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# Bioequivalence study of two brands of Co-amoxiclav 1g tablets (Clavimox® and Augmentin®) in adult healthy volunteers

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**ABSTRACT**

*A bioequivalence study of two oral formulations of Co-amoxiclav 1g, Clavimox® (Pharmacare International Manufacturing Co., Yemen) as the test and Augmentin® (GlaxoSmithKline, UK) as the reference product was carried out in 24 healthy male & female volunteers following a single dose, 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 Co-amoxiclav by a sensitive, reproducible and accurate high pressure liquid chromatography (HPLC) method. Various pharmacokinetic parameters $AUC_{0-t}$, $AUC_{0-\infty}$, Cmax, Tmax, $T_{1/2}$ and $K_{el}$ were determined from Serum concentrations of both formulations and found to be in good agreement with reported values. $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ 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 was found within bioequivalence acceptance range of 80 – 125%. Based on these statistical inferences, it was concluded that Clavimox® is bioequivalent to Augmentin®. Both products were well tolerated.*

**Key words**: Co-amoxiclav, Bioequivalence, Clavimox, Amoxicillin, Augmentin

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## INTRODUCTION

Bioequivalence of two formulations of the same drug is concluded based on the absence of significant difference in the rate ($C_{max}$) and extent of absorption (*AUC*) of two drug formulations [1]. In the present study, the bioequivalence of two Co-amoxiclav tablets was evaluated by comparing those pharmacokinetic parameters.

Co-amoxiclav consists of amoxicillin with the beta-lactamase inhibitor Clavulanic acid. Clavulanic acid itself has no significant antibacterial activity, but by inactivating beta-lactamases, it makes the combination active against beta-lactamase-producing bacteria that are resistant to amoxicillin [6].

After oral administration, Co-amoxiclav is absorbed from the gastrointestinal tract to blood stream.

Amoxicillin, are generally effective against such Gram-negative genera as *Escherichia*, *Klebsiella*, *Haemophilus*, *Salmonella*, *Shigella*, and non–indole-producing *Proteus* [10].

Co-amoxiclav 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[19]. Co-amoxiclav is an oral antibiotic that combines amoxicillin with the β-lactamase inhibitor clavulanate. It is useful for treating complicated sinusitis and otitis media and for prophylaxis of

human or animal bites after appropriate local treatment, cellullitis , Lower uniray tarct infection & diabetic foot infection . [7,9,11]

This combination may be cause a higher incidence of diarrhea and other gastrointestinal symptoms than amoxicillin alone, and less costly alternatives are available (11,20)

Amoxicillin is completely absorbed, with about 85 to 93% bioavailability because of a small first-pass effect. Serum concentrations are greater than those after equal doses of Ampicillin; postabsorptive pharmacokinetics are identical to those of Ampicillin.[4,7,18]

Pharmacokinetics of amoxicillin and Clavulanic acid are broadly similar and neither appears to affect the other to any great extent. [8, 10]

About 20% is bound to plasma proteins and plasma half-lives of 1 to 1.5 hours have been reported.

Amoxicillin is metabolised to a limited extent to penicilloic acid which is excreted in the urine. About 60% of an oral dose of amoxicillin and 40%to 65% of Clavulanic acid are excreted unchanged in the urine in 6 hours by glomerular filtration and tubular secretion [5,8]. Probencid inhibits the secretion of amoxicillin. [12,17]

**Objective of the study**

Primary: To assess the bioequivalence of test product of Co-amoxiclav ( Clavimox® 1g tablets, Pharmacare International Manufacturing Co., Yemen) relative to a reference product (Augmentin® 1g 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). 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 Clavimox 1g tablets (Co-amoxiclav 1g, batch No.: 1405208, Expiry date: 05/2016) manufactured by Pharmacare International Manufacturing Company, Yemen. The reference product was Augmentin 1g tablets (Co-amoxiclav 1g, batch No.:661533, Expiry date: 11/2016) 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 media of water; phosphate buffer pH4.5 and phosphate buffer pH6.8 were used. Temperature was maintained at 37 ± 0.5 $^0$C. Samples of aliquot were collected after 8, 15, 23 and 30 minutes. Concentration of Co-amoxiclav was determined by HPLC (Jasco, Pump2089 &UV 2070 Japan).

**3. Subjects**

All 24 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), blood sample analysis (basic profile, 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, chewing Qat, history of alcohol or drug abuse, consumption of any medication within two weeks prior to study commencement, participation in a clinical trial, history of clinically important illness or major surgery in the 3 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., malabsorption, 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 3 months before enrollment.

Their mean Age was 27.6 ± 4.14 year with a range of 20-35 year and mean body weight was 59.6 ± 7.63 kg with a range of 49.5-79 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.

**4. Study Design**

The study was designed according to the Food and Drug Administration (FDA), European Medicines Agency (EMA) and Gulf cooperation council(GCC) guidelines on bioequivalence investigation.[2,3,13 ] It was conducted

between May 2014 and June 2014 in compliance with the International Conference of Harmonization (ICH) guideline for Good Clinical Practice (GCP) and the Declaration of Helsinki and its amendments. [21,22] 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 1g of either product (Clavimox 1g or Augmentin 1g) 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 of alcohol, grapefruit juice, and beverages was not permitted for 24 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 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 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 20 min after sampling, the blood samples were centrifuged at room temperature at approximately 13000 $\pm$100 rpm for 10 min. the separated Serum was decanted in coded polypropylene tubes and stored frozen at -70$^0$C until analysis.

**6. Determination of Co-amoxiclav Concentrations in serum**

Serum Co-amoxiclav concentrations were measured to assess bioequivalence of studied products. Serum samples were analyzed separately for Amoxicillin and Clavulanic acid according to sensitive, selective and accurate high pressure liquid chromatography (HPLC) methods, which were developed and validated before the study. All solvents used were of HPLC grade; while other chemicals and reagents were of analytical grade; Amoxicillin and Clavulanic acid (Reference standard) were purchased from European Pharmacopeia reference standard (CRS&BRP) and Paracetamol and Metronidazole (internal standards) were purchased from Ningbo(China) and Shandong (China) respectively. The HPLC system was from Jasco, Japan, and it consisted of a solvent delivery pump (PU-2089 ), a system controller (LC-NetII/ADC) and a variable ultraviolet/Visible detector (UV-2070). Chromatographic separation was performed using a ODS(BDS) C18 (100X4.6 mm, 3µm) stainless steel column. A guard column of the same material was used (Capital Analytical, UK). The mobile phase consisted of 7% Methanol in 0.05 M potassium dihydrogen phosphate buffer (pH 3.5), and eluted at a flow rate of 1.2 mL/min; effluent was monitored at a wavelength of 313 nm for Clavulanic acid and 230nm for Amoxicillin. Each analysis required a maximum of 6 min. Quantitation was achieved by measurement of the peak height ratio of the drug to the internal standard. The method was validated by international guidelines. [14,15]

**6.1. Clavulanic acid Bioanalytical method validation**

Clavulanic acid lower limit of quantification was 0.10µg/ml in Serum. A standard curve was generated by preparing seven non-zero Serum standards over the range of 0.1 – 6 µg/ml. The average peak height ratios were plotted against the concentration. The linear regression of Clavulanic acid assay in Serum was characterized as having a mean slope of 1.7044, a mean intercept 0.00325 and coefficient factor ($r$ = 0.9998). Intra- day coefficient of variation (CV %) ranged from 1.97% to 5.44% and inter-day (CV %) ranged from 2.25% to 6.49% at four different concentrations (0.1, 0.3, 3.0 and 5.5µg/ml).The mean absolute recovery 90.65%. Stability tests shown that Clavulanic acid is stable in Serum for at least 45 days when stored at –70°C.

A 100 uL of 16.25% imidazole solution was added to 400 uL sample (standard sample, control sample) and put in water bath at 30C for 13minutes then 100ul of internal standard solution was added to derivatized sample. Add 250ul of 5% perchloric acid and vortex for 30 seconds. Centrifuge the sample and the supernatant was injected and chromatographed. Clavulanic acid and the internal standard were separated from endogenous plasma substances.

**6.2. Amoxicillin Bioanalytical method validation**

Amoxicillin lower limit of quantification was 0.20µg/ml Serum. A standard curve was generated by preparing seven non-zero Serum standards over the range of 0.2 – 24 µg/ml. The average peak height ratios were plotted against the concentration. The linear regression of Amoxicillin assay in Serum was characterized as having a mean slope of 0.3733,a mean intercept 0.0144 and coefficient factor ($r$ = 0.9999). Intra- day coefficient of variation (CV %) ranged from 1.95 % to 2.38 % and inter-day (CV %) ranged from 2.04 % to 4.40 % at four different concentrations (0.2, 0.6, 12.0 and 22.8µg/ml).The mean absolute recovery 96.22%. Stability tests shown that Amoxicillin is stable in Serum for at least 120 days when stored at –70°C. A 100ul of internal standard

solution was added to 400 uL sample (standard sample, control sample) & add 250ul of 5% perchloric acid and vortex for 30 seconds. Centrifuge the sample and the supernatant was injected and chromatographed. Amoxicillin and the internal standard were separated from endogenous plasma substances

**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 versus time curve ($AUC_{0-t}$), the area under the serum concentration versus time curve extrapolated to the infinity ($AUC_{0-\infty}$) and the maximum plasma concentration of the drug ($C_{max}$). The time to reach maximum serum concentration of the drug ($T_{max}$), the elimination half- life ($T_{1/2}$) was selected as secondary parameters. The maximum Co-amoxiclav 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 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 versus 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 21 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 $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}$ and $T_{1/2}$ 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 $\mu T/\mu R$ (ratio of 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 1g per study period;
- Only healthy adult volunteers with no history of hypersensivity reactions to Co-amoxiclav or other related molecules were enrolled;
- Each volunteers checked well being prior to his/her discharge from the clinic.

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. 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 results are presented in Figures 3,4,5 ,6,7& 8.

Both formulations were well tolerated by the volunteers; unexpected incidents that could have influenced the outcome of the study did not occur. All 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, selectivity, extraction recovery, limit of quantification, linearity, accuracy and precision, stability. The validation parameters were defined according to the EMA and the FDA [14,15]. All parameters met predefined acceptance criteria.

Both formulations were readily absorbed from the gastrointestinal tract and Co-amoxiclav was measurable at the first sampling time (0.33 h) in nearly all volunteers. The mean concentration-time profile of the two formulations is shown in the Figures 1&2. All calculated pharmacokinetic parameter values were in good agreement with previously reported studies. Table 2 shows the pharmacokinetic parameters for the two brands

of Co-amoxiclav 1g tablets. The 90% CIs for the natural log-transformed data were also calculated as per the FDA guidelines [16] and the results are shown in Table 3&4.

**Table (1) Demographic data of the population included in the study (n = 24)**

| Variables | | Data | Range |
|---|---|---|---|
| Total volunteer | Male | 17 | |
| | Female | 7 | |
| Mean Age (year) | | 27.6 | 20 to 35 |
| Mean Height (cm) | | 160.6 | 147 to 175 |
| Mean Weight (kg) | | 59.6 | 49.5 to 79 |
| Mean BMI ($kg/m^2$)** | | 23.1 | 18.7 to 29.6 |

** *Mass Body Index (weight/height$^2$)*

**Table (2) Pharmacokinetic Parameters Summary of Clavulanic acid & Amoxicillin for both Test and Reference products (n=24, mean ± SD)**

| Pharmacokinetic parameters | Clavimox 1g® (Test) | | Augmentin 1g® (Reference) | |
|---|---|---|---|---|
| | Clavulanic acid | Amoxicillin | Clavulanic acid | Amoxicillin |
| $AUC_{0-t}$ (µg .h/ml) | 6.648 ± 2.35 | 38.334 ± 8.36 | 6.847 ± 2.27 | 35.597 ± 9.75 |
| $AUC_{0-\infty}$ (µg .h/ml) | 6.858 ± 2.37 | 39.020 ± 8.46 | 7.051 ± 2.28 | 36.443 ± 9.80 |
| $C_{max}$ (µg/ml) | 3.206 ± 1.28 | 12.723 ± 2.90 | 3.389 ± 1.26 | 12.999 ± 3.81 |
| $T_{max}$(h) | 1.262 ± 0.24 | 1.882 ± 0.42 | 1.375 ± 0.37 | 1.723 ± 0.41 |
| $K_{el}$ ($h^{-1}$) | 0.716 ± 0.11 | 0.669 ± 0.15 | 0.698 ± 0.12 | 0.613 ± 0.21 |
| $T_{1/2}$(h) | 1.001 ± 0.24 | 1.085 ± 0.24 | 1.021 ± 0.19 | 1.248 ± 0.41 |
| $AUC_{0-t}$ / $AUC_{0-\infty}$ (%) | 96.55 ± 2.18 | 98.199 ± 1.22 | 96.804 ± 1.21 | 97.584 ± 2.17 |

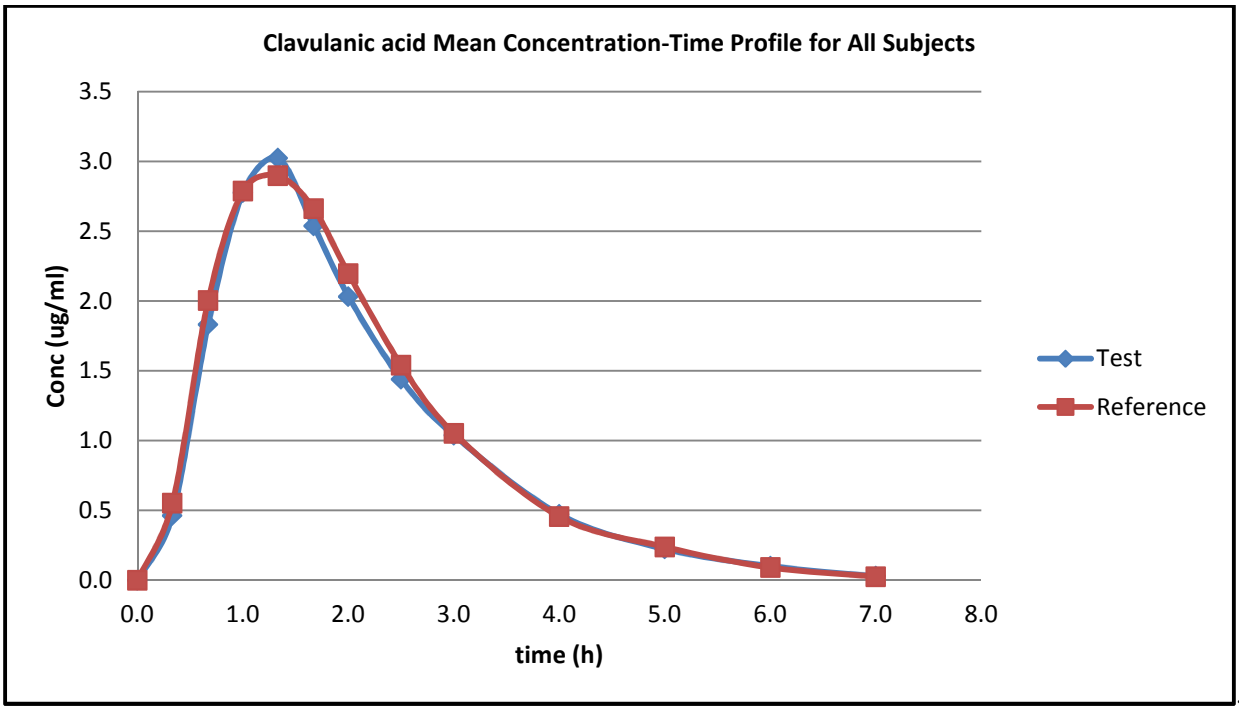


**Figure (1) Clavulanic acid Mean Serum Concentration-Time Profile for All Subjects**

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 Clavimox are comparable to those produced by Augmentin. 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 that the ratios of $AUC0_{-t}$, $AUC_{0-\infty}$ and $C_{max}$ of the two formulations and for the two periods lie within the FDA acceptable range (80-125%).

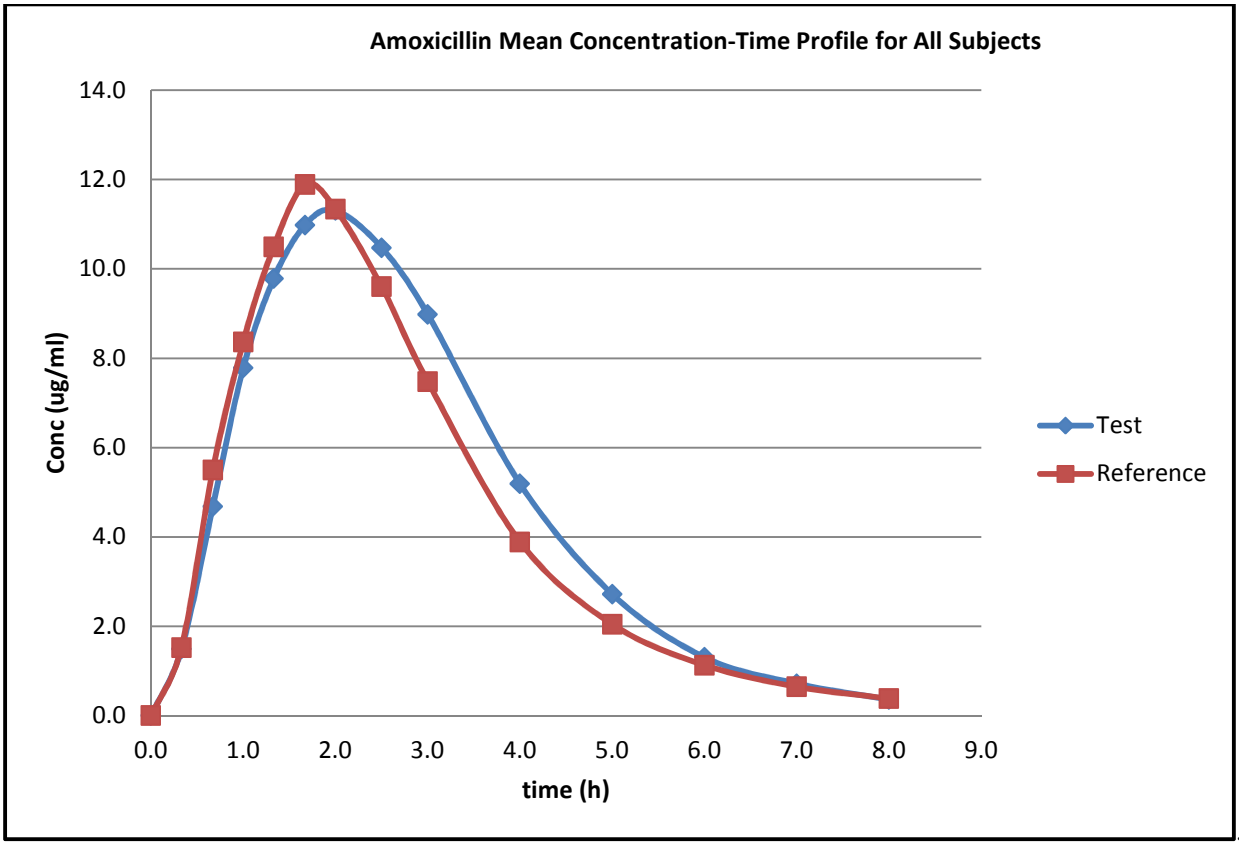


**Figure (2) Amoxicillin Mean Serum Concentration-Time Profile for All Subjects**

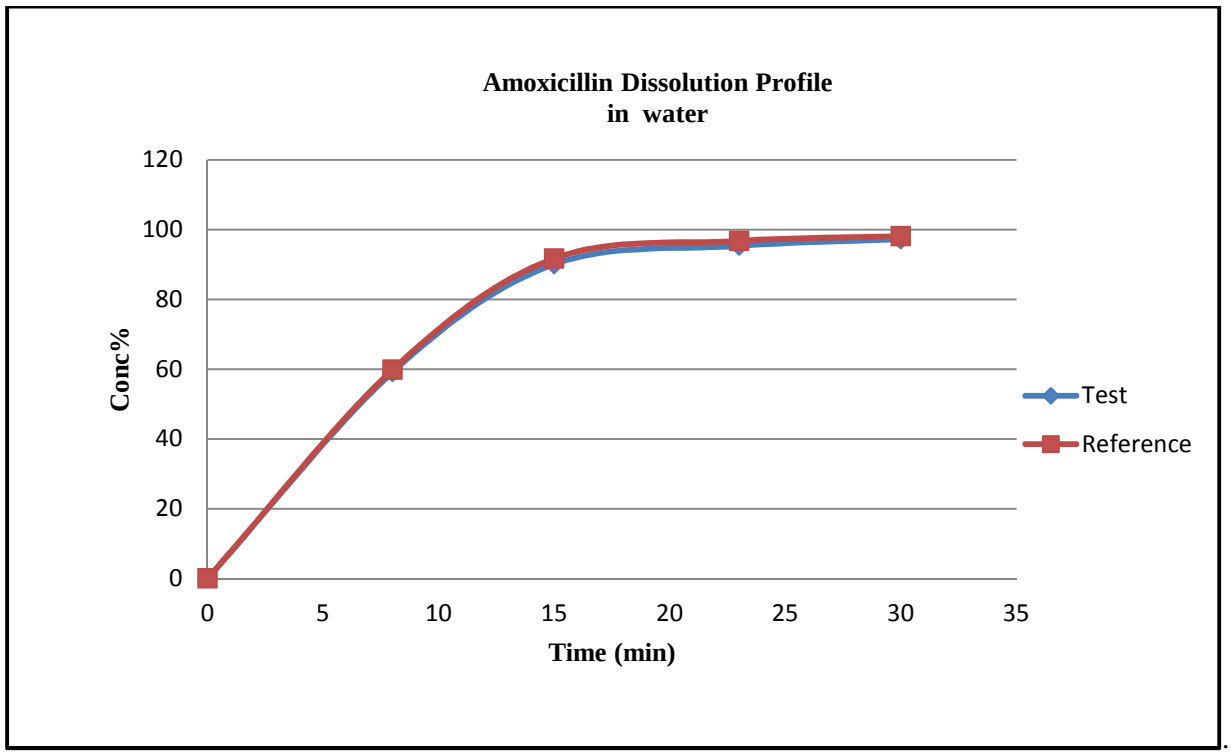


**Figure (3) Dissolution profiles of Amoxicillin in water medium**

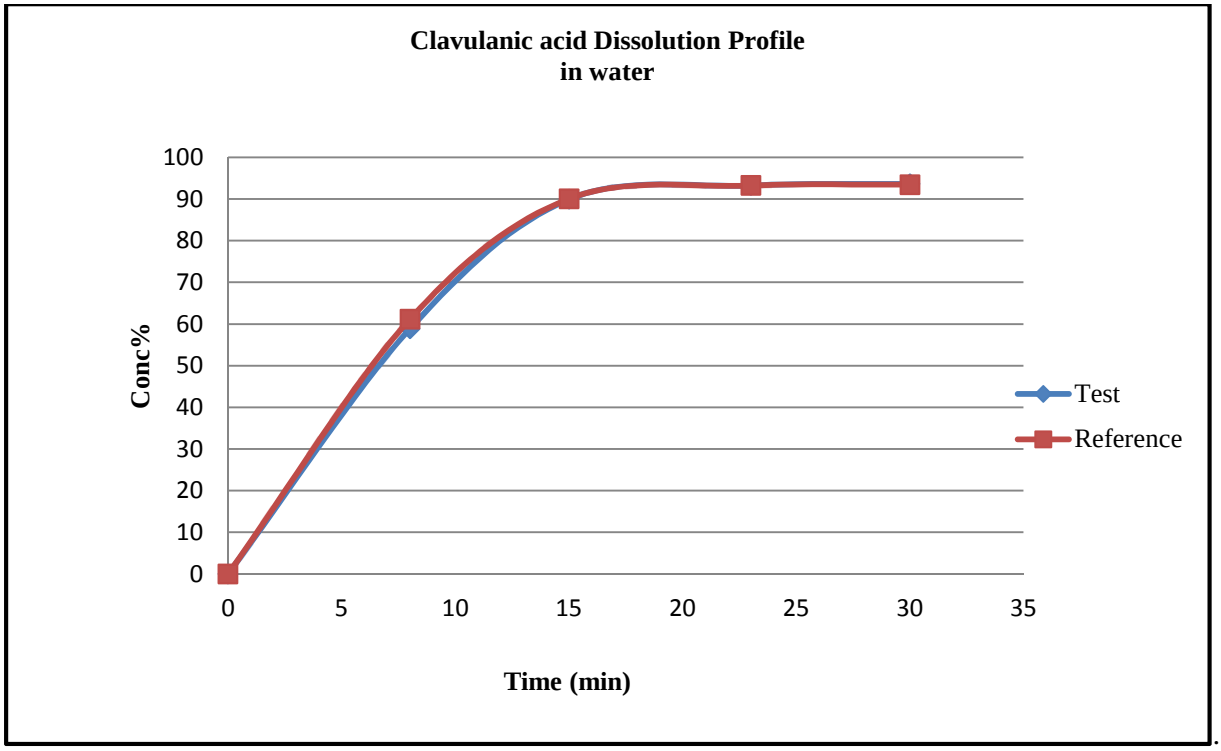


**Figure (4) Dissolution profiles of Clavulanic acid in water medium**

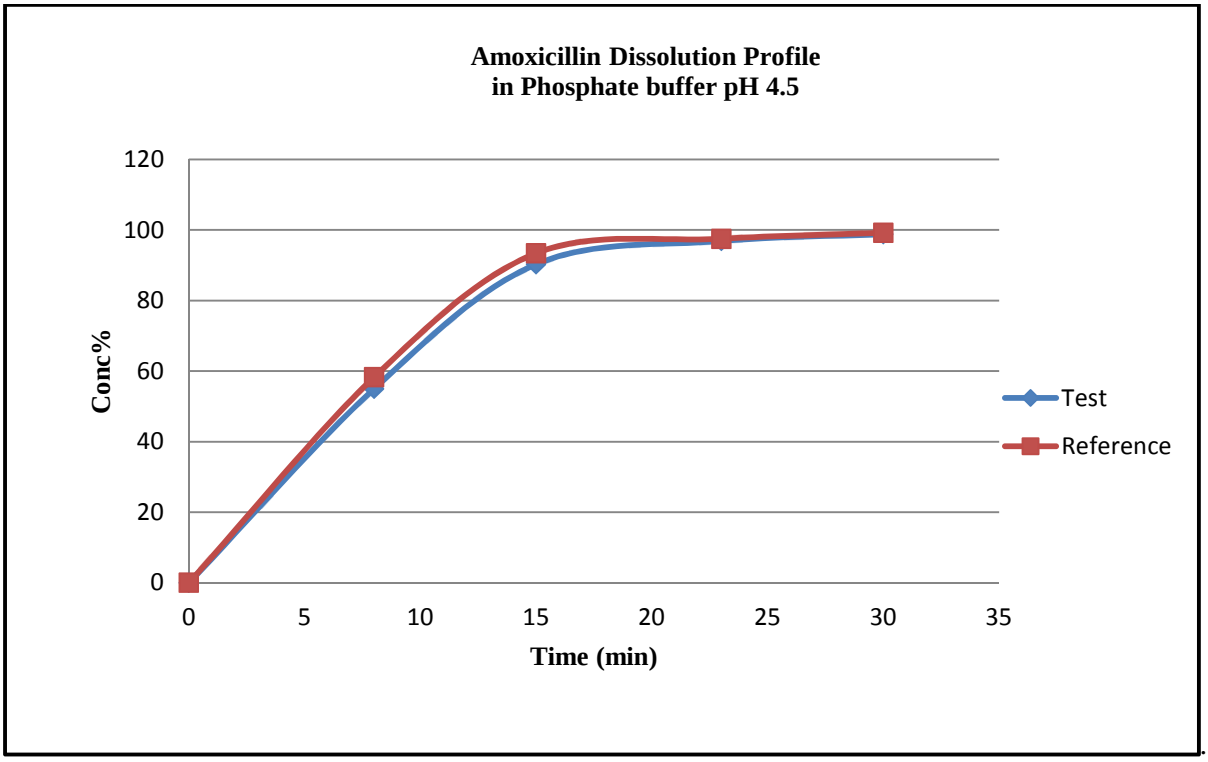


**Figure (5) Dissolution profiles of Amoxicillin in phosphate buffer pH 4.5**

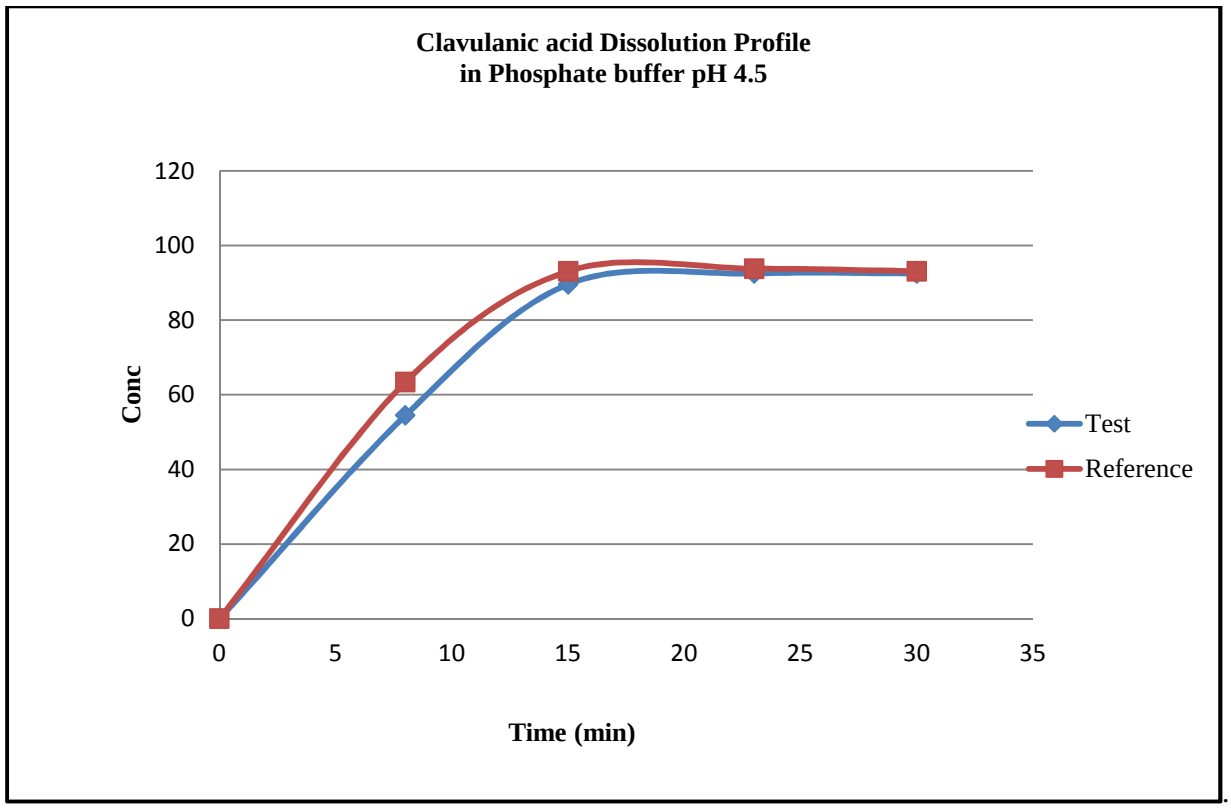


**Figure (6) Dissolution profiles of Clavulanic acid in phosphate buffer pH 4.5**

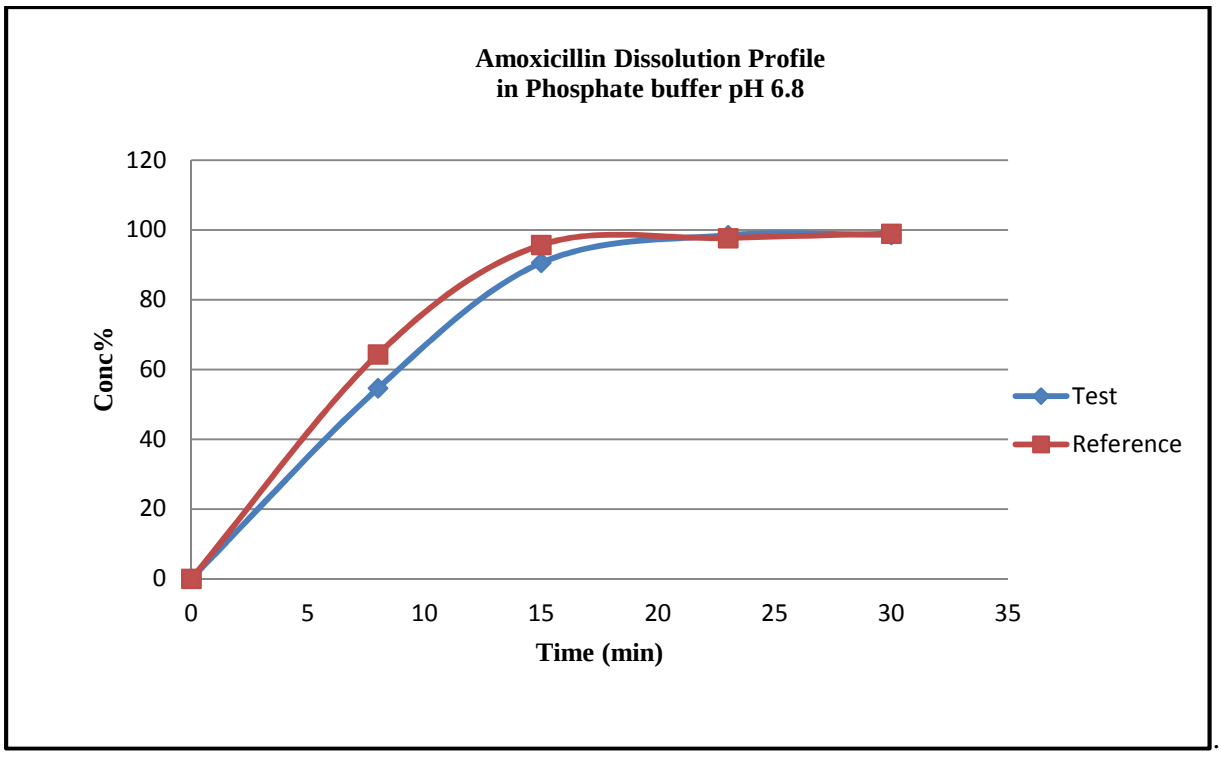


**Figure (7) Dissolution profiles of Amoxicillin in phosphate buffer pH 6.8**

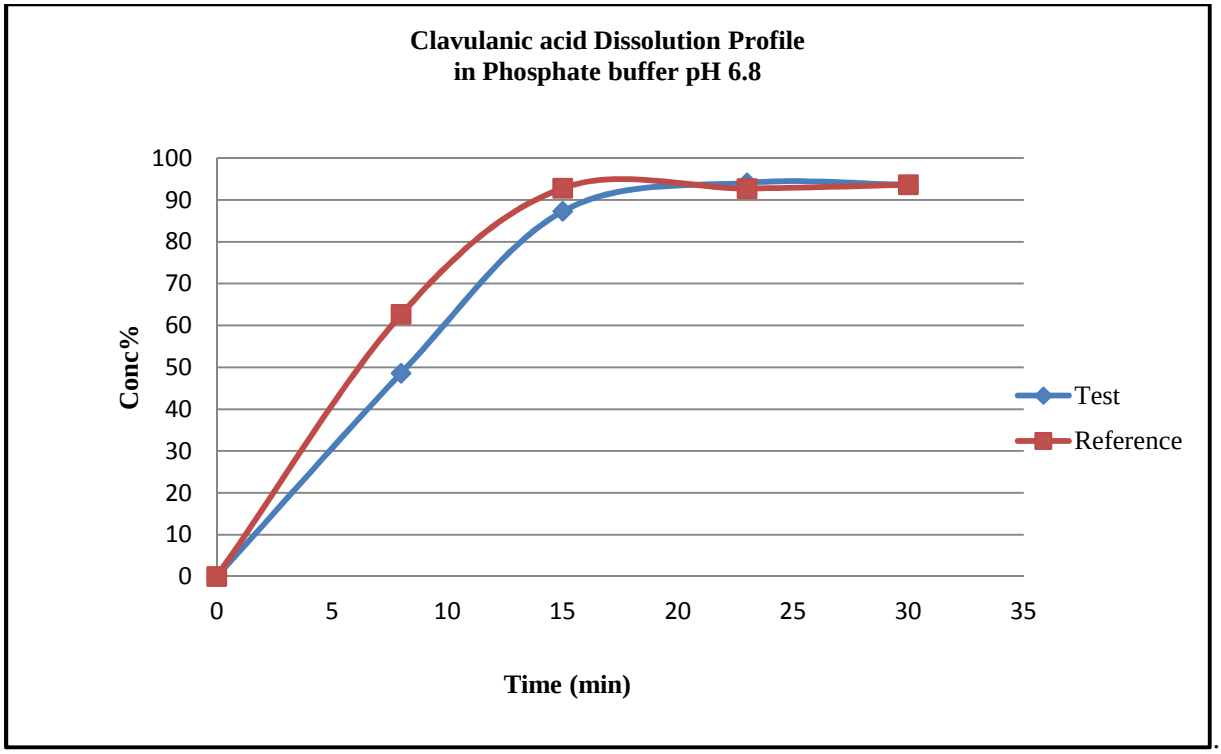


**Figure (8) Dissolution profiles of Clavulanic acid in phosphate buffer pH 6.8**

**Table (3) pharmacokinetic -Bioequivalence parameters of Clavulanic acid**

| Parameter | Treatments Mean± SD | | 90% confidence intervals | | | | *P* value for product effect |
|---|---|---|---|---|---|---|---|
| | Test Product | Reference Product | Point estimator % | Lower limit % | Upper limit % | CV% of CI | |
| $AUC_{0-t}$ (ug.h/ml) | 1.826±0.399 | 1.868±0.351 | 99.5 | 91.7 | 107.3 | 22.4 | 0.415 |
| $AUC_{0-\infty}$ (ug.h/ml) | 1.861±0.384 | 1.900±0.340 | 101.5 | 89.9 | 113.2 | 32.7 | 0.597 |
| $C_{max}$ (ug/ml) | 1.083±0.427 | 1.150±0.396 | 103.7 | 85.3 | 122.2 | 50.8 | 0.625 |

**Table (4) pharmacokinetic -Bioequivalence parameters of Amoxicillin**

| Parameter | Treatments Mean± SD | | 90% confidence intervals | | | | *P* value for product effect |
|---|---|---|---|---|---|---|---|
| | Test Product | Reference Product | Point estimator % | Lower limit % | Upper limit % | CV% of CI | |
| $AUC_{0-t}$ (ug.h/ml) | 3.621±0.242 | 3.539±0.262 | 102.5 | 100.4 | 104.6 | 5.9 | 0.053 |
| $AUC_{0-\infty}$ (ug.h/ml) | 3.639±0.239 | 3.563±0.257 | 102.3 | 100.3 | 104.4 | 5.7 | 0.066 |
| $C_{max}$ (ug/ml) | 2.516±0.250 | 2.523±0.297 | 100.2 | 97.2 | 103.3 | 8.7 | 0.917 |

## CONCLUSION

The results of this study in healthy volunteers indicated that Clavimox 1g tablets manufactured by Pharmacare International Manufacturing Company, Yemen (test product) are bioequivalent to Augmentin 1g manufactured by GlaxoSmithKline, UK (reference product). Both products were well tolerated.

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