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Assessment of bioequivalence profile of locally produced flucloxacillin sodium capsules via urinary excretory rates

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Abstract

The assessment of pharmacokinetic parameters constitutes the basic approach to determining bioequivalence of pharmaceutical products in order to ensure safety, efficacy and potency of the administered drug. This study has examined a locally manufactured flucloxacillin (FLUXACIN 500) and the reference product (FLUCLOXACILLIN 500mg CAPSULES) for therapeutic equivalence in a cross–over non–replicate bioequivalence study using urinary excretion data. The study was conducted with twelve consenting healthy male volunteers in a fasting state using single–dose, randomized, two–period, two–treatment cross over study design. A seven-day washout period was allowed between treatments. Urine specimen collected were analysed by means of HPLC with UV detection while pharmacokinetic parameters were analysed using Microsoft☐ Excel Spreadsheet 2013. fFluxacin 500 reached a maximum excretion rate at an average time of 1.42 hours while flucloxacillin 500 mg reached its maximum excretion rate at an average time of 1 hour. There was no statistical significant difference between the two times. The 90% Confidence Interval of the ratio of the Area under the Excretion Rate–time Curve of fluxacin 500 to flucloxacillin 500 mg was 96.20% to 101.24% while those of the Cumulative Amount of flucloxacillin excreted, and the Rate of Maximum Excretion were 102.92% to 116.85% and 72.59% to 94.61% respectively. Fluxacin 500 demonstrated bioequivalence relationship with flucloxacillin for all the four parameters: Maximal Rate of Excretion, Area under Excretion Rate–Time Curve, Cumulative Amount Excreted and the Time for Maximum Excretion. The test and reference drugs are therefore interchangeable.

Keywords: Flucloxacillin Sodium, Bioequivalence, Pharmacokinetic, *In vitro* dissolution.

INTRODUCTION

A drug that is discovered and manufactured by a pharmaceutical company after having been tested through the various phases of design and clinical testing, and is finally approved for usage on the market is patented and registered as an innovator product. The innovator company, therefore with its exclusivity rights, becomes the sole producer of the product until the patent expires unless some other company is licensed by the innovator company to produce the drug. Upon the expiration of the patent of the innovator drug, other companies are free to produce the same drug without necessarily obtaining an authorisation from the innovator company [1]. The drug produced by the non–innovator company is described as a generic product. A generic drug product usually contains the same therapeutic moiety as the innovator drug but may be in the form of a salt, an ester or even a complex of the active pharmaceutical ingredient.

It is expected of the generic drug product to elicit similar pharmacological activity as the innovator product. However, the formulation of a drug impacts the rate and extent of drug absorption into the systemic circulation since the innovator and generic companies may have deployed different excipients in the preparation of a particular dosage form. As a result of changes in composition, release mechanisms, shape, packaging and the techniques deployed in manufacturing a drug in a particular dosage form by the two companies, variations in bioavailability may occur, raising the need for bioequivalence testing to establish the validity of the manufacturing process.

Bioequivalence testing is a study conducted to measure the *in vivo* performance of two pharmaceutical equivalents or pharmaceutical alternatives through the assessment of their rate and extent of absorption

(bioavailability) into the systemic circulation after they are administered in the same dose strength [2]. These pharmaceutical equivalents/alternatives are considered bioequivalent if their bioavailability fall within an acceptable range of 0.80 to 1.25 (80% to 125%) for logarithmically transformed data [3].

Bioequivalence studies have earned a remarkable attention in the past few decades due to the resurgent manufacturing of generic and brand–name pharmaceuticals globally. This phenomenal rise in the production of generic drugs led to the formulation of policies and regulatory codes by various regulatory institutions the world over. As a result, a lot of progress has been made in the execution of these medicinal research concepts.

Bioequivalence study is deemed a viable pillar in the endorsement of generic pharmaceuticals in the international domain leading to a drastic cut down of costs expended in obtaining prescriptions of these drug formulations. Continuous strides are still being made by regulatory institutions and the scientific world at large to find out new and improved methods to assessing bioequivalence of the several formulations, including the compound formulations that are continuously being churned out by the global pharmaceutical industry [4].

In the light of strengthening the quality assurance of pharmaceutical formulations regarding their pharmacokinetic parameters, bioequivalence studies are conducted to police the safety and quality of drug formulations from their clinical stage through their marketing period to consumers, from the switch of one dosage form to another say capsules to tablets, in the design of generic dosage forms or when there is a change in manufacturing procedure or the site of processing.

Prescriptions meant to be given to patients need to be appropriately done regarding the particular medical condition. The selected medicine of choice is to be centered on an evidence based approach to clinical practice and assured to prove compatibility with any other medicines or alternative therapies the patient may be given [5].

Quite a number of bioequivalence studies have been performed using blood, plasma and serum concentrations of drugs to estimate their pharmacokinetics parameters. The use of urinary pharmacokinetic data is also used to assess the bioequivalence of generic drug products where the particular drug can be excreted significantly unchanged in urine say about forty percent and above. Moreover, the use of blood plasma and serum can be quite complex to handle [3]. Over 65.5% of flucloxacillin is excreted in urine unchanged after an orally administered dose and 76.1% for parenteral administered dose of the drug [6]. It is therefore convenient to assess the bioequivalence profile of the flucloxacillin using the urinary excretion data. The collection of urine samples which is a non–invasive approach of biological sampling makes it much easier for recruiting and enrolling participants on the study and to assure their total commitment in participating in the study.

The product under study is FLUXACIN 500 which is a local generic product from a Ghanaian based Pharmaceutical Company compared with the reference product FLUCLOXACILLIN 500 mg, which is a product of Medreich, Plc, a Marketing Authorisation Holder and Manufacturer in the United Kingdom. [7] This study sought to establish whether there is a therapeutic equivalent relationship between the test and reference drugs.

MATERIALS AND METHODS

Materials

Analytically pure flucloxacillin sodium pure powder, a gift from Ernest Chemists Limited (Ghana) and the two marketed drug products used for the study were flucloxacillin 500 mg a product from Medreich Plc, a Marketing Authorisation Holder and Manufacturer in the United Kingdom and fluxacin 500, a locally manufactured product were used in the study.

The reagents used in the study included 99.5% to 100.5% potassium dihydrogen orthophosphate AnalaR (KH_2PO_4), 99% w/w sodium hydroxide (NaOH) and 90% w/v formic acid (HCOOH)

In Vitro Dissolution Test

A dissolution test was carried out on both products at a temperature of $37 \pm 0.5°C$ to mimic the normal human body temperature condition. The dissolution medium used was phosphate buffer with pH 6.8 to mimic the pH of the small intestine. The dissolution tester was set with baskets to rotate at 100 revolutions per minute (rpm) over a one-hour time period. Ten mL of the dissolution medium was withdrawn at 15, 30, 45 and 60 minutes for each of the capsules for both products. T90+ UV/VIS Spectrometer by PG Instruments Limited and quartz cuvettes were used to measure the absorbances of flucloxacillin dissolved in the dissolution medium for the test and reference products. The basket apparatus was employed for the dissolution testing of the drugs using Erweka DT6 Dissolution bath.

Recruitment of Participants

Twelve healthy male subjects (aged 19–32) with body mass indices ranging from 18.55 to 28.04 kg/m^2 were recruited for the study. The age range of the subjects met the required age range of 18 to 55 years and had body mass indices that fell within the required range of 18.5 to 30kg/m^2 [8].

The exclusion criteria were history of being hypersensitive to any penicillins (such as amoxicillin, ampicillin, cloxacillin and ciprofloxacin), history of any clinically significant diseases of the liver, kidney, heart, pancreas and the lungs. Volunteers who may have been undergoing any concurrent medical treatment of any form were not included in the study. Nonsmokers and subjects who may have undergone some other studies within or three months before the commencement of the study were not permitted to participate in the study. Blood transfusion within or three months to the commencement of the study was not allowed. Tea, coffee, caffeine or any xanthine–containing beverages were forbidden from being taken by the subjects during each phase of the study. The volunteers were adequately briefed on the details of the study and were given a Participant Information Leaflet and Consent Formand a questionnaire each ofwhich they read and filled. Ethical clearance (CHRPE/AP/206/15) was obtained from the Committee on Human Research Publications and Ethics (CHRPE) of the School of Medical Sciences, KNUST and KomfoAnokye Teaching Hospital in Kumasi, Ghana.

Dose Administration, Specimen Collection and pH analysis

The design for treatment was carried out in a single–dose, randomized, two–period, two–treatment crossover design with a seven-day washout period between treatments. Subjects were asked to fast at least ten hours the previous night before they were administered with the drugs and four hours post dose. Each subject was orally administered 500 mg of

either the test or the reference drug with about 150mL of portable water. Sufficient washout duration of seven hours was allowed between treatments to permit effective elimination of the previously administered dose. After the first treatment, subjects were administered the other product (either test or reference drug) depending on which they were administered in the first treatment. Subjects were served a standard meal each four hours post dose during each treatment phase.

Before subjects were administered with the drugs, they were asked to provide drug–free urine samples each at time **t** = 0 after they were asked to drink about 250 mL of portable water (loading dose) about an hour before the drug administration. Urine specimens were collected at times **t** = 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 and 6.0 hours after drug administration. In all, ten specimens were collected per subject per treatment. After each specimen collection, subjects were asked to drink about 100 mL of water. The pH value of each urine specimen was determined immediately after collection for a study of the effect of pH on concentrationof drug excreted unchanged.

Sample Preparation and Chromatographic Analysis

A standard blank urine sample was prepared by a two–fold dilution of an already drug–free urine sample collected with the mobile phase. A 1000 μg/mL stock solution was prepared by dissolving an accurately weighed mass of the reference compound in the blank urine sample. Standard solutions of 10, 20, 40, 80, 100, 200 and 400 μg/mL were prepared from the stock solution by serial dilution.

All samples were analysed on the day of collection. The samples were prepared for analysis by pipetting 5 mL each and diluting to 10 mL (two–fold) with the mobile phase in a 10 mL volumetric flask and then subjecting them to chromatographic analysis.

A developed and validated chromatographic method [9] was employed for the study. Briefly; the samples were analysed on Phenomenex® Synergi 4u Polar–RP column (80A, 50 x 2.00 mm, 4 micron) with a mobile phase system of water, methanol and formic acid 45:54.6:0.4 % (v/v) at a flow rate of 1mL/min and a detection wavelength of 240 nm. The injection volume used was 20μL. Spectra Series P100 Isocratic LC–Pump by Thermo Scientific, Waltham, MA, USA with eDAQPowerchrom 280 Integrator by eDAQ Inc. Colorado Springs, USA and Jasco UV 2075 plus Detector, Essex, UK were used for the chromatographic analysis of the samples.

Pharmacokinetic and Statistical Analyses

The non–compartmental model was used to estimate the pharmacokinetic parameters and Micorsoft® Excel Spreadsheet was used for the analysis. Pharmacokinetic (PK) parameters such as the Maximum Excretion Rate (Rmax), the Area Under the Excretion Rate–Time Curve (AURC), the Cumulative Amount of drug excreted ($Du_{(0-6)}$) for every subject were computed using the non–compartmental model approach and were logarithmically transformed using In(pharmacokinetic parameter). Analysis of variance (ANOVA) was carried out to estimate some of the variations in the study.

The 90% Confidence Interval (CI) of the ratio of the test and reference formulations of the drug was determined by using the Error Mean Sum of Squares (MSE) which was computed for each PK parameter. The formula for computing the 90% CI is given below:

90% CI for the ratio of the cumulative amount of Flucloxacillin excreted is given by the following expression:

$$\text{EXP}^{[(U_T - U_R) \pm S\sqrt{\frac{2}{nt}}(0.05)v]} \quad \text{..................Equation 1}$$

Where:

U_T = the mean of the PK parameter of the test product (fluxacin 500)
U_R = the mean of the PK parameter of the reference drug (flucloxacillin 500mg) n = the number of subjects involved in the treatment per period.
$S = \sqrt{MSE}$
MSE = Error mean sum of squares (obtained from ANOVA analysis)
t (0.05)v = critical value of **t** at α = 0.05
v = the number of degrees of freedom associated with the MSE

The lower and upper CIs were converted to percentages.

RESULTS

In vitro dissolution test

Figure 1 shows the dissolution profile of the test and reference drugs. Fluxacin 500 exhibited an average percent dissolution of 71%, 84% and 100.66% in 15, 30 and 60 minutes respectively. The reference drug, flucloxacillin 500mg also profiled average dissolution percentages of 77%, 88.39% and 102% for time points 15, 30 and 60 minutes respectively.
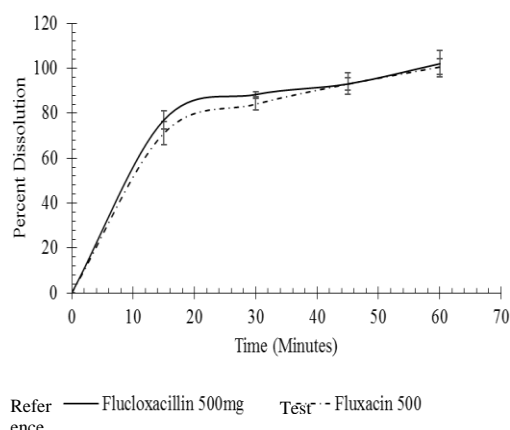

Reference ——Flucloxacillin 500mg   Test - - Fluxacin 500

Figure 1: Dissolution Profiles of Flucloxacillin 500 mg and Fluxacin 500

Dose Administration, Specimen Collection and pH analysis

The total number of urine specimens collected was two hundred and forty (240), twenty (20) per subject. The pH range obtained for flucloxacillin 500 mg was 5.48 to 8.68 and that for fluxacin 500 was 5.65 to 7.70. The median pH for flucloxacillin 500mg and fluxacin 500 were 6.64 and 6.42 respectively and their overall mean pH were 6.68±0.38 and 6.49±0.24 respectively. The concentration-pH graphs of Flucloxacillin 500 mg and Fluxacin 500 are shown in figures 2 and 3 respectively.
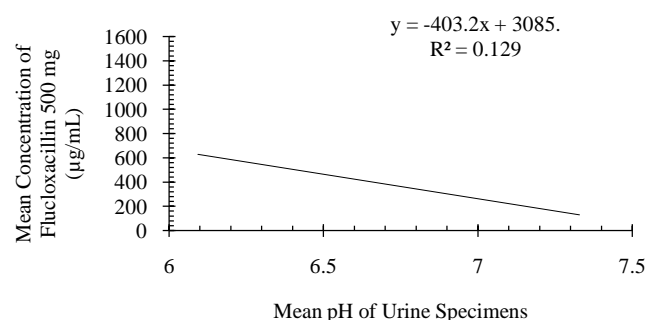

y = -403.2x + 3085.
R² = 0.129

Figure 2: Concentration–pH Graph of Flucloxacillin 500 mg
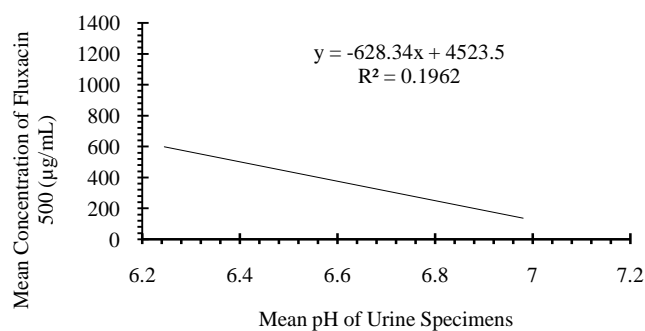
Figure 3: Concentration - pH Graph of Fluxacin 500

$$y = -628.34x + 4523.5$$
$$R^2 = 0.1962$$

Pharmacokinetic and Statistical Analysis

The mean Urinary Pharmacokinetic parameters of the test and reference drugs are presented in Table 1. Flucloxacillin 500 mg recorded 4120.59±43.81 µg/mL as the mean cumulative amount of flucloxacillin excreted unchanged in urine while 4461.00±53.66 µg/mL is the mean amount of fluxacin 500 excreted unchanged in urine. The p–values obtained for inter–subject and intra–subject variability at 5% level of significance were 0.02943 and 0.3014 (Table 1). These p–values being greater than 0.05 shows no significant difference statistically between the cumulative amounts of the two drugs excreted unchanged. Figure 4 graphically illustrates The Area Under the Excretion Rate Time Curve for the test and reference drugs.
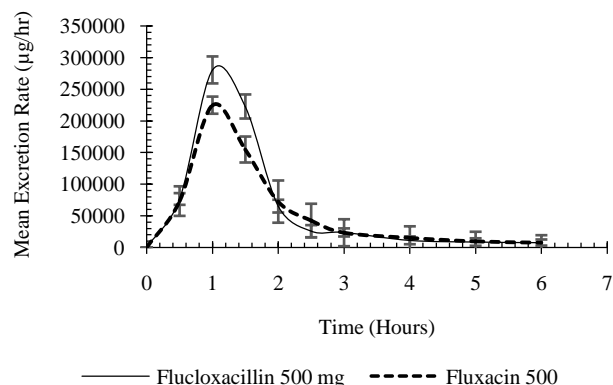

Figure 4: Area Under the excretory Rate –Time Curve (AURC of Flucloxacillin 500 mg and Fluxacin 500

Table 1: Mean urinary pharmacokinetic parameters of test and reference drugs

Parameter	Mean		p–value		90% Confidence Interval (CI)
	Flucloxacillin 500 mg (Ref)	Fluxacin 500 (Test)	Intra–subject Variation	Inter–subject Variation	
AURC (µg/hr)	222729±49.98	220922±43.77	<0.0002	0.7059	96.20–101.24%
Du(0-6) (µg/mL)	4120.59±43.81	4461.00±53.66	0.29428	0.30138	102.92–116.85%
Rmax (µg/hr)	273152±62.33	247012±54.11	0.09689	0.31246	72.59–94.61%
Tmax (hr)	1±.36	1.41667±1.23	0.50	0.26909	–
Acceptance Criteria: p>0.05; 90% CI = 80 – 125%					

DISCUSSION

By compendial requirements, an in vitro dissolution should precede the performance of *in vivo* bioequivalence study and this was done in compliance. The similarity dissolution profile for the test and reference drugs was estimated by means of mathematical comparison in which their f_2 statistic function was computed and found to be 61.5960. The f_2 is a similarity factor which is a "logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent dissolution between the two curves" [10]. To accord a similarity index to a dissolution profile, it is required to obtain an f_2 statistic of more than 50 which should usually lie in the range 50 to 100 [2]. The f_2 statistic of 61.5960 therefore confers a similar dissolution profile index on both drugs. In addition, the difference factor in the dissolution profiles of the two drugs was 3.3106 which fall in the required range of 0 to 15. An f_1 factor that falls in the 0 to 15 range accords sameness of dissolution on the two drugs being compared. Fluxacin 500 has therefore demonstrated in vitro bioequivalence with flucloxacillin 500 mg.

The normal pH range for a urine sample collected from the bladder is usually 4.6 to 8.0 [9]. The pH of urine is usually influenced by circadian rhythms and the kind of food ingested [11]. Despite the pH fluctuations, there was no statistical difference between the mean pH values and the respective median pH values. The mean pH of the urine specimens collected for both drugs were plotted against their respective concentrations (figures 2 and 3) and their ANOVA was also computed. The correlation coefficient (R^2) obtained for the plot of pH against the mean concentrations of flucloxacillin 500 mg was 0.1293 and that of fluxacin 500 was 0.1962. The two R^2 values showed a very poor degree of correlation between the pH and the concentrations of the drug in urine. The p–values obtained for flucloxacillin 500 mg and fluxacin 500 at 5% level of significance were 0.010928 and 0.00059 respectively. Both p–values were less than 0.05, proving that there was a statistical significant difference between the pH and concentrations of the drugs. The R^2 and the p–values show that the pH of urine did not affect the concentrations of the drugs that were excreted.

A 90% Confidence Interval was computed for the cumulative amount of flucloxacillin excreted unchanged in urine for fluxacin 500 with respect to flucloxacillin 500 mg for all the twelve subjects to give an interval of 97.1165% to 116.8462%. This interval fell in the required range of 80% to 125% thereby conferring bioequivalent relationship on the test drug according to the Code of Federal Regulations (CFR) [3]. All the statistical tests were performed after the logarithmic transformation of the respective data.

Flucloxacillin 500 mg recorded an average maximum excretion rate (Rmax) of 273152±62.33 µg/hr while an average of 247012±54.11 µg/hr was the excretion rate estimated for fluxacin 500. The logarithmically treated mean excretion rates were 12.44181 and 12.25391 for flucloxacillin 500mg and fluxacin 500 respectively. The p–values as shown in the Table 1 for inter–subject and intra–subject variations (0.0969 and 0.312, respectively) are all greater than 0.05 at 5% level of significance demonstrating bioequivalence relationship for the two drugs for the maximal rate of excretion.

A 90% Confidence Interval for the Rmax ratio of the two drugs gave a range of 72.59% to 94.61%. The lower limit of the range falls below the lower limit of the required 80 to 125% bioequivalence criterion while the upper limit of 94.61% falls within the required range. Drugs with wide therapeutic index, which are usually described as safe drugs, are permitted an acceptance interval of 70 to 130% at 90% CI qualifying fluxacin 500 as demonstrating bioequivalence with the reference drug pertaining to this pharmacokinetic parameter [12].

The mean area under the excretion rate time curves for the two drugs were 222729.4 ± 49.98 µg/hr for flucloxacillin 500 mg and 220922 ± 43.77 µg/hr for fluxacin 500. The p–value (about 0.0002) obtained for the intra–subject differences is less than 0.05 at 5% level of significance. However, for inter–subject variation, the p–value of 0.7059 does not show any statistical significant difference for the systemic exposure of the drug for the two formulations, being greater than 0.05. A range of 96.20% to 101.24% was obtained at 90% Confidence Interval for the AURC ratio of fluxacin 500 to flucloxacillin 500 mg. This interval falls within the required range of 80–125% at 90% CI, signifying a bioequivalent relationship between the systemic exposures of the two drugs. The mean time to maximum excretion rate for fluxacin 500 and flucloxacillin 500 mg were 1.42 ± 1.23 hours and 1 ± 0.36 hour respectively. There were instances where a subject recorded a Tmax of 0.5 hours and another 1.5 hours for flucloxacillin 500 mg. Fluxacin 500 also recorded Tmaxs at 0.5, 2.5 and 5 hours for some subjects. The other subjects for both drugs recorded Tmaxs at 1 hour. There was however no observed statistical difference between the Tmax for the two drugs since p–values for intra and inter–subject variations (0.50 and 0.27 respectively) were all greater than 0.05.

CONCLUSION

By the in vitro dissolution performed in accordance with the United States Pharmacopoeia requirements, the drugs used demonstrated good dissolution profiles, proving them as having demonstrated the right release properties in vitro. Fluxacin 500 (test drug) demonstrated bioequivalent relationship with flucloxacillin 500 mg (Reference drug) for the pharmacokinetic parameters such as the Cumulative amount of drug excreted unchanged [$Du_{(0-6)}$], the Rate of maximum excretion (Rmax), the Time to maximum excretion (Tmax) for all statistical tests performed on them. Fluxacin 500 also demonstrated bioequivalence with the reference drug as regards its Area under the Excretion Rate–Time Curve (AURC) at 90% Confidence Interval according to the US Food and Drugs Administration requirements. Conclusively, fluxacin 500 demonstrated bioequivalence with flucloxacillin 500 for all pharmacokinetic parameters with no inter–subject significant statistical variation for the two drugs. Fluxacin 500 can therefore be used interchangeably with the reference drug flucloxacillin 500 mg.

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