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COMPARATIVE STUDY OF THE QUALITY OF SOME BRANDS OF DIAZEPAM TABLETS AVAILABLE IN NIGERIA

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ABSTRACT

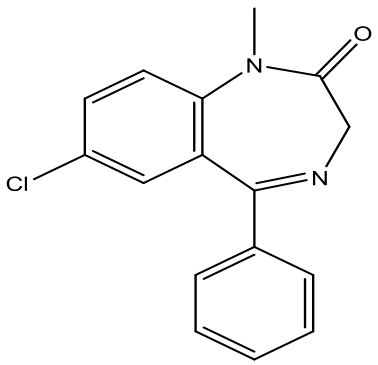
Quality assurance and control of pharmaceutical products is very important for achieving a therapeutically active and standard product. This can be achieved by monitoring some parameters that are specified in the monograph of the drug or product .This study was aimed at evaluating the pharmaceutical profile of different brands of diazepam available in some Nigerian markets. These brands of diazepam were subjected to official tests as specified in USP and BP. The tests were weight uniformity, disintegration, friability, dissolution and crushing strength/hardness test. The content of active ingredient was determined using the U.V visible spectrophotometer at a maximum wavelength of 302 nm. Beer's law was obeyed at a concentration range of 0.2-1.2 µg/ml. The result showed that all the brands of diazepam analyzed passed disintegration, friability and weight uniformity test except D1.Only two brands of diazepam D2 & D3 passed content of uniformity test. Also, all brands of diazepam analyzed passed dissolution test. Fit factor (*F2*) was calculated according to FDA which was used to determine bioequivalence of the brands with the innovator brand. Although all the brands analyzed had *F2* values greater than 50, and hence could be used interchangeably with the innovator brand, the non-conformity of the diazepam generics to some of the quality control test standards called for caution in their substitution clinically.

KEYWORD: Comparative quality study, Diazepam, Nigerian markets, innovator brands, bioequivalence.

INTRODUCTION

To guarantee product stability and safety, pharmaceutical companies strive under Good Manufacturing practice (GMP) to ensure that quality is built into the products. Quality of pharmaceutical product is very important for achieving a therapeutically active and standard drug. Dosage forms, such as tablets, with same drug content do not always give same therapeutic response as there might be differences of formulation additives in the tablet, physical form of the drug and varying of manufacturing process which are responsible for variation in the observed dissolution profile and therapeutic effect in different manufacturing company and process.[1] Quality control can be achieved by following some parameters that are specified in the respective monograph of the drug. United States pharmacopoeia (USP) and British pharmacopoeia (BP) are such two pharmacopoeias that provide the necessary specifications for drug quality generally as it relates to performance, features, reliability, conformance, durability, and aesthetics.[2]

Diazepam is a drug belonging to the benzodiazepine family which acts mainly as an anxiolytic. The benzodiazepine family of depressants is used therapeutically to produce sedation, to induce sleep, to relieve anxiety, muscle spasms and to prevent seizures.[3] In general, benzodiazepines act as hypnotics in high doses, anxiolytics in moderate doses, and sedatives in low doses.[4] They all act by enhancing the actions of a natural brain chemical, GABA (gamma-aminobutyric acid). Chemically, diazepam is known as 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4 benzodiazepin-2-one with the chemical formula, $C_{16}H_{13}ClN_2O$, and molecular formula 284.7445.[5] The chemical structure of diazepam is given in Fig. 1.



7-chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-benzo[*e*][1,4]diazepin-2-one
Chemical Formula: $C_{16}H_{13}ClN_2O$
Molecular Weight: 284.74

Fig. 1: Chemical structure of diazepam.

As a controlled substance, there is an urgent need to strictly regulate the quality of diazepam tablets in circulation to ensure that they comply with standard specifications in order to prevent drug abuse or addiction, and promote predictability of therapeutic outcome in patients. This study was, thus, aimed at evaluating the degree of compliance to quality and safety standards by some brands of diazepam tablets sold in Nigeria, and to equally determine the basis for the utilization of multisource generics of the drug in patients.

MATERIALS AND METHODS

2.1 Sample collection and Assessment

Samples: The respective brands of diazepam tablets (encoded as D1, D2, and D3) used for this study were procured from various pharmacy premises in Port Harcourt and Onitsha, all in the southern states of Nigeria. Information about the various brands such as brand name, manufacturer's address, country of manufacture, manufacturing / expiry dates, batch /lot number, label claim of potency, and registration with the National Agency for Food, Drug Administration and Control (NAFDAC) were assessed .The tablets were also physically examined for shape, color, packaging and overall dosage form conformity.

Reference Drug: Pure sample of diazepam was procured from the company of manufacture, WKG Germany.

METHODS

2.2.1 Preparation of 0.1N HCl

A 10 ml volume of concentrated hydrochloric acid was added to little quantity of distilled water in a 1000 ml volumetric flask then distilled was added to make up to 1000 ml.

2.2.2 Preparation of standard stock solution

A 100 mg of pure sample of diazepam powder was dissolved in 100 mL of methanol in a 100 mL volumetric flask (1000 µg/mL). Serial dilution from the stock solution was made using methanol to obtain concentrations at 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 µg/mL of solution.

2.2.3 Determination of maximum wavelength (λ_{max}) of absorption

An aliquot from the stock solutions was scanned in the UV-Visible Spectrophotometer at different wavelengths and the maximum wavelengths of absorption obtained were 302 nm for Diazepam.

2.2.4 Determination of standard calibration curve

The serial dilutions (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 µg/mL of solution.) obtained from diazepam stock solution were passed through the UV-Visible spectrophotometer and their absorbance was read at 302 nm. A plot of concentration against absorbance was made and the coefficient of determination (r^2) was calculated.

2.2.5 Uniformity of Weight

Twenty tablets from each brand of diazepam was selected and weighed with Acculab analytical balance (ALC210.4, Germany) individually. The weights were recorded in triplicates and the mean, standard deviation, and percentage standard deviation calculated and recorded.[6]

2.2.6 Hardness/Crushing strength test

Ten tablets were randomly selected from each brand of Diazepam. One tablet was placed between the jaws in the hardness tester and adjusted by pushing forward the movable jaw inside, turning the plunger clockwise. The value on the scale that coincides with the pointer was noted and pressure was applied till the tablet breaks. The value on the scale was recorded. This same procedure was repeated for all tablets.[6]

2.2.7 Friability test

Ten tablets of Diazepam were selected at random. Each batch of ten tablets was weighed and their respective weights recorded. The tablets are placed in the friabilator and rotated for 4 min at 25 revolutions per minute (rpm). The tablets were removed, dusted and reweighed.[6]

2.2.8 Disintegration test

The disintegration test for the different brands of diazepam was carried out according to the method described in the BP.[6] A 700ml of distilled water was placed into the beaker in the disintegration apparatus (Erweka disintegration machine, Germany). The temperature of immersion fluid was maintained at 37 ^{o}C. Six tablets were randomly selected from each brand of diazepam. One tablet was placed in each of the six tubes and the tubes were immersed into the fluid. The disintegration time was recorded and average time and percentage deviation were calculated. This was repeated for all six tablets.

2.2.9 Dissolution test

The dissolution test for the different brands of diazepam tablets were carried out according to United States Pharmacopoeia using Erweka dissolution apparatus Germany (paddle type).[7] The 0.1N HCl (900 ml) was placed in each of the vessels of the dissolution apparatus and the medium was maintained at 37^{o}C. The paddles were rotated at a rotational speed of 50 rpm. A tablet from each brand was placed in the vessel containing 0.1 N HCl and the dissolution apparatus was operated for 30 min. A 5 ml of dissolution medium was withdrawn using a pipette for each brand at 5, 10, 15, 20, 25 and 30 min and replaced immediately with 5 ml of 0.1N HCl after each withdrawal. The withdrawn samples were filtered and assayed using UV-Visible spectrophotometer at 302 nm to determine the release of diazepam from the tablets.

2.2.10 Assay of active ingredient

Ten tablets from each brand of diazepam were crushed to powder in a mortar. A 5 mg equivalent of diazepam was weighed, transferred into a volumetric flask and dissolved in 100 ml of 0.1 N HCl. The solution was filtered through a Whatman® filter paper. A 2 ml volume of filtrate was withdrawn and diluted to 10 ml. The absorbance of the resulting solution was measured at 302 nm against a solvent blank. The mean percentage drug content was determined for each brand.[7]

2.2.11 Bioequivalence Determination using Dissolution profile

Similarity factor (f2) was calculated to compare the dissolution efficiency of the various brands. *F2* is a logarithmic reciprocal square root transformation of the sum of square error and is a measurement of the similarity in the percent % dissolution between the two curves.[8]

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

Where:

n = number of time points,

R_t =dissolution value of reference product at time t

T_t =dissolution value of the test product at time t.

3.0 RESULTS AND DISCUSSIONS

Diazepam is a highly regulated drug product which is used therapeutically to produce sedation, induce sleep, and relieve anxiety, muscle spasms and to prevent seizures. It can act as hypnotics in high doses, anxiolytic in moderate doses, and sedative in low doses.[9] In other to eradicate fake, counterfeit and substandard drugs in the Nigerian health system, and to ensure standard product quality, it is very necessary to conduct regular analysis in order to ascertain the quality of multisource drug product in clinical use.[10]

The result of physical assessment of different brands of commercially available diazepam tablets used in this study showed that they were all registered by the National Agency for Food, Drug, Administration, and Control (NAFDAC) with batch numbers, manufacturing dates as well as expiry dates clearly indicated (Table 1). All the tests under this study were carried out before the expiry dates of the samples. The tablets were filmed coated and evenly yellow-colored (Table 2). The overall appearance of tablets determines consumer acceptability and can affect the level of compliance to the dosage regimen by the patient.[11] Thus, the diazepam tablet samples complied with official standards for organoleptic presentations and packaging of pharmaceutical dosage forms.

Table 1: Result of the labeling and Inspection Test.

S/N	Sample	Label Claim	Batch No	Manufacturing date	Expiry date	Country of origin
1	Reference	Pure diazepam powder (2g)	5/12-14-169	06/2014	10/2018	Germany
2	D1	Diazepam 5mg tablets	0286	09/2016	08/2019	Nigeria
3	D2	Diazepam tablets, 5mg	N108501	11/2015	10/2018	Nigeria
4	D3	Diazepam Tablets, 5mg	1511194	11/2016	10/2019	UK.

Table 2: Results for general appearance for the tested brands of Diazepam.

Product code	Colour	Coating type	Dosage form
D1	Yellow	Film coated	Tablet
D2	Yellow	Film coated	Tablet
D3	Yellow	Film coated	Tablet

Weight variation test was carried out to ensure that each of the tablets contained the proper amount of drug. From Table 3 results, the sample with the least mean weight (155.5 mg) was brand D3 while brand D2 had the highest mean weight (232.0 mg). The weight variation of tablets within the compendia limit is a primary indication of content uniformity of the tablet batches. If the variation is out of the compendia limit, it is quite impossible to maintain the content uniformity. From the outcome of the weight variation analysis, it was observed that

all the brands of diazepam (except sample D3) were within the compendia limit of less than 7.5% variation.[7]

Table 3: Weight uniformity test result for diazepam.

Product code	Mean weight (mg)	Coefficient of variation %	Remarks (less than or equal to 7.5%)
D1	211.50±14.60	6.9	Passed
D2	232±10.56	4.55	Passed
D3	155.50±13.56	8.92	Failed

Tablets require a certain amount of strength to withstand mechanical shocks of handling in manufacturing, packaging and shipping. The hardness tests evaluated (Table 4) showed that samples D1 and D3 failed to comply with specifications, while sample D2 fell within the stated limit of 4-8 kgf. Hardness is the load required to crush the tablet when placed on its edge. This showed that most of the tablets in the samples tested might not be able to withstand rigorous treatment in the course of packaging and transportation. Factors affecting the hardness of tablets include compressive force applied during the compression process, the amount of binder and method of granulation.[7]

Table 4: Hardness test of different brands of diazepam.

S/N	Brands	Average kgf	Remark
1	D1	1.45	Failed
2	D2	4.04	Passed
3	D3	2.92	Failed

Friability test was performed to evaluate the ability of the tablets to withstand abrasion in packing, handling and transporting (Table 5). The USP states that the percentage friability permitted is less than 1%, and from the result carried out, all brands passed the friability test.[7] This implied that the samples possess enough robustness to withstand packaging and handling pressures that would make the tablets to maintain the desired weight and content uniformity.[12]

Table 5: Friability test of different brands diazepam.

Brands	% friability	Remark (< 1%)
D1	0.29	Passed
D2	0.11	Passed
D3	0.25	Passed

The rate of absorption of drug as well as the therapeutic efficacy is dependent upon the disintegration time. According to the USP, capsules and uncoated tablets are expected to

disintegrate within 15 minutes.[7] To satisfy the disintegration test, the tablets should disintegrate completely or break up into granules. From the results (Table 6), all the samples passed this test with the shortest disintegration time recorded for sample D1 (0.27 min), while sample D2 had the highest disintegration time of 8.02 minutes. Since disintegration is an important determinant of the dissolution process, the result showed that all the samples are expected to release their active ingredients within specified time limits, having given acceptable disintegration time values. Several factors that affect disintegration process in tablets include effect of fillers, binder lubricants and surfactant. [13]

Table 6: Disintegration time of different brands of Diazepam tablet.

S/N	*DRUG SAMPLE*	*AVERAGE DISINTEGRATION TIME (min)*	*REMARK*
1	D1	0.27	Passed
2	D2	8.02	Passed
3	D3	1.0	Passed

The calibration curve (Fig. 2) for the determination of quantity of diazepam in various drug dilutions used in the study followed Beer's Lambert plot at concentrations between 0.2 to 1.2 µg/ml of the pure diazepam reference sample.

Dissolution is an important factor to be considered in solid dosage formulation as poor dissolution would affect the bioavailability of the drug. Thus, the effectiveness of solid dosage forms relies on its ability to dissolve in the fluids of the gastrointestinal tract prior to absorption into systemic circulation For diazepam, the USP standard for dissolution test profile specified that not less than 85% of the drug should dissolved in 30 min, and from the research all the 3 brands of diazepam analyzed were able to release more than this amount in the stipulated time (Fig. 3). The result implied that the samples could achieve the desired therapeutic goal when administered, including timely onset of action and attainment of adequate peak plasma concentrations.[14]

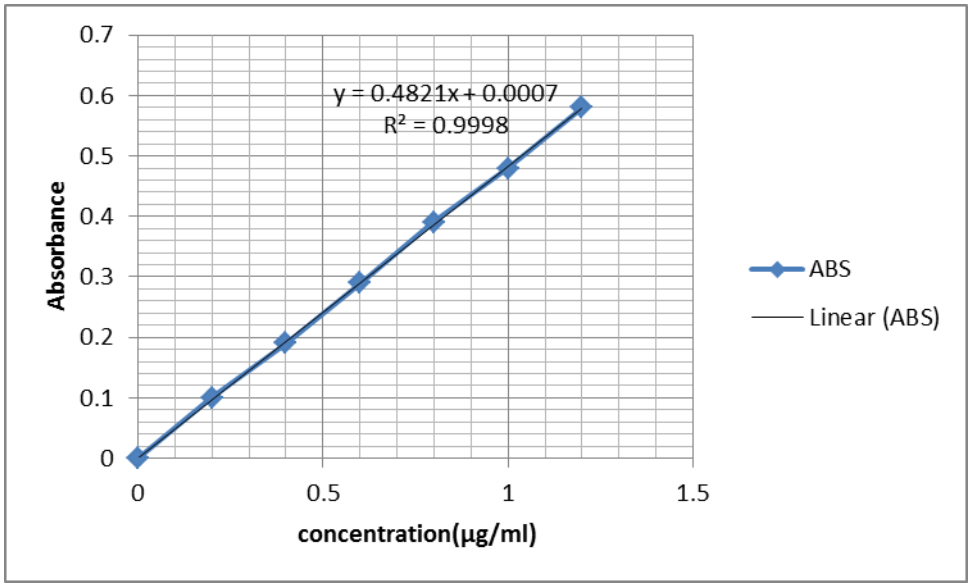


Fig 2: Standard calibration curve for diazepam.

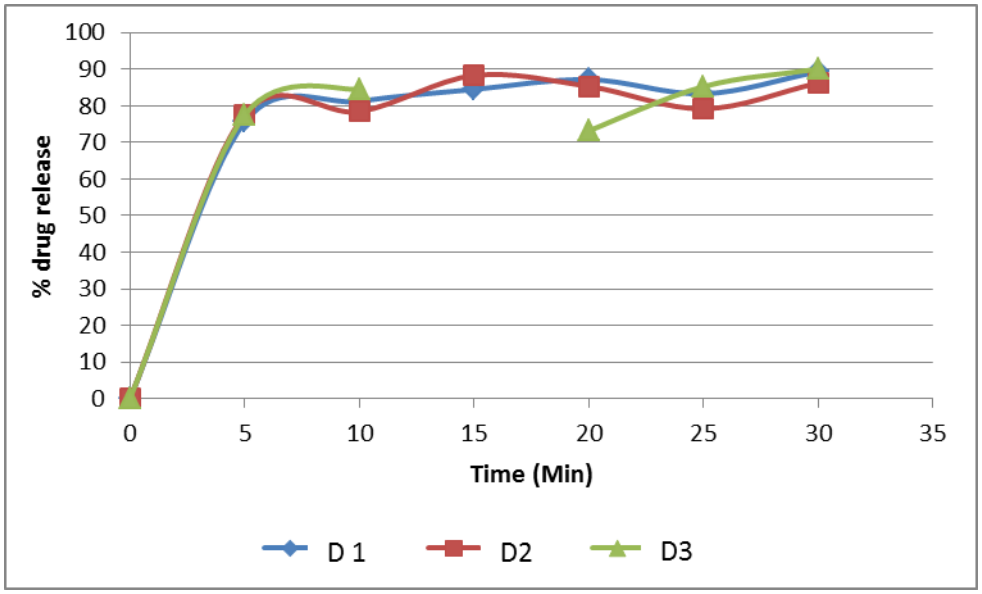


Fig 3: Percentage drug release versus time for the 3 brands of diazepam.

The label claim for diazepam in the samples was 5 mg per tablet. Sample D1 had the least percentage drug content (92.0%) while the other two samples (D2 and D3) gave similar percentage drug content of 96% each. Content uniformity test is a very important assessment for oral solid dosage forms as it ensures the consistency of dosage units, such that each unit in a batch should have an active drug content within a narrow range around the label claim. Hence, for the content of active ingredient, the USP specification for diazepam oral dosage forms is within the range of 95-105%. [7] Thus, only two brands (D2 and D3) passed this test (Table 7).

Table 7: Result of content uniformity test for different brands of diazepam.

Code	(Label claim) (mg)	Drug content (mg)	% content	Remarks (B.P. standard for Diazepam is 95-105%)
D1	5	4.6	92	Failed
D2	5	4.8	96	Passed
D3	5	4.8	96	Passed

Fit or similarity factor (*F2*) was calculated according to FDA to determine bioequivalence of the brands as compared with the innovator brand.[8] It was carried out to determine the bio-pharmaceutical equivalence between the reference brand (D1), and the other samples of diazepam using their drug release profiles. The standard specification by the FDA for *F2* values ranges from 50 to 100, however, where the percentage release profiles of the samples showed values equal or greater than 85%, the FDA specified that the calculation of F_2 factor might not be necessary for the samples. [8] Thus, the *F2* results (Table 8), and that of the dissolution profiles (Fig. 3) suggested that the generic samples of diazepam could be bioequivalent with the innovator D1 and are interchangeable.[15]

Table 8: Fit Factor (f2) for diazepam samples.

Pair comparison	F_2	Remark
D2 VS D1	87.12	Passed
D3 VS D1	88.20	Passed

CONCLUSION

The current study showed that all the brands of diazepam except (D2) passed the weight variation, friability, disintegration test, and dissolution test, while only two brands (D2 and D3) passed content uniformity and hardness test. Although all the brands analyzed had *F2* values greater than 50, and hence could be used interchangeably with the innovator brand, the non-conformity of the diazepam generics to some of the quality control test standards called for caution in their substitution clinically.

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