COMPARATIVE IN-VITRO BIOEQUIVALENCE STUDY OF BRANDED AND GENERIC AMOXICILLIN CAPSULE IN FOUR SIMULATED PHYSIOLOGICAL FLUIDS

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Abstract

Comparative in-vitro bioequivalence study using dissolution profile data of immediate release oral solid dosage form of Biopharmaceutics class 1 and 3 has been advocated by World Health Organization (WHO), Food drugs Administration (FDA) and other international regulatory agencies. In-vitro dissolution offered many benefits compared to conventional in-vivo bioequivalence studies because, it reduces the cost and time of product release as well as avoiding unnecessary use of human volunteers thereby improving access to drugs from multisource for all persons as encouraged by WHO, to guarantee right to health for all.To evaluate and compare the in-vitro dissolution profiles of branded and generic brand of amoxicillin capsules available in Dutse, Jigawa, Nigeria.Dissolution profiles of branded and generic amoxicillin capsules containing amoxicillin 500 mg which are available in Dutse market were determined using the developed UV spectrophotometric method. The obtained dissolution profile data were assessed and comparedusing two different statistical methods: the fit factors (F1&F2) and the dissolution efficiency (D.E.) model. The tested generic brand of amoxicillin capsule can be interchangeable with the innovator brand. The computed F1 factor for the Generic brand are3.35, 6.12, 8.83 and 7.94 for simulated physiological pH (1.2, 4.5, 6.8 and 7.4) respectively which are within the acceptable limit of (0-15). While the F2 factor values are 69.00,57.15, 42.67and 46.76 for simulated physiological pH (1.2, 4.5, 6.8 and 7.4) where at SGF (pH 1.2) and Buffer (pH 4.5) were within the recommended range of ≥50 while values at SIF and Blood (pH 6.8 and 7.4) falls short of the accepted limit of ≥50. Similarly, the mean dissolution efficiencies (D.E.) were 4.00, 6.885.19 and 2.05 which are within the acceptable limit of ±10 for pH (1.2, 4.5, 5.19 and 7.4 respectively).Therefore, tested generic brand of amoxicillin capsule was found to be similar with the innovator brand, thus can be interchangeable with each other in clinical use.

Keywords: Amoxicillin, Simulated pH, Dissolution efficiency, in-vitro and bioequivalence

Introduction

Amoxicillin is a semi-synthetic oral b-lactam antibiotic used for the treatment of bacterial infections caused by susceptible microorganisms (Abrue *et al.*, 2003). It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other b-lactam antibiotics (Ashanagar and Naseri 2007). Amoxicillin is susceptible todegradation by b-lactamase producing bacteria, and is sometimes given with structurally related but pharmacologically in active clavulanic acid and/or Sulbactam to decrease itsdeactivation by the enzyme. It acts by inhibiting the synthesis of bacterial cell

walls, it inhibits cross linkagebetween the linear peptidoglycan polymer chains that make up a major component of the cell wall of gram-positive bacteria (Kassaye and Genete, 2013). Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract, for that reason, the importance of dissolution tests and dissolution profile for the establishment of pharmaceutical equivalence must be stressed (Ferraz *et al.*, 2007). Furthermore, dissolution has been considered in *in-vitro in-vivo* correlation and associations, involved in Biopharmaceutics Classification System (BCS), and has been used forbiowaivers, which is the absence of clinical bioequivalence testing in humans (Krämer *et al*, 2005). Amoxicillin belong toBiopharmaceuticsClassification System(BCS) class 1 category which implies that they are highly soluble and highly permeable active pharmaceutical ingredients (API) and are expected to release 85% or more oftheir drug in 15 min (very rapidly dissolving) in three differentbuffer solutions or need to be compared using F1 and F2 statisticswhen more than 85% are releases in 30 min (rapidly dissolving) (WHO, 2006). Generic drugs substitution from multisource has been advocated by WHO with aim of maximizing population health, consequent to constraint budgetary allocation (Simeon, 2011). This as a whole can result in the overall improvement of healthcare delivery system (Simeon, 2011). For example, in US, generic drugs represent 47% of all prescription dispensed in 1999, 61% in 2006 and 69% in 2008 (Frank, 2007; Mubarak *et al.*, 2012). Generics drugs approval in US led to an average savings of 77% of the product cost within one year (Kozlowski *et al*, 2011). In the same vain generics substitution in UK was highly successful and accounts for 83% (Kamerow, 2011). This rise has ensued because any drug product that is considered bioequivalent must be equal in quality (active ingredient, strength, purity, content uniformity, disintegration and dissolution rates) (Adegbolagun *et al.*, 2007). Nevertheless, this has been attended by a multiplicity of problems of which the most serious is the wide spread distribution of substandard generics, fake drug products. As a consequence, health care providers are usually concerned when selecting one drug among several bioequivalent during treatment regime (Adegbolagun *et al.*, 2007 and Almeri *et al.*, 2011).

The study was set up to evaluate andcompare the *in-vitro* dissolution profiles of branded and generic brand of amoxicillin capsules available in Dutse market. Below is the chemical structure of amoxicillin.

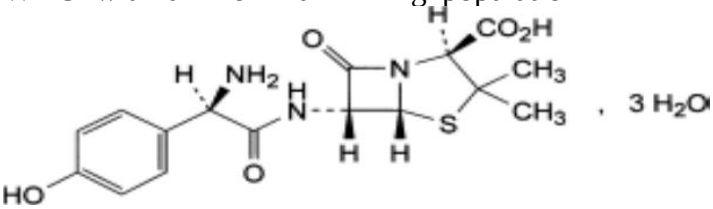

Fig. 1: Chemical structure of amoxicillin trihydrate.

Methodology: Materials and Equipments

Amoxicillin reference standard, two brands of amoxicillin capsules asshown in table 1,Purified distilled water, Conc. HCl, sodium hydroxide pellets, iodine crystal, 96%ethanol, sodium thiosulphate monobasic potassium phosphate, sodium acetate, analytical weighing balance (Mettler Analytical Balance Phillip Harris., England),euweka disintegration time test apparatus (Type ZT3, GmbH, Germany), pH meter (Fisher Scientific, Singapore), UV double beam spectrophotometer(MNF, Helious Zeta, Thermo Scientific England),dissolution tester(Tianjin Guoming Medicinal Equipment Co. LTD.,China), water bath, (model BJE 750A Gallen Kamp, England), Gallen Kamp hot air oven (Philip Harries Ltd, England), porcelain pestle and mortar, thermometer (Mc Donald Scientific International, England), stop watch (Smith England clock system), SPSS 20 and Microsoft excel were employedin the statistical analysis.

Standard preparation

Stock standard solution (1000µg/ml) was preparedby dissolving 10mg of amoxicillin reference standard in 10 ml of the prepared dissolution media pH (1.2, 4.5, 6.8 and 7.4). respectively. Six different concentration levels were prepared by serial dilution from the stock solution for calibration curves preparation in the range of (10-60 µg/ml) in volumetric flasks.

Dissolution test and sample preparation:

Dissolution tests were conducted in all the four simulated physiological pH 1.2(SGF), pH 4.5(buffer),pH 6.8 (SIF) and pH 7.4 (Blood) using USP apparatus 1 (basket) with dissolution test machine, the basket speed was maintained at 100rpm, and 900ml of dissolution medium was used to test all the samples. The dissolution medium was preheated to 37 ± 0.5 ^{o}C. One capsule was placed in the basket of the dissolution apparatus,the machine was operated and 2ml sample was then withdrawn at the time interval of 5, 15, 25, 35, 45 and 55 minutes. After each withdrawal, an equal volume of the medium was used to replace the withdrawn volume in order to maintain the total volume of the sink medium constant. One (1 ml) of the sample solution was quantitatively taken into 10 ml beaker and diluted to volume with the dissolution medium and absorbance measured spectrophotometrically using UV-vis spectrophotometer at (λ229nm) the calibration curves range was 10-60µg/ml while regression equations were y=0.0133x+0.1847,y=0.0137x+0.1967,y=0. 0143x+0.2113 and y=0.0162x+0.0807 and (r^2) were 0.9983,0.9934, 0.9947 and 0.9916 respectively for all the media used.

Statistical analysis of the dissolution profile data:

Fit factors; TheF1 factor, is the average % differenceover all time points in the amount of test branddissolved as compared to the reference brand. TheF1 value is 0 when the test and the reference profilesare identical and increases proportionally with the dissimilarity between the two profiles. The F2 value is between 0 and 100. The value is 100 when the testand the reference profiles are identical andapproaches zero as the dissimilarity increases (Ngwuluka *et al.*, 2009; Anderson*et al.*, 1999; Polli *et al.*,1997). They are computed using the formulas.

$$f_1 = \left\{ \left[\sum_{t=1}^{n} |R_t - T_t| \right] \Big/ \left[\sum_{t=1}^{n} R_t \right] \right\} \times 100$$

$$f_2 = 50 \cdot \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

Where R_t is the percentage of dissolved productfor a reference at time point t, T_t is thepercentage of dissolved product for the test brand,n is the number of time points. For each brand, theanalysis was performed on the mean values of three replicates withdrawals.

Dissolution efficiency:

$$DE = \frac{\int_{t_1}^{t_2} y \cdot dt}{y_{100} \times (t_2 - t_1)} \times 100$$

Where, y is the percentage of dissolved product. D.E. is the area under the dissolution curve between time points t_1 and t_2 expressed as a percentage of the curve at maximum dissolution, y100, over the same time period. For a capsule product, t_1 wassetto correspond to disintegration of the capsule shell. The integral of the numerator, i.e. the area under the curve is calculated by a model independent method, the trapezoidal one. The area under the curve is the sum of all the trapeziums defined by:

$$AUC = \sum_{i=1}^{i=n} \frac{(t_i - t_{i-1})\ (y_{i-1} + y_i)}{2}$$

Where t_i is the i^{th} time point, y_i is the percentage ofdissolved product at time t_i (Anderson, *et al.*, 1999; Ngwuluka, *et al., 2009*).

Results:

Table 1 shows the label information of the tested brands, while table 2 shows the result of weight variation, assay and disintegration tests (BP and USP, 2009).

Table 1: Label information of the Amoxicillin capsules (500mg)

Code	BatchNAFDAC	Country of origin	Date of Mng.	Date of Expiry
Sample A	150211	04-2481	India	Feb.,2015	Jan., 2020
Sample B	15116	04-2898	India	Jan. 2015	Dec., 2019

Table 2: The result of weight variation, assay and disintegration tests were shown in the table blow

S/№	Brands	**Weight variation (mg)± SD(n=20)**	**%mean deviation**	**% Content (min) ± SD (n=6)**	**Disintegration**	**Remark**
1	Sample A	680 ± 0.0083	0.485	108.30	7.02±1.47	passed
2	Sample B	679 ± 0.0085	0.900	108.90	5.62 ±0.92	passed

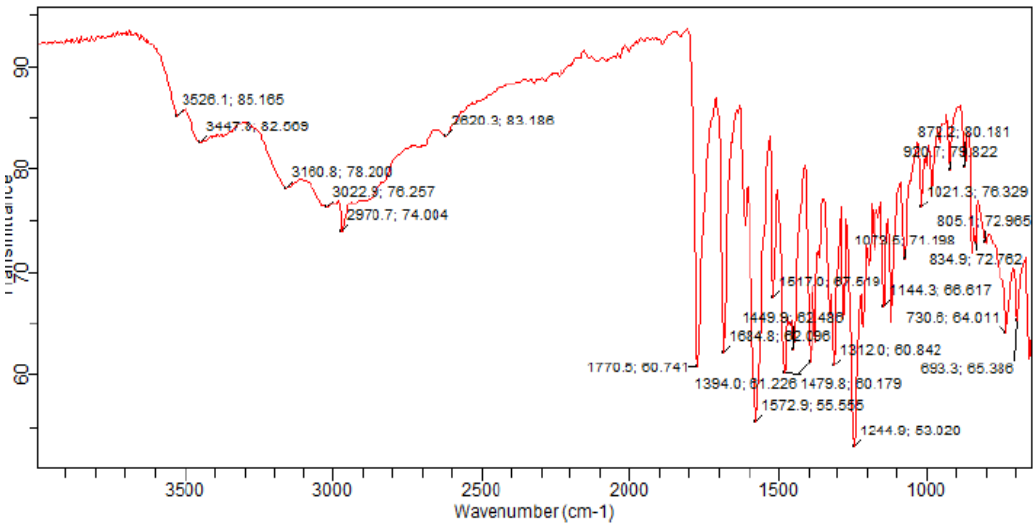

Fig. 2: IR spectrum of standard amoxicillin powder indicating finger print and functional group region

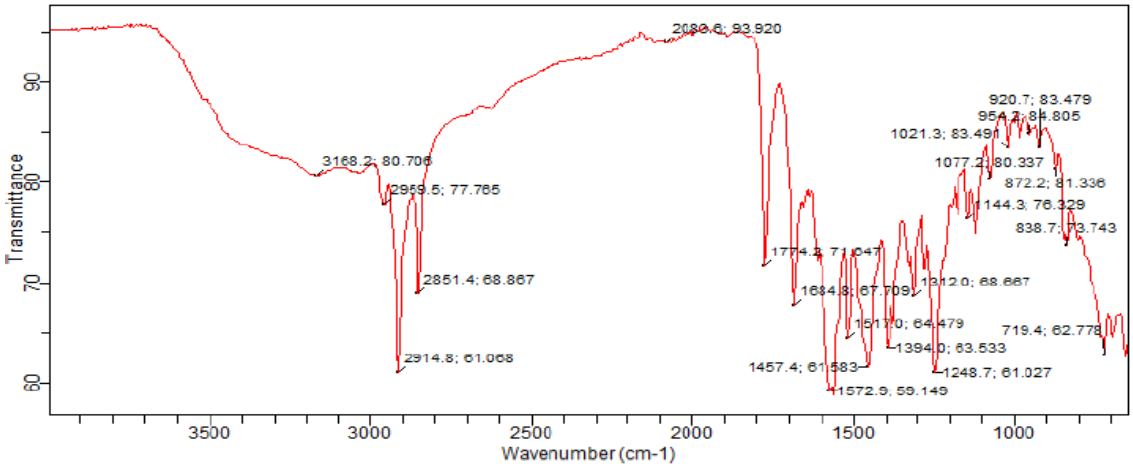

Fig. 3: IR spectrum of the amoxicillin capsule indicating finger print and functional group region.

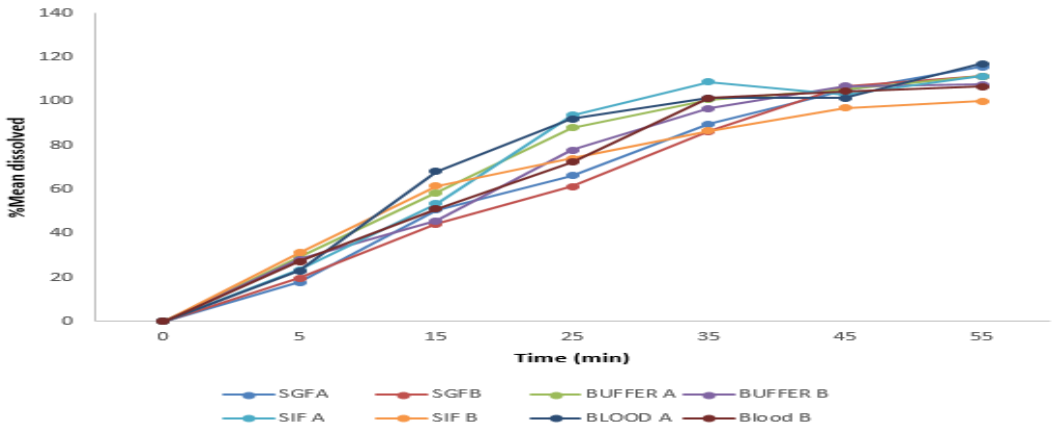

Fig. 4: Dissolution profile of reference and generic amoxicillin in four simulated physiological pH (1.2, 4.5, 6.8 and 7.4)

Table 5: Dissolution profile result of (F1, F2) and D.E.

B BB B			
pH 1.2	pH 4.5	pH 6.8	pH 7.4
F1= 3.35	F1=6.12	F1=8.83	F1=7.94
F2= 69.00	F2=57.15	F2=42.67	F2=46.76
D.E.=1.37	D.E.=-6.86	D.E.=5.19	D.E.=2.03

F1= Difference factor, F2= Similarity factor, D.E. = % Dissolution efficiency

Discussion:

The use of generics drugs and substitution from multisource has been advocated by the World Health Organization (WHO) which prescribed that all persons have the "right to health" and shouldhave access to essential medicines, which is defined as"medicines that satisfy the priority health care needs of apopulation" (WHO, 2010). The main aim of multisource substitution is to ensure that drugs were made affordable to all without compromising the quality and therapeutic efficacy of the branded drug. However routine quality evaluation of the generics to ensure interchangeability with branded should be carried out to guarantee the choice of best and affordable brand thus achieving better pharmacological outcome compared with the branded. The compendial methods include weight variation, disintegration, assay, and dissolution test. More than twenty (20) brands of amoxicillin capsules are in circulation in Dutse Market posing difficulties among healthcare providers on which brand to select to achieve the desired therapeutic outcome. The two brands selected passed both the BP 2009 and USP 2009 identification test as their IR spectra were superimposable with the standard Amoxicillin Spectrum as shown in figure 3 and 4. Assay results for the brands were within the specified limit set by USP, 2009, (90-120%). Similarly,weight variation test result was within the official range of standard deviation not exceeding 7.5% specified by (BP,2009). Furthermore, disintegration test timewas less than 30 minutes, as specified in British Pharmacopoeia (BP,2009). Kassaye and Genete 2013 conducted similar study on nine brands of amoxicillin and reported that all the tested brands complied with official requirement for disintegration. Furthermore, Benmoussa *et al.,* 2012 carried out similar study on three brands of amoxicillin tablets and all were found to pass the test. Dissolution of solid oral dosage form determines its absorption into systemic circulation and serves as predictor of *in-vivo* bioavailability, it is employed to assess the bioequivalence between the branded drug and generic counterpart. For any brand to be considered *in-vitro* bioequivalent with the innovator, US FDA, WHO and EMEA requires that the dissolution profile should be similar in three simulated physiological pH usually (1.2,4.5 and 6.8 or 7.4). Diverse methods of dissolution profiles data comparison have been reported in the literature, however, in this study the two most essential and extensively applied methods have been engaged: the fit factors and dissolution efficiency (D.E.).The fit factors can be expressed by two approaches:F1 (the difference factor) and F2 (the similarity factor). The two dissolution

profiles would be considered similarand bioequivalent, if F1 values lies between 0 and 15whereas, F2 should be between 50 and 100 based on the assumption of a maximum permissible difference of ±10%.The dissolution profiles of Brand B F2 values was 69.00, 57.15, 42.57 and 46.26 in pH (1.2, 4.5, 6.8 and 7.4) respectively, with F2 values below 50 at pH 6.8 and 7.4. However, the F1 values were 3.35, 6.12, 8.83 and 7.94 respectively, in all the four media, since the F1 values were less than 15 in all the simulated media brand B was assumed to be similar with the innovator brand A using the F1 and F2 factor therefore can be considered bioequivalent. The second comparison method employedin this study was dissolution efficiency (D.E.) model. the mean D.E. for the branded and the genericobtained was comparedby measuring the difference between the mean D.E.of the innovator brand andthe test brands. If the differences of the mean dissolution efficienciesare within acceptable limits of (±10%), it can be concluded that the reference and test dissolution profiles are equivalent and can be used interchangeably. As indicated in table 3; D.E. valuesfor simulated pH (1.2, 4.5, 6.8 and 7.4) were 1.37, 6.86, 5.19 and 2.03 respectively, hence Brand B achieved the acceptable limit in all the media used. Therefore, the dissolutionprofiles of Brand B aresimilar withthat of the innovator based on dissolution efficiency method this further confirmed the results obtained from fit factor (F1and F2) calculations which shows similarity of B with the branded (A).Even though F2 issomewhat closely interrelated with D.E., it is more difficultto infer F2 than D.E. without reference todissolution data or curves, as it relates todifferences between curves, and because of its nonlinear behaviour.In this study, all the comparison methodshave proven the similarity of the dissolution profileof Brand B with the innovator A.Thus A and B, can be used interchangeably.

Conclusion

This study indicated that simple, cheap accurate methods should be developed to routinely evaluate bioequivalence of the generics drugs with branded products particularly, the antibiotics which are prone to counterfeiting and/or faking. The results obtained shows that dissolution test can be successfully employed to establish if *in-vitro* performance of drug would predict *in-vivo* activity of the drug. The cheap, selective and reproducible UV spectrophotometric method developed and validated in accordance with ICH guideline for the four different simulated dissolution media pH (1.2,4.5,6.8 and 7.4) can be used successfully to compare the dissolution profile of amoxicillin capsule both the generic and branded. The generic Brand B and innovator brand A were found to have similar results in all the simulated media using F1 and F2 comparison as well as using dissolution efficiency model. Thus brand B can be used interchangeably with brand A (innovator brand).

Recommendation

The result obtained should further be validated using *in-vivo* study to ascertained the interchangeability between the innovator brand A and generic brand B thus,*in-vitro* and *in-vivo* correlation study should be conducted. There is urgent need to continuously undertake dissolution testing of BCS class 1 drugs especially amoxicillin which is among the commonest prescribed penicillin antibiotics to ensure that consumers of this drugs get what they need and also reduce the risk of resistance emergence which is threatening the future use of antibiotics and the public health generally. It is also imperative for the drug regulatory agencies at national and state levels to put more stringent measures of quality assurance of the drugs before given them marketing authorization as well as post market monitoring through in vitro bioequivalent testing, in vivo bioequivalent studies as well as other established method of quality assurance.

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