SPECTROSCOPIC DETERMINATION AND *INVITRO*BIOEQUIVALENCE STUDIES OF DIFFERENT AMOXICILLIN CAPSULE BRANDS

ABSTRACT

Amoxicillin is an aminopenicillin antibiotic, used in the treatmentofseveral infections resulting from susceptible Gram-positive and Gram-negative, such aspneumonia, acute otitis media, acne vulgaris,bacterial endocarditis, streptococcal pharyngitis, urinary tract, *Lyme borreliosis*, *Salmonella*, *Helicobacter pylori*, chlamydia, and odontogenic infections. The study aimed to determine the *in-vitro* bioequivalence of amoxicillin capsules from various brands using physicochemical and spectroscopic parameters. Weight uniformity, friability, disintegration, dissolving tests, and UV spectroscopy wereused to determine the in-vitro bioequivalence of different brands of amoxicillin capsules.The weight variation for all the capsules showed compliance with the USP specifications(≤10% weight deviation). All brands complied with the USP/BP specification for the disintegration test, as disintegration time ranged from 7 to 12 minutes. The dissolution profile of the innovator and the generic brands showed a percentage release from 80.81 – 89.11% within 30 minutes. Thefirst-order kinetics, $t_{1/2}$ and *k* of amoxicillin released ranged from 16.01 – 27.11 min and 2.56 – 4.33 x 10^{-2} min^{-1} respectively. A straight-line graph was obtained asa calibration curve of amoxicillin. UV absorption spectrum showed four distinctive peaks at 240, 275, 320, and 360 nm, with the maximum at 240 nm, while the percentage content of amoxicillin ranged from 90.58 ± 1.38 to 98.74 ± 0.97%. This result complied with the BP and USP specifications. Hence, there was a strong correlation between the release rate constant, *k*, and time since values were ≥ 0.8087 compared with the innovator and other brands.

Keywords*: physicochemical, bioequivalence; antibiotics, amoxicillin, spectroscopy, capsules,*

INTRODUCTION

β-lactam antibiotics including Amoxicillin (an oral semi-synthetic agent) are active compounds used in the treatmentof bacterial infections following susceptible microorganism infections. Amoxicillin is widely accessible in capsules andhas bioavailability issues due to the absorption process [1]. Amoxicillin, with a molecular formulae $C_{16}H_{19}N_3O_5S•3H_2O$, is known chemically as (2S,5,R,6,R)-6-[(,R)-(-)-2-amino-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate (Figure 1) [2].

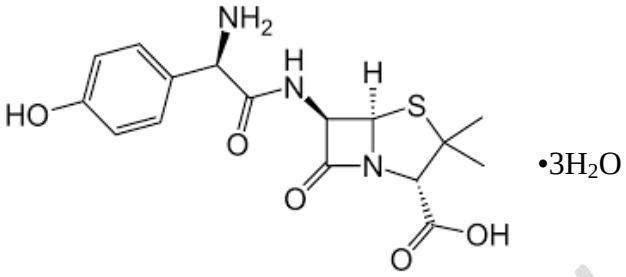

Figure 1. Structure of Amoxicillin trihydrate.

Some previous studies on humans found no significant food impact on the pharmacokinetic parameters of amoxicillin, especially absorption [3], whereas others found a substantial food effect with a 50% reduction in peak plasma levels in non-fasted individuals [4]. An energy-dependent efflux pathway was proposed as evidence for a putative dietary effect on amoxicillin absorption [5]. A thorough investigation of Amoxicillin utilizing healthy volunteers after intravenous, oral, and intramuscular injections of 250, 500, and 1000mg doses revealed changes in pharmacokinetic characteristics. The AUC for IV absorption was 93%, with an 86% urine recovery. Oral and intramuscular treatment produced complete and consistent absorptions, with maximum drug concentrations, AUCs, and urine recoveries comparable to oral dosing [6]. The kinetics of both IM and oral treatment showed dose-related absorption (absorption rate constant at 1.3/h for 250 mg and 0.7/h for 1,000 mg). This resulted in significantly later and lesser peak serum levels as dosages increased. Total absorption, on the other hand, had no dosage dependence, as seen by urine recovery and an AUC change of less than 10% [6].

Bioequivalence is regarded as the absence of significant differences in the degree and rate to which the active ingredient or active portion in pharmaceutical alternatives or

pharmaceutical substitutes becomes available at the site of therapeutic effect if administered at the same molar dose under similar circumstances in a well-designed study [7, 8].Over the decades, several in vitro methods have been employed to qualitatively quantify and compare the amount of substance present in pharmaceutical products and their suitability to serve as effective alternatives in place of the actual innovator brands [9, 10]. Many frequently used techniques are the physicochemical [11], chromatographic [12], electrochemical [13],and spectroscopic [14, 15], methods of analysis respectively. Chromatographic techniques are not only useful in in-vitro bioequivalence studies but are very useful methods in the quantification of active pharmaceuticalmetabolites from biological fluids [16, 17].

The World Health Organization (WHO) has pushed the use of generic brands to reduce the cost of medicines [18]. Generic substitution may be considered when a generic version of an innovator brand comprises the same active component in the same dosage form and route of administration. However, the occurrence of generic items that are incompatible with the innovator's product and/or each other has been documented [1].Antimicrobial resistance and antibiotic drug efficacy can be linked to using generic drugs with subtherapeutic doses or inadequate in vivo drug release [19]. As a result, the study aimed to determine the in vitro bioequivalence analysis of amoxicillin capsules from various brands using spectroscopic techniques, as it has been established that independent investigations such as the present one serve as a significant instrument for revealing potential inferior or non-compliant products that could eventually find an entry into the pharmaceutical market.

MATERIALS AND METHODS

Materials

Reagent and Equipment

All the chemicals used were of analytical grade. Pure Amoxicillin trihydrate powder (99.68% secondary standard, wasdonated byPrimex Nigeria Ltd., Ikeja, Lagos. Hydrochloric acid (37%) manufactured by Riedel-DeHaan Sigma-AldrisChemical Germany. All reagents were prepared using distilled water.Spectrumlab 752pro UV-VIS spectrophotometer, analytical weighing balance, disintegration chamber, dissolution tester.Four brands of Amoxicillin coded - A1, A2, A3, and A4were purchased from KETO DEVINE Pharmacy, Amassoma, Bayelsa state, Nigeria. Brands A1, A2, and A3 are generics, while A4 was the innovator.

Methods

Determination of weight uniformity

Twenty capsulesfrom each of the four brands of amoxicillin were randomly weighed individually using an analytical balance (Ohaus Adventure, USA),and weight values were recorded. The average weights and deviations were also calculated.

Disintegration test

Six capsules from each brandwere placed in separate chambers in a freshly prepared 0.1 M HCl medium at 37°Cusing a Disintegration Apparatus (VSI-19Model). The disintegration time was taken to be the time no particle remained on the basket of the apparatus.

Determination of maximum Wavelength for Amoxicillin

To 50 mg equivalent amoxicillin standard in a 50 mL volumetric flask, 25 mL 0.1M HCl solution was added to dissolve the powder, shaken gently, and made to mark with the 0.1 M HCl to give a concentration of 1000 µg/mL (Stock solution). An aliquot of 0.1 ml of this solution was then transferred to a 10 ml volumetric flask and made to 50 mL with the same

0.1M HCl to obtain a concentration of 10ug/mL. This was then scanned in the UV region, 200-380 nm.A five-point calibration curve of concentrations:1, 2, 4, 8, and 16 ug/mL was prepared from the stock solution of 1000ug/mL. The absorbance of these concentrations wasmeasured at the wavelength of 240 nm (being the $\lambda_{max}$for amoxicillin in this study). Values obtained were used to plot a calibration curve.The calibration curve of amoxicillin is a straight-line graph; Y=0.0143x + 0.0149; R=0.9962

General procedure for application of the method topharmaceutical preparations

An equivalent of 50 mg of amoxicillin powder was transferred into a 100 mL volumetric flask. This was dissolved with 50 mL of 0.1M Hydrochloric acid, shaken gently, and made to mark (solution A). Aliquots of 0.1, 0.2, and 0.3 mLwere then transferred into separate 10 ml volumetric flasks and diluted to mark with 0.1M HCl acid (representing 5, 10, and 15 µg/mL). The absorbance of these solutions wasobtained at 240 nm. This procedure was repeated twice and the amount of drug in capsuleswascalculated from the calibration curve.

Dissolution test and drug release

The dissolution test was carried out followingthe BP method using the USP apparatus 1 (Basket method) in 6 replicates for each brand [20]. The dissolution medium was 900ml 0.1M HCl which was kept at 37 ± 0.5 ^{o}C, 5 mL of dissolution sample was withdrawn at intervals of 0, 5, 10, 15, 30, 45, and 60 minutes and replaced with equal volume to maintain sink condition. Sample aliquots were filtered, and diluted with the dissolution medium and the absorbance was measured by spectrophotometry at 240 nm. The concentration of analyte in each aliquot was determined from the calibration curvefor amoxicillin.

Bioequivalence and similarity of generics to innovator brand

TheUS FDA performance verification test requirements [21], for comparison of dissolution profile between innovator and generic drugs, was employed in conjunction with the USP and BP stipulated limit of not less than 80% of the drug released within 30 minutes. Comparative performance was assessed using graphical and first-order methods.

Data analysis

The weight uniformity was analyzed using simple statistics, while dissolution profiles of the generics and innovator weredone graphically and by kinetics to determine drug release variables – release rate constant k, half-life ($t_{1/2}$), correlation coefficient (R^2), etc., using Microsoft Excel, 2016.

RESULTS AND DISCUSSION

Weight uniformity

Weight variation is an indicator of good manufacturing practices (GMP) used in conjunction with the amount of active pharmaceutical ingredient (API) present in the formulation by manufacturers [22]. There is no significant deviation from the stipulated limit of deviation is ±10% for capsules, values ranged from 0.15 – 3.55 percentage deviation. The highest value was obtained in the A3 brand. The weight variation for all the capsulesshowed compliance with the USP stipulated specifications(≤10% weight deviation) [23].

Disintegration time

All brands of amoxicillin complied with the USP/BP specification for the disintegration test [23, 24], as disintegration time ranged from 7 to 12 minutes.The disintegration study of the solid dosage form is important for the evaluation of drug release and could be used as an indicator to ascertain the lack of batch uniformity and inconsistency in solid dose formulations [25].

Determination of maximum absorption wavelength

The UV absorption spectrum for amoxicillin gave four distinctive peaks at 240, 275, 320, and 360 nm were observed, with the maximum at 240 nm (Figure 2).

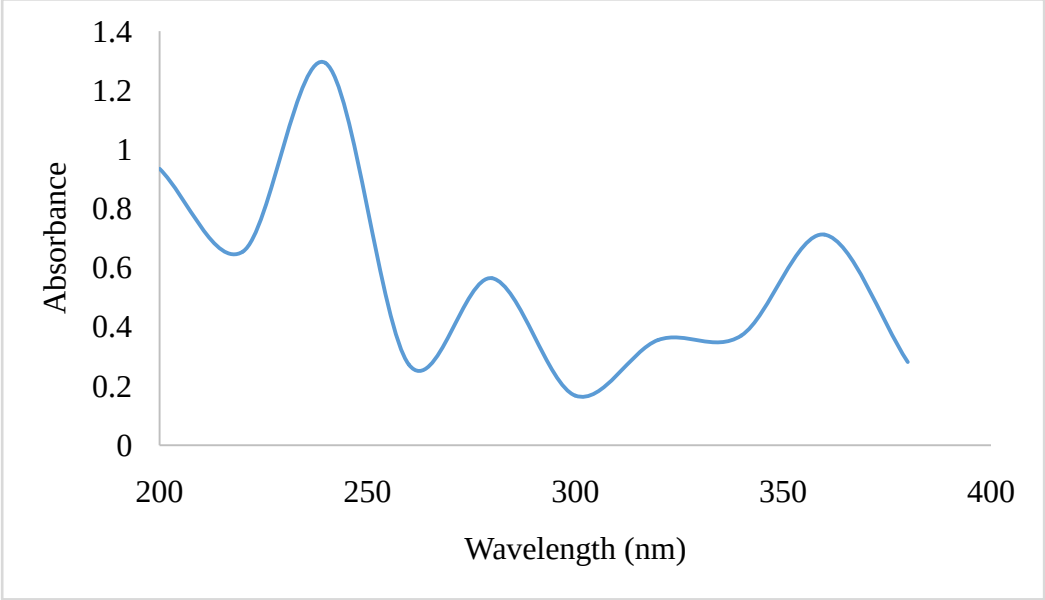

Figure 2. UV-Spectrum of Amoxicillin

Quantification of amoxicillin in capsules

The percentage content of amoxicillin ranged from 90.58 ± 1.38 to 98.74 ± 0.97 (Table 1). All brands except A1 were found satisfactory to the BP specificationwhich ranges from 95 to 105%. [20], while all brands met the USP stipulated standard of content, which should not be less than 90 and not more than 110 %, respectively [23]. The innovator brand A4 was found to contain 98.74 ± 0.12% of the label claim of 500 mg/capsule, while generics ranged from 90.58 ± 0.46 - 96.16 ± 0.37%. The student-t tests for accuracy and precision between the label claim and amount found in brands ranged from 1.400 – 1.496 (Table 1), while values between A4 (innovator) and generics - A1, A2, and A3 were 1.4815, 1.4750 and 1.2947 respectively – all test values were < 3.18 (tabulated) at 95% confidence level for 3 replicates. This suggested that there was no significant difference between label claimsof theamoxicillin brands and assay values obtained, in addition to the A4 (innovator) and A1, A2, and A3 (generics) respectively [26]. Furthermore, the relative standard deviation (%RSD, n = 3) and standard error of the mean (SEM) ranged from 0.38 to 0.98and 0.34 to 1.83respectively, with the least values recorded by the innovator brand for both properties.These values indicated high reproducibility and reliability, with satisfactory precision and accuracy of method.

Table 1. Assay of different brands of amoxicillin

Sample ID	Label claim (mg/capsule)	Amt found ± Sd (mg/capsule)	%RSD	SEM	Drug Content (%)	Student –t test for A4
A1	500	452.91 ± 2.30	0.51	1.33	90.58 ± 0.46 t=1.496	1.4815
A2	500	476.08 ± 3.16	0.66	1.83	95.22 ± 0.63 t= 1.460	1.4750
A3	500	480.78 ± 1.84	0.38	1.07	96.16 ± 0.37 t = 1.408	1.2947
A4	500	493.70 ± 0.59	0.12	0.34	98.74 ± 0.12 t = 1.400	0.0

Dissolution test

The dissolution profile of the innovator and the generic brands showed a percentage release of amoxicillin ranging from 80.81 – 89.11% within 30 minutes. This result complied with the BP and USP specifications for the dissolution rate for the innovator and generic brands, with the order of percentage released at t_{30}as A4 >A3>A2>A1. The innovator brand recorded the highest percentage of amoxicillin released at t_{60} with the generic brands comparable (Figure 3). All test brands were considered equivalent from the*in vitro* drug release profile[20, 23].

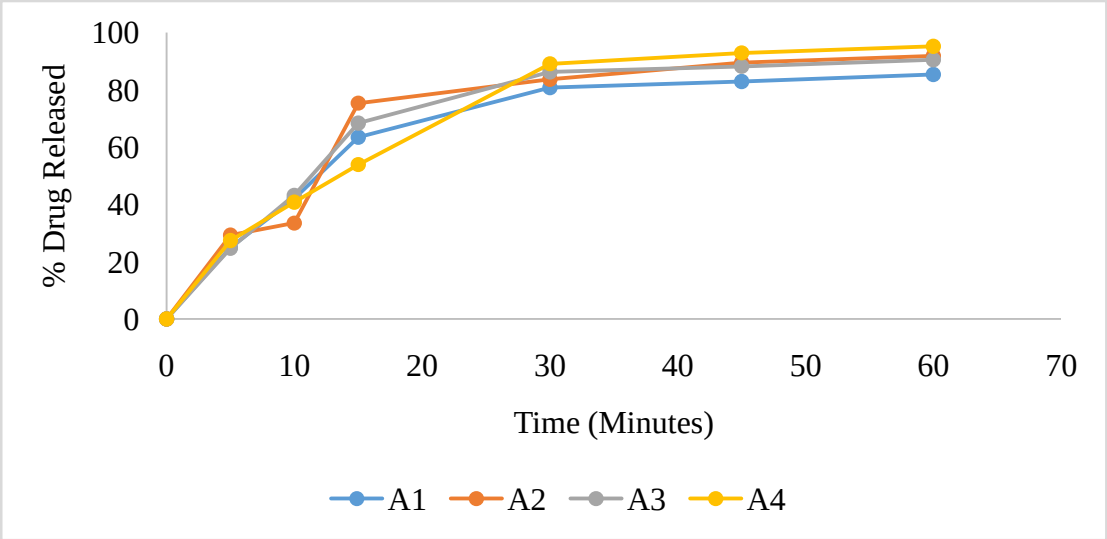

Figure 3.The dissolution profile of 4 brands of Amoxicillin (Innovator and 3 generics, A1, A2, and A3).

Aplot for the *in vitro* release rate constants (*k*) of amoxicillin overtime intervals, $t_5 - t_{60}$ - assuming a first-order kinetic was obtained (Figure 4). This is a comparative-dependent model for bioequivalent studies[27, 28].The mean rate constant *k*, ($t_5 - t_{60}$), ranged from 2.56 to 4.33 x 10^{-2} $min^{-1}$for innovator and brands (Table 2), while other kinetic variablessuch as half-life ($t_{1/2}$) and correlation coefficient (R^2) were from 16.01 – 27.11 min and 0.8087 – 0.9156respectively.The order of release rate constant *k* was A4 (Innovator) >A2 > A3 > A1, while the time taken for half the amount of the drug to be released from the tablet followed the same trend. This implied that the innovator is released fastest amongst all the brands. There was a strong correlation between the release rate constant and timesince R^2values were ≥ 0. 8087 - ditto the innovator and other brands (Table 2).

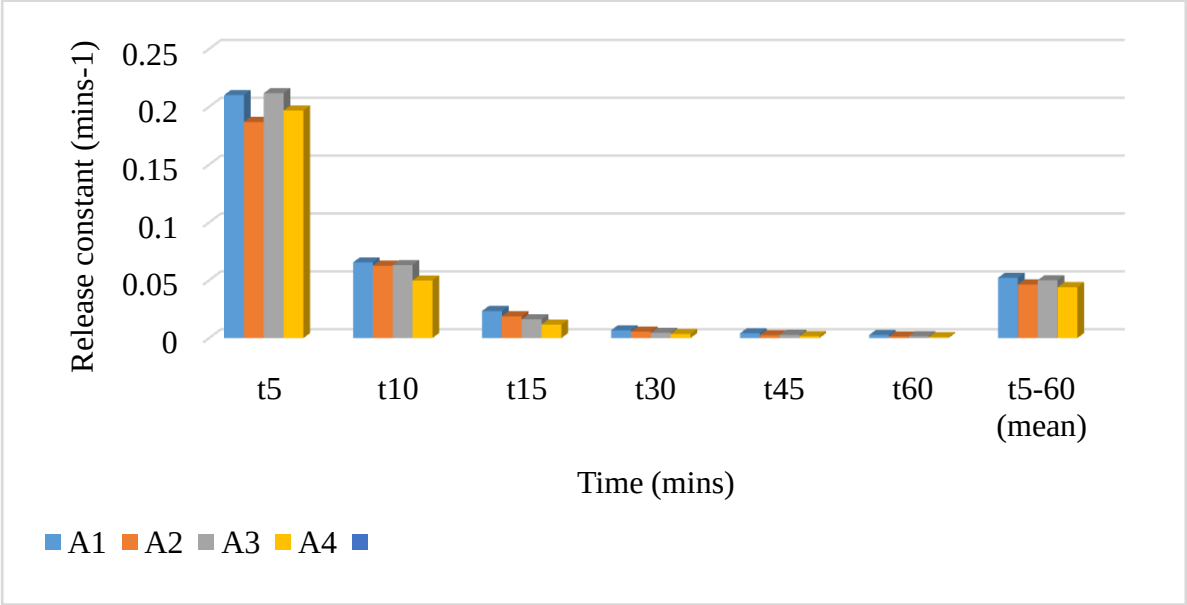

Figure 4.Drug release constant*k*,over time amplitude

Table 2.*In vitro* kinetic variables for innovator and generic brands

Sample code	Rate constant (*k*)	Half-life ($t_{1/2}$) (min)	Correlation coefficient (R^2)
A1	2.56 x 10^{-2}	27.11	0.8293

A2	3.57×10^{-2}	19.41	0.9156
A3	3.29×10^{-2}	21.04	0.8087
A4	4.33×10^{-2}	16.01	0.8738

CONCLUSION

All of the amoxicillin brands tested in this study met the disintegration test requirements set by the USP and BP. The dissolution profiles of the innovator and generic brands demonstrated a relative percentage release pattern within 30 minutes. The UV absorption spectra revealed our unique peaks, with the greatest at 240 nm. The percentage content achieved was all within the standard values mentioned in BP and USP for the brands. The overall in-vitro bioequivalence results in all samples are consistent with the standard, indicating that these brands can be used as alternatives to innovator brands.

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