

**COMPARATIVE *IN-VITRO* BIOEQUIVALENCE STUDIES OF SOME BRANDS OF
METRONIDAZOLE TABLET MARKETED IN ZARIA, KADUNA STATE**

BY

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DECEMBER, 2019

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**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
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ZARIA**

DECEMBER, 2019

DECLARATION

I declare that the work in this dissertation titled "Comparative *In-vitro* Bioequivalence Studies of Some Brands of Metronidazole Tablet Marketed in Zaria, Kaduna State" has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma in this or any other institution.

Mujitapha Fatima Malumfashi

Date

CERTIFICATION

This dissertation titled “Comparative *In-vitro* Bioequivalence Studies of some Brands of Metronidazole Tablet Marketed in Zaria, Kaduna State” by Mujitapha Fatima Malumfashimeets the regulations governing the award of the Degree of Master of Science in Pharmaceutical Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to my late brother Umar Mujitapha, May Almighty Allah, the creator of all and life itself, grant his soul eternal peace.

ACKNOWLEDGEMENT

All praise and worships are for Allah, the creator and sustainer of all and the universe, may His peace, mercy and blessings be upon His noble prophet, his household, and those who follow their path till the last day (the day of resurrection). I am thankful to almighty Allah for His unlimited guidance and blessings upon me to accomplish this work under the supervision of Dr. M. A. Usman and Prof. Ibrahim A. Yakasai. My special appreciation goes to them. Thank you for your support and commitment. My sincere appreciation also goes to Dr. A. Musa Head of Department of Pharmaceutical and Medicinal Chemistry and Dr. SalisuAwwalu for their help and guidance. I wish to thank and express my gratitude to all my lecturers, colleagues and friends who helped me one way or the other. My utmost appreciation goes to my parents Alh. MujitaphaUwais and Haj. SaffiyatMujitapha for they are the building blocks of my success. I sincerely appreciate the effort of my family, especially aunty Hadiza, aunty Bilkisu, Aisha, Asma'u, Hafsat, RukkayyaJuwairiyya, Abubakar, Halimatu, Zainab, UmmaKhulsum, Aliyu, Mohammad and Sulaiman. To my nephews and nieces, thank you all. I am sincerely indebted to my understanding husband, Ibrahim Garba, for his love, physical, emotional and financial support during the course of this work. I, also appreciate my children Moh'd, Abdullahi, Alqasim (Imam), Hauwa'u(Nana), Halimatu and Ibrahim (khaleel) for their understanding. To the technical and non-technical staffs of the Department of Pharmaceutical and Medicinal Chemistry, and other well-wishers, thank you all.

ABSTRACT

Metronidazole is an antibacterial and antiprotozoal drug used in the treatment of amebiasis, giardiasis, trichomoniasis and other microbial diseases. It is an essential drug commonly found in Nigerian market and has so many generics and dosage forms under various trade names by different manufacturers. The purpose of the study was to evaluate and compare the *in-vitro* bioequivalence of branded and generic metronidazole tablet in three different physiological media (pH 1.2, 4.5 and 6.8). Six brands of metronidazole tablet were randomly selected from different areas of Zaria town and evaluated for quality via identification test, assay, weight variation test, disintegration time test, friability, crushing strength and dissolution. Three UV spectrophotometric methods were developed, validated and used for determining the amount of metronidazole release during the *in-vitro* dissolution studies. The percentage of metronidazole released was then subjected to *in-vitro* bioequivalence comparison using the model independent method of comparison. The results showed that all brands passed identification an assay test except brand MD and ME that failed the assay test. The tested brands passed weight variation test, disintegration time test, friability and hardness except brand ME which failed the hardness test. Wavelength of maximum absorbance (λ_{max}) of 295nm, 345nm and 350nm were recorded for metronidazole in pH media 1.2, 4.5 and 6.8 respectively, with a linear relation at concentration range of 2-64 μ g/mL. The correlation coefficients were close to unity with percentage recoveries ranging from 98-102% and precision (% CV) <15%. The *in-vitro* dissolution profile studies showed that brand MB and MC are similar with brand MA at difference factor (f_1) ≤ 15 and similarity factor (f_2) > 50 ; therefore, can be used interchangeable with brand MA in all the three simulated physiological media. Also, brand MF had similar dissolution profile with MA in two physiological media (pH 1.2 and 4.5) but not in pH 6.8. However, the dissolution

profile of brand MD and brand ME were not similar with brand MA in all the three media. The percentage dissolution efficiency (DE) of all the brands in all the three media were within the acceptable limit of $\pm 10\%$. It was concluded that the branded MA could be interchanged with brand MB and brand MC in all the three media and with brand MF in pH 1.2 and pH 4.5 but not in pH 6.8 and also not with brand MD and brand ME in all the three media.

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ABBREVIATIONS

AUC	Area under the curve
BA	Bioavailability
BCS	Biopharmaceutical Classification System
BE	Bioequivalence
BP	British pharmacopeia
CV	coefficient of variation
DE	dissolution efficiency
EMA	European Medicine Agency
ER	error
F1	difference factor
F2	similarity factor
FDA	food and drug administration
FTIR	Fourier Transform Infrared
g	gram
ICH	International Conference for Harmonization
IR	infrared

L	litre
mL	millilitre
NAFDAC	National Agency for Food and Drug Administration and Control
nm	nanometre
°C	Degree Celsius
PR	Percentage Recovery
Rpm	Revolution per Minute
RSD.	Relative Standard Deviation
SD	Standard Deviation
UV	Ultraviolet
WHO	World Health Organization
μ	Microgram

CHAPTER ONE

1.0 INTRODUCTION

The term "bioequivalence" refers to the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives become available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study (WHO, 2005). Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent or pharmaceutically alternatives and their bioavailability in terms of peak (C_{max} and T_{max}) and total exposure (AUC) after administration of the same molar dose under the same conditions are similar to such a degree that their effects can be expected to be essentially the same (Birkett, 2003; WHO, 2005). Generic pharmaceutical equivalents should contain the same active ingredient(s) manufactured in the same dosage form and administered in the same pathway (WHO, 2006; Sally and Heba, 2016). Additionally, they should meet the same compendial or other applicable standards and be similar in strength or concentration. (Sally and Heba, 2016). However, they may differ in shape, excipients, release mechanisms, packaging, scoring configuration, and expiration time. (WHO, 2006, Sally and Heba, 2016). Pharmaceutical alternative is same molar amount of the same active pharmaceutical ingredient (API) but differ in dosage form (e.g., tablets vs. capsules), and/or chemical form (e.g., different salts, different esters) and deliver the same active moiety by the same route of administration (WHO, 2016). The concept of interchangeability also includes not only the equivalence of the dosage form but also the indications and instructions for use (WHO, 2005).

In accordance with WHO recommendations, bioequivalence (BE) can be determined by *in-vivo* pharmacokinetic method and, in some cases, by *in-vitro* dissolution test. Human pharmacokinetic

in-vivo studies are often presumed to serve as the 'gold standard' to assess product bioequivalence of immediate release oral solid dosage forms (Polliet *al.*, 2008). However, in some situations *in-vitro* assays are as good as *in-vivo* tests to determine the bioequivalence of an oral solid dosage form and sometimes better in terms of direct evaluation of product performance due to the fact that *in-vitro* studies serve as the better method that leads to reduced costs, directly assesses product performance, offers benefits in terms of ethical considerations (Polliet *al.*, 2008). *In-vitro* studies directly assess product performance than do conventional human pharmacokinetic BE studies, since *in-vitro* studies focus on comparative drug absorption from the two products (Polliet *al.*, 2008).

Dissolution is the main *in-vitro* method used in quality control and recently to determine bioequivalence between certain drug products (Arlene *et al.*, 2014). Hence, dissolution procedure has played many roles including its contribution in drug development, quality assurance and investigation of similarity between the different brands of the same active pharmaceutical ingredient (API) in a formulation (Arlene *et al.*, 2014).

1.1 Biopharmaceutical Classification System

The Biopharmaceutical Classification System (BCS) is a scientific framework that is based on the aqueous solubility and intestinal permeability of a drug substance (Amidon *et al.*, 1995) and has been used as a criterion for biowaiver. When combined with the dissolution of the pharmaceutical product the BCS takes into account three major factors that govern the rate and extent of drug absorption from immediate release oral solid dosage form viz: dissolution, solubility, and intestinal permeability (WHO, 2005). The original purpose of the system was to aid in the regulation of post-approval changes and generics, providing approvals based solely on *in-vitro* data when appropriate (Shravan *et al.*, 2013). Importantly, the system was designed around

oral drug delivery since the majority of drugs are and remained as oral dosage forms (Shravanet *al.*, 2013).

Pharmacological therapy is essential in treatment of many diseases and it is important that the medicine policy is intended to offer safe and effective treatment with affordable price to the population. One way to achieve this is through biowaiver, defined as the replacement of *in-vivo* bioequivalence studies by *in-vitro* studies (Arrunátegui *et al.*, 2015), that is *in-vivo* bioavailability and/or bioequivalence studies may be waived (not considered necessary for product approval) (Kurdi and Karam, 2015). Instead of conducting expensive and time consuming *in-vivo* studies, a dissolution test could be adopted as surrogate basis for the decision as to whether two pharmaceutical products are equivalent (Kurdi and Karam, 2015). Waivers or permission to skip *in-vivo* bioequivalence studies, are reserved for drug products that meet certain requirements around solubility and permeability and that are also rapidly dissolving (Arrunátegui *et al.*, 2015). The aim of biowaiver guidance is to reduce the risk of bioinequivalence to an acceptable level (Kurdi and Karam, 2015). Moreover, biowaiver eliminates unnecessary exposure of healthy subjects to *in-vivo* studies, reduces the burden of evaluating petitions for registration requiring BE studies, and provides economic relief, maintaining the quality standard of dispensed medicines to public health and thus ensuring therapeutic equivalence (Cook *et al.*, 2002). The BCS provides insight into the most important steps in the oral absorption process of drug substances. For instance, low solubility often indicates that the dissolution may be a rate-limiting step, while low permeability suggests a likely challenge for the molecule to cross the intestinal membrane (Shravanet *al.*, 2013). Thus, different formulation strategies are required to address different problems in achieving bioavailability objectives.

The criteria for biowaiver include:

- Immediate-release solid oral dosage form
- Rapid and similar dissolution.
- High solubility and high permeability.
- Wide therapeutic window.
- Excipients used in dosage form are same as those present in approved drug product

The work of Amidonet *al.*,(1995) revealed that the fundamental events controlling oral drug absorption are the permeability of the drug through the gastrointestinal tract (GI) membrane and the solubility/dissolution of the drug dose in the GI environment. These key parameters are characterized in the Biopharmaceutics Classification System (BCS) by three dimensionless numbers:

1. Absorption Number (A_n): It is the ratio of permeability (P) and the gut radius (R) times the residence time (T) in the small intestine, which can be written as the ratio of residence time and absorptive time (t) (Vikaarset *al.*,2012).
2. Dissolution Number (D_n): It is the ratio of the mean residence time (T) to the dissolution time (t), which includes solubility, diffusivity, density and the initial particle radius (Vikaarset *al.*, 2012).
3. Dose number (D_o). It is defined as the mass divided by the product of the uptake volume (250mL) and solubility of the drug (Rohillaet *al.*, 2011).

Table 1.1 Four possible combination categories for a drug according to the BCS

Class	Solubility	Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

BCS class I drugs are well absorbed (>90%) because they are highly permeable and go rapidly into solution. They exhibit high absorption number and high dissolution number (Siyet *al.*, 2015). The rate-limiting step is drug dissolution, and if dissolution is very rapid, then the gastric-emptying rate becomes the rate-determining step (Siyet *al.*, 2015). The drugs dissolve rapidly when presented in immediate release form, and are also transported across the gut wall. Poor absorption of drugs here is only expected if they are unstable or if they undergo reaction (such as binding or complexation) in the intestine that inactivates them (Esperanza and Guillermina, 2014). They are eligible for a biowaiver based on the BCS. Examples include Abacavir, Acetaminophen, Captopril, Metronidazole, Misoprostol (Arcot, 2011).

The requirement of waiver is that the *in-vitro* dissolution reaches at least 85 % within 15 min for both the test and the innovator product in three different buffer solutions or may need to be compared using difference statistics when greater than 85 % is released in 30 min (rapid release) (WHO, 2006). However, a longer time to achieve at least 85% dissolution (not exceeding 30 minutes) is acceptable if the dissolution profiles are similar and the product composition (test and innovator) is very similar (WHO, 2009).

BCS class II drugs are those with solubility too low to be consistent with complete absorption even though they are highly membrane permeable. These drugs have a high absorption number but a low dissolution number (Siya *et al.*, 2015). *In-vivo* drug dissolution is a rate limiting step for absorption except at a very high dose number. The drugs can exhibit variable bioavailability and need enhancement in dissolution for increasing the bioavailability (Siya *et al.*, 2015). The absorption of class II drugs is usually slower than class I and occurs over a longer period of time. Examples include Phenytoin, Ketoconazole, Azithromycin, Carbamazepine, Ciprofloxacin (Arcot, 2011).

BCS class III drugs are highly soluble but have low permeability. In other words, they are unable to permeate the gut wall quickly enough for absorption to be complete. These drugs may receive a biowaiver only if 85% or more of drug content is released in 15 min (very rapid release) in three different buffer solutions (FDA, 2000; WHO, 2006). Examples include; Ranitidine, Amiloride, Amoxicillin, Lisinopril, Metformin (Arcot, 2011).

Drugs in BCS class IV exhibit low dissolution rate and low permeability property and also have slow or low therapeutic action and are not eligible for biowaiver i.e. drugs of this class are problematic for effective oral administration (Siya *et al.*, 2015). Examples: Taxol, Amphotericin B, Furosemide, Hydrochlorothiazide, Neomycin (Arcot, 2011).

A drug substance is considered highly soluble when the highest dose strength is soluble in ≤ 250 ml water over a pH range of 1 to 7.5 (The volume estimates a glassful i.e. 8 ounce). High solubility ensures that solubility is not likely to limit dissolution and therefore, absorption.

A drug substance is considered highly permeable when the extent of absorption in humans is determined to be 85% or more of an administered dose, based on mass-balance or in comparison to an intravenous reference dose. Initial recommendation in the BCS Guidance suggested an

absorption value of $> 90\%$ as a prerequisite for classification as highly permeable. However, successive scientific discussions and scientific publications suggested relaxing the criteria to 85% (WHO, 2005). High permeability ensure that drug is completely absorbed during the limited transit time through the small intestine.

A drug product is considered to be rapidly dissolving when $\geq 85\%$ of the labeled amount of drug substance dissolves within 30 minutes using USP apparatus I or II in a volume of ≤ 900 mL buffer solutions (WHO, 2005). Rapid dissolution ensures that *in-vivo* dissolution is not likely to be the "rate determining" step.

In general, BCS-based biowaiver can be used to address the questions of bioequivalence between a test and a reference product encountered throughout the product development lifecycle, including investigating bioequivalence between early clinical trial products and to-be-marketed products, generic and innovator products, and in the case of post-approval changes that require bioequivalence testing.

1.2 Research Problem

There is growing universal concern regarding counterfeit medications (Nsimba, 2008). In particular, counterfeit antimicrobial drugs are a threat to public health with many devastating consequences for patients; increased mortality and morbidity and emergence of drug resistance (Kelesidis *et al.*, 2007). According to WHO, up to 10% of the drugs worldwide may be counterfeits of which 50% of them involved antimicrobial drugs, and 78% were from developing countries. (Kelesidis *et al.*, 2015). Also, a WHO study of drug product quality in Africa found that 7.6% of major antibiotic formulations contained no active ingredient, whereas 17.8% of antibiotics and 13% of Antiparasitic products were substandard. (Kelesidis *et al.*, 2015). Metronidazole is one of the most prescribed antimicrobial and antiprotozoal medications and has so many generics some of which has been reported to be of inferior quality to the branded (Buowari, 2012). Also, human BE studies are expensive, time consuming and involved use of human volunteers (Polliet *et al.*, 2008).

1.3 Justification

Metronidazole is one of the first line drugs in the WHO model list of essential medicine used for treating various infection. There are many brands of metronidazole in the Nigerian market from different manufacturers with a reliable evidence that some of them are fake, adulterated or substandard (Buowari, 2012). The availability of numerous generics in the market leads to concern by healthcare professionals on selection among available brands to interchange for the reference brand. Hence, the need to evaluate the quality and bioequivalence of the numerous generics available. Metronidazole belong to class I according to BCS hence is a candidate for *in vivo* biowaiver studies.

1.4 Aim and Objectives

1.4.1 Aim

The aim of the study is to evaluate the *in-vitro* dissolution pattern of various brands of metronidazole tablet in comparison to a reference brand as a surrogate to *in-vivo* bioequivalence.

1.4.2 Objectives

To randomly select different brands of metronidazole tablet.

To conduct quality control studies on the selected brands of the metronidazole tablet.

To develop and validate UV method for the determination of metronidazole in three different simulated physiological media (pH 1.2, 4.5 and 6.8).

To carry out dissolution studies on the selected brands in the three physiological media.

To determine the equivalence of the various brands using similarity factor (f_2), difference factor (f_1) and dissolution efficiency (DE).

1.5 Research hypothesis

The various brands of metronidazole tablet available in Zaria are chemically and pharmaceutically equivalent.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Metronidazole

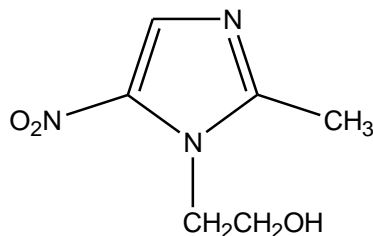


Figure 2.1. Chemical Structure of metronidazole

Metronidazole is a representative antibacterial and antiprotozoal drug that has been synthesized in various laboratories throughout the world. Metronidazole is chemically (2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol) (BP, 2009). It is one of the rare examples of a drug developed against a parasite which has since gained broad use as an antibacterial agent (Samuelson, 1999). In 1953, Hamao Umezawa and colleagues at the University of Tokyo isolated 2-nitroimidazole (azomycin) a fermentation product which was subsequently found by researchers at Rhone-Poulenc in Paris to be active against *Trichomonas vaginalis*, the causative parasite of trichomoniasis (Wright *et al.*, 2014). Azomycin was toxic and difficult to prepare by chemical synthesis, but synthesis and evaluation of a variety of nitroimidazoles led to the discovery in 1957 of a fully synthetic 5-nitroimidazole (metronidazole) which became the first effective drug for the treatment of trichomoniasis. (Wright *et al.*, 2014). In 1962, a woman receiving metronidazole for this indication reported an unexpected side effect to her dentist; clearance of her gum infection. This serendipitous discovery eventually led to the use of metronidazole for the treatment of infections caused by a variety of anaerobic bacteria (including *C. difficile*), for which it is still prescribed today. (Wright *et al.*, 2014). Metronidazole was shown to be

efficacious against *Entamoebahistolytica*, the cause of amoebic dysentery and liver abscess, in 1966. *Giardia lamblia* (also known as *G. duodenalis*) was treated with metronidazole after this luminal parasite was recognized as a cause of malabsorption and epigastric pain in the 1970s (Samuelson, 1999).

2.1.1 Physicochemical properties of metronidazole

IUPAC name: 2-(2-methyl-5-nitroimidazol-1-yl) ethanol

Molecular formula: $C_6H_9N_3O_3$

Molecular Weight: 171.156 g/mol

Dried substance: 99-101.0 per cent

Odour: odourless.

Melting Point: 159-163°C

Appearance: White or yellowish, crystalline powder.

Solubility: slightly soluble in water, in acetone, in alcohol and in methylene chloride.

Stability Stable in air but darkens on exposure to light

pH of saturated aqueous solution: 5.8 metronidazole.

2.1.2 Antimicrobial Mechanism of Action

It is generally agreed that metronidazole is a pro-drug (Thomas *et al.*, 2007) and enters the cell by passive diffusion. It is then activated in either the cytoplasm in bacteria, *Entamoeba*, and *Giardia*, or in a specialized organelle called hydrogenosome in *Trichomonas* (Amber *et al.*, 2015) and that anaerobic organisms reduce the nitro group in metronidazole to a hydroxylamine, as shown in figure 2.2 during which a reactive derivative or reactive species are produced that cause destructive effects on cellular components (i.e., DNA, proteins, and

membranes) (Thomaset *al.*, 2007).

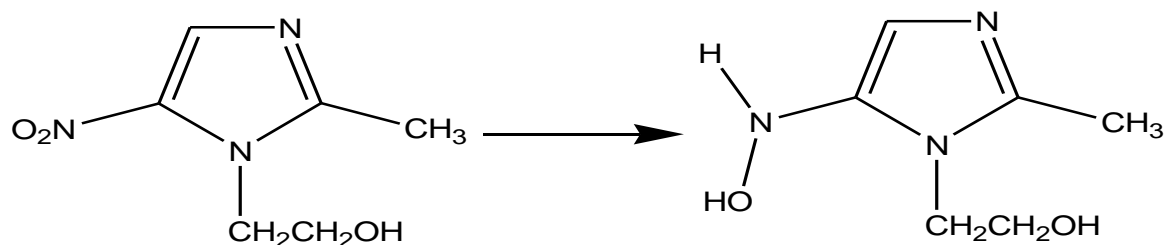


Figure 2.2: Metabolic activation of metronidazole

DoCampo has reported that nitroaryl compounds (nitroimidazoles, metronidazole; nitrofurans, nifurtomox) are reduced to nitro radical anions, which in turn react with oxygen to regenerate the nitroaryl and the superoxide radical anion. (Thomaset *al.*, 2007).

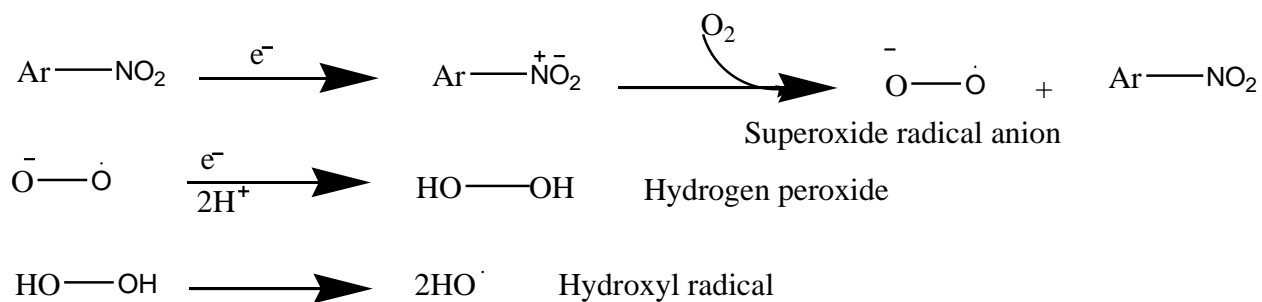


Figure 2.3: Formation of reactive oxygen species (ROS) from nitro aryl compound

Further reduction of superoxide radical anion leads to hydrogen peroxide and homolytic cleavage of the latter leads to hydroxyl radical formation. Superoxide radical anion, hydrogen peroxide, and hydroxyl radicals are referred to as reactive oxygen species (ROS) and are the reactive substances that are implicated in damage to critical cellular components of the parasite (Thomaset *al.*, 2007).

2.1.3 Pharmacokinetic Properties

2.1.3.1 Absorption

Metronidazole is available in a variety of dosage forms, including IV, oral, rectal, and vaginal suppositories. The bioavailability of metronidazole is nearly 100% when administered orally but is significantly less when administered via the rectal route (67-82%) or the vaginal route (19-56%) (Thomas *et al.*, 2007). Following oral administration, Metronidazole is well absorbed, with peak plasma concentrations occurring between one and two hours after administration. Plasma concentrations of Metronidazole are proportional to the administered dose. Studies reveal no significant bioavailability differences between males and females; however, because of weight differences, the resulting plasma levels in males are generally lower (Ashutosh, 2004).

2.1.3.2 Distribution

Metronidazole is the major component appearing in the plasma, with lesser quantities of metabolites also being present. Less than 20% of the circulating metronidazole is bound to plasma proteins (Thomas *et al.*, 2007). Metronidazole appears in cerebrospinal fluid, saliva, and breast milk in concentrations similar to those found in plasma. Bactericidal concentrations of metronidazole have also been detected in pus from hepatic abscesses (Ashutosh, 2004)

2.1.3.3 Metabolism

Liver metabolism of metronidazole leads to two major metabolites: hydroxylation of the 2-methyl group to 2-hydroxymethylmetronidazole (HM) and oxidation to metronidazole acetic acid (MAA), both compounds possess biological activity (Thomas *et al.*, 2007). HM is found in the urine as glucuronide and sulfate conjugates. In addition, a small amount of metronidazole is oxidized to acetamide, a known carcinogen in rats but not in humans, and to the oxalate derivative (Thomas *et*

al., 2007) as shown in Figure 2.4.

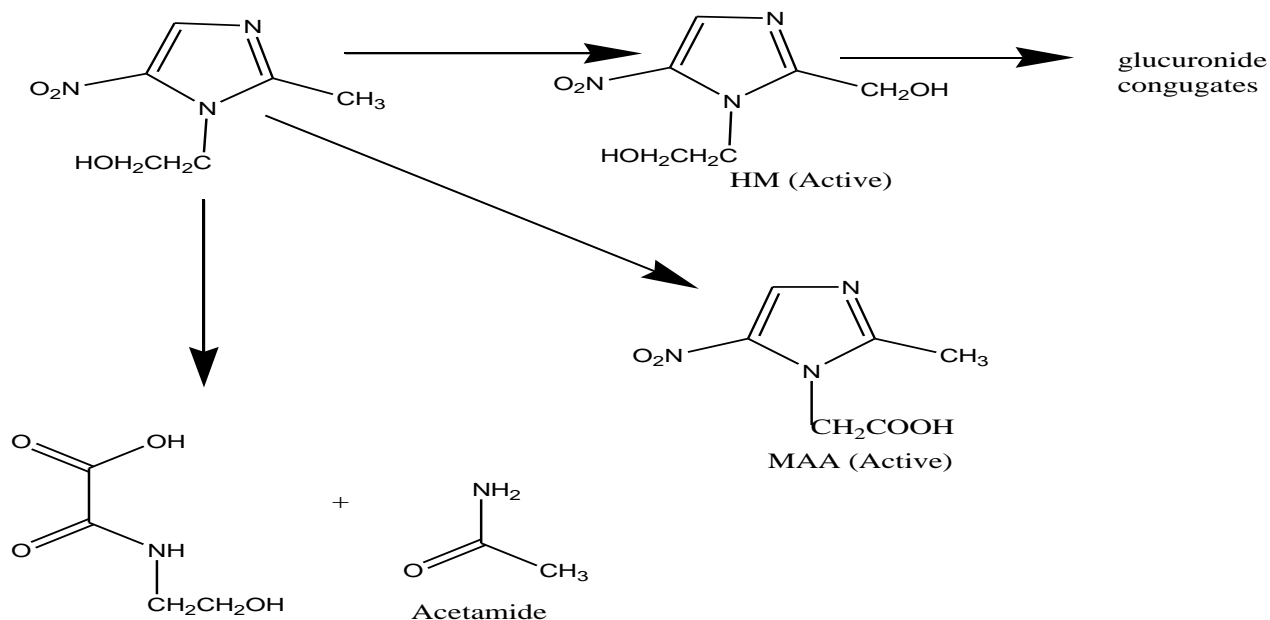


Figure 2.4: Metabolism of metronidazole

2.1.3.4 Elimination

The major route of elimination of Metronidazole and its metabolites is via the urine (60-80% of the dose), with faecal excretion accounting for 6-15% of the dose. The metabolites that appear in the urine result primarily from side-chain oxidation [1-(3-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and 2-methyl-5-nitroimidazole-1-yl-acetic acid) and glucuronide conjugation, with unchanged metronidazole accounting for approximately 20% of the total. Both the parent compound and the hydroxyl metabolite possess *in-vitro* antimicrobial activity. The average elimination half-life of metronidazole in healthy subjects is eight hours.

2.1.4 Uses and Dosage

Metronidazole at a dose of 500-750mg every 8 hours for 5-10 days is considered to be the drug of choice for treatment of protozoal infections amoebiasis (intestinal and extra intestinal), giardiasis (500 mg twice daily for 5-7 days), and trichomoniasis (Oral: 250 mg every 8 hours for 7 days or 375 mg twice daily for 7 days or 2 g as a single dose or 1 g twice daily for 2 doses on same day). It is the drug of choice for treatment of the Gram-positive Bacilli, *Clostridium difficile* and in combination is an alternative therapy for *Helicobacter pylori* infections (Oral: 250-500 mg with meals and at bedtime for 14 days; requires combination therapy with at least one other antibiotic and an acid-suppressing agent (proton pump inhibitor or H₂ blocker)(Thomaset al., 2007). It is used in pelvic inflammatory disease (Oral: 500 mg twice daily for 14 days (in combination with a cephalosporin and doxycycline)), Dracunculus (guinea worm) and as alternative drug to treat, balantidiasis, blastocystitis, and infections by *Entamebapolecki*. It is also used widely for the treatment and prophylaxis of infections caused by anaerobic bacteria(at a dose of 500 mg every 6-8 hours, not exceeding 4g per day). It is a drug of choice against GI strains of *Bacteroidesfragilis* and vaginal infections caused by *Gardnerellavaginalis*(Oral: 500 mg twice daily or 750 mg once daily for 7 days). It has been used successfully in the treatment of antibiotic-associated pseudomembranous colitis(500mg orally 3 times/day for 10-14 days)for mild-to-moderate infection or intravenous formulation (I.V) with oral vancomycin for severe complicated infection. And very useful in Crohn's disease (I.V; 10-20 mg/kg/day)and surgical prophylaxis (Ashutosh, 2004).

2.1.5 Toxic Effects

The common side effects exhibited by metronidazole include abdominal distress, a metallic taste, and a disulfiram-like effect if taken with alcohol (Thomaset al., 2007). Other gastro-intestinal

disturbances (including nausea and vomiting), furred tongue, oral microsites, anorexia; very rarely hepatitis, jaundice, pancreatitis, drowsiness, dizziness, headache, ataxia, psychotic disorders, darkening of urine, thrombocytopenia, pancytopenia, myalgia, arthralgia, visual disturbances, rash, pruritus, and erythema multiforme; on prolonged or intensive therapy peripheral neuropathy, transient epileptiform seizures, and leucopenia (BNF 65, 2013). The drug is reported to be carcinogenic in mice, possibly related to the metabolite acetamide, and as a result should not be used during the first trimester of pregnancy (Thomas *et al.*, 2007).

2.1.6 Synthesis

2.1.6.1

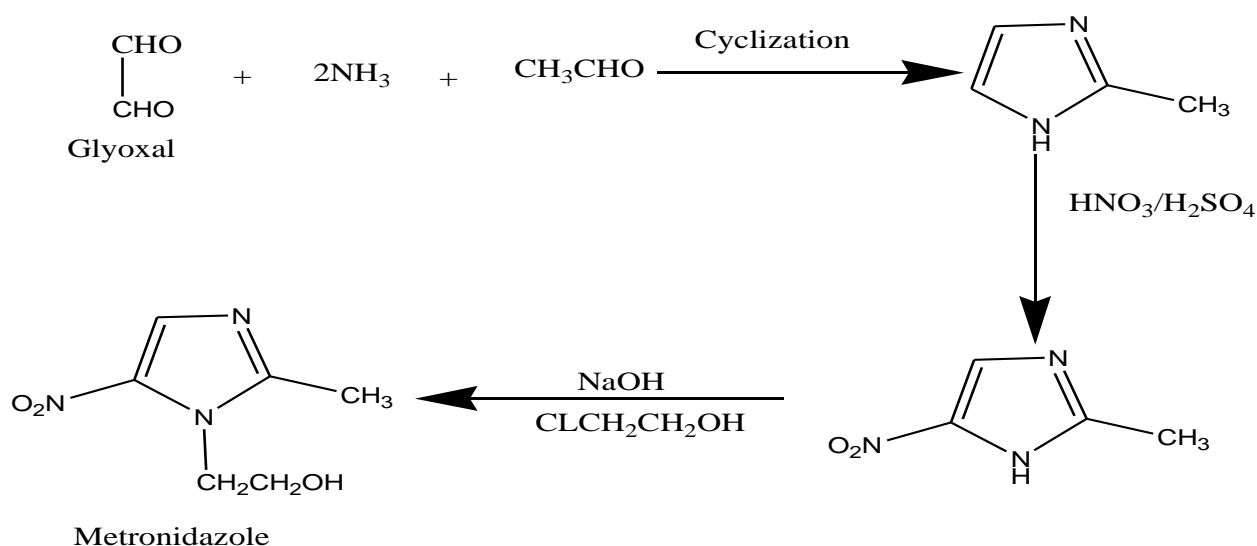


Figure 2.5: Synthesis of metronidazole from Glyoxal(Alagarsamy, 2010)

2.1.6.2 The reaction between 2-methyl-5-nitroimidazole and ethylene chlorohydrin at an elevated temperatures ranging from 128-130°C for a period of 18 hours results into the formation of metronidazole with the elimination of one mole of HCl. (Ashutosh, 2004).

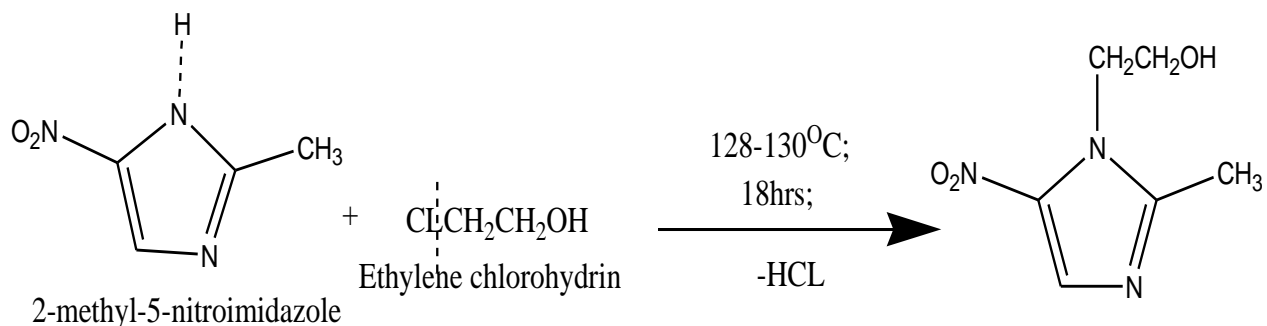


Figure 2.6: Synthesis of metronidazole from 2-methyl-5-nitroimidazole.

2.1.7 Structure Activity Relationship

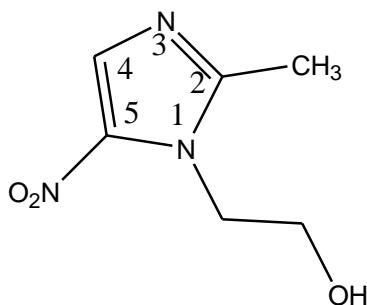


Figure 2.7: Structure activity relationship of metronidazole

The nature of substituents and the position of the nitro group of the nitroimidazole group of antimicrobials are responsible for various pharmacological activities. The presence of different side chains at the position 1 as in Tinidazole and Ornidazole do not differ markedly in their antimicrobial activity (Mital, 2009). A nitro group is essential for both aerobic and anaerobic activities of both 4- and 5-nitroimidazole but the position of the nitro group in metronidazole is important for anaerobic activity (Kim *et al.*, 2009). The compounds with nitro group at position 4 are usually less active than the corresponding 5-nitro derivatives (Mital, 2009). Modifications at the position 2, however, are known to interfere with both the activity

and the microbial spectrum. A Compound which has an imidazolidinone ring structure for example at the position 2 exerts stronger antitrichomonal activity than metronidazole and is highly active against anaerobic bacteria (Mital, 2009). Thus, the modification of the 5-nitroimidazole at the position 2 increases not only its antitrichomonal activity but also its antibacterial activity (Mital, 2009).

The key determinants of aerobic activity in the 4-nitroimidazoles include the bicyclic oxazine, the lipophilic tail, and the oxygen at position 2 (Kim *et al.*, 2009). For the 5-nitroimidazoles, neither the corresponding bicyclic analogs, nor addition of a lipophilic tail conveyed aerobic activity (Kim *et al.*, 2009).

2.1.8 Quantitative Methods for Analysis of metronidazole

2.1.8.1 Official method

Metronidazole is officially reported in British Pharmacopoeia (BP, 2009), United State Pharmacopeia (USP, 2007), Indian Pharmacopeia (IP, 2007), Japanese Pharmacopeia (JP, 2006) and other Pharmacopoeias. The USP, Japanese and Indian Pharmacopoeias described infrared absorption spectrophotometry, ultraviolet spectrophotometry, melting point, thin layer and potentiometric titration. The BP described infrared absorption spectrophotometry as the first identification test for metronidazole followed by melting point, UV and HPLC.

Ultraviolet spectroscopy:

The BP recommend dissolving 40mg of metronidazole in 0.1M hydrochloric acid (HCL) and then diluting it to 100mL. From the solution, 5mL will again be diluted to 100mL and then examine between 230-350nm, the solution shows absorbance maximum at 277nm and a minimum at 240nm (BP, 2009).

2.1.8.2 Reported UV spectroscopic methods of analysis from literature

Literature review showed that several UV spectroscopic methods have been developed for quantifying or estimating metronidazole in bulk, pharmaceutical formulation and biological fluids. Mastanamma *et al.*, 2015 reported measuring absorbance of metronidazole in acidic (0.1N HCL) and basic (0.1N NaOH) medium, with maximum absorbance at 278nm and 320nm respectively and the calibration curves were linear at concentration range of 2-14µg/L. Metronidazole has a maximum absorbance at 318nm in Methanol and Water (50:50) solvent with linear relation at concentration range of 1-13µg/mL as reported by Niravet *et al.*, 2012. Naveed and Qamar (2014) also reported assay of metronidazole based on the UV absorbance maxima at about 340nm using water as solvent. Several other methods have been accounted, for example spectrophotometry (mostly in visible region) and polarography (Naveed *et al.*, 2014)

Metronidazole can be determined alone or in addition to its mixture with other drugs or in the presence of its degradation product or in the presence of its metabolites with the help of HPLC method (Naveed and Qamar, 2014).

2.2 Brand-named and Generic Drugs

Generic medicines are those produced without a license from the innovator company when the patent or other market exclusivity rights on the innovator product has expired (WHO, 2016) i.e. it is a copy that is the same as brand name (Innovator) product. A generic drug is identical or bioequivalent to a brand name drug in dosage form, safety, strength, route of administration, quality, performance characteristics and intended use (Medsafe, 2013). Once innovator patents and exclusivity periods expires, generic companies can market their product by proving equivalence of bioavailability (bioequivalence/relative bioavailability) with innovator. Generic drugs

contained the same active ingredients present in brand-named drug, but often differ in peripheral features that do not impact their bioavailability (Chawla *et al.*, 2014). While generic drugs have been noted to be comparable to brand-named drug in their ability to treat conditions, their bioavailability, or the concentration of the drug that reaches site of action has continued to be debated (Chawla *et al.*, 2014). The possible difference between generic and innovator product is drug particle size, polymorphic form, excipients, manufacturing process equipment, site of manufacturing, batch size etc. (Arcot, 2011). Many experts continue to believe that generic and brand-named drug are bioequivalent and equally viable option for effective drug treatment (Chawla *et al.*, 2014). While both brand-named and generic drug companies in the US must apply for FDA approval before being allowed to sell their drugs to the public, the former is required to undergo pre-clinical and costly three phase clinical testing in order to portray drug safety and efficacy (Chawla *et al.*, 2014). However, the latter is only required to undergo bioequivalence testing, or testing of pharmacokinetic properties, accounting for a significant discrepancy in expenditures between brand-named and generic drug and a subsequent inflation in the pricing of brand-named equivalents (Chawla *et al.*, 2014). Pre-formulation studies include drug-excipient compatibility, polymorphic studies to be conducted to ensure that the generic product possesses equivalent and sometimes even superior stability characteristic to the innovator brand. Dissolution specification should be same between generic and innovator.

2.3 Substandard/Counterfeit/Fake Drug

The counterfeiting of all manner of products is on the rise globally (Kelesidiset *al.*, 2007). In Nigeria today, there is counterfeiting of documents and currency amongst many others. However, no other product has the capacity to harm, as much as kill its consumers, as do illicit

pharmaceuticals (Akinyandenu, 2013). The loose control system in the Nigerian economy has contributed to the circulation of these fake and counterfeit drugs. Counterfeit medicines are part of the broader phenomenon of substandard pharmaceuticals – medicines manufactured below established standards of safety, quality and efficacy. They are deliberately and fraudulently mislabeled with respect to identity and/or source (WHO, 2011). Substandard medicines may be described as genuine drug products which do not meet the required quality specifications. (Glass, 2014). Counterfeiting can apply to both brand-named and generic products and may include products with the correct ingredients but fake packaging, with the wrong ingredients, without active ingredients or with insufficient active ingredients (Kelesidiset *al.*, 2007). The World Health Organization has reported that counterfeit medicines potentially make up more than 50% of the global drug market, with a significant proportion of these fake products being encountered in developing countries (Glass, 2014). This occurrence is attributed to a lack of effective regulation and a weak enforcement capacity existing in these countries, with an increase in this trade resulting from the growing size and sophistication of drug counterfeiters (Glass, 2014). Antibiotics, antituberculosis drugs, antimalarial and antiretroviral drugs are frequently targeted, with reports of 60% of the anti-infective drugs in Asia and Africa containing active pharmaceutical ingredients outside their pharmacopoeial limits (Glass, 2014). Counterfeit drugs in Nigeria include preparations without active ingredients, toxic preparations, expired drugs that are relabeled, drugs issued without complete manufacturing information and drugs that are unregistered with the National Agency for Food and Drug Administration and Control (NAFDAC) (Akinyandenu, 2013). The most common type of substandard/counterfeit drugs have reduced amount of the active drug, and the majority of them are manufactured in Southeast Asia and Africa. The work of Akinyandenu (2013) revealed an estimate of 10% prescription drugs

worldwide as counterfeits, fake or contaminated, and in parts of Africa and Asia, the figures exceed 50%. Most genuine drugs are expensive and counterfeiters take advantage of supplying cheap fake drugs to consumers, especially those who cannot afford the high priced good quality version (Chiwendu, 2008). The high cost of drugs allows for the proliferation of counterfeit drugs in Nigeria and poses a major challenge to both patient and public health. Fake drug proliferation has led to treatment failures, organ dysfunction or damage, worsening of chronic disease conditions, side effect and death of many Nigerians (Kelesidiset *al.*, 2007). Even when patients are treated with genuine drugs, no response is seen due to resistance caused by previous intake of fake drugs (Akunyili, 2004). Fake drugs also deny the Nigerian people the right to safe, effective and quality medicines. These effects of counterfeit drugs on patient are difficult to quantify and are mostly hidden in public health statistics because there are no reliable data on the mortality and morbidity resulting from the consumption of counterfeit drugs in Nigeria (Erhunet *al.*, 2001). Also, patients may lose confidence in health care professionals including their physician and pharmacist, and potentially modern medicine or the pharmaceutical industry in general. Hence, it is a threat to public in terms of trade relations, economic implications, and global pandemics (Nsimba, 2008).

2.4 Dissolution

Dissolution is defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition, i.e. mass transfer from the solid surface to the liquid phase (Guoet *al.*, 2000). It is the process of extracting the API out of the dosage form solid-state matrix into solution within the gastrointestinal tract (Courtney and Bethlehem, 2010).

Dissolution testing is a requirement for all solid oral dosage forms and is used in all phases of development for product release and stability testing. It is a key analytical test used for detecting physical changes in an active pharmaceutical ingredient (API) and in the formulated product (Courtney and Bethlehem, 2010.). Another aim of dissolution testing is to guarantee the quality of the pharmaceutical product and prove consistency from one batch to another and that no important change occurs during the stability study. Any change in dissolution could impact on the efficacy of the pharmaceutical product (Adilet *al.*, 2016). Active pharmaceutical ingredients included in a dosage form must be released and dissolved prior to absorption (Esperanza and Guillermina, 2014). Thus, dissolution studies may be related to the bioavailability of the drugs in the body. Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilisation of the drug under physiological conditions, and the permeability, across the gastrointestinal tract (Courtney and Bethlehem, 2010.). Because of the critical nature of the first two of these steps, *in vitro* dissolution may be relevant to the prediction of *in vivo* performance. The rate at which poorly water-soluble drugs are dissolved in the gastrointestinal tract from the dosage form is correlated with the rate of systemic absorption. Therefore, the *in vitro* dissolution test has become the most suitable tool to predict the way a drug product behaves *in vivo* (at least for highly permeable drugs) (Esperanza and Guillermina, 2014).

The compendial apparatus for dissolution as per United States Pharmacopeia (USP) are: Apparatus 1 (rotating basket), Apparatus 2 (paddle assembly), Apparatus 3 (reciprocating cylinder), Apparatus 4 (flow-through cell), Apparatus 5 (paddle over disk), Apparatus 6 (cylinder), Apparatus 7 (reciprocating holder) (Tiwari *et al.*, 2016). But the most commonly employed dissolution test apparatus are;

- The basket method (Apparatus 1)
- The paddle method (Apparatus 2)

Dissolution medium volume of 900 ml or less in each of the following:

- 0.1N HCl or simulated gastric fluid (SGF) USP
- A pH 4.5 buffer
- A pH 6.8 buffer or simulated intestinal fluid (SIF) USP

The dissolution profile comparison may be carried out using one of the following;

2.4.1 Model-independent methods

These methods were proposed by Moore and Planner to compare the dissolution profiles between pharmaceutical products (Arcot, 2011). The methods compare dissolution profiles without fitting the data to an equation that represents them. This includes mathematical methods like the difference factors (f1) and similarity (f2) or the Rescignoindexes along with the statistical comparisons of parameters obtained from the profiles, such as the area under the curve (AUC) and dissolution efficiency (DE). (Esperanza and Guillermina, 2014). The difference factor (f1) calculates the Percent (%) difference between the two curves at each time point and is a measurement of the relative error between the two curves (Hassouna *et al.*, 2012). The similarity factor (f2) 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 (Hassouna *et al.*, 2012). The f1 value should be >15 to indicate difference between two dissolution profiles, f2 value should be between 50-100 to indicate similarity between two dissolution profiles (Arcot, 2011) and when the two profiles are identical, f2 is equal to 100. An average difference of 10% at all measured time point's results in an f2 value of 50 (Wagh and Patel, 2010).

2.4.2 ANOVA-based statistical methods

These methods treat the percentage dissolved as a random variable to perform the analysis of variance, if the formulation is considered as a single class variable (one-way ANOVA) a time-to-time comparison can be performed, or when formulation and the time are considered as class variables (two-way ANOVA) under the null hypothesis of similarity.

2.4.3 Model-dependent methods

These methods for profile comparison rely on a previous stage of fitting dissolution data to an equation that describe its temporal evolution. After the data have been fit, they can be compared with several statistical methods, such as Hotelling's T square test and the "Regions of Similarity" method.

$$T^2 = \left[(\bar{X}_1 - \bar{X}_2) \cdot \left(S_p \cdot \left(\frac{1}{n_1} + 1/n_2 \right) \right)^{-1} \cdot (\bar{X}_1 - \bar{X}_2) \right]$$

$$F = \{[n_1 + n_2 - p - 1]/[p \cdot (n_1 + n_2 - 2)]\} \cdot T^2$$

$$\approx F_{(p; n_1 + n_2 - p - 1)}$$

CHAPTER THREE

3.0 MATERIAL AND METHOD

3.1 Materials

3.1.1 Drugs

Standard metronidazole powder obtained from Juhel Pharmaceutical Ltd.

Six brands of metronidazole tablets (200mg) purchased locally.

3.1.2 Glass wares and other accessories

250ml Extraction tubes (Pyrex England)

250ml conical flasks (Pyrex England)

100ml conical flasks (Pyrex England)

100ml measuring cylinders (Pyrex England)

50ml beakers (Pyrex England)

25ml beakers (Pyrex England)

10ml Test tubes (Pyrex England)

100ml volumetric flasks (Pyrex England)

50ml volumetric flasks (Pyrex England)

25ml volumetric flasks (Pyrex England)

Porcelain pestle and mortar

Stop watch (from mobile phone)

Filter papers

Aluminum foil

Tissue paper

3.1.3 Equipment and Instruments

Analytical weighing balance (Mettler Analytical Balance Phillip Harris., England)

Erwekafriabilator (Type TA-3R, GmbH, Germany)

Monsanto Hardness tester (Manesty Machines Liverpool, England),

Erweka Disintegration Time Test apparatus (Type ZT3, GmbH, Germany)

pH meter (Fisher Scientific, Singapore)

Dissolution test Machine (Tianjin Guoming Medicinal Equipment co. LTD., China)

UV spectrophotometer model (MNF, HeliosZeta, Thermo Scientific England)

Infrared spectrophotometer (Model. Cary 630, Agilent Technology Germany)

Melting point apparatus (Electro thermal UNID, England)

3.1.4 Reagents

Distilled water

Concentrated HCl (BDH Chemical, England)

Sodium hydroxide pellets (BDH Chemical, England)

Chloroform (BDH Chemical, England)

Sulphuric acid (BDH Chemical, England)

Acetone (BPH Chemical, England)

Acetic acid anhydride (Sigma Aldrich)

Monobasic potassium phosphate (BDH Chemical, England)

Sodium acetate (BDH Chemical, England)

Picric acid solution

0.1M perchloric acid

Brilliant green (1% w/v)

3.2 Methods

3.2.1 Sampling and coding of metronidazole tablet (200mg)

A survey of Metronidazole (200mg)tablets available within Zaria metropolis in Kaduna State was conducted in two hospital pharmacies, three community pharmacies and five patent medicine vendor shops. A total of twenty-six brands of Metronidazole were found to be available out of which six brands were randomly selected. The selected samples were coded as MA, MB, MC, MD, ME & MF with MA representing the reference brand. The label information on each sample such as manufacturer's addresses, batch number, NAFDAC registration number, manufacturing and expiry dates were examined and recorded.

3.2.2Identification test of pure metronidazole powder

3.2.2.1 Melting point determination

Small quantity of the standard metronidazole powder was filled into capillary tube by tapping and placed into the melting point determination machine and examined until the powder begins to melt (BP, 2009). The initial and final temperature were then recorded.

3.2.2.2 Infrared spectrophotometry

A small quantity of the standard metronidazole powder was analyzed in the mid IR region (650-4000 cm^{-1}) at a resolution of 8 cm^{-1} with 16 scans using FTIR Carry Agilent technologies (Appendix ii)

3.2.3Identification test for metronidazole tablets.

3.2.3.1 Melting point determination of the tablet

Twenty tablets from each brand were powdered and a quantity equivalent to 0.2g of metronidazole was weighed and extracted with 4mls of 0.5M sulfuric acid and filtered. Picric acid (10ml) was added to the filtrate and allowed to stand for precipitate formation. The precipitate was then washed with distilled water and dried. Small quantity of the extracted powder from all

the brands was filled into capillary tube by tapping and placed into the melting point determination machine and examined until the powder melted, the initial and final temperature were then recorded (BP, 2009).

3.2.3.2 Infrared spectrophotometry of metronidazole tablets

Metronidazole (200mg) tablets were powdered and a quantity equivalent to 0.1g metronidazole was weighed and extracted with 40mls of chloroform for 15mins. This was then filtered and the filtrate evaporated to dryness (BP, 2009). A small quantity of the dried powder (filtrate) was analyzed in the mid IR region ($650\text{-}4000\text{ cm}^{-1}$) at a resolution of 8 cm^{-1} with 16 scans using FTIR Carry Agilent technologies (Appendix iii-ix).

3.2.4 Assay of metronidazole tablet

Metronidazole (200mg) tablets were powdered and a quantity equivalent to 0.2g metronidazole was weighed and transferred into a conical flask and extracted with six 10ml of hot acetone. The combined extract was then cooled and reconstituted with 50ml anhydrous acetic acid. Two drops of brilliant green indicator were added followed by titration using 0.1M perchloric acid to a yellowish green end point. The process was repeated without the powdered tablet, and the difference between the titrations was reported as the amount of perchloric acid used (BP, 2009).

3.2.5 Uniformity of weight test

Twenty tablets from each sample were randomly selected and the individual weight of each tablet was measured in milligram using digital electronic balance (USP, 1995), and from these data mean weight for each brand as well as the percentage deviation from the mean value were calculated using excel 2016.

3.2.6 Crushing strength test

Five (5) tablets from each sample were randomly selected and the force required to break each tablet using Monsanto Hardness tester was recorded. Mean hardness and standard deviation were calculated using excel 2016.

3.2.7 Friability test

Ten (10) tablets from each sample were carefully weighed and subjected to abrasion by means of Erwekafriabilator at 25 revolutions per minute for 4 minutes. The tablets were then de-dusted, reweighed and the difference in tablet weight was determined. Percentage friability was then calculated using the formula;

$$\text{Percentage friability} = \frac{(\text{Initial weight} - \text{final weight})}{\text{Initial weight}} \times 100$$

3.2.8 Disintegration test

Six tablets from each sample were randomly selected and subjected to disintegration by means of Erweka disintegration apparatus in distilled water (approx. 900mL), at $37 \pm 0.5^\circ\text{C}$. The time taken for each tablet to break up and pass completely through the mess was recorded.

3.2.9 UV Spectrophotometric methods development

3.2.9.1 Preparation of simulated physiological media

Preparation of simulated gastric pH (pH1.2):

A volume (100mL) of 2M hydrochloric acid was measured using a measuring cylinder. It was then transferred into volumetric flask containing sufficient amount of distilled water and the volume made up to 2L with distilled water.

Preparation of acetate buffer (pH 4.5):

Sodium acetate 2.99g was weighed and transferred into a volumetric flask containing sufficient amount of distilled water. 14mL of acetic acid solution was measured and added, and then sufficient quantity of water was added with shaking. The volume was finally made up to 1L with distilled water to obtain solution of pH 4.5 (USP 35- NF 30).

Preparation of phosphate buffer (pH 6.8):

Monobasic potassium phosphate 6.8g was weighed and dissolved in sufficient amount of distilled water and was transferred to volumetric flask. 77mL of 0.2 sodium hydroxide was measured and added into the same container and the volume made up to 1L. The pH was adjusted with 0.2N NaOH and 0.1N HCl.

3.2.9.2 Preparation of stock solutions

Stock solutions (100 μ g/mL) were prepared by dissolving 10mg of standard metronidazole powder in 100 mL of each of the prepared simulated physiological media (pH 1.2, 4.5 and 6.8).

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

Solutions [16 μ g/mL) were prepared from each of the stock solutions and then scanned at 400-200 nm in order to obtain the wavelength of maximum absorption in each media.

3.2.9.4 Construction of calibration curve

A six point's calibration curve of metronidazole in each of the media was constructed by preparing solutions of concentration range 2-64 μ g/mL by serial dilution of each stock solution. The absorbances obtained after scanning (using the λ_{max} of each media) were plotted against their concentrations on Microsoft excel 2016.

3.2.10 Validation of the developed methods

Each of the developed method was validated for linearity, precision, accuracy and percentage recovery, LOD and LOQ in accordance with ICH guideline.

3.2.10.1 Linearity

This was established by least square, coefficient of determination (r^2) and standard deviation (SD) at the intercept on y-axis were computed using LINEST function on Microsoft office Excel 2016.

Limit of detection (LOD) and limit quantification (LOQ):

LOD and LOQ were determined using the following formulae;

$$\text{LOD} = 3.3 \sigma / S \quad \text{LOQ} = 10 \sigma / S$$

Where σ = standard deviation at intercept on y-axis and S = slope of the curve

3.2.10.2 Precision

This was carried out by determining the absorbance of a 16 μ g/ml solution of metronidazole in each of the simulated physiological media six times at an hour interval within the same day for intraday and daily for three consecutive days (inter day).

3.2.10.3 Accuracy and percentage recovery

Five milliliters (5mL) of a 10 μ g/mL solution of Metronidazole was measured and transferred into four labeled 10 ml volumetric flask A-D. Volumetric flask B, C, and D were spiked with 2.6, 3 and 3.4mL of the stock solution to obtain three concentration levels of 80,100 and 120 %respectively and A was left unspiked. Absorbance was taken in triplicates and the mean percentage recovery was calculated using percentage recovery and percentage relative error.

$$\text{Percentage recovery} = \frac{(\text{Conc. of spiked} - \text{Conc. of unspiked})}{\text{Conc. of spiked}} \times 100$$

$$\% \text{ Relative error} = \frac{\text{amount added} - \text{amount obtained}}{\text{amount added}} \times 100$$

This was carried out for solutions of metronidazole in each of the simulated physiological media.

3.2.11 *In-vitro* dissolution studies

One tablet from each sample was subjected to the dissolution medium (900ml) after preheating it to $37 \pm 0.5^\circ\text{C}$. The basket speed was maintained at 100 revolutions per minute (rpm). Two (2mL) sample was withdrawn and was replaced by equal volume of the medium at time intervals of 5, 10, 15, 20, 30, 45 and 55 minutes. One (1 ml) of the aliquot solution was quantitatively taken in to 10 mL volumetric flask and diluted to volume with the dissolution medium and absorbance was measured using UV spectrophotometer.

The absorbance values obtained from each of the simulated physiological media were converted to concentrations and percentage content release. The percentage content released from each media was statistically analyzed for *in-vitro* bioequivalence using the model-independent methods: difference factor (f1), similarity factor (f2), Dissolution efficiency (D.E) using the following formulas; (where R_t and T_t are the average percentages dissolved at time t of the reference and test products, respectively where $\%D_t$ is the percentage dissolved at time t, $\%D_{\max}$ is the maximum dissolved at the final time T, and AUC_{0-T} is the area under the curve from zero to T).

$$f_1 = 100 \cdot \left[\frac{\sum_{t=t_1}^{t_n} (R_t - T_t)}{\sum_{t=t_1}^{t_n} R_t} \right], f_2 = 50 \cdot \log \left\{ \left(1 + \frac{1}{n} \cdot \sum_{t=t_1}^{t_n} (R_t - T_t)^2 \right)^{-0.5} \cdot 100 \right\}$$

$$DE = \left\{ \left(\int_{t_1}^{t_2} \%D_t \cdot dt \right) / (\%D_{\max} \cdot (t_2 - t_1)) \right\} \cdot 100 =$$

$$[AUC_{0-T} / \%D_{\max} \cdot T] \cdot 100$$

CHAPTER FOUR

4.0 RESULT

4.1 Quality control of samples

4.1.1 Label information and physical characteristic of metronidazole

The label information of the various brands and their physical appearances are presented in table 4.1 and 4.2 respectively.

Table 4.1: Label information of six brands of metronidazole tablet (200mg)

Code	Source	NAFDAC Reg. no	Batch number	Manufacturing date	Expiry date
MA	Nigeria	04-0283	AI 0071	Jan., 2017	Dec., 2021
MB	Nigeria	04-0412	4511W	Nov., 2017	Nov., 2020
MC	Nigeria	04-0963	A7330	Nov., 2017	Oct., 2020
MD	Nigeria	04-9936	1711	Oct., 2017	Oct., 2022
ME	Nigeria	04-8426	UGT6349	Sep., 2016	Aug., 2019
MF	Nigeria	04-0386	0164	Aug., 2016	Aug., 2020

Table 4.2: Physical appearance of metronidazole tablet

Brand code	Colour	Shape	Nature of surface	Lustre
MA	White	Round	Smooth	Dull
MA	White	Round	Smooth	Dull
MC	Yellow	Round	Smooth	Dull
MD	White	Round	Smooth	Dull
ME	Yellow	Round	Smooth	Dull
MF	White	Round	Smooth	Dull

4.1.2 Identification test of metronidazole standard powder

The melting point of the standard metronidazole powder was found to be 159-161°C and the superimposed FTIR spectra of the standard metronidazole powder and the reference metronidazole (BP, 2009) is shown in figure 4.1.

4.1.3. Identification of metronidazole tablets

The melting point of the various brands of metronidazole tablet are shown in table 4.3 while the superimposed FTIR spectra of the standard metronidazole powder and the reference (BP, 2009) is shown in figure 4.2.

4.1.4 Assay and biopharmaceutical studies of metronidazole tablets

The percentage content of the various brands of metronidazole are shown in table 4.4 while the weight variation test is shown in table 4.5. The friability and hardness test results are shown in table 4.6 and that of disintegration time is shown in table 4.7.

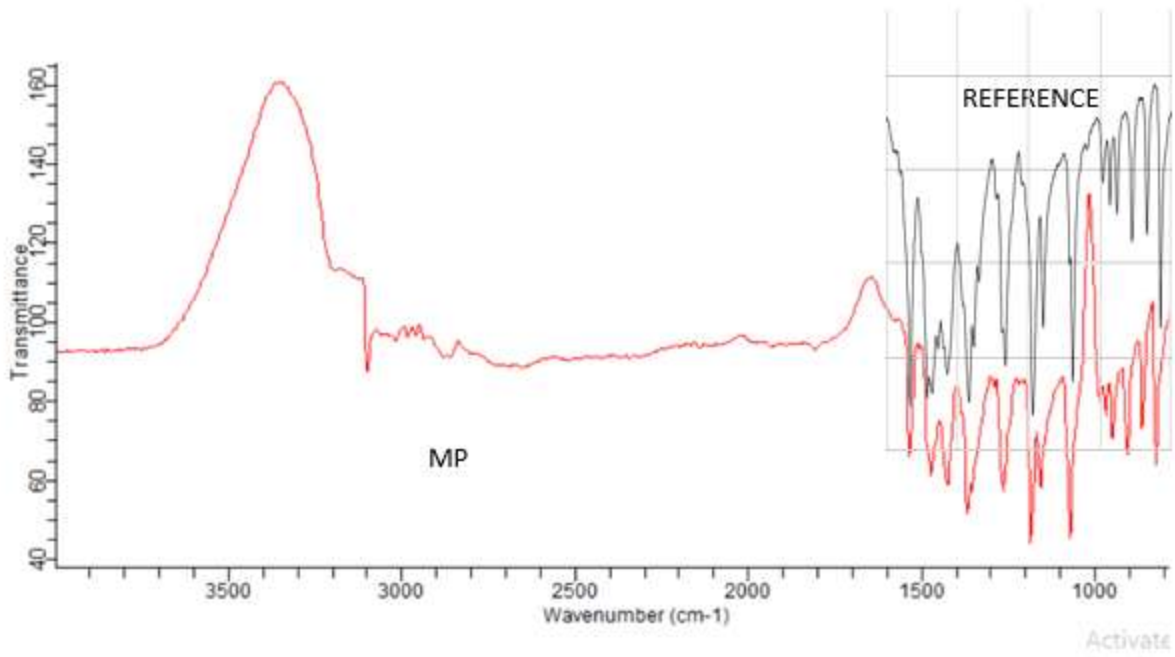


Figure 4.1: Superimposed FTIR spectra of reference and standard metronidazole powder

Table 4.3: Melting point of metronidazole tablets

S/N	Brand	Melting point(°C)
1	MA	160-162
2	MB	159-161
3	MC	159-162
4	MD	160-163
5	ME	160-162
6	MF	159-161

limit 159-163°C as stated in BP 2009

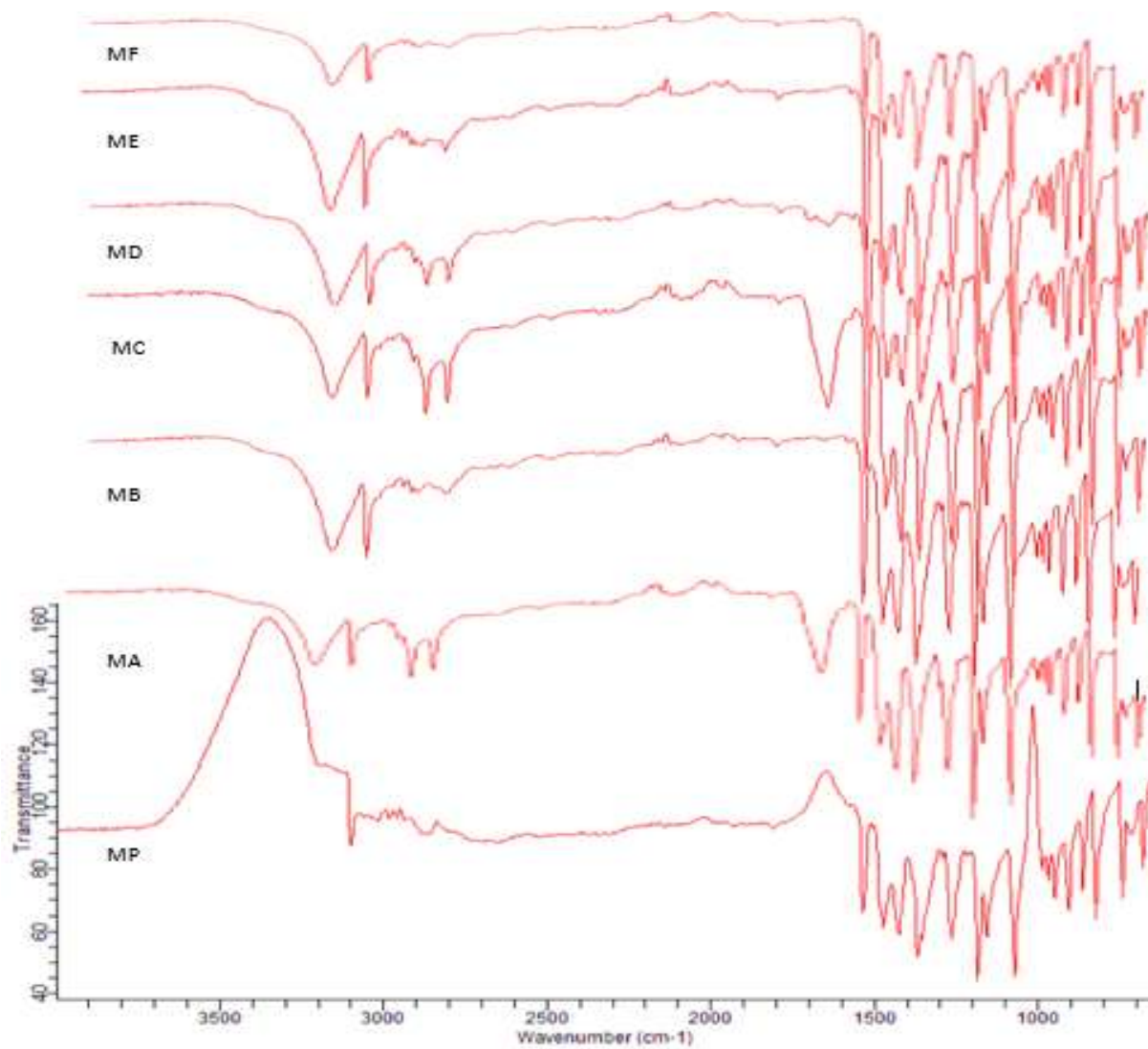


Figure 4.2: Superimposed FTIR Spectra of metronidazole standard powder with the various brands.

Table 4.4: Percentage content of metronidazole assayed in the sample

Brand code	Labeled content (mg)	Quantity taken for assay (g)	Quantity for present (mg)	Percentage content (%)
MA	200	0.2	203.7	102
MB	200	0.2	195.2	98
MC	200	0.2	196.9	99
MD	200	0.2	179.8	90*
ME	200	0.2	183.2	92*
MF	200	0.2	193.5	97

*outside the official limit 95-105%

Table 4.5 Weight variation (uniformity of weight) of metronidazole tablets

Brand code	Mean weight(g) (n=20)± percentage deviation	Remark
MA	0.491±2.1	Passed
MB	0.498±0.84	Passed
MC	0.3285±0.81	Passed
MD	0.315±1.65	Passed
ME	0.432±2.16	Passed
MF	0.312±0.96	Passed

% deviation not to be >7.5 or >5 for tablet weighing between 130-324mg or >324mg respectively (USP, 1995).

Table 4.6: Friability and hardness of metronidazole tablets

Brand code	Hardness± SD	Friability
MA	7.0±1.17	0.00
MB	6.3±0.97	0.25
MC	4.5±0.35	0.30
MD	9.8±0.57	0.31
ME	11.9±0.65*	0.00
MF	7.0±0.61	0.33

Hardness 4-10kg (Orgahet *al*, 2002), friability ≤1%

Table 4.7: Disintegration time (min) of metronidazole tablets

Brands	1	2	3	4	5	6
MA	00:25	00:50	00:50	00:51	1:10	1:10
MB	00:25	00:25	00:30	00:30	00:30	00:30
MC	1:40	1:55	1:55	2:50	2:50	2:25
MD	1:00	1:15	1:20	1:30	1:30	1:35
ME	10:20	10:50	11:40	12:00	14:00	14:00
MF	00:22	00:25	00:25	00:30	00:35	00:35

Disintegration time of uncoated tablet in water at 37°C should not exceed 15min while 30min for coated tablet (BP, 2009)

4.2 Analytical Method

The wavelengths of maximum absorption of metronidazole in the three simulated physiological media are presented in table 4.8 while the calibration curves are shown in figure 4.3, 4.4 and 4.5. The summary of the calibration curve parameters of the developed methods is presented in table 4.9 while validation parameters are shown in table 4.10 and 4.11.

Table 4.8: Wavelength of maximum absorption of 16µg/mL solution of metronidazole in different simulated physiological media.

Medium	pH	Wavelength of maximum absorption (nm)
0.1N HCL	1.2	295
Acetate buffer	4.5	345
Phosphate buffer	6.8	350

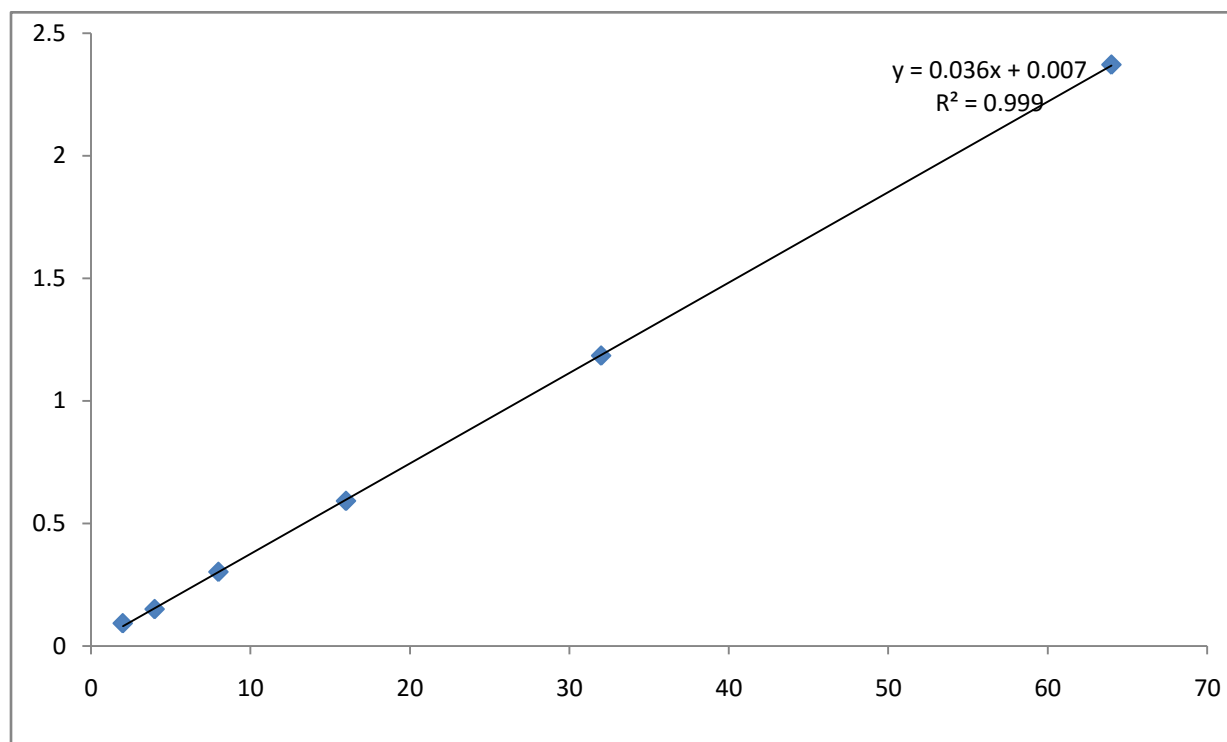


Figure 4.3: Calibration curve of metronidazole in pH 1.2

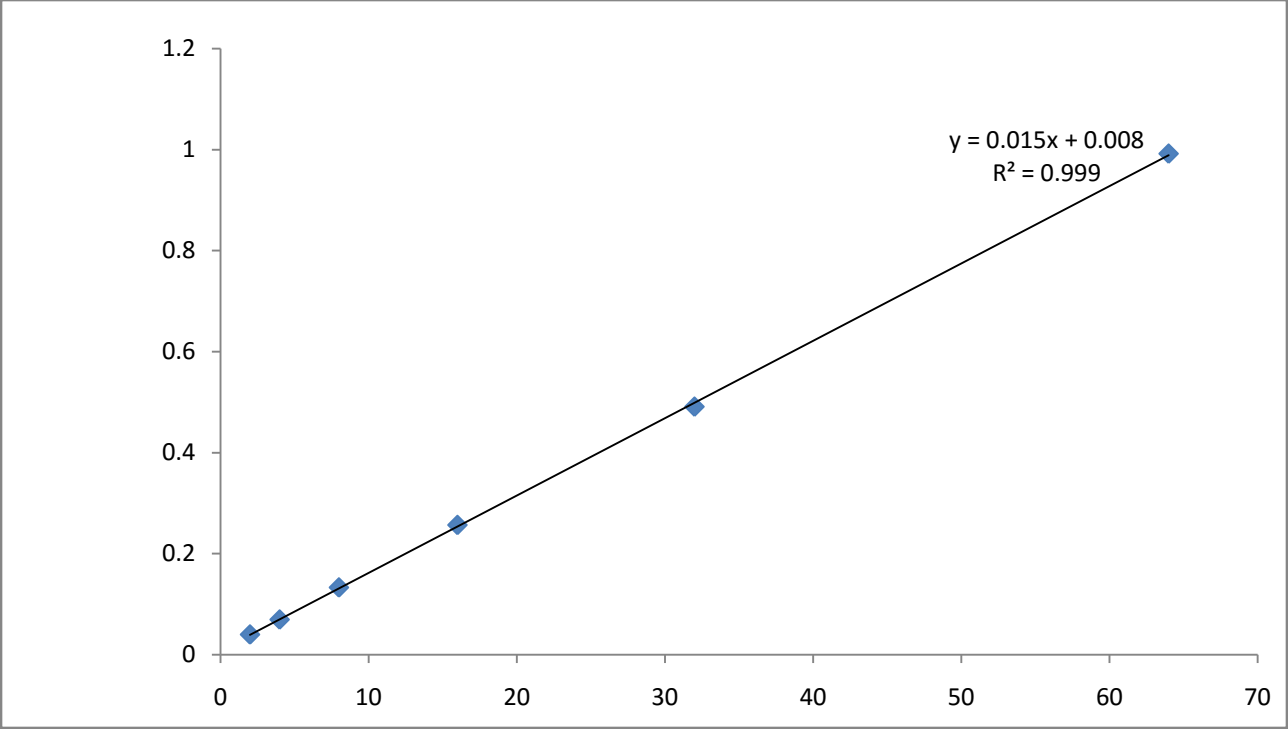


Figure 4.4: Calibration curve of metronidazole in pH 4.5

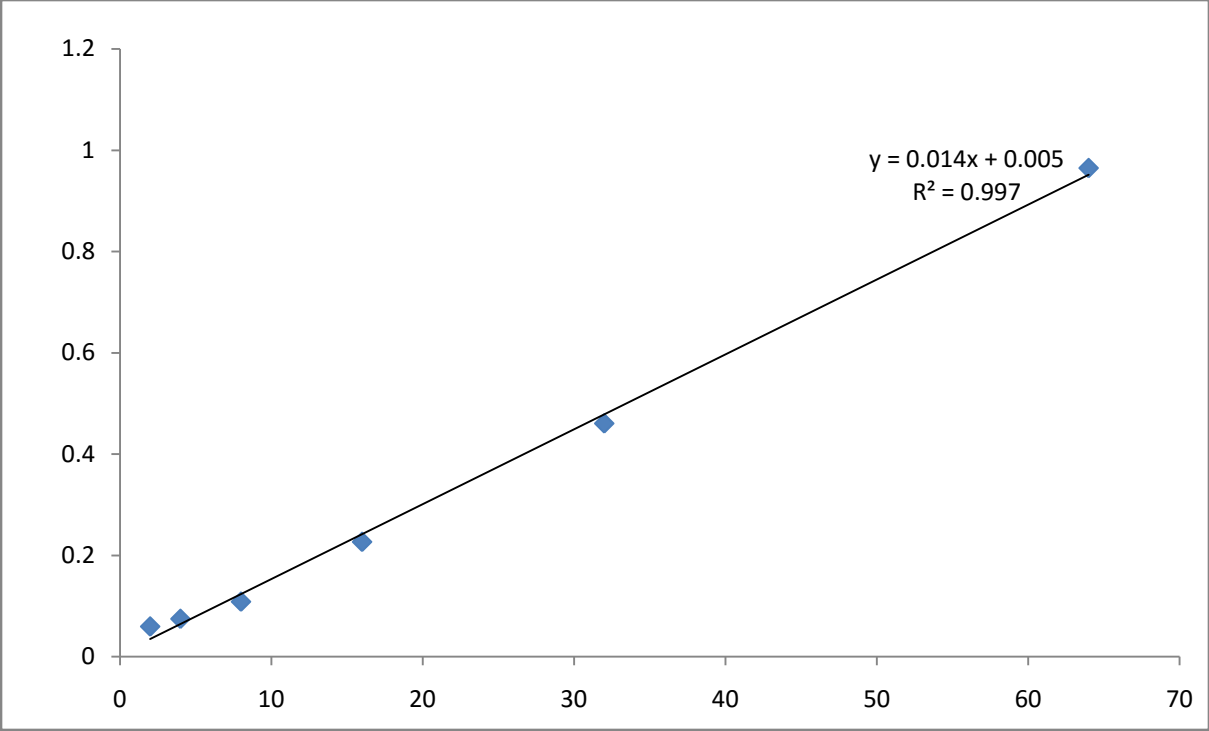


Figure 4.5: Calibration curve of metronidazole in pH 6.8

Table 4.9: Summary of the calibration curve parameter of the developed method

Serial no	Parameter	pH 1.2	pH 4.5	pH 6.8
1	λ_{max} (nm)	295	345	350
2	Concentration range ($\mu\text{g/mL}$)	2-64	2-64	2-64
3	Regression equation	$Y=0.0369X+0.0076$	$Y=0.0153X+0.0086$	$Y=0.0148X+0.0053$
4	Coefficient of correlation	0.9999	0.9999	0.9973
5	Intercept	0.0076	0.0085	0.0053
6	LOD	0.0122	0.0185	0.0858
7	LOQ	1.1156	1.6935	0.26026

Table 4.10: Intra and Interday precision of 16µg/mL solution of metronidazole

Medium	pH 1.2	pH 4.5	pH 6.8
Intraday (%RSD)	1.35	2.95	3.38
Interday (%RSD)	9.42	6.70	4.08

Table 4.11: Accuracy and percentage recovery of the methods

pH	Amount added	Amount obtained	% recovered	% Relative error
1.2	8	7.89	98.64	1.35
	10	10.05	100.51	0.51
	12	11.89	99.12	0.88
4.5	8	8.20	102.45	2.45
	10	10.18	101.76	1.76
	12	11.75	97.93	2.07
6.8	8	7.68	96.05	3.91
	10	9.61	96.15	3.75
	12	11.39	94.93	5.07

4.3 *In-vitro* bioequivalence studies

The percentage content of metronidazole for the three simulated physiological media are shown in figure 4.6, 4.7 and 4.8 while their difference factor (f1), similarity factor (f2) and dissolution efficiency (DE) are presented in table 4.12, 4.13 and 4.14.

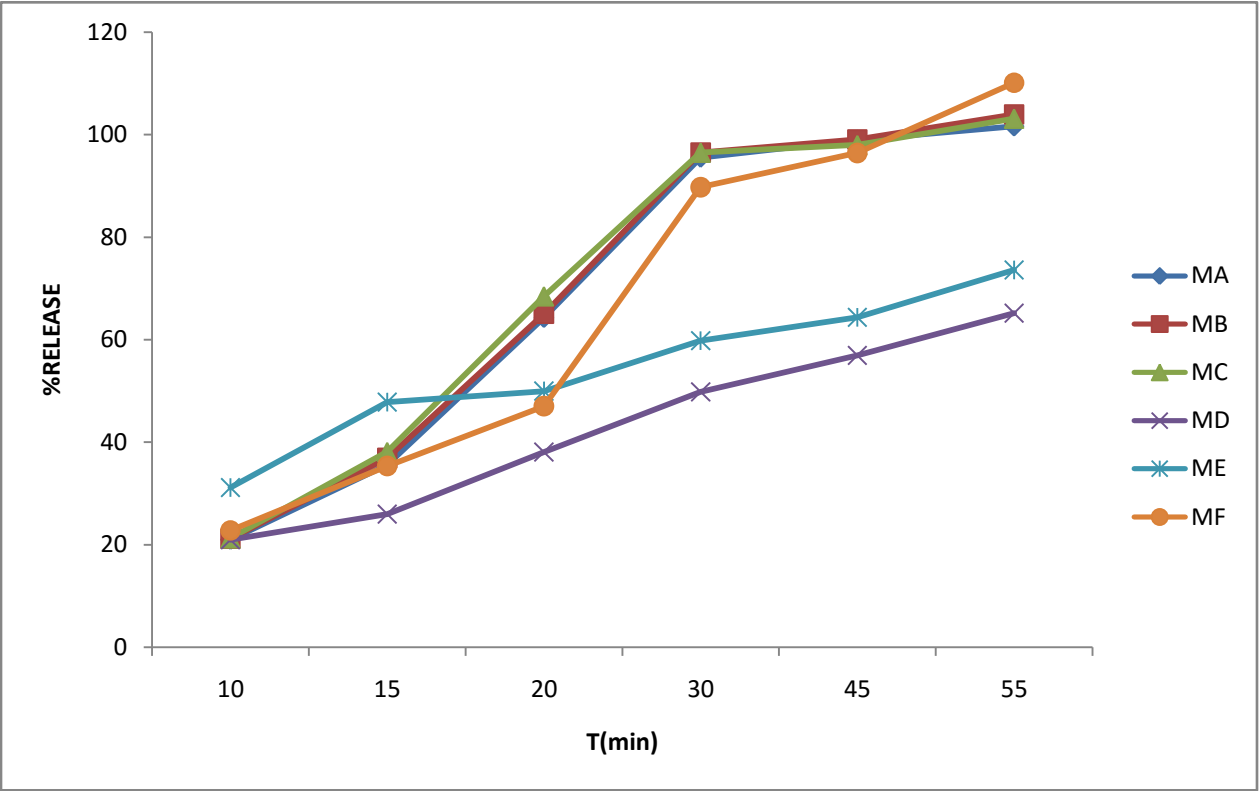


Figure 4.6Percentage content of metronidazole released at various time points in pH 1.2

Table 4.12: Difference factor (f1), similarity (f2) and dissolution efficiency (DE) of metronidazole in pH1.2

Code	F1	F2	DE (%)
MA	0.00	100	31.1
MB	1.34	90.37	31.4
MC	6.67	81.21	31.2
MD	38.36	25.05	33.5
ME	32.38	30.17	31.0
MF	8.1	54.77	34.1

Acceptable limit of f1 is ≤ 15 , f2 is ≥ 50 and % D.E is $\pm 10\%$ (WHO, 2014, FDA, 2015; EMEA, 2001).

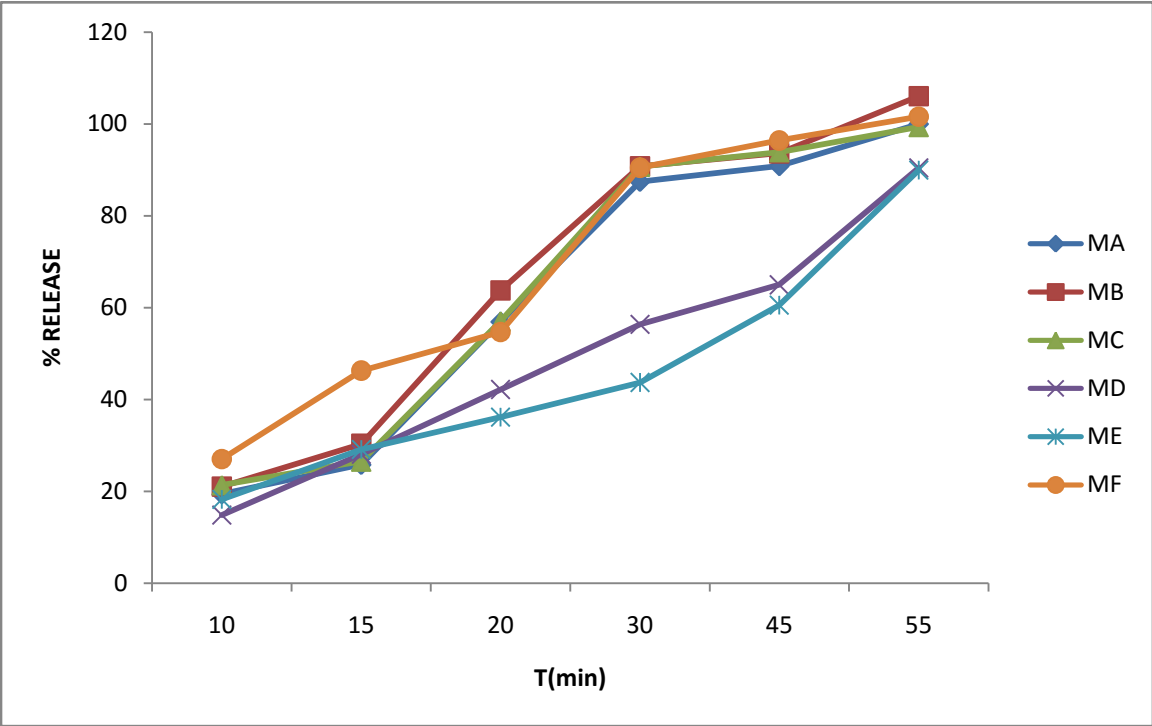


Figure 4.7 Percentage content of metronidazole released at various time points in pH 4.5

Table 4.13: Difference factor (f1), similarity (f2) and dissolution efficiency (DE) of metronidazole in pH4.5

Code	F1	F2	DE (%)
MA	0.00	100	33.2
MB	6.41	66.83	33.5
MC	2.33	82.77	30.2
MD	23.06	37.07	32.4
ME	28.62	31.26	42.2
MF	10.42	51.85	31.6

Acceptable limit of f1 is ≤ 15 , f2 is ≥ 50 and % D.E is $\pm 10\%$ (WHO, 2014, FDA, 2015; EMEA, 2001).

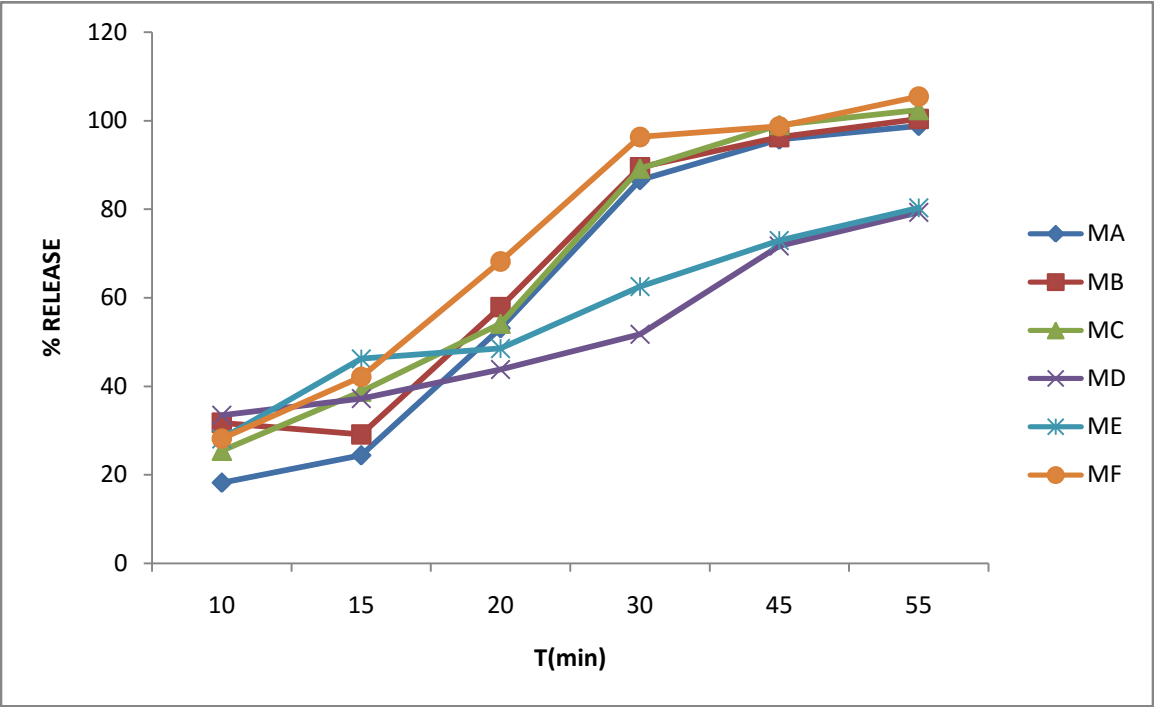


Figure 4.8 Percentage content of metronidazole released at various time points in pH 6.8

Table 4.14: Difference factor (f1), similarity (f2) and dissolution efficiency (DE) of metronidazole in pH6.8

Code	F1	F2	DE (%)
MA	0.00	100	32.7
MB	7.47	59.68	32.0
MC	8.58	57.57	32.0
MD	30.72	33.83	41.7
ME	26.96	36.72	31.8
MF	16.56	42.88	31.3

Acceptable limit of f1 is ≤ 15 , f2 is ≥ 50 and % D.E is $\pm 10\%$ (WHO, 2014, FDA, 2015; EMEA, 2001).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Quality control

Metronidazole standard powder passed the identification test as its melting point was 159-161°C which agrees with the B.P 2009 specification (159-163°C), this was further confirmed with the FTIR spectrum obtained. The IR spectrum was superimposable with the IR spectrum of reference metronidazole at the finger print region (BP, 2009) (appendix 1). All the brands of metronidazole sampled showed the presence of metronidazole API using the melting point as none of the brands melted outside the official specification and their spectra were superimposable with the spectrum of metronidazole standard powder.

The assay of drug content in a formulation is important to ensure the integrity of the formulation and this depends on the amount of the drug contained in it. The percentage content of metronidazole in the sampled tablets ranged from 89 to 102% (table 4.4). The BP limits percent content to be between 95-105% (BP 2009), therefore brands MA, MB, MC and MF with percent contents 102, 98, 99 and 97% respectively have passed the test but brand MD and brand ME with percent content 90 and 92% respectively failed.

Tablets were designed to contain specific amount of drug in a specific amount of formula, which are routinely measured to ensure that the tablet contains the proper amount of drug. This study revealed that all the brands sampled passed the weight variation test, as none of the individual weight of the tablets was outside the official limit (Table 4.5). The USP states that for a tablet weighing between 130mg to 324mg and more than 324mg to pass weight variation test,

no more than two of the tablets should differ from the average weight by 7.5 %, and 5% respectively. And no tablet will differ by more than double that percentage (15 % and 10%). Brand MA, MB, MC and ME have average weight more than 324mg and brand MD and MF have average weight more than 130mg but less than 324mg. All the samples complied with the official specification (Table 4.5) as the percentage deviation of all the brands were satisfactory. The disintegration test is recommended in pharmacopoeia (USP) as an *in vitro* quality control test that determines the possibility of a solid dosage form to disintegrate in the gastro intestinal tract (GI) fluids. All the brands complied with official specification for disintegration test, as all disintegrated in less than 15 minutes as presented in (table 4.7).

Hardness test is an important process in assessing whether the tablets being produced are firm enough to withstand breakage, chipping or crumbling, consumer handling and yet not so hard as to delay disintegration and dissolution time (Adilet *al.*, 2016).). From the result, all the samples passed the hardness test except ME (11.9 KgF) which was above the recommended range of 4-10 KgF (Ogahel *al.*, 2002). Friability test measures the tendency for a tablet to chip, crumble or break, during handling, tumbling motion, transportation, coating, packaging and storage (Adilet *al.*,2016). This can be caused by a number of factors including poor tablet design (too sharp edges), low moisture content, insufficient binder, etc. For obvious reasons, tablet is formulated to withstand such stresses without damage but friable enough that it can disintegrate in the gastrointestinal tract. According to USP, the friability value of tablets should be less than 1%. In all the samples studied, none had a weight loss up to 1% (w/w), with the maximum percentage being 0.33%.

5.2 Analytical Method

Three simple and accurate Spectrophotometric methods were developed and validated according to ICH guideline for the determination of metronidazole. Wavelengths of maximum absorption (λ_{max}) of 295nm, 345nm and 350nm were recorded for pH 1.2 (method 1), pH4.5 (method 2) and pH 6.8 (method 3) respectively (Table 4.8). Beer lambert law was obeyed at the range of 2-64 $\mu\text{g/mL}$ in all the methods as their correlation coefficient (Table 4.9) were closed to unity. The developed methods were precise as both the intra-day and inter-day precisions (Table 4.10) were within the acceptable limit of <15 % Coefficient of variation (FDA, 2006). This shows that the developed methods showed good repeatability. The percentage recoveries were within the acceptable range of (99-102%) as indicated in table 4.11. Hence, the developed methods were simple, precise, accurate and reproducible. Therefore, they can be used for *in-vitro* analysis of metronidazole in both bulk and dosage form.

5.3 *In vitro* dissolution studies

Dissolution is another *in vitro* test that determines the capability of the drug to dissolve in the GI fluids. The *in vitro* dissolution test (as recommended by the USP) measures the amount of drug dissolved in the dissolution medium after a definite time interval. When More than 85 % of labeled amount is released within 15minufes or less from the test and the reference product at pH 1.2, 4.5 and 6.8 using the paddle apparatus at 75 rpm or the basket apparatus at 100 rpm then the profile comparison is not-needed (WHO, 2005). But when more than 85% of labeled amount is released within 30 minutes or less from the test and the reference product under the above-mentioned conditions then the profile comparisons using e.g. f1, f2 and DE arerequired (WHO, 2005).

Comparison of therapeutic performance of two medicinal products containing the same active substances is a critical means of assessing the possibility of alternative between them. When three to four or more-time point are available the model independent method of analysis is preferred for the reference and any essentially similar medicinal product (WHO, 2014). Therefore, in this study the independent method of comparison have been utilized i.e. the f_1 , f_2 and dissolution efficiency (% D.E.).

The different factor (f_1) values in each of the three simulated physiological media (pH 1.2, 4.5 and 6.8) for brand MB (1.3, 6.4 and 7.47) and brand MC (6.67, 2.33 and 8.58) and were within the acceptable range of 0-15 (table 4.12, 4.13 and 4.14), while brands MF has f_1 value of 3.1, 10.42 and 16.56 in pH1.2, pH4.5 and pH6.8 respectively. Hence, brand MB and brand MC are bioequivalent and can therefore be interchangeable with MA in all the three media. Brand MF has identical profile with MA in pH 1.2 and pH 4.5 but not in pH 6.8 as seen in the f_1 value. The f_1 values of brands MD and ME were above the acceptable limit (0-15) and are thus not bioequivalent with the reference brand.

The similarity factor (f_2) values in each of the simulated physiological media were within the acceptable limit of ≥ 50 for brands MB (90.37, 66.83 and 59.68) and MC (81.21, 82.77 and 57.57) while brand MF has similarity value of ≥ 50 in simulated physiological media pH 1.2 (54.77) and pH4.5 (51.85) but not in pH6.8 (42.88) (table 4.14). Brand MD and ME failed to achieve the acceptable limit in each of the simulated physiological media (tables 4.12, 4.13 and 4.14.). Therefore, from the f_2 results brand MB and MC passed the requirement of ≥ 50 in all the simulated physiological, thus can be considered similar with brand MA since its f_1 values were within normal range of 0-15 in each media. Likewise, brand MF achieved similar dissolution with

MA in simulated physiological media (pH 1.2 and 4.5) and since its f_1 is within the acceptable limit is said to be similar with MA, but failed to achieve similar dissolution in (pH6.8) as its f_2 values was less than the acceptable limit of ≥ 50 . Thus MF was similar with MA in two simulated physiological media only, hence, is not considered bioequivalent with MA based f_2 approach. Whereas, brands MD and ME were not similar with brand MA in each of the simulated physiological media (pH 1.2, 4.5 and 6.8) as indicated in tables 4.3, 4.14 and 4.15, there f_2 values were far below 50. Therefore, Brand MD and ME were not bioequivalent with brand MA according to f_2 bioequivalence prediction methods.

Similarly, the extend of absorption or the area under concentration time curve (AUC) as indicated by dissolution efficiency (% D.E.) was also used to compare the released profile of the tested brands against the innovator brand. If the difference in the dissolution efficiencies is within acceptable limits of ($\pm 10\%$), it can be concluded that the reference and tested brand are similar and can be used interchangeably. However, if the %DE is outside the limit ($\pm 10\%$), then the tested brand is not similar with the innovator brand and therefore cannot be interchanged. In the dissolution efficiency (% D.E.) study approach, all the tested brands i.e. MB, MC, MD, ME and MF achieved the acceptable limit of $\pm 10\%$ in each of the simulated physiological media (pH 1.2, 4.5 and 6.8) as shown in tables 4.12, 4.13 and 4.14 respectively. This showed that all the brands have similar extend of absorption.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendations.

6.1 Summary

Metronidazole (200mg) tablet was carefully sampled after a market survey and coded successfully. The selected brands were within their shelf life and met the minimum label information such as NAFDAC registration number, address of manufacturer, date of manufacture and expiry date etc. The identification tests (FTIR) conducted confirmed the presence of metronidazole active pharmaceutical ingredients (APIs) and biopharmaceutical tests carried out confirmed the brands passed weight variation test, disintegration time test, friability and hardness (crushing strength) test, except brand ME which failed the hardness test. In the assay, only brand MD and ME failed to meet the official specification.

The developed and validated UV-spectrophotometric methods have good precision, accuracy and high percentage recovery thus used successfully for *in-vitro* bioequivalence studies of metronidazole in pharmaceutical formulation. The dissolution profile data generated using the developed methods were used in calculating the difference factor, similarity factor and dissolution efficiency, from which the *in-vitro* bioequivalence of metronidazole was predicted. About 60% of the tested brands were found to be bioequivalent with reference brand MA whereas 40% were not bioequivalent.

6.2 Conclusion

In all the three simulated physiological media, brands MB and MC were found to be bioequivalent to the reference brand MA. While brand MF was found to be bioequivalent with the reference brand in only two media (pH 1.2 and 4.5). Brands MD and ME are not bioequivalent with the reference brand in all the three simulated physiological media.

6.2 Recommendations

1. There is need for routine quality control (QC) of drugs especially those in the essential drug list so as to ensure quality as well as conformity with official specification.
2. There is a need for extension of this study to other drugs that fall under BCS class I and class III, so that the concern by healthcare professionals on selection among available brands for interchangeability can be reduced.
3. There is need for drug regulatory agency (NAFDAC) at national level to put more measures of quality assurance of these drugs before given marketing authorization.

REFERENCES

- Adil, M., Awan, A., Abbas, S., Chishti, K., Ahmad, L. (2016). Pharmaceutical evaluation and comparison of different brands of metronidazole available in the local market of Peshawar (Pakistan). *Journal of Coastal Life Medicine*. 4(8): 639-644.
- Akinyandenu, O. (2013). Counterfeit drugs in Nigeria: A threat to public health. *African Journal of Pharmacy and Pharmacology*. 7(36): 2571-2576.
- Akunyili, D. (2004). Fake and Counterfeit Drugs in the Health Sector: The Role of Medical Doctors. *Annals of Ibadan Postgraduate Medicine*. 2(2): 19-23
- Alagarsamy, V. (2010). Text book of medicinal chemistry volume II. Read Elsevir India private limited. 403-404
- Amber, N., Samina A. and Nousheen S. (2015). *In-vitro* dissolution study of metronidazole Film-Coated tablet 400 mg (flagyl) in the presence of Different tea. *World journal of pharmacy and pharmaceutical sciences*. 4 (12): 102-115.
- Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R. (1995). A theoretical basis for a biopharmaceutical drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharmaceutical Research* 12 (3): 413-420.
- Arcot R. C. (2011). Importance of *in-vitro in-vivo* studies in pharmaceutical formulation development. *Pelagia Research Library*. 2 (4): 218-240.
- Arlene, V. S., Jieyu, Z. and Raimar, L. (2014). Investigating the dissolution profile of Amoxicillin, metronidazole and zidovudine formulations used in Trinidad and Tobago west India. *American association of pharmaceutical scientists*.15(5):1060-1069.
- Arrunátegui, L. B., Silva-Barcellos, N.M., Bellavinha, K.R., Ev, L.S., Souza J. (2015). Biopharmaceutics Classification system: importance and inclusion in biowaiver guidance. *Brazilian Journal of Pharmaceutical Sciences*. 51(1): 143-153.
- AshutoshKar, (2004). Advance practical medicinal chemistry. New Age International (P) Ltd. 277-278
- Birkett D. J (2003). Generics-equal or not? *Australian Prescriber*. 26:85-7
- British pharmacopoeia. (2009). Volume I, II and III Monographs: medicinal and pharmaceutical substance Metronidazole. Her majesty stationary office London: 3952-3954.
- British National Formulary 65 March 2013-september 2013. *Royal pharmaceutical society*. 385

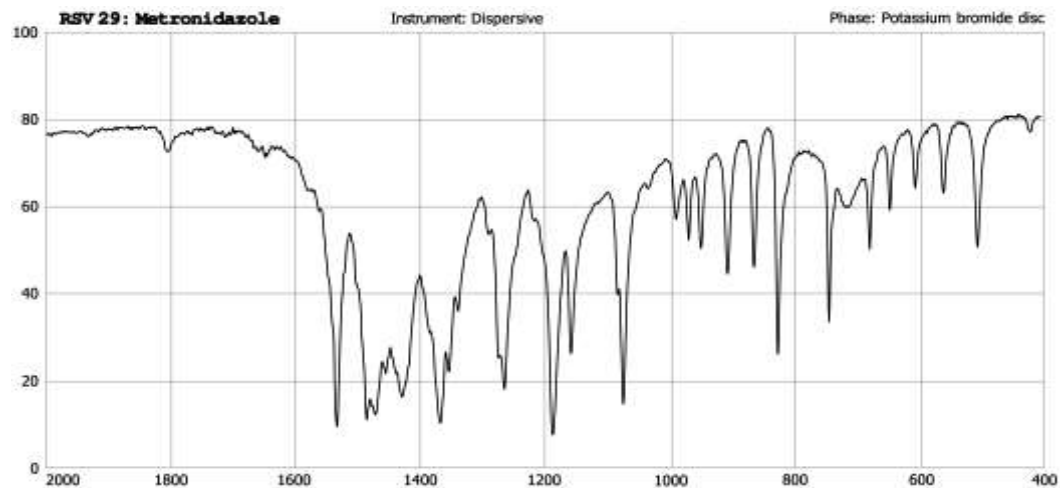
- Buowari O.Y (2012). Fake and Counterfeit Drug: A review article. *African Medical Journal*. 3(2) 1-4
- Chawla A. K., Tofghib,T. Agarwalb, A., Thomasc J. and Mondald T. (2014). A Global Comparison Between Brand Name and Generic Drugs. *Indian Journal of Pharmacy Practice*. 7: 23-28.
- Chiwendu, O. (2008). The fight against fake drug by NAFDAC in Nigeria.44th national course in Health development (ICHHD) September 24, 2007-September 12, 2008:1-67
- Courtney ST., Bethlehem, P.A. (2010). In Vitro Dissolution Testing for Solid Oral Dosage Forms. Technical. Brief. *Particle science drug development services*.5.
- EMA, (2010). Guideline on the investigation of bioequivalence Doc ref: CPMC/EWP/QWP1401/98 ReV. 1/Corr.
- Erhun, W.O., Babalola, O.O., Erhun, M.O. (2001). Drug Regulation and Control in Nigeria: The Challenge of Counterfeit Drugs. *Journal of Health & Population in Developing Countries*. 4(2):23-34.
- Esperanza, R. M., and Guillermina, V. M. (2014). Biopharmaceutical Relevance of Dissolution Profile Comparison: Proposal of a Combined Approach. *Dissolution Technologies*.32-43
- FDA. (2000). Guidance for Industry: Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System. Available from (www.fda.gov/cder/guidance/3618fn)
- Glass, B. D. (2004). Counterfeit drugs and medical devices in developing countries. *Research and Reports in Tropical Medicine*. 5(1): 11-12
- Hassouna M.E.M, Issa Y.M, Zayed A.G. (2012). A comparative study of the *in-vitro* dissolution profiles of paracetamol and caffeine combination in different formulations using HPLC. *Journal of applied pharmaceutical science*. 02(05): 52-59.
- Kahaliw, W. and Ashenef, A. (2013). Comparative quality evaluation of some metronidazole tablets and Metronidazole benzoate oral suspensions available in retail outlets of Addis Ababa, Ethiopia.*International journal of pharmaceutical sciences research journal*. 4(4): 1384-1391
- Kelesidis, T., Kelesidis, I., Rafailidis P. I., and Falagas, M.E. (2007). Counterfeit or substandard antimicrobial drugs: A Review of the scientific evidence. *Journal of Antimicrobial Chemotherapy*. 60 (2): 214–236

- Kim, P., Kang S., Helena I. B., Jiricek J., Collins M., Singh R., Clifton E. (2009). Structure Activity Relationships of Antitubercular Nitroimidazoles. II. Determinants of aerobic activity and quantitative structure-activity relationships. *Journal of Medicinal Chemistry*. 2(5):1329–1344.
- Kumar, S. A., Das S. (2003). A Review Article on Bioavailability and Bioequivalence Studies. *International Journal of Pharmaceutical technology research*. 5(4): 1711-1721
- Kurdi, M., Karam, R. (2015). Biowaivers: Criteria and Requirements – Ministry Of Public Health Qatar. 1-8
- Mastanamma S.K, Sravani K., Anil T. (2015). UV Differential Spectrophotometric Method for The Estimation of Metronidazole in Bulk and Pharmaceutical Formulation. *Rasayan journal of chemistry*. 8 (3): 303-309.
- Mital, A. (2009). Synthetic Nitroimidazoles: Biological Activities and Mutagenicity Relationships. *Scientia Pharmaceutica*. 77: 497–520.
- Naveed S., Qamar F. (2014a). Simple UV Spectrophotometric Assay of Metronidazole. *Open Access Library Journal*. 1(5): 1-4.
- Naveed S., Waheed N. and Nazeer S. (2014b). Degradation Study of Metronidazole in Active and Different Formulation by UV Spectroscopy. *Open access journal*. 6(4): 124-127.
- Nsimb S. E. D., (2008). Problem associated with substandard and counterfeit drugs in developing countries. A review article on global implication of counterfeit drugs in the era of Antiretroviral (ARV) in a free market economy. *East African journal of public health*. (5)3: 205-210
- Patel N.B., Jansari S.K., Arvadiya A.C. Desai H.T (2012). Validation of Cleaning Procedure for Elimination of Ofloxacin and Metronidazole Benzoate from Mixing Equipment by Using UV Spectroscopy. *International Journal of Pharmaceutical Sciences and Research*. 3 (7): 2306-2309
- Rohilla S., Rohilla, A., Marwaha., R.k., Nanda, A. (2011). Biopharmaceutical classification system. A strategic tool for classifying drug substance. *International research journal of pharmacy*. 2(7): 53-59
- Samuelson, J. (1999). Why Metronidazole Is Active against both Bacteria and Parasites. Antimicrobial agents and chemotherapy. *American society for microbiology*. 43 (7): 1533–1541.

- Sally A. H. and Heba M. E. B. (2016). In Vitro Dissolution Similarity as a Surrogate for in Vivo Bioavailability and Therapeutic Equivalence. *Dissolution technology*. 32-39
- Sharvan, P., Singh, S., Ajmal, F. A., Kumar R. S., Ranjan A. G. (2013). Bioavailability: criteria for approving a drug product for Marketing. *Indian Journal of Research in Pharmacy and Biotechnology*. 1(3): 351-359.
- Siya D. S. K., Bhilegaonkar, S., Ajeet M. G., Gajre, P. (2015). Biopharmaceutical Classification System: A Brief Account. *International journal of research methodology*. 1 (1): 20-46.
- Thomas L.L., Williams, D. A., Roche, V.F., William, S.Z., (2007). Foye's principles of medicinal chemistry. *Lippincott Williams and Wilkins*. China. 1131-1133
- Thulasamma P. and Venkateswarlu, P. (2009). Spectrophotometric method for the Determination of metronidazole in Pharmaceutical pure and dosage forms. *Rasayan journal*. 2(4): 865-868.
- Tiwari, R., Tiwari, G., Rai, A.K, Srivastawa, B. Pandey, P., Pandey, S., Bhat, L. (2016). In vitro in vivo correlation and biopharmaceutical classification system. *Chronicles of Young Scientists*. 2(3): 126-233.
- Usman, MA , Eniolorunda, RO , Sule, MI , Hassan, HS , Salisu, IM , Sallah, A , Bashir, DB.(2010). Chemical equivalence studies of four brands of metronidazole tablets. *Nigerian Journal of Pharmaceutical Sciences*. 9 (1): 90-95.
- USP 28- NF 23. 2005. The United States Pharmacopeia the National Formulary. Rockville. The United States Pharmacopoeial Convention. 243
- Vikaars, B., Arun, N., (2012). The biopharmaceutical classification system (BCS): present status and future prospective. *International research journal of pharmacy*. 3(9): 7-11.
- Wagh, M. P., and Patel, J. S. (2010). Biopharmaceutical classification system: Scientific basis for Biowaiver extensions. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2(1):12-19.
- WHO. (2005). Multisource (generic) pharmaceutical products: Guidelines on registration requirements to Establish interchangeability. Working document QAS/04.093/Rev.
- WHO. (2006). Proposal to waive in vivo bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms. Technical Report Series, No. 937.
- WHO. (2006). Counterfeit medicines. Available via <http://www.who.int/mediacentre/factsheets/fs275/en/print.html>

APPENDICES

Appendix i: IR spectra of reference Metronidazole (BP, 2009)

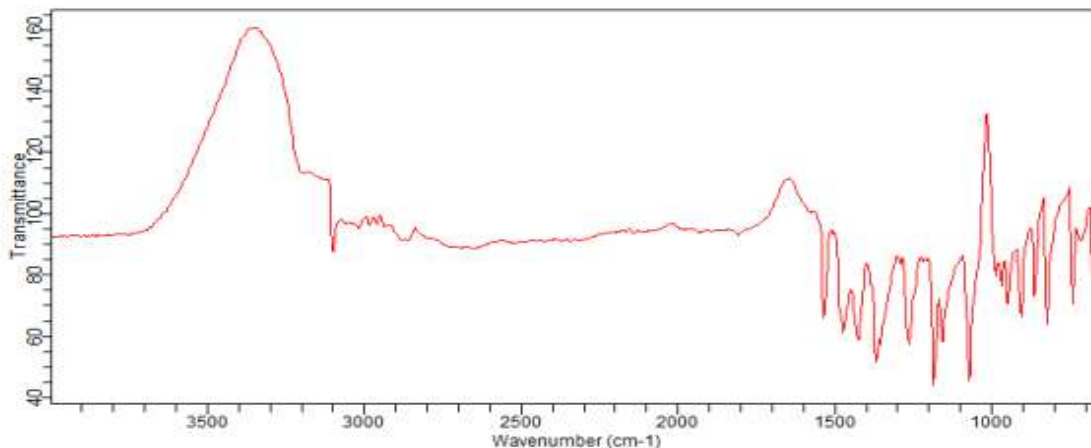


Appendix ii: IR spectra of metronidazole powder



Sample ID:MP
Sample Scans:30
Background Scans:16
Resolution:8
System Status:Good
File Location:C:\Program Files\Agilent\MicroLab PC\Results\MP_2018-03-14T13-55-49.a2r

Method Name:Transmittance Method
User:Admin
Date/Time:2018-03-14T13:55:49.855+01:00
Range:4000 - 650
Apodization:Happ-Genzel

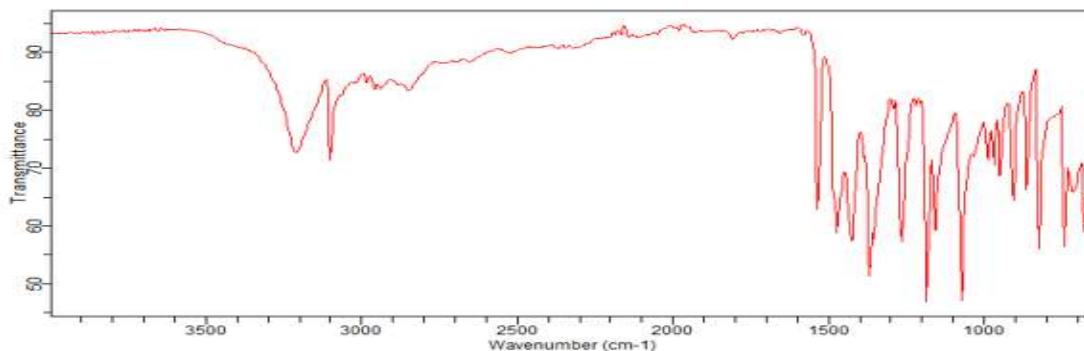


Appendix iii: IR spectra of brand MA



Sample ID:MA
Sample Scans:30
Background Scans:16
Resolution:8
System Status:Good
File Location:C:\Program Files\Agilent\MicroLab PC\Results\MA_2018-03-14T14-10-29.a2r

Method Name:Transmittance Method
User:Admin
Date/Time:2018-03-14T14:10:29.931+01:00
Range:4000 - 650
Apodization:Happ-Genzel

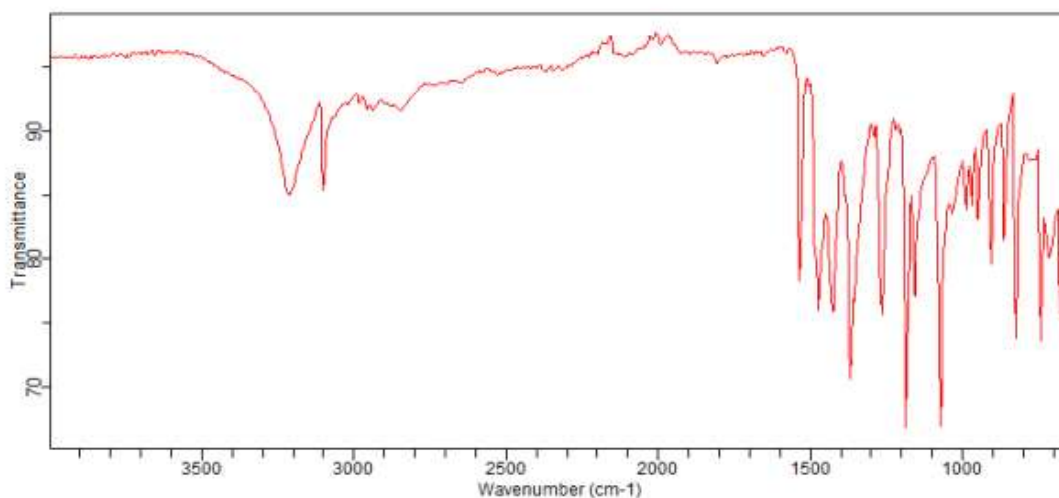


Appendix iv: IR spectra of brand MB



Sample ID:MB
Sample Scans:30
Background Scans:16
Resolution:8
System Status:Good
File Location:C:\Program Files\Agilent\MicroLab PC\Results\MB_2018-03-14T14-09-12.a2r

Method Name:Transmittance Method
User:Admin
Date/Time:2018-03-14T14:09:12.305+01:00
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Apodization:Happ-Genzel

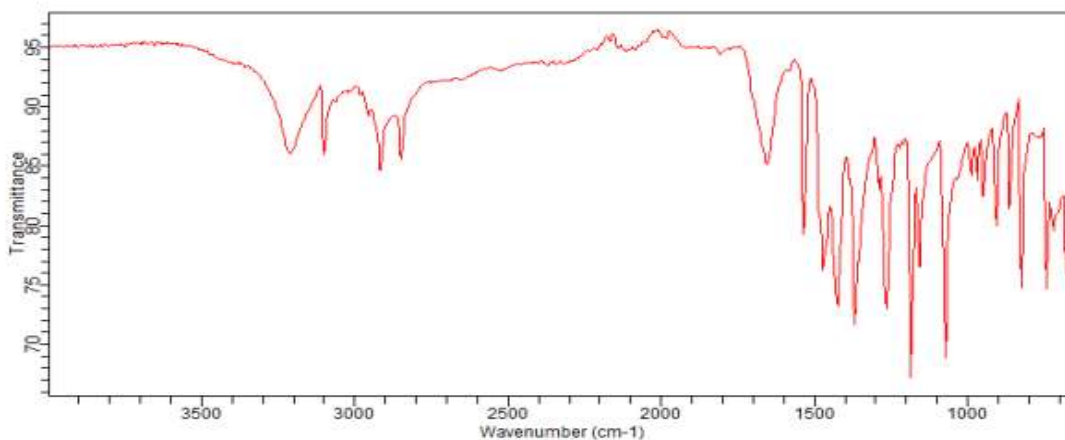


Appendix v: IR spectra of brand MC



Sample ID:MC
Sample Scans:30
Background Scans:16
Resolution:8
System Status:Good
File Location:C:\Program Files\Agilent\MicroLab PC\Results\MC_2018-03-14T14-05-23.a2r

Method Name:Transmittance Method
User:Admin
Date/Time:2018-03-14T14:05:23.702+01:00
Range:4000 - 650
Apodization:Happ-Genzel

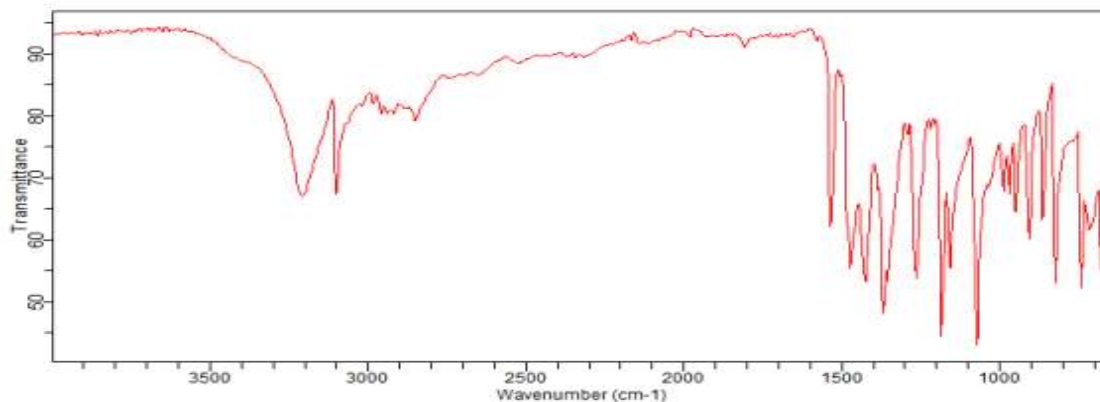


Appendix vi: IR spectra of brand MD



Sample ID:MD
Sample Scans:30
Background Scans:16
Resolution:8
System Status:Good
File Location:C:\Program Files\Agilent\MicroLab PC\Results\MD_2018-03-14T13-58-35.a2r

Method Name:Transmittance Method
User:Admin
Date/Time:2018-03-14T13:58:35.511+01:00
Range:4000 - 650
Apodization:Happ-Genzel



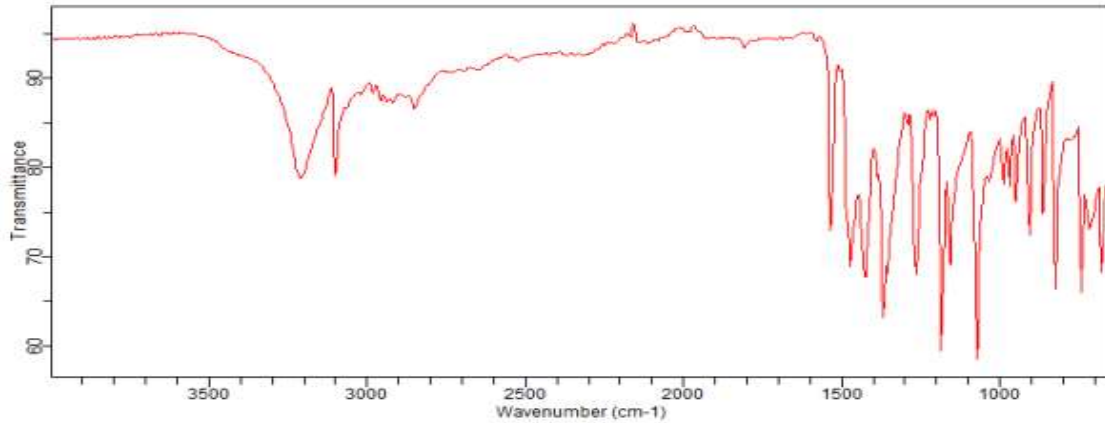
Appendix vii: IR spectra of brand ME



Agilent Technologies

Sample ID:ME
Sample Scans:30
Background Scans:16
Resolution:8
System Status:Good
File Location:C:\Program Files\Agilent\MicroLab PC\Results\ME_2018-03-14T14-03-17.a2r

Method Name:Transmittance Method
User:Admin
Date/Time:2018-03-14T14:03:17.607+01:00
Range:4000 - 650
Apodization:Happ-Genzel



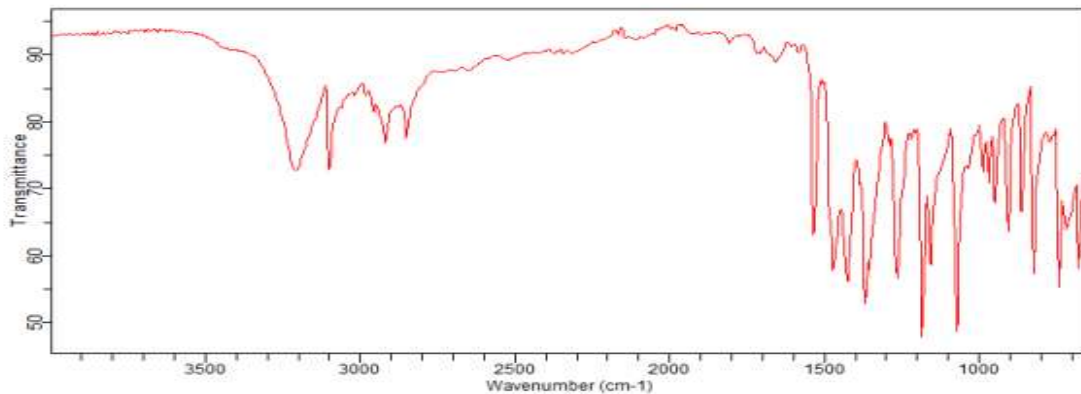
Appendix viii: IR spectra of brand MF



Agilent Technologies

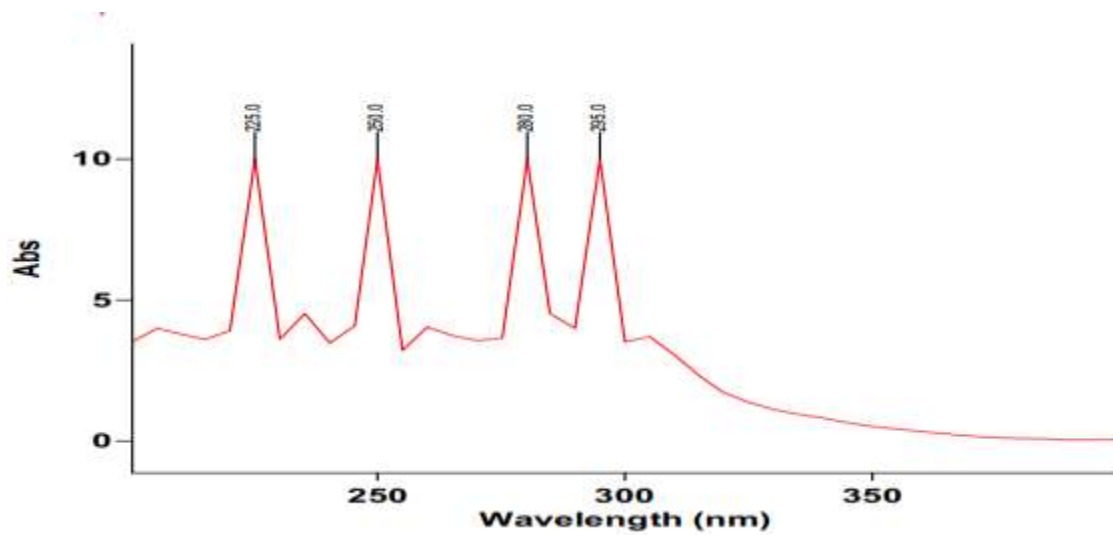
Sample ID:MF
Sample Scans:30
Background Scans:16
Resolution:8
System Status:Good
File Location:C:\Program Files\Agilent\MicroLab PC\Results\MF_2018-03-14T14-11-50.a2r

Method Name:Transmittance Method
User:Admin
Date/Time:2018-03-14T14:11:50.914+01:00
Range:4000 - 650
Apodization:Happ-Genzel



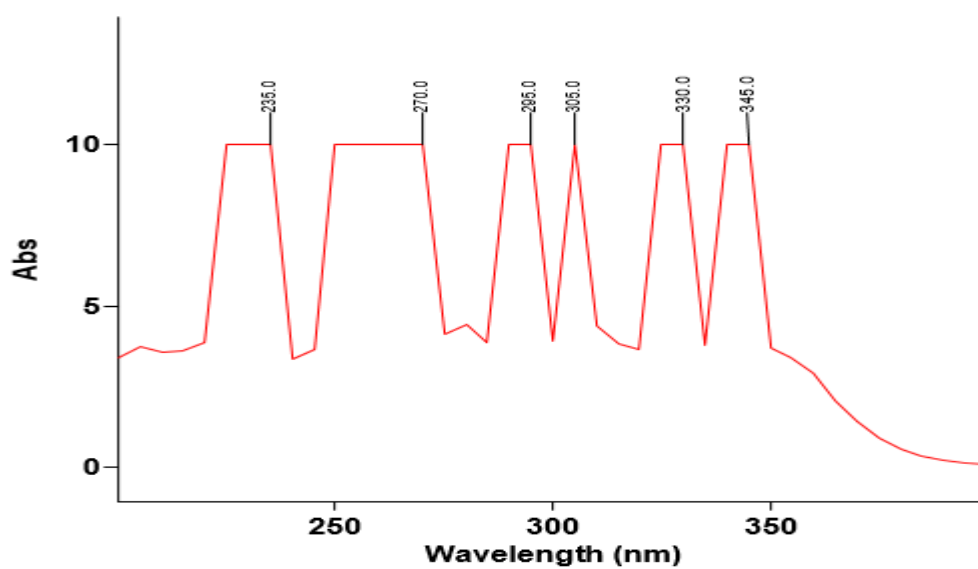
Appendix ix: wavelength of maximum absorption in various pH

pH 1.2



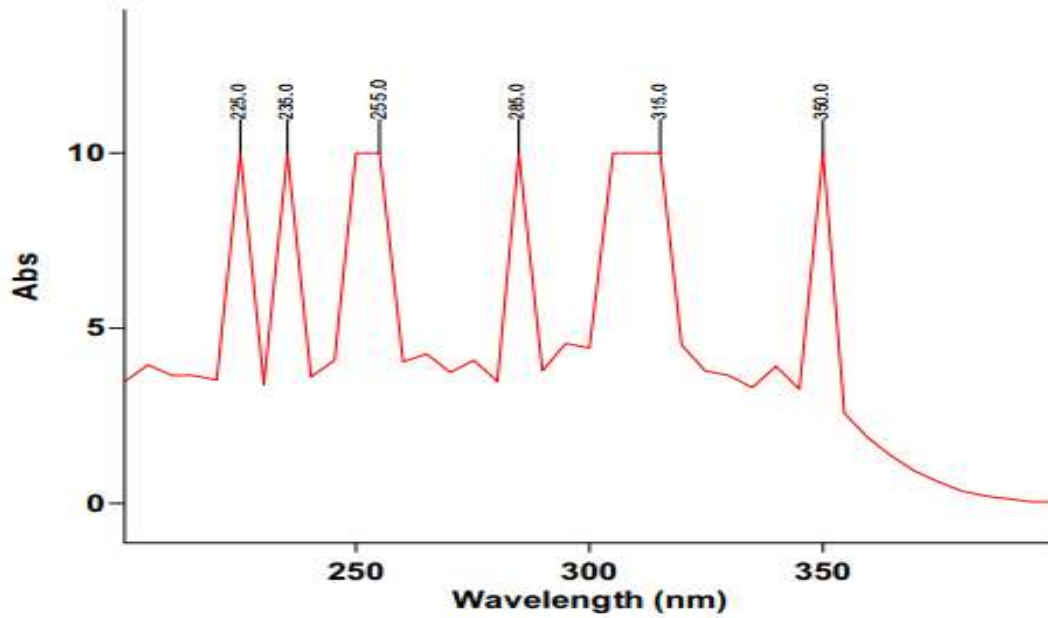
ixa

pH 4.5



ixb

pH 6.8



ixc

Appendix x: Raw data for precisions of the methods

Intraday			
	PH 1.2	PH 4.5	PH6.8
	0.2205	0.1868	0.06154
	0.2156	0.2035	0.6199
	0.2156	0.1952	0.6232
	0.232	0.1915	0.6339
	0.2144	0.1912	0.6329
	0.2112	0.1909	0.6146
MEAN	0.218216667	0.193183333	0.623317
SD	0.007384962	0.005713639	0.008417
%RSD	3.384233803	2.957625047	1.350279

xa

Interday			
	PH 1.2	PH 4.5	PH6.8
	0.279	0.2432	0.8964
	0.2625	0.2517	0.9068
	0.2715	0.2559	0.9294
	0.2703	0.2457	0.9985
	0.2668	0.2533	1.0035
	0.258	0.2478	0.9667
	0.2426	0.2082	0.7285
	0.2559	0.2706	0.9918
	0.25	0.2343	0.9998
MEAN	0.262244	0.245633	0.935711
SD	0.010711	0.017192	0.088111
%RSD	4.084219	6.999229	9.416516

xb

Appendix xi: Percentage recovery raw data in various pH

pH1.2					
THEO.C	Abs	A.conc	amt R	%R	%ER
10	0.3632	9.636856			
18	0.6544	17.52846	7.891599	98.64499	1.355014
20	0.7341	19.68835	10.05149	100.5149	-0.51491
22	0.8021	21.53117	11.89431	99.11924	0.880759

xia

pH4.5

THEO.C	Abs	A,conc	amt R	%R	%ER
10	0.1644	10.18301			
18	0.2898	18.37908	8.196078	102.451	-2.45098
20	0.3201	20.35948	10.17647	101.7647	-1.76471
22	0.3442	21.93464	11.75163	97.93028	2.069717

Xib

pH6.8

THEO.C	Abs	A.conc	amt R	%R	%ER
10	0.1567	10.22973			
18	0.2704	17.91216	7.682432	96.03041	3.969595
20	0.299	19.84459	9.614865	96.14865	3.851351
22	0.3253	21.62162	11.39189	94.93243	5.067568

xic

Appendix xii: Percentage release of metronidazole in various pH

pH 1.2

Time (min)	MA	MB	MC	MD	ME	MF
10	21.01	21.15	21.26	21.00	31.16	22.80
15	35.60	36.90	38.10	25.99	47.81	35.37
20	64.29	65.06	68.47	38.10	49.96	47.03
30	95.54	96.52	96.49	49.83	59.81	89.72
45	98.96	99.08	97.97	56.94	64.37	96.40
55	101.64	103.94	103.12	65.19	73.58	110.08

xiiia

pH4.5

Time (min)	MA	MB	MC	MD	ME	MF
10	19.38	21.03	21.33	14.79	18.21	27.00
15	25.77	30.33	26.49	28.05	29.10	46.26
20	56.89	63.73	57.01	42.15	36.12	54.67
30	87.37	90.76	90.70	56.32	43.68	90.46
45	90.79	93.61	93.85	64.96	60.55	96.37
55	99.94	106.03	99.34	90.43	89.95	101.56

xiib

pH 6.8

Time (min)	MA	MB	MC	MD	ME	MF
10	18.21	21.75	25.45	33.45	28.13	28.19
15	24.39	29.13	38.80	37.19	46.19	42.15
20	53.09	57.87	54.16	43.76	48.53	68.21
30	86.54	89.46	89.19	51.72	62.52	96.33
45	95.70	96.27	99.04	71.67	72.92	98.77
55	98.77	100.38	102.42	79.24	80.28	105.46

xiic

Appendix xiii: preparation of 0.1M NaOH.

8g NaOH was weight and dissolved in sufficient quantity of distilled water contained in 2L volumetric flask. The volumetric flask was finally made to volume with the distilled water to produced 0.1M NaOH

Appendix xiv: Preparation of 0.1M HCL

16.95ml of HCL was measured and added in sufficient quantity of distilled water contained in 2L volumetric flask. The volumetric flask was finally made to volume with the distilled water to produced 0.1M HCL