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Evaluation of the bioequivalence of capsules containing 150 mg of fluconazole

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Abstract

Fluconazole is an antifungal agent. The purpose of this study was to evaluate bioequivalence of two commercial 150 mg capsule formulations of fluconazole available in the Brazilian market. The study was an open, randomized, two-period, two-group crossover trial with a 2-week washout interval. Blood samples were collected throughout a 96-h period after administration of reference product (R) and test product (T) to 28 fasting volunteers. A simple, accurate, precise and sensitive high-performance liquid chromatographic (HPLC) method with ultraviolet detection was developed and validated for quantification of fluconazole in plasma samples after liquid–liquid extraction. Bioequivalence between the products was determined by calculating 90% confidence intervals (90% C.I.) for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the test and reference products, using logarithmic transformed data. The 90% confidence intervals for the ratio of C_{max} (101.06–105.45%), AUC_{0-t} (97.11–104.69%) and $AUC_{0-\infty}$ (97.96–103.36%) values for the test and reference products are within the 80–125% interval, proposed by FDA and EMEA. It was concluded that the two fluconazole formulations are bioequivalent in their rate and extent of absorption.

Keywords: Bioequivalence; Capsule; Fluconazole; HPLC; Plasma; Quantification

1. Introduction

Fluconazole is an antifungal agent used in the treatment of oropharyngeal, esophageal, or vulvovaginal candidiasis and in the treatment of other serious systemic candidal infections. The drug is also used for the treatment of meningitis caused by *Cryptococcus neoformans* (Bennets, 1996).

In the Brazilian market, several pharmaceutical laboratories currently sell fluconazole-based products for treating vaginal candidiasis and dermatomycosis in the form of 150 mg capsules.

The bioavailability of a drug product is defined as the rate and extent to which the active ingredient or therapeutic moiety is absorbed and becomes available at the site of drug action. Two drug products are con-

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sidered to be bioequivalent if they are pharmaceutical equivalents (i.e., similar dosage forms made, perhaps, by different manufacturers) or pharmaceutical alternatives (i.e., different dosage forms) and if their rates and extents of absorption do not show a significant difference when administered at the same molar dose of the therapeutic moiety under similar experimental conditions (Chow and Liu, 2000).

Bioequivalence or comparative bioavailability has gained increasing attention during the last 40 years after it became evident that marketed products having same amounts of the same drug may exhibit marked differences between their therapeutic response. In many instances, these differences were correlated successfully to dissimilar drug blood levels caused mainly by impaired absorption (Abdou, 1989).

Fluconazole is an antifungal drug, used in severe systemic infections. Consequently, it is important that the dosage form provides effective plasma concentration, thereby assuring the elimination of the microorganism which causes the infection. Should the plasma concentration fall the level required to ensure efficacy, the infection will not be eradicated and the risk of developing resistance to the drug may increase.

The purpose of this study is to evaluate bioequivalence of two commercial 150 mg capsule formulations of fluconazole available in the Brazilian market.

2. Materials and methods

2.1. Samples

Samples of hard capsules containing 150 mg of fluconazole, produced by Solvay Farma Ltda, Brazil (Flunazol, test product) and by Laboratórios Pfizer Ltda, Brazil (Zoltec, reference product), were used.

2.2. Fluconazole quantification in human plasma

The published HPLC methods for fluconazole quantification in plasma through high-performance liquid chromatography (HPLC) (Hosotsubo et al., 1990; Inagaki et al., 1992; Koks et al., 1995; Wallace et al., 1992; Cociglio et al., 1996; Hülsewede and Dermoumi, 1996; Ng et al., 1996; Majcherczyk et al., 2002) bear disadvantages which hinder their application on bioequivalence trials.

These disadvantages are related to the use of not commercially available internal standard, to the internal standard low resolution at adopted chromatographic conditions, to the intricate or expensive extraction procedures or to a low sensitivity.

Accordingly, a simple method was developed as part of this paper, which is exact, accurate and sensitive to fluconazole quantification in plasma through HPLC.

2.2.1. Extraction procedure

Sample preparation was performed by extracting plasma fluconazole with an organic solvent in an alkaline environment. Twenty-five microlitres of sodium hydroxide 5 M was added to 15 ml glass tubes containing 500 μl of plasma. The samples were extracted with 4.0 ml of dichloromethane by vortex mixing for 60 s. After centrifugation at 3000 rpm for 20 min, the aqueous phase was discarded and the organic phase filtered through a HV Millex® polypropylene unit with 0.45 μm hydrophilic PVDF Durapore® membrane. Three millilitres of the filtrate were transferred to a clean glass tube and evaporated to dryness under a nitrogen stream at 37 °C. The residue was dissolved in 500 μl of mobile phase and injected into the chromatographic system.

2.2.2. High-performance liquid chromatography

The chromatographic system consisted of the following components: two Shimadzu LC-10ADVP pumps, a Shimadzu DG14A degasser, a Shimadzu SIL-10ADVP autosampler, a Shimadzu SPD-10AVP variable-wavelength detector and a Shimadzu SCL-10AVP system controller. Chromatographic analysis was performed at ambient temperature, using a Shimadzu Shim-Pack G-ODS pre-column (10 mm × 4 mm i.d., 5 μm particle size) and a Supelco Supelcosil LC-18 column (150 mm × 4.6 mm i.d., 5 μm particle size). The mobile phase consisted of water–acetonitrile (70:30, v/v) and was pumped at a flow rate of 1.0 ml/min. The analytes were detected at 210 nm.

2.3. Method validation

Validation was accomplished through determination of recovery, linearity, quantification limit, precision, accuracy, specificity and stability (Causon, 1997; Bressolle et al., 1996).

2.4. Bioequivalence trial

Twenty eight healthy volunteers, 12 males and 16 females, 19–45 years (mean ± S.D., 27 ± 6 years), height of 151–180 cm (mean ± S.D., 166 ± 7 cm) weight of 50–79 kg (mean ± S.D., 61 ± 8 kg), and within 15% of their ideal body weight, were enrolled. The clinical protocol was approved by the local Ethics Committee and the volunteers gave written informed consent to participate in the study. Volunteers were healthy and had no history of heart, kidneys, neurological or metabolic diseases, no history of drug hypersensitivity, were not undergoing any pharmacological treatment and female volunteers were not pregnant.

The study was an open, randomized, two-period, two-group crossover trial with a 2-week washout interval. During the first period, volunteers from group A received a single 150 mg dose of Zoltec® (reference product), while volunteers from group B received a single 150 mg dose of Flunazol® (test product). During the second period, the procedure was repeated on the groups in reverse.

The capsules were administered to the volunteers in the morning, after an overnight fast, with 200 ml of water. Volunteers received standard lunch and afternoon snacks, respectively, 5 and 8 h after drug administration.

Volunteers did not ingest any alcoholic drink, coffee or other xanthine-containing drinks during the trial. Furthermore, they did not take any other drug, 1 week before the study and during its execution.

Blood samples were collected at 0 (pre-dose) and at 1, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 96 h post-dose. The samples were centrifuged and the plasma was stored at −20 °C until fluconazole quantification.

2.5. Fluconazole quantification in plasma samples

Calibration standards of 0.25, 0.5, 0.75, 1.00, 1.25, 1.50, 3.00 and 5.00 μg/ml and quality-control samples of 0.50, 2.00 and 5.00 μg/ml were prepared by spiking blank human plasma with standard solutions of fluconazole.

The HPLC injection sequence was as follows: calibration standards, volunteers' plasma samples (in duplicate) and quality-control samples throughout all sequence (in triplicate).

2.6. Bioequivalence evaluation

Non-compartmental analysis was performed to estimate pharmacokinetic parameters. C_{max} (maximum observed plasmatic 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 trapezoidal rule.

The terminal first order constant (k_{el}) was determined by a least squares fit of the terminal plasma concentrations (using Excel® for Windows®).

The constant k_{el} 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 $AUC_{t-\infty}$ (Ritschel, 1992).

Bioequivalence between the products was determined by calculating 90% confidence intervals (90% C.I.) for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the test and reference products, using logarithmic transformed data. Analysis of variance (ANOVA) was used to assess group and period effects.

3. Results

3.1. Development of the analytical method for fluconazole quantification in plasma

The proposed method is suitable for fluconazole quantification in plasma samples, showing specificity (Fig. 1), 91% recovery, linearity between 0.25 and 5.00 μg/ml ($y = 5.8998 - 0.3032$, $r^2 = 0.9989$), quantification limit of 0.25 μg/ml, intra-assay precision between 5.5 and 9.0% and inter-assay precision between 3.5 and 5.2%, accuracy between 95.9 and 108.0%, samples stability for 180 days at −20 °C temperature and stability of the organic extracts after reconstitution with mobile phase for 24 h at room temperature.

3.2. Bioequivalence evaluation

Average concentration versus time curves after administration of reference (Zoltec® 150 mg, Pfizer) and

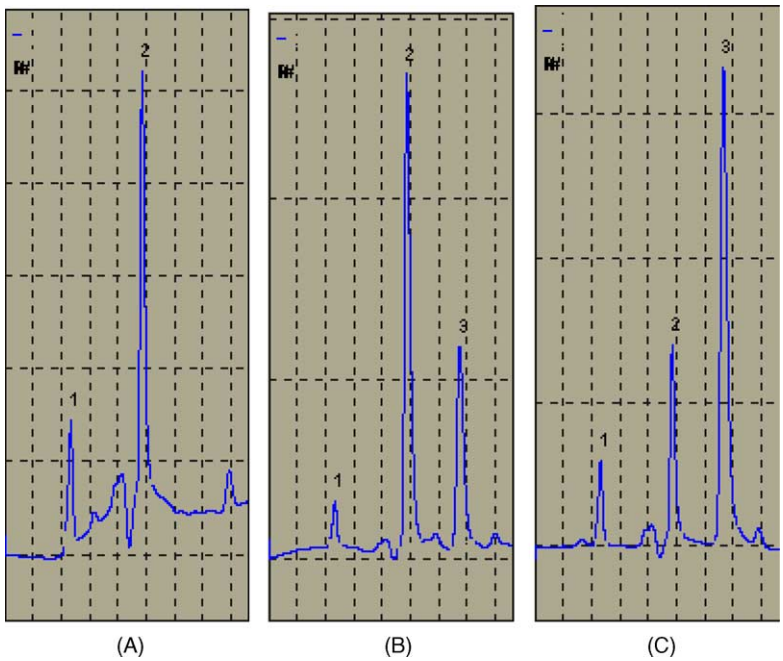

Fig. 1. Chromatograms of: (A) blank plasma obtained from healthy volunteers; (B) blank plasma spiked with fluconazole (2.00 μg/ml); (C) plasma from healthy volunteer, 3 h after administration of single dose of fluconazole (150 mg). Peak 3 = fluconazole, retention time = 3.3 min.

test (Flunazol® 150 mg, Solvay Farma) products to 28 healthy volunteers are shown in Fig. 2.

Table 1 shows the average values of pharmacokinetic parameters after administration of reference (Zoltec® 150 mg, Pfizer) and test (Flunazol® 150 mg, Solvay Farma) products to 28 healthy volunteers.

The results of the analysis of variance (ANOVA) for the assessment of product, group and period effects and the 90% confidence intervals (90% C.I.) for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the test and reference products, using logarithmic transformed data, are shown in Table 2.

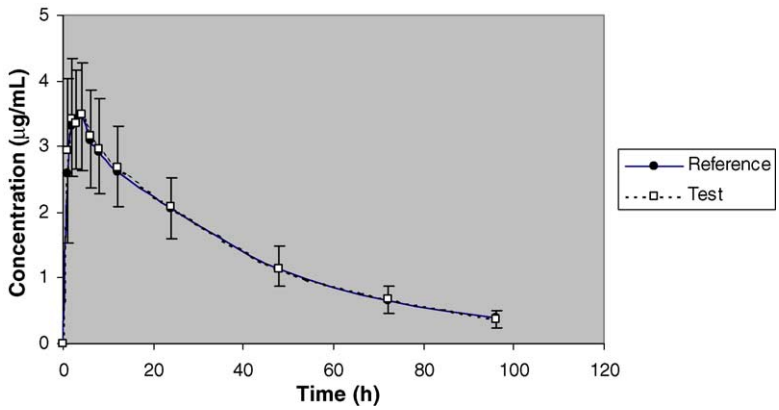

Fig. 2. Average plasma concentrations of fluconazole after administration of reference (Zoltec® 150 mg, Pfizer) and test (Flunazol® 150 mg, Solvay Farma) products to 28 healthy volunteers. Bars indicate standard deviations (lower bars for reference product and upper bars for test product).

Table 1
Pharmacokinetic parameters after administration of reference (Zoltec® 150 mg, Pfizer) and test (Flunazol® 150 mg, Solvay Farma) products to 28 healthy volunteers

	Zoltec® 150 mg					Flunazol® 150 mg				
	C_{max} (μg/ml)	t_{max} (h)	AUC_{0-t} (μg h/ml)	$AUC_{0-\infty}$ (μg h/ml)	$t_{(1/2)el}$ (h)	C_{max} (μg/ml)	t_{max} (h)	AUC_{0-t} (μg h/ml)	$AUC_{0-\infty}$ (μg h/ml)	$t_{(1/2)el}$ (h)
Average	3.64	2.96	135.72	153.33	29.99	3.75	2.79	137.30	154.45	29.99
S.D.	0.79	1.00	29.52	35.96	4.84	0.75	1.26	31.94	36.81	4.34
C.V. (%)	21.70	33.78	21.75	23.45	16.14	20.00	45.16	23.26	23.83	14.47

S.D., standard deviation; C.V., coefficient of variation.

Table 2
Analysis of variance (ANOVA) for the assessment of the product, group and period effects, and 90% confidence intervals (90% C.I.) for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the test and reference products, using logarithmic transformed data, after administration of reference (Zoltec® 150 mg, Pfizer) and test (Flunazol® 150 mg, Solvay Farma) products to 28 healthy volunteers ($\alpha = 0.05$)

Pharmacokinetic parameter	ANOVA (*P*-value)			C.I. 90%
	Variation source			
	Product	Group	Period	
C_{max}	0.087975	0.396559	0.164812	101.06–105.45
AUC_{0-t}	0.715861	0.140079	0.930681	97.11–104.69
$AUC_{0-\infty}$	0.784755	0.125261	0.884423	97.96–103.36

4. Discussion

The analytical method developed for fluconazole quantification in plasma samples showed good specificity, sensitivity, linearity, precision and accuracy, thereby enabling its use in bioequivalence trials. Furthermore, it showed several advantages over other published methods.

At first, the absence of internal standard represents an important simplification, since the internal standards used in other methods are not commercially available (Hosotsubo et al., 1990; Inagaki et al., 1992; Wallace et al., 1992; Hülsewede and Dermoumi, 1996). Only two methods describe the use of phenacetin (Koks et al., 1995) or amphotericin B (Ng et al., 1996) as internal standard, but these substances present problems in the extraction procedure or chromatographic resolution under the analytical conditions used. The high recovery of extraction (91%) obtained with the proposed method allows the non-use of the internal standard, without compromising precision and accuracy.

The chromatographic analysis time of each sample was 5 min. The quantification limit (0.25 μg/ml) was lower than that obtained by Hosotsubo et al., Hülsewede and Dermoumi, Ng et al. and Majcherczyk et al. and close to that obtained by Inagaki et al. and Koks et al.

The average plasma decay curves (Fig. 2) obtained for the test product (Flunazol® 150 mg, Solvay Farma) and reference product (Zoltec® 150 mg, Pfizer) were similar as were the pharmacokinetic parameters (Table 1).

Previous studies (Brammer et al., 1990) reported that following oral administration of 50 mg of fluconazole, the maximal drug concentration in the plasma varies between 0.8 and 1.0 μg/ml. As fluconazole exhibits linear pharmacokinetic in doses up to 3.0 mg/kg (Brammer et al., 1990), it can be stated that these values are comparable with the present study, in which the oral administration of 150 mg of fluconazole provided maximal plasma concentrations of around 3.7 μg/ml. The values obtained for $AUC_{0-\infty}$ were similar to those reported in that study.

The fluconazole pharmacokinetic evaluation after oral and rectal administration, accomplished by Pfaff et al. (1993), revealed that following oral administration of capsules with 200 mg of fluconazole, the $AUC_{0-\infty}$ (174.4 μg h/ml), C_{max} (3.4 μg/ml) and t_{max} (4.9 h) values were similar to those in the present study, considering the dose difference. The same

can be stated with regard to the results obtained by Thorpe et al. (1990) following oral administration of 100 mg of fluconazole ($AUC_{0-\infty}$ = 93.00 μg h/ml, C_{max} = 1.70 μg/ml and t_{max} = 4.3 h).

According to FDA and EMEA regulations, the sampling schedule should be planned to provide a reliable estimate of the extent of absorption. This is generally achieved if AUC_{0-t} is at least 80% of $AUC_{0-\infty}$.

Usually, the sampling time should extend to at least three terminal elimination half lives of the active drug ingredient, beyond t_{max}. Time periods between sampling should not exceed one terminal half life (Nation and Sansom, 1994).

The values obtained for plasma decay half life in the present study were 29.99 h for both the reference and test products and were similar to other authors' reports (Debruyne and Ryckelynck, 1993); t_{max} values were 2.96 h for reference product and 2.79 h for test. Thus, the total sampling time (96 h) and time between sampling (≤24 h) were adequate to estimate the extent of absorption. The average $AUC_{0-t}/AUC_{0-\infty}$ value was 0.89 for both test and reference and individual values ranged from 0.82 to 0.97.

The multivariate analysis, accomplished through analysis of variance (ANOVA) for assessment of period, group and product effects, revealed the absence of any of these effects in the present study.

The 90% confidence intervals for the ratio of C_{max} (101.06–105.45%), AUC_{0-t} (97.11–104.69%) and $AUC_{0-\infty}$ (97.96–103.36%) values for the test and reference products are within the 80–125% interval proposed by FDA and EMEA. It was concluded that the two fluconazole formulations are bioequivalent in their rate and extent of absorption.

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