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Original Article

# Bioequivalency Study of Two Formulations of Ketoconazole Tablet in Healthy Volunteers

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**Abstract**

In this study, the pharmacokinetic parameters of two marketed tablet formulations of ketoconazole were studied, and the relative bioavailability of the test formulation was compared with a reference formulation. A single dose (12x2) double blind randomized cross-over study of a generic formulation of ketoconazole tablet (2x200 mg), and a commercial brand, Nizoral tablet (2x200 mg, Janssen Pharmaceutica Beerse Belgica) was carried out. All of the tablets met the United States Pharmacopoeia dissolution specifications. The plasma level of ketoconazole was determined by using a modified rapid and selective reverse phase HPLC method. Plasma data was used to evaluate the relative bioavailability and other pharmacokinetic parameters characterizing rate [peak plasma concentration ($C_{max}$) and time of peak concentration ($T_{max}$)] and the extent of absorption (AUC). The mean peak plasma concentration ($C_{max}$) of ketoconazole of the two different formulations, A (reference) and B (test), were 7.08±2.81 and 6.74±2.20 mg/l at 1.70±0.48 h and 1.73±0.75 h, respectively. The mean $AUC_{0-\infty}$ of the two products, were 39.07±16.25 and 31.85±14.64 for A and B, respectively. Statistical analysis showed no significant differences between various pharmacokinetic parameters of the two different formulations. The 90% parametric confidence intervals for the mean of test/reference ratios of $C_{max}$, $AUC_{0-12}$, $AUC_{0-\infty}$ and $C_{max}/AUC_{0-\infty}$ were within the bioequivalence acceptable range (80-125%). Results of this study showed that the extent and rate of absorption of ketoconazole tablets tested are comparable and the generic formulation is bioequivalent to the commercial product.

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## 1. Introduction

Ketoconozole (*cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1-H-imidazole-1-yl methyl)-1,3-dioxolan-4-yl] methoxy] phenyl] piperazine) is an imidazole antifungal agent, which is administered either topically or orally [1, 2]. It is given by mouth in chronic mucocutaneous candidiasis, fungal infection of gastro-intestinal tract, dermatophyte infections of skin and fingernails, and for the treatment of systemic blastomycosis,

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candidiasis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis [2-4]. The absorption of ketoconazole from the gastro-intestinal tract is variable and increases with the decrease of stomach pH. Mean peak plasma concentration of about 3.5 μg/ml has been obtained 2 h after the oral administration of 200 mg ketoconozole. Ketoconazole is more than 90% bound to plasma proteins, mainly albumin. It is widely distributed but its penetration into the CSF is poor. The elimination of ketoconazole is reported to be biphasic, with an initial half-life of 2 h and a terminal half-life of about 8 h [2-7]. Ketoconazole is metabolized in the liver to inactive metabolites. It is excreted as metabolites and unchanged drug mainly in the feces and some in the urine [2, 3].

Formulation and processing changes may directly influence the dissolution and bioavailability of a pharmaceutical formulation during development, manufacture, and product optimization. The process of scale-up may also alter dissolution and bioavailability [8, 9].

Many countries have established procedures for the introduction of generic pharmaceutical products. In order to protect consumers, these generic products must be demonstrated to be therapeutically equivalent to a previously approved product, typically an innovator product. The therapeutic equivalence of a generic and an innovator product is most commonly based on the demonstration of bioequivalence, i.e. clinically insignificant differences in the rate and extent of drug absorption usually assessed from pharmacokinetic measurements [10-13].

Bioequivalency studies on generic drugs manufactured in Iran have been conducted to evaluate the possible effects of formulation and processing changes in generic products. The aim of this study was to determine the bioequivalency of a generic formulation of ketoconozole tablet containing 200 mg

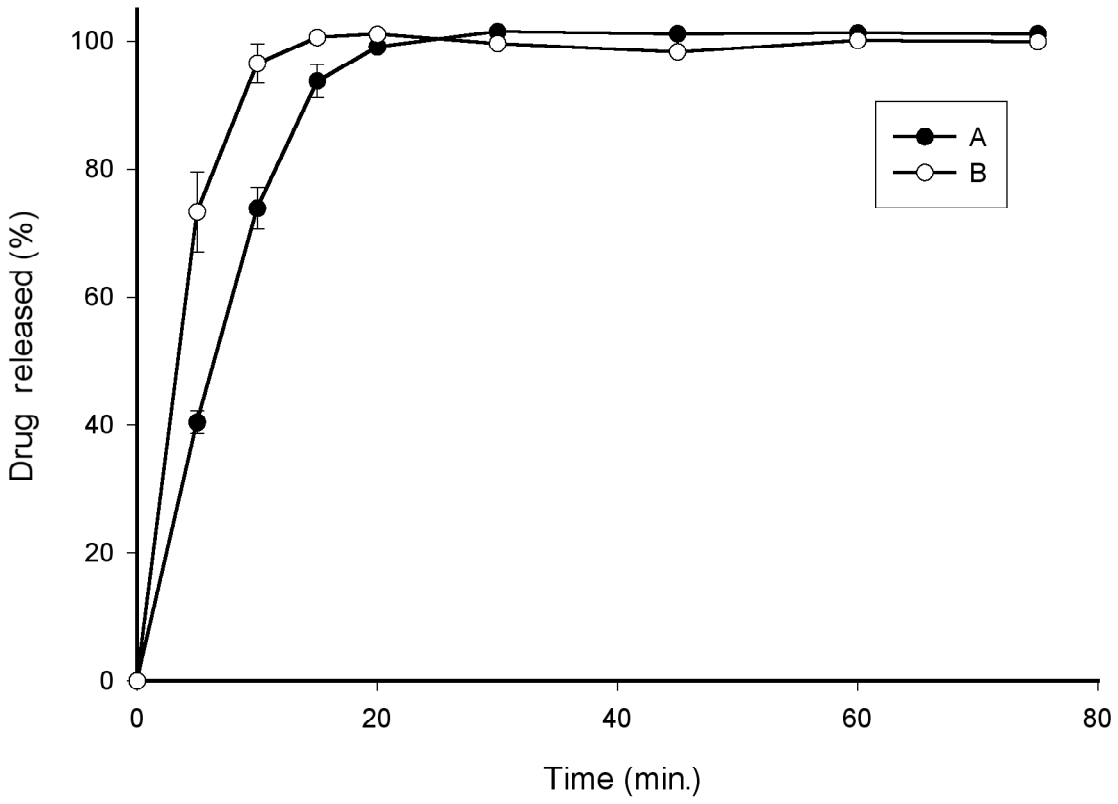


**Figure 1.** Mean dissolution profiles of two different 200 mg ketoconazole tablet formulations, A (reference) and B (test)

ketoconazole in comparison with a brand dosage form (reference product, nizoral tablet, Janssen Pharmaceutica Beerse, Belgica) of the same strength, in healthy volunteers.

## 2. Materials and methods

*2.1. In vitro study*

Dissolution data was obtained on 6 samples of each product using rotating paddles (apparatus II) at 50 rpm according to the specifications of the United States Pharmacopoeia XXIV (three flasks dissolution tester, Shimifan, Iran). The dissolution medium was 900 ml of 0.1 N HCl at 37 $^{o}$C [14]. Aliquots (5 ml) were taken for analysis at 5, 10, 15, 20, 30, 45, 60 and 75 min. and measured with spectrophotometer at 225 nm. The mean percentage of the drug released from the two different formulations at each sampling time were calculated and compared statistically.

*2.2. In vivo study*

Twelve normal healthy male volunteers 23 to 29 years of age weighting 59 to 78 kg were employed in this investigation. All subjects had no liver dysfunctions, acute or chronic disease and had not received any medicine two weeks prior to the study. Informed written consent was obtained from each subject. The permission of the ethic committee was obtained.

*2.3. Experimental design*

The study was designed as a randomized double blind cross-over investigation. All subjects were fasted over night for 10 hours before each experiment and 3 hours after the drug administration. Each volunteer received a single dose of 400 mg ketoconazole (two tablets) of two different tablet formulations (A and B) on two separate occasions with one week wash out period. Formulation A

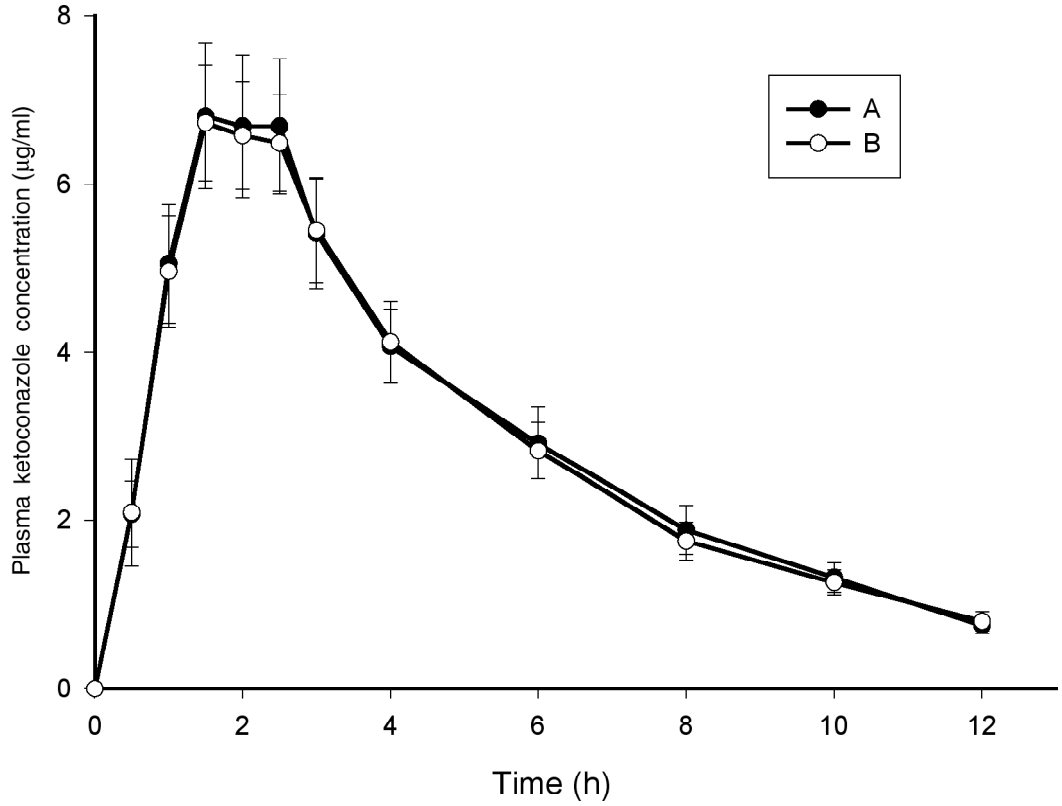


**Figure 2.** Mean (±SEM) plasma ketoconazole concentration in twelve healthy male volunteers after oral administration of 400 mg of two different formulations of ketoconazole tablets, A (reference) and B (test).

**Table 1.** The mean pharmacokinetic parameters of ketoconazole after a single oral administration of 400 mg of two different formulations (A and B) to twelve healthy volunteers.

| | Parameters | | | | | |
|---|---|---|---|---|---|---|
| **Formulation** | **$C_{max}$ (mg/l)** | **$T_{max}$ (h)** | **$k_e$ (1/h)** | **$t_{1/2}$ (h)** | **AUC $_{0-12}$ (mg.h/l)** | **$AUC_{0-\infty}$ (mg.h/l)** |
| A (Reference) | 7.08±2.81 | 1.70±0.48 | 0.28±0.05 | 2.55±0.43 | 36.30±15.21 | 39.07±16.25 |
| B (Test) | 6.74±2.20 | 1.73±0.75 | 0.31±0.06 | 2.37±0.56 | 29.64±13.65 | 31.85±14.64 |
| Statistical analysis | NS | NS | NS | NS | NS | NS |

NS= not significant

(Nizoral, Janssen Pharmacetica Beerse, Belgica), a commercial dosage form, was used as standard for comparison with local generic formulation, labeled B, (Rooze Daru Pharmaceutical Corporation, Tehran, Iran).

*2.4. Sampling*

Blood samples were collected into heparinized glass tubes just prior and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hours after the administration of the drug. Plasma was separated immediately after collection from heparinized blood and kept frozen at -20 $^{o}$C until analyzed.

*2.5. Assay*

Plasma samples were analyzed for ketoconazole by using rapid and selective reversed phase high performance liquid chromatographic method [15]. The method was modified to achieve a better separation, sharper peaks and shorter retention times for the drug and internal standard. The liquid chromatography comprised of a Model 600 E Waters pump (Waters Association, Milford, MA, U.S.A.), a variable wavelength, model 484 Waters UV detector and a U6K, Waters sample injection system. The chromatograms were recorded on a model 745B Waters recorder. The mobile phase was a mixture of potassium dihydrogen phosphate (0.05 M)-methanol-acetonitrile (40:30:30). The pH of the mobile phase was adjusted to 4.9 with 85% phosphoric acid. The mobile phase was filtered under vacuum, degassed and pumped through the column at a flow rate of 1 ml/min. The Novapak $C_{18}$ column (150x309 mm i.d.) was used for the analysis of the samples. All the chemicals were Fluka analytical grade except ketoconazole and clotrimazole, which were obtained from Janssen Pharmactica and Sigma, respectively.

*2.6. Sample preparation and calibration curve*

For the analysis of ketoconozle in plasma, 20 μl of the internal standard solution (200 μg/ml clotrimazole in methanol) was added to 0.5 ml plasma in a centrifuge tube and vortex mixed for 1 min. To the mixed sample, 1 ml acetonitrile was added in order to precipitate the plasma proteins. After vortex-mixing for 1 min. and centrifugation at 4500 rpm for 15 min., 10 μl of the supernatant was injected into the HPLC column. Standard samples containing 0.2 to 8 μg/ml of ketoconazole in plasma were prepared daily to construct the standard curve.

*2.7. Pharmacokinetics and statistical analysis*

Plasma data were analyzed for appropriate pharmacokinetic parameters using non-compartmental method. Elimination rate constant ($k_e$) was estimated by least-square regression of the terminal end of the plasma concentration-time profile. The area under the plasma concentration-time curves from time zero to the last measurable concentration at time, t, and ($AUC_{0-t}$) was calculated using trapezoidal rule. The area was extrapolated to infinity ($AUC_{0-\infty}$) by adding $C_t/k$ to the $AUC_{0-t}$, where $C_t$ is the last detectable drug concentration [13, 16]. The maximum plasma concentration ($C_{max}$) and the time of the peak plasma concentration ($T_{max}$) were determined using individual subject plasma concentration-time curves. The elimination half life ($t_{1/2}$) of ketoconazole was calculated by using the following equation:

$$t_{1/2} = \ln 2 / k_e$$

**Table 2**. Parametric 90% confidence intervals (CI) for the mean pharmacokinetic parameters of ketoconazole formulations.

| Parameter | Test/Reference | |
|---|---|---|
| | **Geometeric mean** | **CI** |
| $AUC_{0-12}$ ($mghl^{-1}$) | 0.94 | 87.7-101.8 |
| $AUC_{0-\infty}$ ($mghl^{-1}$) | 0.94 | 87.6-102.3 |
| $Cmax/AUC_{0-\infty}$ ($h^{-1}$) | 1.01 | 94.2-122.7 |
| Cmax ($mg\ l^{-1}$) | 0.95 | 83.4-110.1 |
| | **Test/Reference** | |
| | **Arithmetic mean** | **CI** |
| $T_{max}$ (h) | -0.04 | -0.41-0.49 |

The relative bioavailability of the test dosage form was estimated as the $AUC_{0-\infty}$ ratio of the test to the reference formulation [13].

The *in vitro* dissolution data of two formulations were compared by two-tailed student t-test at each sampling time. Parametric bioequivalence testing and ANOVA (for a crossover design) were performed using SAS for Windows V6.10 (SAS Institute Inc., Cary, NC, USA). $C_{max}$, $AUC_{0-12}$, $AUC_{0-\infty}$ and $C_{max}/AUC_{0-\infty}$ were log transformed prior to statistical data evaluation followed by 90% parametric confidence interval test for the mean of test/reference individual ratios of each parameter [1, 13]. For these parameters 0.8-1.25 was used as the acceptance range. For $T_{max}$, ±20% of the reference mean was used as acceptance range. The formulations were defined as bioequivalent with respect to a certain parameter if the 90% confidence interval was fully contained within the acceptance range for the parameter, according to the recommendations of the International Harmonization and Consensus DIA Meeting on bioavailability testing requirements and standards [17] and current Federal Drug Agency guidelines [18].

## 3. Results

Ketoconazole was well tolerated following oral administration of a 400 mg single dose of test and/or reference products and no adverse effects were reported. Formulation A, a commercial dosage form, was used as a reference product while formulation B, a generic dosage form, was used as a test product in two separate occasions with one week washout period. The plasma concentrations of ketoconazole were determined using a rapid and sensitive reverse phase HPLC method. The chromatographic method yielded sharp, symmetrical and well-resolved peaks for ketoconazole and internal standard. The calibration curves for determination of ketoconazole in plasma were obtained by plotting the peak-height ratios of ketoconazole to clotrimazole for seven different concentrations.

The standard curves of ketoconazole in plasma were linear over the concentration range of 0.2-8 μg/ml of ketoconazole. The regression equation of the calibration curve was found to be $Y= 0.2035X + 0.0008$. The correlation coefficient of the calibration curve was 0.9995. The coefficient of variation of within day and day-to-day variations of the assay were 3.57% and 4.86%, respectively.

The *in vitro* dissolution data indicated that all the tablets met the United States Pharmacopoeia dissolution specifications [14]. As indicated in Figure 1, the total amount of ketoconazole was released from the two products in 20 min. Content uniformity of the two formulations was evaluated and both of them had the same content uniformity. Statistical analysis showed no significant differences between dissolution data of two dosage forms.

The mean plasma concentration-time curves after oral administration of 400 mg of two different formulations (A and B) to the twelve healthy volunteers are shown in Figure

2. There was no significant difference between ketoconazole plasma concentrations at each sampling time, following oral administration of two formulations. The mean pharmacokinetic parameters calculated from individual plasma level-time are shown in Table 1. The mean peak plasma concentration ($C_{max}$) of ketoconazole after single oral administration of 400 mg of ketoconazole of two different formulations, A and B, were 7.08±2.81 and 6.74±2.20 mg/l at 1.70±0.48 h and 1.73±0.75 h, respectively. The mean $AUC_{0-\infty}$ of two products, A and B, were 39.07±16.25 and 31.85±14.64 mg.h/l, respectively.

Statistical analysis of these data showed no significant differences between the pharmacokinetics parameters of two formulations. No sequence or period effects were found for any of the parameters tested. The $C_{max}$, $AUC_{0-12}$ and $AUC_{0-\infty}$ were log transformed prior to statistical data evaluation [13]. The 90% parametric confidence intervals for the mean of ratios (the test to the reference formulation) of $C_{max}$, $AUC_{0-12}$, $AUC_{0-\infty}$ and $C_{max}/AUC_{0-\infty}$ of drug were calculated and were 83.4-110.1%, 87.7-101.8%, 87.6-102.3% and 94.2-122.7%, respectively (Table 2). These values were within the conventional bioequivalence range (80-125%). As shown in Table 2, the mean difference (test-reference) of $T_{max}$ was 0.04 h with a 90% confidence interval of 0.41-0.49. The stipulated bioequivalence range for the difference of $T_{max}$ is ±20% of the reference $T_{max}$ mean which in this case corresponds to ±0.34. Taking formulation A as a reference, (100% bioavailability assumed), the relative bioavailability of the test formulation B for each volunteer were calculated and were 87.82%. Statistically no significant differences between the bioavailability of the ketoconazole tablets of two products were observed. However significant inter-subject variation was observed.

## 4. Discussion

The pharmacokinetic parameters and bioequivalence of two different ketoconazole tablet formulations (A and B) following oral administration of a single dose of 400 mg to twelve healthy male volunteers were studied. This study was designed in a randomized, double blind, cross-over investigation.

Ketoconazole serum profiles obtained from the two formulations were comparable. The mean pharmacokinetic parameters of two products were also in the same order of magnitude as reported values [2, 4-6]. Moreover, bioequivalence could be shown for both rate ($C_{max}$ and $C_{max}/AUC_{0-\infty}$) and extent ($AUC_{0-24}$ and $AUC_{0-\infty}$) of absorption dependent parameters. The 90% confidence interval for the mean difference of $T_{max}$ between two formulations (test and reference) was 0.41-0.49. Although this interval is slightly wider than stipulated bioequivalence range of ±0.34, it could not affect the conclusion of bioequivalence in the rate of absorption since the other rate-dependent parameters ($C_{max}$ and $C_{max}/AUC_{0-\infty}$) are similar for both products (Table 2). Moreover, the *in vitro* dissolution profiles of ketoconazole from two formulations were also similar.

In conclusion based on the pharmacokinetic and statistical results of this study, the two formulations of ketoconazole seem to be bioequivalent regarding the rate and extent of absorption.

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## References

[1] Shargel L, Mutnick AH, Souney PF, Swanson LN. *Comprehensive pharmacy review.* 4th ed. Baltimor: Lippincott Williams & Wilkins, 2001: 131-40, 794, 798.

[2] Parfitt K. *Martindale, the extra pharmacopoie*.

33th ed. vol. 1, London: The Pharmaceutical Press, 2002; pp. 389-90.

[3] Daneshmand TK, Wornock DW. Clinical pharmacokinetics of ketoconazole. *Clin Pharmacokin* 1988; 14: 13-34.

[4] *Drug facts and comparisons.* 58th ed. Missouri: Fact and Comparisons Inc., 2004; 1564-5.

[5] Gennoro AR, Chase GD. *Remington: the science and practice of pharmacy.* 20th ed. Pennsylvania: Mack Publishing Company, 2000; 1552-3.

[6] *Physicians' desk reference, PDR.* 58th ed., NJ: Medical Economics Company, 2004; 1757-9.

[7] Scheifele DW, Hunter MI, Bortolussi RA. Ketoconazole. *Can Med Assoc J* 1985; 133: 1117-20.

[8] Skelly JP, Van Burskirk GA, Savello DR, Amindon Gl, Arbit HM, Dighes, Fawzi MB, Gonzalez MA, Malick AW, Malinowski H, Nedich R, Peck GE, Pearce DM, Shah V, Shangraw RF, Schwartz JB, Truelove J. Workshop report, scale-up of immediate release oral solid dosage forms. *Pharm Res* 1993; 10: 313-6.

[9] Immediate release solid oral dosage forms; scale-up and postapproval changes: chemistry, manufacturing and controls: *in vitro* dissolution testing; *in vivo* bioequivalence documentation guidance. *Fed Reg* 1995; 60: 61638-43.

[10] Schroeder TJ, Cho MJ, Pollack GM, Floc'h R, Moran HB, Levy R, Moore LW, Pouletty P. Comparison of two cyclosporine formulations in healthy volunteers: Bioequivalence of the new Sang-35 formulation and Neoral. *J Clin Pharmacol* 1998; 38: 807-14.

[11] Shah A, Liu MC, Vaughan D, Heller AH. Oral bioequivalence of three ciprofloxacin formulations following single-dose administration: 500 mg tablet compared with 500 mg/10 mL or 500 mg/5 mL suspension and the effect of food on the absorption of ciprofloxacin oral suspension. *J Antimicrob Chemother* 1999; 43 (Suppl. A): 49-54.

[12] Foroutan SM, Shafaati AR, Zarghi A, Khoddam A. Bioequivalence studies of two formulations of baclofen tablet in healthy volunteers. *Iranian J Pharm Res* 2003; 2: 153-5.

[13] Shargel L, Yu A. *Applied biopharmaceutics an d pharmacokinetics.* 4th ed., Appleton and Lange, 1999; pp. 247-79.

[14] *The united state pharmacopoeia, U.S.P. 26 and N.F. 21.* vol.1 and 2, Rockville: United state pharmacopoeia convention, 2003; pp. 1046-8, 2155-6.

[15] Chin TWF, Loeb M, Fong IW. Effects of an acidic beverage (Coca-Cola) on absorption of ketoconazole. *Antimicrob Agents Chemother* 1995; 39: 671-5.

[16] Boroujerdi M. *Pharmacokinetics.* New York: McGraw-Hill, 2002; pp. 153-80.

[17] Cartwright AC. International harmonization and consensus DIA meeting on bioavailability testing requirements and standards. *Drug Inform J* 1991; 25: 471-82.

[18] Chen ML, Patnaik R, Hauck WW, Schuirmann DJ, Hyslop T, Williams R. An individual bioequivalance criterion: regulatory considerations. *Statistics Med* 2000; 19: 2821-42.