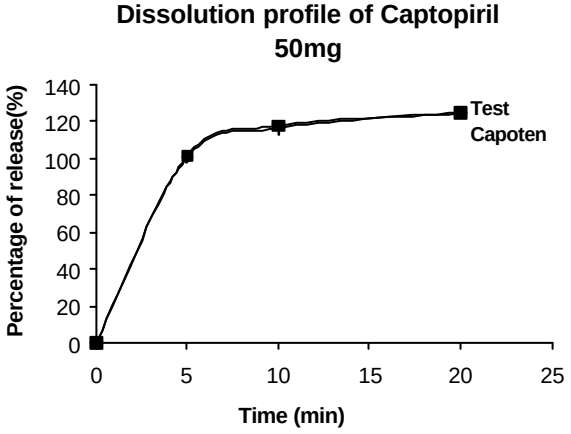


**Figure 2.** Dissolution behaviors of Capoten tablets and captopril tablets at each 50 mg.

**Table 1.** Pharmacokinetic data for captopril (dose of 2 x 25 mg captopril tablets; *n* = 12).

| | | Capoten® (reference) | | | Captopril (test) | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **Unit** | **Mean** | **SD** | **RSD%** | **Mean** | **SD** | **RSD%** |
| $C_{max}$ | ng/mL | 459.8 | 63.1 | 13.7 | 466.6 | 51.3 | 11.1 |
| $T_{max}$ | hr | 1.0 | 0.2 | 20.0 | 1.1 | 0.3 | 27.3 |
| $AUC_{0-t}$ | ng.hr/mL | 1292.9 | 244.7 | 18.9 | 1281.5 | 207.1 | 16.2 |
| $AUC_{0-}$ | ng.hr/mL | 1392.4 | 277.8 | 19.9 | 1403.3 | 234.1 | 16.7 |
| $K_e$ | $hr^{-1}$ | 0.50 | 0.06 | 13.24 | 0.45 | 0.16 | 12.48 |

SD = standard deviation; RSD = relative standard deviation.

standard and 20 ìL NaOH 1M and 20 ìL 2-bromo 2-acetonaphtone 0.02% solution in acetone. The mixture was left for 30 min. After adding 20 ìL phosphoric acid 20% and 1 mL acetonitrile and mixing for 5 min, the samples were centrifuged for 10 min and 500-ìL aliquots were evaporated to dryness under nitrogen. The residue was reconstituted in 50 ìL acetonitrile and 20 ìL was injected onto the HPLC column (Nucleosil-NH2, 5 ìm, 250 mm × 4 mmID; Knauer, Germany). Detection was at 245 nm. The analytical method was fully validated in terms of intra- and inter-day variations, linearity, accuracy and percent recovery according to USP guidelines.[5]

### Pharmacokinetic variables

To compare the rate and extent of absorption of captopril in both studies, the following pharmacokinetic variables were calculated for each volunteer and product, using actual blood sampling times. The areas under the plasma concentration curves ($AUC_{0-t}$) were calculated using the linear trapezoidal rule. The maximum plasma concentration ($C_{max}$) and time to reach maximum plasma concentration ($T_{max}$) were obtained directly from the plasma-concentration data. The $AUC_{0-\infty}$ was calculated by dividing the last measured concentration ($C_t$) by the elimination rate constant and adding the result to the $AUC_{0-t}$. The elimination rate constant was calculated by least-squares regression using the last points of each curve.

### Statistical Analysis

The test and reference treatments in the two phases of each study were compared with respect to relevant pharmacokinetic variables using analysis of variance with volunteer, product and period effects after a logarithmic transformation of the data. Point estimates and 90% confidence intervals (CIs) for the test/reference mean ratios of these variables were calculated. Bioequivalence of the test and reference products was assessed on the basis of these CIs, in relation to the conventional bioequivalence range of 80 – 125%. In addition, a non-parametric point estimate and 90% CI for the test-reference median difference in $T_{max}$ was calculated.

## Results

### *In vitro* studies

All products met the pharmacopoeial specifications for weight variation, content assay and content uniformity assay. Dissolution behaviors of the two brands studied are shown in Figures 1 and 2. The results represent the mean of 6 units (± standard error of the mean). All tablets met the USP 24 dissolution specifications which indicates that not less than 80% of the labeled amount of captopril dissolved in 20 minutes.

### Validation of the Analytical Method

The method was linear over a range of 20 – 800 ng/mL of drug in plasma. The between-day coefficients of variation determined from quality control samples processed together with each batch of samples were between 5.2% and 8.9% for concentrations ranging between 20 ng/mL and 500 ng/mL and the accuracy was 95 – 102%. The limit

**Table 2.** Pharmacokinetic data for captopril (dose of 1 x 50 mg captopril tablet; *n* = 12).

| | | Capoten® (reference) | | | Captopril (test) | | |
|---|---|---|---|---|---|---|---|
| **Variables** | **Unit** | **Mean** | **SD** | **RSD%** | **Mean** | **SD** | **RSD%** |
| $C_{max}$ | ng/mL | 535.5 | 76.9 | 14.4 | 517.2 | 517.2 | 14.9 |
| $T_{max}$ | hr | 1.0 | 0.3 | 30.0 | 1.1 | 0.3 | 27.3 |
| $AUC_{0-t}$ | ng.hr/mL | 1388.2 | 206.7 | 14.9 | 1309.5 | 174.4 | 16.3 |
| $AUC_{0-}$ | ng.hr/mL | 1518.8 | 256.8 | 16.9 | 1444.5 | 194.1 | 13.4 |
| $K_e$ | $hr^{-1}$ | 0.45 | 0.10 | 22.40 | 0.44 | 0.10 | 23.96 |

SD = standard deviation; RSD = relative standard deviation.

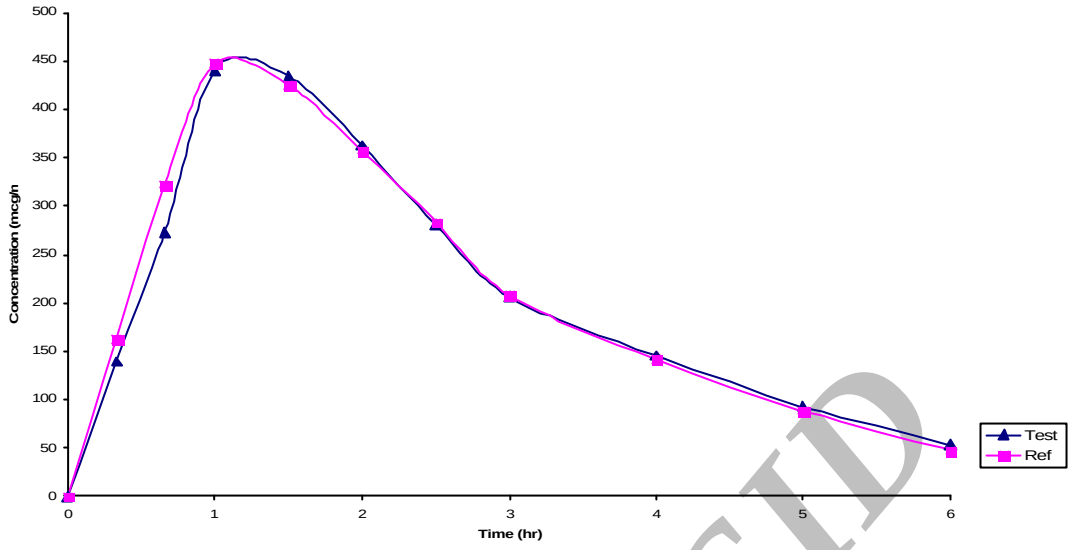


**Figure 3.** The mean plasma captopril concentrations (*n* = 12) following single-dose administration of 2 X 25 mg Capoten and captopril tablets.

of quantification was found to be 10 ng/mL on the basis of signal-to-noise ratio.

**Pharmacokinetic results**

Pharmacokinetic variables for studies 1 and 2 are summarized in Tables 1 and 2, respectively. The mean plasma captopril concentrations are represented in Figures 3 and 4, respectively.

In the first study, the point estimates (90% CI) of the test/reference mean ratio for $C_{max}$, $AUC_{0-t}$ and $T_{max}$ were 102% (98.6 – 105.4%), 99.7% (97.1 – 102.3%) and 108.5% (98.2 – 118.7%), respectively. In the second study, the point estimates (90% CI) of the test/reference mean ratio for $C_{max}$, $AUC_{0-t}$ and $T_{max}$ were 96.8% (92.9 – 100.7%), 94.9% (90.3 – 99.5%) and 108.5% (98.2 – 118.7%), respectively.

## Discussion

The 90% CIs for the test/reference mean ratios of the plasma captopril pharmacokinetic variables

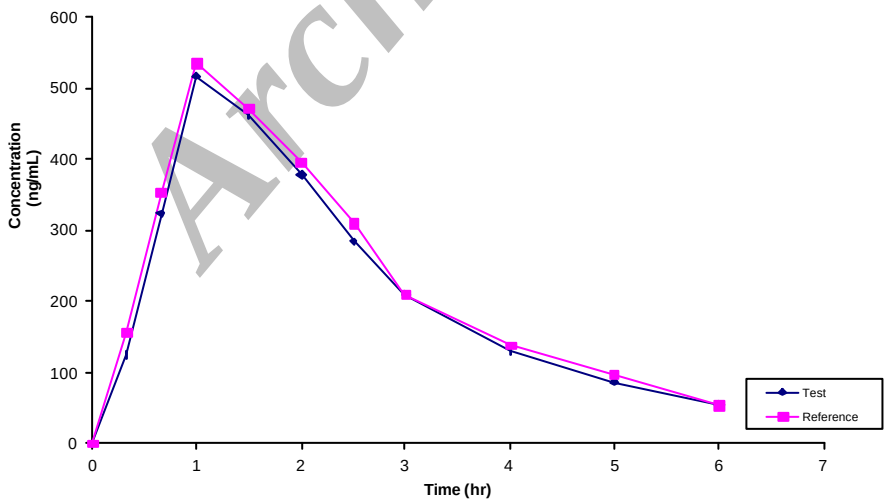


**Figure 4.** Mean plasma captopril concentrations (*n* = 12) following single-dose administration of 1 x 50 mg Capoten and captopril tablets.

$C_{max}$, $AUC_{0-t}$ and $T_{max}$ (as measures of the rate and extent of absorption of captopril, respectively), all fell within the conventional bioequivalence range of 80 – 125%. The test products (captopril HCl) are therefore bioequivalent to the reference product (Capoten®) with respect to the rate and the extent of absorption of captopril for both 25 mg and 50 mg tablets.

In conclusion, the results of these studies, which are similar to the results of a previously reported study,[4] showed that captopril tablets manufactured in Iran are comparable to foreign brands and can produce acceptable plasma concentrations.

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