RESEARCH ARTICLE

Bioequivalence and Pharmacokinetic Comparison of Two Levofloxacin Oral Formulations in Arabic Healthy Men

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ABSTRACT

Levofloxacin is a fluoroquinolone antibacterial agent with a broad-spectrum activity. A comparative bioavailability (bioequivalence) study was conducted between a newly developed tablet formulation containing 500 mg levofloxacin against the brand product Tavanic® tablet. Both drug products were given as a single dose to 32 healthy male adult Arabic subjects applying fasting, two-treatment, two-period, two-sequence, randomized cross-over design, separated by one-week washout interval between dosing. Nineteen serial blood samples were collected from each subject before drug intake (zero time) and up to 36 hours post-dosing. Levofloxacin concentrations were measured in the plasma samples obtained from each subject to determine the pharmacokinetic parameters C_{max}, T_{max}, AUC0–t, AUC0–∞, Thalf, and mean residence time (MRT) applying non-compartmental data analysis. The parameters mentioned above were statistically analyzed by analysis of variance (ANOVA) test. Ln-transformed values of the primary parameters used for bioequivalence testing, namely $C_{max,}$ AUC0–t, and AUC0–∞ were also statistically analyzed by ANOVA and 90% confidence interval tests. Based on functional data analysis (FDA) and european medicines evaluation agency (EMEA) criteria on bioequivalence, the results obtained from the current investigation demonstrated bioequivalence between the newly developed levofloxacin tablet and the brand product Tavanic® tablet. All subjects well tolerated both drug products. Therefore, both drug products can be considered interchangeable in clinical practice.

Keywords: Arabic Healthy Men, Bioequivalence, Levofloxacin, Pharmacokinetics.

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INTRODUCTION

Levofloxacin is a quinoline carboxylic acid derivative. It is the synthetic L-isomer of the racemic quinolone ofloxacin. Levofloxacin is a fluoroquinolone antibacterial agent indicated for the treatment of a wide range of infections, including nosocomial pneumonia, community-acquired pneumonia, acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, complicated/uncomplicated skin and skin structure infections, chronic bacterial prostatitis, acute pyelonephritis, complicated and uncomplicated urinary tract infections, and post-exposure inhalational anthrax. Levofloxacin is available in tablet dosage forms containing 250 and 500 mg of the drug.[1,2]

Levofloxacin is rapidly and almost completely absorbed from gastrointestinal tract (GIT) after oral intake. The time to reach maximum plasma level (T_{max}) ranges from 1 to 2 hours, and the oral bioavailability approaches 100%. Food has little effect on the rate and extent of levofloxacin oral bioavailability. Levofloxacin follows linear (dose-proportional) two compartments pharmacokinetics with first-order elimination over a wide dose range. Single oral doses of 50 to 1000 mg produce average maximum plasma concentration (C_{max}) and area under plasma concentration-time curve (AUC) ranging from about 600 to 9400 ng/mL, and 4700 to 108000 ng.h/mL, respectively.[3,4]

The pharmacokinetic characteristics of levofloxacin are identical after single and multiple oral dosage regimens.

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Levofloxacin is widely distributed throughout the body and penetrates well into most body tissues and fluids with an average apparent volume of distribution of approximately 1.1 L/kg. Almost 24–38 percent of the drug bound to plasma proteins (mainly albumin) and the degree of binding is not saturable (i.e., concentrations independent). The drug levels in tissues and fluids are generally greater than those found in plasma; however, the penetration into the cerebrospinal fluid is relatively poor.[3,4]

The terminal plasma elimination half-life (T_{half}) of levofloxacin ranges from 6 to 12 hours in subjects with normal renal function. About 80% of the drug is eliminated as an unchanged drug in the urine through glomerular filtration and tubular secretion. Since renal clearance and total body clearance are highly dependent on creatinine clearance, dosage adjustments are needed in patients with significant renal rather than hepatic impairment. Factors including age, gender or race of subjects have no appreciable impact on levofloxacin pharmacokinetics, considering the differences in renal function and body mass and composition due to these factors.[3,4]

Important drug interactions exist between levofloxacin and other fluoroquinolones, with aluminum and magnesium-containing antacids and ferrous sulfate, resulting in a significant reduction in the drug absorption. Therefore, the agents mentioned above should be given at least 2 hours before or after levofloxacin intake.[3,4] Besides, administration of levofloxacin with calcium-fortified orange juice reduce C_{max} and T_{max} values of the drug by about 14–18% and 50%, respectively.[5]

According to FDA and EMEA guidance on bioavailability and bioequivalence, 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 considered to be bioequivalent if they are pharmaceutical equivalents (i.e., similar dosage forms which may be produced 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 same therapeutic moiety and administered under similar experimental conditions.[6]

It is evident from so many clinical observations documented from physicians, pharmacists, and health professionals that drug products of similar dosage form, the identical route of administration, and the same dose of the same drug may demonstrate significant differences between their therapeutic and/or their adverse effects. In most situations, the differences are due to dissimilarities in drug absorption rate and/or extent of drug absorption and consequently on the rate and/or extent of drug bioavailability.[7-10]

Therefore, in the last three decades, bioequivalence studies gained increasing attention and became obligatory for registration and marketing of generic drug products since these studies provide important and vital information that ensure the availability of safe and effective medicines to the patients.[6] Recently, many formulation approaches and technologies are ongoing to enhance the rate and extent of drug absorption in order to improve the rate and extent of drug product bioavailability particularly for Class II drugs which possesses the problem of low water solubility.[11,12]

There are four approaches that can be applied for evaluating bioequivalence between generic and brand drug products including *in-vivo* studies (pharmacokinetic, pharmacodynamic, and studies with a measurable clinical outcome), in addition to *in-vitro* studies. The pharmacokinetic method is mostly and commonly applied for establishing bioequivalence since it is faster, easier, appropriate, reliable, and less costly than the other above-mentioned *in-vivo* methods. Besides, the pharmacokinetic approach can directly assess the rate and extent of drug absorption by estimating C_{max} and T_{max} (which reflect the rate of systemic absorption), and AUC which reflect the extent of drug absorption and total systemic exposure.[6]

The average bioequivalence (relative bioavailability) between a generic and a brand formulation is assessed based on statistical analysis applying ANOVA and 90% confidence interval (90% CI) tests for ln-transformed values of the primary pharmacokinetic parameters used for bioequivalence testing, strictly speaking, C_{max} and AUC. A generic and a brand drug product are considered bioequivalent if 90% CI lie within 80.00–125.00% ranges.[6]

For antimicrobial therapy expressly, documentation of bioequivalence for generic drug products[7-9] are very important and vital and have special concern to achieve the required therapeutic plasma concertation with optimal effect and minimum adverse effect to assure the elimination of the microorganism which causes the infection. On the other extreme, if the antimicrobial plasma concentration falls below the required level to ensure efficacy, the infection will not be eradicated, and the possibility for the risk of developing resistance to the drug may increase. Therefore, several bioequivalence studies were conducted for levofloxacin in different countries to establish the bioequivalence of the marketed generic drug products in their populations.[13-18] Among these, researches were published for levofloxacin bioequivalence for Chinese,[13] Bangladeshi,[14] Mexican,[15] Indian,[16] Korean,[17] and Brazilian populations.[18]

In general, pharmaceutical drug industries and Arabic drug manufacturers are producing so many generic products with prices lower than the corresponding multinational competitors, taking into account the quality of their pharmaceutical products by *in-vitro* and *in-vivo* (bioequivalence) evaluations. Thus, the objects of the current investigation were to compare the pharmacokinetic characteristics and assess the bioequivalence between a newly devolved tablet formulations containing 500 mg levofloxacin as a test product with the reference brand product Tavanic® tablet after administering both drug products to healthy male adult Arabic subjects under fasting state.

MATERIALS AND METHODS

Dissolution Testing

As recommended by FDA guidance on dissolution, as a prerequisite for bioequivalence testing of a test/generic drug formulation against the reference/brand drug formulation,

a promising in-vitro dissolution performance between the test and the reference formulations and under discriminating dissolution conditions (including type of dissolution apparatus, speed of rotation, volume and pH of the dissolution media) should be obtained. The similarity factor (F2) between the dissolution profiles of the test versus the reference drug formulations should be measured. For dissolution profiles of two drug formulations (e.g., test versus reference) to be considered similar, the F2 values should be more than 50 (50–100), confirming pharmaceutical similarity between both formulations and may reflect sameness or equivalence of the in-vivo performance of them.[19]

Study Products

The test formula was a newly developed tablet containing 500 mg levofloxacin. The reference formula was the brand Tavanic® tablet containing 500 mg levofloxacin manufactured by Pfizer USA.

Study Protocol

The study was conducted according to a study protocol prepared by the principal investigator and approved by the clinical investigator and the Institutional Review Board (IRB). The protocol involved all details of the study, including the informed consent form, the study's design, the clinical procedures, bioanalytical phase, all pharmacokinetic and statistical data analysis, and eventually documentation and submission of the final report. No changes and/or amendments to the study protocol that significantly influence the conduct of the study were implemented without obtaining the principal investigator, the clinical investigator and IRB written approval and favorable opinion, except when necessary to eliminate an immediate risk to the subject or if the change is minor such as logistical or administrative amendments. The study protocol was prepared following and to adhere with FDA and EMEA guidance and criteria in bioequivalence.[6]

Ethical Considerations

The study was conducted according to ICH guidelines for good clinical practice[20,21] and the declaration of Helsinki. [22] The subjects provided their informed consents at the screening stage before the commencement of the study. The subjects were thoroughly informed about all details of the study, including the risks, benefits, procedures, objectives, and rights as research subjects. The consent form was written in clear, easy and understandable English and Arabic languages using non-technical terms. The investigators did not force the subjects by any means to participate or to continue participation against their will. The subjects voluntarily provided written informed consent before performing the screening and all required examinations proving the eligibility of the participants. The consent procedures were achieved under the supervision of the principal and clinical investigators. The clinical investigator signed the consent form of each subject, and by the subject and two witnesses. Two original copies of the consent forms were signed, one copy was given to the participant to know and preserve his rights, and the other copy was saved in the study file. All the screened subjects were compensated even those who were not selected to be enrolled in the study.

Study Design

A single-dose, fasting, two-treatment, two-period, two-sequence, randomized cross-over design was applied. [6] The study was open-labeled but laboratory-blind. An equal number of subjects were randomly assigned to each dosing sequence of the treatments (test and reference formulas). Thus, half of the participants received a single 500 mg dose of the test drug product in the period I, while the other half received a single 500 mg dose of the reference drug product according to the randomization schedule set in the study protocol. In period II, the order of drug products administration was reversed. The treatments were separated by a one-week washout interval between dosing (i.e., period I and period II).

Inclusion Criteria

The subjects were considered healthy and eligible for participation in the current research based on the following inclusion criteria: 1) adult male with age between 18-48 years, and within the limits for his height and weight as defined by the body mass index (BMI) range from 18 to 30 kg/m^2; 2) the subject is willing to adhere with the conditions of the study and undergo the pre- and post-study medical examinations stated in the protocol; 3) non-smokers or light smokers (not more than10 cigarettes per day), and no illicit drug or alcohol abuse; 4) no history of contraindication and/or allergy to levofloxacin and any related agents; 5) no history for the need of chronic medication(s); 6) normal physical checkup and clinical examinations including vital signs, ECG, hepatic, renal, respiratory, cardiac, gastrointestinal and psychiatric; 7) normal clinical laboratory tests including biochemistry, hematology, routine urine analysis, negative for HIV and for hepatitis B and C; 8) no medications were taken for the last two weeks prior the study, no hospitalization or blood donation or participation in any clinical trials such as pharmacokinetic, bioavailability or bioequivalence within the last 2 months prior to the present study.

Study Conduct

The subjects were confined in the clinical site in each period before about 14 hours of drug product administration until 24.0 hours after dosing. The subjects were admitted to the clinical site at about 6 PM. They served a traditional dinner at 8 p.m. In the morning of the next day at 8 AM and after overnight fasting of 12 hours, the investigational drug products were administered with 240 mL of tepid water. Drug administration was carried out by qualified clinical staff under the supervision and monitoring of the clinical investigator and the quality assurance (QA). No water was allowed 2 hours before and after dosing. Standard lunch, snack, and dinner were served 4-, 8- and 12-hours post-dosing, respectively. The meals were identical in both study periods, and the subjects were asked to have the entire meal.

The subjects were not allowed to take any medication for any reason(s) other than the investigational drug products unless according to the clinical investigator's decision. The

subjects were not allowed to have any meals and fluids other than those served in the study. Xanthine-containing products were prohibited 12 hours before and 12 hours after dosing. Grapefruit juice or beverages containing grapefruit were not allowed within the past week before period I of the study until the completion of the whole study, which is the time of discharge after the end of period II. The subjects were ambulatory but were prohibited from strenuous activity. They were not allowed to sleep or lie during the first four hours of drug administration and they remained seated upright.

Blood Sampling

An intravenous cannula was placed into the forearm anticubital vein of each subject. The cannula was left in place until 24 hours after blood sampling. Immediately after each blood sample withdrawal, the cannula was flushed with 0.5 mL of normal saline containing 10 units heparin to keep the cannula patent and prevent blood clotting. Besides, to get rid of residual blood in the cannula, about two drops blood was discarded before the next blood sample withdrawal. During each period of the study, 5 mL blood samples were obtained from each subject before dosing (zero time) and then at 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 24.0, 30.0 and eventually at 36.0 hours after dosing. A total of 19 blood samples were collected from each subject at each period. The whole volume obtained from each participant was 210 mL, including the blood taken for screening and clinical laboratory tests pre- and post-dosing. The blood samples were directly transferred to heparinized tubes and then immediately centrifuged for 5 minutes at 4000 rpm. The plasma samples were separated by polypropylene disposable tips and then directly transferred to Eppendorf tubes and then immediately stored at -30 ± 5°C until the day of analysis to determine levofloxacin concentrations in the plasma samples. All the tubes used to collect blood and plasma samples were labeled according to the in-house coding system. The labeling/coding system were confidential, the principal investigator and the QC personal solely have access to the labeling/coding system.

Safety Evaluation

The subjects were judged healthy by thorough examination in the screening stage by physical, medical, and clinical laboratory examinations. Besides, after each study period, clinical examinations and clinical laboratory tests, including hematology, biochemistry, and urinalysis were repeated. Vital signs including blood pressure, pulse, and temperature were recorded at 1.0 hours before drug administration and then at 2, 4, 8, 12, 24 and 36 hours post-dosing. The clinical investigator, together with the clinical staff and QC responsible, were available during the whole study to observe, monitor, and document the occurrence of any adverse events (AEs), adverse drug reactions (ADR), and serious adverse effects (SAE). Besides, the clinical investigator interviewed the participants and was asked to spontaneously report any AEs/ ADR/ SAE which may occur at any time during the study, including the washout period.

Participant's Termination

The clinical investigator terminates the participation of any subjects from the study according to the following cases:

- Poor cooperation, imperfect compliance, and violation of the subject in any procedure related to the study protocol
- The occurrence of illness that continued participation may threaten and jeopardize the subject's health and well-being,
- Clinically significant changes in the subject's baseline clinical laboratory test and/or vital signs.

Levofloxacin Assay

Measurement of levofloxacin in the plasma samples was achieved following FDA guidance on bioanalytical method validation.[23] A sensitive, specific, accurate, and precise, high-performance liquid chromatography with fluorescence detection method described recently was applied to determine levofloxacin in plasma. [24] The standard calibration curve was linear over the concentration range from 20–20000 ng/mL levofloxacin in plasma with a lower limit of quantification (LLoQ) of 20 ng/mL and a correlation coefficient (r) of 0.9987 using least-squares linear regression analysis. The precision and accuracy were within the acceptable ranges for intra-day and inter-day assay. Each analytical batch/run involved standard calibration curve, quality control (QC) samples (low, mid and high), and the unknown authentic samples containing the drug collected after period II and II. No determination was achieved by extrapolation below the LLoQ or above the upper limit of quantitation (ULoQ) of the standard calibration curve.[23]

Pharmacokinetic Calculations

Kinetica software was used for calculating the pharmacokinetic parameters including C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, $K_{elimination}$ (λ_z), T_{half} and MRT obtained from plasma concentration versus time data of levofloxacin by non-compartmental data analysis applying standard methods.[25,26] Microsoft Excel was utilized for data plotting and descriptive statistics, including arithmetic means, geometric means, ratio of means, maximum values, minimum values, median, standard deviation (SD), and coefficient of variation (CV) relative bioavailability. Microsoft Word 2007 was used for data edition and reporting.

The maximum concentration of drug in plasma (C_{max}) and the time to peak (T_{max}) were determined directly from each subject's concentration versus time curve. The terminal elimination rate constant ($K_{elimination}$ or λ_z) was measured for each subject and for each drug product by linear regression of at least the last three points at the terminal phase of each subject's log-concentration versus time curve. The terminal elimination half-life (T_{half}) was calculated from $0.693/\lambda_z$. The AUC_{0-t} which is the area under plasma concentration versus time curve from time zero up to the time of last blood sample withdrawal (t_{last}), was calculated using the Trapezoidal rule. The $AUC_{t-\infty}$ which is the extrapolated area ($AUC_{extrapolated}$) which is also called residual ($AUC_{residual}$) or tail area (AUC_{tail}) is the area under plasma concentration versus time curve from t_{last} to infinity was calculated as C_{last}/λ_z. The $AUC_{0-\infty}$ which is the area under plasma concentration versus time curve from time zero to infinity, was calculated from the sum of AUC_{0-t}

and $AUC_{t-\infty}$. The %extrapolated AUC was obtained from $(AUC_{t-\infty}/AUC_{0-\infty}) \times 100$. The MRT is the mean residence time estimated from the area under the moment curve (AUMC) divided by AUC. [25,26]

Statistical Analysis

ANOVA tests were performed for all calculated parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, $K_{elimination}$ (λ_z), T_{half}, and MRT. Moreover, ANOVA and 90% confidence interval (90% CI) tests were applied only for the in-transformed parameters C_{max}, AUC_{0-t} and $AUC_{t-\infty}$ since they are considered the primary parameters for bioequivalence evaluation recommended by FDA and EMEA guidance.[6] The non-parametric Friedman design and Kruskal-Wallis testes were utilized to evaluate the differences between each of T_{max} and MRT values against their corresponding values for the reference formulations.[27] The average bioequivalence of two drug products was concluded if 90 % CI interval for each parameter C_{max}, AUC_{0-t} and $AUC_{t-\infty}$ lie within 80.00 to 125.00%. Differences between the test against the corresponding reference drug products parameters are declared statistically not significant at 5% significance level ($\propto = 0.05$) when $p \geq 0.05$. Schuirmann's two one-sided t-tests were utilized as additional confirmation to 90% CI for bioequivalence judgment.[6]

RESULTS AND DISCUSSION

Dissolution Data

It appeared from this investigation that both test and reference formulas entirely dissolved within 45 minutes. The dissolution

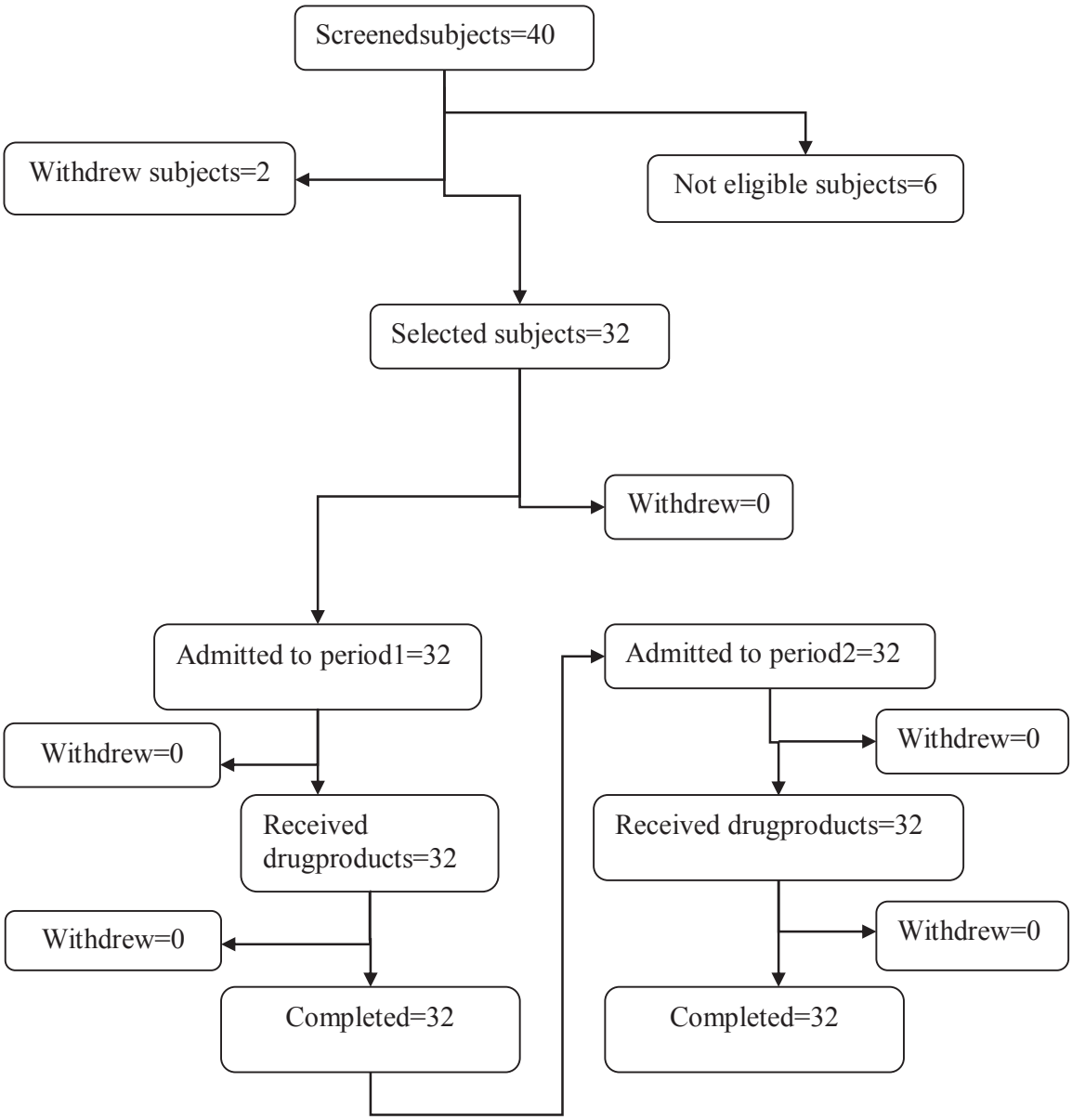

Diagram 1: Subject's flow chart from screening stage to discharged from the study.

profiles of both formulas were almost similar and to a good extent superimposable. Besides, the similarity factor F2 was calculated to be 91, which indicate sameness and pharmaceutical equivalence of both formulas, and thus, the invitro result was so encouraging and promising to proceed for to invivo bioequivalence study as recommended by FDA guidance on dissolution.[19]

Study Description

The proper design for the current study and the schedule of blood sampling strategy were obtained from previously published investigations after levofloxacin administration for pharmacokinetics, bioavailability and bioequivalence studies.[13-18] According to FDA and EMEA guidelines, cross-over design is recommended in bioequivalence evaluation since in such design the same subject will receive the investigational drug products (e.g., test versus reference) under similar experimental conditions, and consequently the intra-subject variability is expected to have no significant impact on the conclusion of bioequivalence.[6]

Forty subjects were screened to participate in the current research to account for any dropout and withdrawal common in clinical trials. The disposition of the subjects during the study from screening stage until discharge is shown in diagram 1. Six subjects were not eligible to be enrolled in the study according to the inclusion criteria set in the study protocol, therefore, they were excluded from the study. Two more subjects withdrew from the study due to personal reasons before drug products administration at period I. The remaining 32 subjects completed the whole study since no dropout and withdrawal of any subject occurred as illustrated in diagram 1. No violation or deviation from the study protocol was reported during this investigation, including the clinical, bioanalytical, pharmacokinetic, statistical, and bioequivalence evaluation.

Clinical Observations

Potential risks of AEs, ADRs, and SAEs are not likely to occur in this study for many reasons. Among these reasons are: 1) only single oral therapeutic dose of each drug product was administered during each period of the study, 2) the duration of washout interval was one week which is quite enough for almost complete removal of the drug from the body since the average terminal elimination half-life of the drug is about 8 hours, 3) the participants were under medical surveillance and follow up in the clinical site and even during the wash out interval, 4) moreover, adult healthy eligible individuals were selected to participate in this research.

Based on clinical observations, both drug products were well tolerated by all subjects. All the participants left the study without any clinically significant change in their clinical baseline properties, including vital signs and clinical laboratory tests (hematology, biochemistry and urinalysis). No incidences of AEs, ADRs, and SAEs were reported during the whole study. All the 32 subjects who started the study participated to the end of the study. The demographic data and the baseline vital signs of the subjects are summarized in Table 1.

Table 1: Demographic data and baseline vital signs of 32 male subjects participated in the study.

Characteristics	*Mean±SD*	*(%CV)*	*Range*
Age (years)	27.6±5.7	20.7	20-43
Height (m)	1.78±0.052	2.92	1.7-1.87
Body weight (kg)	75.5±8.66	11.5	62-89
BMI (kg/m^2)	23.96±2.55	10.6	19.7-28.7
Systolic blood pressure (mmHg)	118.1±7.7	6.5	110-130
Diastolic blood pressure (mmHg)	74.4±5.4	7.3	70-85
Pulse (beat per minute)	68.4±4.4	6.5	63-76
Temperature (°C)	36.8±0.28	0.008	36.0-37.1

Levofloxacin Plasma Concentrations

The analytical method applied in this study[24] to determine levofloxacin in the plasma was sensitive, precise, accurate, and specific according to FDA bioanalytical method validation criteria.[23] Levofloxacin was not detected (plasma levels below LLoQ 20 ng/mL) in the plasma samples obtained before drug products administration at period II for all subjects and both test and reference drug products indicating the absence of carryover effects and ensuring that one week washout interval between dosing is quite enough for bioequivalence study of levofloxacin. The drug levels were above the LLoQ and detected in the plasma samples obtained from most subjects after 0.33-hour post-dosing and for both products. This indicates rapid appearance of the drug in plasma.

Applying ANOVA tests for the plasma concentrations of the drug at each time point (18 data points from 0.33–36 hours post-dosing) for the test product against the corresponding concentrations for the reference product revealed no significant differences ($p > 0.05$) between the concentration-time profiles of both products. Moreover, both products' concentrations versus time curves are almost overlapping and, to a reasonable extent, superimposable, as shown in Figure 1. Thus, the results mentioned above demonstrate an excellent similarity in both products' concentration-time profiles and disposition characteristics, including drug absorption, distribution, and elimination, as depicted in Figure 1.

Pharmacokinetic Data

Table 2 introduces the pharmacokinetic parameters of levofloxacin after administration of the test and the reference drug products. It is apparent from Figure 1 and Table 2 that for both the test and the reference formulations, levofloxacin tablet is rapidly absorbed, achieving its peak level in plasma within about one hour (range 0.67–2.5 hours), then the drug levels declined monoexponentially with average T_{half} of about 8 hours (range 7–10 hours). The MRT is a secondary pharmacokinetic parameter (which reflects drug residence in the body) that supported the similarity in the estimated T_{half} between both products since MRT was found to be around 10 hours (range 9.5–12.5 hours), as shown in Table 2.

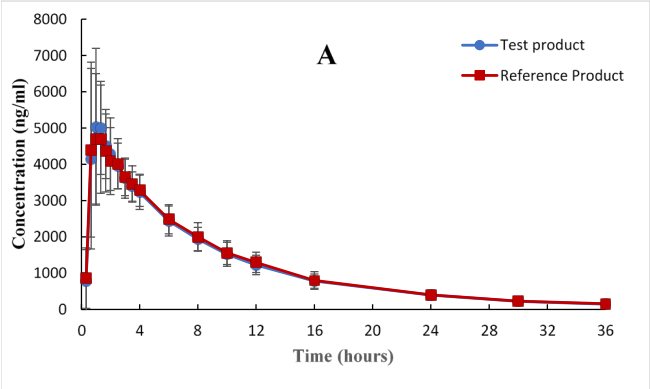

Figure 1: Plasma concentrations-time profiles (Mean±SD) of levofloxacin after a single dose of 500 mg tablet of the test and reference products plotted in (A) linear and (B) semilog scale.

Furthermore, it is evident from Table 2 that both formulations demonstrated nearly identical pharmacokinetic behaviors since the mean and the ranges of their pharmacokinetic parameters reflect the rate and extent of drug absorption and total drug exposure, namely C_{max}, $AUC_{0\text{-}t,}$ and $AUC_{0-\infty}$ are approximately similar. In addition, for both products, low inter-subject variation with %CV ranges from almost 10–20 % were found for all the calculated parameters, as shown in Table 2. A higher %CV of about 50% was observed for T_{max} values and both products (Table 2). The extrapolated AUC had a negligible contribution to the total AUC ($AUC_{0-\infty}$) with a $\%AUC_{extrapolated}$ of about 3% (Table 2). Thus, blood sampling for 36 hours post-dosing after oral levofloxacin applied in this research and using 20 ng/mL as LLoQ of the drug in plasma are adequate for reliable estimation of levofloxacin's pharmacokinetics in humans. Thus, all the findings mentioned above confirm the similarity in pharmacokinetic characteristics of test and reference formulas.

Statistical Analysis

ANOVA tests for all the pharmacokinetic parameters obtained from the test product versus their corresponding values obtained for the reference product (Table 2), including C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, $K_{elimination}$ (λ_z), $T_{half,}$ and MRT, and for the ln-transformed C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$ revealed no significant differences ($p > 0.05$) mainly for the formulation's source of variation (Table 3) which is the primary cause of differences (if any) between formulations in bioequivalence evaluation.[6] The non-parametric Friedman design and Kruskal-Wallis testes exhibited no significant ($p > 0.05$) differences

Table 2: Pharmacokinetic parameters of levofloxacin after a single dose of 500 mg tablet of the test and the reference products.

Test product								
Statistics	*C_{max} (µg/mL)*	*T_{max} (hr)*	*$AUC_{0\text{-}t}$ (µg.hr/mL)*	*$AUC_{0\text{-}\infty}$ (µg.hr/mL)*	*% AUC_{extra}*	*λ_z (hr^{-1})*	*$T_{0.5}$ (hr)*	*MRT (hr)*
Mean	5.97	1.22	42.00	43.55	3.5	0.0939	7.43	10.2
±SD	1.37	0.59	8.01	8.52	0.89	0.0072	0.61	0.77
%CV	23.0	48.4	19.1	19.6	25.4	7.7	8.2	7.5
Minimum	3.76	0.67	26.54	27.55	2.1	0.0760	6.62	9.12
Maximum	8.08	3.0	58.72	61.95	5.6	0.1047	9.12	12.53
Geomean	5.81	1.0*	41.24	42.73	**	**	**	**

Reference product								
Statistics	*Cmax (µg/mL)*	*Tmax (hr)*	*AUC0-t (µg.hr/mL)*	*AUC0-∞ (µg.hr/mL)*	*% AUCextra*	*λz (hr^{-1})*	*T0.5 (hr)*	*MRT (hr)*
Mean	5.75	1.28	42.69	44.38	3.7	0.0938	7.44	10.41
±SD	1.38	0.65	8.76	9.31	0.97	0.0078	0.64	0.90
%CV	24.0	50.8	20.5	21.0	26.2	8.3	8.6	8.6
Minimum	2.98	0.67	24.71	25.83	2.2	0.0796	6.50	8.55
Maximum	7.67	2.5	59.98	63.24	5.7	0.1067	8.71	12.57
Geomean	5.55	1.2*	41.76	4338	**	**	**	**

* Median, **Geomean calculation are not statistically useful for bioequivalence testing for these parameters.

Table 3: ANOVA tests for the pharmacokinetic parameters.

	Source of variations and their p values		
Parameters	*Formulation*	*Sequence*	*Period*
Cmax	0.4173	0.2314	0.7224
AUC0-t	0.1974	0.0272	0.2417
AUC0–∞	0.1466	0.0325	0.2233
Ln Cmax	0.3838	0.2097	0.6568
Ln AUC0-t	0.3299	0.0169	0.3435
Ln AUC0–∞	0.2458	0.0191	0.3172
Tmax	0.6956	0.8639	0.4645
λz	0.9922	0.7786	0.9728
T0.5	0.9384	0.8363	0.9554
MRT	0.0814	0.1856	0.4929

Table 4: Geometric mean ratio, relative bioavailability and 90% confidence interval (90% CI) for the test versus the reference products.

Parameter	*Geomean ratio*	*Relative* bioavailability*	*90% CI lower limit*	*90% CI upper limit*
Cmax	104.7	103.7	96.28	112.79
AUC0 – t	0.99	0.98	96.69	100.89
AUC0 – ∞	0.99	0.98	96.42	100.65

*****Relative bioavailability =Arithmetic Mean Test/Arithmetic Mean Reference.

Table 5: Schuirmann's two one-sided t-test for pharmacokinetic parameters of the test versus reference drug products.

Pharmacokinetic Parameter	*Lower t(30 df)*	*Upper t(30 df)*	*Accepted TL and TU ≥ t(0.05-30 df)*
Cmax	3.9025	5.6702	1.6973
AUC0 – t	16.8050	18.7860	1.6973
AUC0 – ∞	16.4230	18.7910	1.6973

between T_{max} and MRT values against their corresponding values for the reference formulations.

The geometric mean ratio and the relative bioavailability for each of the primary parameters used in bioequivalence decision specifically C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were approaching unity confirming the close similarity in the pharmacokinetics of the test and the reference drug products in term of the rate and extent of drug absorption and total drug exposure as shown in Table 4. Moreover, the ranges of 90% CI for Ln-transformed Cmax, AUC0-t and AUC0–∞ for the test versus the reference product (Table 4) were well within the bioequivalence acceptance criteria as per FDA and EMEA guidance on bioequivalence.[6] Above all, Schuirmann's two one-sided t-tests certify the results obtained from 90% CI tests as demonstrated in Table 5. Therefore, it is concluded from the current results that the newly developed levofloxacin 500 tablet formulation is bioequivalent to the reference brand product Tavanic® tablet manufactured by Pfizer USA. Consequently, both products can be considered interchangeable in clinical practice.

CONCLUSION

The present study presents the pharmacokinetic characteristics of a newly developed generic formula of levofloxacin 500 mg tablet and the reference brand product Tavanic® $^{500\ mg}$ tablet after administering healthy male adult Arabic subjects under fasting state. Moreover, according to FDA and EMEA criteria, bioequivalence was found between both products. Hence, the generic product may be interchangeable with the brand product in therapy with levofloxacin and can be prescribed in clinical practice as a safe and effective alternative to the brand product.

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