Chemical Equivalence of Some Brands of Metronidazole Tablets Marketed in Sagamu Community

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ABSTRACT

The occurrence of fake and substandard medicines has become a global issue that draws the attention of all key players in the sector in all nations of the World. This study was carried out for comparative quality assessment of different brands of metronidazole tablets in Sagamu community in Nigeria to determine their suitability for therapeutic purpose.

Five (5) brands of 200mg metronidazole tablet, marketed in Sagamu community Pharmacies were randomly selected and subjected to physicochemical studies which include uniformity of weight, crushing strength, friability, and disintegration rate, and chemical equivalence study using the high performance liquid chromatography (HPLC) according to official procedures in British Pharmacopoeia (B.P).

All brands except brand D and E passed the weight uniformity test. The friability test was passed by all the brands except brand E according to B.P specification which states that the loss in weight should be less than 1% also all brands except brand B showed satisfactory crushing strength. Also, the disintegration rate of the brands was satisfactory according to the B.P. specification as all the brands disintegrated within 30 minutes. The results of high performance liquid chromatography revealed the percentage content of brands A,B,C,D, and E to be 97.44% w/w, 130.9% w/w, 111.56% w/w, 98.52% w/w, 96.02% w/w respectively. The British Phamacopoeia specification for percentage composition of metronidazole is in the range of 90-105%. Therefore brand A, D and E passed the test while brand B and C did not pass the test.

The results showed that only brand A passed all the analytical procedures and therefore fit or safe for human consumption.

Keywords: Chemical equivalence, Metronidazole and safety.

INTRODUCTION

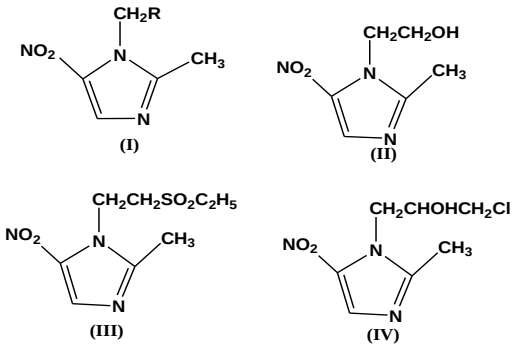
For the past two decades, the menace of sub-standard, fake and adulterated drugs in the Nigerian drug market has been a very big issue and the present situation is alarming. Consequences of sub-standard, fake and adulterated drugs may be fatal as seen in "My pikin" brand of paracetamol in Nigeria some years ago. Prescribers assume that different brands of the same drugs (generics) are pharmaceutically equivalent (comparable quality) and thus interchangeable. However, chemical equivalence studies have shown that this is not always true[1]**.** Although practically all types of pharmaceutical products have been shown to be involved , the existing data suggest that anti-infectious agents, particularly antibiotics and antiparasitic agents like metronidazole, are the most counterfeited products in developing countries[2,3].

The introduction of nitroheterocyclic drugs in the late 1950s and 1960s heralded a new era in the treatment of infections caused by Gram positive and negative bacteria and a range of

pathogenic protozoan parasites[4] .

The antibiotic azomycin (a-2 nitroimidazole), isolated in Japan from streptomycete, was the first active nitroimidazole to be discovered and acted as the main impetus for the systematic search for drugs with activity against anaerobic protozoan. This led to the synthesis of 5-nitroimidazole (I) and its' derivatives such as metronidazole (II), tinidazole (III) and ornidazole (IV)[5,6] . Metronidazole; 1- β – hydroxyethyl -2 – methyl 5 – nitroimidazole is the drug now most widely used in the treatment of anaerobic protozoan parasitic infections caused by *Teania. vaginalis, Giardia duodenalis* and *Entamoeba histolytica*[7,8] . It is remarkably safe compared with the toxic amoebicides, emetin, and is the recommended alternative for the treatment of amoebiasis. Metronidazole and related nitroimidazole (tinidazole)- (which is not available in some countries) are also the only drugs of choice effective for the treatment of giardiasis[8] (Brook,2009). It is also effective against anaerobic Gram – negative bacilli, including most *Bacteroide**s*** species; *Fuscobacterium* and *Veillonella.* anaerobic gram–positive cocci including *Clostridium, Eubacterium, Peptococcus* and *Peptostreptococcus.* Metronidazole is also active against *G. vaginalis* and the protozoa *E .histolytica, Trichomonas Vaginals* and protozoa *lamblia*[7].

Metronidazole acts primarily against the trophozoite forms of *E. histolytica* and has limited activity against the encysted forms.



Metronidazole is thought to act as an electron sink by accepting electron from ferredoxin (the parasite's electron transport protein) and depriving the cell of essential reducing equivalents. Essential co-enzymes such as nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) are formed, leading to the death of the parasite[4].

Furthermore the nitro group of metronidazole becomes reduced in accepting electrons from ferrodoxin. The reduced metabolites are said to be toxic to the parasite. It binds to DNA by distorting its helical structure, preventing DNA from functioning as a template.

The parasite thus "commits suicide" by converting the drug to a metabolite toxic to it. This property is common to nitro aromatics[6].

Metronidazole is selectively toxic to anaerobic microorganisms. After entering the cell by diffusion, its nitro group is reduced by certain redox protein operating in the anaerobic microbes to highly reactive nitro radical which exerts cytotoxic effect by damaging DNA and other critical biomolecules.

It is an essential drug commonly sold in the Nigerian Market. There are different brands and dosage forms under various trade names by different pharmaceutical companies. It is marketed by Pfizer under the trade name Flagyl in the US, by Sanofi-Aventis globally under the same tradename Flagyl, in Pakistan and Bangladesh .It is also available with the brand name of Nidagyl manufactured and marketed by Star Laboratories. In Thailand it is marketed as Mepagyl[6]

Drug quality is currently receiving renewed international attention[9] . Over the past decade, there has been an increase in public awareness of the existence of counterfeit and substandard drugs which have been increasingly reported in developing countries where drug regulations are ineffective[3,10]. The prevalence of fake, counterfeit and substandard drugs has reached an alarming stage that some researchers like Pecoul *et al.,* (1999) [11] thought that it was unachievable to overcome Hence the reason for the assessment of pharmacopoeial quality or chemical equivalence of some brands of metronidazole 200 mg tablets in pharmacies in Sagamu community.

Materials and Methods

Samples collection

Five (5) brands of metronidazole 200 mg tablets were purchased at different pharmaceutical stores in Sagamu community of Ogun state, Nigeria. The samples were coded A to E and their physical examination parameters were recorded as shown in table 1 below.

Table.1: Brands of 200 mg metronidazole tablets used in this study

Brand Code	Place of Manufacture	Description	NAFDAC Number/ Batch Number	Date of Manufacure	Expiry Date
A 200mg	Nigeria	Yellow,smooth, circular with flat surface	04-0980/11048	May-11	May-14
B 200mg	Nigeria	White, smooth, circular,convex surface	04-0238/MA-1349	May-11	Apr-16
C 200mg	Nigeria	Yellow,smooth, Circular with convex surface	04-0272/S24338	Sep-11	Mar-14
D 200mg	Nigeria	White, smooth, circular with flat surface	04-0072/T1-5511	May-11	Apr-16

Determination of Uniformity of Weight

This was carried out according to British pharmacopoeia (B.P), (2003) [12] procedure. Twenty (20) tablets were weighed individually on the analytical balance(OHAUS, India) and the average weight of tablets (Mean) was calculated as Total weight of tablets / number of tablets. The deviation of each weight from the average was then calculated and the standard Deviation (SD) computed.

Determination of Tablet Friability

This was carried out in accordance to B.P, (2003) [12] procedure. Ten (10) pre weighed tablets were placed in the plastic chamber revolving at 24-25 rpm for 4 mins. The tablets were subjected to combined effects of abrasion and shock in the DBK friability test apparatus . The tablets were dropped at a distance of six inches on each revolution. The instrument is operated for 100 revolutions after which the tablets were reweighed.

$$\%\text{weight loss} = \frac{(\text{weight of tablet before fribillation}) - (\text{weight of tablet after fibrillation}) \times 100}{\text{Weight of tablet before friability testing}}$$

Determination of Tablet Crushing Strength

Ten tablets were tested from each of the brands. Each tablet was placed between a fixed and a movable jaw of a hardness tester, a force was applied through a screw driver spring by turning the screw. The average force needed to break the ten tablets from each of the brands was recorded in newton as stated in B.P, (2003)[12]

Determination of Tablets Disintegration Rate

The disintegration test apparatus consists of a basket made of transparent polyvinyl or other plastic material. It had 6 tubes set into the same basket with equal diameter and a wire mesh made of stainless steel with uniform mesh size is fixed to each of these six tubes in which 6 tablets were placed. Small metal discs was used to enable immersion of the dosage form completely. The entire basket-rack assembly was movable by reciprocating motor which was fixed to the apex of the basket-rack assembly moving up and down at 28 to 32 Revolutions per min(rpm) approximately carrying a rigid basket rack assembly. The entire assembly was immersed continously (in and out)in a vessel containing distilled water in which the disintegration test was to be carried out. The

vessel was provided with a thermostat to regulate the temperature of the fluid medium to 37 ˚C. The disintegration of each tablet was timed and recorded as the disintegration process was done[12].

Analysis of Brands of Metronidazole Tablet using High Performance Liquid Chromatography(Hplc)

This was carried out according to standard procedure[12] (BP 2003). High performance liquid chromatographic(HPLC) analysis was carried out using Zorbax XBD C8 150X4.6MM, 5µm column as stationary phase and a mobile phase of methanol and water in ratio 20:80, flowing from a quatenary pump with a flow rate of 1 ml/min at an ambient temperature and ultraviolet detector at wavelength 254nm. After the chromatographic conditions were set and stabilized to obtain a stable base line. A mixed standard solution of the pure metronidazole powder was prepared in mobile phase and filtered Solutions were injected through a manual injector (Rheodya injector of loop size 20 µL) and the chromatograms recorded. The standard chromatogram of metronidazole powder with concentrations 5, 10, 25, 50, 75, and 100 µg/ml was prepared separately.

For analysis of the brands, 15 tablets of each brand of metronidazole (200 mg) were accurately weighed and finely powdered. A stock solution was prepared and and diluted to different concentrations (5, 10, 25, 50, 75, 100 µg/ml). 20µL of the sample was dispensed into the injector using a syringe.The process was done in duplicates and the peak areas were recorded and the concentrations were extrapolated from the standard curve as shown in table 8 .

Statistical Analysis

Statistical analysis was carried out using Student 't' test with 95% confidence level. A significant change was considered acceptable if $p < 0.05$. Results were expressed as mean of duplicates and standard deviation.

RESULTS

Ttable 2 - variation of uniformity of weght of the brands of metronidazole tablets (200 mg) showing the number of deviation outside ±7.5% and ±15% limits.

Brand code	Mean deviation ± Standard Deviation	Number of tablet(s) outside ±7.5 % of the weight	Number of tablet(s) outside ±15 % of the weight
A	0.5789±0.0434	0	**0**
B	0.3501±0.0263	0	**0**
C	0.6076±0.0456	0	**0**
D	0.3712±0.0278	1	**0**
E	0.3899±0.0292	4	**0**

Table 3- Results of friability test for ten randomly selectedtablets from each brand of metronidazole tablets (200 mg)

Brand code	Weight of 10 tablets before friabilation(g)	Weight of 10 tablets after friabilation(g)	% friability
A	5.7978	5.7806	**0.2966**
B	3.5476	3.5422	**0.1522**
C	6.0808	6.0353	**0.7483**
D	3.7173	3.6837	**0.9039**
E	**3.9249**	**3.3806**	**3.3806**

Table 4- summary of disintegration rate for all brands of metronidazole tablets under study

Brand code	Disintegration time(mins) (Mean)	Standard Deviation	% Coefficient of Variation
A	1.03	0.69	**66.99**
B	6.06	3.32	**54.79**
C	1.30	0.53	**40.77**
D	8.77	3.98	**45.38**
E	**6.31**	**2.93**	**46.43**

Table 5 - Results of crushing strength in newton for each of the 10 tablets of each brand of metronidazole (200 mg) tablets.

Brands				
A	**B**	**C**	**D**	**E**
Crushing Strenght (N)				
67.7	59.6	50.5	96.7	**99.9**
86.6	55.6	41.9	93.1	**105.6**
54.0	38.5*	46.7	106.8	**88.2**
60.0	56.4	58.4	122.5	**90.2**
92.3	45.1	41.5	124.1	**54.0**
68.1	39.5*	55.2	84.6	**84.2**
92.7	53.6	64.9	121.3	**62.4**
106.0	49.2	55.6	76.6	**62.0**
86.2	44.3	49.6	47.5	**106.8**
73.3	**35.1***	**59.2**	**76.1**	**112.8**

*Tablets that fell below the minimum force required of 40 N[13]

Table 6. Results of HPLC analysis of the standard metronidazole powder

Concentration µg/ml	Peak Area	Average Peak Area (mAu)
5	28.55	28.55
10	52.43	56.3
	60.35	
25	107.32	119.7
	132.16	
50	218.17	229.1
	239.95	
75	429.98	430.6
	431.399	
100	555.64	555.6

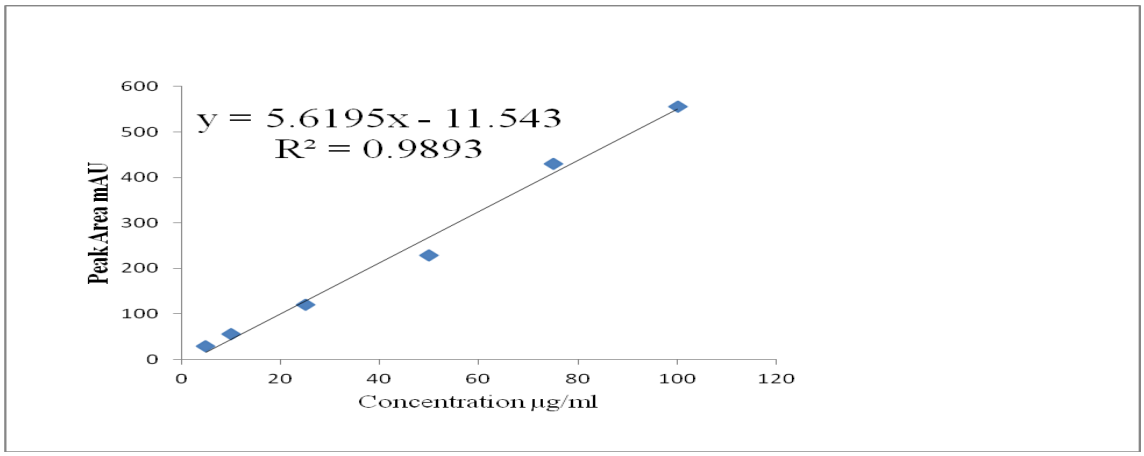


Figure 1. Standard calibration curve of metronidazole powder .

Table 7. Results of hplc analysis of all brands of metronidazole tablets.

Brands	Peak Area (mAu)	Percentage Composition (w/w)	BP.specification 95% to 105%
A	262.21	97.44	Pass
B	356.29	130.9	Fail
C	301.89	111.56	Fail
D	265.27	98.52	Pass
E	258.22	96.02	Pass

The table shows the percentage content or composition of each brand and the brands that failed according to B.P. (2003)[12] standards.

Discussion

The assessments involved the use of both qualitative and quantitative methods of evaluation. Five different brands of metronidazole tablets obtained from different retail pharmacy outlets within Sagamu town were subjected to a number of tests in order to assess their biopharmaceutical and chemical equivalence. All the brands used were within their shelf life as at the time of the study.

The quantitative biopharmaceutical evaluation of five brands of 200mg metronidazole tablet involves the determination of the Uniformity of weight, Friability, Hardness, and rate of disintegration [12, 13] .

The results of physical examination or assessment of the tablets samples tested is listed in Table 1. Most of the samples had good and impressive physical appearance, rough edges, and scratches were absent. No dull appearance noticed.

The significance of the uniformity of weight is to verify that the tablets in each batch falls within the appropriate size range. The results presented in Table 2 show the weight of 20 randomly selected tablets from each brand,calculated percentage deviation and coefficient of variation (a measure of the difference in the uniformity of weight within the batch) for the five selected brands of 200 mg metronidazole tablets under study.

The British Pharmacopoeia (2003) [12] states that for tablets having mean weight of 200 mg not more than 2 tablets are permitted to deviate from the mean by ±7.5% of which brand E did not pass, and no tablet should deviate more than ±15% of which all brands passed as shown in brand D result in Table 2.

According to the British Pharmacopoeia (2003)[12] a maximum weight loss not more than 1% after friabilation is required. All brands except brand E passed the friability test whose percentage loss in weight after friabilation was greater than 1%. This test is used to evaluate the ability of the tablet to withstand abrasion, resist chipping, and break under storage, transportation and handling. The stronger the interparticulate bonding, the lower the friability for each brand that was studied as shown in table 3

The Swiss Pharmacopoeia, way back in 1935, required that a disintegration test should be performed on all tablets and capsules as a criterion of its performance.Table 4 shows the results of the disintegration tests conducted on all the brands of metronidazole 200mg tablets . According to the British Pharmacopoeia (2003)[12], all uncoated tablets are required to disintegrate in water within the first 15 minutes. The disintegration rates ranged from 0.41 to 12.52 minutes , indicating that all the disintegration times were within the BP. Official limit of 30 minutes.

Brand A showed the fastest disintegration time with all six tablets disintegrating completely by 2.01 minutes while brand D had the longest disintegration time with the sixth tablet disintegrating at 12.52 minutes. Brands longer disintegration time may be as a result of, the type and concentration of the diluents, disintegrants, lubricants and binders used, the method of manufacture (wet or dry granulation or direct compression), high compression pressure, hardness of tablet, type and composition of the coat (for enteric coated tablets) .particle size and solubility of the drug[14].

Although all the brands under study passed the disintegration tests, it should be noted that a tablet that fails to disinegrate within the specified disintegration time may result in therapeutic failure of the drug as it may not be available for absorption and distribution within the body system.

Pharmacists involved in manufacturing have been able to manipulate disintegration time of drugs to the advantage of the patients i.e in the production of extended or sustained release tablets to encourage drug adherence and to have the right concentration of a drug in the body at a particular time.

The hardness of a tablet is important in determining its rate of disintegration, and dissolution[15] . It will also affect indirectly the rate of absorption of a drug and the concentration of the drug in the body at a particular time. For the mechanical strength of a tablet to be satisfactory, the minimum force required is 40N[13] . Table 5 shows the hardness of 10 randomly selected tablets from each brand of metronidazole under study. Brands A,C,D,and E passed the test while brand B failed by having two of the tablets that were crushed with a force below 40N hardness[13].

The high performance liquid chromatography method can be used to separate mixtures of compounds, identify ,purify and quanify the components of a mixture. It was applied in this study to determine the content uniformity of the 5 brands of metronidazole 200g under study and the results showed that there was a significant difference in percentage contents of the brands ($p< 0.05$) . Three out of five brands passed the test as shown in table 7. Brand A,D and E passed the test while brand B and C failed the test.The British pharmacopoeia (2003)[12] requires a percentage purity of 90 -105 % for metronidazole 200 mg tablets. This failure could have been as a result of adsorption by the filter paper or degradation of the active ingredient during filteration, decomposition on handling , unfavourable storage conditions or manufacturing errors of improper in-house quality control unit of the manufacturing company or non adherence to Good Manufacturing practice (GMP). Aside these the production run is expected to be monitored under control chart. At regular intervals (10-15 min.) during the course of manufacturing, the operator must sample specified number of tablets for testing (in-process control) e.g. the weight of tablet, tablet thickness, friability, disintegration time etc. so that finished products posses quality assurance. Since chemical equivalence has been established for brand A, D, and E, It will be necessary to establish biopharmaceutical equivalence of these drugs before administering such drugs to patients[2]

The overall result obtained in this study points out to the drug manufacturing companies, that they should always give due attention to their *in vitro* and *in vivo* tests in their manufacturing procedure as only brand A passed all the analytical assessments and therefore fit or safe for human consumption..

Also, manufacturers should have a proper in-house quality control unit that ensures strict adherence to procedures that will enhance better quality of tablets and factors that may affect the stability of metronidazole should be reviewed at specified interval[16] .

Since all the brands under study have National Agency for Food, Drug Administration and Control (NAFDAC) number, and yet not all passed the tests that they were subjected to, it would be proper for Drug regulatory bodies to upgrade and verify the standards of imported raw materials and metronidazole tablets being manufactured in Nigeria regularly and should also do periodic re-evaluation of tablets and capsules of other drugs by random market sampling for quality assessment.

Healthcare professionals especially Pharmacists should make sure that the drugs that are being handed over to their patients are drugs that are not expired, defective or unfit for use[17]. Also they should be convinced beyond reasonable doubt that the drugs being dispensed to patients do not have their active ingredient(s) fraudulently diluted or substituted because a direct consequence of poor pharmaceutical quality product will be a poor biological activity or therapeutic activity. The adverse effect of such product could lead to morbidity[15,18,19] or therapeutic failure as well as causing deterioration of patient's health, or causing economic loss to the patient.

In conclusion the results show that only brand A passed all the analytical procedures and therefore fit or safe for human consumption.

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