

COMPARATIVE BIOEQUIVALENCE EVALUATION OF TWO BRANDS OF OFLOXACIN 200 MG TABLETS (JEDCOFLACIN[®] VS. TARIVID[®]) IN 24 HEALTHY VOLUNTEERS: A RANDOMIZED, SINGLE-DOSE, TWO-PERIOD, TWO-SEQUENCE CROSSOVER STUDY

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This study was performed to investigate the bioequivalence of two brands of ofloxacin, namely, Jedcoflacin[®] 200 mg/tablet (from Jedco International Pharmaceuticals CO., Egypt, as a generic test product 'A') relative to Tarivid[®] 200 mg/tablet (from Aventis Pharma Deutschland GmbH, Germany, as a reference product 'B') after a single dose oral administration of 400 mg (2×200 mg) to 24 healthy adult volunteers under fasting conditions, for the purpose of registration, approval, and marketing stage. The bio-analysis of clinical plasma samples was accomplished by a HPLC method for the determination of plasma ofloxacin concentrations. Pharmacokinetic parameters, determined by standard non-compartmental methods, and analysis of variance (ANOVA) statistics were calculated using statistical analysis system (SAS) software. The parametric 90% Confidence intervals (CIs) of the least squares mean test/reference ratios were found to be within the confidence limits of 80.00-125.00% for AUC_{0→30}, AUC_{0→∞} and C_{max}, *i.e.* 87.10% to 101.95%, 87.49% to 102.19%, and 91.71% to 105.30%, respectively. This single-dose study demonstrated that the test product (A) was found bioequivalent to the reference product (B) following an oral dose of 2x200 mg/ tablet, as per predetermined regulatory criteria for bioequivalence, in the 24 fasting healthy volunteers. Therefore, the two formulations were considered to be bioequivalent.

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1. Introduction

Bioequivalence studies of generic and innovator products are a routine regulatory practice to obtain approval for registration for marketing of generic products. Ofloxacin is a synthetic broad-spectrum antimicrobial agent for oral administration [1-4]. Chemically, ofloxacin (Figure 1), is a fluorinated carboxyquinolone, namely, (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid and is marketed as a racemic mixture. The empirical formula of ofloxacin C₁₈H₂₀FN₃O₄, and its molecular weight is 361.4 [5]. The mechanism of action of ofloxacin and other fluoroquinolone antimicrobials involves inhibition of bacterial topoisomerase IV and DNA gyrase (both of which are type II topoisomerases), enzymes required for DNA replication, transcription, repair and recombination [5-7].

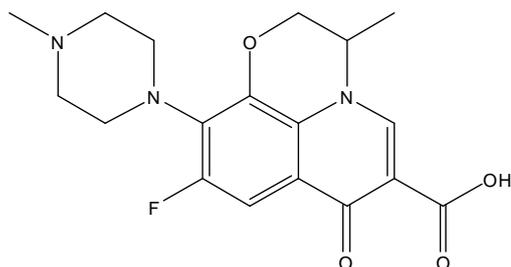


Fig. 1: Chemical structure of ofloxacin

Following its oral administration, bioavailability of ofloxacin in the tablet formulation is approximately 98% [2, 8]. Maximum serum concentrations are achieved 1-2 hours after an oral dose [9]. Absorption of ofloxacin after single or multiple doses of 200 to 400 mg is predictable, and the amount of drug absorbed increases proportionately with the dose [10]. Following multiple oral doses at steady-state administration, the half-lives are approximately 4-5 hours and 20-25 hours. However, the longer half-life represents less than 5% of the total AUC. Accumulation at steady-state can be estimated using a half-life ($t_{1/2}$) of 9 hours. The total clearance and volume of distribution are approximately similar after single or multiple doses. Both single dose and steady-state plasma profiles of ofloxacin injection were comparable in extent of exposure to those of ofloxacin tablets when the injectable and tablet formulations of ofloxacin were administered in equal doses (mg/mg) to the same group of volunteers. Food alters the onset and/or rate of ofloxacin absorption, but not the extent of absorption or the elimination rate following oral administration. Thus, food reduces peak ofloxacin concentrations (C_{max}) by 20% compared to fasting conditions. However, the extent of absorption and the half-life of ofloxacin were the same after each treatment [11]. In the current study, the bioequivalence (or rate and extent of absorption) of a single dose of ofloxacin 400 mg (2×200 mg/tablet) of Jedco, Egypt (Jedcoflacin[®] 200 mg/tablet), as a generic formulation, and of Aventis Pharma, Germany (Tarivid[®] 200 mg/tablet), as the reference one, were compared under fasting conditions. Bioequivalence of the two products was assessed based on the plasma concentration data obtained following their administration to 24 healthy adult volunteers in a balanced single center, open-label, randomized, single-dose study with two-way crossover design, to compare the bioequivalence of ofloxacin tablets between two products.

2. Experimental

2.1 Subjects

Twenty-four healthy male adult volunteers participated in the study. The mean age (\pm SD) of the volunteers was 35.46 ± 5.48 years, with a range of 28 - 45 years, mean body weight was 76.25 ± 3.88 kg with a range of 70 - 80 kg, and mean height was 174.04 ± 6.99 cm with a range of 163 - 185 cm. On the basis of medical history, clinical examination and laboratory investigation (hematology, blood biochemistry and urine analysis), no subject had a history or evidence of hepatic, renal, gastrointestinal or hematologic deviations or any acute or chronic diseases or allergy to ofloxacin or any fluoroquinolone antibiotics. The volunteers were asked to abstain from taking any drug including over-the-counter (OTC) for two weeks prior to and during the study. The volunteers were informed about the risk and aim of the study by the clinical investigator and signed a written informed consent statement before entering the study. The volunteers were free to withdraw from the study at any time. The study protocol was approved by the ethics committee of the College of Pharmacy and the Institutional Review Board (IRB) of King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia

2.2 Identity of Study Medications

Test product (A) Jedcoflacin® tablets (200 mg ofloxacin/tablet); Batch No. 1605081114, manufactured by Jedco International Pharmaceuticals Co., Egypt, and the Reference product (B) Tarivid® tablets (200 mg ofloxacin/tablet); Batch No. 40N691, manufactured by Avetis Pharma Deutschland GmbH, Germany.

2.3 Study Design

Bioequivalence evaluation is usually carried out *in vivo* by comparing the rate and extent of drug absorption of the test and reference formulations in healthy volunteers. In a standard *in vivo* bioequivalence study design, study volunteers received test and reference products on separate occasions, in single dose, with random assignment to the two possible sequences of product administration. Samples of plasma were analyzed for drug concentrations, and pharmacokinetic parameters were obtained from the resulting concentration-time curves. These pharmacokinetic parameters were then analyzed statistically to determine if the test and reference products yielded comparable values. Standard statistical methodology based on the two one-sided T-tests procedure to determine whether average values for pharmacokinetic parameters measured after administration of the test and reference products are comparable. This procedure involves the calculation of a 90% confidence interval for the ratio between pharmacokinetic variable averages of the test and reference products. The limits of the observed confidence intervals were within the pre-determined range for the ratio of the product averages. The determination of the confidence interval range and the statistical level of significance were based on the parametric theory. Standard non-compartmental procedure was employed for the analysis of pharmacokinetic data derived from *in vivo* bioequivalence studies. Analysis of variance (ANOVA) was performed on the pharmacokinetic parameters to assess the effect of variables (volunteers, sequence, period and formulation) on the study outcome. On the basis of these considerations, a single-dose, two-treatment, two-period, two-sequence crossover bioequivalence study on healthy normal volunteers was adopted as described in the study protocol.

2.4 Collection and Handling of Blood Samples for Analysis

The administration of the two products to the volunteers was carried out by means of a two-way crossover design with a 1-week washout period. Volunteers were randomly divided into 2 equal groups and assigned to 1 of the 2 sequences of administration. In the morning of study day 1 of each study period and before drugs administration, a cannula was inserted into the subject's forearm vein and remained there until the 16-hour blood sample was collected. The volunteers were returned to the clinical site the next day for the 24- and 30-hour blood samples. Each subject received a single oral dose of (2x200 mg/tablet) of either brand with 240 ml of water after overnight fast for at least 10 hours. Volunteers were allowed to eat a standard meal 4 hours after drug administration. Beverages and food containing caffeine were not permitted over the entire course of the study. Volunteers were ambulatory during the study, but strenuous activity was prohibited. The volume of blood taken for determination of ofloxacin in plasma was 8 ml per sample. The following blood samples for the analysis of ofloxacin in plasma were collected at (-0.50 hour) and at 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 14.00, 16.00, 24.00, and 30.00 hours after drugs administration. The number of blood collections for drug analysis was 20 samples in each study period. Blood samples were collected, protected from light, into evacuated glass tubes containing heparin as an anticoagulant (heparinized vacutainers, Beckton and Dickinson, Rutherford, NJ, USA) through the indwelling cannula placed in the subject's forearm veins, slightly shaken and immediately centrifuged at approximately 3500 r.p.m for 5 minutes. After centrifugation, plasma samples were transferred directly into two labeled 5 ml-plastic tubes protected from light. These samples were immediately stored in a freezer at a nominal temperature of -70°C pending analysis. For each volunteer, the total amount of blood loss during the whole study (including blood for laboratory tests) did not exceed 320 ml.

2.5. Analysis of plasma samples

The procedure involves protein precipitation *via* ultrafiltration of the spiked plasma sample and injecting the clear ultrafiltrate into the chromatographic system. Chromatographic separation was performed on a Waters-Alliance 2695 liquid chromatography system (Waters Associates, USA) consisting of a binary pump, an autosampler, a column oven and a Waters 2475 Multi λ Fluorescence Detector. The chromatographic system and peak data handling was managed by Empower software package version 4.0. After extensive various preliminary experimental trials optimum chromatographic conditions were achieved. Ofloxacin and acebutolol, as the internal standard (IS), were eluted on a Symmetry C18 (3.9×150 mm), $5 \mu\text{m}$ particle size HPLC column, protected by a sentry guard column, Symmetry RP C18 (3.9×20) mm, $5 \mu\text{m}$ particle size HPLC column, column temperature was kept constant at 25 ± 2 °C. The mobile phase consisted of 19% Acetonitril in (0.1% Trifluoroacetic acid containing 1 ml triethylamine per one liter of water). The solvents were filtered prior to use, under vacuum, using $0.22\text{-}\mu\text{m}$ and $0.45\text{-}\mu\text{m}$ membrane filters (Millipore, Milford, MA), respectively. An injection volume of $10 \mu\text{L}$ with a flow-rate of $1.0 \text{ mL}\cdot\text{min}^{-1}$ was used for separation of ofloxacin and internal standard with fluorescence detector operated at an excitation and emission wavelengths of 320 and 485 nm, respectively. Retention times of 4 and 6 minutes for ofloxacin and IS, respectively. Fluorescence detector was chosen as it provides high sensitive detection and selectivity for ofloxacin. The first step was to determine a combined excitation and emission wavelengths for both drug and IS.

2.6 Pharmacokinetic analysis

The pharmacokinetic parameters of ofloxacin were estimated using standard non-compartmental methods. The analysis procedure followed the scaled bioequivalence limits imposed by the FDA [12]. All parameters were determined from the true (actual) sample collection times and assayed plasma concentrations at these times. The maximal plasma concentration (C_{max}) and the time to peak plasma concentration (T_{max}) of ofloxacin were taken directly from the measured data. The area under the plasma concentration-time curve ($\text{AUC}_{0 \rightarrow 30}$) was calculated from measured data points from time of administration to time of last quantifiable concentration (C_{last}) by the linear trapezoidal rule. The area under the plasma concentration-time curve extrapolated to infinity ($\text{AUC}_{0 \rightarrow \infty}$) was calculated according to the following formula:

$$\text{AUC}_{0 \rightarrow \infty} = \text{AUC}_{0 \rightarrow 30} + C_{\text{last}} / [\text{Ln}(2) / T_{1/2}]$$

Where, C_{last} is the last quantifiable concentration. The ratio $\text{AUC}_{0 \rightarrow 30} / \text{AUC}_{0 \rightarrow \infty}$ as a percent, was determined as an indicator for the adequacy of sampling time. The elimination half-life ($T_{1/2}$) was calculated as:

$$T_{1/2} = \text{Ln}(2) / (-b)$$

Where, b was obtained as the slope of the linear regression of the Ln-transformed plasma concentrations versus time in the terminal period of the plasma curve. At least 3 non-zero plasma concentration-time points were used in the calculation. The extent of absorption is determined by $\text{AUC}_{0 \rightarrow t}$ and $\text{AUC}_{0 \rightarrow \infty}$. The rate of absorption is determined by C_{max} . For the parametric analysis of bioequivalence for Ln-transformed data, the acceptance boundaries were set at 80.00-125.00% for $\text{AUC}_{0 \rightarrow 30}$, $\text{AUC}_{0 \rightarrow \infty}$ and C_{max} .

2.7 Statistical analysis

Statistical analyses were performed by the two-way analysis of variance (ANOVA) for crossover design at an $\alpha = 0.05$ using the general linear modeling (GLM) procedure of the statistical analysis system (SAS) software (SAS Institute, Inc., Cary, NC, USA). The model contained the main effects of subject within sequence, period and formulations. Sequence effects were tested against the mean square term for volunteers within sequence. All other main effects were tested against the mean square error term. The pharmacokinetic parameters: $\text{AUC}_{0 \rightarrow 30}$, $\text{AUC}_{0 \rightarrow \infty}$, C_{max} , T_{max} , K_{el} and $T_{1/2}$ were analyzed assuming multiplicative model. Drug concentrations at each sampling time point were also analyzed statistically using analysis of variance. Bioequivalence of the two formulations were assessed by calculating the 90% confidence intervals based on the ANOVA (parametric) of the mean Test/Reference ratios of $\text{AUC}_{0 \rightarrow 30}$,

$AUC_{0 \rightarrow \infty}$ and C_{max} using log-transformed data. In addition, bioequivalence between the two formulations was also assessed by Schuirmann's two one-sided t-tests procedure [13]. Ofloxacin is a drug with an intermediate to low intra-subject variability (ANOVA-CV of C_{max} and $AUC \leq 20\%$). The method of Hauschke *et al.* [14] for sample size determination for bioequivalence assessment using multiplicative model was used.

3. Results

During this study, 25 volunteers were screened, and 24 volunteers completed both periods of the study and were discharged in good health. The two formulations were well-tolerated in all 24 volunteers, unexpected incidents that could have influenced the outcome of the study did not occur. Both of the formulations of ofloxacin were readily absorbed from the gastrointestinal tract of the volunteers. Ofloxacin was measurable at the first sampling time (0.33 h) in most volunteers following the administration of the test and reference formulations.

3.1. Analysis of plasma samples

Validation of the analytical method including, linearity, recovery, specificity, stability, precision and accuracy was previously reported [15]. The validation procedure followed the FDA international guidelines [16]. Standard curves for the analyte in plasma were generated daily and were linear (r^2 of 0.9980) in the range of 25-4500 ng/ml over the entire period of the study. Quantization was achieved by measurement of the peak area ratio of the drug to the internal standard, and the limit of quantification for ofloxacin in plasma was 25 ng/ml. A typical chromatogram for ofloxacin and IS (acebutolol) is shown in Figure 2. The intra-day accuracy of the method for ofloxacin ranged from 93.5- 110.3%, while the intra-day precision ranged from 5.51 - 8.46%. The inter-day accuracy ranged from 96.5- 111.9%, while the inter-day precision ranged from 5.76 - 6.60%. The absolute analytical recovery of ofloxacin was 88.6% and for the internal standard (acebutolol) was 89.2%. The relative analytical recovery of ofloxacin ranged from 100.4 - 101.4%. Stability study showed that ofloxacin is stable in plasma for 6 hours at room temperature and for more than 4 months when stored at -70°C . Ofloxacin is stable for 4 cycles of freeze and thaw in 28 days, when stored at -70°C and thawed at room temperature. The stock solutions of ofloxacin and the internal standard are stable at room temperature for 6 hours and at -70°C for 26 days. The mean plasma concentration time curves for the two brands are demonstrated in Figure 3.

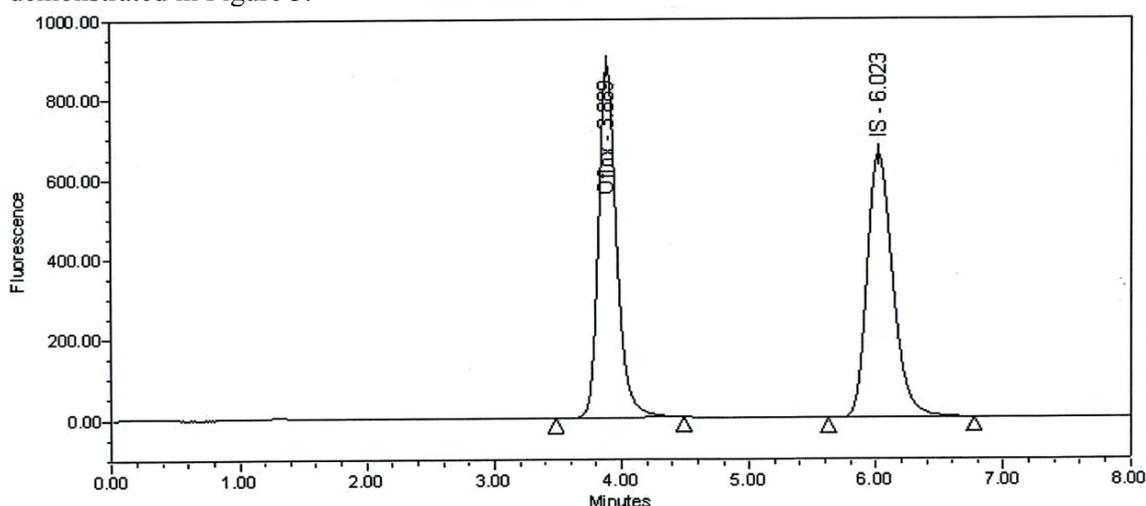


Figure 2: HPLC chromatogram showing human plasma sample containing ofloxacin 1750.0 ng/ml and internal standard (acebutolol) 71.4 $\mu\text{g/ml}$.

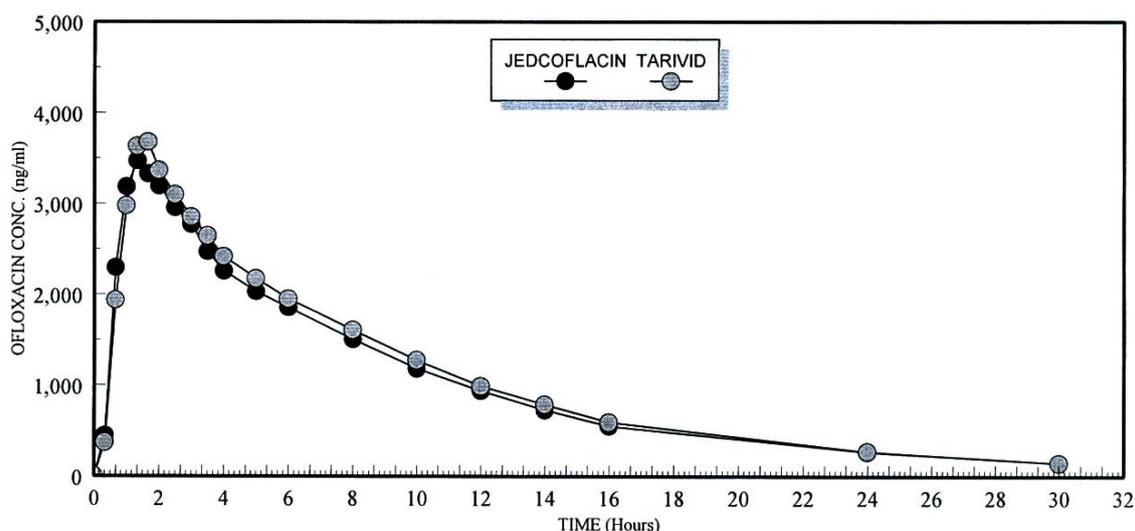


Fig. 3. Mean plasma concentration-time profiles of ofloxacin (ng/ml) after oral administration of (2x200 mg/tablet) of the two brands to 24 healthy volunteers.

The relative bioavailability of the generic formulation was found to be 94.23%, 94.56% and 98.27% based on $AUC_{0 \rightarrow 30}$, $AUC_{0 \rightarrow \infty}$ and C_{max} , respectively. Nineteen ANOVAs were performed to compare ofloxacin plasma concentration produced by the two formulations at each sampling time. Those concentrations were statistically higher following administration of Jedcoflacin[®] relative to Tarivid[®] at 0.33, 0.67 and 1.00 hours. There was no statistical difference between the two formulations at the sixteen remaining time points.

Table 1 shows the geometric mean values and the range for the above parameters ($AUC_{0 \rightarrow 30}$, $AUC_{0 \rightarrow \infty}$, C_{max} , and $AUC_{0 \rightarrow 30} / AUC_{0 \rightarrow \infty}$) along with K_{el} and $t_{1/2}$. Table 2 shows the parametric 90% confidence intervals of the mean values of the pharmacokinetic parameters: $AUC_{0 \rightarrow 30}$, $AUC_{0 \rightarrow \infty}$, C_{max} , and $AUC_{0 \rightarrow 30} / AUC_{0 \rightarrow \infty}$, respectively, as well as the point estimates for test/reference ratio assuming multiplicative models (using log-transformed data).

Table 1: Mean Pharmacokinetics Parameters of Ofloxacin 400 mg (Jedcoflacin[®] ofloxacin Tarivid[®] tablets) following their administration to 24 volunteers.

Pharmacokinetic Parameter	Treatment (Mean \pm SD)	
	Test Product	Reference Product
C_{max} (ng/mL)	4107.31 \pm 1170.88	4128.20 \pm 908.16
$AUC_{0 \rightarrow t}$ (ng.hr/mL)	29880 \pm 6167	31400 \pm 4889
$AUC_{0 \rightarrow \infty}$ (ng.hr/mL)	31297 \pm 6540	32753 \pm 5006
T_{max} (h)	1.38 \pm 0.59	1.48 \pm 0.53
K_{el} (1/h)	0.104 \pm 0.011	0.110 \pm 0.010
$T_{1/2}$ (h)	6.716 \pm 0.724	6.363 \pm 0.540
$AUC_{0 \rightarrow 30} / AUC_{0 \rightarrow \infty}$ %	0.96 \pm 0.01	0.96 \pm 0.01

Table 2: Bioequivalence confidence intervals of ofloxacin formulations (Jedcoflacin[®] versus Tarivid[®] tablets) following their oral administration to 24 healthy volunteers

Pharmacokinetic Parameter	90% Confidence Intervals		
	A/B Point estimate %	Lower limit %	Upper Limit %
C _{max}	98.27	91.71	105.30
AUC _{0→t}	94.23	87.10	101.95
AUC _{0→∞}	94.56	87.49	102.19

4. Discussion

Table 2 demonstrated that the confidence limits for the mean AUC_{0→30}, AUC_{0→∞}, C_{max}, and AUC_{0→30}/AUC_{0→∞} indicate that these values are entirely within the bioequivalence acceptable boundaries of 80-125% for AUC_{0→30}, AUC_{0→∞} and C_{max}. There was no statistical difference between the two formulations at the nineteen time points. It can be seen that the mean plasma concentration-time profiles from brand A and B are almost superimposable. The parameters used to measure bioavailability were AUC_{0→30} and AUC_{0→∞} and C_{max} for the extent of absorption, and T_{max}, and C_{max} for the absorption rate and they were calculated in a model independent manner. The pharmacokinetic characteristic T_{max} is presented as mean (± SD). Results of the ANOVA of the bioavailability data showed that there were no significant differences between the two formulations on all of the pharmacokinetic parameters. Furthermore, periods and sequence effects did not influence the outcome of the statistical analysis. The intra-individual variations in the pharmacokinetic parameters - AUC_{0→30}, AUC_{0→∞}, and C_{max} estimated from the coefficients of variation (CV %) as determined by ANOVA- were, 15.98%, 15.76%, and 14.10%, respectively. The assessment of bioequivalence between the two formulations were carried out according to the statistical tests procedures (90% CI and the two one-sided T-tests procedure) currently recommended by the FDA and EMEA. Selecting the most variable metric (C_{max}) and mean (Test/Reference) ratio = 0.98, the required sample size was determined to be 16 volunteers. Data values shown in that the confidence limits for the mean AUC_{0→30}, AUC_{0→∞}, C_{max}, and AUC_{0→30}/AUC_{0→∞} are entirely within the bioequivalence acceptable boundaries of 80-125% for AUC_{0→30}, AUC_{0→∞}, and C_{max}.

5. Conclusion

In conclusion, based on the statistical results of this study, the test product, Jedcoflacin[®] tablets

(200 mg ofloxacin/tablet) of Jedco International Pharmaceuticals Co., Egypt, investigated in this study was shown to be bioequivalent with the reference product, Tarivid[®] tablets (200 mg ofloxacin/tablet) of Aventis Pharma, Germany, following an oral dose of 400 mg (2x200 mg ofloxacin/tablet). Plasma levels may be used as surrogate parameters for clinical activity. Therefore, the data obtained in this study prove, by appropriate statistical methods, the essential similarity of plasma levels of ofloxacin from the test product Jedcoflacin[®] tablets (200 mg ofloxacin/tablet and from the reference product Tarivid[®] tablets (200 mg ofloxacin/tablet) suggesting equal clinical efficacy of these two products.

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