COMPARATIVE BIOEQUIVALENCE STUDIES OF SOME BRANDS OF METRONIDAZOLE TABLETS MARKETED IN ZARIA, NIGERIA

BY

ShuaibuALIYU, B. PHARM. (A.B.U.) 2010
M.SC/PHARM-SCI/12150/2010-2011

A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER DEGREE IN PHARMACEUTICAL CHEMISTRY

DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY, FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA

OCTOBER, 2014

DECLARATION

I declare that the work in this thesisentitled "Comparative Bioequivalence Studies of some brands of Metronidazole Tablets Marketed in Zaria, Nigeria" 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 thesiswas previously presented for another Degree or Diploma in this or any other Institution.

_____ _____

Shuaibu Aliyu Date

CERTIFICATION

This Thesis entitled "COMPARATIVE BIOEQUIVALENCE STUDIES OF SOME BRANDS OF METRONIDAZOLE TABLETS MARKETED IN ZARIA, NIGERIA" by Shuaibu ALIYU meets the regulations governing the award of the Degree of Master of Science in Pharmaceuticalof the Ahmadu Bello University, and is approved for its contribution toknowledge and literary presentation.

_____	_____
Chairman, Supervisory Committee Dr. M.A. Usman	Date
_____	_____
Member, Supervisory Committee Prof I.A. Yakasai	Date
_____	_____
Head of Department Prof M.A. Musa	Date
_____	_____
Dean, School of Postgraduate Studies Prof H.A. Zoaka	Date

DEDICATION

This work is dedicated to my late parents. May Almighty Allah have Mercy on them as they did to me when, I was helpless.

ACKNOWLEDGMENT

Praise and blessings are due to Allah (SWT) who in His infinite mercy made it possible for me to actualise this work. Peace and blessings of Allah be on His noble Messenger and those who followed their path up to the last hour.

My special appreciation goes to my supervisors, Dr. M.A. Usman and Prof. I.A. Yakasai who served not only as supervisors but also as parents and academic mentors. Thank you for your dedication and generosity to me. Equally, my appreciation also goes to Dr. Aliyu Musa of the Department of Pharmaceutical and Medicinal Chemistry who has contributed immensely to my professional carrier as a Pharmacist.

However, my utmost appreciations go to my late parents for moulding me for what I am today. The prayers, support and encouragement of the parents, family, brothers, sisters, wife, parent-in-laws, friends, colleagues and well-wishers will not be forgotten. My unimaginable appreciation also goes to my wife and my daughter for their dedication and patience while away from them during the course of this study.

Finally, my sincere appreciation goes to all the technical and the non-technical staff of the Department of Pharmaceutical and Medicinal Chemistry as well as anybody who in oneway or the other has contributed to the success of this programme. May the Almighty Allah reward you all.

TABLE OF CONTENTS

Approval Page. i

Tittle Page. ii

Declaration iii

Certification iv

Dedication. v

Acknowledgment. vi

Table of Contents vii

List of Figure xii

Abbreviations xiii

Abstract. xiv

1.0 CHAPTER 1: INTRODUCTION 1

1.1 Post Market Survey 2

1.2 Multisource (Generic) Pharmaceutical Products 3

1.3 Quality Control 4

1.4 Quality Control Requirements of Tablet Dosage Form . . . 4

1.5 Quality of a Drug Product 5

1.6 Fake or Substandard Drug Products 6

1.7 In-Vitro Evaluation Studies of Tablets Dosage Form . . . 6

1.8 Methods for In-vitro Evaluation Studies 7

1.8.1 Identification Test 7

1.8.2 Weight Variation 7

1.8.3 Disintegration 8

1.8.4 Dissolution Rate. 8

1.8.5 Uniformity of Content. 8

1.8.6 Friability of Tablets Testing 9

1.8.7 Hardness Testing 9

1.9 In-vivo Availability Studies 9

1.10 Bioequivalence and Bioavailability 10

1.11 Comparative Bioavailability 10

1.12 Study Design for In-vivo Availability Studies 11

1.13 Validation of Analytical Methods 11

1.13.1 Accuracy 12

1.13.2 Precision 12

1.13.3 Specificity 12

1.13.4 Linearity 13

1.13.5 Range 13

1.13.6 Robustness 14

1.13.7 Detection Limit 15

1.13.8 Quantitation Limit 15

1.14 Application of Validated Method to Routine Drug Analysis . . 16

1.15 Statement of the Research Problem 16

1.16 Justification of the Study 17

1.17 Research Hypothesis. 17

1.17.1 Null Hypothesis 17

1.17.1 Alternate Hypothesis 17

1.18 Aims and Objectives of the Study 17

2.0 CHAPTER 2: LITERATURE REVIEW 19

2.1 History of Metronidazole 19

2.2 Chemistry of Metronidazole 20

2.3 Physicochemical Properties of Metronidazole 21

2.3.1 Salt and Esters Form of Metronidazole 21

2.3.2 Solubility of Metronidazole 21

2.4 Therapeutic Index of Metronidazole 21

2.5 Available Dosage Form Strengths 21

2.6 Analytical Methods for Analysis of Metronidazole from a Dosage Form 22

2.7 Indications 23

2.8 Adverse Effects 23

2.9 Interactions of Metronidazole with some Drugs 24

2.9.1 Alcohol 24

2.9.1 Busulfan 24

2.9.3 Antacids, Kaolin-pectin or Colestyramine 24

2.9.4 Barbiturates 25

2.9.5 Disulfirim 25

2.9.6 Carbamazepine 25

2.9.7 Phenytoin 25

2.15 Pharmacokinetics of Metronidazole 25

2.16 Justification for the use of Saliva as an Analytical fluid . . . 27

3.0 CHAPTER 3: MATERIALS AND METHODS 28

3.1 Materials 28

3.1.1 Reagents and Chemical used 28

3.1.2 Equipment and Glass Wares 28

3.1.3 Samples 28

3.2 Method 28

3.2.1 Sample Collection 28

3.2.2 Assessment of In-vitro Parameters of the Samples 29

3.2.3 Analytical Method 31

3.2.4 Preparation of the Stock Solution 32

3.2.5 Selection of pH of the Medium 32

3.2.6 Construction of Calibration Curve 32

3.2.7 Validation and Optimization of the Analytical Method . . . 33

3.2.8 In-vivo Application of the Method 34

3.2.9 Extraction Procedure 35

3.2.10 Data Analysis 35

4.0 CHAPTER 4: RESULT 36

4.1 Labels of the Samples of Metronidazole 200 mg Tablets Randomly Selected as Samples. 36

4.2 Physical Appearance of the Samples of Metronidazole 200 mg Tablets Randomly Selected as Samples 37

4.3 Identification Test on the sample of Metronidazole Tablets . . 37

4.4 Weight Variation and Disintegration Time for the Samples of Metronidazole 200 mg Tablets Dosage Form 38

4.5 Assay, Dissolution, Friability and hardness Tests of Metronidazole Tablets Dosage Form 39

4.6 Result for the Identification of Metronidazole Reference Standard using UV Spectrophotometry according to BP 1993 method . . . 40

4.7 Effect of pH on the Absorbance of 15 µg/ml stock Solution of Metronidazole Reference Standard 42

4.8 Intra- and inter-day Precisions of the Method using 15 µg/ml of Metronidazole Reference Standard at pH 9. 42

4.9 Accuracy of the Method and Percentage Recovery of Metronidazole Reference Standard spiked into Blank Saliva 43

4.10 Calibration Curve for in-vivo Availability Studies of the Sample of Metronidazole Tablets 44

4.11 Linearity of the Method 45

4.12 Concentration-time Curve for Metronidazole from Saliva Samples . 46

4.13 Mean Bioequivalence Parameters of the Brands of Metronidazole Tablets 47

4.14 Bioequivalence Ratio of Reference and Test 48

5.0 CHAPTER 5: DISCUSSION 50

6.0 CHAPTER 6: CONCLUSION, SUMMARY AND RECOMMENDATIONS56

6.1 Summary 56

6.2 Conclusion 56

6.3 Recommendations 57

References xv

Appendices xxv

LIST OF FIGURES

Figure 2.1: Chemical Formula of Metronidazole 20

ABBREVIATIONS

%	Per Cent
BP	British Pharmacopoeia
F	Force
FDA	Federal Drug Agency
g	Gram
ICH	International Convention/Council on Harmonisation
IP	International Pharmacopoeia
kg	kilogram
Max	Maximum
mg	Milligram
min	Minute
ml	Millimetre
n	Number
nm	Nanometre
RSD	Relative Standard Deviation
s	Second
USP	United State Pharmacopoeia
UV	Ultraviolet
WHO	World Health Organisation
λ	Wave Length

ABSTRACT

Multisource pharmaceutical products are intended to be pharmaceutically equivalent or pharmaceutical alternatives that are bioequivalent and hence are therapeutically equivalent and interchangeable. Thus, this study was aimed at carrying out a comparative *in-vitro* and *in-vivo* bioequivalence studies of some brands of Metronidazole (200 mg) tablets marketed in Zaria, Nigeria. BP 2002 method was use for the assessment of the pharmaceutical and chemical equivalences of the samples of metronidazole tablets. Tablet hardness and friability testing were also carried out to reinforce the official methods for the *in vitro* studies. The analytical method for the *in vivo* studies was adopted from the work of Kolawole and Ameh (2004). The method was validated according to ICH/WHO guideline. Six healthy human volunteers were enrolled for the studies according to the normal protocols for bioequivalence studies. The study yielded the following results: The presence of metronidazole was observed in all the samples with a content uniformity of 92.45 - 106.16 %. All the samples had acceptable level of weight variation, except MT05, MT08 and MT12 with 5, 4 and 14 tablets respectively deviated from the mean weight. All the samples disintegrated within 15 minutes (BP specification) with the exception of MT07 which disintegrated in 18.97 minutes. Each of the samples had at least a dissolution of 70 % within 45 minutes. All the samples had acceptable level of friability, except MT05, MT07, MT08 and MT11 with value of 1.70 %, 1.81 %, 1.62 % and 10.25 % respectively above the 1 % acceptable limit. All the samples of the tablets had acceptable hardness within the limit of 4-10 kgF, except MT10 and MT11 with a value of 10.70 kgF and 10.6 kgF respectively. The validated method was found to have an intra- and inter-day precision of 0.491 and 0.417 % respectively expressed as percentage relative standard deviation. The accuracy of the method was 1.98 % with a mean percentage recovery of 98.3 %. All the samples had acceptable level of bioequivalence

within the range of 0.93-1.05 with reference to MT01® as the reference standard using point estimate ratio of the mean C_{max} and AUC0→8h /µg. In conclusion, the samples of the metronidazole 200 mg tablets analysed can be used interchangeably even though there was some variation in their pharmaceutical equivalences.

CHAPTER 1

INTRODUCTION

Increasing economic activities in many parts of the world have led to the proliferation of pharmaceutical manufacturing industries with the attendant introduction of many brands of the same drug into Market. The availability of different brands of a generic product places many prescribers in difficult situation over choice of an ideal brand (Omeje, *et al*., 2007). Thus, the increased influx of multisource Metronidazole tablets from different countries in the Nigerian drug market coupled with the locally manufactured brands of the drug is rapidly increasing.

A generic medicinal product is a product which has the same qualitative and quantitative composition of active substances and the same pharmaceutical form as the reference medicinal product, and whose bioequivalence with reference medicinal product has been demonstrated by appropriate bioavailability studies. The different salts, esters, ethers, isomers, mixture of isomers complexes or derivatives of an active substance are considered to be same, unless they differ significantly in properties with regards to safety and/or efficacy.

Furthermore, the various immediate release oral pharmaceutical forms shall be considered to be one and the same form (European Medicine Agency, 2010). Analysis of drug and their metabolites in a biological matrix is carried out using samples spiked with calibration (reference) standards and using quality control samples. The purity of the reference standard used to prepare spiked samples can affect study data. For this reason, an authenticated analytical reference standard should be used to prepare solution of known concentrations. If possible, the reference standard should be identical to the analyte. When this is not possible, an established chemical form (free from base or acid, salt or ester) of known purity can be used. Three types of reference standards are usually used:

1. Certified reference standard (e.g. USP compedial standards)
2. Commercially supplied reference source and/or
3. Other materials of documented purity

The source and lot number, expiration date, certificate of analyses when available, and/or internally or externally generated evidence of identity and purity should be furnished for each reference standard (FDA, 2001).

1.1 Post Market Survey

Post market surveillance or monitoring involves all activities undertaken to obtain more data and information about a product after it had been granted marketing authorization and made available for public use. The data and information so obtained could be employed for improvement, development of standards and regulations. Regulatory agencies rely on limited information obtained during clinical trials and to some extent scientific literature as guides to granting marketing of medicines for public use. It is therefore imperative to conduct post market surveillance or monitoring of approved medicines in order to adequately assess the quality, therapeutic effectiveness and safety of medicines for the lager public. Post market monitoring ought not to be a one off event. Rather it should be a continuous event throughout the life of a drug product. Activities of post market monitoring of a drug have been identified to include; review of products condition of approved study evaluation and investigation of reported drug complains; inspection of manufacturer's processes and procedures (Ngwuluka, *et al*., 2009).

1.2 Multisource (Generic) Pharmaceutical Products

Multisource Pharmaceutical products are intended to be pharmaceutically equivalent or pharmaceutical alternatives that are bioequivalent and hence are therapeutically equivalent and interchangeably (WHO, 2005). The marketing of multisource drug products registered by national drug agencies in developing countries, with the view of improving health care delivery through competitive pricing, has its attendant problem of ascertaining their quality and inter-changeability. Variable clinical response to drug presented as generics and batch-to-batch inconsistencies have been reported. Such unacceptable trends were exhibited in some drug products including metronidazole and metformin. Quality control procedures, which are useful tools for batch-to-batch consistency in manufacturing, which should be performed for every

drug products requires analysis for their biopharmaceutical and chemical equivalency. These methods ensure that any of the generic products can be used interchangeably. The observation is that most of the generics have much lower shelf prices than the innovator products, which raises the issue of the likelihood of unequal product performance (Awofisayo, *et al*., 2010 a).

Oral ingestion is the most common method of drug administration. It is the safest, most convenient and most economical (Buxton, 2006). Tablets are the most frequently administered oral dosage form. The increase in the number of generic drug products from multiple sources has placed people involved in the delivery of health care in a position of having to select one from among several seemingly equivalent products. For instance, in 1975 approximately 9% of all prescription drugs dispensed in United State were generic versions. This figure rose to 20% in 1984 and 40% in 1991. Over 80% of the approximately 10,000 prescription drugs available in 1990 were obtained from more than one sources and variable clinical responses to these dosage forms supplied by two or more drug manufacturers ware documented. These variable responses may be due to the formulation ingredients employed, methods of handling, packaging and storage and even the rigors of in-process quality control. Thus, Michael *et al*, (2003) suggested that there is need to determine their pharmaceutical and therapeutic equivalence in order to ensure inter-changeability (Bano, *et al.,* 2011).

1.3 Quality Control

Quality control is the part of good manufacturing practice (GMP) concerned with sampling, specifications and testing, and with the organization, documentation and released procedures which ensure that the necessary and relevant tests are actually carried out and that materials are not released for use, nor products released for sale or supply, until their quality has been judged to be satisfactory. Quality control is not confined to laboratory operations but must be involved in all decisions concerning the quality of the product (WHO, 2006).

The independence of quality control from production is considered fundamental; each manufacturer should have a quality control function. The quality control function should be independent of other departments and under the authority of a person with appropriate

qualifications and experience, who has one or several control laboratories at his or her disposal. Adequate resources must be available to ensure that all the quality control arrangements are effectively and reliably carried out (WHO, 2006).

1.4 Quality Control Requirements of Tablet Dosage Form

Tablets are solid dosage form usually obtained by single or multiple compression of powders or granules. In certain cases tablets may be obtained by moulding or extrusion technique and they are uncoated or coated (WHO, 2011). Considering being the most widely use oral solid dosage form, always available due to its advantages of compactness, stability, portability, blandness of taste and ease of administration. Thus, these dosage forms are convenient for both the manufacturer and the patient. Being so popular, increase the risk of being faked or counterfeited (Rudnick and Schwartz, 2000). Below are the quality control requirements for tablets and the type of test required in evaluating them as stated by WHO:

1. Visual appearance, labelling, odour, taste, texture, hardness and friability.
2. Moisture content where limits are giving in official compendia.
3. Standard and test of identity: Designed to demonstrate unambiguously that the specimens examined contain the active ingredients they purport to contain.
4. Standard and test of homogeneity: apply test for uniformity of weight.
5. Standard and assay for the active ingredients and for degradation product provide quantitatively the permitted range of content of the active ingredient per tablet of average weight.
6. Standard and test of purity: for potentially harmful degradation compounds that may be generated during the production and storage of the dosage from and for contaminants whose presence may indicate a deviation from good manufacturing practice (Olaniyi, 2005).

1.5 Quality of a Drug Product

The aim of any drug therapy is the restoration of health to a patient; drugs should therefore contain medicaments at effective concentrations to achieve this purpose. A good quality drug product is achieved when there is adequate material, man and machine in place. This will give rise to products appropriate for their intended use. For this objective to be achieved it requires

the involvement and commitment of all concerned at all stages of drug development and manufacture. Hence for a drug to be of good quality, it has to conform to standard requirements as prescribed in official (national or international) monographs. These monographs such as British pharmacopoeia, National British Formulary and International Pharmacopoeia, contain laid down procedures for the production of specific items and they also contain details on expected quality of such items. Therefore it is the responsibility of the drug manufacturer to adopt and/or modify such procedures with the aim of producing drugs that are of high quality (Balat, 2006).

1.6 Fake or Substandard Drug Products

A drug can be said to be fake or substandard if the:

1. Drug product contains or excluded other active medicaments in addition to that indicated in the label.
2. Label claims of the product content differs from the actual product content
3. Products have met requirements (a) and (b) but have failed to be available due to poor formulation
4. Products met criterions (a) and (b) however are not capable of maintaining all their characteristics properties during its shelf-life probably due to poor storage conditions. The havoc done by substandard drugs cannot be over emphasized. Drug faking has assumed a worldwide pandemic (Balat, 2006).

1.7 *In-Vitro* Evaluation Studies of Tablets Dosage Form

Pharmaceutical equivalence is the condition in which drug products, containing the identical quantity of active substance (but not necessary containing the same excipients) in an identical comparable dosage form, meet all applicable standards of identical strength, quality, purity and potency. Pharmaceutically equivalent drug products may differ in characteristics such as shape, release mechanism, labelling (to some extent), scoring and excipients (including colours, flavours and preservatives) (Apu, *et al.,* 2011). Also, pharmaceutical equivalence is when the drug products contain the same active ingredients, the same dosage form, and route of administration and are identical in strength or concentration (Mullaicharm, *et al*., 2012). The

standard quality control test such as diameter, size and shape, uniformity of weight thickness, hardness, friability, percentage of medicament (Assay), rate of disintegration, dissolution and solubility can be carried out on compressed tablets for their evaluation (Kishore and Amareshwar, 2012). Assay of potency can also be carried out by microbiological assay (bioassay) (Apu, *et al*., 2011).

However, pharmaceutical equivalence of drugs may be established by *in-vitro* studies based on measurements intended to reflect the rate and extent to which the active pharmaceutical ingredient become available at the site of action. Based on the general consideration that *in-vitro* drug dissolution is predictive of *in-vitro* performance, *in-vitro* drug dissolution test for immediate release tablets and capsules are used among other things, to ensure conformity of drug products with official or set specifications and lot-to-lot quality control (Awofisayo, 2010 b). For products to be pharmaceutical equivalent, the products should be equivalence in pharmaceutical development, stability and in manufacture (Schmauster, 2010).

1.8 Methods for *In-Vitro* Evaluation Studies

1.8.1 Identification test

Identification testes are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g. Spectrum, chromatographic behaviour, chemical reactivity, etc.), to that of a reference standard (European medicine agency, 1995).

1.8.2 Weight variation

Tablet is designed to contain a specific amount of a drug in a specific amount of tablet formula. To check whether tablet contain a proper amount of drug, weight of tablet should be routinely measured (Bano, *et al.,* 2011). BP 2002 states that no two tablets: with an average weight of 80mg or less deviates by 10%, more than 80mg but less than 250mg deviates by 7.5% and 250mg or more should deviates by 5%.

1.8.3 Disintegration

Disintegration test measures the time required under a given set of conditions for a group of tablets to disintegrate into a particles. For compressed uncoated tablets the testing fluid is water at 37^0C, but in some cases the monographs direct that simulated Gastric fluid be used. (Remington, 1975). The BP specification is that uncoated tablets should disintegrate within 15 minutes and film coated in 30 minutes while USP specifies that uncoated and film coated tablets should disintegrate within 30 minutes. (Ngwuluka, *et al*., 2009).

1.8.4 Dissolution rate

Dissolution testing is required for all solid oral dosage forms in which absorption of the drug is necessary for the products to exert the desired therapeutic effect. Exceptions are for tablets meeting a requirement for completeness of solution or for rapid (10 - 15 minutes) disintegration for soluble or radio-labelled drugs. Generally experiments are conducted at 37˚C (USP, 2009). The acceptable limit is that a tablet or capsule should not contain anything less than 70% of the active ingredient when sample is taken at 45 minutes from the dissolution medium (BP, 2002).

1.8.5 Uniformity of content

The test for uniformity of content is based on the assay of the individual content of active ingredient of a number of single dose units to determine whether the individual contents are within limit set with reference to the average content of the sample. The test is not required for multivitamin and trace element preparations and in other justified and authorized circumstances (BP, 2002).

1.8.6 Friability of tablets testing

Friability testing is use to evaluate the ability of tablet dosage form to withstand abrasion in packaging, handling and shipping. (Remington, 1975). A maximum loss of 1% of the mass of the tablets tested is acceptable for most products.

1.8.7 Hardness testing

Hardness testing assesses the ability of tablets to withstand handling without fracturing or chipping (Ngwuluka, *et al*., 2009). It also measures the resistance of tablets to abrasion or

breakage under conditions of storage, transportation and handling before usage (Remington, 1975). Hardness of 4 - 15kgF is the acceptable limit (Ogah, *et al.,* 2002).

1.9 *In-Vivo* Availability Studies

Multisource pharmaceutical products need to conform to the same standards of quality, efficacy and safety as required of the originator's (comparator) product. Specifically, the multisource product should be therapeutically equivalent and interchangeable with the comparator product. Testing the bioequivalence between a product and a suitable comparator (pharmaceutically equivalent or a pharmaceutical alternative) in a pharmacokinetics study with a limited number of subjects is one way of demonstrating therapeutic equivalence without having to perform a clinical trial involving many patients. In such a pharmacokinetics study any statement about the safety and efficiency of the test product will be a prediction based on measurement of systemic concentrations, assuring that essentially similar plasma concentrations of the drug will result in essentially similar concentrations at the site of action, and thus an essentially similar therapeutic outcome. The bioequivalence study provides indirect evidence of the efficacy and safety of a multisource drug product. Often this will be the only evidence that the product is safe and efficacious. It is therefore crucial that the bioequivalence study is performed in an appropriate manner (WHO, 2006).

1.10 Bioequivalence and Bioavailability

Bioequivalence testing is considered as a surrogate for the chemical evaluation of the therapeutic performance of drug product (Awofisayo, 2010a). Bioequivalence shows that two formulations of one drug are equivalent. Bioequivalence testing can be used for drug-drug interaction studies and food effect availability studies (Zheng, *et al*., 2012).

On the other hand bioavailability is the rate and extent of absorption of a drug from its dosage form as determined by its concentration-time curve in the systemic circulation or by its excretion in urine, it is thus obvious that the drug preparation is always being compared to a reference standard. When this reference standard is an intravenous dose, it is absolute bioavailability that will be obtained. This is because a fast intravenous dose (bolus dose) of the

drug is assumed to introduce the whole dose (i.e. 100%) of the drug administered into the body at zero time after administration. However, relative bioavailability is the bioavailability of a drug determined by comparing the blood level and/or urinary excretion after administration of the test drug and a reference form (usually the innovator product or an established product) in the formulation, utilizing the same route of administration (Olaniyi, 2005).

1.11 Comparative Bioavailability

Most bioavailability studies, whether for a new or generic product, possess a common theme. A test was conducted to identify the quantitative nature of a specific product comparison. This comparison for a new drug may be, for example, to assess the performance of an oral formulation relative to the performance of a modified-release formulation in comparison to a conventional capsule. For a generic product, it is typically a comparison of a competitive formulation with a reference product. Such commonality surrounding comparative bioavailability studies suggests a universal experimental approach (Thiessen, 2004).

1.12 Study Design for *In-Vivo* Availability Studies

The study should be designed in such a way that the formulation effect can be distinguished from other effects. (European Medicine Agency, 2010). The study should be open label, balanced, and randomized, two sequences, two treatments, two period, single dose and crossover study with a washout period of two weeks (Bhoir, *et al*., 2012). The subjects should abstain from food and drinks, which may interact with circulatory, gastrointestinal, hepatic or renal function e.g. alcoholic drinks or certain fruit juices such as grape fruit juice) during a suitable period before and during the study. Subjects should not take any other concomitant medication (including herbal remedies) for an appropriate interval before as well as during the study (European Medicine Agency, 2010).

1.13 Validation of Analytical Methods

There are many reasons for the need to validate analytical procedures. Among them are regulatory requirements, good science, and quality control requirements. The code of federal regulations explicitly states that the accuracy, sensitivity, specificity and reproducibility of test methods employed by the firm should be evaluated and documented (Chan, 2008). The

objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose (European Medicine Agency, 1995).

1.13.1 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the values which is accepted either as a conventional true or an accepted reference value and the value found. This is sometimes termed trueness. (European Medicine Agency, 1995). The international convention on Harmonization (ICH) defines the accuracy of an analytical procedure as the closeness of agreement between the values that are accepted either as conventional true values or an accepted reference value and the value found (Chan, 2008). Accuracy of an analytical procedure is also express as recovery (Ghante, 2012). It is expresses as a percentage and relative standard deviation (RSD). Acceptable limit is 1 - 5% (Harvey, 2000).

1.13.2 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of the same homogenous samples under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision may be investigated using homogeneous, authentic samples. However, it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements (European medicine Agency, 1995).

1.13.3 Specificity

The ICH defines specificity as the ability to assess unequivocally an analyte in the presence of components that may be expected to be present. In many Publications, Selectivity and specifically are often used inter changeability. However, there are debates over the use of specificity over selectivity and same authorities, for example, the international union of pure and applied chemistry (IUPAC), have preferred the term selectivity, reserving specificity for

those procedures that are completely selective. For pharmaceutical application, the above definition of ICH will be used (Chan, 2008).

1.13.4 Linearity

ICH defines linearity of an analytical method as the ability (within a given range) to obtain test results of variable data (e.g. absorbance and area under the curve) which are directly proportional to the concentration (amount of analyte) in the sample. The data variables that can be used for quantization of the analyte are the peak areas, peak heights, or the ratio of peak areas (height) of analyte to the internal standard peak. Quantitation of the analyte depends on it obeying Beer's law for the spectroscopic method over a concentration range. Therefore the working sample concentration and samples tested for accuracy should be on the linear range. There are two general approaches for determining the linearity of the method. The first approach is to weigh different amount of standard directly to prepare linearity solutions at different concentrations. However, it is not suitable to prepare solution at very low concentration, as the weighing error will be relatively high. Another approach is to prepare a stock solution of high concentration. Linearity is then demonstrated directly by dilution of the standard stock solution. This is more popular and the recommended approach. Linearity is best evaluated by visual inspection of a plot of the signals as a function of analyte concentration. Subsequently, the variable data are generally used to calculate a regression line by the least square method. At least five concentrations levels should be used. Under normal circumstances, linearity is acceptable with a coefficient of determination (r^2) of ≥ 0.997. The slope, residual sum of squares, and y intercept should also be reported as required by ICH (Chan, 2008).

1.13.5 Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range is normally expressed in the same units as test result (e.g. per cent, part per million) obtained by the analytical procedure. For content uniformity, a normal range would cover 70 - 120 % of the nominal concentration. For

dissolution testing, a normal range is ±20 % over the specified range. If the acceptable criterion for a controlled release product covers a region from 20 % after 1h, and up to 90 % after 24h, the validated range would be 0 - 110 % of the labelled claim. In this case, the lowest appropriate quantifiable concentration of analyte will be used as the lowest limit as 0 % is not appropriate (Chan, 2008).

1.13.6 Robustness

Robustness of an analytical procedure is a measure of the analytical method to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The evaluation of robustness is normally considered during the development phase and depends on the type of procedure under study. Experimental design (e.g., Fractional factorial design or placket-Burman design) is common and useful to investigate multiple parameters simultaneously. The result will help to identify critical parameters that will affect the performance of the method. Common method parameters that can affect the analytical procedure should considered based on the analytical technique and properties of the samples (Chan, 2008).

1.13.7 Detection limit

The detection limit (DL) is a characteristic for the limit test only. It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The detection is usually expressed as the concentration of the analyte in the sample, for example, percentage, parts per million (ppm) or parts per billion (ppb).

Detection limit can be estimated from the standard deviation of the response and the slop of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard deviation of the intercepts of the regression lines

done in the range of the DL. This estimate will need to be subsequently validated by the independent analysis of a suitable number of samples near or at the DL.

$$DL = \frac{3.3\delta}{S}$$

Where δ is the standard deviation of the response and S is the slope of the calibration curve (Chan, 2008).

1.13.8 Quantitation limit

The quantitation limit (QL) is a characteristics of quantitative assay for low levels of compound in a sample matrices, such as impurities in bulk drug substance and degradation products in finished pharmaceuticals QL is defined as the concentration of related substance in the sample that will give a signal to noise ratio of 10:1. The QL of method is affected by both the detector sensitivity and the accuracy of sample preparation at low concentration of the impurities. In practice, QL should be lower than the corresponding ICH report limit.

$$QL = \frac{10\delta}{S}$$

Where δ = standard deviation of response and S = slope of the calibration curve (Chan, 2008).

1.14 Application of Validated Method to Routine Drug Analysis

Assay of all samples of an analyte in a biological matrix should be completed within the time period for which stability data are available. In general, biological samples can be analysed with a single determination without duplicated or replicate analysis if the assay method has acceptable variability as defined by validation data. This is true for procedures where precision and accuracy variability routinely fall within acceptable limits. For a difficult procedure with a labile analyte where high precision and accuracy specifications may be difficult to achieve, duplicate or even triplicate analysis can be performed for a better estimate of analyte. A calibration curve should be generated for each analyte to assay samples in each analytical run and should be used to calculate the concentration of the analyte in the unknown samples in the run. The spiked samples can contain more than one analyte. An analytical run can consist of QC samples, calibration standards, and either all the processed samples to be analysed as one batch or a batch composed of processed unknown samples to concentration range in addition to a calibrator sample at lower limit of quantification, LLOQ. Estimation of concentration of

unknown samples by extrapolation of standard curves below LLOQ or above the highest standard is not recommended. Instead, the standard curve should be redefined or samples with higher concentration should be diluted and re assayed. It is preferable to analyse all study samples from a subject in a single run (Centre for drug evaluation and research, 2001).

1.15 Statement of the Research Problem

The concern about the quality of drugs sold increases every year, not only in commercial terms, but also in legal and ethical aspects, since the health of patients depends on the quality and effectiveness of drugs (Nascimento, *et al*., 2011). Metronidazole is not an exception as there are many brands of locally and imported products of the drug in various drugs outlets in Nigeria. There are reliable evidence that some of them are fake, adulterated and substandard (Musa, *et al*., 2011).

1.16 Justification of the Study

In compliance to the problem stated above, WHO states the guidelines for analysis of multi-generic source of pharmaceutical products However, various regulatory agencies around the world are demanding validated method for the registration of new drugs to ensure the quality of drugs marketed (Nascimento, *et al*., 2011). In the same vein, drugs having more than three generic products require analysis for their biopharmaceutical and chemical equivalency. These methods ensure that any of the generic products can be used interchangeably (Chandrasekaran, *et al.,* 2011).

1.17 Research Hypothesis

1.17.1 Null hypothesis

There is no significant difference in the pharmaceutical, chemical and bio equivalences between Loxagyl® and other multi-generic sources of metronidazole tablets marketed in Zaria metropolis.

1.17.2 Alternate hypothesis

There is significant difference in the pharmaceutical, chemical and bio equivalences between Loxagyl® and other multi-generic sources of metronidazole tablets marketed in Zaria metropolis.

1.19 Aims and Objectives of the Study

1. To validate and modified the adopted UV spectrophotometric method for the analysis of metronidazole in a dosage form from a biological samples.
2. To establish the pharmaceutical, chemical and bioequivalence as well as interchangeability of various brands of metronidazole tablets marketed in Zaria metropolis with reference to comparator products (Loxagyl).

CHAPTER 2

LITERATURE REVIEW

2.1 History of Metronidazole

Metronidazole is one of the examples of drug developed against parasite, which has since gained broad use as an antibacterial agent. Briefly, at Rhone-Poulenc in France, extracts of *Streptomyces* species were screen for activity against *Trichomonas vaginalis*, a cause of vaginal itching. A Nitroimidazole, *Azomycin*, was identified, and a synthetic derivative, metronidazole, was used to treat chronic trichomonad infections, beginning in 1959. The antibacterial activity of metronidazole discovered by accident in 1962 when metronidazole cured a patient of both *trichomonad vaginitis* and *bacterial gingivitis*. However, it was not until the 1970s that metronidazole was popularized (Samuelson, 1999).

Also, the isolation of the antibiotic, azomycin (2-nitroimidazole) from a Streptomyces by Maeda and collaborators in 1953 and the demonstration of its trichomonocidal properties by Horie in 1956 led to the chemical synthesis and biological testing of many nitroimidazole. One compound, 1-(β-hydroxyethyl)-2-methyl-5-nitroimidazole, now called *metronidazole* (FLAGYL®). It was observed that metronidazole has high activity *in-vitro* and *in-vivo* against the anaerobic protozoa Trichomonas viginalis and Entamoeba histolytica. Dunel and associates (1960) reported that oral doses of the drug imparted trichomonacidal activity to semen and urine and that high cure rates could be obtained in both male and female patients with trichomoniasis (Phillips and Stanley, 2006). Later studies revealed that metronidazole has extremely useful clinical activity against varieties of anaerobic pathogens that include both gram positive and gram negative bacteria, in addition to the protozoan Giardia lamblia. In 1964, a dentist known as Shinn noted that patient with gingivitist was cured with flagyl (Phillips and Stanley, 2006). It was also the first drug to have a cure rate approaching 100 % per cent with systemic treatment (Cudmore, *et al.,* 2004).

Presently, metronidazole which is inexpensive has good penetration and produces relatively few side effects, is on the formulary at most hospitals for prophylaxis against anaerobic infection after bowel surgery, for treatment of wound abscess and for treatment of antibiotic associated colitis caused by clostridium difficile (Samuelson, 1999).

2.2 Chemistry of Metronidazole

Metronidazole is (2- (2 methyl-5- nitromidazole-1-yl) ethanol) (BP, 1993; Kolawole, 2004 and Usman *et al*, 2011). However it can be named as [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] (Alves *et al.*, 2007; Houghton, *et al*., 1982 and Phillips and Stanley, 2006). It is a synthetic antimicrobials agent with activity against obligate anaerobic bacterial and protozoa (Kolawole, 2004 and Mustapha, *et al.,* 2006). It is a prototype of nitroimidazole class of antimicrobials (Ezzeldin and El-Nahhas, 2012). It is white or yellowish, odourless crystals or crystalline powder. It darkens on exposure to light. Its formula is $C_6H_9N_3O_3$ with a molecular mass of 171.2 and chemical formula is represented in the figure below

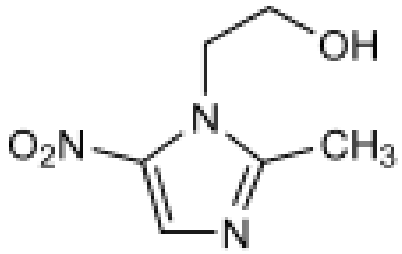


Figure 2.1: Chemical formula of metronidazole (Martindale, 1999)

2.3 Physicochemical Properties of Metronidazole

2.3.1 Salt and esters form

The British pharmacopoeia, the European pharmacopoeia, the International pharmacopoeia, and the US pharmacopoeia have monographs for metronidazole base and metronidazole benzoate. The Brazilian pharmacopoeia has monograph for metronidazole base only. Metronidazole base is used only for gel, injections, tablets and suppositories, whereas metronidazole benzoate is formulated as oral suspensions. Metronidazole hydrochloride is used for injections (Rediguieri, *et al.,* 2011).

2.3.2 Solubility of metronidazole

Metronidazole's solubility in water has been reported as 10mg/ml at 20 ˚C. Another source reported a solubility of 64.8 mg/ml at room temperature and at pH 1.2, decreasing to around 10 mg/ml at pH values between 2.5 and 8. Metronidazole was considered soluble at a dose of 500 mg (Rediguieri, *et al*., 2011). On the other hand, it is sparingly soluble in water and in alcohol, slightly soluble in chloroform and ether (Martindale, 1999). However, according to BP specification; metronidazole is slightly soluble in acetone and methylene chloride (BP, 2002).

2.4 Therapeutic Index of Metronidazole

Metronidazole is in general, very well tolerated, has a wide therapeutic index and its serum and tissue concentrations do not required routine determination (Rediguieri, *et al*; 2011).

2.5 Available Dosage Form Strengths

The WHO Model List of Essential Medicine mentioned 200 mg to 500 mg of metronidazole tablets dosage form, 200 mg/5ml metronidazole (as benzoate) oral suspension, 500 mg in 100ml vial for I.V, 100 ml bag for intravenous infusion; and 0.5 g and 1 g metronidazole suppositories (WHO, 2011) as well as 10 %w/w metronidazole vaginal cream available in tube of 60 g with applicator (Sonofi-aventis, 2012).

2.6 Analytical Methods for Analysis of Metronidazole from a Dosage Form

Metronidazole can be determined with microbiological techniques (Ralph, et al; 1974), Spectrophotometric methods; thin layer Chromatography (TLC), Gas Chromatography (GC) and High Pressure Liquid Chromatography (HPLC). Early detection of metronidazole was based on bioassay and gas liquid chromatography. These methods were time consuming and failed to determine the concentrations of the metabolites. UV (ultraviolet) and IR (infrared) spectrophotometry and HPLC, especially for analysis from biological fluids, were preferred with the development of the other techniques. HPLC has advantage over the other methods as an accurate and sensitive method. It was reported that HPLC has sensitivity at nanogram/gram level to detect drug content from vaginal tissue and as little as 5ng of the drug was detected from the serum. HPLC methods are able to determine hydroxyl and acetic acid metabolite detection is usually carried out at 320 nm (Turgut and Ozyazici, 2004). However, metronidazole

can be determined by titrimetry and potentiometry. Indian pharmacopoeia describes the non-aqueous titration method using perchloric acid as titrant and malachite green as indicator for the assay of tinidazole and metronidazole. British pharmacopoeia describes potentiometric and non-aqueous titration method using perchloric acid as titrant. United States pharmacopoeia describes HPLC and non-aqueous titration method for the assay of metronidazole only (Dinesh, *et al.,* 2003)

2.7 Indications

Metronidazole is used in the treatment of susceptible protozoal infections such as amoebiasis, balantidiasis, Blastocystis hominis infections, giardiasis and trichomonisis; it has also been tried in leishmaniasis and microsporidisis. Metronidazole is also used in the treatment and prophylaxis of anaerobic bacterial infections. Specific bacterial infections treated with, metronidazole includes bacterial vaginosis, acute necrotizing ulcerative gingivitis, pelvic inflammatory disease, tetanus and antibiotic colitis. Metronidazole is used to eradicate Helicobacter pylori in peptic ulcer disease. (With other antimicrobials, and either bismuth compounds or proton pump inhibitors) and in the management of malodorous tumours and ulcers where there is anaerobic infection. It is also use in the treatment of rosacea and dracunculiasis (guinea-worn infection) and has given in the treatment of perional crohins disease and hepatic encephalopathy. It has also been tried as an adjunct to the radio therapy of malignant neoplasms (Martindale 1999).

2.8 Adverse Effects

The adverse effects of metronidazole are generally dose related. The most common are gastrointestinal disturbances, especially nausea and unpleasant metallic taste. Vomiting and diarrhoea, glositis, and stomatitis may be associated with overgrowth of candida. There are rare reports of antibiotic-associated colitis associated with metronidazole (it is also used in the treatment of this condition). Weakness, dizziness, ataxia, headache, drowsiness, insomnia, and change in mood or mental state such as depression or confusion have also been reported. Peripheral neuropathy usually presenting as numbness or tingling in the extremities, and epileptiform seizures are serious adverse effects on the nervous system that have been

associated with high doses of metronidazole or prolonged treatment. Temporary moderate leucopenia and thrombocytopenia may occur in some patients receiving metronidazole. Skin rashes, urticaria, and pruritus occur occasionally and erythema multiforme, angioedema and anaphylaxis have been reported rarely. Other side effects include urethral discomfort and darkening of the urine. Raised liver enzyme values, homeostatic hepatitis and Jaundice have occasionally been reported. Thrombophlembitis may follow the intravenous administration of metronidazole. Studies have shown metronidazole to be mutagenic in bacteria and carcinogenic in some animals (Martindale, 1999).

2.9 Interactions of Metronidazole with Some Drugs

The following are some of the drug interactions of metronidazole.

2.9.1 Alcohol

Metronidazole may provoke a disulfiram like reaction in some individuals when given with alcohol; reactions have occurred after the use of preparations formulated with alcohol, including injections, as well as after drinking alcohol. Acute psychosis or confessional state was reported in 6 of 29 alcoholic patients who were also receiving disulfiram. However an analysis of published reports and a study in healthy subjects both found that there was no convincing evidence of a disulfiram-like reaction between metronidazole and alcohol although caution was still advised (Martindale, 1999).

2.9.2 Busulfan

The use of busulfan with metronidazole significantly increased plasma concentrations of busulfan and the degree of associated toxicity, including elevations of liver function tests, veno-occlusive disease and mucositis (Martindale, 1999).

2.9.3 Antacid, kaolin- pectin or colestyramine

The absorption of metronidazole from the gut is unaffected by kaolin- pectin, but a small reduction occurs if either an aluminium hydroxide antacid or colestyramine is given concurrently (Stockley's drug interactions, 2005).

2.9.4 Barbiturates

Phenobarbital markedly increases the loss of metronidazole from the body so that larger doses are needed. Conventional doses of metronidazole in the presence of Phenobarbital failed to clear up trichomoniasis in a woman, and giardiasis or amoebiasis in children (Stockley's drug interactions, 2005).

2.9.5 Disulfiram

Acute psychosis and confusion can result from the concurrent use of metronidazole and disulfiram (stockley's drug interaction, 2005).

2.9.6 Carbamazepine

A patient receiving carbamazepine for bipolar disorder developed dizziness, diplopia and nausea 4 days after the addition of metronidazole for diverticulitis (Martindale, 1999).

2.9.7 Phenytoin

In addition to conflicting reports on the effect of metronidazole on the metabolism of phenytoin, increased metabolism of metronidazole was reported in patient during treatment with phenytoin (Martindale, 1999).

2.15 Pharmacokinetics of Metronidazole

Metronidazole is readily and almost completely absorbed after oral doses. Peak plasma concentrations of about 6 and 12 µg/ml are achieved, usually within 1 to 2 hours, after a single dose of 250 and 500 mg respectively. Some accumulation occurs and consequently there are higher concentrations when multiple doses are given. Absorption may be delayed, but is not reduced overall by food. Metronidazole benzoate given by mouth is hydrolysed in the gastrointestinal tract to release metronidazole, which in turn is than absorbed. Peak steady- state plasma concentration of about 25 µg/ml with trough concentrations of about 18 µg/ml have been reported in patients given an intravenous loading dose of 15 mg/kg following by 7.5 mg/kg every 6 hours. The availability of metronidazole from rectal suppositories is 60 to 80 %; peak plasma concentrations are half those achieve with equivalent oral doses and effective concentrations occurs after about 5 to 10 hours. Absorption from vaginal passerines is poor with a reported bioavailability of about 20 to 25 %; absorption is gradual producing peak plasma

concentrations of about 2 mg/ml after a dose of 500 mg. An intra-vaginal gel formulation providing a dose of 37.5 mg metronidazole produce peak plasma concentrations of 0.3 mg/ml at 8 hours, with a bioavailability of 56 % metronidazole is widely distributed. It appears in most body tissues and fluids including bile, bone, breast milk, cerebral, CSF, liver and liver abscesses, saliva, seminal fluid, and vaginal secretions, and achieves concentrations similar to those in plasma. It also crosses the placenta and rapidly enters the foetal circulation. No more than 20% is bound to plasma proteins.

Metronidazole is metabolized in the liver by side-chain oxidation and glucoronide formation. The principal oxidative metabolites are 1- (2- hydroxyl ethyl) -2 hydroxymethyl-5-nitroinidazole (the hydroxyl metabolite), which has antibacterial activity and is detected in plasma and urine, and 2-methyl-5-nitroinitrozole -1- acetic acid (the acid metabolite), which has virtually no antibacterial activity and is often not detected in plasma, but is excreted in urine. Small amounts of reduced metabolites, acetamide and N-(2- hydroxyl ethyl) oxamic acid (HOA), have also been detected in urine and are probably formed by the intestinal flora (Martindale, 1999).

The elimination half-life of metronidazole is about 8 hours that of the hydroxyl metabolite is slightly longer. The half-life of metronidazole is reported to be longer in neonates and in patients with severe hepatic impairment; that of the hydroxyl metabolite is prolonged in patients with substantial renal impairment. The majority of a dose of metronidazole is excreted in the urine, mainly as metabolites; a small amount appears the faeces (Martindale, 1999).

2.16 Justification for the Use of Saliva as an Analytical Fluid

In recent years saliva has attracted much attention, in particular among people interested in the determination of drug concentrations, who suggest that saliva might be substituted for plasma in the areas of pharmacokinetic studies and drug monitoring. The traditional biological samples for the qualitative and quantitative measurement of most drugs are blood, plasma and urine. Previous publications have made it clear that for many drugs, the monitoring of saliva is a real alternative for determining plasma levels because saliva lacks “the drama of blood” (Hold, *et*

al., 1999) in comparison to blood saliva has the following advantages. Non-invasive, easy to collect, cost effective, no need for highly skilled personnel, no risk of contracting infection agents such as HIV, hepatitis B and C (Punyadeera, 2011).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The following were the materials used for the study.

3.1.1 Reagents and chemicals used

Analytical grade chemicals and reagents (BDH) were used.

3.1.2 Equipment and glass wares

Various types of glass wares, Analytical weighing balance (Mettler Analytical Balance Phillip Harris., England), Centrifuge Machine (Gallenkamph, England), Dissolution test apparatus (DT 80 - GmbH, Germany), Euweka Disintegration Time Test apparatus (Type ZT3, -GmbH, Germany), Euweka friabilator (Type TA-3R, GmbH, Germany)

Monsanto Hardness tester (Manesty Machines Liverpool, England), pH meter (Fisher Scientific, Singapore), Rotor mixer (Gallankamp, England), UV double beam spectrophotometer (MNF, Helious Zeta, Thermo Scientific England) and Water bath (model BJE 750A Gallenkamp, England)

3.1.3 Samples

Standard metronidazole powder from J. Link China with a batch number 10090704, manufacturing date of 9 September, 2010 and expiry date of 9 September, 2014.

Different brands of metronidazole tablets collected from Zaria metropolis.

3.2 Method

3.2.1 Sample collection

Different brands of metronidazole tablets were randomly purchased at different retail pharmacies and drug outlets in Sabon Gari, Zaria City, Tudun Wada, Samaru and Shika in the month of July, 2011. All the tablets were labelled to contain 200 mg of metronidazole as the active ingredient. The samples were coded and stored in the recommended conditions specified by the manufacturer in their original packs prior to the research. The details about the name, manufacturer, production and expiry dates, NAFDAC registration number and batch number were given in appendix I.

3.2.2 Assessment of *in-vitro* parameters of the samples

3.2.2.1 Physical Examination

A sachet containing10 tablets for each of the samples was randomly taken, the tablets were removed and examined for lustre, colour, texture, nature of surface and presence of score (Musa*, et al*., 2011).

3.2.2.2 Weight Variation

Twenty tablets were randomly taken. The tablets were collectively weighted as described in BP 2002. The mean weight was calculated and the deviation from the mean for each tablet was calculated.

3.2.2.3 Identification Test

A quantity of the powered tablets containing 10 mg of metronidazole, 10 mg of zinc power, 1 ml of deionised water and 0.25 ml of hydrochloric acid in a test tube was heated on a water bath for 5 minutes. The test tube was cool, then 0.5 ml of sodium nitrite solution was added and the excess nitrite was removed with sulphamic acid. Then 0.5 ml of 2-naphthol solution and 2 ml of 5 M sodium hydroxide were added. The formation of orange-red colour indicates the presence of metronidazole. The procedure was repeated for all the samples (BP, 2002).

3.2.2.4 Friability Test

Ten tablets of each sample were initially weighed and then placed in friabilator which operated at 25 revolutions per minute. After 4 minutes run, the remaining intact tablets were removed, de-dusted with a tissue paper and reweighted. The loss in weight was noted and the percentage lost calculated (BP, 2002).

3.2.2.5 Disintegration Test

Six tablets were selected at random for each brand. The disintegration time for each brand was determined using Euweka disintegration apparatus. Water was used as the medium maintained at 37 ± 1^{o}C. One table was placed in each of the six tubes/units of the machine. The time taken for each of the individual tablet to pass through the tube was recorded. The average time for six tablets was taken as the disintegration time for a brand (BP, 2002).

3.2.2.6 Dissolution Rate Test

The dissolution rates of the active ingredient from the tablet dosage form were determined using Euweka dissolution apparatus. The dissolution medium was 0.1 N Hydrochloric acid solution. The medium was maintained at 37± 0.5 ^{0}C. The paddles of the apparatus were maintained at a speed of 100 rpm, 1ml of a sample was withdrawn with a syringe at 45minutes and diluted to 10 ml with the medium. The absorbance of metronidazole from the solution was determined using double beam UV spectrophotometer at 277 nm. The percentage of metronidazole released in the dissolution medium was determined. The procedure was repeated three times for each of the brands (BP, 2002). The absorbance was converted to concentration in % w/v from which percentage released was calculated using E 1 %, 1 cm of 380 (BPC, 1978).

3.2.2.7 Hardness Test

This is an unofficial method of ascertaining the quality of a tablet. Five tablets were selected randomly. A tablet was placed between the Jaws of a Monsanto Hardness tester. The orientation of the tablets was in the same way with respect to the direction of applied force. The force required to crush each tablet was noted and recorded in kilograms force (Muhammed*, et al.*, 2012).

3.2.2.8 Assay

Twenty tablets of metronidazole were randomly selected. The tablets were crushed in a mortar with a pestle. A quantity of the powder containing 0.2 g of metronidazole was taken into a separating funnel and extracted with six 10ml quantities of acetone. The solution was cool and 50ml of acetic anhydride was added to the combined extracts. 0.1 ml of 1 % w/v solution of brilliant green in anhydrous acetic acid was also added and titrated with 0.1 M Perchloric acid VS to a yellowish green end point. The operation was repeated without the powered tablets. The difference between the titrations represents the amount of Perchloric acid VS required. Each ml of 0.1 M per chloric acid VS is equivalent to 17.12 mg of $C_6H_9N_3O_3$. The procedure was repeated for all the brands (BP, 2002).

3.2.2.9 Identification of Standard Metronidazole Powder

This was done according to BP (1993) method. 0.02 % w/v of metronidazole in 0.1 M HCl was prepared by dissolving 100 mg of standard metroninidazole powder in 100ml of 0.1 M HCl in Erlenmeyer volumetric flaks. Then 20 ml of the solution was taken with a pipette and the volume made up to 100 ml with 0.1 M HCl in Erlenmeyer volumetric flaks. Finally 10 ml of the resulting solution was taken and the volume made up to 100 ml with 0.1 M HCl. The resulting solution was 0.02 %w/v of metronidazole powder. A portion of 0.02 %w/v of the metronidazole solution was scanned at the UV region of 230 - 350 nm (BP, 1993).

3.2.3 Analytical Method

UV spectrophotometric method developed by Kolawole and Ameh, (2004) was adopted and modified.

3.2.4 Preparation of the Stock Solution

100 mg of the standard metronidazole powder was accurately weighed with the aid of an analytical weighing balance. The powder was carefully transferred into 100 ml cleaned and dried Erlenmeyer volumetric flask. 20 ml of deionised water was added. The flask was vigorously shaken to dissolve the powder and the volume made up to the mark with deionised water. The resulting solution was 1 mg/ml from which a stock solution of 15 µg/ml was formed through serial dilution method.

3.2.5 Selection of pH medium

1 ml of the stock solution was spiked into 1 ml of saliva in different centrifuge tubes; then 1 ml of the different buffer medium with pH 4, 5, 6, 7, 8 and 9 was individually added into the centrifuge tubes. The resultant solution was than vortex mixed for 1 minute and centrifuged at 3 g for 10 minutes. The supernatants were extracted with 2 x 5 ml of chloroform. The chloroform layer was collected, then 4 ml of 0.1 M HCL was added to all the tubes and the absorbance of each aqueous layer was taken at 277 nm using double beam UV spectrophotometer. The pH given the highest absorbance was noted and recorded

3.2.6 Construction of calibration curve

Metronidazole has a peak plasma concentration of 12 mg/ml following oral administration of 500 g in 1 to 2 hours (Martindale, 1999). With respect to this, working concentrations of 2.5 to 15 µg/ml were prepared from the stock solution. About 20 ml of saliva was collected with the aid of chewing gum to stimulate salivation from healthy human volunteers. The saliva was centrifuged at 3 g for 10 minutes. The supernatant was collected and 1 ml of the supernatant was transferred to a test tube. 3 ml of buffer solution with pH 9 was added followed by the spiking of different concentrations of 1 ml of 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, 12.5 µg/ml and 15 µg/ml of metronidazole to each of the test tubes. Each of the test tubes was vortex mixed with a rotor mixer for 1 minute. The solutions were extracted with 2 x 5 ml of chloroform. The chloroform layer was collected, then 4 ml of 0.1 M HCL was added and the absorbance of the aqueous layer was taken at 277 nm (as obtained from 3.2.5 as the maximum absorbance wavelength of metronidazole) using double beam UV spectrophotometer. The absorbance were plotted against concentrations (µg/ml); and the linearity (coefficient of correlation, r^2; intercept on the vertical axis, C; slope of the graph, S and standard deviation, δ) was established using method of least square with the aid Microsoft Excel 2010, limit of detection, LOD and limit of quantitation LOQ were determined using the formula LOD = 3.3δ/S and LOQ = 10δ/S (where S is slope of the graph) respectively.

3.2.7 Validation and optimization of the analytical method

3.2.7.1 Linearity

The linearity of the proposed method was established by using method of least square and regression analysis.

3.2.7.2 Precision

Precision was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was determined by taking the absorbance of 1ml of 15µg/ml stock solution with 3ml buffer solution with a pH 9. The absorbance was taken six times at an interval of 1 hour in the first day under the same experimental condition. Inter-day precision was evaluated by taking

the absorbance of 1ml of 15 µg/ml stock solution with 3 ml of buffer solution with a pH 9 three times in three consecutive days.

3.2.7.3 Accuracy and Percentage Recovery

This was done by spiking 1 ml each of 8 µg/ml, 10 µg/ml and 12 µg/ml of metronidazole corresponding to concentration of 80 %, 100 % and 120 % of a nominal concentration of 10 µg/ml solution of metronidazole into 1 ml of supernatant portion of saliva (Ghante, *et al.,* 2012; Nascrimento, *et al.*, 2011; Sundaraganapathy *et al*, 2011 and Wrasse-Songoi, *et al.*, 2010). 3 ml of the pH buffer was added, vortex mixed for 1 minute and extracted with 2 x 5 ml chloroform, followed by addition of 4 ml of 0.1 M HCl. The aqueous layer was collected and the absorbance for each concentration was taken at 277 nm.

3.2.8 *In-vivo* application of the method

3.2.8.1 Selection of subjects

Six healthy human volunteers were enrolled for the study. Their health status was confirmed by urinalysis. The subjects were non-smokers, non-alcohol drinkers or Kola nut consumers. The protocols of the study were explained to them and informed consent sought. No drug was used two weeks prior to study. Their mean age was 26.33±0.95 years and mean body mass indices was 22.08±1.11 kg/m^2 which were within the acceptable normal range of 18 – 55 years (European Medicine Agency, 2010).

3.2.8.2 Study design

The study was single dose, randomized, crossover design study with a washout period of two weeks.

3.2.8.3 Drug administration

400 mg (2 tablets of 200 mg each) of metronidazole was taken orally by each of the subjects following overnight fasting of not less than 10 hours. The drugs were administered with about 200 ml of water. No food or drink allowed 3 hours after administration of the drugs.

3.2.8.4 Saliva collection

Saliva was collected with the aid of chewing gum into a 5 ml sample bottles. About 2 ml of saliva samples were collected. The samples were centrifuged at 3 g for 10 minutes. 1 ml of the supernatant was taken followed by the addition of 3 ml of pH buffer. The solutions were vortex mixed for 1minute and stored in a freezer at - 4 ^{O}C prior analysis.

3.2.8.5 Sampling time

Blank saliva was collected prior to administration of the drugs to each subject which corresponds to time zero; and subsequently at 30 minutes, 1 hour, 2hour, 3hour, 4 hours, 6hour and 8 hour after administration.

3.2.9 Extraction procedure

The extraction procedure was adopted from the work of Kolawole and Ameh (2004). The frozen samples were melted with the aid of water bath maintained at a temperature of 37 ^{O}C. The samples were extracted with 2 x 5ml chloroform in a separating funnel for about 10 minutes. 4 ml of 0.1 M hydrochloric acid was added to the combined chloroform extract and agitated for about 2 minutes in a separating funnel. The aqueous layer was taken and the absorbance at 277 nm was noted and recorded against blank saliva as base line using double beam UV spectrophotometer.

3.2.10 Data analysis

The calibration curve and the regression analysis were done using Microsoft excel 2010. The absorbance were converted to concentration terms using the validated calibration curve. The mean AUC, the mean C_{max} and the mean T_{max} were calculated with the aid of a graphpad prism version 5. The point estimate ratio was calculated manually with reference to the mean value for sample MT01 as the reference standard. The results were presented as mean ± SEM and percentage where appropriate.

CHAPTER 4

RESULT

The Results for both *in-vitro* and *in-vivo* studies of the samples of metronidazole were shown below:

4.1 The Labels of the Samples of Metronidazole (200 mg) Tablets Randomly Selected as Samples

Table 4.1: Labels of the Samples of Metronidazole (200 mg) Tablets Randomly Selected as Samples.

Brand	Country of Origin	Batch No.	NAFDAC No.	Manuf. Date	Expiry Date
MT01	Nigeria	MA1048	04-0283	01/2011	12/2015
MT02	Nigeria	UGT0290	04-8426	12/2010	11/2013
MT03	Nigeria	21590	04-4012	05/2011	05/2014
MT04	Nigeria	1109	04-9936	03/2011	03/2016
MT05	Nigeria	0015	04-7473	01/2011	05/2013
MT06	Nigeria	14	04-1308	12/2010	05/2013
MT07	Nigeria	0160	04-0386	06/2011	02/2014
MT08	Nigeria	153311	04-0072	03/2011	09/2016
MT09	Nigeria	524136	04-0275	03/2011	02/2013
MT10	Nigeria	11015	04-0980	02/2011	02/2014
MT11	Nigeria	MT470	04-4283	04/2011	05/2014
MT12	Nigeria	142	04-7612	05/2011	05/2014

4.2 Physical Appearance of the Samples of Metronidazole (200 mg) Tablets Randomly Selected as Samples

Table 4.2: Result for the Physical Appearance of the Samples of Metronidazole (200 mg) Tablets

Brand	Colour	Shape	Score	Lustre	Nature of surface
MT01	White	Circular, convex surface	+	Dull	Smooth
MT02	Yellow	Circular, convex surface	+	Shiny	Rough
MT03	White	Circular, convex surface	+	Dull	Smooth
MT04	White	Circular, convex surface	-	Dull	Smooth
MT05	Yellow	Circular, flat surface	-	Dull	Smooth
MT06	Yellow	Circular, flat surface	+	Dull	Rough
MT07	White	Circular, convex surface	+	Shiny	Smooth
MT08	Yellow	Circular, flat surface	+	Dull	Smooth
MT09	Yellow	Circular, flat surface	-	Dull	Smooth
MT10	Yellow	Circular, flat surface	+	Dull	Smooth
MT11	Yellow	Circular, flat surface	+	Dull	Smooth
MT12	Yellow	Circular, flat surface	+	Dull	Rough

4.3 Identification Test on the Samples of Metronidazole Tablets

All the Brands of Metronidazole (200 mg) Tablets revealed the presence of Metronidazole in the Tablet Dosage Form using BP 1993 method.

4.4 Weight Variation and Disintegration Time for the Samples of Metronidazole (200 mg) Tablets Dosage Form

Table 4.3: Result for Weight Variation and Disintegration Time for Metronidazole (200 mg) Tablet Dosage Form

	n=20		**n=6**
Brand	**Mean weight/mg(SEM)**	**No. of tablets deviated by≥5%**	**Mean disintegration time/min/(SEM)**
MT01	347.35 (3.00)	None	3.09 (0.51)
MT02	484.75 (2.52)	None	2.42 (0.14)
MT03	338.50 (0.74)	None	0.95 (0.08)
MT04	506.80 (2.55)	None	9.20 (1.00)
MT05	589.50 (5.34)	5	3.57 (0.36)
MT06	581.15 (2.21)	None	10.95 (0.29)
MT07	334.85 (3.03)	2	18.97 (1.33)
MT08	372.10 (3.94)	4	1.01 (0.18)
MT09	609.25 (2.59)	1	0.74 (0.07)
MT10	585.00 (3.43)	2	1.20 (0.16)
MT11	600.70 (2.16)	1	0.62 (0.13)
MT12	666.70 (13.06)	14	6.48 (0.49)

Normal values: weight variation, no more than 2 tablets deviate by ≥ 5 % (BP, 2002). Disintegration time: BP ≤ 15minutes (BP, 2002)

4.5 Assay, Dissolution, Friability and Hardness Tests of Metronidazole Tablets Dosage Form

Table 4.4: Result for Assay, Dissolution, Friability and Crushing Strength Tests of Metronidazole Tablets Dosage Form.

Brand	**n=6**	**n=6**	**n=6**	**n=5**
	Assay (%)	**Mean dissolution (%)**	**Friability (%)**	**Mean hardness (KgF) (SEM)**
MT01	102.72	84.17	0.29	5.50 (0.28)
MT02	95.87	96.09	1.00	5.00 (0.20)
MT03	102.72	89.61	0.90	6.50 (0.40)
MT04	106.16	108.50	0.40	6.70 (0.52)
MT05	99.30	87.44	1.70	5.50 (0.28)
MT06	102.72	79.29	0.90	5.70 (0.84)
MT07	102.72	79.29	1.81	9.40 (0.54)
MT08	106.14	103.02	1.62	7.10 (0.30)
MT09	99.30	74.30	0.66	6.30 (0.23)
MT10	92.45	72.00	0.86	10.70 (0.46)
MT11	102.72	80.39	10.25	10.60 (0.17)
MT12	92.45	73.60	0.77	7.60 (0.68)

Normal values: Assay (BP: 85-115 %, IP: 90-110 %) Mean dissolution rate (BP not < 70% at 45 minutes), Mean friability (BP and USP ≤ 1 %) and Mean hardness 4-10 Kg

4.6 Identification of Metronidazole Reference Standard Using UV Spectrophotometry According to BP 1993 Method

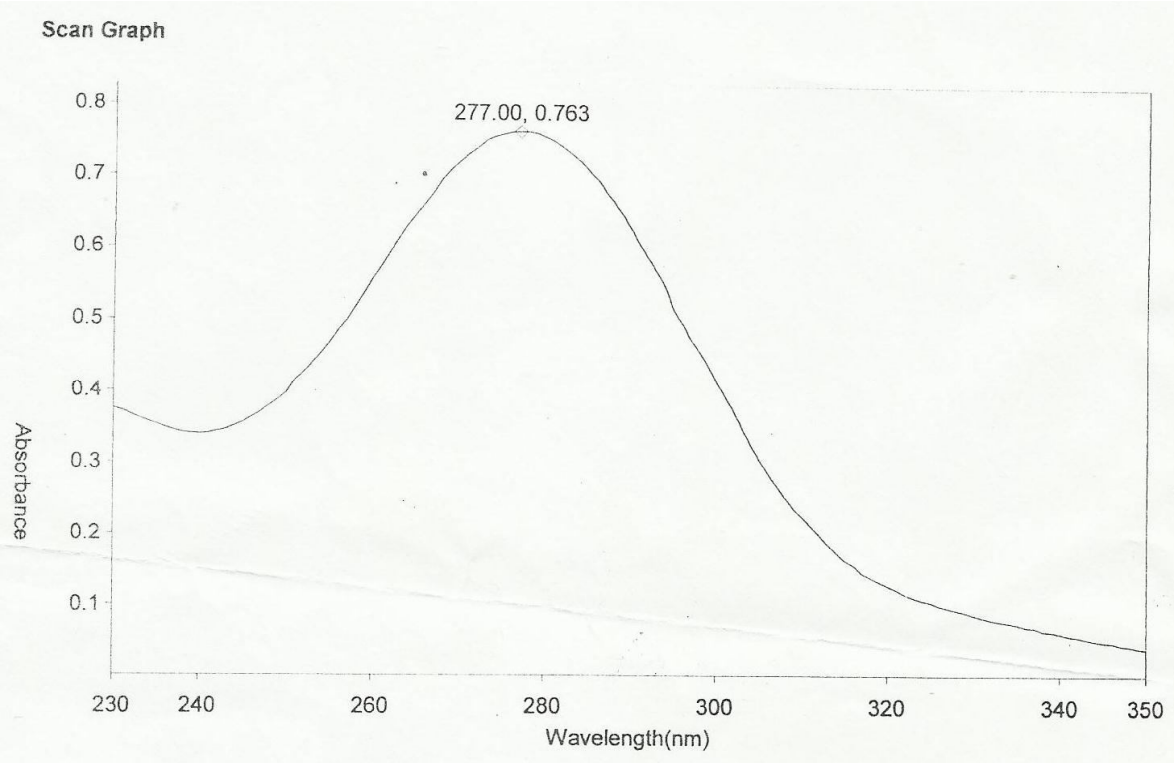


Figure 4.1: Absorbance of 0.02 %w/v Solution of Metronidazole Standard Powder in 0.1 M HCl scanned at 230-350 nm.

4.7 Effect of PH on the Absorbance of 15 µg/ml Stock Solution of Metronidazole Reference Standard Spiked into Blank Saliva

Table 4.5: Effect of pH on the Absorbance of 15 µg/ml stock Solution of Metronidazole Reference Standard spiked into blank saliva.

S/n	pH medium	Absorbance
1.	4	0.0320
2.	5	0.0471
3.	6	0.0471
4.	7	0.0500
5.	8	0.0505
6.	9	0.0506

4.8 Intra- and Inter-Day Precisions of the Method

Table 4.6: Absorbance for intra- and inter-day Precisions of 15 µg/ml of Metronidazole Reference Standard at pH 9 spiked into blank Saliva

s/n	**Absorbance**	
	Intra-day precision	**Inter-day precision**
1.	0.0506	0.0509
2.	0.0509	0.0512
3.	0.0507	0.0512
4.	0.0513	0.0514
5.	0.0510	0.0513
6.	0.0512	0.0508
Mean	0.0510±0.0001	$0.0511 \pm 8.7 \times 10^{-5}$
SD	2.5×10^{4}	2.13×10^{-4}
RSD (%)	0.491	0.417

NOTE: Acceptable limit, RSD ≤ 2 %

4.9 Accuracy of the Method and Percentage Recovery of Metronidazole Reference Standard Spiked into Blank Saliva

Table 4.7: Accuracy of the Method and Percentage Recovery of Metronidazole Reference Standard spiked into Blank Saliva

S/N	n = 3 Amount added (µg/ml)	n = 3 Amount found(µg/ml)	Percentage recovery (%)	Accuracy (%Er)
1.	8	7.9	98.75	1.25
2.	10	9.7	97.00	3.00
3.	12	11.8	98.33	1.70
Mean value			98.30	1.98

The mean value for percentage recovery was 98.03% (acceptable limit of 98 – 102 %; Chan, 2008) and accuracy of the method was 1.98 %Er (acceptable range: 1-5 %Er)

4.10 Calibration Curve for *In-Vivo* Availability Studies of the Samples of Metronidazole Tablets

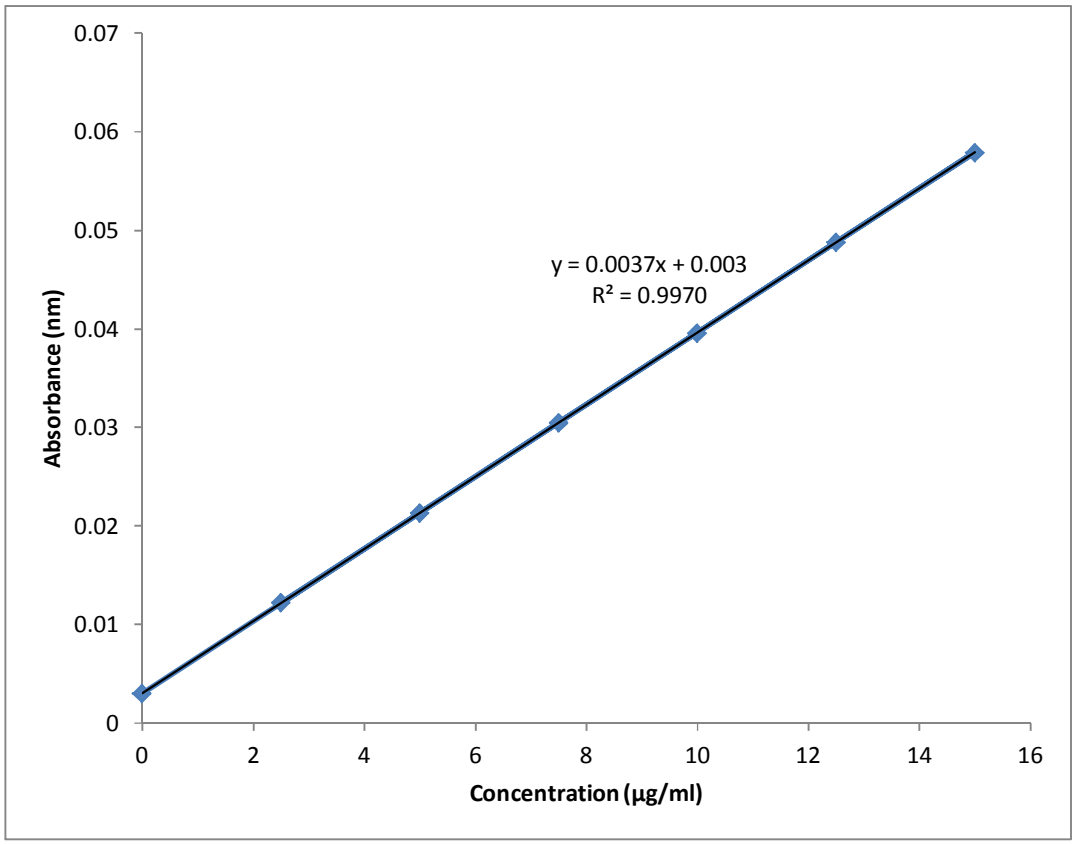


Figure 4.2: Calibration Curve for *in-vivo* Availability Studies of the Samples of Metronidazole Tablets

4.11 Linearity of the Method

Table 4.8: Result for Linearity of the Method

S/N	Parameter	Value
1.	Maximum wavelength, λ_{max}	277 nm
2.	Calibration curve range	2.5-15 µg/ml
4.	Regression equation	$Y = 3.66\times10^{-3}X + 3.02\times10^{-3}$
5.	Intercept on the vertical axis, C	0.0030
6.	Correlation coefficient, r^2	0.9970
7.	Limit of detection, LOD	1.1928 µg/ml
8.	Limit of quantitation, LOQ	3.6145 µg/ml
9.	Standard deviation, σ^2	1.3229

4.12 Concentration-Time Curve for Metronidazole from Saliva Samples

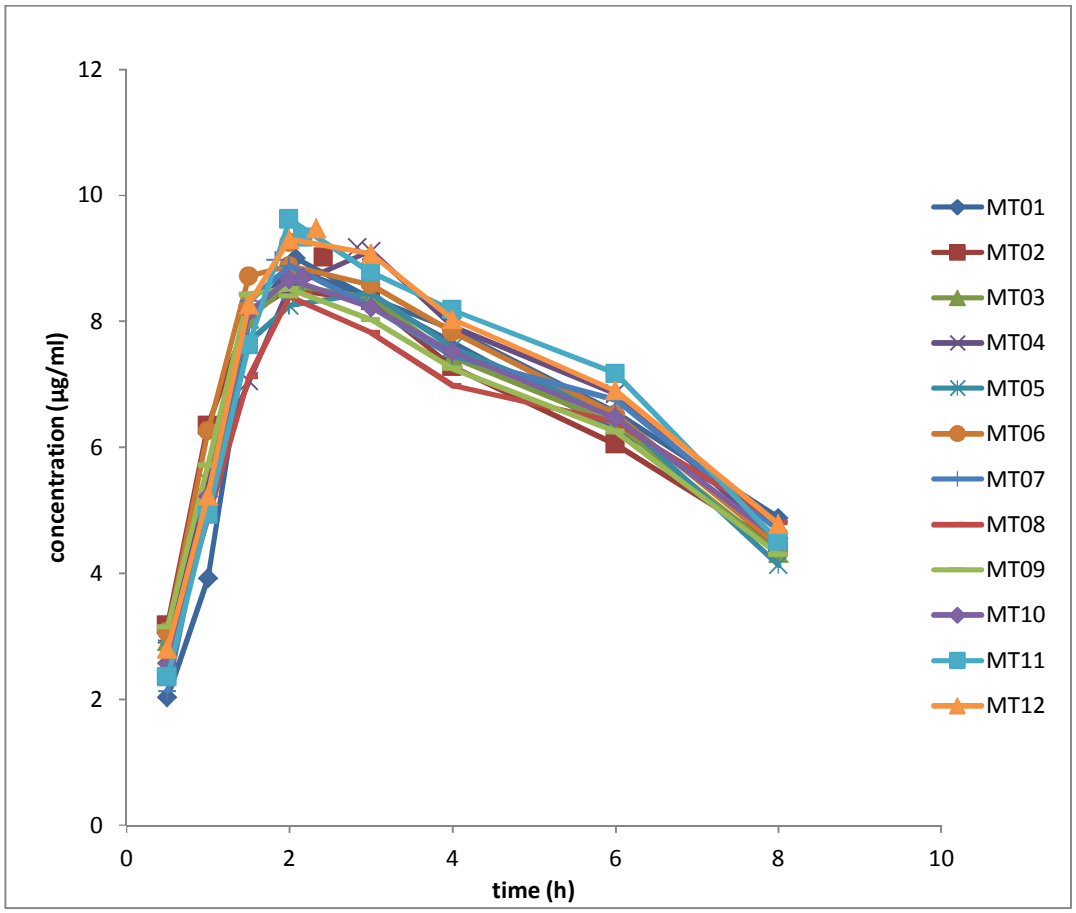


Figure 4.3: Concentration-time Curve for the *in-vivo* Administration of Various Brands of Metronidazole (200 mg) Tablets in six Healthy Human Volunteers

4.13 Mean Bioequivalence Parameters of the Various Brands of Metronidazole Tablets

Table 4.9: Mean Bioequivalence Parameters of the Various Brands Metronidazole Tablets from Saliva Samples with the SEM in brackets

Brand	**C_{max} µg/ml (SEM)**	**T_{max} h (SEM)**	**$AUC_{0\rightarrow 8h}$/µg.h/ml(SEM)**
MT01®	9.00 (0.14)	2.08 (0.20)	75.01 (2.86)
MT02	9.02 (0.28)	2.42 (0.27)	72.14 (0.24)
MT03	8.83 (0.30)	2.08 (0.30)	72.38 (1.03)
MT04	9.17 (0.12)	2.83 (0.17)	71.54 (0.69)
MT05	8.65 (0.23)	2.17 (0.28)	71.70 (0.96)
MT06	9.25 (0.23)	2.00 (0.32)	71.63 (0.85)
MT07	8.43 (0.15)	1.83 (0.11)	72.19 (0.67)
MT08	8.40 (0.15)	2.17 (0.17)	73.35 (0.68)
MT09	8.40 (0.15)	2.16 (0.28)	72.56 (0.78)
MT10	8.70 (0.26)	2.17 (0.28)	72.04 (1.38)
MT11	9.48 (0.25)	2.17 (0.17)	70.80 (0.56)
MT12	9.48 (0.19)	2.50 (0.22)	72.72 (0.24)

4.14 Bioequivalence Ratio of Reference and Test

Table 4.10: Bioequivalence Ratio of Reference and Test

Brand	**C_{max} (µg/ml)**	**$AUC_{0\rightarrow 8h}$ (µg.h/ml)**
MT01®	9.00	75.01
MT02 (Test)	9.02	72.14
Point estimate ratio of the mean	1.00	0.96
Remark	Bioequivalent	Bioequivalent
MT03 (Test)	8.83	72.38
Point estimate ratio of the mean	0.98	0.99
Remark	Bioequivalent	Bioequivalent
MT04 (Test)	9.17	71.54
Point estimate ratio of the mean	1.02	0.95
Remark	Bioequivalent	Bioequivalent
MT05 (Test)	8.65	71.70
Point estimate ratio of the mean	0.96	0.96
Remark	Bioequivalent	Bioequivalent
MT06 (Test)	9.25	71.63
Point estimate ratio of the mean	1.03	0.96
Remark	Bioequivalent	Bioequivalent
MT07 (Test)	8.43	72.19
Point estimate ratio of the mean	0.94	0.96
Remark	Bioequivalent	Bioequivalent

Acceptable range for point estimate ratio of the mean: 0.8-1.25

Continuation for table 4.10

Brand	**C_{max} (µ/ml)**	**$AUC_{0\rightarrow 8h}$ (µg.h/ml)**

MT01® Reference	9.00	75.01
MT08 (Test)	8.40	73.35
Point estimate ratio of the mean	0.94	1.00
Remark	Bioequivalent	Bioequivalent
MT09 (Test)	8.40	72.56
Point estimate ratio of the mean	0.93	1.03
Remark	Bioequivalent	Bioequivalent
MT10 (Test)	8.70	72.04
Point estimate ratio of the mean	0.97	1.00
Remark	Bioequivalent	Bioequivalent
MT011 (Test)	9.48	70.80
Point estimate ratio of the mean	1.05	1.00
Remark	Bioequivalent	Bioequivalent
MT012 (Test)	9.48	72.72
Point estimate ratio of the mean	1.05	1.01
Remark	Bioequivalent	Bioequivalent

Acceptable range for point estimate ratio of the mean: 0.8-1.25

CHAPTER 5

DISCUSSION

All the samples of metronidazole tablets examined were registered with the NAFDAC and have reasonable shelf life as shown in table 4.1. This was an indication that the drug products met NAFDAC requirements for pharmaceutical products in Nigeria. All the samples have impressive appearance even though there was variation in organoleptic properties of the samples as shown in table 4.2. However, some of the tablets were scored and some were not scored. The scoring permit accurate subdivision of the tablet in order to provide doses of less than one tablet and also facilitate breaking of the tablet for ease of swallowing a dose consisting of one or more whole tablet (WHO, 2011).

All the brands of metronidazole tablets show the presence of metronidazole in each of the samples using the procedure states in BP (2002). The assay test was done according to BP 2009 and the content uniformity was found to be in the range of 92.45 - 106.14 % (table 4.4). The values were in agreement with the BP (2002) specification of 85 - 115 % for tablets dosage form. International Pharmacopoeia specification for tablet dosage form is 90 - 110 % (IP, 2003). This shows that all the samples of metronidazole tablets collected were chemically equivalent to each other.

All the brands of the samples with the exception of MT05, MT08 and MT12 complied with the BP (2002) specification for uniformity of weight which states that for tablet weighing 250 mg or more, weight of not more than 2 two tablets should differ from the average weight by more than 5 %. Number of more than 2 tablets deviated by 5 % for sample MT05, MT08 and MT12 was an indication of deviation from the compendial specifications (table 4.3). The uniformity of weight might be as a result of non-uniformity of active ingredient and pressure different during the compression process (Mullaicharan, *et al.*, 2012). However, the difference in inter batches could be attributed to variations in percentage of excipients especially diluents, or bulking agents, which is usually the decision of formulation pharmacist (Ibezim, *et al.*, 2008).

Uniformity of weight does serves as a pointer for good manufacturing practice (GMP) as well as amount of the pharmaceutical ingredient (API) in a product (Ngwuluka, *et al*., 2009).

Tablet friability is used to evaluate tablets resistance to abrasion (BP, 2002). The acceptable limit maximum is 1 % (BP, 2002). All the brands of the samples had acceptable friability values with the exception of 1.70 %, 1.81 %, 1.62 % and 10.25 % for samples MT05, MT07, MT08 and MT11 respectively (table 4.4). The high friability value for MT11 is an indication of using binder with low adhesive strength or absence of binder, as well as tableting done under low compressing force (Ibezim, 2008).

Hardness testing though not official, is an important in-process means of assessing whether the tablets being produced are firm enough to withstand breakage, chipping or crumbling and yet not so hard as to delay disintegration (Mbah, *et al*., 2012). It is also influences friability. The greater the pressure applied, the harder the tablet (Mullaicharam, *et al.,* 2012). The acceptable limit is 4 - 10 kgF (Mohammed, *et al.,* 2012. Moses, *et al*., 2010). All the brands with the exception of brands MT10 had values for hardness testing within the acceptable limit (table 4.3).

Disintegration test for the various brands of metronidazole tablet was conducted using Vanderkamp tablet disintegration tester. All the brands of metronidazole with the exception of MT07 disintegrated within the acceptable limit of not more than 15 minutes as specified in BP (2002) for uncoated tablets (table 4.3). However, all the brands met USP requirements which states that uncoated and film coated tablets should disintegrate within 30 minutes (USP, 2009).

In-vitro dissolution testing is considered as one of the most important quality control test which can provide valuable information about the biological availability as well as batch consistency (Kumar, *et al*., 2000). However, in vitro dissolution testing offers a convenient and inexpensive means of predicting absorption and bioavailability difference among capsule and tablet formulation of the same drug (Azam and Haider, 2008). The accomplishment of dissolution profile is recommended as support in the development and optimization of drug formulation as

well as in the establishment of *in-vitro/in-vivo* correlation. All the brands of metronidazole tablets examined using BP (2002) method were found to have dissolution profile of at least 70 % of the active ingredient taken at 45 minutes from the dissolution medium as stated in BP (2002). However, there was marked variation in their dissolution behaviour in 0.I N HCl. This is because an oral dosage form is normally composed of drug substance and excipients and the proportion between them, the type of excipients and the manufacturing method of the final product are chosen based on the content, the physiochemical and the bulk properties of the drug and its absorption characteristics, taken as a whole, this gives each product certain dissolution characteristics which varies from one brand to the other (Esimone, *et al.,* 2008).

The method was adopted from the work of Kolawole and Ameh (2004); and validated according to ICH guideline. The absorbance was recorded at 277 nm as found by scanning 0.02 % w/v solution of metronidazole in 0.1 M HCl at 230 to 350 nm. Absorption of 0.763 was obtained at a maximum wave length of 277 nm as described in BP (1993). The absorbance of the aqueous solution was highest at pH 9; which was similar to reported value by Kolawole and Ameh (2004) and opposed a pH 7 used by Mishra, *et al* (2010). Correlation coefficient of 0.9970 between the absorbance and the concentration was found from the calibration curve, which shows a good correlation between the absorbance and the concentration within the range of 2.5 - 12.5 µg/ml. precision of the method was done by assaying 6 determinations of 15 µg/ml solution of metronidazole (Wrasse-Sangoi, *et al.*, 2010) for both intra and inter-day precisions. Intraday and inter-day precisions for the developed method expressed as relative standard deviation were 0.491 % and 0.411 % respectively (table 4.6). The values were within the normal limit of <1.5-2 % (Schmauser, 2010). This shows that the method has good precision for intra and inter day and as well as its capability for repeated measurement. The mean value of the percentage recovery was 98.30 % (table 4.7) which falls within the acceptable limit of 98-102 % (Chan, 2008). However, the mean accuracy of the method expressed as percentage error was 1.98 % (table 4.7) which was within the acceptable value of 1-5 % as reported by Harvey (2000). This was an

indication that the method has good reproducibility for analysis of metronidazole from biological samples.

Use of blood for bioequivalence study is complex (Ullah *et al*, 2009). Pahkla *et al (2005)*, in their work show a good correlation of metronidazole in blood and saliva which justifies the use of metronidazole in the treatment of some types of periodontal diseases such as aggressive periodontitis and chronic progressive periodontitis that does not react favourably with conventional treatment (Pahkla *et al*, 2005). Six healthy human volunteers were enrolled for the study.

Bioequivalence and bioavailability of drug products are usually assessed by means of univariate statistical analysis of the important parameters, such as the peak drug concentration (C_{max}), the time to reach this concentration (T_{max}), and the area under the drug concentration-time curve (AUC) (Alves, *et al.,* 2007). The bioequivalence parameters were found from saliva samples following oral, single dose administration of 2 tablets of 200 mg (400 mg) of metronidazole tablets to overnight fasted human volunteers. UV visible spectrophotometer was used for the analysis at 277 nm. *In-vivo* study showed that the mean C_{max} for the various brands of metronidazole tablets ranged 8.1-10.3 µg/ml. The values were in agreement with the values reported by other researchers on similar topic on healthy human volunteers revealed the following: Usman *et al,* (2007) reported C_{max} of 7.00±0.43 – 7.20±0.58 µg/ml for test and reference sample of 400mg metronidazole tablet respectively from saliva samples using UV visible spectrophotometer. Kolawole and Ameh, (2004) reported 8.72 - 9.02 µg/ml for 400 mg metronidazole tablet from saliva samples using UV visible spectrophotometer. Usman *et al,* (2011) reported C_{max} 8.041±0.41 µg/ml for 400 mg of metronidazole tablet from blood samples using HPLC method. The point estimate ratio of C_{max} for all the brands of metronidazole with reference to brand MT01 as the comparator drug ranged 0.93 - 1.05, which were within the bioequivalence acceptance limit of 0.8-1.25

The mean T_{max} for the various samples of metronidazole tablets extrapolated from the concentration-time curve were within the range of 1.83 – 2.83h. The values were in harmony with the reported values of 0.25 - 4h for non-pregnant patients by Zeng *et al,* (2010) for 500mg of metronidazole from blood samples using HPLC method. However, Rediguiri *et al*, (2011) reported T_{max} of 0.25-4h for oral doses of 250, 500, 750 and 2000 mg of metronidazole tablets. Ezzeldine and El-Nahhas, (2012) reported T_{max} of 2.75-3 h from blood on healthy human volunteers using HPLC method. Kolawole and Ameh, 2004 reported T_{max} of 2.03±0.052 - 2.77±0.96 h for 400 mg of metronidazole from saliva on healthy human volunteers using metronidazole samples with UV visible spectrophotometer. Also, Usman *et al*, (2007) reported T_{max} of 65.00±0.49 - 70.00±0.41 minutes respectively for test and reference samples of 400 mg of metronidazole tablets from saliva samples on healthy human volunteers using UV visible spectrophotometer. While Usman *et al* (2011) reported 1.774±0.06 h for 400 mg of metronidazole from blood samples using HPLC method

Furthermore the $AUC_{0 \rightarrow t}$ should cover at least 80% of the $AUC_{0 \rightarrow \infty}$ (European Medicine Agency, 2010). The mean $AUC_{0 \rightarrow 8h}$ for all the brands of the samples were within the range of 70.80 - 75.01 µgh/ml. The values were in harmony with the reported values of $AUC_{0 \rightarrow \infty}$ of 82.0 - 300.7 µg/ml ($AUC_{0 \rightarrow 8h}$, 80 % = 65.6 – 240.56 µg/ml) by Ezzeldine and El-Nahhas, (2012) from blood samples for 500 mg of metronidazole using HPLC method. Other researchers on similar topic using 400 mg of metronidazole on healthy human volunteers revealed the following results: Usman *et al,* (2011) reported $AUC_{0 \rightarrow \infty}$ of 103.17±6.25 µg.h/ml ($AUC_{0 \rightarrow 8h}$, 80 % was 82.4 µg.h/ml) versus 87.37±2.56 µg.h/ml ($AUC_{0 \rightarrow 8h,}$ 80 % was 69.90 µg.h/ml) in patients from blood samples using HPLC method. Houghton *et al,* (1982) reported $AUC_{0 \rightarrow \infty}$ of 82±17 µg.h/m ($AUC_{0 \rightarrow 8h}$, 80 % was 65.6 µg.h/ml) from blood samples using HPLC method. *Usman et al*, 2007 reported $AUC_{0 \rightarrow 6h}$ of 69.93±1.15 for reference drug and 65.50±0.89 µg.h/ml test drug from saliva samples using UV spectrophotometry. Kolawole and Ameh, 2004 reported $AUC_{0 \rightarrow \infty}$ of 73.69 to 82.76 µg.h/ml ($AUC_{0 \rightarrow 8h}$, 80 was 58.95 – 66.21 µg.h/ml). On the other hand, Emani et al, 2006 reported $AUC_{0 \rightarrow \infty}$ of 136.73 µg.h/ml ($AUC_{0 \rightarrow 8h}$, 80 % was

(109.38 µg.h/ml) for test drug and 137.45 µg.h/ml ($AUC_{0 \rightarrow 8h}$, 80% was 109.96 µg.h/ml) for reference drug. Turgut and Ozyazici, (2004) reported AUC of 100 – 159 mg.h/L for single studies with oral and intravenous dose of 500 mg of metronidazole using HPLC method of analysis. The point estimate ratio for the mean $AUC_{0 \rightarrow 8h}$ for the all brands of metronidazole with respect to MT01 as the comparator product were ranged 0.97 – 1.00. The range was within the acceptable limit of 0.8 – 1.25 for bioequivalence studies.

CHAPTER 6

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

1. All the samples of metronidazole 200 mg tablets used for the study had good physical appearance.

2. The presence of metronidazole was observed in each tablet of the samples.

3. Analysis of pharmaceutical equivalence revealed that all the samples of metronidazole tablets had acceptable level of weight variation, except samples MT05, MT08 and MT12 which deviated from the compendial specification. All the samples disintegrated within the BP, 2002 specification except MT07 with a value of 18.97 minutes. All the tablets had acceptable value for active ingredient with not less than 70% value at 45 minutes as specified in the BP, 2002. However, for the unofficial methods, all the sample except MT05, MT07, MT08 and MT11 passed the friability test. Only MT10 and MT11 failed the hardness test.

4. The validated method adopted had good precision and accuracy with high percentage recovery which is an indication that the method is robust and capable for bioequivalence studies of metronidazole from saliva samples.

5. All the samples of metronidazole 200 mg tablets dosage form were bioequivalent with a point estimate ratios within 0.8-1.25 for bioequivalence studies.

6.2 Conclusion

It has been shown from the validation parameters of the method that it was robust and reproducible with high degree of accuracy for detection of metronidazole from saliva samples. All the samples of metronidazole tablets collected revealed the presence of metronidazole in each of the tablets dosage form, the samples had acceptable level of bioequivalence and thus can be used interchangeably. This shows that there is no difference in the pharmaceutical, chemical and bio equivalences between Loxagyl® and other brands of metronidazole marketed in Zaria metropolis during the course of this research. However, there was variation in the pharmaceutical parameters of the samples. Sample MT05, MT08 and MT12 failed weight variation test. MT05, MT07, MT08 and MT11 failed friability test; while MT10 and MT11

failed the hardness test. However, statistical analysis of bioequivalence parameters shows no significant statistical difference. Thus, the samples have comparable bioavailability.

6.3 Recommendation

1. More precise analytical methods such as HPLC, may be used to reassess the studies using a larger sample size.

2. the Considering the recently introduced, Mega drug distribution system by federal government in the country, there is need to establish and maintain quality control laboratories in all state of the federation to ensure distribution of safe and effective drugs in all the distribution chain.

3. There is need to have a guideline for bioequivalence study in the country by the regulatory agency as most of the drugs in use in the country are imported.

4. There should also be a need for routine post market surveillance of pharmaceuticals by the regulatory agencies to ensure the quality of drugs at different channels of distribution.

REFERENCES

Alves, A.J., Aquino, T.M., Neto, J.L.C., Filho, S.D.S., Junor, H.J., Gasper, F.L., Luna, M.C.M.M., Alves, C.J., Alves, A.Q., Oliveira, C.F., and Goes, A.J.S. (2007). Bioequivalence between two Metronidazole Formulations. The *Latin American Journal of Pharmacy;* 26(2), 266-269.

Apu, A.S., Khan, N.H., Karim, M., Bola, N.G., Sirazule, M.K. Jamaluddin, A.T.M., and Rahman, Z. (2011). In-vitro Evaluation of the Pharmaceutical Equivalence of Phenoxymethylphenicillin Tablet Formulations Available in Bangladesh. *The Journal of Pharmacy Research*, 4(5), 1445-1447.

Awofisayo, S.O., Awofisayo, O.A., Eyen, N., and Udoh, I.E. (2010 a). Comparative Assessment of the Quality Control Measurements of Multisource Ofloxacin Tablets Marketed in Nigeria. *Journal of Dissolution Technologies,* 16(16), 20-31. Retrieved from http://www.dissolutiontech.com

Awofisayo, S.O., Willie, E., and Umoh, E. (2010 b). Quality Control Evaluation of Multisource Arthemether-Lumefantrine Tablets Prescribed for Uncomplicated Multi drug Resistant Malaria. *The Indian Journal of Novel Drug Delivery*, 2(4), 153-157.

Azam, G., and Haider, S.S. (2008). Evaluation of Dissolution behaviour of Paracetamol Suspensions. *The Dhaka University Journal of Pharmaceutical Sciences,* 1(1), 53-58.

Balat, L. (2006). *Quality Control Assessment and Physicochemical Interaction Studies of Some Brands of Chloroquine Phosphate Tablets*. (Unpublished master's thesis). Ahmadu Bello University, Zaria, Nigeria.

Bano, R., Gauhar, S., Shyum-Naqvl, S.B., and Mahmood, I.S. (2011). Pharmaceutical Evaluation of different Brands of Levofloxacine Tablets (250 mg) Available in Local

Market of Karachi (Pakistan). *The International Journal of Current Pharmaceutical Research*. 3(1), 15-22.

Bhoir, S., Gaikwad, P., Bhagwat, A., and Jathar, S. (2012). Steady-state Pharmacokinetics of Immediate-release and Controlled-release Metronidazole Tablets. *The International Journal of Pharmacy and Pharmaceutical Sciences,* 4(3), 353-356.

British Pharmaceutical Codex (1978). *Product Monograph: Metronidazole.* Her Majesty's Stationary Office, University Press Cambridge.11 ed. pp.567

British Pharmacopoeia (1993). *Product Monograph: Metronidazole.* Volume I and II Her Majesty's Stationary Office, University Press Cambridge. pp. 374.

British Pharmacopoeia (2002). Volume I and II Her Majesty's Stationary Office, University Press Cambridge. A soft copy.

Buxton, I.L.O. (2006). Pharmacokinetics and Pharmacodynamics: The Dynamics of Drug Absorption, Distribution, Action and Elimination. In L.L. Brunton (11 ED). *Goodman & Gilman's the Pharmacological basis of Therapeutics* (pp 4). United States: McGraw-Hill Medical Publishing Division.

Centre for Drug Evaluation and Research (2001). *Guidance for bioavailability and Bioequivalence Studies.* Ministry of Health and Family Welfare, Government of India, New Delhi

Chan, C.C. (2008). Analytical Method Validation: Principles and Practices. In *Pharmaceutical Manufacturing Hand Book: Regulations and Quality* (pp 727-742). *John Willey and Sons, inc.*

Chandrasekaran, A.R., Jia, C.Y., Theng, C.S., Muniandy, T., Muralidharan, S., and Dhanaraj, S.A. (2011). In-vitro Studies and Evaluation of Metformin Marketed Tablets-Malaysia.

Journal of Applied Pharmaceutical Science, 1(5), 214-217. Available at http://www.japsonline

Cudmore, S.L., Delgaty, K.L., Hayward-McClelland, S.F., Petrin, D.P., and Garber, G.E. (2004). Treatment of Infections Caused by Metronidazole-Resistant Trichomonas vaginalis. *Clinical Microbiology Review*, 17(4), 783-793. Available at http://cmr.asm.org/content/17/4/783#ref-list-1

Dinesh, N.D., Nagaraja, P. (2004). A Sensitive Spectrophotometric Assay for Tinidazole and Metronidazole using a Pd-C and Formic acid Reduction System. The *Turkish Journal of Chemistry,* 28(1), 335-343.

Esimonea, C.O., Okoye, F.B.C., Onah, B.U., Nworu, C.S., and Omeje, E.O. (2008). In-vitro Bioequivalence Study of Nine Brands of Artesunate Tablets Marketed in Nigeria. The *Journal of Vector Borne Disease,* 45(1), 60-65.

European Medicine Agency (1995). Validation of Analytical Procedures: Text and Methodology. Westferry Circus, Conary Wharf, London E14 4HB, UK: European Medicine Agency

European Medicine Agency (2010). Guidance on the Investigation of Bioequivalence. (CPMP/EWP/QWP/1401/98 Rev. 1/Corr.). 7 Westferry Circus, Conary Wharf, London E14 4HB, UK: European Medicine Agency

Ezzeldin, E., and El-Nahhas, T.M (2012). New Analytical Method for the Determination of Metronidazole in Human Plasma: Application to Bioequivalence Study. *Tropical Journal of Pharmaceutical Research,* 11(5), 799-805. Retrieved from http://www.tjpr.org http://dx.doi.org/10.4314/tjpr.v11i5.14

FDA (2001). Bioanalytical Method Validation. Guidance for industries, U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Drug

Evaluation and Research (CDER), Centre for Veterinary Medicine. Retrieved from http://www.fda.gov/cvm

Ghante, M.R., Pannu, H.K., Loni, A., and Shivsharan (2012). Development and Validation of a RP-HPLC Method for Simultaneous Estimation of Metronidazole and Norfloxacin in Bulk and Tablet Dosage Form. The *International Journal of Pharmacy and Pharmaceutical Sciences,* 4(4), 241-245.

Harvey, D. (2000). Spectroscopic Methods of Analysis. In Modern Analytical Chemistry (pp 409). United States: McGraw-Hill Higher Education.

Hold, K.M., Douwe de Boer, J. Z., and Maes, J.R. (1996). Saliva as an Analytical Tool in Toxicology. *International journal of drug testing,* 1, 1-29. Retrieved from http://big.stpt.usf.edu/~journal/volume1.htm

Ibezim, E.C., Attama, A.A., Obitte, N.C., Onyishi, V.I., and Brown, S.A. (2008). In vitro Prediction of in vivo Bioavailability and Bioequivalence of Brands of Metronidazole Tablets in Eastern Nigerian Drug Market. The *Science Research and Essays,* 3(11), 552-558. Available at http://www.academicjournals.org/SRE

International Pharmacopoeia (2003). Tests and General Requirements for Dosage Forms, Quality Specifications for Pharmaceutical Substances and Tablets. World Health Organization, Geneva, Vol. 5 PP. 147-184.

Kishore, K.A., and Amareshwar, P. (2012). Quality Evaluation and Comparative Study on Tablet Formulations of different Pharmaceutical Companies. The *Journal of Current Chemical and Pharmaceutical Sciences*, 2(1), 24-32.

Kolawole, J.A., and Ameh, I.U. (2004). Chronopharmacokinetics of Metronidazole in Healthy Human Volunteers. *The Journal of Pharmacy and Bioresources, 1(1), 29-34.*

Kumar, M.S., Gupta, B.K., and Ghosal, S.K. (2000). Assessment of Bioavailability of Experimental Control Release Microcapsules of Nifedipine. *The Journal of Drug Research,* 7(3), 175-180.

Mbah, C.C., Emosairue, C.O. Builders, P.F., Isimi, C.Y., and Kunle, O.O. (2012). Effect of Process Parameters on the Properties of some Metronidazole Tablet and Capsule Formulations. *The African Journal of Pharmacy and Pharmacology*, 6(24), 1719-1725. Available at http://www.academicjournals.org/AJPP

Mishra, A.K., Yadava, R., Mishra, A., Verma, A., and Chattopadhyay, P. (2010). Development and Validation of UV Spectrophotometric Method for the Determination of Metronidazole in Tablet Formulation. The *International Journal of Pharmacy Research and Development,* 2(6), 1-5. Available at www.ijprd.com

Mohammed, F.A., Arunachalam, A., Venkatarami, R.G., Pallavi, V., Moulali, S.K., and Rama Raju, T.V.T. (2012). Formulation and Evaluation of Carbamazepine Extended Release Tablets USP 200mg. The *international Journal of Biology and Pharmacy Research;* 3(1), 145-153.

Moses, P., Subramanian, L., Palanichamy, S., Jeganath, S., and Thirupathi, A.T (2010). Formulation and Evaluation of Ciproflouxacine controlled release Matrix Tablets. *Scholars Research Library*, 2(2), 237-243. Retrieved from http://www.scholarsresearchlibrary.com/archive.html

Mullaicharam, A.R., Ahmed, J.J., and Halligudi, N. (2012). Evaluation of Pharmaceutical Equivalence of different Brands of Ranitidine Tablets from Multinational in Oman. *International Journal of Nutrition, Pharmacology and Neurological Diseases,* 2 (1), 40-44. Available at http://www.ijnpnd.com.

Musa, H., Sule, Y.Z., and Gwarzo, M.S. (2011). Assessment of Physicochemical Properties of Metronidazole Tablets Marketed in Zaria, Nigeria. The *International Journal of Pharmacy and Pharmaceutical Sciences,* 3(3), 27-29.

Mustapha, K.B., Odunola, M.T., Garba, M., and Obodozie, O. (2006). Rapid, Cost-Effective Liquid Chromatograghic Method for the Determination of Metronidazole in Biological Fluids. The *African Journal of Biotechnology*, 5(13), 1188-1190. Available http://www.academicjournals.org/AJB

Nascimento, G.N.L., Rosa, D., Nishijo, H., and Aversi-Ferreira, T.A. (2011). Validation of a Spectrophotometric Method to determine Ciprofibrate Content in Tablets. The *Brazilian Journal of Pharmaceutical Sciences,* 47(1), 23-29.

Ngwuluka, N. C., Lawal, K., Olorunfemi, P.O., and Ochekpe, N.A. (2009). Post-market In-vitro Bioequivalence Study of Six Brands of Ciprofloxacin Tablets/caplets in Jos, Nigeria. The *Scientific Research and Essay*, 4(4), 298-305. Accessed June 18, 2012 from http://www.academicjournals.org/SRE

Ogah, C.O., Falade, O.M., and Erorini, O.C. (2002). Quality of Chloroquine Phosphate and Paracetamol Tablets Preparations in Lagos, Nigeria. The *West African Journal of pharmacy*. 20(1), 58-63.

Olaniyi, A.A. (2005). Chemical and Physicochemical Methods. In Principles of Quality assurance and Pharmaceutical Analysis (pp 137-206). 5 Oleware Obasa street Ibadan: Mosuro publishers.

Omeje, E.O., Nwodo, N., and Uzochukwu, I. (2007). Quality Control Assessment and the Possibility of Interchange Ability Between Multisourced Norfloxacine Tablets Marketed in Nigeria. *The Scientific Research and Essay.* 2(8), 348-352. Available online at http://www.academicjournal.org/SRE

Pahkla, E.R., Koppel, T., Saag, M., and Pahkla, R. (2005). Metronidazole Concentration in Plasma, Saliva and Periodontal Pockets in Patients with Periodontitis. *The Journal of Clinical Periodontol*, 32(1), 163-166.

Phillips, M.A. and Stanley, S.L. (2006). Chemotherapy of Protozoal Infections: Amebiasis, Giardiasis, Trichomoniasis, Trypanosomiasis, Leishmaniasis, and other Protozoal infections. In L.L. Brunton . *Goodman and Gilman's the Pharmacological basis of Therapeutic*. CD ROM. United States: McGrow

Punyadeera, C. (2011). Diagnostic Potential of Saliva: Current State and future Applications, *American Association of Clinical Chemistry*

Ralph, E.D., Clarke, J.T., Libke, R.D., Luthy, R.P., and Kirby, W.M.M (1974) Pharmacokinetics of Metronidazole as determined by Bioassay. *The Antimicrobial Assay and Chemotheraphy,* 6(6), 601-696.

Rediguieri, C.F., Porta, V., Nunes, S.G., Nunes, T.M., Junginger, H.E., Kopp, S. and Barends, D.M. (2011). Biowaiver manographs for immediate release Solid Oral Dosage Forms: Metronidazole. The *Journal of Pharmaceutical Sciences*; 100(5), 1618-1627.

Remington's Pharmaceutical Sciences (1975). The Science of Pharmaceutical dosage forms (ed 15). Easton, Pennsylvania, U.S.A.: Mack Publishing Company.

Martindale, (1999). Monograph on Metronidazole. In Royal Pharmaceutical Society of Great Britain (32 Ed) *the Complete Drug Reference* (PP 585-588). Great Britain, England: Pharmaceutical press.

Martindale (2007). Monograph on Metronidazole. In: Royal Pharmaceutical Society of Great Britain, *the Complete Drug Reference*. CD ROM. Great Britain, England: 6Pharmaceutical Press.

Samuelson, J. (1999). Why Metronidazole is active against both Bacteria and Parasites. *Antimicrobial Agents' Chemotherapy,* 43(7), 1533-1541. Retrieved from http://aac.asm.org/content/43/7/1533#ref-list-1

Sanofi-aventis (2012). *Product monograph: Flagyl. Sanofi-aventis* (Submission No. 155394). West Laval Quebec, Canada: Sanofi-aventis.

Schmauster, B. (2010). Analytical method development. (Training workshop Pharmaceutical development with focus on paediatric formulations). Beijing: World Health Organisation. Available at http://www.who.int/prequal/trainingresources/pqores/workshop_china2010/english/23/006-Analytical_method_development.pdf

Stockley's Drug Interaction (2005). Interaction Monographs: Metronidazole. (CD ROM). London: *The Publications of the Royal Pharmaceutical Society of Great Britain,* Pharmaceutical Press.

Sundaraganapathy, R., Jambulingam, M., Ananda, T.S., and Subasini, U. (2011). Development and validation of UV Spectrophotometric Method for the Determination of Venlafaxine Hydrochloride in Bulk and Solid Dosage Forms. The *International Journal of Pharmaceutical and Industrial Research,* 1(1), 28-31.

Thiessen, J.J. (2000). *Bioavailability and Bioequivalence.* (Doctorate Thesis, Leslie Dan Faculty of Pharmacy, University of Taranto, Taranto, Ontario M5S2S2 Canada). Retrieved from www.iuphar.org

Turgut, E.H., and Ozyazici, M. (2004). Bioavailability file: Metronidazole. The *FABAD Journal of Pharmaceutical Science,* 29(1), 39-49.

Ullah, A., Azad, M.A., Sultana, R., Kabir, E.R., Mahbub-Latif, A.H.M., and Abul, H. (2009). Pharmacokinetics Study of Amoxicilline Capsules in Healthy Bangladeshi Subjects

using urinary excretion data. *The Dhaka University Journal of Pharmaceutical sciences,* 8(1), 53-59.

Usman, M., Ashiq, I., Ashraf, M.O., Khokhar, M.I., and Saeed-ul-Hassan, S. (2011). Comparative Pharmacokinetics of Metronidazole in Healthy Volunteers and in Patients Suffering from Amoebiasis. *The Pakistan Journal of Pharmacy;* 24 (1 & 2), 41-46.

Usman, M.A., Sule, M.I., Ahmadu, A.A., and Ojochenemi, D.A. (2007). Bioequivalence evaluation of two brands of Metronidazole Tablets in Healthy Human Volunteers. *The Nigerian Journal of Pharmaceutical Sciences;* 6(2), 55-58.

World Health Organisation. (2011). *Model list of Essential Medicines.* (Report No. EDL17). Geneva: World Health Organisation. http://www.who.int/medicines/publications/essentialmedicines/en/index.html

World Health Organisation. (2011). *Revision of Monograph on Tablets: Final text for Addition to the International Pharmacopoeia.* (Report No. QAS/09. 324/final). Geneva: World Health Organisation. Retrieved from http://www.who.int/entity/.../Tabs-GeneralMono-rev-FINAL_31032011.pdf

World Health Organization (2005). *Multisource (Generic) Pharmaceutical Products: Guidelines on Registration Requirements to Establish Interchangeability*, (Report No. QAS/04.093/Rev.4). Geneva: World Health Organisation. Retrieved from http://www.int/medicines/areas/quality_safety/quality_assurance/multisource/pharmaProductsGuidelinesRegistraionRequirements/InterchageabilityTRS937Annex7.pdf

World Health Organization (2006). Additional guidance for organizations performing in-vivo bioequivalence studies. (Technical Report No. 937). Geneva: World Health Organisation. Retrieved from http://www.who.int/medicines/areas/quality_safety/quality_assurance/AdditionalGuidanceOrganisation/performanceBioequivalence/StudiesTRS937

Wrasse-Sangoi, M., Secretti, L.T., Diefenbach, I.F., and Rolim, C.M.B. (2010). Development and Validation of an UV Spectrophotometric Method for the Determination of Aliskiren in Tablets. *Quim. Nova*, 33(6), 1330-1334.

Zheng, C., Wang, J., and Zhao, L. (2012). Testing Bioequivalence for Multiple Formulations with Power and Sample Size Calculations. *The Journal of Pharmaceutical Statistics;* 11(1), 334-341. DOI: 10.1002/pst.1522.

APPENDICES

APPENDIX I: Addresses of the Manufacturers of the various brands of Metronidazole Tablet Samples marketed in Zaria, Nigeria

Table I: Addresses of the Manufacturers of the various brands of Metronidazole Tablet Samples marketed in Zaria, Nigeria

SAMPLE	BRAND NAME	ADDRESS OF THE MANUFACTURER
MT01	Loxagyl®	May and Baker Nig. Plc, Ikaja Lagos
MT02	Unigyl®	Unique Pharmaceutical Ltd, Ogun State
MT03	Emgyl®	Emzor Pharmaceutical Industries Ltd, Lagos State
MT04	Metrotab ®	SKG Pharma Ltd, Lagos State.
MT05	Vamis Metronidazole®	Tuyil Pharm. Ind. Ltd 22 New Yidi Road, Ilorin, Nigeria.
MT06	Stargyl®	Associated Pharmaceutical Ltd, Plot 10-12, EziamaIndust, Estate, Eziama, Aba, Abia State, Nigeria.
MT07	Jugyl®	Juhel Nig. Ltd, 35 Nkadoor Road, Emes. Inugu, Nigeria.
MT08	Metrozo®	Vitabiotic Nig. Ltd, Ikaja Lagos
MT09	Metrozam ®	Sam pharmaceutical Ltd, 2 Western Reservoir Road, Ilorin, Kwara State, Nigeria.
MT10	Bonagyl®	Bond Chemical Ltd, Adeshina Layout Awe Oyo State Nigeria.
MT11	Lemetro®	Me Care Ind. Ltd. Debo Ind. Compound Plot No. 6 Blocj H Oshodi Ind. Scheme Lagos. Nigeria.
MT12	Palagyl®	Pal Pharm. Ind. Ltd. Plot 101 Magande Road BapaiInd Area Kano, Nigeria.

APPENDIX II: Raw result for quality control tests on the samples of metronidazole tablets dosage form

1.0 **FRIABILITY TEST**

$$\text{Friability} = \frac{\text{(Initial weight of tablets - finals weight after 4minutes)}}{\text{Initial weight}} \times 100\%$$

Table II: Raw result for friability test

Brand	Initial weight of 10 Tablets	Final weight	Percentage	Remark
MTO 1	3.48	3.47	0.30	Satisfactory
MTO 2	4.86	4.81	1.00	Satisfactory
MTO 3	3.38	3.35	0.90	Satisfactory
MTO 4	5.05	50.3	0.40	Satisfactory
MTO 5	5.93	5.83	1.70	Unsatisfactory
MTO 6	5.80	5.75	0.90	Satisfactory
MTO 7	5.32	3.26	1.81	Unsatisfactory
MTO 8	3.70	3.64	1.62	Unsatisfactory
MTO 9	6.10	6.06	0.70	Satisfactory
MT 10	5.81	5.76	0.90	Satisfactory
MT 11	5.95	5.34	10.25	Unsatisfactory
MT12	6.50	6.45	0.8	Satisfactory

2.0 **HARDNESS TEST**

Table III: Raw result for hardness test

Brand	**Tablet Hardness/KgF**					**Mean hardness/ KgF (SEM)**	**Remark**
1	**1**	**2**	**3**	**4**	**5**		
MT01	6.5	6.0	5.0	5.0	5.0	5.5 (0.28)	Satisfactory

MT02	4.5	5.5	5.0	4.5	5.5	5.0 (0.20)	Satisfactory
MT03	7.5	7.0	5.0	6.0	7.0	6.5 (0.40)	Satisfactory
MT04	10.0	11.0	9.0	12.5	11.0	10.7 (0.52)	Unsatisfactory
MT05	6.0	6.0	4.5	6.0	5.0	5.5 (0.28)	Satisfactory
MT06	9.0	5.0	4.0	6.5	4.0	5,7 (0.84)	Satisfactory
MT07	10.0	8.0	11.0	10.0	8.0	9.4 (0.54)	Satisfactory
MT08	6.0	7.0	8.0	7.5	7.0	7.1 (0.3)	Satisfactory
MT09	6.5	7.0	5.5	6.0	6.5	6.3 (0.23)	Satisfactory
MT10	9.5	9.5	12.0	11.5	11.0	10.7 (0.46)	Satisfactory
MT11	6.5	7.0	6.0	6.5	7.0	6.6 (0.17)	Satisfactory
MT12	9.0	6.0	8.5	9.0	5.5	7.6 (0.68)	Satisfactory

3.0. **DISINTEGRATION TEST**

Table IV: Raw result for disintegration test

Brand	**Disintegration Time (minute)**						**Mean**	**Remark**
	1	**2**	**3**	**4**	**5**	**6**		
MT01	0.75	2.33	3.42	3.47	4.08	4.5	3.09	Satisfactory
MT02	1.92	2.09	2.25	2.17	2.33	3.75	2.42	Satisfactory
MT03	0.67	0.75	0.92	1.03	1.17	1.18	0.95	Satisfactory
MT04	5.33	7.40	8.92	9.17	12.08	12.25	9.20	Satisfactory
MT05	2.33	3.13	3.33	3.58	3.75	5.28	3.57	Satisfactory
MT06	9.50	10.92	10.92	11.17	11.42	11.75	10.95	Satisfactory
MT07	15.17	165	16.5	19.33	22.17	24.17	18.97	Unsatisfactory
MT08	0.50	0.0	0.92	1.08	1.33	1.75	1.01	Satisfactory
MT09	0.50	0.50	0.70	0.82	0.87	0.97	0.74	Satisfactory
MT10	0.58	0.83	1.25	1.25	1.58	1.7	1.20	Satisfactory
MT11	0.33	0.3	0.2	0.58	0.77	1.25	0.62	Satisfactory

MT12	4.92	.25	.33	6.75	7.13	8.5	6.48	Satisfactory

4.0. **DISSOLUTION TEST**

4.1. Table V: Absorbance for various brands of metronidazole tablet taken at 45 minutes from the dissolution medium.

Brand	**Absorbance (nm) at 45minutes**					
	1	**2**	**3**	**4**	**5**	**6**
MTO1	0.646	0.5770	0.5160	0.6300	0.6910	0.7020
MTO2	0.7517	0.7610	0.6001	0.7501	0.7684	0.7501
MTO3	0.679	0.6917	0.6756	0.6780	0.6717	0.6776
MTO4	0.7752	0.7387	0.8831	0.8847	0.7529	0.9120
MTO5	0.6844	0.6612	0.6673	0.6840	0.6050	0.6916
MTO6	0.5474	0.5502	0.5701	0.6247	0.5472	0.5518
MTO7	0.6171	0.5951	0.5928	0.6012	0.6081	0.6012
MTO8	0.7828	0.8786	0.7250	0.8375	0.7927	06810
MTO9	0.5297	0.5445	0.5784	0.5282	0.5328	0.5472
MT10	0.5472	0.5495	0.5784	0.5282	0.5328	0.5472
MT11	0.6156	0.6088	0.6027	0.6019	0.6285	0.6082
MT12	0.5290	0.5548	0.5601	0.577	0.6088	0.5335

4.2. Table VI: Percentage of metronidazole released from the tablet dosage form at 45 minutes in the dissolution medium

Brand	Percentage of (%) metronidazole release					Mean value (SEM)		Remark
	1	2	3	4	5	6		
MT01	85.00	75.92	67.89	82.89	90.92	92.37	84.17(1.02)	Satisfactory
MT02	98.91	100.13	79.02	98.70	101.10	98.70	96.09(3.14)	Satisfactory
MT03	89.34	91.01	88.90	89.23	90.01	89.16	89.61(0.29)	Satisfactory
MT04	102.06	97.20	116.20	116.41	99.06	120.00	108.5(3.80)	Satisfactory
MT05	90.05	87.00	87.80	90.00	79.6	91.00	87.44(1.56)	Satisfactory
MT06	72.03	72.40	75.10	82.20	72.00	72.06	75.39(1.49)	Satisfactory
MT07	81.20	78.30	78.00	79.10	80.01	79.10	79.29(0.44)	Satisfactory
MT08	103.00	115.60	95.40	110.20	104.30	89.60	103.03(3.5)	Satisfactory
MT09	69.76	72.30	76.10	69.50	70.10	72.00	74.30(1.77)	Satisfactory
MT10	72.00	72.30	76.10	69.50	70.10	72.00	72.00(0.86)	Satisfactory
MT11	81.00	80.10	79.3	79.20	82.70	80.02	80.39(0.49)	Satisfactory
MT12	69.60	73.00	73.7	75.00	80.10	70.20	73.60(1.42)	Satisfactory

APPENDIX III: Mean Absorbance Values for in-vivo Administration of 400 mg dose of Metronidazole to Healthy Human Volunteers

1. Table VII: Absorbance for first administration

Time (h)	5	1.0	1.5	2.0	3.0	4.0	6.0	8.0
MT01	0.0093	0.0189	0.0326	0.0370	0.0348	0.0333	0.0278	0.0222
MT02	0.0152	0.0267	0.0337	0.0356	0.0363	0.0322	0.0252	0.0182
MT03	0.0104	0.0215	0.0322	0.0356	0.0363	0.0330	0.0296	0.0211
MT04	0.0152	0.0263	0.0322	0.0352	0.0374	0.0319	0.0267	0.0204
MT05	0.0104	0.0204	0.0308	0.0385	0.0348	0.0337	0.0322	0.0163
MT06	0.0145	0.0282	0.0359	0.0333	0.0311	0.0285	0.0245	0.0189
MT07	0.0108	0.0226	0.0359	0.0348	0.0330	0.0311	0.0285	0.0185
MT08	0.0108	0.0208	0.0356	0.0359	0.0345	0.0337	0.0296	0.0208
MT09	0.0156	0.0256	0.0322	0.0345	0.0326	0.0282	0.0263	0.0189
MT10	0.0100	0.0222	0.0326	0.0367	0.0348	0.0326	0.0289	0.0211
MT11	0.0123	0.0222	0.0322	0.0400	0.0370	0.0341	0.0278	0.0215
MT12	0.0137	0.0222	0.0341	0.0374	0.0359	0.0319	0.0285	0.0204

2 Table VIII: Absorbance for two weeks after first administration

Time (h)	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0
MT01	0.0104	0.0215	0.0322	0.0356	0.0363	0.0330	0.0296	0.0211
MT02	0.0152	0.0263	0.0322	0.0352	0.0374	0.0319	0.0267	0.0204
MT03	0.0111	0.0215	0.0319	0.0359	0.0385	0.0326	0.0278	0.0182
MT04	0.0123	0.0237	0.0326	0.0400	0.0367	0.0337	0.0278	0.0211
MT05	0.0119	0.0193	0.0319	0.0359	0.0426	0.0374	0.0296	0.0197
MT06	0.0141	0.0278	0.0363	0.0352	0.0333	0.0315	0.0267	0.0185

MT07	0.0093	0.0219	0.0330	0.0367	0.0352	0.0311	0.0289	0.0215
MT08	0.0108	0.0226	0.0359	0.0348	0.0330	0.0311	0.0285	0.0185
MT09	0.0141	0.0241	0.0363	0.0352	0.0333	0.0315	0.0267	0.0185
MT10	0.0093	0.0204	0.0330	0.0363	0.0356	0.0333	0.0319	0.0208
MT11	0.0126	0.0234	0.0337	0.0404	0.0374	0.0341	0.0282	0.0215
MT12	0.0148	0.0226	0.0337	0.0385	0.0411	0.0367	0.0300	0.0211