Pharmacokinetics and Bioequivalence Study of Two Ciprofloxacin Hydrochloride Tablets in Chinese Healthy Volunteers Under Fasting and Fed Conditions: A Randomized, Open-Label, Two-Formulation, Two-Sequence, Two-Period, Single-Dose Crossover Study.

Fei Qin
Baiyunshan Pharmaceutical General Factory
Gan-Mi Wang
Baiyunshan Pharmaceutical General Factory
Jin-Ying Huang
Baiyunshan Pharmaceutical General Factory
Jia-Rong Wu
Baiyunshan Pharmaceutical General Factory
Wen-Jie Song
Baiyunshan Pharmaceutical General Factory
Jun Deng
Baiyunshan Pharmaceutical General Factory
Ying Xiao
Baiyunshan Pharmaceutical General Factory
Jian-Song Wang
Baiyunshan Pharmaceutical General Factory
Ke-Wei Zhu (zhukew2018@outlook.com)
Baiyunshan Pharmaceutical General Factory

Research article

Keywords: ciprofloxacin, bioequivalence, pharmacokinetics, fasting, fed, safety

Posted Date: November 12th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-103736/v1

Version of Record: A version of this preprint was published at Int. Journal of Clinical Pharmacology and Therapeutics on December 1st, 2021. See the published version at https://doi.org/10.5414/cp204032.

Abstract

Background

Ciprofloxacin is a broad-spectrum fluoroquinolone antibiotic which is active against a wide range of Gram-positive and Gram-negative bacteria. The study mainly aimed to determine the bioequivalence of two branded ciprofloxacin hydrochloride tablets (250 mg) under the fasting and fed conditions.

Methods

The study was carried out in 48 healthy Chinese subjects under fasting and fed conditions with a randomized, open-label, two-formulation, two-sequence, two-period, single-dose crossover design. In each period of the study, the subjects were assigned to receive a single oral dose of 250 mg of ciprofloxacin hydrochloride. Blood samples were collected from an hour before dosing to 36 h after administration with 16 time points in total. The bioequivalence analysis was performed after ln-transformation of the ciprofloxacin pharmacokinetic parameters including maximum concentration (C_{max}), area under the plasma concentration–time curve from time 0 to time t (AUC_{0-t}), area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$). Two formulations are considered bioequivalent if the 90% confidence intervals (CIs) for the test/reference geometric mean ratios (GMRs) for the ln-transformed pharmacokinetic parameters fall within the standard acceptance range of 80% – 125%.

Results

In total of 48 subjects were enrolled in the fasting and fed studies, and one of the subjects was excluded before the administration. In the fasting study, the 90% CIs for the test/reference GMRs of the ln-transformed data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 85.41% to 100.97%, 95.40% to 100.27%, and 95.48% to 100.30%, respectively. For the fed study, the 90% CIs for the test/reference GMRs of the ln-transformed data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 90.15% to 113.75%, 99.10% to 103.77% and 99.11% to 103.80%, respectively. A total of 8 of 47 subjects experienced AEs in the fasting and fed studies.

Conclusions

In the study, the generic (test) product of ciprofloxacin hydrochloride 250 mg was bioequivalent to the innovator (reference) product after a single oral dose administration under the fasting and fed conditions. Both two brands of ciprofloxacin tablets were safe and well tolerated.

Trial registration

The clinical trial was registered at Center for the Drug Evaluation of the National Medical Products Administration (registration number: CTR20171152; date of registration September 25, 2017;

http://www.chinadrugtrials.org.cn/clinicaltrials.searchlistdetail.dhtml).

1 Background

Ciprofloxacin is a broad-spectrum fluoroquinolone antibacterial agent which exhibits excellent antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria. Ciprofloxacin is one of the few broad-spectrum antibacterial agents clinically effective after both oral and intravenous administrations. Like other quinolones, the antibacterial mechanism of ciprofloxacin is inhibition of bacterial topoisomerase II that belongs to a DNA gyrase and introduces negative supercoils into bacterial DNA. It is believed that quinolones act on the A subunits of DNA gyrase to inhibit resealing of the DNA double-strand, and promote the exonucleolytic degradation of bacterial single-strand DNA to play a bactericidal role [1–3].

Ciprofloxacin has a wide range of clinical uses owing to its broad antibacterial spectrum, and is efficacious in the therapy of various infections, especially those caused by Gram-negative pathogens [4]. In addition to complicated urinary tract infections [5], gastrointestinal infections [6], gonorrhea [7], ciprofloxacin was widely used in the therapy of lower respiratory tract infections [8], skin and soft tissue infections [9], sinusitis [10], otitis media [11], sepsis [12], etc. Ciprofloxacin exhibits excellent antimicrobial activity against many pathogens, such as some Gram-positive organisms including *staphylococci*, *streptococci*, and most of Gram-negative organisms consisting of *Hemophilus influenzae*, *Escherichia coli*, *Enterobacter*, *P. aeruginosa* species. Furthermore, ciprofloxacin exhibits potent bactericidal activity against many of enteric pathogens, and *Chlamydia trachomatis*, *Neisseria gonorrhoeae* resistant to penicillin [13].

After a single-dose oral administration of 250-mg ciprofloxacin, mean peak serum concentrations of 1.35 to 1.42 ug/ml for ciprofloxacin were achieved at 1-1.5 h in a dose-dependent model, the mean value (± SD) for area under the serum curve from 0 to 12 h was 5.43 ± 0.54 h·ug/mL; the terminal serum half-live was in the range of 3.8 to 4.3 h, and the percent of ciprofloxacin recovered in urine 0 to 12 h after administration ranged from 30–45% [14]. The mean absolute bioavailability after oral administration of ciprofloxacin ranges from 69–85% [1]. Food could delay the absorption of ciprofloxacin, but without significant impact on pharmacokinetics profile [15].

Most of drug-related adverse reactions of ciprofloxacin are mild to moderate, and the total incidence of adverse drug reactions of ciprofloxacin is 10.2%. Ciprofloxacin was well-tolerated according to pooled data across worldwide clinical trials consisting of 8861 courses of ciprofloxacin, and the most frequently adverse drug reactions involved gastrointestinal symptoms (5%), metabolic and nutritional symptoms (4.6%) [16].

The main objective of this study was to determine the bioequivalence of ciprofloxacin hydrochloride formulation manufactured by Baiyunshan Pharmaceutical General Factory (Guangzhou, China) with a branded innovator product after oral administration with single 250-mg dose in healthy Chinese volunteers. We investigated the pharmacokinetic properties and intra-individual variation coefficients of ciprofloxacin under fasting and fed conditions by determining the ciprofloxacin plasma concentrations. The validation of rationality involved the method of analyzing the ciprofloxacin plasma concentrations,

and the time interval setting of blood collection time and cleaning period. The secondary object of the study was to evaluate the tolerance and safety of ciprofloxacin in healthy volunteers.

2 Methods

2.1 Ethics

The study was performed as a randomized, open-label, two-formulation, two-sequence, two-period, single-dose crossover bioequivalence study with a wash-out period of 7 days, and conducted at the Guangdong Province Traditional Chinese Medical Hospital, Guangzhou, China. The study was approved by the independent Ethics Committee of the hospital (registration number: A2017-002-1-02) on the basis of the principles of some international guidelines including the CIOMS (Council for International Organizations of Medical Sciences) International Ethical Guidelines for Biomedical Research Involving Human Subjects, the Good Clinical Practice, the World Medical Association's Declaration of Helsinki, and WHO Operational Guidelines for Ethics Committees for Biomedical Research Review. The signed informed consent was acquired from every enrolled volunteer before formal screening.

2.2 Test and Reference Products

The ciprofloxacin hydrochloride tablet (Kuinuoxian®, drug specification: 250 mg; purity: 98.1%; batch number: 01170801; expiry date: July, 2020) used as the test product w manufactured by Baiyunshan Pharmaceutical General Factory (Guangzhou, China), which attached to Baiyunshan Pharmaceutical Holdings Limited (GPHL), Guangzhou, China.

We chose the ciprofloxacin hydrochloride tablet (Ciprobay®, drug specification: 250 mg; purity: 98.4%; batch number: BXN14RC; expiry date: August, 2020) manufactured by Bayer Vital GmbH, Leverkusen, Germany, as the reference product, in accordance with the Procedures for Selection and Determination of Reference Product for Generic Chemicals, which was published by the National Medical Products Administration (NMPA), Beijing, China. The reference product was in the recommended list of NMPA.

2.3 Inclusion and Exclusion Criteria

Subjects who met all the following requirements could be included:

- Subjects aged at least 18 years old, at most 45 years old, with appropriate sex ratio
- Male subjects weighed ≥ 50 kilogram and female subjects weighed ≥ 45 kilogram, with a body mass index (BMI) between 19-26 kg/m^2
- All medical examination results showed no abnormality, or abnormality without clinical significance
- Female subjects with negative pregnancy tests and male subjects had no fertility planning from two weeks before administration to the next six months, they and their partners used effective contraception and had no egg or sperm donation programs

- Subjects were fully aware of the purpose, nature, methods, and possible adverse events of the study, volunteered to serve as subjects, and signed informed consent before the study began
- Subjects were able to communicate well with the researchers, and understood and complied with the strict requirements of the study.

Subjects who met any of the following criteria would be excluded:

- Allergic physique, or allergy to the food and drug, especially for penicillin, clarithromycin, cephalosporin
- Surgical or medical history, illness with clinical significance, which would endanger the safety of subjects or have an impact on study results
- Difficulty for swallowing, or gastrointestinal disease, which affected drug absorption
- Positive test results for hepatitis B surface antigen (HBsAg), anti-HIV antibodies, hepatitis C antibody, or treponema pallidum antibody
- Gestation or lactation for the female subjects
- Diarrhea after drinking milk in fed study
- Positive urinary drug screen for morphine, methamphetamine, ketamine, dimethylene dioxyamphetamine, tetrahydrocannabinolic acid, cocaine
- Habitual use of Chinese herbs or functional vitamins
- Participation in another clinical trials within three months prior to the screening
- Blood donation or blood loss exceeded 400 mL within three months prior to the screening, or intention to donate blood during the clinical trial within three months after the end of the clinical trial
- Use of any medication or health products within three months prior to the screening
- Smoking > 5 cigarettes per day within three months prior to the screening, or disagreement to avoid using any tobacco products during the trial
- Alcohol drinking averaged > 14 units per week or > 2 units per day within 3 months prior to the screening, 1 unit was equivalent to 17.7 mL ethanol, or disagreement to avoid using any ethanol products during the trial
- Values for alcohol breath test results were > 0.0mg /100 mL
- A long history of consuming excess caffeinated beverages, which were more than eight drinks a day, 1 drink was 250 mL, or intake of food or drinks rich in methyl xanthine purines or caffeine (coffee, strong tea, cola, chocolate, etc) within 48 hours prior to the administration, and failure to stop the intake during the clinical trial
- Intake of special diet, such as pitaya, mango, pomelo, the food and drinks prepared from these fruits, or strenuous exercise within 48 hours prior to the administration, or other factors that could affect the absorption, distribution, metabolism, or excretion of ciprofloxacin
- Intolerableness to venipuncture blood collection or history of dizziness
- Special requirements for diet, and failure to follow the uniform diet

- Occurrence of acute illness during the screening period, or before the administration
- Any other factors the researchers considered inappropriate for volunteers to participate in the study.

2.4 Subjects

After signing the informed consent forms, all enrolled volunteers would undergo a series of inquiries and examinations, such as demographic data, medical history, allergy, the history of smoking and drinking, physical examination, weight and height measurement, vital signs, 12-lead electrocardiogram (ECG), chest radiography, laboratory analysis of blood routine, urine routine and blood biochemistry, serum pregnancy test (female only) and disease markers for syphilis, HIV (human immunodeficiency virus), and hepatitis B and C viruses. The screening tests were performed within 7 days prior to the first period of administration.

The volunteers who met all the inclusion criteria and did not meet any of the exclusion criteria during screening period were notified to enroll in the Phase Clinical Research Center of the hospital for a uniform lunch and dinner one day before the trial. In the fasting study, a total of 107 volunteers were recruited, 93 of whom were excluded. For the fed study, 26 of 90 volunteers were eligible, among whom 24 volunteers were assigned to receive a randomized order and the remaining 2 volunteers were excluded because the number of subjects had exceeded the predetermined number (24).

The following assessments were performed again before administration: health survey (recent medical history, medication history, smoking and drinking), vital sign measurement, female pregnancy test, alcohol breath test, drug screening in urine. In accordance with these assessments, the researchers checked the inclusion/exclusion criteria again and made the final decision, and all enrolled subjects must guarantee to fast for at least 10 hours before administration.

Under the terms of the informed consent, the subject had the rights to withdraw from the study and revoke the informed consent without any reason at any time, also without any impact on their rights, interests, and entries into other clinical studies. The researchers would let the subjects withdraw from the study if the subjects were unfit to continue the study in the following cases:

- Necessity to stop the study from the point of view of medical ethics
- Occurrence of serious adverse events (SAEs)
- Poor compliance involved failure to accept the examination and administration according to regulations, use of drug or food affecting safety assessment and pharmacokinetic analyses
- Other behaviors that could affect the test results

2.5 Study Drug Administration

Subjects were randomly assigned to receive a single oral dose of the test (T) or reference (R) product of ciprofloxacin hydrochloride 250 mg, in light of a balanced randomization schedule generated by SAS 9.4

(SAS Institute Inc., Cary, NC, USA), and the study drug was administrated in the order of T-R or R-T during each period.

In the fed study, 24 subjects were randomly assigned to two groups at a 1:1 ratio. After an overnight fast of ≥10 hours. Ciprofloxacin hydrochloride tablets were administered with 240 mL of water after standardized high-fat and high-calorie (800-1000 kilocalorie) meals, the meals were eaten up within 30 minutes, and chewing drugs was forbidden during administration. Subjects were granted a free access to water until 1 hour before study drug dosing and 1 hour after administration, and fasted until 4 hours after administration. Standard light meals were provided 4 and 10 hours after administration of ciprofloxacin hydrochloride tablets. Two dosing periods were separated by a 7-day washout period, and two groups were cross-administered on the seventh day. For the fasting study, the only difference from the fed study was that subjects fasted until 4 hours after administration.

Vital signs (pulse, blood pressure, body temperature) were assessed prior to dosing and at 2 (± 0.5) h, 4 (± 0.5) h, 12 (± 0.5) h, 24 (± 0.5) h after administration. The assessments should delay until sampling was completed, if there was a scheduling conflict between the assessments and sampling. Adverse events (AEs), concomitant medication usage and non-drug treatments were tracked through the whole study. Subjects were permitted to leave the Phase Clinical Research Center of the hospital until all blood sample collections were completed. Snacks, areca nuts, alcoholic drinks and smoking were forbidden from the enrollment to the final follow-up visit.

2.6 Sample Collection

Subjects were admitted to the Phase Clinical Research Center of the hospital one day prior to each period. After an overnight fast (≥10 hours), the baseline blood samples were collected within one hour before study drugs dosing. Collection of postdose blood samples were within two minutes of the specified times. Five hours after administration of study drug, the time deviation could be extended to three minutes. Venous blood samples of upper extremities were collected into labeled ethylene diamine tetraacetic acid (EDTA) tubes for pharmacokinetic analyses before administration and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0 and 36.0 h after administration in each period, and there were 16 time points in total.

Within 1 hour after collection, blood samples of 4 mL were centrifuged at 3000 rpm for 10 minutes at 4 °C to separate plasma in a precooled centrifuge. Each plasma sample was divided into two aliquots, then transferred to corresponding labeled tubes, and one of which was in reserve. The plasma samples were transferred to -60 °C within 1 hour after centrifugation. After packaging with dry ice by professional cold chain logistics company (Shengsheng Supply Chain Management s.a, Shanghai, China), the plasma samples were transferred to the analytical facility (MicroConstants., Inc, Beijing, China) for assay, and temperature monitoring and adequate dry ice were supplied during transportation.

2.7 Analytical Method and Validation

Ciprofloxacin plasma concentration was determined by a means of liquid chromatography-tandem mass spectrometry method. A XSelect HSS T3, 5.0 μm, 100 x 2.1 (Waters corporation. Milford, Massachusetts, U.S.A) mm column was used for separation at a flow-rate of 0.5 mL/min with column temperature of 30°C. The mobile phase consisted of water with 20 mM ammonium formate (Acros Organics, Belgium) and 0.1% formic acid (ROE, U.S.A): methanol (Honeywell, U.S.A) with 0.1% formic acid (65:35, vol/vol), all chemicals and reagents were HPLC (High Performance Liquid Chromatography) grade. Ciprofloxacin (batch number: 130451-201203, purity: 84.2%, expiry date: March 7, 2018) used as a reference substance was obtained from the National Institutes for Food and Drug Control, Beijing, China. Ciprofloxacin hydrochloride-D8 (batch number: 1519-039A3, purity: 99.5%, expiry date: October 11, 2019), as an internal standard (IS), was purchased from TLC Pharmaceutical standards Ltd, Shanghai, China. HPLC instrumentation (model number: 1100) was purchased from Agilent Technologies Inc (California, U.S.A). Mass spectrometric detection was performed in a multiple reaction monitoring (MRM) mode using electrospray ionization source, and the mass spectrometer was interfaced to a computer workstation running MassLynx v.4.1 (Waters, Milford, Massachusetts, U.S.A). The standard curve was found to be linear over the concentration range of 1.87 - 935 ng/mL, with lower limit of quantification (LLOQ) of 1.87 ng/mL, and the LLOQ of quality control (QC) was also 1.87 ng/mL. The inspection contents of analytical methodology validation included between-run precision and accuracy, within-run precision and accuracy, recovery rate, selectivity (specificity), matrix effects, dilution reliability, stepwise dilution reliability, residual effect of HPLC injection, analytical batch length, reproducibility for reinjection of 72 hours, placement stability of processed samples, freeze-thaw stability, stability of samples, matrix effects, hemolysis effects, recovery rate, accuracy and precision, reproducibility for reinjection of 72 hours.

2.8 Follow-up

After the end of the two period of sampling, the subjects underwent a series of safety assessments before hospital discharge, which included physical examination, vital signs, 12-lead ECG, chest radiography, laboratory analysis of blood routine, urine routine and blood biochemistry, serum pregnancy test. The subjects would be permitted to leave the Phase Clinical Research Center of the hospital, if their assessments were satisfactory.

The subjects were followed up 3-7 days after hospital discharge, and inquired whether there were any subsequent AEs, occurrence of which would be recorded, the follow-up would be continued until the disappearance of the AEs. If the subjects experienced AEs during the study, they would be followed up until the AEs stabilized, disappeared, or lost to follow-up.

2.9 Safety Assessments

Safety Set (SS) consisted of all subjects who had received the study drug at least once after enrollment and undergone safety assessments, the role of SS was to evaluate the safety of the study drug. The incidence, time, severity, and relationship to the study drug of AEs were recorded by nursing and observations of the medical staff, and results of laboratory and pathology throughout the study. Clinical parameters for the safety assessments were obtained from physical examination, vital signs, 12-lead

ECG, and laboratory analysis of blood routine, urine routine and blood biochemistry. The researchers calculated the amount and frequency of abnormal clinical parameters in the study, then detailly listed the abnormal clinical parameters and corresponding clinical explanations, and concretely described the changes in vital signs.

2.10 Pharmacokinetic and Statistical Analyses

Chromatogram collection and chromatographic peak integral of ciprofloxacin and IS was performed by MassLynx v.4.1 (Waters, Milford, Massachusetts, U.S.A). Linear fitting of the peak area ratio of ciprofloxacin to IS and the concentration of the reference substance of ciprofloxacin was to obtain a standard curve equation (weight = $1/x^2$). The peak areas and concentrations obtained from MassLynx v.4.1 were imported into the Microsoft Office Excel (Microsoft Corporation, Washington, U.S.A) to be used to calculate the mean, deviation, standard deviation, and variable coefficient (CV). The data were stored on a computer hard drive interfaced to the mass spectrometer for subsequent pharmacokinetic analyses.

Phoenix WinNonlin version 6.4 (Pharsight Corporation, Mountain View, California) were used for calculation of pharmacokinetic parameters, which included C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, $t_{1/2}$, and λz.

- AUC_{0-t} = the area under the plasma concentration–time curve from time 0 to time t, it was calculated by linear trapezoidal method, where time t was the last timepoint to collect blood sample
- $AUC_{0-\infty}$ = the area under the plasma concentration-time curve from time 0 to infinity, it was calculated as $AUC_{0-t} + C_t/\lambda z$, where C_t was the last measurable drug concentration, and λz was apparent elimination rate constant derived from linear regression analysis of ln(concentration-time) curve during the elimination phase
- T_{max} = the time to maximum concentration (C_{max}), both T_{max} and C_{max} were actual measured values
- $t_{1/2}$ = ln2/λz, terminal elimination half-life

2.11 Bioequivalence Analyses

Bioequivalence of the two ciprofloxacin hydrochloride tablets was determined by analysis of variance (ANOVA) after ln-transformation of the C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, which was performed using Statistical Analysis Software (SAS) version (SAS Institute Inc., USA) 9.4 with a non-compartment model. A test product was considered bioequivalent to a reference product if 90% CIs of the test/reference GMRs for ln-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t}, and $AUC_{0-\infty)}$ were within the acceptance range of 80.00% – 125.00%.

3 Results

3.1 Demographic Date

In the fasting study, twenty-four subjects were enrolled between the age of 18–43 years (mean ± SD: 27.9 ± 6.4 years), and consisted of 16 male subjects and 8 female subjects. The mean ± SD (range) height, weight and BMI of these subjects were 167.15 ± 8.46 cm (152.0–183.1 cm), 59.88 ± 8.39 kg (47.0–74.6 kg) and 21.34 ± 1.61 kg/m^2 (19.1–24.2 kg/m^2), respectively. Two subjects smoked occasionally, the other subjects never smoke, and none of the subjects had drinking history. Twenty-two subjects completed the study while two subjects withdrew at the end of first period of the fasting study for personal reasons.

For the fed study, twenty-three subjects completed the whole study, and one subject were excluded, due to the use of penicillin eye drops two days before administration in the first period. The twenty-three subjects consisted of sixteen male subjects and seven female subjects, the mean ± SD (range) age, height, weight and BMI of them were 27.8 ± 6.3 years (20–42 years), 164.66 ± 7.57 cm (147.1–176.4 cm), 60.65 ± 8.08 kg (47.2–75.1 kg) and 22.30 ± 1.85 kg/m^2 (19.1–25.1 kg/m^2), respectively. Five subjects smoked occasionally and the rest of 18 subjects never smoke; 16 subjects did not have drinking history, and the remaining seven subjects drank occasionally.

3.2 Pharmacokinetics

The mean ciprofloxacin plasma concentration–time curves after 250-mg single-dose oral administration of two ciprofloxacin hydrochloride tablets in the fasting and fed studies were shown in Fig. 1 (datasets: Table S1). Comparisons of the pharmacokinetic parameters between the two formulations under the fasting and fed conditions were shown in Table 1 and Table 2, respectively.

The mean (SD) pharmacokinetic properties of the test product under the fasting condition were as follows: C_{max}, 1600.0 (412.96) ng/mL; AUC_{0-t}, 6673.9 (1593.32) h·ng/mL; and $AUC_{0-\infty}$, 6728.2 (1596.93) h·ng/mL, the corresponding median (range) of T_{max} was 0.75 (0.5, 2) hour. With the reference product, the pharmacokinetic properties were: C_{max}, 1727.8 (384.09) ng/mL; AUC_{0-t}, 6831.3 (1533.67) h·ng/mL; $AUC_{0-\infty}$, 6884.7 (1535.64) h·ng/mL, and the median (range) of T_{max} was 1.00 (0.5, 2) hour. The mean (SD) $t_{1/2}$ values for the test and reference product under the fasting condition were 5.574 (0.7064) and 5.475 (0.5707) hours, respectively, the plasma λz values were 0.1258 (0.01767) and 0.1273 (0.01420) h^{-1}.

Under the fed condition, the mean (SD) pharmacokinetic properties of the test product were as follows: C_{max}, 1329.0 (333.15) ng/mL; AUC_{0-t}, 5512.3 (1215.44) h·ng/mL; and $AUC_{0-\infty}$, 5567.3 (1226.47) h·ng/mL. For the reference product, the pharmacokinetic properties were: C_{max}, 1320.7 (347.16) ng/mL; AUC_{0-t}, 5512.3 (1215.44) h·ng/mL; and $AUC_{0-\infty}$, 5567.3 (1226.47) h·ng/mL. The mean (SD) $t_{1/2}$ values

for the test and reference product under the fasting and fed conditions were 6.001 (0.7544) and 5.879 (0.6676) hours, respectively. The plasma λz values were 0.1183 (0.01403) and 0.1187 (0.01576) h^{-1}.

3.3 Bioequivalence

Bioequivalence assessments between the test and reference products using a confidence interval method after a single oral dose of 250 mg of ciprofloxacin in healthy Chinese subjects in the fasting and fed studies were shown in Table 3 and Table 4, respectively.

In the fasting study, the 90% CIs for the test/reference GMRs of the ln-transformed data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 85.41–100.97%, 95.40–100.27%, and 95.48–100.30%, respectively. CVs within intra-subject for ln-transformed data C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 16.12%, 4.77% and 4.72%, respectively. The effects of administration sequence, period, and formulation on ANOVA of ln-transformed data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$were shown in Table S2. In general, the fixed factors (sequence of administration, period, and formulation) had no significant effects on the ANOVA of ln-transformed data for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

For the fed study, the 90% CIs for the test/reference GMRs of the ln-transformed data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 90.15–113.75%, 99.10–103.77% and 99.11–103.80%, respectively. CVs within intra-subject for ln-transformed data C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 23.19%, 4.54% and 4.55%, respectively. The results of ANOVA for effects of the fixed factors including administration sequence, period and formulation on ln-transformed data for the pharmacokinetic parameters (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) were shown in Table S3. No sequence of administration, period and formulation effects were found on ANOVA of ln-transformed data for the pharmacokinetic parameters.

3.4 Safety

In the fasting study, a total of 8 adverse events (AEs) occurred in 6 subjects (Table S4). 5 AEs were reported in 4 of 24 (16.7%) subjects who received a single-dose of the test product, included dermatitis (1/24), hyperuricemia (1/24), elevated heart rate (1/24), elevation of blood pressure (1/24), increased bilirubin (1/24), these AEs were all mild in severity, the outcome of 1 of 5 AEs was loss to follow-up, another one was remission, and the others were recovery. 2 of 24 (8.3%) subjects reported 3 AEs after administration of the reference products, which included dizziness (1/24), strain of lumbar muscles (1/24) and right patella osteomalacia (1/24), the severity of these AEs was mild, and the outcomes were all recovery. Not any subjects withdrew from the fasting study due to the AE. No serious AEs were reported throughout the fasting study.

Only 2 of 23 (8.7%) subjects experienced 2 AEs under the fed condition (Table S4), 1 of 23 subjects who received a single-dose oral administration of the test product reported one AE, which was hypotension accompanying with needlesickness, and severity of it was mild, the outcome was recovery. The other subject experienced AE due to the administration of the reference product, and the AE was also hypotension accompanying with needlesickness, which was mild and transient.

4 Discussion

We performed a clinical study to obtain some important information including pharmacokinetic properties of the test and reference products under the fasting or fed conditions, and within-subject CVs for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, the data for pharmacokinetic properties contributed to judge the accuracy of our study results and determine the bioequivalence between the test and reference products preliminarily, the data for within-subject CVs could be used for estimating the sample size and improving the study scheme in subsequent large-scale study if necessary, and if a test product was not equivalent to the reference product, we tried to change the particle size or formulation technology of the test product.

In accordance with the published studies, after a single dose oral administration of ciprofloxacin of 500 mg in healthy volunteers, within-subject CVs for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ after were in the approximate range of 12.8–19.6%, 11.0–22.0% and 9.95–26.5%, respectively [17–20]. The sample sizes range of 20 to 26 was adequate to ensure a power of 90% to correctly evaluate bioequivalence under the following assumptions: $\alpha = 0.05$, $0.95 < AUC_{0-t}/AUC_{0-\infty} < 1.05$, with within-subject CV of 20.0%, and the bioequivalence criterion was that 90% CIs of the test/reference GMRs for ln-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were completely included in the defined interval (0.80, 1.25) [21–22]. We chose a number of 24 subjects in our study, considering the following factors: the number of subjects was generally 24 in the previous published studies [17–19, 23]; probable withdrawal of subjects from the study; the within-subject CVs data mainly derived from male subjects [17–18, 20], the introduction of female subjects might increase the values for the CVs. For our study, the powers of test calculated for C_{max} under the fasting and fed conditions were 87.3% and 90.3%, respectively, which were both close to 90%, and the powers of test calculated for AUC_{0-t} and $AUC_{0-\infty}$ were all > 99.9%, therefore our choice of the sample size for subjects was appropriate.

Our study mainly aimed to evaluate the bioequivalence of the test and reference products after a single oral dose of 250 mg of ciprofloxacin hydrochloride in healthy Chinese subjects under the fasting and fed conditions. A total of 48 eligible subjects were enrolled in our study, consisted of 24 subjects in the fasting study and 24 subjects in the fed study. 23 of 24 subjects completed the clinical trial in the fed study, because one subject withdrew from the study before administration in the first period, due to personal negligence. In the fasting study, two subjects withdrew at the end of first period for personal reasons, the data of the them obtained from the first period of the fasting study were included in the pharmacokinetic analyses, but not safety and bioequivalence assessments.

Ciprofloxacin exhibited high safety properties with no drug-related severe AEs found through the study. A total of 8 of 47 subjects reported AEs in our study including the fasting and fed studies, all AEs were mild in severity, and outcomes of most AEs were cure or remission without additional medical assistance, most of the AEs were determined as “probable not” to be related to our study drugs.

As shown in Table 3 and Table 4, the results from our study suggest that the test product was bioequivalent to the reference product under the fasting and fed condition in accordance with the defined criteria for bioequivalence, all of the 90% CIs for the GMRs of ln-transformed values for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ fell within the acceptance range of 80–125%.

There were plenty of pharmacokinetics studies about ciprofloxacin after a single dose oral administration of a ciprofloxacin tablet in adult patients or healthy subjects, but most of studies on the pharmacokinetics of ciprofloxacin mainly focused on the dose of 500 mg, the studies about a single dose of 250 mg were rare among them [14, 24–33]. Several previous studies indicated that the 250-mg single-dose of ciprofloxacin was efficacious and safe in the therapy of uncomplicated gonorrhea,equally to the 500-mg dose of ciprofloxacin [7, 34–37], so it’s meaningful to investigate the pharmacokinetics of a single dose of ciprofloxacin 250 mg. The ciprofloxacin pharmacokinetic parameters determined after a single-dose oral administration of ciprofloxacin hydrochloride of 250 mg in our study were somewhat different from those reported in published study [14], maybe due to racial difference [38]. The median (range) of T_{max} after administration of the test and reference products under the fasting condition were 0.75 (0.5, 2) and 1.00 (0.5, 2) hour, respectively; for the fed condition, the corresponding values were 1.25 (0.75, 3) and 1.25 (0.5, 3) hour, demonstrating that ciprofloxacin was rapidly absorbed under both the fasting and fed condition, and food could delay the absorption of ciprofloxacin, the preliminary conclusion about the “food effect” was consistent with a published study [39], of course, the conclusion need further investigation and confirmation. The subjects of our study were all healthy adults; therefore, the findings were not necessarily applicable to patients or other populations.

5 Conclusions

In the study, the generic (test) and innovator (reference) product of ciprofloxacin hydrochloride of 250 mg were bioequivalent after a single oral dose administration under the fasting and fed conditions. Both two formulations were well tolerated.

Abbreviations

C_{max}: maximum concentration; AUC_{0-t}: area under the plasma concentration–time curve from time 0 to time t; $AUC_{0-\infty}$: area under the plasma concentration-time curve from time 0 to infinity; CI: confidence interval; GMR: geometric mean ratio; SD: standard deviation; CIOMS: Council for International Organizations of Medical Sciences; NMPA: National Medical Products Administration; BMI: body mass index; ECG: electrocardiogram; HIV: human immunodeficiency virus; SAEs: serious adverse events; T: test product; R: the reference product; AE: Adverse events; EDTA: ethylene diamine tetraacetic acid; IS: internal

standard; MRM: multiple reaction monitoring; LLOQ: lower limit of quantification; QC: quality control; SS: Safety Set; CV: variable coefficient; T_{max}: the time to maximum concentration; $t_{1/2}$: terminal elimination half-life; λz: apparent elimination rate constant derived from linear regression analysis of ln(concentration-time) curve during the elimination phase; ANOVA : analysis of variance; PK: pharmacokinetics.

Declarations

Ethics approval and consent to participate

The study was approved by independent Ethics Committee of the Guangdong Province Traditional Chinese Medical Hospital (registration number: A2017-002-1-02). Informed consent was obtained from all individual participants included in this study.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was received, and the study was carried out by self-funding.

Authors' contributions

All authors contributed to the study conception and design. QF drafted the clinical study protocol, WJS were responsible for revising the protocol. QF and WGM conducted the execution of the clinical trial. HJY was responsible for determination of clarithromycin plasma concentration, data collection, interpretation and analysis were performed by WGM. SWJ, WJR and DJ were all clinical research associates of this study. XY were responsible for conducting the whole study. The first draft of the manuscript was written by ZKW, QF and WJS were responsible for revising it critically. All authors read and approved the final manuscript, and ZKW was responsible for submission. All authors agree to be accountable for all aspects of the work.

Acknowledgments

The authors thank all of the subjects enrolled in our clinical study, and all the medical staff who participated in the clinical trials, and anyone who provided us more or less help in the whole study. Clinical and analytical facilities were provided by the Guangdong Province Traditional Chinese Medical Hospital (Guangzhou, China) and MicroConstants., Inc (Beijing, China), respectively.

CONSORT

The study adhered to the CONSORT guidelines, and our randomized controlled trials were carried out in strict accordance with the predetermined protocols.

References

1. Campoli-Richards DM, Monk JP, Price A, Benfield P, Todd PA, Ward A. Ciprofloxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs. 1988;35(4):373–447.
2. LeBel M. Ciprofloxacin: chemistry, mechanism of action, resistance, antimicrobial spectrum, pharmacokinetics, clinical trials, and adverse reactions. Pharmacotherapy. 1988;8(1):3–33.
3. Wiseman LR, Balfour JA. Ciprofloxacin. A review of its pharmacological profile and therapeutic use in the elderly. Drugs Aging. 1994;4(2):145–73.
4. Davis R, Markham A, Balfour JA. Ciprofloxacin. An updated review of its pharmacology, therapeutic efficacy and tolerability. Drugs. 1996;51(6):1019–74.
5. Wagenlehner F, Nowicki M, Bentley C, Lückermann M, Wohlert S, Fischer C, et al. Explorative Randomized Phase II Clinical Study of the Efficacy and Safety of Finafloxacin versus Ciprofloxacin for Treatment of Complicated Urinary Tract Infections. Antimicrob Agents Chemother. 2018;62(4):e02317-17.
6. Pichler HE, Diridl G, Stickler K, Wolf D. Clinical efficacy of ciprofloxacin compared with placebo in bacterial diarrhea. Am J Med. 1987;82(4A):329–32.
7. Echols RM, Heyd A, O'Keeffe BJ, Schacht P. Single-dose ciprofloxacin for the treatment of uncomplicated gonorrhea: a worldwide summary. Sex Transm Dis. 1994;21(6):345–52.
8. Ball AP, Tillotson GS. Lower respiratory tract infection therapy–the role of ciprofloxacin. J Int Med Res. 1995;23(5):315–27.
9. Fass RJ. Treatment of skin and soft tissue infections with oral ciprofloxacin. J Antimicrob Chemother. 1986;18 Suppl D:153-7.
10. Falser N, Mittermayer H, Weuta H. Antibacterial treatment of otitis and sinusitis with ciprofloxacin and penicillin V–a comparison. Infection. 1988;16(Suppl 1):51-4.
11. Spektor Z, Pumarola F, Ismail K, Lanier B, Hussain I, Ansley J, et al. Efficacy and Safety of Ciprofloxacin Plus Fluocinolone in Otitis Media With Tympanostomy Tubes in Pediatric Patients: A Randomized Clinical Trial. JAMA Otolaryngol Head Neck Surg. 2017;143(4):341–9.
12. Lipman J, Scribante J, Gous AG, Hon H, Tshukutsoane S. Pharmacokinetic profiles of high-dose intravenous ciprofloxacin in severe sepsis. The Baragwanath Ciprofloxacin Study Group. Antimicrob

Agents Chemother. 1998;42(9):2235–9.

13. LeBel M. Ciprofloxacin: chemistry, mechanism of action, resistance, antimicrobial spectrum, pharmacokinetics, clinical trials, and adverse reactions. Pharmacotherapy. 1988;8(1):3–33.
14. Gonzalez MA, Uribe F, Moisen SD, Fuster AP, Selen A, Welling PG, Painter B. Multiple-dose pharmacokinetics and safety of ciprofloxacin in normal volunteers. Antimicrob Agents Chemother. 1984;26(5):741–4.
15. Ledergerber B, Bettex JD, Joos B, Flepp M, Lüthy R. Effect of standard breakfast on drug absorption and multiple-dose pharmacokinetics of ciprofloxacin. Antimicrob Agents Chemother. 1985;27(3):350–2.
16. Rahm V, Schacht P. Safety of ciprofloxacin. A review. Scand J Infect Dis Suppl. 1989;60:120–8.
17. Valizadeh H, Hamishehkar H, Ghanbarzadeh S, Zabihian N, Zakeri-Milani P. Pharmacokinetics and bioequivalence evaluation of two brands of ciprofloxacin 500 mg tablets in Iranian healthy volunteers. Arzneimittelforschung. 2012;62(12):566–70.
18. Hassan Y, Alfadly SO, Azmin MN, Peh KK, Tan TF, Noorizan AA, Ismail O. Bioequivalence evaluation of two different formulations of ciprofloxacin tablets in healthy volunteers. Singapore Med J. 2007;48(9):819–23.
19. Harahap Y, Prasaja B, Indriati E, Lusthom W, Lipin. Bioequivalence of ciprofloxacin tablet formulations assessed in Indonesian volunteers. Int J Clin Pharmacol Ther. 2007;45(6):373–6.
20. Hammami MM, De Padua SJS, Hussein R, Al Gaai E, Khodr NA, Al-Swayeh R, et al. Generic-reference and generic-generic bioequivalence of forty-two, randomly-selected, on-market generic products of fourteen immediate-release oral drugs. BMC Pharmacol Toxicol. 2017;18(1):78.
21. Hauschke D, Steinijans VW, Diletti E, Burke M. Sample size determination for bioequivalence assessment using a multiplicative model. J Pharmacokinet Biopharm. 1992;20(5):557–61.
22. Ring A, Lang B, Kazaroho C, Labes D, Schall R, Schütz H. Sample size determination in bioequivalence studies using statistical assurance. Br J Clin Pharmacol. 2019;85(10):2369–77.
23. Cuadrado A, Gascón AR, Solinís MA, Ramírez E, Hernández RM, Knie U, Pedraz JL. Bioequivalence of two oral ciprofloxacin tablets formulations. Int J Clin Pharmacol Ther. 2004;42(6):336–41.
24. Crump B, Wise R, Dent J. Pharmacokinetics and tissue penetration of ciprofloxacin. Antimicrob Agents Chemother. 1983;24(5):784–6.
25. Davis RL, Koup JR, Williams-Warren J, Weber A, Smith AL. Pharmacokinetics of three oral formulations of ciprofloxacin. Antimicrob Agents Chemother. 1985;28(1):74–7.
26. Tartaglione TA, Raffalovich AC, Poynor WJ, Espinel-Ingroff A, Kerkering TM. Pharmacokinetics and tolerance of ciprofloxacin after sequential increasing oral doses. Antimicrob Agents Chemother. 1986;29(1):62–6.
27. Guay DR, Awni WM, Peterson PK, Obaid S, Stein D, Breitenbucher R, Matzke GR. Single and multiple dose pharmacokinetics of oral ciprofloxacin in elderly patients. Int J Clin Pharmacol Ther Toxicol. 1988;26(6):279–84.

28. Yeung SM, Walker SE, Tailor SA, Awdishu L, Tobe S, Yassa T. Pharmacokinetics of oral ciprofloxacin in continuous cycling peritoneal dialysis. Perit Dial Int. 2004;24(5):447–53.
29. Lubart E, Berkovitch M, Leibovitz A, Britzi M, Soback S, Bukasov Y, Segal R. Pharmacokinetics of ciprofloxacin in hospitalized geriatric patients: comparison between nasogastric tube and oral administration. Ther Drug Monit. 2013;35(5):653–6.
30. Overholser BR, Kays MB, Forrest A, Sowinski KM. Sex-related differences in the pharmacokinetics of oral ciprofloxacin. J Clin Pharmacol. 2004;44(9):1012–22.
31. Wingender W, Graefe KH, Gau W, Förster D, Beermann D, Schacht P. Pharmacokinetics of ciprofloxacin after oral and intravenous administration in healthy volunteers. Eur J Clin Microbiol. 1984;3(4):355–9.
32. Dixit RK, Satapathy SK, Kumar R, Dhiman RK, Garg SK, Taneja S, et al. Pharmacokinetics of ciprofloxacin in patients with liver cirrhosis. Indian J Gastroenterol. 2002;21(2):62–3.
33. Davis RL, Koup JR, Williams-Warren J, Weber A, Heggen L, Stempel D, Smith AL. Pharmacokinetics of ciprofloxacin in cystic fibrosis. Antimicrob Agents Chemother. 1987;31(6):915–9.
34. Moran JS. Ciprofloxacin for gonorrhea–250 mg or 500 mg? Sex Transm Dis. 1996;23(2):165–7.
35. Hook EW 3rd, Jones RB, Martin DH, Bolan GA, Mroczkowski TF, Neumann TM, et al. Comparison of ciprofloxacin and ceftriaxone as single-dose therapy for uncomplicated gonorrhea in women. Antimicrob Agents Chemother. 1993;37(8):1670–3.
36. Oriel JD. Ciprofloxacin in the treatment of gonorrhoea and non-gonococcal urethritis. J Antimicrob Chemother. 1986;18 Suppl D:129 – 32.
37. Balachandran T, Roberts AP, Evans BA, Azadian BS. Single-dose therapy of anogenital and pharyngeal gonorrhoea with ciprofloxacin. Int J STD AIDS. 1992;3(1):49–51.
38. Zhou HH, Koshakji RP, Silberstein DJ, Wilkinson GR, Wood AJ. Racial differences in drug response. Altered sensitivity to and clearance of propranolol in men of Chinese descent as compared with American whites. N Engl J Med. 1989;320(9):565–70.
39. Ledergerber B, Bettex JD, Joos B, Flepp M, Lüthy R. Effect of standard breakfast on drug absorption and multiple-dose pharmacokinetics of ciprofloxacin. Antimicrob Agents Chemother. 1985;27(3):350–2.

Tables

Table 1. Pharmacokinetic properties of ciprofloxacin after administration of two ciprofloxacin hydrochloride tablets under the fasting condition.

PK parameters	Mean ± SD (n = 24)	
	Test product	Reference product
T_{max} (h)[a]	0.75 (0.5, 2)	1.00 (0.5, 2)
C_{max} (ng/mL)	1600.0 ± 412.96	1727.8 ± 384.09
AUC_{0-t} (h·ng/mL)	6673.9 ± 1593.32	6831.3 ± 1533.67
$AUC_{0-\infty}$ (h·ng/mL)	6728.2 ± 1596.93	6884.7 ± 1535.64
λz (1/h)	0.1258 ± 0.01767	0.1273 ± 0.01420
$t_{1/2}$ (h)	5.574 ± 0.7064	5.475 ± 0.5707

[a] median (minimum, maximum)

Abbreviations: PK, Pharmacokinetic; SD, standard deviation; C_{max}, maximum concentration; AUC_{0-t}, area under the plasma concentration–time curve from time 0 to time t; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; λz, apparent elimination rate constant derived from linear regression analysis of ln(concentration-time) curve during the elimination phase; $t_{1/2}$, terminal elimination half-life.

Table 2. Pharmacokinetic properties of two ciprofloxacin hydrochloride tablets in healthy Chinese subjects under the fed condition.

PK parameters	Mean ± SD (n = 23)	
	Test product	Reference product
T_{max} (h)[a]	1.25 (0.75, 3)	1.25 (0.5, 3)
C_{max} (ng/mL)	1329.0 ± 333.15	1320.7 ± 347.16
AUC_{0-t} (h·ng/mL)	5512.3 ± 1215.44	5443.1 ± 1219.83
$AUC_{0-\infty}$ (h·ng/mL)	5567.3 ± 1226.47	5495.2 ± 1224.30
λz (1/h)	0.1183 ± 0.01403	0.1187 ± 0.01576
$t_{1/2}$ (h)	6.001 ± 0.7544	5.879 ± 0.6676

[a] median (minimum, maximum)

Abbreviations: PK, Pharmacokinetic; SD, standard deviation; C_{max}, maximum concentration; AUC_{0-t}, area under the plasma concentration–time curve from time 0 to time t; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; λz, apparent elimination rate constant derived from linear regression analysis of ln(concentration-time) curve during the elimination phase; $t_{1/2}$, terminal elimination half-life.

Table 3. Bioequivalence assessments between the test and reference products under the fasting condition (n = 22).

PK parameters	GMR	90% CI	CV (%)	Power of test
C_{max}	92.86	85.41 – 100.97	16.2	90.7
AUC_{0-t}	97.81	95.40 – 100.27	4.77	> 99.9
$AUC_{0-\infty}$	97.86	95.48 – 100.30	4.72	> 99.9

Abbreviations: PK, Pharmacokinetic; GMR, geometric mean ratio (Test/Reference); CI, confidence interval; CV, coefficient of variation (within intra-subject); C_{max}, maximum concentration; AUC_{0-t}, area under the plasma concentration–time curve from time 0 to time t; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity.

Table 4. Statistical analysis of results obtained after administration of ciprofloxacin in the fed study (n = 23).

PK parameters	GMR	90% CI	CV (%)	Power of test
C_{max}	101.27	90.15 – 113.75	23.19	87.3
AUC_{0-t}	101.41	99.10 – 103.77	4.54	> 99.9
$AUC_{0-\infty}$	101.43	99.11 – 103.80	4.55	> 99.9

Abbreviations: PK, Pharmacokinetic; GMR, geometric mean ratio (Test/Reference); CI, confidence interval; CV, coefficient of variation (within intra-subject); C_{max}, maximum concentration; AUC_{0-t}, area under the plasma concentration–time curve from time 0 to time t; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity.

Figures

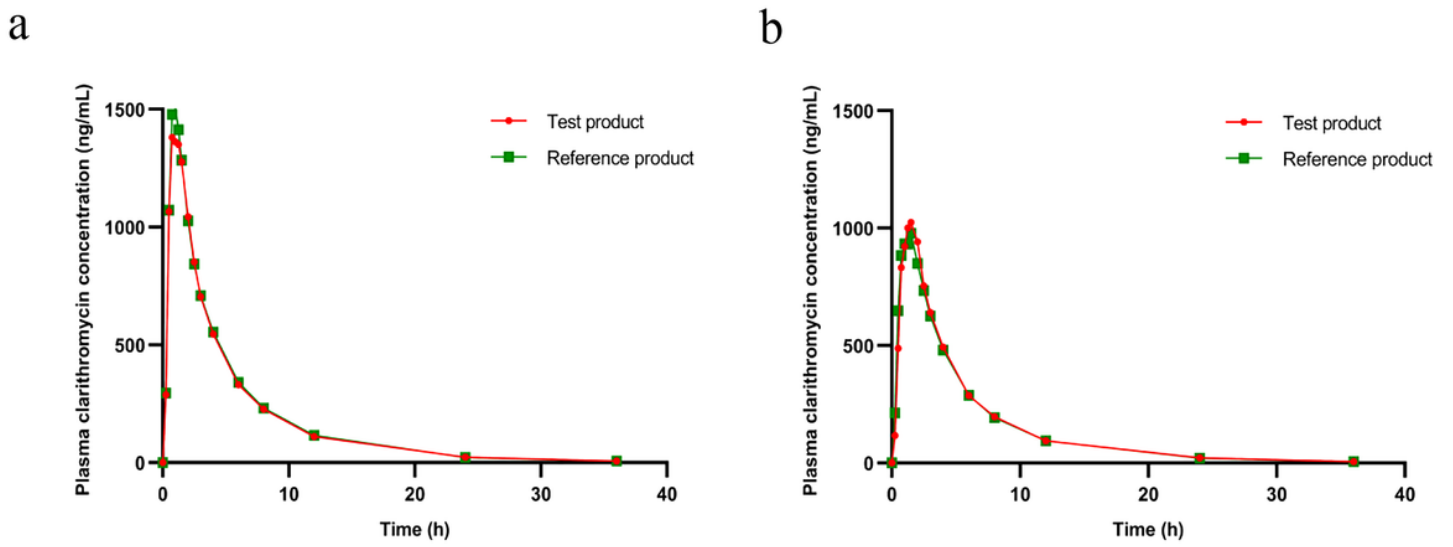

Figure 1

Plasma concentration-time curves of ciprofloxacin in healthy Chinese subjects. a After a single oral dose of 250 mg of the test and reference products under the fasting condition (n = 24). b After a single oral dose of 250 mg of the test and reference products under the fed condition (n = 23).

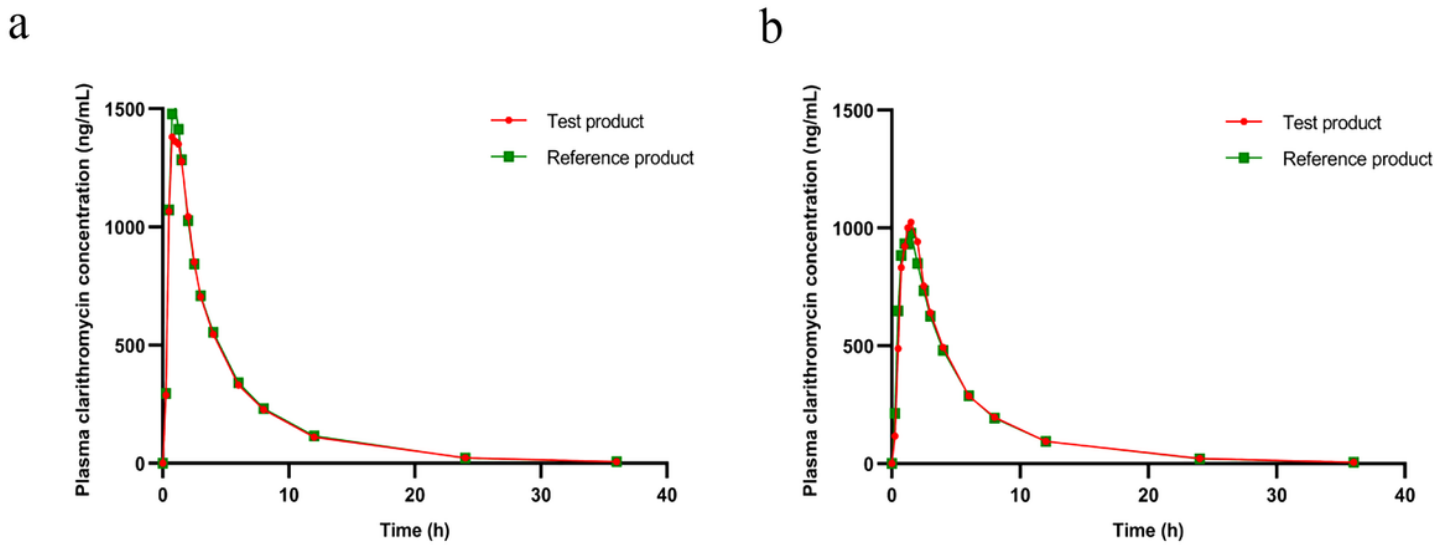

Figure 1

Plasma concentration-time curves of ciprofloxacin in healthy Chinese subjects. a After a single oral dose of 250 mg of the test and reference products under the fasting condition (n = 24). b After a single oral dose of 250 mg of the test and reference products under the fed condition (n = 23).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- TableS1.xlsx
- TableS2.docx
- TableS2.docx
- TableS3.docx
- TableS3.docx
- TableS4.docx
- TableS4.docx
- CONSORT2010FlowDiagram.doc
- CONSORT2010FlowDiagram.doc
- CONSORT2010Checklist.doc
- CONSORT2010Checklist.doc