study of two brands of cefuroxime 500 mg tablets (Bioxime® and Zinnat®) in adults healthy volunteers

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ABSTRACT

A bioequivalence study of two oral formulations of Cefuroxime Axetil 500 mg Bioxime® (Pharmacare International Manufacturing Co., Yemen) as the test and Zinnat® (GlaxoSmithKline, UK) as the reference product was carried out in 26 healthy male & female volunteers (one drop out) following a single dose, two sequence, two period, two-treatment cross-over design. Both test and reference tablets were administered to each subject after an overnight fasting on two treatment days separated by an one-week washout period. After dosing, serial blood samples were collected for a period of 8 h. Serum harvested from blood was analyzed for Cefuroxime by a sensitive, selective, reproducible and accurate high pressure liquid chromatography (HPLC) method. Various pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$ and K_{el} were determined from Serum concentrations of both formulations and found to be in good agreement with reported values. AUC_{0-t}, $AUC_{0-\infty}$, C_{max} were tested for bioequivalence after log-transformation of data. No significant difference was found based on analysis of variance (ANOVA), 90% confidence interval for test/reference ratio of these parameters was found within bioequivalence acceptance range of 80-125%. Based on these statistical inferences, it was concluded that Bioxime® is bioequivalence to Zinnat®. Both products were well tolerated.

Key words: Cefuroxime, Bioequivalence Study.
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INTRODUCTION

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.[4] In the present study, the bioequivalence of two Cefuroxime tablets was evaluated by comparing those pharmacokinetic parameters.

Cefuroxime is a broad-spectrum, β-lactamase-stable cephalosporin. [12] Cefuroxime is a second-generation cephalosporin, proven to be relatively safe. It can be given orally (Cefuroxime Axetil) as well as parenterally (Cefuroxime Sodium)[4]. Cefuroxime Axetil is 1-acetyloxyethyl ester of Cefuroxime [8, 7, 12], which has improved oral bioavailability [2]. After oral administration, Cefuroxime Axetil is absorbed from the gastrointestinal tract and rapidly hydrolyzed by nonspecific esterase in the intestinal mucosa and blood to Cefuroxime. Therefore, Cefuroxime Axetil cannot be measured in human plasma [2].

Cefuroxime Axetil has an *in vitro* antibacterial spectrum against many Gram-positive and Gram-negative organisms. Its beta-lactamase (β -lactam) stability makes it useful in treating a variety of infections caused by β -lactam-producing strains of *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus*[1]. An advantage of Cefuroxime over other second-generation cephalosporin 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, 17].

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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 [7, 4]. Cefuroxime is indicated in the treatment of uncomplicated gonorrhea, respiratory tract infections, urinary tract infections, pediatric infections, preoperative prophylaxis, bone and joint infections and septicemia [4, 17].

Upon oral administration, Cefuroxime Axetil is reported to be rapidly hydrolyzed in intestinal mucosa, with 37-52% of an oral dose reaching to the systemic circulation as Cefuroxime [4]. Peak serum levels occur within 2 – 3.6 h 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 . Approximately 33 – 50% of the circulating Cefuroxime is Protein bound. It is distributed throughout most body tissues and fluids including the gallbladder, 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. 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 [4]. Elimination half-life($T_{1/2}$) is 1 – 2 h [2, 4] in patients with normal renal functions and it increases as renal function declines.

Objective of the study

• Primary: To assess the bioequivalence of test product of Cefuroxime Axetil (Bioxime® 500mg tablets, Pharmacare International Manufacturing Co., Yemen) relative to a reference product (Zinnat®500mg tablets, GlaxoSmithKline, UK) 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)[1].

• Secondary: To assess the tolerability of both products by registration of adverse events and /or adverse drug reactions.

EXPERIMENTAL SECTION

1. Study Products

The test product was Bioxime® 500mg tablets (Cefuroxime Axetil 500mg, batch No.: 1202078, Expiry date: February 2015) manufactured by Pharmacare International Manufacturing Company, Yemen. The reference product was Zinnat® 500mg tablets (Cefuroxime Axetil 500mg, batch No.:C541556, Expiry date: August 2014) manufactured by GlaxoSmithKline, UK.

2. In vitro dissolution

An in vitro dissolution profile comparison was performed using analytical methods according to USP requirements. Samples of test and reference tablets were evaluated (n =12) and Dissolution apparatus -II paddle (Erweka DT70 Germany) was used, 900ml of different medias of deferent pH 1.2, 4.5 and 6.8 was used. Temperature was maintained at 37 ± 0.5 ^{0}C. Samples of aliquot were collected after 15, 30 and 45 minutes. Concentration of Cefuroxime Axetil was determined by UV/VIS Spectrophotometer (Jasco V-230, Japan) at 278mm.

3. Subjects

All 26 male and female healthy subject volunteers enrolled into the study were examined to verify their healthy status. These examinations included medical history, vital sign measurements, electrocardiogram (ECG), laboratory investigations (liver functions test, renal functions test, blood sugar, complete blood cell count, viral serology) and urinalysis (sediment, drugs). Subjects with relevant clinical, analytical, or ECG abnormalities were excluded from the trial. Additional exclusion criteria were as follows: smoking, history of alcohol or drug abuse, consumption of any medication or chewing qat within one month prior to study commencement, participation in a clinical trial in the 3 months before enrollment; history of clinically important illness or major surgery in the 6 months before enrollment; inability to relate to and/or cooperate with the investigators; medication allergy; illness or disorders that could affect the absorption, distribution, metabolism, and/or excretion of drugs (e.g., mal-absorption, edemas, renal and/or hepatic failure); history of positive serology for hepatitis B or C (not due to immunization); or HIV and blood or blood-derivative transfusion in the 6 months before enrollment.

Their mean Age was 27.5 ± 3.6 year with a range of 20-36 year and mean body weight was 59.5 ± 9.1 kg with a range of 47-80 kg. The volunteers were informed about the risk and aim of the study and signed a written informed consent form before entering the study. The volunteers were free to withdraw from the study at any time. The study protocol was approved by Ethics Committee of Pharmacy College, Sana'a University.

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4. Study Design
The study was designed according to the Food and Drug Administration (FDA), European Medicines Agency (EMA) and Gulf cooperation council (GC) guidelines on bioequivalence investigation [1, 5, 6]. It was conducted between September 2013 and December 2013 in compliance with the International Conference of Harmonization (ICH) guideline for Good Clinical Practice (GCP) and the Declaration of Helsinki and its amendments [16, 11]. Ethical approval was received from the Ethics Committee of pharmacy college, Sana'a University.

The study was a single-dose, randomized- sequence, double-blind, two-period cross-over design with 7 day washout period. A single dose 500 mg of either product (Bioxime 500 mg or Zinnat 500 mg) was administered with 200 ml of water to swallow after an overnight fasting (10 h). The order of administration was randomized prior to the start of the study. Food intake was strictly controlled and all subjects received the same food to minimize the effects of food on the study outcomes. The standardized breakfast and lunch were served at 4 and 7 h after drug administration, respectively. The consumption drinks contain caffeine, grapefruit juice, and beverages were not permitted for 48 h prior to the study, or after drug administration, until final blood samples were collected. During the study period, the subjects were ambulatory but prohibited from strenuous activity, they were under medical surveillance. After a period of 7 days, the study was repeated in the same manner to complete the cross-over design.

5. Blood Sample Collection
A 20 GA catheter (Neotec, Singapore) was inserted into suitable forearm vein and 10 ml of blood was withdrawn at different time intervals. Venous blood samples were obtained prior to dosing (baseline) and 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 in each period. The samples were collected in pre-labeled polypropylene tubes. Within 30 min after sampling, the blood samples were centrifuged (Sigma3-18k, Witeg Wised, Germany)at room temperature at approximately 12000 rpm for 10 min. the separated Serum was decanted in coded polypropylene tubes and stored frozen at -70^0C (Ultra-Low Temp. freezer, Witeg Wised, Germany) until analysis.

6. Determination of Cefuroxime Serum Concentrations
Due to rapid de-esterification of Cefuroxime Axetil to Cefuroxime in the intestinal mucosa, Serum Cefuroxime concentrations were measured to assess bioequivalence of studied products. Serum samples were analyzed for Cefuroxime according to a sensitive, selective, reproducible 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 sodium (Reference standard) was purchased from Aurobindo pharma (India) and Acetanilide (internal standard) was purchased from Thomas Baker (India). The HPLC system was from Jasco, Japan, and it consisted of a solvent delivery pump (PU-2089 Plus), a system controller (LC-NetII/ADC) and a variable ultraviolet/Visible (UV/VIS) detector (UV-2070Plus). Chromatographic separation was performed using an Optimal ODS-H C-18 (150X4.6 mm, 5µm) stainless steel column. A guard column of the same material was used (Capital Analytical, UK). The mobile phase consisted of 25% Acetonitrile (Sigma-Aldrich, Germany) in 0.05M Ammonium dihydrogen phosphate (Scharlau, Spain) buffer (pH 3.5), and eluted at a flow rate of 1.0mL/min; effluent was monitored at a wavelength of 273 nm. Each analysis required a maximum of 8 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 [9, 10, 3]. The limit of Quantitation for Cefuroxime was 0.2µg/ml Serum. A standard curve was generated by preparing seven non-zero Serum standards over the range of 0.2 – 10 µg/ml. The average peak height ratios were plotted against the concentration. The linear regression of Cefuroxime assay in Serum was characterized as having a mean slope of 0.249 and a mean intercept of 0.0041 ($r = 0.9999$). Intra- day coefficient of variation (CV %) ranged from 0.973 to 1.400% and inter-day CV ranged from 1.568 to 2.858% at three different concentrations (0.6, 3.5 and 7.0µg/ml). The mean relative recovery 95.85%. Stability tests shown that Cefuroxime is stable in Serum for at least 4 weeks when stored at –70°C. A 400 µl Serum sample was taken and mixed with 100 µl of internal standard (Acetanilide 125µg/ml) and 600 µl of 5% Perchloric Acid (Thomas Baker, India) were added and shaken on a vortex mixer (VM-10, Witeg Wised, Germany) for 30 s and centrifuged at 12000rpm for 10 min. The supernatant solution was loaded in the polypropylene tube and 20µl was then injected to the HPLC system and peak heights were recorded.

7. Pharmacokinetics Analysis
Pharmacokinetic analysis was performed by means of model independent method using MS- Excel software. The parameters selected as primary endpoints of the study were the area under the serum concentration-time curve (AUC_{0-t}), the area under the serum concentration-time curve extrapolated to the infinity ($AUC_{0-\infty}$) and the maximum Serum concentration of the drug (C_{max}). The time to reach maximum plasma concentration of the drug (T_{max}), the elimination half- life ($T_{1/2}$) was selected as secondary parameters. The maximum Cefuroxime concentrations (C_{max}) and the corresponding peak times (T_{max}) were determined by the inspection of the individual drug serum concentration-time profiles. The elimination rate constant (K_{el}) was obtained as the slope of the linear regression of

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the log-transformed serum concentration values versus time data in the terminal phase. $T_{1/2}$ was calculated as $0.693/K_{el}$. Area under the serum-time curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. Area under the serum concentration - time curve extrapolated to the infinity ($AUC_{0-\infty}$) was calculated by equation $AUC_{0-t} + C_t/ K_{el}$, where C_t is the last measurable concentration.

8. Statistical Analysis

The statistical calculations were performed using the SPSS Software (version 22 for Windows). The tests for normality of ln-transformed pharmacokinetic parameters were performed with the use of the Shapiro-Wilk, Kolmogorov-Smirnov tests. The analysis of variance (ANOVA) was performed on the ln-transformed data of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} applying General Linear Models (GLM) procedure to assess the effects of formulations, periods, sequences and subjects on these parameters. The statistical significance of effects was determined on basis of the calculated *p*-values with value larger than 0.05 meaning no statistical significance.

Based on the ANOVA results, 90% CI for the μ_T/μ_R (ratio of geometric means for the test and the reference product) of the analyzed pharmacokinetic parameters was computed. Bioequivalence is assumed when 90% CI of the point estimate (test over reference products) for AUC_{0-t}, $AUC_{0-\infty}$ and for C_{max} falls within the 80 – 125 % range.

9. Tolerability Analysis

In order to prevent the occurrence of adverse events during the study, the following measures have been taken: The drug administration was limited to a single oral dose of 500 mg/study period; only healthy adult volunteers with no history of hypersensitivity reactions to Cefuroxime or other related molecules were enrolled; Each volunteers checked well being prior to his/her discharge from the place of the study.

Tolerability was determined by monitoring vital signs (blood pressure, heart rate, body temperature) at baseline and at the end of each period. The participants were interviewed by the physician as well as nonspecific questioning. All the subjects were advised to report any adverse event or undesirable sign or symptom at any time during the study period.

RESULTS AND DISCUSSION

The dissolution profiles of the test product matched those of the reference product under various pH conditions as per USP requirements and recommendations. Similarity factors calculated for the dissolution profiles in all buffers indicated similarity between dissolution profiles of the test and reference products. The similarity factor (*f2*) values at different pH lie within acceptance range of 50-100.The results are presented in Figure (2).

Both formulations were well tolerated by the volunteers; unexpected incidents that could have influenced the outcome of the study did not occur. There was one drop-out before starting the first period and all the other volunteers who had started the study continued to the end and were discharged in good health. The summary of the demographic data of the population is presented in Table (1).

The method validation covered all required tests, including evaluation of the carry-over effect, sensitivity, selectivity, extraction recovery, limit of quantification, linearity, accuracy, precision and stability. The validation parameters were defined according to the EMA and the FDA [9, 10, 13]. All parameters met predefined acceptance criteria.

Both formulations were readily absorbed from the gastrointestinal tract and Cefuroxime was measurable at the first sampling time (0.5 h) in nearly all volunteers. The Average Serum Concentration-Time Profile for All Subjects of the two formulations is shown in the Figure 1. All calculated pharmacokinetic parameter values were in good agreement with previously reported studies [2, 4]. Table 2 shows the pharmacokinetic parameters for the two brands of Cefuroxime 500 mg tablets. The 90% CIs for the natural log-transformed data were also calculated as per the FDA guidelines [14] and the results are shown in Table (3).

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

The 90% CIs also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two formulations and for the two periods lie within the FDA acceptable range (80-125%).

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**Table (1) Demographic data of the population included in the study (n = 26)**

Variable		Data
Gender	male [n]	13
	female [n]	13[a]
Total volunteers [n]		26
Age [years]*		27.5<20 - 36>
Height [cm]*		159 <148 - 173>
Weight [kg]*		59.5<47 - 80>
BMI[kg/m^2][b]		23.4 <19.1 -29.2>

* Mean <range>   [a] one drop-out
[b] Mass Body Index (weight/height^2)

Table (2) Pharmacokinetic parameters of Cefuroxime 500mg tablets (n=25, mean ± SD)

Pharmacokinetic parameters	Bioxime® (Test)	Zinnat® (Reference)
AUC_{0-t} (µg/ml·h)	17.308 ± 3.588	18.13 ± 5.113
$AUC_{0-\infty}$ (µg/ml·h)	17.802 ± 3.667	18.717 ± 5.257
C_{max} (µg/ml)	5.562 ± 1.394	6.044 ± 1.566
T_{max}(h)	1.780 ± 0630	1.760 ± 0.663
K_{el}(h^{-1})	0.613 ± 0.120	0.632 ± 0.133
$T_{1/2}$(h)	1.169 ± 0.209	1.142 ± 0.235
AUC_{0-t} / $AUC_{0-\infty}$ (%)	97.112 ± 0.754	96.814 ± 1.370

Table (3) Statistical Analysis of natural log-transformed data

Statistical Analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
ANOVA GLM(*p*-value)	0.512 (0.253)[a]	0.468 (0.288)	0.144 (0.592)
90% CI	99.5(96.3 – 102.6)[b]	99.3(96.2 – 102.4)	96.5(90.9 – 102.1)
90% CI (CV)	9.3	9.2	16.9

[a] values in parentheses indicate the analysis of period   [b] Point Estimated (90% CI)
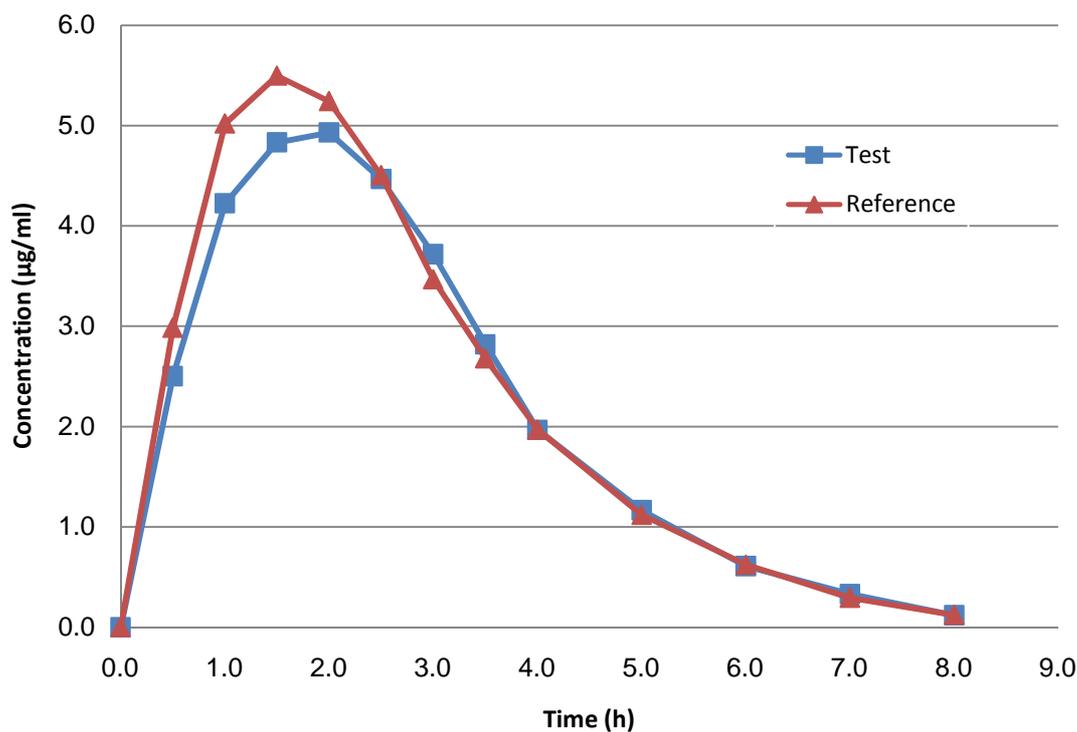
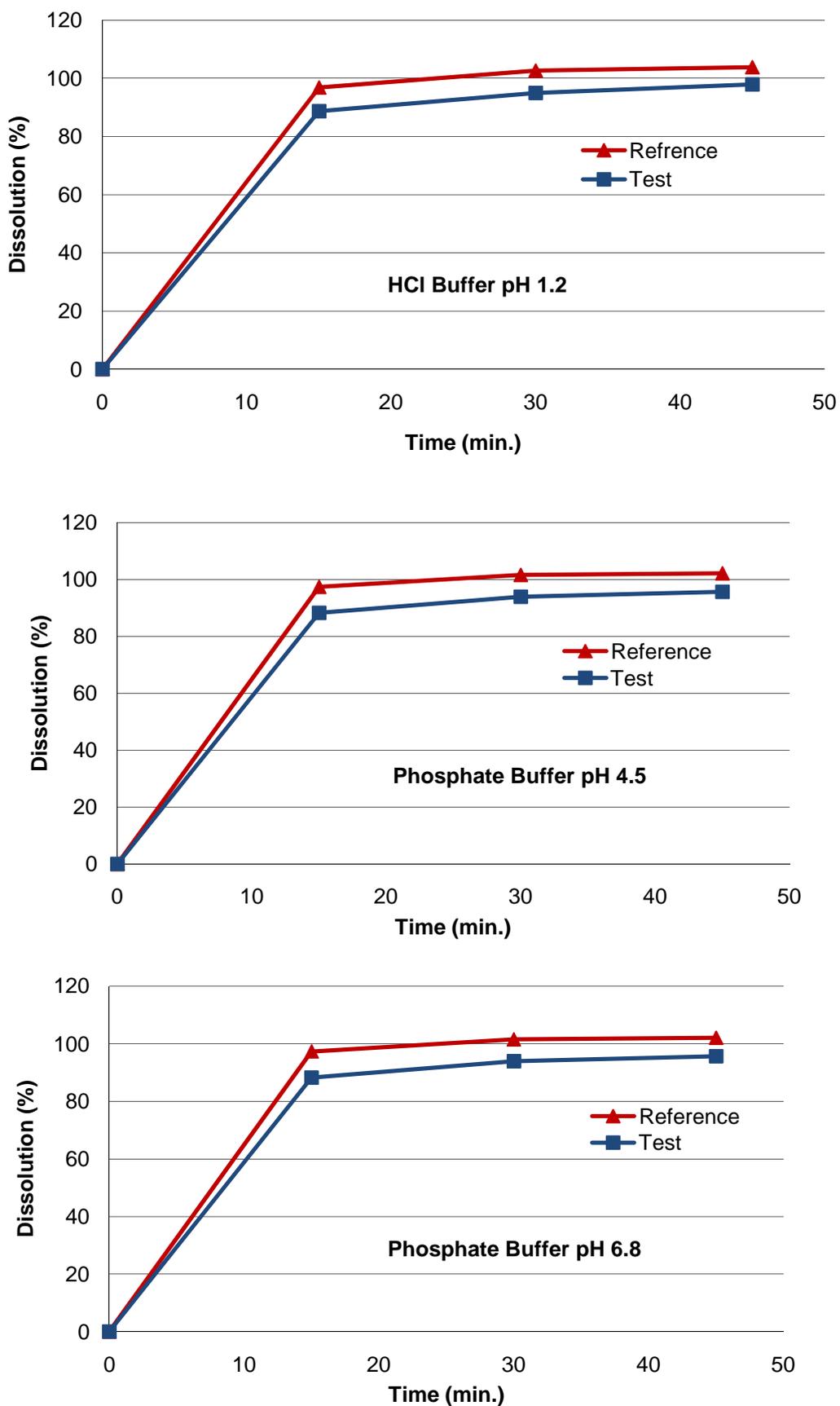**Figure (1) Average Serum Concentration-Time Profile for All Subjects**

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Figure (2) Dissolution profiles of Cefuroxime Axetil in buffer of different pH

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CONCLUSION

The results of this study in healthy volunteers indicated that Bioxime 500 mg tablets manufactured by Pharmacare International Manufacturing Company, Yemen (test product) are bioequivalent to Zinnat 500 mg manufactured by GlaxoSmithKline, UK (reference product). Both products were well tolerated.

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