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RESEARCH PAPER

Bioequivalence of Two Brands of Ciprofloxacin 750 mg Tablets (Sarf® and Ciprobay®) in Healthy Human Volunteers

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ABSTRACT

An open, randomized, two-way crossover study was carried out in 28 healthy volunteers at Gulf Pharmaceutical Industries (Julphar), as a joint venture with Saqr Hospital, Ras Al-Khaimah, UAE. The two commercial brands used were Sarf® (Julphar, UAE) as test and Ciprobay® (Bayer AG, Germany) as reference product. The drug was administered to each subject with 240 mL of water after an overnight fasting in two treatment days separated by a one-week washout period. After dosing, serial blood samples were collected for a period of 24 hr and serum was separated and analyzed for ciprofloxacin using a sensitive, reproducible, and accurate high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection. Various pharmacokinetic parameters, including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $t_{1/2}$, *and* λ_Z, *were determined from ciprofloxacin serum concentration–time profiles for both formulations and found to be in good agreement with reported values. The parameters* AUC_{0-t}, $AUC_{0-\infty}$, *and* C_{max} *were tested for bioequivalence after log-transformation of data. No significant difference was found based on analysis of variance (ANOVA); the 90% confidence intervals (95.73–107.62%, 94.98–108.26%,*

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92.80–103.90% for AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, respectively) for the test/reference ratios of these parameters were within the bioequivalence acceptance range of 80–125%. Based on this data, it is concluded that both formulations are bioequivalent and are interchangeable in medical practice.
Key Words: *Bioequivalence; Ciprofloxacin; Healthy human volunteers; HPLC analysis*

INTRODUCTION

The bioequivalence of two formulations of the same drug is concluded based on the lack of difference in the rate (C_{max}) and extent of absorption (AUC), especially in conventional drug formulations (1). In the present study the bioequivalence of two ciprofloxacin tablets was evaluated by comparing the pharmacokinetic parameters derived from serum ciprofloxacin concentration–time profiles.

Ciprofloxacin is a broad-spectrum fluoroquinolone antibacterial agent active against a wide range of aerobic gram-positive and gram-negative organisms. It acts by inhibiting the bacterial enzyme DNA gyrase, which is needed for DNA synthesis (2). Chemically ciprofloxacin is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid, structurally related to nalidixic acid. Its molecular formula is $C_{17}H_{18}FN_3O_3$ and its molecular weight is 331.35 (3).

In vitro its activity has been demonstrated against gram-negative bacteria including *Escherichia coli*, *Enterobacter* spp., *Haemophilus* spp., and *Neisseria* spp. Most gram-positive bacteria are susceptible or moderately susceptible, including *Staphylococcus* aureus methicillin-susceptible strains, *Salmonella* spp., and most strains of streptococci are moderately susceptible to ciprofloxacin (4). Ciprofloxacin is a good alternative in a wide variety of situations such as lower respiratory tract infections, complicated urinary tract infections, gastrointestinal, skin, and bone infections, and sexually-transmitted diseases (5).

In comparison with other non-fluorinated quinolones, ciprofloxacin has a good oral absorption and a better bioavailability. It has an approximate bioavailability of 70% (6); maximum plasma concentrations (0.8 to 3.9 μg/mL) are achieved within 1 to 2 hr after oral administration of single 250–750 mg doses (5,6). Food has been shown to prolong the time to reach maximum concentration; however, this is thought not to be relevant clinically (6,7). The drug has a large volume of distribution (2.1 to 5 L/kg) and is concentrated in many body tissues and fluids, including bile, kidney, liver, gallbladder, prostate, and lung tissues (5,7–9). Ciprofloxacin is metabolized in the liver to at least four metabolites, three of which have some antimicrobial activity (10–12). When administered orally, ciprofloxacin is largely excreted unmetabolized in the urine (25–30%) and feces (50%), although small amounts of metabolites (22%) have been detected in urine (10,12). Gastrointestinal elimination occurs predominantly via transintestinal elimination, but bile excretion also occurs (13,14). In contrast, following intravenous administration, 57% was recovered in the urine as unchanged ciprofloxacin within 24 hr, with approximately 24% appearing as metabolites (total 81.7%) (10). The elimination half-life of ciprofloxacin is about 3–6 hr (5,6,15,16). Although the pharmacokinetics of ciprofloxacin have been reported in many studies, very few of them focused on the bioequivalence issue; therefore, in the present work, we emphasize this issue.

Objectives of the Study

The aim of this study was to assess the bioequivalence of two commercial 750 mg tablets of ciprofloxacin (Sarf® from Julphar, UAE and Ciprobay® from Bayer AG, Germany) in healthy human volunteers by statistical analysis of the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} as recommended by the Food and Drug Administration (FDA) (17).

EXPERIMENTAL

Study Products

Test Product:	Sarf®—ciprofloxacin 750 mg tablet
Batch No.:	0006, expiry date 08/2002
Manufacturer:	Gulf Pharmaceutical Industries, Julphar, UAE
Reference Product:	Ciprobay®—ciprofloxacin 750 mg tablet

Batch No.: CAWWU1, expiry date 12/2003
Manufacturer: Bayer AG, Germany

In Vitro Studies

Samples of the two brands, Sarf and Ciprobay, were tested and passed the USP compendial requirements with respect to their content uniformity and weight variation. Dissolution studies were performed according to USP 24 (18) using USP apparatus 2 (Erweka DT 70, Erweka International AG, Battwil, Switzerland) in a 37°C thermostated bath. Samples were withdrawn at designated time intervals and analyzed spectrophotometrically (Perkin-Elmer UV/VIS spectrophotometer model Lambda 10, Norwalk, CT) for their ciprofloxacin contents at 276 nm against water as a blank.

In Vivo Study

Study Subjects

Twenty eight healthy adult male volunteers were recruited at Saqr Hospital, Ras Al-Khaimah, UAE, as a joint venture with Julphar. Their mean age was 28.83±5.97 years (range 19–41 years), mean body weight was 67.73±10.62 kg (range 48–93 kg), and mean body height was 168.87±8.70 cm (range 149–178 cm). On the basis of their medical history, physical and clinical examination, and laboratory investigation (hematology, blood biochemistry, and urine analysis) no subject had a history or evidence of hepatic, renal, gastrointestinal, or hematological deviations, or any acute or chronic disease or drug allergy. All subjects were negative for hepatitis B antigen. No consumption of alcohol was permitted for the subjects from 48 hr prior to the first study drug administration until the end of the 24-hr sample period. Similarly beverages and food containing caffeine were not permitted over the entire course of the study. The volunteers were instructed to abstain from taking any medication for at least one week prior to and during the study period. Informed consent were obtained from all volunteers before entering the study. The study protocol was approved by the Institutional Review Board (IRB) of Saqr Hospital, Ras Al-Khaimah, UAE.

Drug Administration and Sample Collection

The study design was a single-dose, randomized, two-way treatment crossover design. The volunteers were hospitalized at 7:00 p.m. and had a standard dinner in hospital. On the morning of phase I, after an overnight fasting (10 hr) subjects were given a single dose of either formulation (reference or test in a randomized fashion) of ciprofloxacin 750 mg tablets with 240 mL of water. No food was allowed until 4 hr after the dose administration. Water was allowed 2 hr after the dose. Water, lunch, and dinner were given to all subjects according to a preplanned schedule. Beverages and food containing caffeine were not permitted over the entire course of the study. During the first 5-hr blood collection period, volunteers were allowed to wander around but prohibited from strenuous activity. They were under direct medical supervision at the study site. Approximately 5-mL blood samples for ciprofloxacin assay were drawn into evacuated glass tubes through indwelling cannula before (0 hr) and at 0.50, 1.0, 1.50, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, and 24.0 hr after dosing. Blood samples were kept at room temperature for coagulation and then centrifuged at 3500 rpm for 10 min; serum was transferred directly into plastic tubes and stored frozen at −20°C pending drug analysis. After a period of 7 days, the study was repeated in the same manner to complete the crossover design.

Chromatographic Conditions

Serum samples were analyzed for ciprofloxacin according to a sensitive, selective, and accurate high-performance liquid chromatography (HPLC) method, which was developed and validated before the study at the Quality Control Laboratory of Julphar. All solvents used were of HPLC grade; other chemicals and reagents were of analytical grade. Ciprofloxacin was obtained from Julphar, UAE; ofloxacin (internal standard) was purchased from Sigma Chemicals, Germany (Desenhopen, Germany).

The chromatographic separations and quantitative determinations were performed on a high-performance liquid chromatograph from Agilent Technologies (formerly Hewlett-Packard, Waldbronn, Germany): Model 1100 series, equipped with a G1322A degassing unit, G1311A quaternary pump, G1313A autoinjector, ALS-G1314A UV/VIS detector, and controlled by HP Chem. Station software version 6.1 (released May 1998). Chromatographic separation was performed using a Symmetry® C_{18} (dimethyloctadecylsilyl bonded

amorphous silica; 150×3.9 mm i.d.; 5 μm particle size) HPLC column (Waters, Milford, MA). The mobile phase consisted of 91% water, 8.5% acetonitrile, and 0.5% triethylamine (pH adjusted to 2.90 using phosphoric acid). The mobile phase was eluted at a flow rate of 1.2 mL/min at ambient room temperature. Detection of ciprofloxacin and the internal standard was achieved by monitoring the absorbance at a wavelength of 280 nm; the peak area was measured, and the peak area ratio of drug to internal standard and the concentration were calculated. Each analysis required a maximum of 25 min. The method was validated by following international guidelines (19). The relationship between concentration and peak area ratio was found to be linear within the range 0.025 to 6.250 μg/mL for ciprofloxacin, with a limit of quantitation of 0.025 μg/mL.

The intra-day precision of the method ranged from 0.53 to 4.60%, while the inter-day precision ranged from 1.15 to 4.94% for ciprofloxacin. The absolute analytical recovery for ciprofloxacin was 94.73%. The stability study showed that ciprofloxacin was stable in serum for six weeks when stored at −20°C.

Sample Preparation for HPLC Injection

Two hundred and fifty microliters of the internal standard working solution (ofloxacin 20 μg/mL) was added to 0.5 mL serum sample. The samples were vortexed for 2 min and precipitation of the serum proteins was accomplished by adding 250 μL each of 4 M phosphoric acid and saturated disodium hydrogen phosphate solution, respectively. The mixture was then shaken on the vortex mixer for 2 min and centrifuged at 3000 rpm for 5 min. The clear supernatant layer was separated and filtered through a 0.45 μm filter. Aliquots were loaded into an autosampler tray and volumes of 50 μL were injected into the column, where ciprofloxacin and internal standard were separated from endogenous substances and their peak areas recorded.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by means of a model-independent method using the Win Nonlin™ computer program (20). The elimination rate constant (λ_Z) was obtained as the slope of the linear regression of the log-transformed serum concentration values vs. time data in the terminal phase. The elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_Z$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated from $AUC_{0-t}+C_t/\lambda_Z$, where C_t is the last measurable concentration.

Statistical Analysis

For the purpose of bioequivalence analysis, AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were considered as the primary variables. Two-way analysis of variance [ANOVA, GLM procedure; using Win Nonlin™ computer program (20)] for crossover design was used to assess the effect of formulations, periods, sequences, and subjects on these parameters. The difference between two related parameters was considered statistically significant for p-values equal to or less than 0.05. Parametric 90% confidence intervals (17) based on the ANOVA of the mean test/reference (T/R) ratios of AUCs and C_{max} were computed.

RESULTS AND DISCUSSION

The cumulative amount (%) of ciprofloxacin dissolved in vitro from both brands of test tablets (Sarf) and reference tablets (Ciprobay) is plotted as a function of time in Fig. 1. The results are within the range of USP 24 dissolution requirements; not less than 80% (Q) of the labeled amount of ciprofloxacin is dissolved in 30 min.

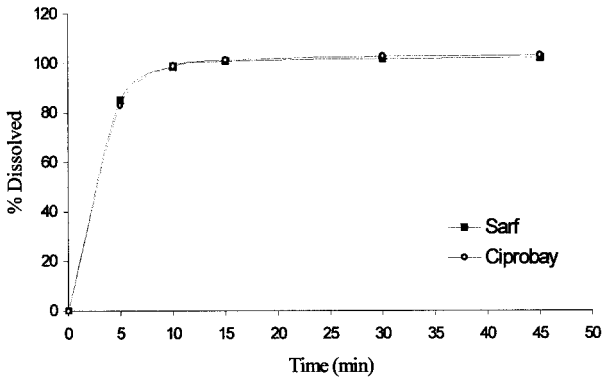

Figure 1. Dissolution profile of Sarf® and Ciprobay® tablets.

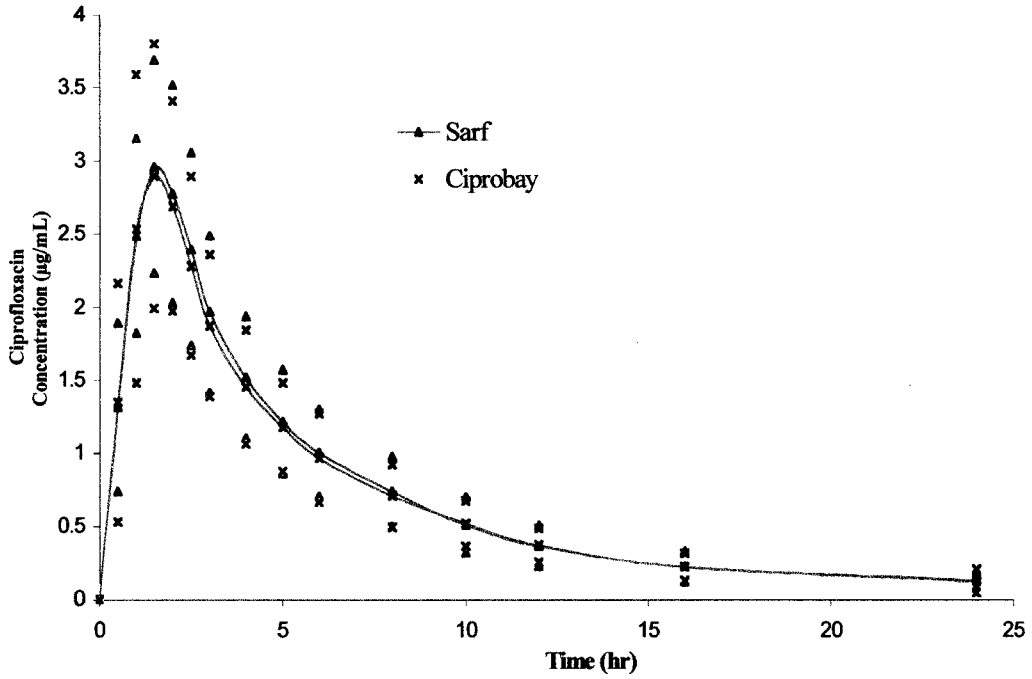

Figure 2. Mean (± SD) serum concentration of ciprofloxacin 750 mg tablets after oral administration of single dose of the two brands to 28 healthy human volunteers.

The two studied products (Sarf and Ciprobay 750 mg tablets) were well tolerated by all subjects; unexpected incidents that could have influenced the outcome of the study did not occur. There was no drop-out and all volunteers who had started the study continued to the end and were discharged in good health.

Both formulations were readily absorbed from the gastrointestinal tract and ciprofloxacin was measurable at the first sampling time (0.50 hr) in almost all the volunteers. Plots of the mean (±SD) of ciprofloxacin serum concentration–time for the two formulations over the 24-hr sampling period are shown in Fig. 2. A non-compartmental approach was used to determine the pharmacokinetic parameters and all the values were in good agreement with reported studies (5,6,15,16). Table 1 shows the pharmacokinetic parameters for the two brands of ciprofloxacin 750 mg tablets.

The 90% confidence intervals for the log-transformed data were also calculated as per the FDA (17) guidelines, and the results are shown in Table 2. On the basis of the serum levels of the 28 volunteers completing this study (Fig. 2), the mean relative bioavailability of Sarf 750 mg tablets was 103.4% for AUC_{0-t}, 103.8% for $AUC_{0-\infty}$, and 99.7% for C_{max}.

Table 1

Pharmacokinetic Parameters of Ciprofloxacin 750 mg Tablets (mean±SD, n = 28)

Pharmacokinetic Parameters	Sarf® (Test)	Ciprobay® (Reference)
AUC_{0-t} (µg/mL hr)	16.549±4.461	16.237±3.870
$AUC_{0-\infty}$ (µg/mL hr)	17.785±4.699	17.549±4.356
C_{max} (µg/mL)	3.204±0.593	3.295±0.775
T_{max} (hr)	1.518±0.419	1.536±0.508
$t_{1/2}$ (hr)	5.501±1.944	5.506±1.657
λ_Z (hr^{-1})	0.139±0.041	0.136±0.037

Area Under the Curve (AUC_{0-t} and $AUC_{0-\infty}$)

The mean and standard deviation of AUC_{0-t} and $AUC_{0-\infty}$ of the two products did not differ significantly, suggesting that the serum profiles generated by Sarf are comparable to those produced

Table 2

Statistical Analysis of Log-Transformed Data

Statistical Analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
ANOVA GLM (p-value)	0.6681 (0.0384)[a]	0.7191 (0.0349)	0.5871 (0.2599)
90% CI	95.73–107.62%	94.98–108.26%	92.80–103.90%

[a]Values in parentheses indicate analysis for periods.

by Ciprobay. After log-transformation of the data, ANOVA for these parameters showed no statistically significant difference between the two formulations either in periods, formulations, or sequences, having p-value greater than 0.05. The 90% confidence intervals also demonstrated that the ratios of AUC_{0-t} or $AUC_{0-\infty}$ of the two formulations and for two periods lie within the FDA acceptable range of 80–125%.

Peak Plasma Concentration (C_{max})

Analysis of variance was performed on C_{max} values of both products and showed that the two formulations were not statistically different. Furthermore, there was no statistically significant difference with regard to periods and sequences ($p > .05$). The 90% confidence intervals also demonstrated that the ratio of C_{max} of the two formulations was within the FDA acceptable range of 80–125%.

The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent (21). To demonstrate bioequivalence certain limits should be set depending on the nature of the drug, patient population, and clinical end-points.

It is generally accepted that for basic pharmacokinetic characteristics, such as AUC and C_{max}, the standard equivalence range is 0.8–1.25 (17,22). The confidence intervals (CI) obtained in our study were 95.73–107.62%, 94.98–108.26%, and 92.80–103.90% for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}, respectively. Therefore, the CI for the main parameters reflecting the extent and rate of absorption are within the acceptance range, confirming the bioequivalence of both brands.

CONCLUSION

Statistical comparison of the AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} clearly indicated no significant difference between Sarf and Ciprobay, 750 mg tablets, in any of the calculated pharmacokinetic parameters. The confidence intervals for the ratios of mean AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} indicated that these values are entirely within the bioequivalence acceptance range of 80–125% (using log-transformed data). Based on the above data we can conclude that Sarf, manufactured by Gulf Pharmaceutical Industries, UAE, is bioequivalent to Ciprobay, manufactured by Bayer AG, Germany, and that both products can be considered equally effective in medical practice.

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