RESEARCH ARTICLE

Bioequivalence Study of a Newly Developed Azithromycin Suspension Versus Zithromax® Following a Single Dose to Healthy Fasting Adult Subjects

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Received: 19th November, 2020; Revised: 07th January, 2021; Accepted: 25th January 2021; Available Online: 25th March, 2021

ABSTRACT

Introduction: Azithromycin is an orally administered semisynthetic azide, a subclass of macrolide antibiotics. It is structurally related to erythromycin with similar spectrum of activity. Azithromycin has a prolonged elimination half-life (2–4 days) due to extensive tissue penetration, making the drug suitable for once-daily dosing. Azithromycin is widely prescribed to adults and pediatric patients.

Objective: The present study aimed to compare the rate and extent of bioavailability (bioequivalence) of a newly developed generic azithromycin suspension (200 mg azithromycin/5 mL) as a test drug product with azithromycin suspension (Zithromax®, Pfizer, USA) as the reference brand drug product.

Methods: Both drug products were administered as a single dose of 500 mg (12.5 mL suspension) to 28 healthy adult male Arabic subjects applying fasting, randomized, two-period, two-sequence crossover design with three weeks washout interval between dosing. Serial blood samples were obtained from each subject before drug dosing (Zero time) and then up to 72 hours post-dosing. The concentrations of azithromycin in the plasma sample of each subject were determined by a validated High-performance liquid chromatography-mass spectrometry (HPLC-MS)/MS method. The pharmacokinetic parameters C_{max}, truncated $AUC_{0\text{-}72hr}$ and T_{max} were calculated by non-compartmental analysis.

Results: The mean ± SD values of C_{max}, $AUC_{0\text{-}72hr}$ and T_{max} of azithromycin for the test product were 358.2 ± 120.6 ng/mL, 2722.3 ± 1116.2 ng.hr/mL and 2.57 ± 1.19 hours, respectively. The corresponding values of these parameters for the reference product were 315.8 ± 89.5 ng/mL, 2567.2 ± 846.7 ng.hr/mL and 2.63 ± 1.10 hours, respectively. The 90% confidence interval for the primary parameters used in bioequivalence evaluation namely C_{max} and $AUC_{0\text{-}72hr}$ were within Food and Drug Administration (FDA) and European Medicines Evaluation Agency (EMEA) acceptance range of 80-125%.

Conclusion: It can be concluded from the current investigation that the test and reference products are bioequivalent. Both products were well tolerated by all subjects and unexpected adverse events were not observed throughout the study. Thus, the newly developed generic azithromycin suspension may be considered interchangeable to Zithromax® suspension in clinical practice.

Keywords: Azithromycin suspension, Bioequivalence, Healthy fasting adult subjects.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.1.29

How to cite this article: Alani ME, Al-Tamimi DJ, Al-Mahroos MI, Ammoo1 AM, Al-Tamim MJ, Ibraheem JJ. Bioequivalence Study of a Newly Developed Azithromycin Suspension Versus Zithromax® Following a Single Dose to Healthy Fasting Adult Subjects. International Journal of Drug Delivery Technology. 2021;11(1):159-165.

Source of support: Nil.

Conflict of interest: None

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INTRODUCTION

Azithromycin tablets, azithromycin capsules, and azithromycin suspension contain the active ingredient azithromycin, an azalide, a subclass of macrolide antibiotics. Azithromycin is derived from erythromycin, but it differs chemically from erythromycin, a methyl-substituted nitrogen atom is incorporated into the lactone ring. Azithromycin molecular formula is C38H72N2O12, and its molecular weight is 749.00. Azithromycin is indicated for treating many medical conditions, including acute bacterial exacerbations of chronic obstructive pulmonary disease, community-acquired pneumonia, pharyngitis/tonsillitis, uncomplicated skin and skin structure infections, urethritis, cervicitis and genital ulcer disease.[1]

Azithromycin administered orally is rapidly absorbed; however, the absolute oral bioavailability is reported to be about 37%. The extent of plasma protein binding of azithromycin is concentration-dependent and ranges from 7% at low drug level in plasma and up to 50% at high plasma level.[1] Administration of azithromycin tablet with food causes a 23% increase in the maximum concentration of the drug in plasma (C_{max}). However, 56% elevation in C_{max} was found after azithromycin suspension taken with food. The extent of absorption (AUC) and bioavailability is unchanged for both azithromycin tablet or suspension administered with food.[1,2] The time to reach maximum plasma level (T_{max}) is 2–3 hours.[1,3-10] The level of azithromycin in tissue is up to 50 times higher than plasma, indicating that the drug is widely distributed to tissue. Azithromycin exhibits greater tissue penetration than erythromycin and, consequently, longer residence time in the body, making the drug convenient for once-daily dosing. Plasma terminal elimination half-life is reported to be 2 to 4 days.[1,3,4,8,10] Other literature reported a shorter elimination half-life of 30–40 hours.[5-7] Following oral azithromycin intake, a small fraction of the drug is demethylated in the liver. Biliary excretion is the major route of drug elimination from the body. Very high levels of unchanged drug were found in bile together with 10 metabolites that have no significant antimicrobial effects. About 6% of unchanged azithromycin was recovered in urine.[1]

Several approaches can be used to establish and document bioequivalence between generic and brand drug products. These include *in-vivo* studies (pharmacokinetic, pharmacodynamic, and studies with clinical benefit endpoints), in addition to *in-vitro* studies. The pharmacokinetic method is the most appropriate, reliable, and commonly used approach for establishing bioequivalence since it assesses the rate and extent of drug absorption by calculating the primary pharmacokinetic parameters C_{max} and T_{max} (which reflect the rate of systemic absorption), and AUC, which assess the extent of absorption and systemic exposure.[11-16]

Arabic pharmaceutical companies manufacture many generic pharmaceutical products. These products' prices are significantly lower than the corresponding multinational competitors, taking into consideration the quality of the product by *in-vitro* and *in-vivo* (bioequivalence) tests. This study aimed to assess bioequivalence between a newly formulated generic suspension containing 200 mg/5 mL azithromycin against the reference brand product Zithromax® 200 mg/5 mL suspension produced by Pfizer, USA. This aim was achieved by comparing the pharmacokinetic parameters obtained from plasma concentration-time data of each product administered to healthy male adult Arabic subjects under a fasting state as recommended by FDA[11-13] and EMEA[14] guidelines.

MATERIALS AND METHODS

Study Design

The study was conducted according to a study protocol following ICH guidelines on good clinical practice[17], FDA[11-13,] and EMEA[14] guidance on bioavailability and bioequivalence. The study protocol contains all details of the study, including the clinical, the bioanalytical, all approval forms, and the informed consent form. The study protocol was approved by the clinical investigator and the institutional review board (IRB) before study conduct. A written and signed informed consent with two witnesses was obtained from each subject before participation in the current investigation. According to Helsinki's declaration,[18] all details of the study were mentioned in the informed consent form, including benefits and possible risks associated with participation and the right to withdraw at any time during the study. Besides, a session was arranged with the principal and the clinical investigators' subjects to explain all the details of the research and the rights of the subjects mentioned in the informed consent form. The subjects were compensated financially for their participation in the study, even the subjects who were fond to be not eligible for inclusion in the study at the screening stage, or the subjects who dropped out or withdraw from the study at any time.

The study was designed as an open-label, laboratory-blind, fasting, single-dose, two-treatment, two-period, two-sequence, randomized crossover study with three weeks washout period between dosing.[11-14,19] In each study period, an equal number of subjects (14 subjects) were randomly assigned according to a randomization scheme to receive each formulation per sequence. The test product containing 200 mg azithromycin in 5 mL of the suspension (12.5 mL was given equivalent to 500 mg azithromycin) as a single dose was compared against the same dose of the reference product Zithromax® suspension.

Inclusion/Exclusion Criteria

Twenty-eight (28) Arabic healthy male adult subjects were selected to participate in the study. The subjects were considered healthy and eligible for participation in the study based on physical examinations, clinical examinations, and clinical laboratory tests. The inclusion criteria were as follow: the subjects comply with the requirements of the protocol and willing to undergo pre-study and post-study examinations, age between 18-48 years, body mass index (BMI) 18–30, non-smoker or light smoker (less than ten cigarettes a day), have no history of alcohol and drug abuse, did not participate in

any clinical trials (e.g., pharmacokinetics, bioavailability, and bioequivalence studies), no recent surgery, no blood donation within the last 2 months prior to the current study, have normal vital signs (blood pressure, temperature and pulse), normal ECG, have normal physical examinations, clinical examinations and normal clinical laboratory tests including biochemistry, hematology, negative (HIV, hepatitis B, and C), negative drug abuse testing in urine, negative alcohol abuse testing in saliva, and normal routine urine analysis.

The subjects were excluded from participation in this study if they had a medical history of the following conditions: contraindication or hypersensitivity to the study drug and any related compounds, acute infection within the last week prior to the study, diseases including: cardiovascular, respiratory, renal, hepatic, gastrointestinal, epilepsy, bleeding, severe anemia, coagulation disorders, and psychiatric problems.

Study Conduct

The subjects were asked to attend the clinical site 14 hours before dosing to do alcohol and drug abuse tests and vital signs. A standard dinner was served to the subjects 12 hours before dosing. The subjects were confined at the clinical site to ensure the fasting condition after dinner and until drug dosing. The subjects remained at the clinical site for 24 hours post-dosing for blood sampling, returned to the clinical site for blood sampling at 48, and then ultimately at 72 hours post-dosing (end of each study period). The test or the reference product was administered with 240 mL water after overnight fasting of 12 hours per the randomization schedule. The clinical staff performed mouth checks to ensure that the drug product was taken by the subjects as directed. A standard lunch, snack and dinner was served at 4, 8 and 12 hours after dosing, respectively. All the meals in both periods of the study were identical and served at the same time. The subjects were asked to eat all the meal given. No water was allowed 2 hours before and 2 hours after dosing, then water was allowed as desired. The subjects were not allowed to sleep or lie during the first four hours of dosing and they were remained upright standing or sitting. After 3 weeks washout interval, the subjects returned to the clinical site, and the same procedure mentioned above in period 1 was repeated in period 2 of the study to complete the crossover design.

Blood Sampling

Blood samples (5 mL) were drawn via an indwelling cannula placed in the forearm antecubital vein at one hour before dosing (zero time) and then at 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 2.33, 2.67, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 12.0, 16.0, 24.0, 48.0, and finally at 72.0 hours post-dosing. A total of 20 blood samples were withdrawn from each subject at each period. The total volume of blood obtained from each subject during the whole study was 230 mL, including the blood withdrawn for screening and clinical laboratory tests pre- and post-dosing. After each sample withdrawal, the cannula was kept patent by flushing with 1 mL of heparinized saline (0.5 IU heparin per mL saline). About 0.2 mL of blood was discarded from the cannula before each sample withdrawal to ensure the removal of any residual drug in the cannula. The actual sampling time for each blood sample was recorded. The blood samples were directly transferred to heparinized tubes and centrifuged for 5 minutes at 4000 rpm. The plasma was separated by polypropylene disposable tips and immediately transferred into two separate Eppendorf tubes to be stored as two aliquots. One aliquot was used for measuring azithromycin plasma concentration, and the second aliquot was saved for repeating the analysis if needed. The plasma samples were stored in deep freeze at -20 ± 5 °C until analysis. The tubes containing plasma samples were labeled by a confidential in-house labeling system which refers to study protocol number, period, subject number in the randomization scheme, and plasma sample number as per the blood sampling schedule. The principal investigator and the quality assurance (QA) responsible solely had access to the labeling system.

Safety Assessment

The drug safety and tolerability in the subjects were evaluated by vital signs including blood pressure, temperature, and pulse, which were recorded at 1.0 hour before dosing and then at 2.0, 4.0, 6.0, 24.0, and lastly at 72.0 hours post-dosing, which is the time of discharge for periods 1 and 2 of the study. Besides, the safety assessments involved monitoring and interviewing the subjects about the potential adverse events/reactions associated with the use of azithromycins such as diarrhea, nausea, abdominal pain, vomiting, dyspepsia, dizziness, headache, hypersensitivity, allergic reactions, and fatigue. All clinical steps and at each period of the study were monitored by the clinical investigator, assigned physician, nurses, and clinical staff. Clinical facilities were also available to handle emergencies beyond the capability of the clinical site. Subjects were free to leave the study at any time they wish. Besides, withdrawal to protect the health of subjects was also considered according to the clinical investigator's decision.

Determination of Azithromycin in Plasma

Inhouse specific, selective, sensitive, accurate, and precise high-performance liquid chromatography (HPLC) coupled with MS/MS detector was used for the determination of azithromycin in the plasma samples obtained from each subject. The analytical method was validated according to FDA bioanalytical method validation guidelines.[20,21] The standard calibration curve's linearity was evaluated for concentrations range from 10.0–600.0 ng/mL, which assume to cover the plasma concentration range of azithromycin obtained after therapeutic oral doses of the drug. Roxithromycin was used as an internal standard. The lower limit of quantitation (LLOQ) of azithromycin in plasma was 10.0 ng/mL. As recommended by above guidance,[20,21] after completing the clinical phases (at the end of period 2), all plasma samples obtained from each subject for the test and reference products together with standard calibration curve and quality control (QC) samples (low, medium and high) were analyzed as one batch in one analytical run to determine azithromycin concentrations in the

unknown authentic samples. Moreover, azithromycin plasma levels were not measured by extrapolation below the LLoQ or above the upper limit of quantitation (ULoQ) of the standard calibration curve.[20,21]

Pharmacokinetic and Statistical Data Analysis

Kinetica software was used for all pharmacokinetic and statistical data analysis of azithromycin. Excel achieved plotting of plasma concentration versus time data. Individual plasma concentrations at each time point of the test product were statistically compared against the corresponding concentrations for the reference product by analysis of variance (ANOVA) tests to find any significant difference (if any) between the test's concentration-time profiles versus the reference products. The pharmacokinetic parameters were calculated for each subject and each period applying non-compartmental analysis. The primary pharmacokinetic parameters used for bioequivalence testing[11-14] were calculated, including the maximum azithromycin plasma concentration (C_{max}), the time at which C_{max} occurs (T_{max}), and area under plasma concentration versus time curve from time of drug intake (t_0) and up to the last blood sample withdrawal at 72 hours post-dosing (truncated $AUC_{0\text{-}72hr}$). The values C_{max} and T_{max} were obtained directly from the concentration versus time profile of each subject. Truncated $AUC_{0\text{-}72hr}$ was calculated for each subject by trapezoidal rule. The mean ± SD of azithromycin plasma concentration versus time data for each test and the reference products were plotted in rectilinear graphs. Besides, the mean of both the test and the reference products were plotted together in rectilinear and semi-log graphs.

The descriptive statistics including arithmetic means, geometric means, the ratio of means, maximum values, minimum values, standard deviation (SD), coefficient of variation (CV) were determined for all pharmacokinetic parameters. ANOVA test was applied for the pharmacokinetic parameter C_{max}, T_{max}, and $AUC_{0\text{-}72hr}$ and the Ln-transformed values of C_{max} and $AUC_{0\text{-}72hr}$ to demonstrate the effect of the period, subjects nested in sequence, treatment (formulation) and sequence. Differences were declared statistically not significant at 5% significance level ($\propto$=0.05) when p >0.05. [22,23] The test product (T) was declared bioequivalent to the reference product (R) if the 90% CI of the ratio (T/R) of the Ln-transformed C_{max} and $AUC_{0\text{-}72hr}$ lies within 80–125%.[11-14,24] Schuirmann two one-sided t-test was also achieved as further support for 90% CI test.

RESULTS AND DISCUSSION

Study Conduct

The present bioequivalence study between a newly developed formulation (suspension) containing 200 mg azithromycin per 5 mL against the reference brand product Zithromax® suspension containing 200 mg azithromycin per 5 mL was conducted according to FDA and EMEA guidance on bioavailability and bioequivalence.[11-14] As recommended by these guidelines concerning long half-lives drugs as azithromycin, blood sampling for up to 72 hours post-dosing and calculating truncated $AUC_{0\text{-}72hr}$ was used in this study. Table 1 presents the demographic data of the 28 subjects who participated in the study.

Both the test and the reference products were well tolerated by all subjects. Unexpected adverse events/reactions that influence study conduct were not recorded other than those reported in Table 2, which were mild/moderate and recovered without any medical action.

No dropout or withdrawal occurred during the entire study, and all subjects who started the study completed both periods of the study. The subjects were discharged without any significant changes in their baseline clinical data.

Bioanalytical Data

The bioanalytical method applied in the current study was validated according to FDA bioanalytical method validation guidance.[20,21] The lower limit of quantitation (LLoQ) of azithromycin in plasma was 10.0 ng/mL. The developed method of analysis used in this investigation provided accurate, precise, specific, sensitive, and selective assay of azithromycin in human plasma. The correlation coefficients (r) for all standard calibration curves in plasma samples were more than 0.9998. The intra- and inter-batch precision and accuracy for low, mid and high concentrations were less than 15% which is within the accepted range as recommended by the above guidelines.[20,21] The recovery of azithromycin in plasma for three concentration ranges were reproducible, and the average value was 79 ± 8 SD. Therefore, this study's bioanalytical method is suitable for analyzing azithromycin in plasma samples collected from pharmacokinetics, bioavailability, and bioequivalence studies.

Plasma Concentrations Data

The mean ± SD for plasma concentrations versus time profiles of azithromycin plotted in a rectilinear graph for the test and the reference products, respectively, are introduced in Figures 1A and 1B. The mean plasma concentration versus time profiles for both products plotted in rectilinear and semilog graphs, respectively, are depicted in Figures 2A and 2B. It is obvious from these figures that the absorption of azithromycin from suspension is rapid since plasma levels above the LLoQ (10 ng/mL) appeared in most subjects and for both products after 20 minutes post-dosing and it reached maximum levels within 2 hours. The drug's plasma levels declined exponentially as a

Table 1: Demographic data of 28 male subjects who participated in the study

Subjects	*Mean*	*± SD*	*%CV*	*Min*	*Max*
Age (years)	25.1	5.6	22.4	20	46
Weight (Kgs)	78.4	11.9	15.2	63	89
Height (m)	1.72	0.07	4.1	1.59	1.81

Table 2: Adverse events (AE) and adverse drug reaction (ADR) observed after administration of the Test (T) and the reference (R) drug products

Sub. No.	*Period*	*Product*	*AE/ADR*	*Intensity*	*Relation to product*	*Recovery*	*Medical action*
01	1	R	Nausea	Mild	Probable	Complete	No/Audit only
06	1	T	Nausea	Mild	Probable	Complete	No/Audit only
07	1	T	Gastric upset	Mild	Probable	Complete	No/Audit only
11	1	R	Weakness	Mild	No	Complete	No/Audit only
14	1	T	Headache	Moderate	No	Complete	No/Audit only
20	1	R	Dizziness	Mild	Probable	Complete	No/Audit only
26	1	R	Allergy	Mild	Probable	Complete	No/Audit only
27	1	T	Fatigue	Moderate	No	Complete	No/Audit only
03	2	T	Allergy	Mild	Probable	Complete	No/Audit only
05	2	T	Gastric upset	Mild	Probable	Complete	No/Audit only
09	2	R	Nausea	Mild	Probable	Complete	No/Audit only
10	2	R	Gastric upset	Mild	Probable	Complete	No/Audit only
18	2	T	Weakness	Moderate	No	Complete	No/Audit only
19	2	R	Gastric upset	Mild	Probable	Complete	No/Audit only
22	2	R	Headache	Mild	No	Complete	No/Audit only
25	2	T	Dizziness	Moderate	Probable	Complete	No/Audit only
28	2	R	Myalgia	Moderate	No	Complete	No/Audit only

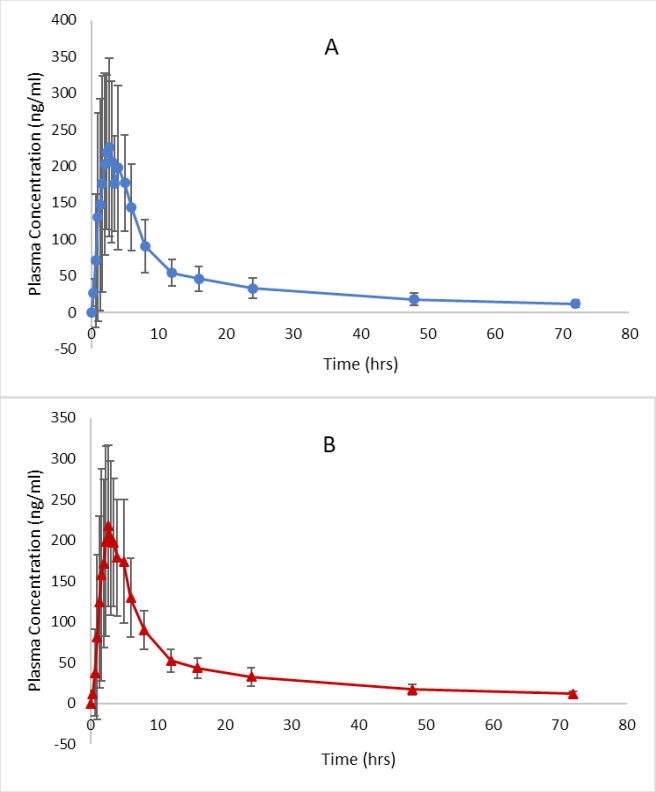

Figure 1: Plasma concentrations versus time profiles of azithromycin suspension (Mean ± SD): A) Test product, B) Reference product

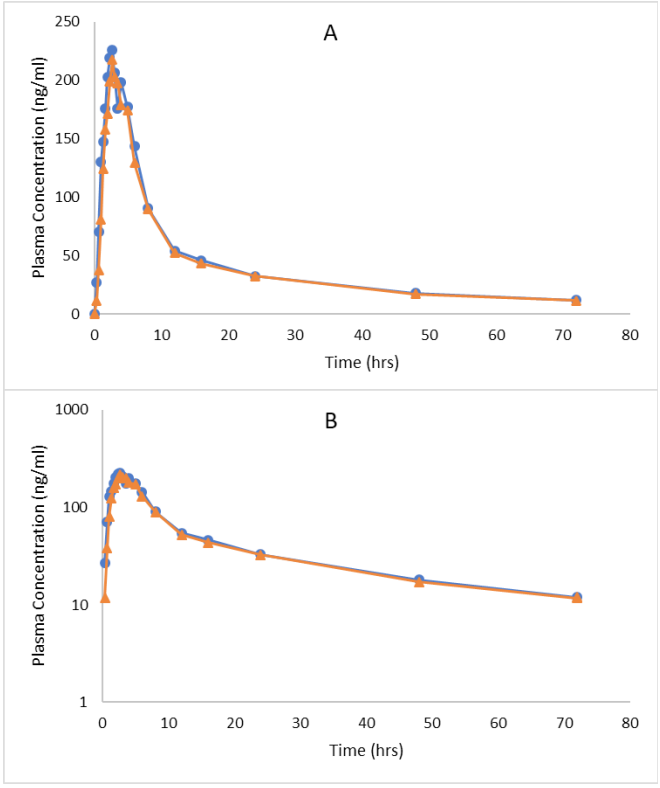

Figure 2: Mean plasma concentrations versus time profiles of azithromycin suspension for both test and reference products: **A:** Rectilinear graph, test product bullet and reference product triangle, **B:** Semilog graph, test product bullet and reference product triangle

slow distribution phase followed by a long elimination phase, as shown in Figure 2B. The same pharmacokinetic behavior of azithromycin found in the current study was reported previously.[1]

Figueres 1 and 2's visual observation shows good agreement between the plasma concentrations-time profiles for both products since both profiles are almost superimposable. ANOVA tests for the individual plasma concentrations at each time point of the test product with the corresponding concentrations for the reference product revealed statistically

Table 3: Pharmacokinetic parameters of azithromycin suspension after a single dose of the test and the reference drug products. Dose 500 mg (12.5 mL) 200 mg/5 mL

Pharmacokinetic parameter	*Test product Mean ± SD (%CV)*	*Reference product Mean ± SD (%CV)*
C_{max} (ng/mL)	358.22 ± 120.56 (33.7)	315.82 ± 89.52 (28.3)
$AUC_{0\text{-}72hr}$ (ng.hr/mL)	2722.3 ± 1116.2 (41.0)	2567.2 ± 846.7 (33.0)
T_{max} (hr)	2.57 ± 1.19 (46.3) (2.67)*	2.63 ± 1.10 (41.8) (2.67)*

*Median

Table 4: Ratio of pharmacokinetic parameters of the test (T) versus reference (R) drug products

Pharmacokinetic parameter	*Ratio of geometric mean T/R*	*Relative bioavailability* (%)*
C_{max}	1.12	113
$AUC_{0\text{-}72hr}$	1.04	106

*Relative bioavailability (%) = Ratio of (arithmetic mean T/R) X100

Table 5: A total of 90% confidence interval (CI) for pharmacokinetic parameters of the Test versus Reference drug products

Pharmacokinetic Parameter	*90 % CI*	*Accepted 90% C I*
Ln C_{max}	102.67–121.69	80.00–125.00
Ln $AUC_{0\text{-}72hr}$	95.81–112.43	80.00–125.00

Table 6: Schuirmann two one-sided t-test for pharmacokinetic parameters of the test versus reference drug products

Pharmacokinetic Parameter	*Lower t(26df)*	*Upper t(26df)*	*Accepted* $T_{L\ and}\ T_U \geq t(0.05\text{-}26\ df)$
Ln C_{max}	2.2453	6.7162	1.7056
Ln $AUC_{0\text{-}72hr}$	3.9650	5.5140	1.7056

insignificant differences. Thus, this result indicates that the absorption and disposition characteristics of azithromycin for the test product are comparable to the reference product and suggest bioequivalence of both products. At zero time (before drug intake of period 2 of the study), azithromycin was below to the LLoQ (10.0 ng/mL) in the plasma samples obtained from all subjects; thus this ensures the absence of carry-over effect and also indicates that 3 weeks washout interval between dosing is quiet enough for bioequivalence study of this drug.

Pharmacokinetic and Statistical Data Analysis

Table 3 presents the descriptive statistics of the pharmacokinetic parameters C_{max}, $AUC_{0\text{-}72hr}$ (truncated $AUC_{0\text{-}72hr}$), and T_{max} obtained from the pharmacokinetic analysis of the individual plasma concentrations versus time data of the test and reference products. Table 3 shows a good similarity between the pharmacokinetic parameters of the test versus the reference product. Moreover, the interindividual differences in C_{max}, $AUC_{0\text{-}72hr,}$ and T_{max} were almost identical between the two products, as shown in Table 3. Measuring truncated AUC up to 72 hours post-dosing for long half-life drug as azithromycin (terminal half-life 2-4 days)[1,3,4,8,10] is recommended by FDA[11-13] and EMEA[14] guidance.

Interestingly, the average C_{max} value presented in the current study (Table 3) demonstrated slight differences to that found in other studies after administration of azithromycin tablet, capsule or suspension.[3-10,25] Besides, the mean T_{max} value found in this study (Table 3) and the mean values published in all literature are similar, which is about 2 hours.[1,3-10,25]

Statistical comparison applying ANOVA test between the test and the reference products for C_{max}, $AUC_{0\text{-}72hr}$ and T_{max} exhibited insignificant differences for period, formulation and sequence effects. However, the subjects nested in a sequence effect was significant due to interindividual variations. The arithmetic and geometric mean ratios in addition to the relative bioavailability for C_{max} and $AUC_{0\text{-}72hr}$ values are demonstrated in Table 4. The range of 90% confidence interval (CI) and the results of Schuirmann two one-sided t-tests for these parameters are shown in Table 5 and 6, respectively. The 90% CI for these parameters lies within the accepted range of 80-125%.[11-14] The non-parameter Kruskal Wallis test and Friedman test demonstrated no significant difference between both products' Tmax values. Thus, according to FDA[11-13] and EMEA[14] guidance on bioequivalence, it can be concluded that the newly developed generic azithromycin suspension is bioequivalent to the reference brand Zithromax® suspension and can be considered interchangeable and prescribable in clinical practice.

CONCLUSION

It was concluded from this study that the newly developed generic azithromycin suspension was bioequivalent to the reference brand Zithromax® suspension. Both formulations were well tolerated by all subjects, and unexpected adverse events were not recorded throughout the study. Therefore, the newly developed generic azithromycin suspension may be interchangeable to Zithromax® suspension and prescribable in clinical practice.

ACKNOWLEDGEMENTS

The authors are very grateful to all subjects participated in the study. Great thanks and appreciations to the staff who conducted the clinical and analytical parts of the study. Miss Zahraa Al-Tamimi and Manar Al-Tamimi are greatly acknowledged for the technical assistance.

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