Bioequivalence Evaluation of Two Brands of Cefuroxime 500 mg Tablets (Cefuzime® and Zinnat®) in Healthy Human Volunteers

Mansour S. Al-Saida, Khalil I. Al-Khamisb, Esmail M. Niazya, Yousry M. El-Sayedb, Khalid A. Al-Rashooda, Sulaiman Al-Bella, Mohd. A. Al-Yamani, Tawfeeq A. Al-Najjarb, Syed M. Alamb, Ruwayda Dhamb, and Q. Zaman Qumaruzamanb

ABSTRACT: A bioequivalence study of two oral formulations of 500 mg cefuroxime axetil was carried out in 24 healthy volunteers following a single dose, standard two-treatment cross-over design at the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, working jointly with King Khalid University Hospital. The two formulations used were Cefuzime® (Julphar, United Arab Emirates) as the test and Zinnat® (Glaxo Wellcome, England) as the reference product. Both test and reference tablets were administered to each subject after an overnight fasting on two treatment days separated by a 1-week washout period. After dosing, serial blood samples were collected for a period of 8 h. Plasma harvested from blood was analysed for cefuroxime by a sensitive, reproducible and accurate high pressure liquid chromatography (HPLC) method. Various pharmacokinetic parameters including \( \text{AUC}_{0\rightarrow t} \), \( \text{AUC}_{0\rightarrow \infty} \), \( C_{\text{max}} \), \( T_{\text{max}} \), \( T_{1/2} \) and \( K_{\text{el}} \) were determined from plasma concentrations of both formulations and found to be in good agreement with reported values. \( \text{AUC}_{0\rightarrow t} \), \( \text{AUC}_{0\rightarrow \infty} \) and \( C_{\text{max}} \) were tested for bioequivalence after log-transformation of data. No significant difference was found based on an analysis of variance (ANOVA); 90% confidence interval for test/reference ratio of these parameters were found within bioequivalence acceptance range of 80–125%. Based on these statistical inferences, it was concluded that Cefuzime® is bioequivalent to Zinnat®. Copyright © 2000 John Wiley & Sons, Ltd.

Key words: bioequivalence evaluation; cefuroxime; pharmacokinetic parameters

Introduction

Bioequivalence of two formulations of the same drug is concluded based on the lack of difference in the rate (\( C_{\text{max}} \)) and extent of absorption (AUC) especially in conventional drug formulations [1]. In the present study, the bioequivalence of two cefuroxime tablets was evaluated by comparing those pharmacokinetic parameters.

Cefuroxime is a second-generation cephalosporin, proven to be relatively safe. It can be given orally (cefuroxime axetil) as well as parenterally (cefuroxime sodium) [2]. Cefuroxime axetil is a prodrug of cefuroxime, which upon absorption undergoes immediate de-esterification to free cefuroxime. Cefuroxime axetil has an \textit{in vitro} antibacterial spectrum against many Gram-positive and Gram-negative organisms. Its beta-lactamase (\( \beta \)-lactam) stability makes it useful in treating a variety of infections caused by \( \beta \)-lactam-producing strains of \textit{Haemophilus influenzae}, \textit{Moraxella catarrhalis} and...
Staphylococcus aureus [3]. An advantage of cefuroxime over other second-generation cephalosporins is that it is effective in the treatment of Neisseria gonorrhoea and H influenzae. It is characterized by being the only second-generation cephalosporin which adequately penetrates into the cerebrospinal fluid (CSF) [4]. Cefuroxime acts by inhibiting bacterial wall synthesis of actively dividing cells by binding to one or more penicillin binding proteins (PBPs), resulting in the formation of a defective cell wall that is osmotically unstable and thus a bactericidal action is exerted [5,6]. Cefuroxime is indicated in the treatment of uncomplicated gonorrhoea, respiratory tract infections, urinary tract infections, paediatric infections, preoperative prophyaxis, bone and joint infections, meningitis, skin, soft tissue infections and septicaemia [7].

Upon oral administration, cefuroxime axetil is reported to be rapidly hydrolysed in the intestinal mucosa, with 37–52% of an oral dose reaching to the systemic circulation as cefuroxime [2,7–9]. Peak serum levels occur within 2–3.6 h [7,10,11] following an oral dose; the reported area under the curve (AUC) is 19.9 μg/mL·h in healthy subjects after administration of a single oral cefuroxime axetil 500 mg dose [10]. Approximately 33–50% of the circulating cefuroxime is protein bound [7,9]. It is distributed throughout most body tissues and fluids including the gall bladder, liver, kidney, bones, uterus, ovary, sputum, bile, and peritoneal, pleural and synovial fluids. It penetrates inflammed meninges and reaches therapeutic levels within the CSF, and it crosses the placenta [2,4]. Cefuroxime is largely (52%) excreted unchanged in the urine and a small percentage is excreted in breast milk; most of the drug is recovered within the first 6 h after administration [7,12,13]. Elimination half-life (T1/2) is 1–2 h [7,10,14,15] in patients with normal renal functions and it increases as renal function declines [2].

Objectives of the Study

The aim of this study was to assess the bioequivalence of a test formulation of Cefuroxime axetil (Cefuzime® 500 mg tablets, Julphar, United Arab Emirates) relative to a reference formulation (Zinnat® 500 mg tablets, Glaxo-Wellcome, UK) by statistical analysis of the pharmacokinetic parameters AUC0–t, AUC0–∞, and Cmax as recommended by the Food and Drug Administration (FDA) [21].

Material and Methods

Study Products

Manufacturer: Test Product: Cefuzime®—Cefuroxime axetil 500 mg tablet Batch No.: 0007, Expiry date: February 2002 (cefuroxime content: 98.48%)

Manufacturer: Gulf Pharmaceutical Industries, Manufacturer: Reference Zinnat®—Cefuroxime axetil 500 Product: mg tablets Batch No.: B4759BA, Expiry date February 2001 (cefuroxime content: 99.78%)

Manufacturer: Glaxo-Wellcome, Greenford, UK

Study Subjects

Twenty-four (24) healthy adult male volunteers participated in this comparative study at King Khalid University Hospital, Riyadh, Saudi Arabia. Their mean age was 35.1 ± 6.8 years with a range of 21–48 years and mean body weight was 75.3 ± 8.6 kg with a range of 55–88 kg. On the basis of medical history, clinical examination and laboratory investigation (haematology, blood biochemistry and urine analysis), no volunteer had a history or evidence of hepatic, renal, gastrointestinal or haematologic deviations or any acute or chronic diseases or allergy to β-lactam antibiotics. The volunteers were instructed to abstain from taking any drug, including over-the-counter (OTC), for 2 weeks prior to and during the study period. 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 Institutional Review Board.
Drug Administration and Sample Collection

After an overnight fasting (10 h) subjects were given single dose of either formulation (reference or test in a randomized fashion) of cefuroxime axetil 500 mg tablet with 240 mL of water. Food and drinks (other than water, which was allowed after 2 h) were not allowed until 4 h after ingestion of the tablets and then water, breakfast, lunch and dinner were given to all volunteers according to a time schedule. Beverages and food containing caffeine were not permitted over the entire course of study. Volunteers were ambulatory during the study but prohibited from strenuous activity, they were under direct medical supervision at study site. Blood samples (10 mL) for cefuroxime assay were drawn into evacuated heparinized glass tubes through an indwelling cannula before (0 h) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0 and 8.0 h after dosing. Blood samples were centrifuged at 1600 g for 10 min; plasma was decanted in coded polypropylene tubes and stored frozen at −70°C pending drug analysis. After a period of 7 days, the study was repeated in the same manner to complete the cross-over design.

Chromatographic Conditions

Plasma samples were analysed for cefuroxime according to a sensitive, selective and accurate high pressure liquid chromatography (HPLC) method, which was developed and validated before the study. All solvents used were of HPLC grade; while other chemicals and reagents were of analytical grade; cefuroxime and cephalaxin (internal standard) were purchased from Sigma-Aldrich Company Ltd., Poole, Dorset, UK.

The HPLC system was from Shimadzu, Kyoto, Japan, and it consisted of a solvent delivery pump (LCD-10AD), a system controller (SCL-10A), an auto-injector (SIL-10A), and a variable ultraviolet (UV) detector (SPD-10A). Chromatographic separation was performed using a Supelcosil™ LC-18DB (5 μm, 4.6 × 150 mm) stainless steel column. A guard column of the same material was used (Waters Associates, USA). The mobile phase consisted of 10% acetonitrile in 0.05 M potassium dihydrogen phosphate buffer (pH 3.0), and eluted at a flow rate of 2.0 mL/min; effluent was monitored at a wavelength of 280 nm. Each analysis required a maximum of 12 min. Quantitation was achieved by measurement of the peak height ratio of the drug to the internal standard. The method was validated by following international guidelines [16]. The limit of quantitation for cefuroxime was 0.25 μg/mL plasma. A standard curve was generated by preparing ten non-zero plasma standards over the range of 0.25–10 μg/mL. Standards were analysed in replicates (n = 12). The average peak height ratios were plotted against the concentration. The weighted linear regression of cefuroxime assay in plasma was characterized as having a mean slope of 0.0456 and a mean intercept of 0.0038 (r = 0.9990). Intra-day coefficient of variation (CV%) ranged from 2.20 to 2.93% and inter-day CV ranged from 1.88 to 2.93% at three different concentrations (0.75, 3.5 and 7.0 μg/mL). The mean relative recovery and absolute recovery were 100.3 and 96.5%, respectively. Stability tests shown that cefuroxime is stable in plasma for at least 4 weeks when stored at −70°C.

Sample Preparation for HPLC Injection

A 400 μL plasma sample was taken in an eppendorf microcentrifuge tube (1.5 mL); 100 μL of internal standard (cephalexin 100 μg/mL) and 100 μL of 12.5% trichloroacetic acid (TCA) were added and shaken on a vortex mixer for 30 s and centrifuged at 5000g for 10 min. The supernatant solution was loaded in the autosampler tray and 50 μL was then injected to column and peak heights were recorded.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by means of model independent method using MS-Excel software. The maximum cefuroxime concentrations (C_max) and the corresponding peak times (T_max) were determined by the inspection of the individual drug plasma concentration-time profiles. The elimination rate constant (K_el) was obtained as the slope of the linear
regression of the log-transformed plasma concentration values versus time data in the terminal phase. \( T_{1/2} \) was calculated as \( 0.693/K_{el} \). Area under the curve to the last measurable concentration (AUC\(_{0-t}\)) was calculated by the linear trapezoidal rule. Area under the curve extrapolated to infinity (AUC\(_{0-\infty}\)) was calculated by equation:AUC\(_{0-t}\) + Ct/K\(_{el}\) where Ct 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 model [17], SAS Institute, NC, USA) for cross-over design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. The difference between two related parameters was

![Figure 1. Mean plasma concentrations of cefuroxime after oral administration (500 mg) of the two brands to 24 healthy human volunteers](image-url)


### Table 1. Pharmacokinetic parameters of cefuroxime 500 mg tablets

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Cefuzime® (test)</th>
<th>Zinnat® (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{0-\infty}$ (μg/mL·h)</td>
<td>13.751 ± 4.928</td>
<td>14.242 ± 4.591</td>
</tr>
<tr>
<td>AUC$_{0-8}$ (μg/mL·h)</td>
<td>14.490 ± 4.938</td>
<td>14.901 ± 4.591</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (μg/mL)</td>
<td>4.280 ± 1.472</td>
<td>4.484 ± 1.189</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.917 ± 0.637</td>
<td>1.688 ± 0.586</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>1.218 ± 0.251</td>
<td>1.134 ± 0.182</td>
</tr>
<tr>
<td>$K_{\text{el}}$ (per h)</td>
<td>0.593 ± 0.128</td>
<td>0.626 ± 0.100</td>
</tr>
</tbody>
</table>

*Mean ± S.D., n = 24.

were also calculated as per the FDA guidelines [21] and the results are shown in Table 2.

The mean and standard deviation of AUC$_{0-\infty}$, AUC$_{0-\infty}$, and $C_{\text{max}}$ of the two products did not differ significantly, suggesting that the plasma profiles generated by Cefuzime® are comparable to those produced by Zinnat®. ANOVA for these parameters, after natural log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, formulations or sequence, having a $p$-value greater than 0.05.

The 90% CIs also demonstrated that the ratios of AUC$_{0-\infty}$ or AUC$_{0-\infty}$ or $C_{\text{max}}$ of the two formulations and for the two periods lie within the FDA acceptable range of 80–125%.

### Conclusion

On the basis of the plasma levels of the 24 volunteers completing this study (see Figure 1), the mean relative bioavailability of cefuzime 500 mg tablets was 99.6% for AUC$_{0-\infty}$, 100.2% for AUC$_{0-\infty}$ and 100.1% for $C_{\text{max}}$. Statistical comparison of the AUC$_{0-\infty}$, AUC$_{0-\infty}$ and $C_{\text{max}}$ clearly indicated no significant difference between Cefuzime® 500 mg tablets and Zinnat® 500 mg tablets in any of the calculated pharmacokinetic parameters. The CIs for the ratios of mean AUC$_{0-\infty}$ AUC$_{0-\infty}$ and $C_{\text{max}}$ indicated that these values are entirely within the bioequivalence acceptance range of 80–125% (using log-transformed data).

Based on the above, we can conclude that Cefuzime®, manufactured by Gulf Pharmaceutical Industries, United Arab Emirates, is bioequivalent to Zinnat®, manufactured by Glaxo-Wellcome, England, and that both products can be considered equally effective in medicinal practice.
References


