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Quality assessment of amoxicillin-clavulanate potassium tablets in Lagos, Nigeria

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ABSTRACT

Amoxicillin clavulanate potassium is a broad spectrum antibiotic used in the management of most bacterial infections. The innovator brand, Augmentin, is expensive, hence the need to substitute them with much cheaper generic brands. This brings about the question of bioequivalency, an essential biopharmaceutic factor that provides option for generic substitution especially in underdeveloped countries such as ours. A study on quality assessment of six generic brands in comparison with the innovator brand was carried to ascertain the quality of the generic brands that are available in Nigerian drug market. Assessment of their bioequivalence was done vis-a-vis the evaluation of physicochemical characteristics such as uniformity of weight, friability, hardness, disintegration, dissolution rate and assay of the brands, using both official and non-official methods. All the brands studied complied with the official specifications on uniformity of weight and friability but the brands AC-2 and AC-7 failed the disintegration time test. Brand AC-7 exhibited a very high crushing strength which consequently affected it's dissolution rate negatively and did not comply with USP specification on dissolution. Out of the six generic brands tested, five of them were considered bioequivalent with the innovator brand with the exception of brand AC-7. Any reported case of therapeutic failure concerning the brands that complied with specification most likely, might be related to bad patient compliance, physiological and pharmacokinetic conditions relating to the patients using the drug and inadequate advice to patients on the part of the healthcare professionals.

Keywords: Amoxicillin clavulanate potassium, bioequivalence, dissolution test, therapeutic failure, patient compliance.

INTRODUCTION

The choice of drugs in the treatment and management of any ailment is an important point of consideration to healthcare practitioners. Innovator drugs in general are known to be medicine of proven therapeutic efficacy, but they turn out to be expensive and out of reach to majority of patients especially in developing countries of Africa. As at 2011, about 70 percent of Nigeria's 150 million people live below the poverty line, the Daily Trust (a Nigerian based daily newspaper) reported, citing Lamido Sanusi, governor of the Central Bank of Nigeria. This does not imply that the poor cannot have access to a good medicare. The availability of generic drugs guarantees optimal healthcare due to their affordability. World Health Organization (WHO) suggest that generic drug substitution should be a key component of a national drug policy in order to address what economics define as "market failure" in the pharmaceutical sector [17]. According WHO, a generic medicine is a pharmaceutical product that is no longer protected by a patent, it is interchangeable with an innovator drug and can be copied by other companies [17].

Generic drugs in drug markets, by deduction from definitions are supposed to be chemically and biopharmaceutically equivalent to the innovator drug. By comparison, they contain same therapeutically active ingredients; contain the same salt, ester or chemical form; are of the same dosage form; identical in strength, concentration and route of administration. Achievement of this level of equivalence is of great concern to the healthcare practitioners where there is the need to balance therapeutic effect of the available drugs with affordability. Pharmaceutical companies in Nigeria cannot meet the drugs requirement of the citizenry, consequently resulting in importation from countries around the world to complement the locally manufactured ones. Majority of the drugs circulating the Nigerian market available to the healthcare system are imported. The National Agency for Food and Drug Administration and Control (NAFDAC), the regulatory body guarantees the quality and efficacy of drugs that go into circulation, nonetheless, fake and counterfeit drugs still find their ways into the market place. Counterfeit medicines account for approximately 68% of the drug market in Nigeria [7]. According to World Health Organisation (WHO) approximately 30% of medicines in circulation in most African countries, Asia and Latin America are counterfeit [18].

There had been reported cases of generic drugs that are not bioequivalent to the standard innovator drugs, sensitive drugs such as antibiotics inclusive; brands of ampicillin and tetracycline capsules [13]. Bioequivalence study conducted on brands of sulphadoxine-pyrimethamine tablets in Nigerian market showed that some of the drugs were not pharmaceutically bioequivalent [14]. The study on ciprofloxacin, by Adegbolagun et al. in 2007, showed that some of the brands in circulation were obviously fake drugs. Comparative evaluation of physicochemical properties of some commercially available brands of Metformin hydrchloride tables in Lagos, Nigeria [1] also indicated that some of the brands investigated in the study were substandard drugs compared to the innovator drug [4]. Frequent observation of therapeutic failures of medicinal products, especially the generics necessitates regular review and study of medicine circulating in the Nigerian drug market and developing countries at large.

Antibiotics are very sensitive medicine and are used in the management of microbial infections. If not properly used as specified, the tendency that the microbes involved may develop resistance against them and render them ineffective is very high [20]. Resistance towards the medicines can equally be developed in cases related to fake antibiotic products, where they are underdosed. This has recently been observed in generic drugs related to Augmentin-like medicines, containing amoxicillin and clavulanic acid and it derivatives as the active ingredients: primary reason for embarking on this study.

Amoxicillin (Fig: 1), the primary API in the amoxicillin/clavulanic acid tablet, is a semisynthetic antibiotic with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms. Like most penicillins, it can be degraded by β-lactamase in the GIT, and therefore, the spectrum of activity does not include organisms which produce these enzymes.

A structurally related β-lactam, clavulanic acid (Fig: 2) possesses the ability to inactivate a wide range of β-lactamase enzymes commonly found in microorganisms resistant to penicillins. The formulation of amoxicillin and clavulanic acid as amoxicillin-clavulanate potassium tablets protects amoxicillin from degradation by β-lactamase enzymes and effectively extends the antibiotic spectrum of amoxicillin to include many bacteria normally resistant to amoxicillin and other β-lactam antibiotics. Thus, amoxicillin-clavulanate potassium tablets possess the properties of a broad-spectrum antibiotic and a β-lactamase inhibitor. Nonetheless, the protective effect of clavulanic acid over amoxicillin does not translate to elimination of resistance by bacteria. Skipping doses or not completing the full course of therapy may decrease the effectiveness of the immediate treatment, and increase the likelihood of bacterial resistance not treatable by amoxicillin/clavulanic tablets or other antibacterial drugs in the future.

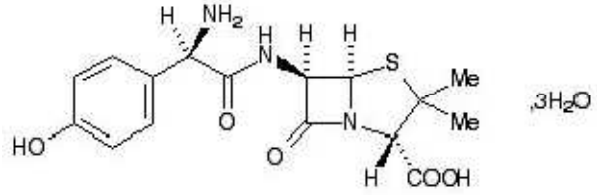

Fig: 1. Amoxycillin trihydrate

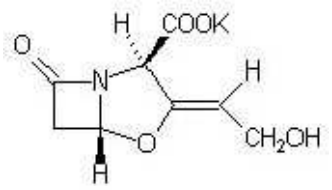

Fig: 2. Potassium clavulanate (derivative of Clavulanic acid)

Therapeutic failure has been reported in some of the brands that are available in the Nigerian drug market. This failure may occur not only due to insufficient active ingredients but also due to inclusion of wrong ingredients or non inclusion of the active pharmaceutical ingredient (API) [16,19]. It is equally a note of importance that the quantity of API is not the only factor that can affect the bioequivalence of medicines. Formulation excipients and methods are factors of consideration in the release and dissolution profile of the API [3,4]. In view of these facts bioequivalence investigation becomes inevitable for drug quality assessment. This is supported by estimation of the physicochemical properties of medicine at specific interval of time within and after the shelf life [2,6].

Therapeutic equivalence of medicines is determined through the evaluation of the chemical and biopharmaceutical equivalence [12]. The medicines must be identical in strength, quality, purity and in the same dosage form for the same route of administration [1,4]. Dissolution profile of oral medications is of great importance: significant variation within the generics is a pointer to deficiency in drug formulation and the delivery system which can ultimately affect the therapeutic effect of the medicine. Dissolution test is known to be an important parameter in predicting the *in-vivo* bioavailability of most oral preparation [4,5,8,10,15].

Medicines of unquestionable quality and therapeutic efficacy are supposed to be available to all classes of people in a society irrespective of their economic status. Hence, the objective of this study was to evaluate the physicochemical properties of seven brands of amoxicillin/clavulanic acid tablets available for use in Nigeria, using official and unofficial compendia.

EXPERIMENTAL SECTION

Seven brands of Amoxicillin/Clavulanate 625 mg tablets were purchased from reputable pharmacies in Lagos for the purpose of the study and designated AC-1, AC-2, AC-3, AC-4, AC-5, AC-6 and AC-7, where AC-1 is the innovator medicine. The study was performed within the expiration dates of the products. All the reagents used were of analytical grades while the Methanol was of HPLC grade. Freshly prepared distilled water was used at every step of the project where necessary.

Uniformity of weight - Weighed on an analytical balance individually 20 tablets selected from each brand and the average weight calculated. In accordance with IP/BP and USP, not more than two of the individual weights should deviate from the average weight by more than the percentage given in the pharmacopoeia and none deviates by more than twice that percentage. IP/BP & USP limits for tablet weight variation for tablets over 250 mg should not be more than 5%.

$$Average\ weight = \frac{\sum(x_1 + x_2 \cdots x_n)}{n},$$

where, x - weight of each tablet; n - total number of tablets.

$$\%\ deviation = \frac{Weight\ of\ tablet - Average\ weight\ of\ tablets}{Weight\ of\ tablet} \cdot 100$$

Hardness Test - From each brand, ten tablets were taken, each placed between the spindle of Erwerka tablet testing machine. By tuning the knurled knot, pressure was applied to hold the tablet in place and was gradually increase until the tablet breaks. Records were taken at the breaking points.

Friability Test - Ten tablets from each brand were placed in a friabilator and were carefully dedusted prior to testing. Tablet samples were accurately weighed, and place the friabilator's drum. The drum was rotated 100 times after which tablets were removed, dedusted and reweighed accurately again. A maximum mean weight loss from the three samples of not more than 1.0% is considered acceptable.

$$\%\ Friability = \frac{Initial\ weght - Final\ weight}{Initial\ weight} \cdot 100$$

Disintegration Test - Randomly selected six tablets of each brand were placed in the plastic tubes of a multi-unit disintegration tester at 37 ^{o} C in distilled water. The apparatus was switched, with the vertical oscillatory motion, the tablets were left in the tubes and the disintegration time was taken to be the time no more granules from the tablets were left on the mesh. This experiment was carried out in triplicate.

Dissolution Test - The dissolution studies were carried out using the apparatus USP Type II (Paddle) with the RPM of 75 and the dissolution medium is 900 ml of water and the temperature 37±0.5°C was maintained. The parameters of dissolution apparatus were set, added one tablet into each of six dissolution vessels. The dissolution apparatus was immediately started. 10mL of the sample solution was withdrawn at the end of 5, 10, 15, 30, 45 and 60 minutes from each dissolution vessel, and replaced with 10mL of dissolution medium after each sample withdrawal to maintain sink conditions. This solution was filtered through 0.45µ membrane filter, filled into vials and labeled appropriately.

Assay of Tablets - Estimation of Amoxicillin and clavulanate Potassium tablets by HPLC method. The assay was carried out using official monograph of Amoxicillin and clavulanate tablet as reported USP HPLC method, revision bulletin of March 1, 2009.

Data Analysis - A model independent approach using a similarity factor (f_2) to compare dissolution profiles was applied as described by the US FDA (Moore). The similarity factor (f_2) 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 curves. This is presented in the following equation:

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

where n is the number of time points, R_t is the dissolution value of the reference at time t, and T_t is the dissolution value of the test at time t. Using the mean dissolution values from reference and test curves at each time interval, similarity factor (f_2) from the above equation is of consideration where it is greater than 50 (50-100), ensure sameness or equivalence of the curves and, thus, of the performance of the test and reference products. Situation where (f_2) is less than 50 in an indication of difference in dissolution profile relative to the reference drug (innovator drug) and are not interchangeable.

RESULTS AND DISCUSSION

Evaluation of the tablets was based on basic physicochemical parameters [2,3,21]: uniformity of weight, hardness, friability, disintegration time and dissolution study as shown in Table 1 and Table 2. Table 3 shows the f_2 statistical similarity factor comparing the dissolution curve of the six generic brands (AC-2, AC-3 AC-4, AC-5, AC-6 and AC-7) with the innovator brand (AC-1).

From Table 1, result on weight uniformity from all the brands shows that they all conform to the official requirement of most pharmaceutical compendia. None of them deviated in weight with a difference of more than 5 % from the mean value.

Official requirement on friability is a maximum mean weight loss of not more than 3 $\%^{w}/_{w}$ (BP & USP $\leq 1\%^{w}/_{w}$; Russian Pharmacopoeia $\leq 3\%^{w}/_{w}$. From indications, the friability result shows that all the tablets were strong enough to withstand abrasion as none of them has a friability value of more than the specified. The friability of all the

brands ranges between 0.27% and 0.60%. From the obtained results, it can be concluded that all the brands passed the friability test.

Indication of tablet hardness is normally presented in the crushing strength. It assesses the resistance of the tablet to permanent deformation. In the non-official test carried out, 5 brands (AC-1, AC-2, AC-3, AC-4, AC-5 and AC-6) passed while the brand AC-7 failed with a value of 45.54±0.65 kgf.

The importance of tablet disintegration cannot be overemphasized as it is an integral part of drug release from the dosage form, consequently a factor of consideration in bioavailability of drug: it is directly proportional in rate to the dissolution of the dosage form. Official specifications for disintegration of tablets is given as 15 minutes maximum time for uncoated tablets (Russian Pharmacopoeia,). However brands AC-2 and AC-7 gave disintegration time of 16.60 and 65.20 minutes respectively while the disintegration time of brands AC-1, AC-3, AC-4, AC-5 and AC-6 fell within the specified limit.

The dissolution rate profile according to BP for a conventional tablet requires not less than 70% $^{w}/_{v}$ of the API in solution in 45 minutes. In the case of Amoxicillin clavulanate tablets according to USP revision bulletin not less than 85% $^{w}/_{v}$ of the labeled amount of amoxicillin and not less than 80% $^{w}/_{v}$ of the labeled amount of the clavulanate are expected to be dissolved in 30 minutes. Table 2 and Figure 3 show that brands AC-1, AC-2, AC-3, AC-4, and AC-5 had over 85 % $^{w}/_{v}$ of amoxicillin in solution and brand AC-6 with a value of 84.5 % $^{w}/_{v}$. Meanwhile, over 80 % $^{w}/_{v}$ of clavulanate (Figure 4) went into solution in 30 minutes in all the brands tested except brand AC-7. The brand AC-7 had values of 65.7% $^{w}/_{v}$ of amoxicillin and 50.5% $^{w}/_{v}$ of clavulanate in solution in 30 minutes.

Table 2 shows that all the brands tested (AC-1, AC-2, AC-3, AC-4, AC-5, AC-6 and AC-7, in relation to the content of the active ingredients, had contents that falls within the USP requirement of 90-110%.

Basic qualitative analysis on all the brands tested showed the presence of the active pharmaceutical ingredients. This analysis also includes the visual observation of the colour, size and shape of the tablets as labeled. The result showed that the tablet conform in physical appearance and content. The quantitative evaluations of the tablets were based on uniformity of weight, friability, hardness, disintegration, dissolution as well as API content [2,3,21]. From the results, uniformity of weight of all the brands showed compliance with official specification. The same compliance was also observed as related to the friability but the same cannot be implied on all the brands when it concerns tablet hardness and the disintegration time. Result showed that brand AC-7 had a value of approximately 46 kgf, which can serve as an explanation to the high disintegration time of the same brand (65.20 minutes). Brand AC-2 had a hardness of 10.44 kgf with a disintegration time of 16.60 minutes. These brands clearly failed the specifications on disintegration time despite the fact that the percentage content of active ingredients from the assay result showed that they are within limit. By implication, this explains why the dissolution value of brand AC-7 failed dissolution test, since value less than specification were released within the specified time. The brand AC-2 despite the slight deviation in disintegration time still passed dissolution test. Inference can be made on formulation problem which may be related to the binder effect as well as pressure of compression, making it difficult for the active ingredient to be readily available.

This is equally reflected in the dissolution profile curve, where all the brands were compared using similarity factor, f_2 statistical value. The result obtained showed that the generic brands AC-2, AC-3 AC-4, AC-5 and AC-6 had f_2 value greater than 50 with the exception of the generic brand AC-7 with a relative value of 41. This shows that all the brand tested are interchangeable with the innovator brand with the exception of AC-7 brand.

Table 1: Physicochemical Characteristics of Amoxicillin Clavulanate Potassium Tablets

Code	Uniformity of weight Deviation (%) SEM	Friability (%)	Hardness Kgf±SEM	Disintegration time (min)
AC-1	0.75 ± 0.15	0.27	8.52 ± 0.54	7.50
AC-2	0.95 ± 0.22	0.48	10.44 ± 0.62	16.60
AC-3	1.74 ± 0.17	0.54	7.25 ± 0.96	8.40
AC-4	1.22 ± 0.32	0.36	7.94 ± 0.85	6.00
AC-5	1.64 ± 0.18	0.44	11.24 ± 0.78	8.20
AC-6	1.87 ± 0.19	0.60	10.55 ± 1.05	5.60
AC-7	1.05 ± 0.25	0.30	45.54 ± 0.65	65.20

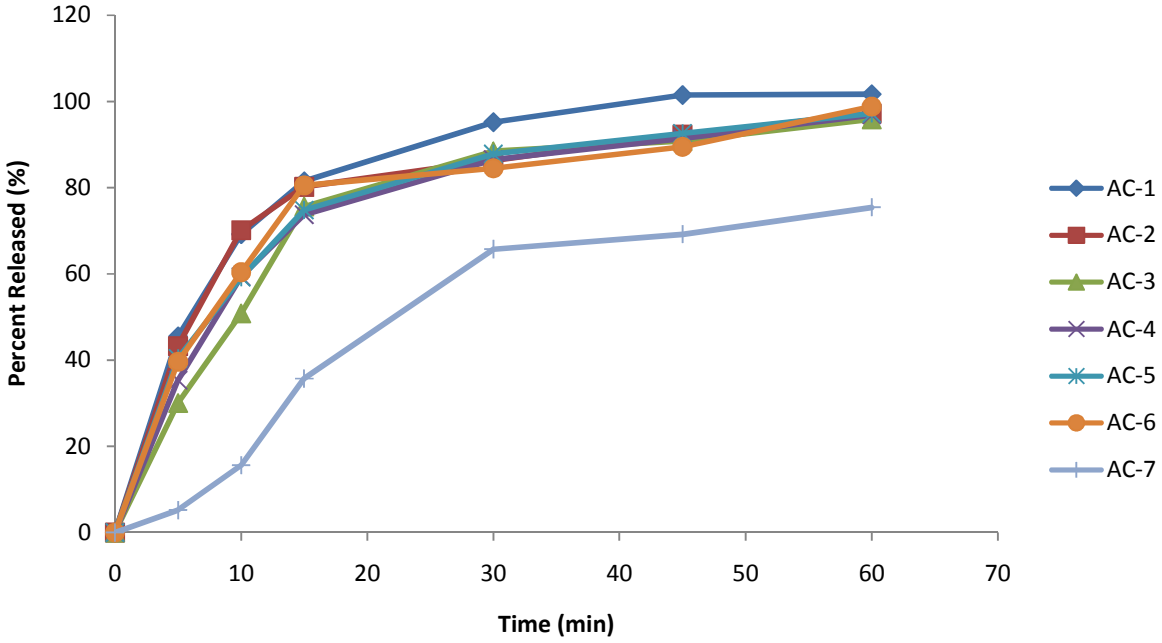

Fig 3: Dissolution profile of Amoxicillin from seven brands of Amoxicillin/clavulanate tablets

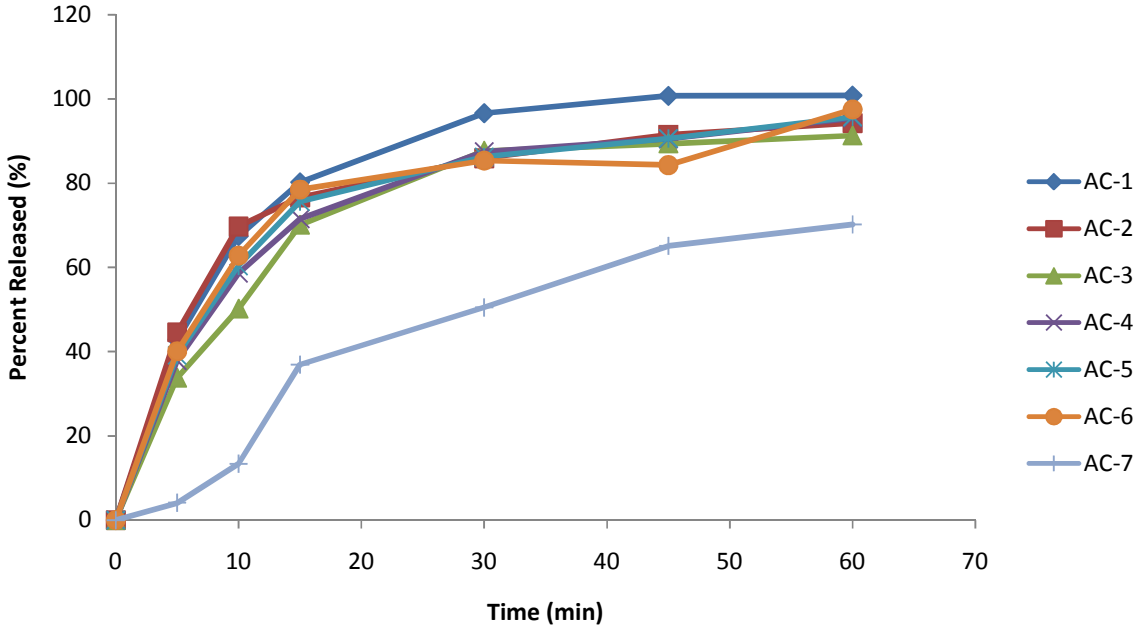

Fig 4: Dissolution profile of Clavulanate from seven brands of Amoxicillin/clavulanate tablets

Table 2: The Dissolution and Assay of Amoxicillin Clavulanate Potassium tablets

Code	Amoxicillin			Clavulanate Potassium		
	(Drug released, %w/v)		Assay	(Drug released, %w/v)		Assay
	30 min	45 min	(%)	30 min	45 min	(%)
AC-1	95.2 ± 0.2	101.5 ± 0.3	102.5	96.6 ± 0.4	100.7 ± 0.1	99.6
AC-2	86.3 ± 0.1	92.3 ± 0.2	97.5	85.9 ± 0.1	91.5 ± 0.4	96.8
AC-3	88.4 ± 0.3	90.8 ± 0.4	98.4	87.7 ± 0.3	89.4 ± 0.2	97.6
AC-4	86.5 ± 0.2	91.4 ± 0.8	95.6	87.5 ± 0.4	90.5 ± 0.6	95.1
AC-5	87.8 ± 0.4	92.6 ± 0.2	97.3	86.2 ± 0.4	90.6 ± 0.5	96.7
AC-6	84.5 ± 0.3	89.5 ± 0.7	96.8	85.4 ± 0.5	84.3 ± 0.2	95.1
AC-7	65.7 ± 0.6	69.2 ± 0.2	95.4	50.5 ± 0.5	65.1 ± 0.2	95.9

Table 3: f_2 Values of generics relative to the innovator drugs

Generic Products	AC-2	AC-3	AC-4	AC-5	AC-6	AC-7
f_2 Values	75	71	67	60	74	41

CONCLUSION

Deducing from the above results, it is easier to justify the therapeutic failure of some of the Amoxicillin clavulanate potassium tablets in the Nigerian drug market. The generics studied showed that apart from the innovator drug AC-1, the generic brands AC-2, AC-3, AC-4, AC-5 and AC-6 are chemically and biopharmaceutically equivalent to the innovator drug. The brand AC-7 obviously may result in therapeutic failure from all indications. In any case, where therapeutic failure is observed in the other brands that passed in the course of this study, it can only be adduced to failure on the part of patient compliant, other physiological and pharmacokinetic conditions relating to the patients using the drug or inadequate advice on the part of the pharmacist to the patient on the appropriate use of the antibiotics.

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