AN ASSESSMENT OF THE INTERCHANGEABILITY OF GENERIC METRONIDAZOLE TABLETS UNDER BIO- WAIVER CONDITIONS

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ABSTRACT

Background: Generic medicines significantly reduce cost of medicines to governments and individuals. They are more affordable than innovator medicines and thus improve availability of life savings medicines in many resource limited settings.

A number of generic metronidazole formulations are available for substitution in practice. However, the interchangeability of the different generic versions with one another or with the reference product is not common knowledge to health practitioners. This study assessed the interchangeability of innovator brand of metronidazole tablets (Flagyl®) with some generic metronidazole tablets using the biowaiver criteria.

Methods: Interchangeability of samples of metronidazole was assessed under biowaiver conditions, as approved for the industry by World Health Organization (WHO) and many regulatory bodies. Pharmacopeia methods were used to ascertain pharmaceutical equivalence. Dissolution profiles of metronidazole tablets at pH 1.2, 4.5 and 6.8 were compared using the similarity factor, to establish *in vitro* bioequivalence.

Results: The results showed that all metronidazole samples passed physicochemical assessments, while the innovator and five out of six generic samples passed the assay of drug content. All the brands of metronidazole assessed were rapidly dissolving (≥85% at 30 minutes) at pH 1.2 and pH 4.5 buffer but were not at pH 6.8 buffer. The dissolution profiles showed they were not superimposable.

None of the generics assessed have a similarity factor greater than 50 in the three media, meaning they are not equivalent to the innovator based on their dissolution profiles.

Conclusion: *In vivo* bioequivalence studies are required to ascertain therapeutic equivalence for these products. For Nigerian manufacturers to avail themselves of the cost saving effect of biowaivers, design and formulation of immediate release generic formulation must factor in appropriate excipients.

Keywords: generic, biowiaver, *In vitro* equivalence, metronidazole

INTRODUCTION

Generic medicines significantly reduce cost of medicines to governments and individuals [1]. They are more affordable than innovator medicines, therefore, improving availability of life savings medicines in many resource limited settings[2]. Generic medicines have the same active ingredient, in the same dose and dosage form, as the reference medicine but may differ in excipients, color and shape. But, for generic medicines to be interchangeable with reference medicines or with another generic there must be evidence of therapeutic equivalence to a reference product through bioequivalence studies[1].

In clinical practice, it is paramount that generic medicines provide equivalent therapeutic outcome as innovator medicines, for a responsible generic substitution practice. *In vivo* bioequivalence studies that are used to established therapeutic equivalence are often expensive and invasive in nature. However, Biopharmaceutics classification system (BCS) have established that some active pharmaceutical ingredient (API) meeting criteria of high permeability and solubility will behave as solutions *in vivo* therefore qualifies for biowaiver. A biowaiver means that *in vivo* bioavailability and/or bioequivalence studies may be waived (i.e. not considered necessary for product approval[3].Therefore, national authorities may accept *in vitro* dissolution studies based on BCS as surrogates for *in vivo* bioequivalence studies, to reduce time and cost of product approval thereby improving access to good quality essential generic medicines[1]. Major advantages of the biowaiver procedure include simplification of the product approval process and the reduction of the time required, thus reducing the cost of bringing new products to market[3]. BCS is now an established and a valid approach to waive *in vivo* bioequivalence studies for class I and III solid immediate oral dosage forms[4].

The responsibility for the proper use of generics lies with national bodies, but registration of generics and generic drug policies are still under development in many low- to middle- income countries[1]. To date, there is no generic drug policy in Nigeria; however, the Nigerian national drug policy (NDP) recommends prescribing and dispensing using generic names of medicines. The NDP is silent on generic substitution practice, but generic substitution is known to occur in practice. There are often many generic versions for the same therapeutic moiety available for use in practice, making it possible for patients to receive a different generic preparation each time they present a prescription in the pharmacy[5]. Often times, pharmacist do not have access to data that assures bioequivalence, therefore generic substitution occurs without empiric data that interchangeability is appropriate. When patients are switched between different generic formulations, there is potential for greater variation in drug pharmacokinetics and therapeutic outcome.

Metronidazole, an anti-amoebic and antibacterial, is an important medicine in practice because of its activity against anaerobic bacteria. Metronidazole is eligible for biowaiver because of its high solubility and high permeability (BCS Class I medicines)[6]. A number of generic metronidazole formulations are available for substitution in practice, however, the interchangeability of the different innovator products with generic is not common knowledge to practicing health workers. This study assessed the interchangeability the innovator brand (Flagyl®) with some generic metronidazole tablets using the biowaiver criteria.

MATERIALS AND METHODS

Metronidazole samples and Comparator pharmaceutical product.

The innovator brand of metronidazole, Flagyl® Sanofi Aventis, was used as the comparator pharmaceutical product (CPP). Six readily available generic brands

of metronidazole tablets (Table 1) were purchased from registered and reputable pharmaceutical distribution outlets in Lagos, Nigeria. All the metronidazole samples were immediate release uncoated tablets that qualifies for biowaiver. They were all manufactured in Nigeria and registered by National Agency for Food and Drug Administration and Control (NAFDAC).

Reagents and reference standards

All reagents and solvents used were of analytical grade, all dissolution media were always freshly prepared with pH appropriately adjusted prior to use. Metronidazole standard was purchased from Fluka Anaytical (Sigma Aldrich Chemical Corps, St. Louis, MO, USA), CAT number PHR1052-1G, Lot: P500652.

METHODS

The British Pharmacopeia methods and validated equipment were used to assess the following physico- chemical parameters; uniformity of weight, friability, hardness and disintegration and assay test for metronidazole samples collected for this study[7].

Uniformity of weight

Twenty tablets randomly selected from each of the seven brands were weighed individually with an analytical weighing balance (Ohaus® Adventure USA). The mean weights for each brand and percentage deviation from the mean value were determined.

Hardness test

This was assessed using ten tablets randomly selected from each brand. They were individually placed between the platens of a tablet hardness tester (Electrolab tablet hardness tester). The pressure in kg/cm^{2}at which each tablet got crushed was recorded.

Friability test

Erweka® (GMBH) friabilator was employed to assess the friability of twenty tablets of each brand of metronidazole tablets at a rotation speed of 25 revolutions per minute for 4 minutes. The tablets were then re-weighed and the percentage friability was calculated.

Disintegration test

Six randomly selected tablets from each brand were placed in the mesh of a tablet disintegration tester (Campbell® Model TD-400) filled with distilled water and maintained at 37±0.5^{0}C. The tablets were considered completely disintegrated when all the particles passed through the wire mesh and time was recorded.

Preparation of dissolution media

The dissolution media: 0.1N HCL (pH 1.2), acetate buffer (pH 4.5), and phosphate buffer (pH 6.8) were prepared as described by British Pharmacopoeia[7].

Preparation of standard curve and drug content determination

Metronidazole standard (10 mg) was weighed and transferred into a 100ml volumetric flask, 20 ml of mobile phase – filtered and degassed mixture of water and methanol (80:20)[8]– was added and sonicated for 10 minutes after which the

solution was made up to 100 ml with the mobile phase to obtain a concentration of 100µg/ ml. Gradient calibration range of 20 – 100 µg/ ml was used to obtain the calibration curve used for assay of drug content.

Chromatographic conditions

The chromatographic procedure was carried out using an Agilent® 1200 series Infinitely Better HPLC-UV with a reverse phase Zobrax Eclipse XDB C-18 (150 mm X 4.6 mm, 5.0 µm) column, quaternary pump with auto sampler injector set at 20 µL. The mobile flow rate was operated at 1.2ml/ min and UV detector wavelength set at 254 nm. The mobile phase consists of water and methanol in the ratio 80:20 respectively.

***In vitro* dissolution study**

The innovator brand of metronidazole Flagyl® coded (CPP) and two generic brands G3 and G4 were selected for evaluation of interchangeability using *in-vitro* methods. The generic samples selected were based on the content assay results and availability at time of the study. The *in vitro* dissolution profiles were carried in three media; 0.1 N HCL (pH 1.2), acetate buffer (pH 4.5), and phosphate buffer (pH 6.8). Using an Intech Model DA-6D dissolution apparatus, USP apparatus II (Paddle) maintained at 37±0.5^{O}C at a speed of 50 rpm in 900 ml of dissolution media, six dosage units of each brand was assessed in line with B.P. specifications. 5 ml of dissolution samples were simultaneously withdrawn at predetermined time intervals and replaced with fresh 5 ml of appropriate medium in order to maintain sink condition. The withdrawn samples were filtered through a 0.45 µm syringe filters, diluted appropriately with the respective dissolution medium, and analyzed using a UV spectrophotometer at 275nm wavelength.

The actual concentrations of metronidazole in the respective brands at the different sampling times were determined using metronidazole reference standard calibration curve in the respective dissolution media.

Statistical analysis of dissolution data

Percentage dissolved data was calculated using Microsoft Excel 2010. Dissolution profiles were analyzed using similarity factor (f_2) was used to compare the similarity or dissimilarity between the dissolution profiles of the innovator and generic brands.

$$f_2 = 50 \text{ Log } \{[1 + \frac{1}{n} \sum_{t=1} n(R_t - T_t)^2]^{-0.5} x\ 100\} \qquad \text{Equation 1}$$

where, R_t and T_t are the cumulative percentage dissolved at time point t for reference and test products, respectively, and n is the number of pool points.

Table 1: Metronidazole tablets samples tested

Brand Code	Strength	Manufac. Date	Expiry date	Batch Number	NAFDAC Number
CPP	400 mg	02-2014	02-2016	T106	04-5566
G1	400 mg	03-2014	03-2019	No Number	A4-4689
G2	400 mg	12-2013	11-2016	20131201	A4-9727
G3	400 mg	04-2014	04-2017	2116T	04-79536
G4	400 mg	08-2013	07-2018	A130072	04-5566
G5	400 mg	09-2013	09-2018	4613	04-9101
G6	400 mg	07-2014	06-2017	UT008	A4-0945

RESULTS

The results showed that all metronidazole samples passed physico-chemical assessments, while the innovator and five out of six generic samples passed the assay (Table 2).

Table 2: Physicochemical characteristics of the different metronidazole brands

Brand code	Uniformity of weight (% deviation)	Hardness (Kg/cm^2) ± SD	Friability (%)	Disintegration (mins) ± SD	Assay (%) Average ±SD
CPP	3.5 ± 0.02	5.05 ± 0.56	0.24 ± 0.12	1.42 ± 0.20	95.2 ± 0.21
G 1	4.2 ± 0.01	11.30 ± 0.33	0.15 ± 0.30	0.33 ± 0.09	99.0 ± 0.32
G 2	2.4 ± 0.02	12.06 ± 0.17	0.31 ± 0.16	1.07 ± 0.31	93.6 ± 0.50
G 3	1.8 ± 0.01	5.50 ± 0.54	0.22 ± 0.32	0.80 ± 0.18	105.4 ± 0.23
G 4	3.7 ± 0.01	10.52 ± 0.62	0.18 ± 0.14	0.67 ± 0.11	91.1 ± 0.10
G 5	3.9± 0.03	9.93 ± 0.38	0.91 ± 0.21	0.27 ± 0.18	88.8 ± 0.41
G 6	3.1 ± 0.12	7.71 ± 0.50	0.12 ± 0.02	0.67 ± 0.11	93.9 ± 0.42

All the brands of metronidazole assessed were rapidly dissolving (≥85% at 30 minutes) at pH 1.2 and pH 4.5 buffer but were not at pH 6.8 buffer. The dissolution profiles showed they were not superimposable (Figures 1-3).

Table 3: Results of f_2 calculation for metronidazole tablets in different dissolution media

	CPP	G 4	G 3
Time (min)	**Percentage dissolved (% ±SD)**		

pH 1.2			
5	105.95	90.67	84.40
10	106.65	105.97	96.90
15	108.93	109.48	102.70
30	109.35	110.12	109.30
45	111.38	110.68	109.40
60	114.16	111.01	111.70
		f_2 = 12	f_2= 45
pH 4.5			
5	102.26	57.05	57.96
10	103.59	81.34	65.83
15	104.26	92.88	78.55
30	106.94	103.21	89.49
45	107.08	104.39	97.32
60	109.58	106.21	98.62
		f_2 = 29	f_2 = 23
pH 6.8			
5	35.33	26.22	28.49
10	49.25	43.62	37.62
15	55.38	60.55	46.29
30	63.07	80.60	58.45
45	66.93	81.18	64.76
60	70.81	81.67	70.58
		f_2 = 43	f_2 = 54

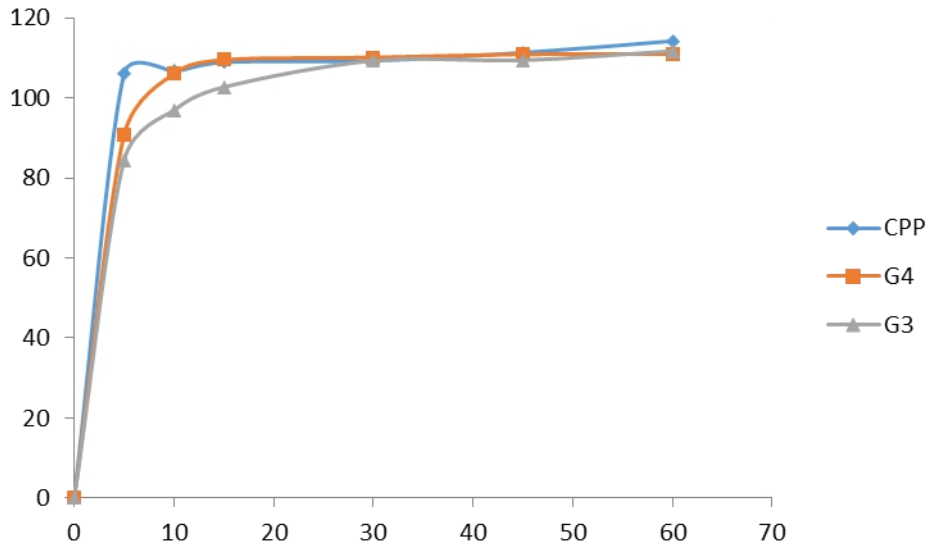

Figure 1: Dissolution profile of innovator brand and two generic brands of metronidazole tablets in pH 1.2

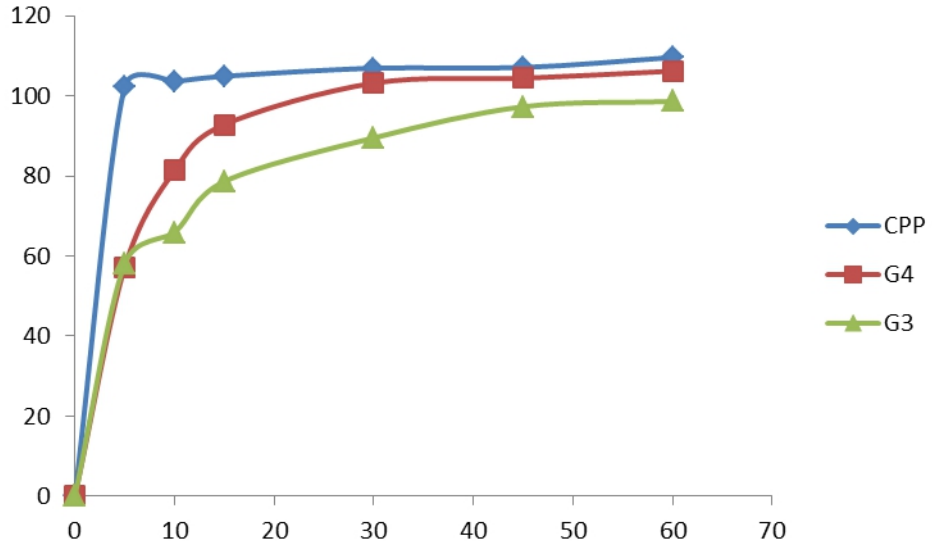

Figure 2: Dissolution profile of innovator brand and two generic brands of metronidazole tablets in pH 4.5

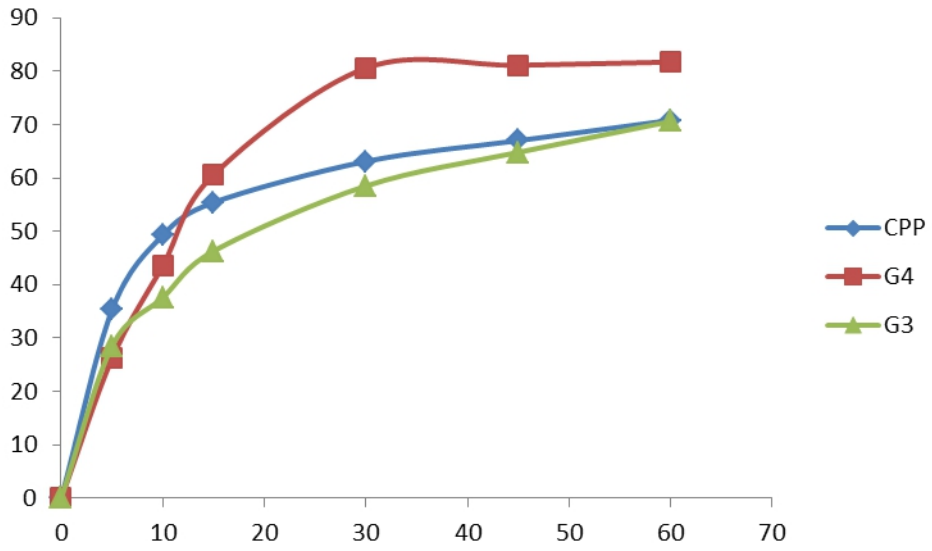

Figure 3: Dissolution profile of innovator brand and two generic brands of metronidazole tablets in pH 6.8

None of the generics assessed have a similarity factor greater than 50 in the three media, meaning they are not equivalent based on their dissolution profiles.

DISCUSSIONS

Biowaivers are regulatory procedures, which allow national authorities like NAFDAC, to make informed decision whether a generic medicine requires *in vivo* bioavailability studies or not. This study used biowaiver criteria to assess therapeutic equivalence of some metronidazole tablets that are in circulation in Lagos, Nigeria. Therapeutic equivalence for this study encompasses pharmaceutical equivalence as well as bioequivalence by *in vitro* method. Therefore, the result will be discussed first with regard to pharmaceutical equivalence of metronidazole tablets evaluated and then their bioequivalence.

According to WHO, products are pharmaceutical equivalents if they contain the same molar amount of the same APIs in the same dosage form, if they meet comparable standards and if they are intended to be administered by the same route[3]. Most of the samples of metronidazole assessed, six out of seven

including the innovator brand, are considered pharmaceutical equivalents. This study result is similar to that of another study where five out of seven brands and of metronidazole met the BP standards, confirming them as chemically and physically equivalent[9]. In another studies, four out of five brands of four hundred milligram metronidazole passed assay of active constituents and other physico-chemical properties which is suggestive of pharmaceutical equivalence[10]. Drugs that do not contain the same molar amount of the same API cannot be compared chemically and therapeutically.

Based on the *in vitro* dissolution results of this study, none of the two generics compared are considered therapeutic equivalents to the CPP. Our study have comparable results to that of Lobenberg, *et al.,* where none of the metronidazole assessed in the Americas was equivalent to the CPP[11]. But, differed from the result of Ilomuanaya *et al.,* where eight out of twelve brands were considered therapeutic equivalents to the CPP[10]. However, their study[10] used two dissolution media whereas regulatory guidance for biowaivers recommends three media, as used by our study. Also, other studies from different parts of the world showed that generic metronidazole, especially the higher dosage form do not often qualify for biowaiver[11-13]. These products will need more expensive, time consuming and invasive *in-vivo* studies to confirm bioequivalence. Though the consequence of bio-inequivalence of generic metronidazole to CPP may not result in serious side effects, because of its wide therapeutic index, there is a potential for treatment failure and development of microorganism resistance and the consequent necessity to use more expensive antibiotics.

All the generic metronidazole tablets assessed in this study did not list the excipients used in formulation, therefore, the impact of excipients on the formulation is unknown. The nature and characteristics of excipients in a formulation are contributing reasons BCS class I API may not achieve *in vitro*

equivalence with CPP. Another reason is the CPP used in this study, there is evidence from the literature that there are two patent holders for Flagyl®, Pfizer and Sanofi Aventis, and the two products were not therapeutically equivalent based on *in vitro* studies[7]. There is need to standardize the CPP for metronidazole tablet for future *in vitro* studies and industry guidance.

The problem of generic medicines available in Lagos not being equivalent to a CPP have also been documented for other drugs apart from metronidazole[14, 15]. I*n vivo* bioequivalence studies are required to ascertain therapeutic equivalence for these products. For Nigerian manufacturers to avail themselves of the cost saving effect of biowaivers, design and formulation of immediate release generic formulation must factor in appropriate excipients.

The scientific basis for BCS is sound and it is regulatory utility is improving every day as well as becoming a norm in developed countries for marketing authorization. The WHO is encouraging developing countries to adopt it by providing industry guidance. More developing countries need to adopt biowaiver for marketing authorization purposes so as to improve access to qualitative generic medicines. Manufacturers of generic pharmaceutical products, especially in developing countries, owe a duty of care to their consumers to develop products that are interchangeable with a CPP. BCS Class I and III APIs qualify for biowaiver in industry guidance developed by WHO. Manufacturers of generic essential medicines in these classes should consider adopting biowaiver criteria, very rapid dissolving (≥ 85% in 15minutes) at pH 1.2, 4.5 and 6.8, like compendia standard. This will help to assure dispensing health practitioners of interchangeability of these products.

A limitation of this study is the number of metronidazole samples used. As at the time of the study only few metronidazole products were available for purchase in

the distribution chain in Lagos. Also, there was no reliable sampling frame for registered metronidazole products such that random sampling could not be used to select products for this study. Therefore, the generalizability of the study is hampered. Notwithstanding this limitation, the results of this study conforms with similar studies on *in vitro* equivalence of immediate release solid oral dosage forms, thereby strengthening our conclusion.

CONCLUSION

Though most of the generic metronidazole tablets assessed were considered pharmaceutically equivalent with the comparator pharmaceutical product, none of them was therapeutically equivalent with the CPP based on *in vitro* equivalence assessment.

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