Original Article

Clinical pharmacokinetics and bioavailability study between generic and branded fluconazole capsules

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ABSTRACT

Fluconazole is an effective bis-triazole oral antifungal agent indicated for the treatment of systemic and superficial candidiasis. The clinical pharmacokinetics and relative bioavailability (bioequivalence) of a single dose of new generic formula (capsule) containing 150 mg fluconazole as a test product was compared against the brand product Diflcan® 150 mg capsule, Pfizer, as a reference product. Both products were administered to 28 healthy Arabic adult males applying fasting, a two-sequence, two-period, two-treatment, single-dose, randomized crossover design with two weeks washout interval between dosing. Eighteen blood samples were taken from each subject before dosing (time zero) and then at 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, 96, and eventually at 120 hours post-dosing. From plasma concentrations obtained from each participant, the pharmacokinetic parameters; $K_{elimination}$ (λ_z), AUC_{0-t}, T_{max}, C_{max}, $AUC_{0-\infty}$, T_{half} and MRT were calculated by non-compartmental analysis and statistically analyzed using ANOVA. Ln-transformed values of the parameters used for bioequivalence evaluation namely C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were also statistically analysed by ANOVA and 90% confidence interval tests. For T_{max} and MRT, nonparametric tests were performed. Based on EMEA and FDA criteria on bioequivalence, the results showed bioequivalence of the 2 products. Both drug products were well tolerated by all participants. Thus, the new generic fluconazole 150 mg capsule can be interchangeable with Diflcan® capsule and may be prescribable in clinical practice.

Keywords: Fluconazole, Pharmacokinetics, Relative bioavailability, Fasting men

Introduction

Today, infection due to fungal pathogens has become more frequent [1]. Fluconazole is prescribed for prevention and treateatment of different and many types of superficial and systemic fungal and yeast infections in adults and neonates [2-4].

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It is available as oral tablets containing 50, 100,150, or 200 mg fluconazole, as a powder for oral suspension containing 350 or 1400 mg fluconazole, and as a sterile solution for intravenous use containing 2 mg/ml fluconazole [3]. The drug is also indicated in severe burn injury [5] and for therapy in diabetes mellitus [6].

Many clinical advantages of fluconazole are due to its unique pharmacokinetic properties. The pharmacokinetics of the drug are almost similar after administration by the oral or intravenous routes (tablets and suspension). All the oral drug dose reaches the systemic circulation due to low first-pass metabolism resulting in absolute bioavailability of more than 90%, therefore, the daily dose of fluconazole is almost identical for intravenous, oral tablets and suspension. In fasting healthy subjects, fluconazole maximum plasma concentration (C_{max}) is

attained within 1-3 hours after oral administration. The terminal elimination half-life (T_{half}) of fluconazole is about 30 hours with a range of 20-50 hours and is prolonged greatly in patients with renal impairment. The relatively long half-life of the drug offers the basis for once-daily dosing in treating fungal infections. The apparent volume of distribution of fluconazole approximates the volume of total body water. Besides, the drug has a low degree of binding to plasma protein, which ranges between 11%-12%. This degree has no clinical significance. Fluconazole is mainly excreted (about 80%) unchanged in the urine. Fluconazole demonstrated linear (dose proportional) pharmacokinetics in term of its elimination rate constant $K_{elimination}$ (λ_z), the area under plasma concentration-time curve (AUC) and the C_{max} [3, 7].

Fluconazole is widely used worldwide for severe systemic fungal infections. Therefore, it is important to avoid therapeutic failure and adverse effects of fluconazole formulation by achieving the required therapeutic plasma concertation with optimal effect and minimal adverse effect which can be assured by bioequivalence assssement. If a fluconazole formula yield plasma levels below the required therapeutic concertation to ensure efficacy, the infection will persist, and the risk of developing resistance is expected [8-10]. Hence, many bioequivalence researches were conducted in different countries to examine the bioequivalence of their marketed generic fluconazole in their nations [11-18]. These researches include Chinees [11], Arabic [12], Thia [13], Brazilian [14], Spanish [15], Serbian [16], Mexican [17], and Bangladeshi [18] populations.

This investigation aimed at studyning the pharmacokinetics of a newly developed generic capsule formula containing 150 mg fluconazole in healthy male adult Arabic subjects and to find out whether the generic formula is bioequivalent with the reference brand Diflcan® 150 mg capsule produced by Pfizer, by comparing the pharmacokinetic characteristics of both formulations. Besides, as a secondary purpose, the tolerability of both formulas was also assessed.

Materials and Methods

Dissolution test

As a prerequisite for bioequivalence testing of a generic against the brand drug formulations, a promising in vitro dissolution performance between both formulas should be obtained [19-22]. As recommended by FDA guidelines on dissolution [19], the similarity factor (F2) between the dissolution profiles of both products should be more than 50 (50-100), which confirm the pharmaceutical similarity between both products and may reflect sameness or equivalence of the in vivo performance of both products [19-22].

Study design

This research was conducted as per a study protocol applying ICH guidelines on good clinical practice [23, 24], the latest version of the Declaration of Helsinki [25], and international guidelines on bioavailability and bioequivalence [26-28], including FDA and EMEA. The study protocol involved all details of this study, i.e., clinical, bioanalytical, pharmacokinetics, biostatistics, consent forms, and approvals from the principal investigator, the institutional review board (IRB), and the clinical investigator. All subjects recruited for the study gave their signed and written informed consent together with two witnesses and the clinical investigator before screening the subjects. Twenty-eight Arabic healthy male adult subjects were given each investigational drug product applying an open-label, single-dose, fasting, two-period, crossover design, two-sequence, two-treatment, randomized, with two-week washout interval between dosing. In each period (i.e., period 1 and 2) of this research, an equal number of participants (14) were randomized based on a randomization scheme established in the study protocol in order to receive each formula per sequence.

Investigational drug products

The generic formula was a capsule containing 150 mg fluconazole and the brand product was Diflcan® 150 mg capsule, Pfizer, Canada as a reference product.

Selection of eligible subjects

Arabic healthy adult men were selected to participate in this research according to the inclusion/exclusion criteria stated in the study protocol. The subjects age was between 18-48 years, body mass index (BMI) 18-30, non-smoker or light smoker (less than 10 cigarettes a day), have no history of alcohol and drug abuse, did not participate in any clinical trials, no recent surgery and blood donation within the last 2 months prior to the current study.

The subjects were regarded healthy based on personal interviews, complete physical examinations, clinical laboratory tests, and clinical examinations involving normal vital signs (temperature, blood pressure, and pulse), normal ECG, no medical history or diseases including cardiovascular, respiratory, renal, hepatic, gastrointestinal, epilepsy, bleeding, severe anemia, coagulation disorders, and psychiatric problems. The subjects should not have a history of hypersensitivity and contraindication to fluconazole and any related compounds. The clinical laboratory tests included normal biochemistry and hematology, negative (HIV, hepatitis B and C), negative drug abuse by urine examination, negative alcohol abuse by salivary testing, and normal routine urine analysis.

Clinical experimental conditions

The subjects attended the clinical unit before about 14 hours of drug product administration to perform vital signs, alcohol and drug abuse examinations. The subjects provided standard dinners 12 hours prior to dosing. The subjects were confined at the clinical unit 24 hours post-dosing for blood sampling and returned to the clinical unit for blood sampling at 48, 72, 96,

and eventually at 120 hours post-dosing (end of each study period). As per the randomization plane, the test or the reference product was administered to each subject with 240 ml water after overnight fasting of 12 hours. A standard lunch was provided at 4 hours followed by a standard snack at 8 hours and a standard dinner at 12 h after dosing. No water was allowed 2 h prior and 2 h after dosing, then water was allowed as desired. All the meals given for both periods of the research were identical and provided at the same time. The subjects were not allowed to sleep or lie during the first hours of drug product administration and they were stayed upright sitting or standing. After 2-weeks washout interval, the subjects returned to the clinical unit, and the same above-mentioned procedure was repeated as in the first period to complete the crossover design.

Collection of blood samples

Venous blood samples (5 ml) were obtained from subject's forearm antecubital vein at zero time (about one hour prior dosing) and then at 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, 96, and eventually at 120 hours post dosing. Each blood sample was directly transferred to heparinized tube and immediately centrifuged for 5 minutes at 4000 rpm for separation of plasma. The plasma samples were directly placed into Eppendorf tubes and saved in deep freezer at -20±10 °C until assessing fluconazole concentrations.

Tolerability and safety

Fluconazole tolerability and safety of each test and reference formula administered to each individual and at each period were assessed by observing, monitoring, and interviewing each individual about any potential adverse events (AEs), adverse drug reactions (ADR), and serious adverse effects (SAE) by the clinical investigator and the clinical staff. In addition to that, the vital signs were recorded at approximately 1 hour prior to drug administration, and then at 1, 2, 4, 6, 12, 24, 48, 72, 96, and eventually at 120 hours after drug intake, which is the time of discharge for periods 1 and 2 of this study.

Quantification of fluconazole concentrations in plasma

A high-performance liquid chromatography with UV detection at 210 nm was used for the quantification of fluconazole in human plasma applying modified analytical methods described previously [29-31]. Phenacetin was utilized as the internal standard. The analytical method was validated according to the current recommended FDA bioanalytical method validation guidelines [32]. The linearity of the standard calibration curve was established for concentrations range from 50-10000 ng/ml, which ought to cover the plasma concentrations range of fluconazole obtained after therapeutic oral doses of the drug as shown in previous pharmacokinetics [3, 7], bioavailability, and bioequivalence researches [11-18]. The lower limit of quantitation (LLOQ) of fluconazole in plasma was 50 ng/ml.

After completing the clinical phase (i.e., at the end of period 2), all plasma samples collected from each subject for the test and reference formulas together with the standard calibration curve, in addition to the quality control (QC) samples (low, medium & high) were analyzed together as one batch and in one analytical run to quantify fluconazole concentrations in the unknown authentic plasma samples. In addition to that, fluconazole plasma concentrations were not calculated by extrapolation below the LLOQ or above the upper limit of quantitation (ULOQ) of the established standard calibration curve [32].

Pharmacokinetic parameters

Kinetica software was used for the estimation of all pharmacokinetic parameters of fluconazole including C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $K_{elimination}$ (λ_z), T_{half}, and MRT of each subject and for each period applying non-compartmental analysis [33, 34]. Plasma concentration versus time data was plotted by Excel software. The C_{max} and the time at which C_{max} occurs (T_{max}) were obtained without interpolation from the concentration versus time curve of each subject. The area under the plasma concentration versus time curve from the time of pre-dosing (t_0) up to the time of last blood sample withdrawal (t_{last}) at 120 hours post-dosing (AUC_{0-t}) was calculated by Trapezoidal rule. The extrapolated area under the plasma concentration versus time curve from t_{last} to infinity ($AUC_{t-\infty}$) was calculated from the ratio of C_{last}/λ_z. The C_{last} is the last quantifiable fluconazole plasma level that meets or exceeds the LLOQ. The terminal elimination rate constant $K_{elimination}$ (λ_z) was determined by least-square linear regression fitness of not less than three data points at the terminal phase of the log-concentration versus the time curve of each subject. The terminal elimination half-life (T_{half}) was calculated from the ratio of $0.693/\lambda_z$. The area under the plasma concentration versus time curve from t_0 to infinity ($AUC_{0-\infty}$) was calculated as AUC_{0-t} plus $AUC_{t-\infty}$. The % extrapolated AUC was calculated from ($AUC_{t-\infty}/AUC_{0-\infty}$) ×100. The mean residence time (MRT) was derived from the ratio of area under the moment curve (AUMC)/AUC [33, 34]. The mean±SD fluconazole plasma concentration versus time data for the test and the reference formulas were plotted in rectilinear and semi-log graphs.

Statistical data analysis for bioequivalence judgment

In order to evaluate the presence of statistical difference (if any) between the concentration-time profiles of fluconazole obtained from the test formula given to each subject against the corresponding profiles obtained from the reference formula, fluconazole plasma levels at each time point of the test formula were statistically compared against the corresponding concentrations for the reference formula by ANOVA tests.

The descriptive statistics involving geometric means, arithmetic means, ratio of means, coefficient of variation (CV), standard deviation (SD), minimum and maximum values were calculated for all the determined pharmackinetic parameter C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, λ_z, T_{half} and MRT. ANOVA tests were carried out for all the above-mentioned parameters of the test formula against the corresponding values obtained from the reference formula to demonstrate the impact of treatment (test versus reference formula), period, sequence, and subjects nested in sequence sources of variations. Moreover, ANOVA tests were also carried out for the Ln-transformed values of the primary parameters utilized for bioequivalence evaluation, namely C_{max}, $AUC_{0-\infty}$, and AUC_{0-t}. The test formula (T) was declared bioequivalent to the reference formula (R) if the ranges of 90% Cl of the (T/R) ratio for the Ln-transformed values of $AUC_{0-\infty}$, AUC_{0-t}, and C_{max} are 80.00-125.00 % [26]. Besides, the non-parametric Wilcoxon signed-rank test was used to evaluate the difference between T_{max} values of the test versus reference formulas [35].

Results and Discussion

Dissolution data

The dissolution profiles of both formulas were almost similar and to a good extent superimposable. Besides, the similarity factor F2 was calculated to be 95.6, which indicated sameness and pharmaceutical equivalence of both formulas. Thus, the dissolution result obtained in this study was so encouraging and promising to proceed to in vivo bioequivalence study [19-22].

Study conduct

Figure 1 shows the disposition of study subjects from the screening until the completion and discharged from the study at the end of period 2. As per previous fluconazole bioequivalence studies conducted in different populations [11-18], it appeared that sample size of 28 subjects is enough for achieving adequate power for bioequivalence judgments [11-18]. Thus, 38 subjects were recruited to be screened in order to end up with a minimum of 28 eligible subjects to be selected for participation in the study. Eight subjects were found to be not eligible to participate in this study according to the inclusion/exclusion criteria stated in the study protocol. Two more subjects withdrew from the study at the screening phase due to personal reasons. Hence, 28 eligible subjects were enrolled in this research as depicted in **Figure 1**. Since no dropout and withdrawal took place during the entire study, thus, all the 28 participants who started this research completed both periods of the study.

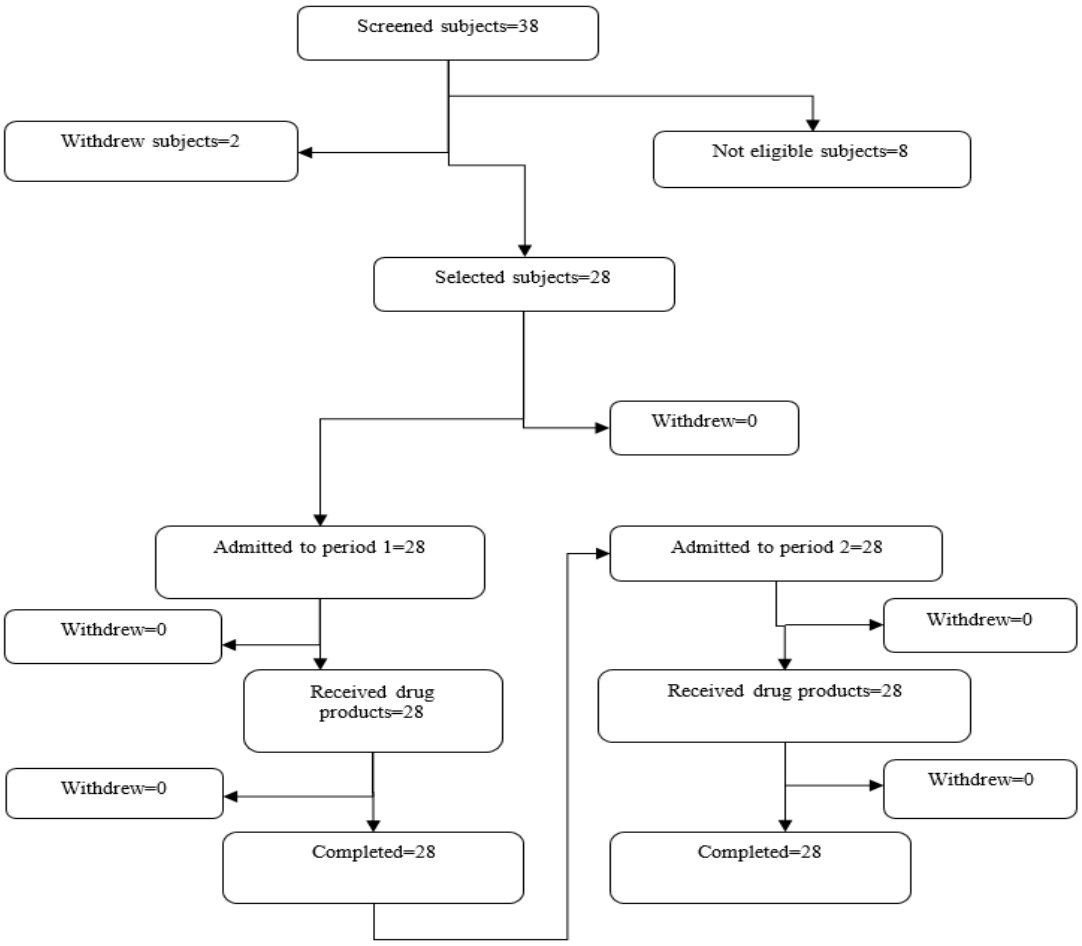


Figure 1. Disposition of the study subjects from screening until discharged

Tolerability and safety data

Both the test and the reference formulas were well tolerated by all subjects. Unexpected AEs, ADR, and SAE that possess a significant impact on study conduct were not documented. All the participants were discharged with no significant change in their baseline clinical data including vital signs and clinical laboratory tests involving biochemistry, haematology, and routine urine analysis which were done before drug intake of period 1, at the end of period 1, before drug intake of period 2, and eventually at the end of period 2 upon subjects discharged from the study.

Bioanalytical data

The analytical method used in the current study for determination of fluconazole plasma concentrtions was found to be accurate, precise, specific, selective, sensitive, and met the acceptance criteria according to the current recommendations of FDA bioanalytical method validation guidance [32]. In addition to that, the ranges for quantification of fluconazole from 50-10000 ng/ml was quite enough for covering the plasma concentrations range of fluconazole obtained as shown in **Figure 2**.

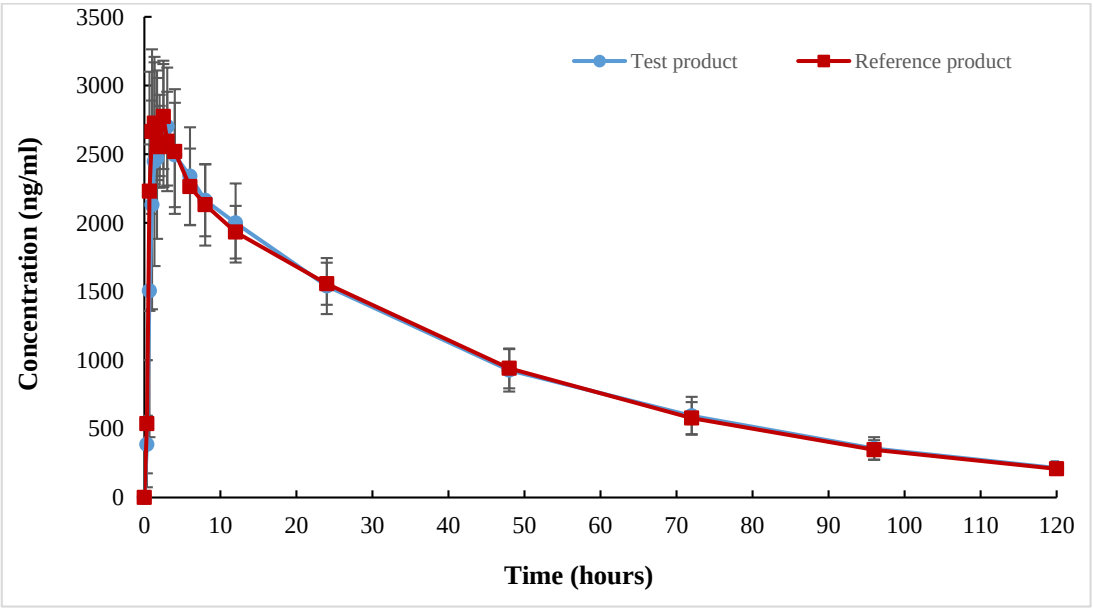
The inter- and intra-batch precision and accuracy for fluconazole at low, mid, and high concentrations were less than 9%, which is well within the accepted ranges of 15-20% as recommended by the above guidelines [32]. The recovery of the drug in all plasma concentration ranges was reproducible and the average value was 87±7. The LLOQ was reproducible and identifiable at 50 ng/ml. No interfering peaks with fluconazole were detected in the chromatograms. The correlation coefficients (r) were greater than 0.9988. Therefore, the bioanalytical method applied in this research is suitable for analysing fluconazole in plasma samples collected from bioavailability, pharmacokinetic, and bioequivalence studies.

Plasma concentrations of fluconazole

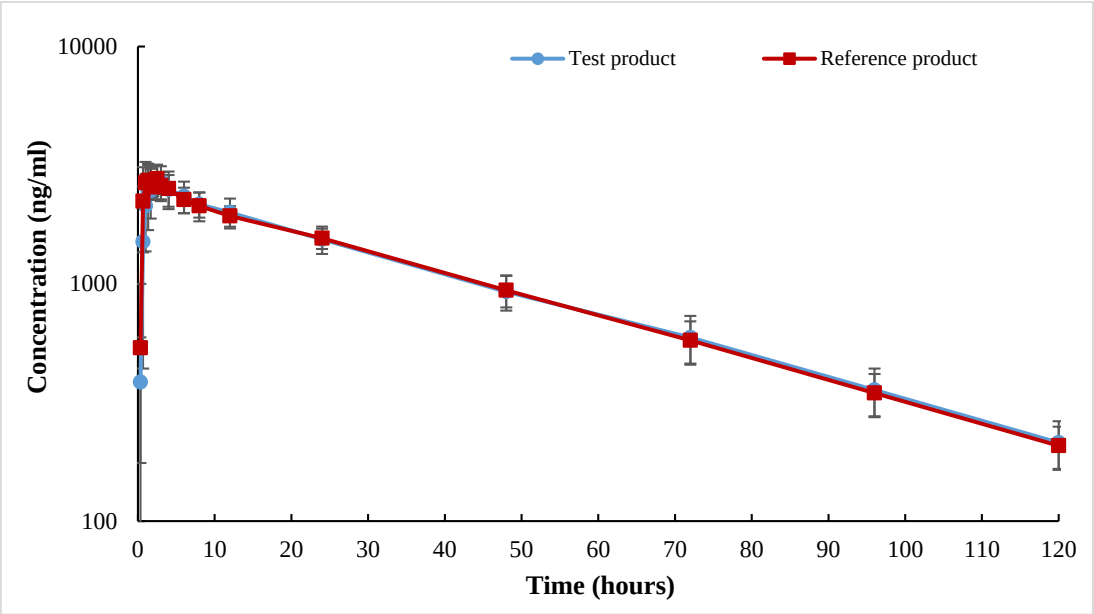
Plasma concentrations versus time profiles of fluconazole (mean±SD) which were obtained after the administration of the test and the reference formulas are plotted in rectilinear and semilog graphs as shown in **Figure 2**. It is apparent from **Figure 2** that fluconazole is rapidly absorbed from the administered capsule since plasma levels above the LLOQ (50 ng/ml) appeared in all the 28 subjects and for both formulas at the first blood sample withdrawal (at 0.33 minutes post-dosing), and fluconazole reached its maximum levels within 2 hours of drug intake. The plasma levels of fluconazole then declined mono-exponentially with a long terminal elimination phase as shown in **Figure 2**. The present finding is supported by several previous pharmacokinetics [3, 7], bioavailability and bioequivalence studies [11-18].

Visual observation of **Figure 2** demonstrates excellent agreement between the plasma concentration-time profiles for both formulas since both profiles are nearly superimposable. ANOVA tests for the individual plasma concentrations at each blood sampling time point (18 blood samples from 0.33 and up to 120 hours post-dosing) of the test formula versus the corresponding concentrations for the reference formula revealed no significant differences ($p>0.05$). Hence, this finding indicates that the absorption and disposition behaviors for the test formula are identical to the reference formula and suggest bioequivalence of both formulas.

The current research showed that for both formulas and for all participants, fluconazole levels were not detected (below the LLOQ of 50 ng/ml) in plasma samples obtained prior to drug intake at period 2 of the study. Hence, this observation ensures the absence of carry-over effect and indicates that two weeks washout interval between dosing is enough for the bioequivalence study of fluconazole.



a)



b)

Figure 2. Plasma concentrations (Mean±SD) versus time profiles of fluconazole after administration of 150 mg capsules of the reference and test products to 28 healthy men. a) Rectilinear graph, b) Semilog graph

Pharmacokinetic data

Table 1 presents the descriptive statistics of all the calculated pharmacokinetic parameters including $K_{elimination}$ (λ_z), $AUC_{0-\infty}$, AUC_{0-t}, T_{max}, C_{max}, T_{half}, and MRT. **Table 1** demonstrate good agreement between all the above-mentioned parameters of both formulas. The % extrapolated AUC was less than 9 % as shown in **Table 1**, which clearly indicates that 120 hours blood sampling after fluconazole intake was very enough for reliable measurements of fluconazole levels for pharmacokinetics, bioavailability, and bioequivalence studies.

Interestingly, the average and the ranges of all the current pharmacokinetic parameters obtained after the administration of fluconazole capsule to the Arabic population **(Table 1)** are almost similar to the corresponding pharmacokinetic parameters obtained after the administration of fluconazole capsule or tablets to other populations including Thai [13], Brazilian [14], Spanish [15], Serbian [16], Mexican [17], and Bangladeshi [18]. Thus, it can be concluded that ethnicity may possess no significant impact on the pharmacokinetics of fluconazole, i.e., absorption (rate and extent), and disposition.

Statistical data analysis for bioequivalence judgment

Statistical comparison applying ANOVA test between the test and the reference formulas for all pharmacokinetic parameters introduced in **Table 1** demonstrated no significant differences for the period, formulation, and sequence sources of variation. The non-parameter Wilcoxon signed-rank test exihibited no significant variations between the T_{max} values of both formulas. The arithmetic and geometric mean ratios and the relative bioavailability of the test/reference formulas approach 100% as shown in **Table 2**. Moreover, the ranges of 90%CI **(Table 2)** were well within bioequivalence accepted ranges of 80.00%-125.00% [26]. Hence, it is concluded from the current research that the newly developed generic fluconazole 150 mg capsule is bioequivalent to the brand product Diflcan® 150 mg capsule, Pfizer, and can be considered interchangeable with Diflcan® and prescribable in clinical practice.

Table 1. Pharmacokinetic parameters of fluconazole 150 mg capsule after administration of the reference and test products to 28 healthy men

	Test product							
Statistics	**C_{max} (ng/ml)**	**T_{max} (h)**	**AUC_{0-t} (ng.h/ml)**	**$AUC_{0-\infty}$ (ng.h/ml)**	**% AUC_{extra}**	**λ_z (h^{-1})**	**$T_{0.5}$ (h)**	**MRT (h)**
Mean	3068.5	2.16	112215.8	122810.2	8.6	0.021	33.8	48.8
±SD	512.9	0.75	14854.9	17445.8	2.1	0.002	2.5	5.1
%CV	16.7	34.7	13.2	14.2	24.6	9.6	7.5	10.4
Min	2271	0.67	90081	97932	6	0.016	31	43
Max	3891	3	132416	148688	14	0.023	39	62

Geomean	3029.0	2.25*	111301.9	121661.5	**	0.021	33.7	48.6
Reference product								
Statistics	**C_{max} (ng/ml)**	**T_{max} (h)**	**AUC_{0-t} (ng.h/ml)**	**$AUC_{0-\infty}$ (ng.h/ml)**	**% AUC_{extra}**	**λ_z (h^{-1})**	**$T_{0.5}$ (h)**	**MRT (h)**
Mean	3117.3	1.625	112876.4	122710.8	8.5	0.021	33.8	48.5
±SD	472.6	1.21	11682.9	15802.8	2.54	0.002	3.98	5.02
%CV	15.2	74.7	10.4	12.9	29.9	10.3	11.8	10.3
Min	2345	0.67	95533	92133	5	0.016	29	41
Max	3691	4	128753	141911	15	0.024	44	61
Geomean	3083.2	1.17*	112319.7	121733.2	**	0.021	33.6	48.3

* Median, **Geomean was not calculated

Table 2. Geometric mean ratio, relative bioavailability, and 90% CI for the test versus the reference products

Parameter	Geomean ratio	Relative bioavailability*	90% CI lower limit	90% CI upper limit
C_{max}	0.98	0.98	94.22	102.43
AUC_{0-t}	0.99	0.99	96.30	103.83
$AUC_{0-\infty}$	1.00	1.00	95.50	104.77

***Relative bioavailability=arithmetic mean test/arithmetic mean reference

Conclusion

This research displays the pharmacokinetic characteristics of fluconazole 150 mg capsule for a newly developed generic product in comparison to the brand product Diflcan® 150 mg capsule, Pfizer after administration to Arabic adult male healthy individuals. The ranges of 90% CI for the primary parameters used in bioequivalence judgment namely C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were well within the accepeted interval as per FDA and EMEA guidinace. Hence, the new generic formula of fluconazole 150 mg capsule was considered bioequivalent to the brand formula and may be prescribable and interchangeable with the brand Diflcan® 150 mg capsule in clinical practice.

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Conflict of interest: None

Financial support: None

Ethics statement: The current research was achieved in accordance with ICH guidelines for good clinical practice (GCP), and adhering with the ethical principles for medical research involving human subjects as per the declaration of Helsinki.

References

1. Mathew S, Victório CP. Antifungal Properties of Rhizomes of Alpinia calcarata Roscoe from Western Ghats, South India. Int J Pharm Phytopharmacol Res. 2020;10(5):1-7.
2. Najjar AA, Alosaimi EH, Abduljabbar HS, Hashim HA, Alem MA, Ahmed MM, et al. Prevalence of Fungi in Human Follicular Fluid and Its Potential Impact on In Vitro Fertilization Process. Arch Pharm Pract. 2020;11(4):75-82.
3. Diflcan® product monograph, Pfizer Canada, Submission Control No: 229321, Date of Revision: July 25, 2019.
4. Gian MP. Clinical pharmacology of fluconazole in neonates: effects and pharmacokinetics. Int J Paediatr. 2016;4(3):1475-89.
5. Jason MC, Alireza F, Matthew PR, Kevin KC, Clinton KM, Kevin SA. Intravenous antibiotic and antifungal agent pharmacokinetic-pharmacodynamic dosing in adults with severe burn injury. Clin Ther. 2016;38(9):2016-31.
6. Sazlyna MSL, Mahipal S, Fekade S. Challenges in antifungal therapy in diabetes mellitus. J Clin Med. 2020;9(2878):1-9.
7. Debruyne D, Ryckelynck JP. Clinical pharmacokinetics of fluconazole. Clin Pharmacokinet. 1993;24(1):10-27.
8. Tyson ED, Carolyn JP, Kelsey EA, Scott JB. Antifungal dosing strategies for critically ill patients. Curr Fungal Infect Rep. 2017;11(1):5-15.

9. Javier MG, Carlos AR, Maria A, Andres FZ, Omar V. Antifungal pharmacodynamics: Latin America's perspective. Braz J Infect Dis. 2016;21(1):79-87.
10. Athina P, Elias I, Charalampos A, Emmanuel R. Antifungal drug dosing adjustment in critical patients with invasive fungal infections. J Emerg Crit Care Med. 2019;3(37)1-16.
11. Li Z, Liu J, Qiu W, Chen H. Bioequivalence of fluconazole capsules in healthy Chinese subjects. Chin J Clin Pharmacol Ther. 2020;25(8):910-5.
12. Muhammad MH, Sophia JSDP, Rajaa H, Eman AlG, Nesrine AK, Reem AlS, et al. Generic-reference and generic-generic bioequivalence of forty-two, randomly selected, on-market generic products of fourteen immediate-release oral drugs. BMC Pharmacol Toxicol. 2017;18(78):1-20.
13. Wattananat T, Akarawut W. Bioequivalence evaluation of two fluconazole capsule formulations in healthy Thai volunteers. J Health Sci. 2007;16(5):683-9.
14. Porta V, Chang KH, Storpirtis S. Evaluation of the bioequivalence of capsules containing 150 mg of fluconazole. Int J Pharm. 2005;(288):81-6.
15. Portolés A, Almeida S, Terleira A, de Pablo I, Filipe A, Caturla MC, et al. Truncated AUC in the Evaluation of Fluconazole Bioequivalence. Arzneimittelforschung. 2004;54(11):752-6.
16. Du~an J, Vesna KB, Slavica V, Danica S, Midhad V, Nermina P. A randomized, open-label pharmacokinetic comparison of two oral formulations of fluconazole 150 mg in healthy adult volunteers. Clin Ther. 2005;27(10):1588-95.
17. Jose APA, Mireya LG, Teresita DJCS, Roxana HG, Julian MC, María DLA MB, et al. Bioequivalence of two oral fluconazole formulations in healthy subjects: a single dose, open-label, randomized, two-period crossover study. J Bioequivalence Bioavailab. 2010;2(2):023-7.
18. Sarker UK, Misbahuddin M, Hossain MA. Bioequivalence study of Flunac™ and Diflucan™ in healthy Bangladeshi male volunteers. KYAMC J. 2012;2(2):159-63.
19. Zhang Z. FDA's current practice and challenges in the use of dissolution similarity testing for demonstration of bioequivalence – case studies, Bioequivalence Office of Generic Drugs, CDER, FDA dissolution similarity workshop, May 2019.
20. Sally H, Heba ElB. In vitro dissolution similarity as a surrogate for in vivo bioavailability and therapeutic equivalence. Dissolut Technol. 2016;23(3):32-9.
21. Anand PK, Nileen M, Arati P. Comparative in vitro release study of some commonly available fluconazole tablets. Int Res J Pharm. 2016;7(6):63-5.
22. Ukwueze SE, Rufus M. Quality assessment and in vitro bioequivalence studies on some generic brands of fluconazole capsules commonly dispensed in Nigeria pharmacies. World J Pharm Res. 2021;10(4)122-30.
23. International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), ICH Harmonized Guidance: Guidance for Good Clinical Practice E6(R2), Current Step 4 version dated 9 November 2016.
24. E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1), Guidance for Industry, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), March 2018.
25. WMA Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects, 64th WMA General Assembly, Fortaleza, Brazil, October 2013.
26. Elambharathy E. Bioequivalence guidelines requirements for orally administered generics (IR products) in Gulf Cooperation Council Countries, European Union and United States of America. J Bioequivalence Bioavailab. 2019;11(1):6-13.
27. Henrike P. Generic drug workshop: current EMA regulatory perspectives on bioequivalence study and biowaiver. Federal Institute for Drugs and Medical Devices. Seoul. 2017.
28. Casimir AA, Amine C, Yahia C, Mustapha B, Jean MC. Bioequivalence regulation in emerging countries: example of Moroccan regulations on immediate release formulations and comparison with international guidelines. J Appl Pharm Sci. 2019;9(11):28-35.
29. Sung-Su K, Ho-Taek I, Il-Mo K, Hyun-Su L, Heon-Woo L, Sung-Hee C, et al. An optimized analytical method of fluconazole in human plasma by high-performance liquid chromatography with ultraviolet detection and its application to a bioequivalence study. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;852(1-2):174-9.
30. Jumpei S, Ayano T, Yuka K, Hidehiko M, Tetsuya I, Kensuke S, et al. A sensitive method for analyzing fluconazole in extremely small volumes of neonatal serum. J Pharm Health Care Sci. 2020;6(14):1-7.
31. Narender M, Sri BK, Balakrishna M, Padmalatha K. A simple liquid chromatographic method for simultaneous estimation of azithromycin, fluconazole and ornidazole in bulk pharmaceutical dosage forms. Int J Pharm Pharm Sci. 2019;11(8):26-34.
32. Bioanalytical Method Validation Guidance for Industry, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2018.
33. Leon S, Andrew BCY, Murray D. Shargel and Yu's Applied Biopharmaceutics & Pharmacokinetics, 8th ed. McGraw-Hill Education/Medical: New York, NY, USA. 2021.
34. Hartmut D, Stephan S. Rowland and Tozer's Clinical Pharmacokinetics and Pharmacodynamics: Concepts and

Applications, 5th ed. LWW: Philadelphia, PA, USA. 2019.

35. Patrick S, Thomas RV. Nonparametric statistical methods in medical research. Anaesth Analg. 2020;131(6):1862-3.