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An Ultraviolet-Visible (UV-vis) Spectrophotometric Determination of Pharmaceutical Equivalence of Some Enalapril Maleate Tablet Generics Marketed in Nigeria

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Abstract Anultraviolet-visible (UV-vis) spectrophotometric determination of enalapril maleate content and other biopharmaceutical parameters using five tablet generics of the drug marketed in Nigeria were conducted to access the quality and compliance of these commercial brands with pharmacopeia standard. The quality of the brands were evaluated using methods described in USP and BP which included, weight uniformity test, dissolution test, disintegration test, friability test and hardness test. Content of active ingredient was evaluated by means of the UV/Vis spectrophotometer at 240 nm. Beer Lambert's law was obeyed over the concentration of 10-60 μg/mL with a correlation coefficient (R^2) of 0.984. The different brands used complied with the standard specifications for the weight uniformity, disintegration, dissolution and content of active ingredient tests. Only three of the brands passed the friability and hardness tests. The *in vitro* bioequivalent results, however, showed that only two brands (ENP-2 and ENP-3) could be considered bioequivalent and interchangeable with the innovator brand (ENP-5), while ENP-1 and ENP-4 should be subjected to constant drug monitoring and pharmacovigilance when prescribed in place of the innovator brand.

Keywords Quality Assessment, Innovator Brand, Enalapril maleate, Tablets, Nigeria, UV

Introduction

Pharmaceutical analysis is an analytical method used to determine the quality and quantity of pharmaceutical products. It also gives the information about the purity and safety of the products. Briefly described, it identifies, determines, quantifies purifies and separates the active compound or other components from mixtures [1]. In practice, separation, identification or quantification, may constitute the entire analysis or be combined with another method. Pharmaceutical analysis has been routinely deployed in drug quality assurance and validation techniques of pharmaceutical products.

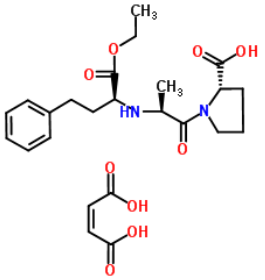

Figure 1: Structure of enalapril maleate (Source: ChemSpider®)

Enalapril is a dicarbocyl-containing peptide which functions as an angiotensin-converting enzyme (ACE) inhibitor with antihypertensive activity [2]. It is a prodrug that is converted by de-esterification into its active form enalaprilat. The sulfhydryl group of enalapril is replaced by carboxylate group to produce enalaprilat in the liver by enzymes called esterases [3]. Enalapril Maleate (Fig. 1) is the maleate salt form of enalapril which could be formulated into tablet and other dosage forms.

Enalapril maleate is chemically known as N-(1-Ethoxy-1-oxo-4-phenyl-2-butanyl)-L-alanyl-L-proline (2Z)-2-butenedioate (1:1),with the chemical formula $C_{24}H_{32}N_2O_9$.Enalapril, after biotransformation into its active form (enalaprilat), competitively binds to and inhibits ACE, thereby blocking the conversion of angiotensin I to angiotensin II. This blockade prevents the potent vasoconstrictive actions of angiotensin II and results in vasodilation [4]. Enalapril also decreases angiotensin II-induced aldosterone secretion by the adrenal cortex, which leads to an increase in sodium excretion and subsequently increases water outflow [5].

Several research works have been carried out on quality control of enalapril maleate tablets in some countries across the globe. In one of such studies, the application of visible spectrophotometer in determination of enalapril maleate content in tablets and estimation of Ester group stability was conducted in Poland [6]. The result obtained showed that the ester group of enalapril maleate was stable in a period of time studied (50 days) at 700c in the solid state. This implies that storage conditions and other in-house manufacturing processes should determine to a large extent the quality, efficacy and interchangeability of various generic products of enalapril maleate tablets in circulation. Thus, the need for routine assessment of these dosage forms in circulation cannot be overemphasized, especially for highly patronized and life-saving drug products that are marketed under various generic brands in many developing countries that are deficient in requisite infrastructure and logistics for standard drug distribution, storage and dispensing [7, 8]. The aim of the present investigation was to evaluate some physico-chemical properties (crushing strength, weight variation, friability, drug content, disintegration and dissolution profiles) of commonly available formulations of enalapril maleate tablets marketed in southern Nigerian cities, with a view to determining the level of compliance of these brands with pharmacopoeial standards.

Materials and Methods.

Sample Procurement and Assessment

a) **Samples:** The respective brands of enalapril maleate tablets (encoded as ENP-1, ENP-2, ENP-3, ENP-4 & ENP-5) used for this study were procured from various pharmacy premises in some Nigerian cities located at the south-south region of the country around June/July 2017. 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 tablets were also physically examined for shape, color, packaging and overall dosage form conformity with regulations.

b) **Reference Drug:** Standard enalapril maleate was procured from AfrabChem Lab Lagos, Nigeria.

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 enalapril maleate. 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 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. The specification for the disintegration of uncoated tablet in phosphate buffer (pH 7.2) is within 30 min according to British Pharmacopoeia [10].

Dissolution Test

Drug release studies were carried out using an Erweka® 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 240 nm and the percent drug release was calculated [10].

Content of active ingredient

Ten tablets from each brand of enalapril maleate were crushed to powder in a mortar. A 10-mg equivalent of enalapril 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 240 nm against a solvent blank using a Jenway® UV/Vis Spectrophotometer (Model 6405). The mean percentage drug content was determined for each brand [10].

Bioequivalence Determination using Dissolution profile

Similarity Factor (F_2) was calculated 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 the two curves.

The following equation was used to calculate F_2.

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

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.

Similarity factor has been adopted by [11], the European Agency for the Evaluation of Medicinal Products [12] and the Committee for Proprietary Medicinal Products (CPMP) to compare dissolution profiles. Two dissolution profiles are considered similar and bioequivalent, if F_1value is between 0 and 15 while F_2 value is between 50 and 100 [11].

Results and Discussion

The results of the physical examination of the respective brands of enalapril tablets used for this study are presented in Table 1, showing label claim, date of manufacture/expiration and registration status with the National Agency for Food, Drug Administration and Control, NAFDAC in Nigeria. All the brands of enalapril tablets studied had all the required information and were registered with NAFDAC. The Nigerian drug statutes stipulate that any drug product in the country that is not registered with NAFDAC is fake. The actual brand name, batch number, manufacturer and country of manufacture of the samples were not presented in the Table due to ethical considerations.

Table 1: Product information for various brands of enalapril maleate tablets studied

Tablet Brand	Date of manufacture	Expiry Date	NAFDAC Registration status	Label drug content (mg)
ENP-1	06/2014	05/2018	Registered	10.00
ENP-2	05/2015	04/2018	Registered	10.00
ENP-3	11/2015	10/2018	Registered	10.00
ENP-4	11/2016	10/2019	Registered	10.00
ENP-5	06/2016	05/2019	Registered	10.00

Table 2: Physical assessment of the various brands of enalapril maleate tablets studied

Brand name	Color	Packaging	Dosage form
ENP-1	White	Aluminum foil blister	Film coated tablet
ENP-2	White	Aluminum foil blister	Film coated tablet
ENP-3	Brown	Aluminum foil blister	Film coated tablet
ENP-4	White	Aluminum foil blister	Film coated tablet
ENP-5	Brown	Aluminum foil blister	Film coated tablet

The USP [9] specified that the amount of active ingredient for enalapril maleate tablets should fall within 90-110%. Table 3 showed the results of actual and total percentage drug content for the respective brands of enalapril maleate tablet studied as against the label claim of 10 mg. All the brands met the specification for total drug content.

Table 3: Results of drug content for the respective brands of enalapril maleate tablets

Brand name	Label content (mg)	Actual content (mg)	Actual content (%)
ENP-1	10.00	9.40	94.02
ENP-2	10.00	9.71	97.10
ENP-3	10.00	9.75	97.50
ENP-4	10.00	10.62	106.20
ENP-5	10.00	10.84	108.40

Table 4 showed the data on the tablet uniformity of weight. The United States Pharmacopoeia [9] specified that for tablets or capsules that weigh between 130-324 mg, standard deviation of weight should not exceed 7.5% and for tablet or capsules that weigh more than 325mg, standard deviation of weight should not exceed 5%. The various brands thus passed the test for uniformity of weighthaving percentage deviation within the ranges of 1.3-2.56%.The pharmacopeia compliance with regard to uniformity of weight of each brand studied is important since the uniformity of dosage unit can be demonstrated by either weight variation or content uniformity study [13]. These either reflect indirectly or measure directly the amount of drug substance in the tablet [14].

Table 4: Results of uniformity of tablet weight for the respective brands of enalapril maleate

Brand name	Mean weight (mg)	Coefficient of variation (%)	Remarks
ENP-1	125.80±1.79	1.42	Passed
ENP-2	176.85±2.30	1.30	Passed
ENP-3	200.65±3.05	1.52	Passed
ENP-4	135.70±3.47	1.50	Passed
ENP-5	174.65±2.62	1.50	Passed

Table 5 shows the results of crushing strength or hardness, friability and disintegration time tests for the respective brands of enalapril tablets. Hardness or crushing strength test assesses the ability of tablets to withstand handling without fracturing or chipping. It can also influence the disintegration and friability of tablet dosage forms. The USP recommends a crushing strength of 4-8 kgf for uncoated tablets [9]. However, the brands of product under consideration are all film-coated and might give slightly higher crushing strength. The crushing strength recorded in the study, however, ranged from 1.62±0.5–4.92kgf, with ENP-1 showing the least value. These values complied with official specifications, hence the tablets were expected to withstand abrasion or fragmentation when subjected to mechanical shock. Friability test is used to evaluate the tablet resistance to abrasion. The USP [9] states that the percentage friability permitted is less than 1%. From the results obtained, three of the brands (ENP-2, EN-3 & ENP-5) gave a percentage friability of less than 1% while the other two (ENP-1 & ENP-4) gave percentage friability of 1.33% and 1.47% respectively.

Table 5: Tablet Crushing strength, Friability and Disintegration time for the respective brands of enalapril maleate studied.

Brand name	Hardness/Crushing strength (kgf)	Friability (%)	Disintegration time (min.)
ENP-1	1.62	1.330	4.521
ENP-2	4.35	0.157	2.150
ENP-3	4.73	0.215	2.090
ENP-4	1.80	1.470	8.650
ENP-5	4.92	0.164	3.170

For disintegration test, the USP [9] states that film coated tablets are meant to disintegrate within 30 minutes and other coated tablets (sugar coated) in 60 minutes. The enalapril brands used in this study all passed the disintegration test with an average disintegration time ranging from 2.09-8.65 minutes. The result showed that some of the brands had higher disintegration time than the others. Disintegration is a necessary step in determining the pharmacokinetic properties of the drug and mainly applies to tablet dosage forms. This process will lead to the dissolution of the drug which releases the active ingredient and then the pharmacokinetic processes of absorption, distribution, metabolism and excretion take place.

Table 6: Dissolution profile of enalapril tablet brands

Time	% drug release				
	ENP-1	ENP-2	ENP-3	ENP-4	ENP-5
0	0	0	0	0	0
5	60.33	69.00	52.11	79.15	79.60
10	68.13	75.03	59.72	83.08	80.31
15	76.26	80.16	63.79	87.30	85.04
20	84.00	86.37	72.63	90.51	92.05
25	92.47	92.10	86.12	95.00	96.62
30	96.18	97.42	98.41	98.46	99.50

Table 6 represented the dissolution profile for the various brands of enalaprilin phosphate buffer. Dissolution is the major factor to be considered in solid dosage forms because without a good dissolution profile, the pharmacokinetic properties and the bioavailability of the drug would be affected. The USP states that not less than 80% of enalapril should dissolve in 30minutes [9]. From the result obtained, all the brands of enalapril released 100% of their active ingredient within 30 minutes, with each brand giving percentage release of more than 80% in 30 minutes. 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 [15-17].

Table 7 was the results of bioequivalence testing of different brands of enalapril maleate tablets via the determination of their similarity/difference factor. 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. It focuses on the difference or comparison in percentage dissolved between reference product and test product at various time intervals [18].

Table 7: Results of bioequivalence test for the various brands of enalapril maleate tablets.

Product	F_2
ENP-1	39.00
ENP-2	77.70
ENP-3	54.60
ENP-4	31.00
ENP-5	Reference product

Hence, F_2 factor was used to show the similarity between a particular brand and the innovator brand. Two dissolution profiles are considered similar and bioequivalent, if F2 is between 50 and 100 [11]. From their respective F_2values, only two of the tested brands of enalapril (ENP-2 & 3) could be said to be bioequivalent with the innovator brand (ENP-5) as their values were within the standard acceptable range (F_2> 50) and thus can be interchanged with the innovator brand.

Conclusion

The results of this study indicated that the different brands of enalapril maleate used complied with the standard specifications for the weight uniformity, disintegration, dissolution and content of active ingredient tests, but, only three of the brands passed the friability and hardness tests. The *in vitro* bioequivalent results, however, showed that only two brands (ENP-2 and ENP-3) could be considered bioequivalent and interchangeable with the innovator brand (ENP-5), while ENP-1 and ENP-4 should be subjected to constant drug monitoring and pharmacovigilance when prescribed in place of the innovator brand.

Conflict of Interests

The authors declare no conflict of interest in the course of this study.

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