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ULTRAVIOLET SPECTROPHOTOMETRIC EVALUATION OF DIFFERENT BRANDS OF AZITHROMYCIN DIHYDRATE TABLETS AND CAPSULES SOLD IN NIGERIA.

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ABSTRACT

The aim of this study was to carry out a spectrophotometric and other pharmaceutical quality evaluation of different brands of azithromycin dihydrate tablets and capsules from Nigerian markets and to predict their bioequivalence using *in vitro* dissolution tests. The evaluation was done by carrying out tests such as weight uniformity, friability, active drug content, disintegration and dissolution tests using standard procedures. The similarity factor was determined for the brands. All the brands complied with the weight uniformity test, friability (brands AZ1-AZ9) and active drug content test. All brands (except AZ8) complied with the official specification for disintegration time test with no disintegration at T_{30} and dissolution test with T_{30} less than 85%. With dissolution profile of 85% within 15 minutes for samples AZ1. AZ8 displayed very low dissolution rate, which would likely result in poor bioavailability. From the results of the bioequivalence determination, all the tested brands (except AZ-8) could be said to be bioequivalent with the innovator brand (AZ9) and thus interchangeable. The similarity factor (F_2) value for sample AZ8 was determined to be 10.7, suggesting that (AZ8) was not bioequivalent with the innovator product as its F_2 value fell outside the acceptable standard. In conclusion, the results of the study showed that there is the need for constant monitoring of different brands of azithromycin introduced into the drug market to ascertain bioequivalence and conformity with pharmacopoeia standards.

KEYWORDS: Azithromycin dihydrate, Tablets, Capsules, Comparative study, Bioequivalence.

INTRODUCTION

Azithromycin, chemically known as (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)- 11-[(2S,3R,4S,6R)-4- (dimethylamino)-3-hydroxy-6-methyloxan-2-yl] oxy-2-ethyl-3,4,10-trihydroxy-13-[(2R,4R,5S,6S)-5- hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,6,8,10,12,14-heptamethyl-1-oxa-6-azacyclopentadecan-15-one, is a 15-atom lactone macrolide ring compound. It is a semi-synthetic derivative of erythromycin obtained by the addition of methylated nitrogen into the lactone ring of erythromycin. Thus, it is a macrolide antibiotic which inhibits 50S ribosomal subunit formation and elongation at transpeptidation step in gram-positive and gram-negative organisms.[1] Azithromycin being a newer agent of the macrolide class of antibiotics suggests that it possesses better tissue penetration, gastrointestinal tolerability and improved pharmacokinetic properties than erythromycin and its widespread popularity arises primarily from such pharmacokinetic properties which allow many infections to be treated with 3-5 days of once daily administration, compared to 3-4 times a day for up two weeks for erythromycin.[2,3]

The driving interest in carrying out quality evaluation on the drug arises partly from the facts that azithromycin is the first line treatment for cholera in children and pregnant women[4] and can also be used for treatment of uncomplicated typhoid fever[5], shigellosis, STIs due to *Chlamydia trachomatis* (or *Neisseria gonorrhea*), *Hemophilus ducreyi*, prophylaxis and treatment of disseminated *Mycobacterium avium* complex disease in HIV patients.[6]

The National Agency for Food, Drug Administration and Control (NAFDAC) which is the regulatory authority responsible for the regulation and control of drugs in Nigeria, has released standards for quality, efficacy and safety in line with WHO guidelines which were aimed at getting quality and safe drugs to the consumers. Also, drug products that are chemically equivalent must be identical in strength, quality and purity, while as pharmaceutical equivalents, they are assessed by being similar in terms of content uniformity, disintegration and dissolution rates.[7] There is therefore urgent need to regularly evaluate the quality of available brands of drug preparations in the healthcare delivery system so as not to jeopardize the expected therapeutic outcome.[8]

The extensive spectrum of activity of azithromycin and its unique pharmacokinetic properties when compared to earlier classes of macrolides have led to its increased use against susceptible organisms in Nigeria. Clinicians and pharmacists are faced with the challenge of interchangeability among numerous brands of the drug in the market. The objective of this study was to evaluate the chemical and pharmaceutical equivalency of the commercial brands of azithromycin dihydrate tablets and capsules available in some Nigerian drug markets at the time of this study and predict their interchangeability in clinical use.

MATERIALS AND METHODS

Sample Procurement and Assessment

a) Samples: The respective brands of azithromycin dihydrate tablets (encoded as AZ-1, AZ-2, AZ-3, AZ-4 & AZ-5) and azithromycin dihydrate capsules (encoded as AZ-6, AZ-7, AZ-8, & AZ-9) used for this study were procured from various pharmacy premises in some Nigerian cities located at the southern regions of the country in August, 2015. Information about the various brands such as brand name, producer's name, country of manufacture, manufacturing/expiry dates, batch/or lot number, label claim of potency of the drug and product registration status with the National Agency for Food and Drug Administration and Control (NAFDAC) were assessed. The samples were also physically examined for shape, color, packaging and overall dosage form conformity.

b) Reference Drug: Standard azithromycin dihydrate was procured from GPHF- Global Pharma Health Fund e.v.; Assembled by TTM e.v., Germany (Lot Number: L25168P).

METHODS

Preparation of simulated intestinal fluid (phosphate buffer), pH 7.2

This was prepared as follows: A 34 g quantity of potassium dihydrogen phosphate was dissolved in 500 ml of distilled water. The pH was adjusted to 7.2 using 0.1 N NaOH and the volume was made up to 1000 ml with distilled water.[9]

Preparation of simulated gastric fluid (SGF), pH 1.2 (without enzyme)

A 12.0 g quantity of sodium chloride was dissolved in about 5.3 L of distilled water and the pH adjusted to 1.2 using 0.1 N concentrated hydrochloric acid. The volume was made up to 6.0 L.[9]

Weight Variation

Twenty (20) tablets were selected randomly and weighed individually. The average weight was calculated and individual weight was compared to the average weight. The tablet batches pass the test if not more than two of the individual weights deviate from the average weight by more than ± 7.5% and none deviated by twice ± 7.5%.[10]

Crushing strength

Ten tablets were randomly selected from each brand of azithromycin dihydrate. The tablet crushing strength was determined using Monsanto tablet hardness tester (Monsanto, India).[10]

Friability test

The percentage friability of the tablets from each brand was determined using Erweka® friabilator. It should be less than 1%. Ten tablets taken from each brand were selected randomly and weighed, then placed in the friability test apparatus and rotated about 100 times. The tablets were then carefully dusted and reweighed to ascertain weight loss.[10]

Disintegration Test

The disintegration test was performed according to pharmacopoeial procedure. Six tablets from each formulation were weighed and placed in the baskets. The apparatus (Erweka® ZT122) was operated using SGF, pH 1.2 as immersion fluid at 37± 1°C for 2 h. The tablets were observed for any sign of disintegration, cracking or softening. The tablets were then removed and the immersion fluid replaced with SIF (phosphate buffer; pH 7.2). The apparatus was operated on same condition as SGF for 1h.[10]

Dissolution Test

Drug release studies were carried out using an Erweka® DT600 dissolution test apparatus set at 100 rpm for 1 h in simulated gastric fluid (pH 1.2) and after that, for 1h in intestinal fluid (phosphate buffer, pH 7.2) as dissolution medium at 37°C ± 1°C. After an interval of 10, 20, 30, 40, 50 and 60 min respectively, 10 ml of the samples were taken out and 10 ml of fresh phosphate buffer pH 7.2 added to keep the volume of dissolution medium constant. The sample was analyzed using UV spectrophotometer at 205 nm for simulated gastric fluid and 230 nm for simulated intestinal fluid and the percent drug release was calculated.[10]

Content of active ingredient

Ten tablets/capsules from each brand of azithromycin dihydrate were crushed to powder in a mortar (or poured out of the capsules). A 10-mg equivalent of azithromycin was weighed, transferred into a volumetric flask and dissolved in 100 ml of phosphate buffer. The solution was filtered through a Whatman® filter paper. A 2 ml volume of the filtrate was withdrawn and diluted to 10 ml. The absorbance of the resulting solution was measured at the 225 nm against a solvent blank using a Labtech® UV/Vis Spectrophotometer. The mean percentage drug content was determined for each brand.[10]

Bioequivalence Determination using Dissolution profile

Similarity Factor (f_2) was determined to compare the dissolution efficiency of the various brands. 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 two curves at each point.[11]

F_2 was determined using the equation:

$$f_2 = 50\log\left\{\left(1+\frac{1}{n}\sum_{i=1}^{n}(R_t - T_t)^2\right)^{-0.5} X100\right\}$$

or

$$f_2 = 50 \bullet \log\left[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{t=n}\left[\overline{R}(t)-\overline{T}(t)\right]^2}{n}}}\right]$$

Where
n = number of time points,
R_t =dissolution value of reference product at time t and
T_t =dissolution value for the test product at time t.

RESULTS AND DISCUSSION

The results of the product information and physical examination of the respective brands of azithromycin dihydrate samples used for this study are presented in Tables 1 & 2. The preliminary evaluation showed that the samples complied with basic physical assessment requirements by displaying the label claim, batch number, date of manufacture and expiration, manufacturer, country of manufacture and registration status with the National Agency for Food and Drug Administration and Control, NAFDAC in Nigeria. All the brands were within their shelf life as at the time of the study. The samples were subjected to both qualitative and quantitative evaluation methods to assess their pharmaceutical and chemical equivalence. Qualitative evaluation included describing the tablet color, size and shape which was carried out by visual inspection.

Table 1: Product information for various brands of azithromycin dihydrate samples.

Sample Brand	Date of manufacture	Expiry Date	NAFDAC Registration status	Label drug content (mg)
AZ-1	06/ 2014	05/2016	Registered	500
AZ-2	09/2014	08/ 2016	Registered	500
AZ-3	03/ 2015	02/ 2018	Registered	500
AZ-4	10/ 2013	09/ 2016	Registered	500
AZ-5	08/ 2013	07/ 2016	Registered	250
AZ-6	08/ 2013	07/ 2016	Registered	250
AZ-7	06/ 2015	05/ 2018	Registered	250
AZ-8	09/ 2014	08/ 2017	Registered	250
AZ-9	02/ 2015	02/ 2020	Registered	250

Table 2: Physical assessment of the various brands of azithromycin dihydrate samples.

Brand name	Color	Packaging	Dosage form
AZ-1	White	Aluminum foil blister	Tablet
AZ-2	White	Aluminum foil blister	Tablet
AZ-3	White	Aluminum foil blister	Tablet
AZ-4	Blue	Aluminum foil blister	Tablet
AZ-5	Pink	Aluminum foil blister	Tablet
AZ-6	Cream	Aluminum foil blister	Capsule
AZ-7	White & pink	Aluminum foil blister	Capsule
AZ-8	Cream	Aluminum foil blister	Capsule
AZ-9	Cream	Aluminum foil blister	Capsule

The results of the pharmaceutical tests for weight uniformity, friability, disintegration and percentage drug content are presented in Table 3. All the brands complied with the uniformity of weight determination by not deviating up to 5% of their mean value (Table 3). All the tablet brands passed the disintegration test of less than 30 minutes for coated tablets[10], but for the capsules, all (except AZ8) passed the disintegration test of less than 30 minutes for hard gelatin capsules (Table 3). The percentage friability was less than 1%.

Table 3: Results of some pharmaceutical tests of the samples.

Samples	Uniformity of weight (mg)	Disintegration test (mins)	% drug content (% w/w)
AZ1	744.45±0.70	1.28	109.7
AZ2	718.95±0.70	1.90	103.6
AZ3	813.60±0.87	2.04	109.7
AZ4	738.75±1.17	2.06	109.1
AZ5	357.50± 0.99	9.30	104.9
AZ6	383.10±3.42	6.09	99.4
AZ7	356.00±1.79	5.28	104.9
AZ8	498.60±3.34	57.00	100.0
AZ9	567.40±1.41	11.99	100.0

The release profile of the samples at different time intervals are presented in Table 4. All the tablet brands showed a dissolution profile that met the official specification of 85% w/v dissolution at 30 minutes[9], however, for the capsules, all, but AZ8, showed a dissolution profile of less than 85% and this may be related to its disintegration test value which was the longest, the nature of excipients used, the formulation process, the polymers used in capsule shell production or gelatin-plasticizers ratio used in capsule formulation.[12] It could also be due to the effect of heat and/ humidity in the storage of the product which may have caused cross-linking. Generally, the observed differences in drug release pattern of generic brands have been attributed to product formulation technology used by different manufacturers, which might also have to do with excipients used in the formulations.[12,14]

Table 4: Release rates of the various Samples.

Samples % release / Time(mins)	AZ1	AZ2	AZ3	AZ4	AZ5	AZ6	AZ7	AZ8	AZ9
10	97.5	85.9	84.7	85.9	85.9	89.0	94.51	29.3	85.9
20	98.1	89.0	97.5	89.0	89.0	99.4	97.56	35.4	99.4
30	99.4	100.0	99.4	100.0	99.4	104.0	98.78	43.3	105.0

The bioequivalence testing of different brands of azithromycin samples was to be determined via the calculation of their similarity factor (F2). As the name implies, similarity factor (F_2) stresses on the comparison of closeness of two comparative formulations. The F_2 parameter is commonly used to establish similarity of two dissolution profiles. Samples AZ1-AZ7, did not need any mathematical determination for bioequivalence and interchangeability as they had dissolution profiles of ≥ 85%.[15] Thus, all the tested brands (except AZ-8) could be said to be bioequivalent with the innovator brand. The F_2 value for sample AZ8 was determined to be 10.7. This suggested that the sample is not bioequivalent with the innovator product as its F_2 value fell outside the standard acceptable range (0 - 15 for F_1 and > 50 for F_2).

Similarity factor has been adopted by FDA[11], the European Agency for the Evaluation of Medicinal Products[16] and the Committee for Proprietary Medicinal Products (CPMP) to compare dissolution profiles. Two dissolution profiles are considered similar and bioequivalent, if F_1 value is between 0 and 15 while F_2 value is between 50 and 100.[11]

CONCLUSION

This study indicated that all brands of azithromycin dihydrate tested conformed to the USP standards for drug content. Based on the *in vitro* tests, AZ1, AZ2, AZ3, AZ4, AZ5, AZ6, AZ7, were considered bioequivalent and interchangeable with the innovator brand (AZ9). AZ8 has very low dissolution rate, which will likely result in poor bioavailability. The results showed that there is still the need for constant monitoring of various brands of azithromycin introduced into the drug market to ascertain bioequivalence and conformity with pharmacopoeia standards.

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