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QUALITY ASSESSMENT AND IN VITRO BIOEQUIVALENCE STUDIES ON SOME GENERIC BRANDS OF FLUCONAZOLE CAPSULES COMMONLY DISPENSED IN NIGERIAN PHARMACIES

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ABSTRACT

Fluconazole is an antifungal agent commonly used for several fungal infections bedeviling many developing countries across Africa. Quality assurance of multi-source pharmaceutical products ensures that the manufacturing processes of the products comply with required specifications thereby assuring product efficacy when used interchangeably. This study was aimed at investigating the in-vitro dissolution profiles and other physicochemical parameters of different generics of fluconazole capsules that are available in some Nigerian markets to determine their biopharmaceutical equivalence and interchangeability. The quality and physicochemical equivalence of five different brands of fluconazole capsules (coded F1-F5) were subjected to official tests as specified in the USP (2009) and BP (2012), including physical examination, weight variation, disintegration, drug content and dissolution profiles. The content of active ingredient was determined using the UV-visible spectrophotometer at a maximum wavelength of 262 nm. Similarity factor was calculated to determine the bioequivalence of the brands against the innovator brand F1. All the brands passed the official standards for uniformity of weight (< 5.0 mg), disintegration (< 10 min), and dissolution (> 95% at 30 min) tests, while only F5 failed the test for content of active ingredient (< 90%). The Fit factor analysis showed that only brands F3 and F4 had a similarity factor greater than 50. In conclusion, some of the brands of fluconazole capsules analyzed were found not to be bio-pharmaceutically equivalent and interchangeable with the innovator brand, thereby underscoring the need for constant quality assurance of drug products in circulation.

KEYWORDS: Fluconazole, interchangeability, similarity factor, disintegration time, innovator brand.

INTRODUCTION

Antifungals, also known as antimycotics, are pharmaceutical fungicides or fungistatic agents used to treat and prevent mycosis such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as *cryptococcal meningitis*, and others.[1-3] Fluconazole is an antifungal medicine used for a number of fungal infections. As the first of a new subclass of synthetic triazole antifungal agents, fluconazole is available as tablets and capsules for oral administration. It is designated chemically as 2-(2, 4-difluorophenyl)-1, 3-bis (1H-1, 2, 4-triazol-1-yl) Propan-2-ol with a molecular weight of 306.3. The structural formula of fluconazole USP is shown in figure 1.

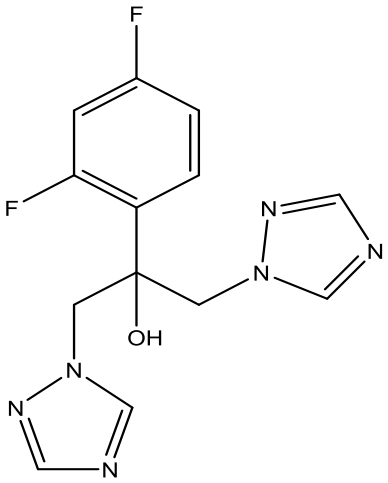

Fig 1: Fluconazole structure.

Fluconazole is a white crystalline solid which is slightly soluble in water, soluble in alcohol and acetone, readily soluble in methanol, and very slightly soluble in toluene.[3] It has a chemical formula of $C_{13}H_{12}F_2N_6O$. Fluconazole tablets/capsules USP contain 50 mg, 100 mg, 150 mg or 200 mg of fluconazole. The oral capsule is available as both generic brands, and as the innovator brand-name, Diflucan®.

Drug quality control is necessary and intended to ensure the efficacy, safety and quality of medicines and other pharmaceutical products.[4] Fluconazole, one of the highly patronized and readily affordable antifungal drug products, is marketed under various generic brands in many developing countries that are deficient in requisite infrastructure and logistics for standard drug distribution and storage. The need for routine quality assessment of its dosage

forms in circulation cannot be overemphasized. The aim of this study was to investigate the *in-vitro* dissolution profiles and other physicochemical parameters of different generics of fluconazole capsules that are available in some Nigerian markets, and also determine their biopharmaceutical equivalence and interchangeability with the innovator brand.

MATERIALS AND METHOD

The different brands of fluconazole capsules ((coded F1-F5)) were procured from retail pharmacy outlets in Enugu state, Rivers state and Lagos state, all in southern part of Nigeria. A pure sample of Fluconazole (15 g) was sourced from Emzor Pharmaceuticals Ltd, Lagos, Nigeria. This was used as the primary standard.

Reagents used

Potassium dihydrogen phosphate, sodium hydroxide pellet, distilled water and solvents such as methanol. All the reagents used were of analytical grade.

Physical assessment

The packaging and labeling for each of the brands were carefully checked for information such as manufacturer's address, manufacturing dates of the drugs, expiry dates, batch numbers, amount of labelled active ingredients and the National Agency for Food, Drug, Administration, and Control (NAFDAC) registration numbers. Also, the color and appearance of the capsules were noted as also described by the innovator brand, Diflucan®.[5]

Capsule weight uniformity test

Ten (10) intact capsules of fluconazole were randomly selected and one capsule each was weighed. The capsule was opened and the contents were removed as completely as possible. The emptied shells were weighed. The net weights of their contents were determined by subtraction. The determinations were done in triplicate. The procedure was repeated with the other 9 capsules of each brand.

Preparation of simulated intestinal fluid (phosphate buffer), pH 6.8

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 6.8 using 0.1 N NaOH and the volume was made up to 1000 ml with distilled water.[6]

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.[7]

Capsule disintegration test

Six capsules were randomly selected from each brand and placed in each of the cylindrical mesh of the disintegrator apparatus tubes. The disintegration media used was 700 ml of phosphate buffer of pH 6.8 maintained at 37 ± 1°C while the equipment was operated at 50 revolutions per minute. The time taken for each capsule to disintegrate was recorded and compared with the standard specified for capsules in the B.P.[8]

Preparation of calibration curve

Preparation of fluconazole pure sample solution for calibration curve: Fluconazole stock solution was freshly prepared by dissolving 100 mg pure sample in 10 ml of methanol solvent and diluted up to100 ml with phosphate buffer in 100 ml volumetric flask. This was the first stock solution and contains 1000 mg/ml of drug. From the first stock solution 10ml was taken to another 100 ml volumetric flask and diluted up to the mark with phosphate buffer and contains 100 mg/ml of drug concentration. From the second stock various other concentrations were prepared like 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1.0 mg/ml. An aliquot from fluconazole stock solution was scanned in the UV-Visible Spectrophotometer using distilled water as blank. The stock was scanned within the range of 200 nm to 270 nm wavelengths and the highest absorbance was obtained at a wavelength of 262 nm. Absorbance values of these concentrations were measured by UV double beam spectrophotometer at 262 nm against the reagent blank. The corresponding absorbance versus concentration values were used to generate the calibration curve for fluconazole.

Content of active ingredient

The contents of ten capsules for each brand of fluconazole were emptied and thoroughly mixed in a mortar. A 50-mg equivalent of fluconazole was weighed, transferred into a volumetric flask and dissolved in 100 ml of phosphate buffer with 30 min sonication. The solution was filtered through a Whatman® filter paper. Five standard solutions were prepared from this stock solution in different concentrations (1.0, 0.5, 0.25, 0.125, and 0.0625 mg/mL) by dilution with the same solvent. The absorbance of the resulting solutions was measured at 262 nm against a solvent blank using a Jenway® UV/Vis Spectrophotometer (Model 6405).

The process was repeated for each brand and the mean percentage drug content for each brand was calculated using Beer Lambert's plot as determined from the calibration curve.

Dissolution test

In-vitro dissolution studies were carried out using a dissolution apparatus USP (paddle type) at a paddle speed of 50 rpm. The dissolution medium was 900 ml of phosphate buffer, pH 6.8, which was maintained at 37 ± 1ºC. One capsule randomly selected from each of the brands was placed in the dissolution media and 5 ml sample withdrawn at the intervals of 5, 10, 20, 30, 40, 50 and 60 min for the fluconazole brands. 5ml of the fresh dissolution medium was used to replace each of the withdrawn samples immediately. The withdrawn samples were filtered and their absorbance was determined at maximum wavelength of 262 nm using a UV-Vis Spectrophotometer.

Amount of drug release = Concentration x volume of dissolution medium x dilution factor

$$\% \text{ content} = \frac{Actual\ Content}{Amount\ of\ API} \times 100$$

Bioequivalence determination using dissolution profile

Similarity Factor (f2) was calculated to compare the dissolution efficiency of the various brands. It is a measurement of similarity in the (%) of dissolution between two curves (usually that of generic and innovator brand) and is calculated as a logarithmic reciprocal square root of the sum of squared error.[9]

The following equation was used to calculate f2.

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

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.

RESULTS AND DISCUSSIONS

Table 1: Result of physical assessment of various fluconazole brands.

Brand	Label claim (mg)	Manufacturing date	Expiry date	Nafdac Reg. Status
F1 (Innovator)	50	09/2018	08/2023	Yes
F2	50	02/2018	09/2021	Yes
F3	50	07/2019	02/2022	Yes
F4	50	09/2017	08/2020	Yes
F5	150	06/2018	05/2021	Yes

The result of physical assessment of different brands of commercially available fluconazole 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.

Weight variation test was carried out to ensure that each of the capsules contained the proper amount of drug. The sample with the least mean weight (115.9 mg) was brand F2 while brand F5 had the highest mean weight (483.9 mg). None of the samples deviated more than 7.5% for capsules weighing more than 300 mg and 10% for capsules weighing less than 300mg, thus indicating that all the samples studied fell within the specified USP[6] standard (Table 2).

According to the USP, capsules and uncoated tablets are expected to disintegrate within 15 minutes. To satisfy the disintegration test, the capsules should disintegrate completely into a soft mass having no palpably firm core and only some fragments of the gelatin shell.[10] All the samples passed this test with the shortest disintegration time recorded for sample F2 (2.85 min), while sample F5 had the highest disintegration time of 6.77 minutes.

Table 2: Results of weight variation, disintegration, percentage content and fit factor.

Sample	Weight variation mean (mg) ± SEM	Disintegration Mean (min) ± S/D	% drug content	Fit Factor (f2)
F1	118.0 ± 0.64	5.17 ± 1.22	95.2	-
F2	115.9 ± 0.65	2.85 ± 0.66	104.4	42.3
F3	223.1 ± 2.14	5.21 ± 1.12	86.0	56.8
F4	240.2 ± 2.02	3.54 ± 0.77	94.2	53.9
F5	483.9 ± 1.69	6.77 ± 1.11	46.4	41.5
Official Specification	≤ 5-7.5 (USP)	5-30 (USP)	95-105 (USP)	> 50 (FDA)

The label claim for fluconazole in the samples were 50mg and 150mg. Sample F5 had the least percentage drug content (46.4%) and sample F2 (104.4%) had the highest percentage drug content. Content uniformity test is a very important assessment for oral solid dosage (OSD) forms. It ensures the consistency of dosage units, such that each unit in a batch should have a drug substance content within a narrow range around the label claim. Hence, for the content of active ingredient, the USP specification for fluconazole oral dosage forms is within the range of (95-105%). Two brands (F3 and F5) failed this test (Table 2).

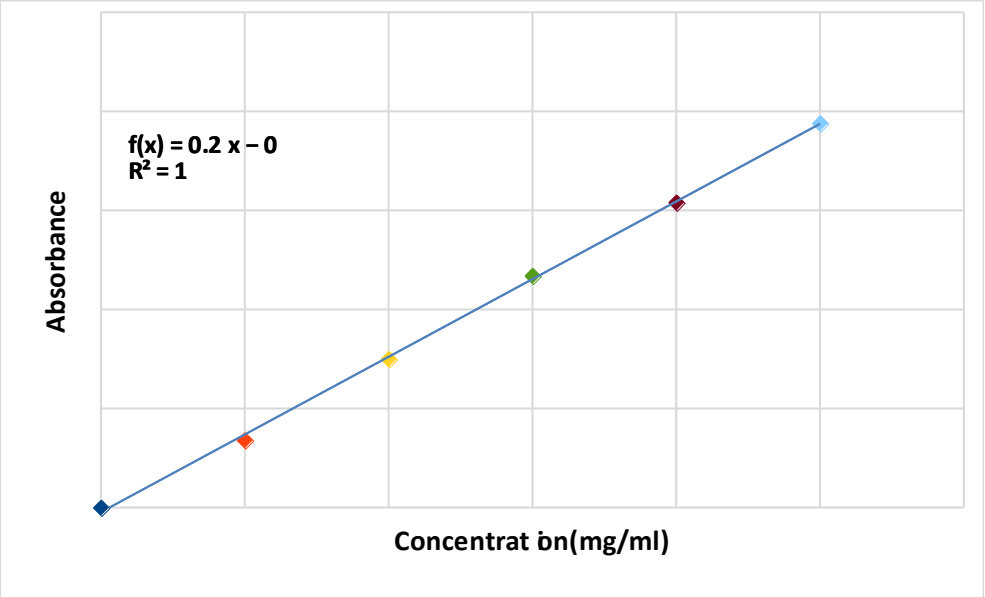

Fig. 2: Standard calibration curve of fluconazole at 262nm.

The standard calibration curve for fluconazole was carried out and it yielded the equation **y =** 0.196x - 0.002, R^2 = 0.999 which is a straight line as represented in figure 2. The equation was found to obey Beer Lamberts law, having an intercept of 0 and starting from the origin.

Drug dissolution test is routinely carried out to provide critical *in vitro* drug release information for quality control, by assessing batch-to-batch consistency of solid oral dosage forms such as capsules. It helps to predict *in vivo* drug release profiles of these dosage forms when administered to patients.[3,11] According to the USP, immediate release drug should have not less than 85% of its drug content dissolved within 30 minutes. The results showed that at 30 minutes, all the samples studied passed the dissolution test (Figure 3).

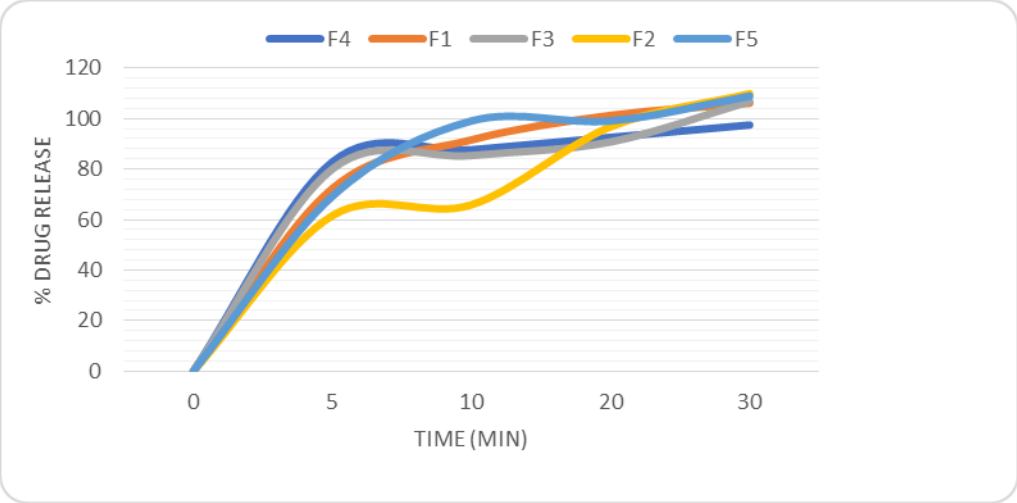

Fig. 3: Graph of percentage drug release of fluconazole capsules.

For comparison of *in-vitro* dissolution profiles, the difference and similarity factors (f1and f2) were emphasized by US FDA.[11] Similarity factor (f2) emphasizes the comparison of the relative closeness of generic to innovator brand of the drug product. The f2 parameter is commonly used to establish similarity between two dissolution profiles of generic and innovator brand, and by extension the bioequivalence of the products.[12] The similarity factors of the various brands of fluconazole were within the range of 41.49 to 56.84 except F2 and F5 that were less than the standard range (Table 2). F2 had a similarity factor of 42.26 and F5 a factor of 41.49 while the reference range is 50 to 100.[111] Comparing the brands of fluconazole used in this analysis, brands F3 and F4 may be prescribed interchangeably with each other and with the innovator except F2 and F5.

CONCLUSION

The results of the comparative and quality control studies of five different brands of fluconazole capsules in Nigerian pharmacies showed that not all the brands met the compendia standards for the formulation of the dosage form. From the brands assayed, only two brands, F3 and F4, with similarity factors of 56.84 and 53.94 respectively, could be said to be bio-pharmaceutically equivalent, and thus could be prescribed interchangeably with the innovator brand, F1.

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