# COMPARATIVE BIOEQUIVALENC E STUDIES OF SOMEBRANDS OF METRONIDAZOLE TABL ETS 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 BELL O UNIVERSITY , ZARIA

# IN PARTIAL FULFILMENT OF THE REQUIREMENT S FOR THE AWARD OF A

MASTER DEGREE IN PHARMACEUTICAL CHEMISTRY

# DEPARTMENT OF PHARMACE UTICAL AND MEDICINAL CHEMISTRY , FACULTY OF PHARMACEUTICAL SCIENCES ,

AHMADU BELLO UNIVERSITY, ZARIA

# OCTOBER, 2014

# DECLARATION

# I declarethat the work in thisthesisentitled “Comparative Bioequivalence Studies of some brandsof MetronidazoleTabletsMarketed in Zari,aNigeria” has been carried out bmye in

the Departmentof Pharmaceuticaal ndMedicinal Chemistry. The information derived from the literaturehas been duly acknowledged in the text and a lisret foefrencesprovided. No part of this thesiswas previousplyresented for anotheDregreeor Diploma in this or any otherInstitution.

# 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 ergulations governing the award of thDe egreeof Master of Science in Pharmaceuticaol f the Ahmadu Bello University, andis approved for its contribution toknowledgeandliterary presentation.

# Chairman, Sueprvisory Committee Date

Dr. M.A. Usman

# Member, Supervisory Committee Date

Prof I.A. Yakasai

# Head of Department Date

Prof M.A. Musa

# Dean, School of Postgraduate Studies Date

Prof H.A. Zoaka

# DEDICATION

This work isdedicated to my lateparents. May Almighty Allah have Mercy on them as theydid to me when, I washelpless.

# ACKNOWLEDG M ENT

Praise and blessings are due to Allah (TS)Wwho in His infinite mercymade it possiblefor me to actualise this work. Peaec and blessings of Allah be oHnis noble Messengerand thosewho 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. Equamllyy, appreciation also goes to Dr. Aliyu

Musa of theDepartmentof Pharmaceuticaal nd Medicinal Chemistrywho has contributed immensely to my professional carrier as a Pharmacist.

# However, my utmost appreciations go to my late parents for moulding me fot rI wahma today. The prayers, support and encouragement of the parents, fabmroitlyh,ers, sisters, wife, paren-tin-laws, friends, colleagues and w-welilshers will not be forgotten. My unimaginable appreciation also goes to my wife and my daughter for thdeiciratdioen and

patience whlei away from them during the cosuer of this study.

# Finally, my sincere appreciation goes to all the technicalthaendnon-technical staff of the Departmentof Pharmaceutical and Medicinal Chemistry as well as anybody who in onewayor the other has contributed to the success of this programmyet.hMe aAlmighty

Allah reward you all.

# TABLE OF CONTENTS

ApprovalPage. . . . . . . . . . i

Tittle Page. . . . . . . . . . . ii

[Declaration . . . . . . . . . . iii](#_TOC_250005)

[Certification . . . . . . . . . . iv](#_TOC_250004)

[Dedication. . . . . . . . . . . v](#_TOC_250003)

[Acknowledgment. . . . . . . . . . vi](#_TOC_250002)

[Table ofContents . . . . . . . . . vii](#_TOC_250001)

List of Figure . . . . . . . . . . xii

Abbreviations. . . . . . . . . . xiii

[Abstract. . . . . . . . . . . xiv](#_TOC_250000)

* 1. CHAPTER 1: INTRODUCTION . . . . . . 1
  2. Post Market Survey . . . . . . . . 2
  3. Multisource (Generic) Pharmaceutical Products . . . . 3
  4. Quality Control . . . . . . . . 4
  5. Quality Control Requi rements of Tablet Dosage Form . . . 4
  6. Quality of a Drug Product . . . . . . . 5
  7. Fake or Substandard Drug Products . . . . . 6
  8. In-Vitro Evaluation Studies of Tablets Dosage Form . . . 6
  9. Methods for In-vitro Evaluation Studies . . . . . 7
     1. Identification Test . . . . . . . . 7
     2. Weight Variation . . . . . . . . 7
     3. Disintegration. . . . . . . . . 8
     4. Dissolution Rat.e . . . . . . . . 8
     5. Uniformity of Conten.t . . . . . . . 8
     6. Friability of Tablets Testing. . . . . . . 9
     7. Hardness Testing . . . . . . . . 9
  10. In-vivo Availability Stu dies . . . . . . . 9
  11. Bioequivalence and Bioavailability. . . . . . 10
  12. Comparative Bioavailability . . . . . . 10
  13. Study Design forIn-vivo Availability Studies . . . . 11
  14. Validati on 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. Detection Limit . . . . . . . . 15
    2. Quantitation Limit . . . . . . . . 15
  1. Application of Validated Method to Routine Drug Analysis . . 16
  2. Statement of the Research Problem . . . . . 16
  3. Justification of the Study . . . . . . . 17
  4. Research Hypothesis. . . . . . . . 17
     1. Null Hypothesis . . . . . . . . 17

1.17.1 Alternate Hypothesis. . . . . . . . 17

* 1. Aims and Objectives of the Study . . . . . . 17
  2. CHAPTER 2: LITERATURE REVIEW . . . . . 19
  3. History of Metronidazole . . . . . . . 19
  4. Chemistry of Metronidazole . . . . . . 20
  5. Physicochemical Properties oMf etronidazole . . . . 21
     1. Salt and Esters Formof Metronidazole . . . . . 21
     2. Solubility of Metronidazole . . . . . . . 21
  6. Therapeutic Index of Metronidazole . . . . . 21
  7. Available Dosage Form Strengths. . . . . . 21
  8. Analytical Methods for Analysis of Metronidazole from a Dosage Form 22

2.7 Indications . . . . . . . . . 23

* 1. Adverse Effects . . . . . . . . 23
  2. Interactions of Metronidazole with someDrugs . . . . 24

2.9.1 Alcohol . . . . . . . . . 24

2.9.1 Busulfan . . . . . . . . . 24

* + 1. Antacids,Kaolin-pectin orColestyramine . . . . . 24
    2. Barbiturates . . . . . . . . . 25

2.9.5 Disulfirim . . . . . . . . . 25

2.9.6 Carbamazepine . . . . . . . . 25

2.9.7 Phenytoin . . . . . . . . 25

* 1. Pharmacokinetics of Metronidazole . . . . . 25
  2. Justification for the use of Saliva as an Analytical fluid . . . 27

3.0 CHAPTER 3: MATERIALS AND METHODS . . . . 28

3.1 Materials . . . . . . . . . 28

* + 1. Reagents and Cheicmal used. . . . . . . 28
    2. Equipment and Glass Ware.s . . . . . . 28

3.1.3 Samples . . . . . . . . . 28

3.2 Method . . . . . . . . . 28

* + 1. Sample Collection . . . . . . . . 28
    2. Assessment of I-nvitro Parameters of the Sample.s . . . 29
    3. Analytical Method . . . . . . . . 31
    4. Preparation of the Stock Solution . . . . . . 32
    5. Selection of pHof theMedium . . . . . . 32
    6. Construction of Calibration Curve . . . . . . 32
    7. Validation and Optimiaztion of the Analytical Metohd . . . 33
    8. In-vivo Application of the Method . . . . . . 34
    9. ExtractionProcedure. . . . . . . . 35
    10. Data Analysis. . . . . . . . . 35
  1. CHAPTER 4: RESULT . . . . . . . 36
  2. Labels of the Samples of Metronidazole 200 mg Tablets Ranodmly Selected as Samples. . . . . . . . . 36
  3. Physical Appearance of the Samples of Metronidazole 200 mg Tablets Randomly Selected as Samples . . . . . . 37
  4. Identification Test on the sample ofMetronidazole Tablets . . 37
  5. Weight Variation and Disintegration Time for the Samples of Metronidazole 200 mg Tablets Dosage Form . . . . . . 38
  6. Assay, Dissolution, Friability and hardness Tests of Metronidazole Tablets Dosage Form. . . . . . . . . 39
  7. Result for the Identification of Metronidazole Reference Standard using UV Spectrophotometryaccording to BP 1993 method . . . 40
  8. ( I I H F W R I S + R Q W K H $ E V Reference Standard. . . . . . . . 42
  9. Intra - and inter-day Precisions of the Method usin J — J P Reference Standard at pH 9. . . . . . . 42
  10. Accuracy of the Method and Percentage Recovery of Metronidazole Reference Standard spiked into Blank Saliva . . . . . . 43
  11. Calibration Curve for in -vivo Availability Studies of the Sample of Metronidazole Tablets . . . . . . . 44
  12. Linearity of the Method . . . . . . . 45
  13. Concentration-time Curve for Metron idazole from Saliva Samples . 46
  14. Mean Bioequivalence Parameters of the Brands of MetronidazeolTablets 47
  15. Bioequivalence Ratio of Reference and Test . . . . 48

5.0 CHAPTER 5: DISCUSSION . . . . . . 50

6.0 CHAPTER 6: CONCLUSION, SUMMARY AND RECOMMENDATIONS 56 6.1 Summary . . . . . . . . . 56

6.2 Conclusion . . . . . . . . . 56

* 1. Recommendations . . . . . . . . 57

References . . . . . . . . . . xv

Appendices . . . . . . . . . . xxv

LIST OF FIGURES

Figure 2.1:ChemicalFormula of Metronidazole . . . . 20

|  |  |
| --- | --- |
|  | ABBREVIATIO NS |
| % | Per Cent |
| BP | British Pharmacopoeia |
| F | Force |
| FDA | Federal Drug Aegncy |
| 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 Phamracopoeia |
| UV | Ultraviolet |
| WHO | World Health Organisation |
|  | 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. Thutsh,is study was aimed actarrying out a comparativien-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 samplesmoeftronidazole tablets. Tablet hardness and friability testing were also carried out to reinforce the official methods forinthveitro studies. The analytical method for thien vivo studies was adopted from the work of Kolawole and Ameh (2004). The methodwas 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 al lthe samples with a content uniformity of 92.4-5 106.16 %. 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 awliuthe v

of 1.70 %, 1.81 %, 1.62 % and 10.25 % respectively above the 1 % acceptable limit. All the samples of the tablets had accepetahbal rdness within thleimit of 4-10 kgF, except MT10

# and MT11 with a value of 10.70 kgF and 10.6 kgF respectively. Theavteadlidmethod was found to have an intr-aand inte-rday 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 hadpt**a**cbcleelevel of bioequivaelnce

# within the range of 0.9-31.05 with reference toMT01® as the reference standard using point estimate ratio of the meanmCax and AUC0 : K — J , Q F metronidazole 200 mg tablets analysed can be used interchangeably even though there was some variation in tehir pharmaceutical equivalences.

CHAPTER 1 INTRODUCTION

Increasing economic activities in manyartps 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 dfiifcult situation over choice of an ideal brand (Om**e**jte,al., 2007). Thus, the increased influx of multisource Metronidazole tablets from different countries in the Nigerian drug market coupled with theclaolly manufactured brands of thderug is rapidly ni creasing.

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 medicinaul cptrohdas 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 whit 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 criaerd 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 standrda should be used to prepare solutoiofnknown 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 canedb.eTuhsree 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,tifcicearte 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. Post Market Survey

Post market surveillance or monitoring involves all actisvituiendertaken 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 raetigounls. 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 ormonitoring 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 througehloifuetothf 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 ancdedpurores N( gwuluka,et al., 2009).

* 1. Multisource (Generic) Pharmaceutical Products

Multisource Pharmaceutical products are intended to be pharmaceutically equivalent or pharmaceutical alternatives that are bioequivalent and hence are therapeuticivaalllyenetqaund 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 ofrtaasinceing their quality and inte- r

changeability. Variable clinical response to drug presented as generics an-dto-bbaattcchh inconsistencies have been reported. Such unacceptable trends were exhibited in some drug products including metronidazole and memtfoinr. 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 anofythe 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 (Awofiseat yaol.**,** 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 produmctsmfuroltiple 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 Stwaetere 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 tw**o** r 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 o-fpirnocess quality control. Thus, Michaeetl al, (2003) suggested

that there is need to determine their pharmaceutical and therapeutic equivalence in order to ensure inte-rchangeability (Banoe, t al.,2011).

* 1. 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 supplyt,hueinrtiql uality 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 cdoenrseid 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 sevceornatrl ol 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. Quality Control Requirements of Tablet Dosage Form

Tablets are oslid 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 usoeliodradlosage

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, incrtehaesreisk 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, hasrdannedsfriability.
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 nad 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. 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 qaudaete 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 Pharmaaco, pc**o**enitain

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 prondgudcirugs that are of high quality (Balat, 2006).

* 1. 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 maintainingirall the

characteristics properties during its s-hliefelf 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. 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 stradnsdoaf 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 preservavtei s) (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 (Mullaicheatrmal.**,** 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 theiruaetvioanl (Kishore and

Amareshwar, 2012). Assay of potency can also be carried out by microbiological assay (bioassay) (Apue, t al., 2011).

However, pharmaceutical equivalence of drugs may be establishine-dvibtryo studies based on measurements intended rtoeflect the rate and extent to which the active pharmaceutical ingredient become available at the site of action. Based on the general consideratinio-n that

vitro drug dissolution is predictive oinf -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-tolo-ltot quality control (Awofisayo, 2010

b). For products to be pharmaceutical equivalent, the productusldshboe equivalence in pharmaceutical development, stability and in manufacture (Schmauster, 2010).

* 1. Methods for In-Vitro Evaluation Studies
     1. Identification test

Identification testes are intended to ensure the identity of an analyte in a samhpisle.is T

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. Weight variation

Tablet is designed to conintaa 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 (Banoe,t al., 2011). BP 2002 states that no two tablets: with an average woefight

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. Disintegration

Disintegration test measures the time required under a given set of conditions for a group of tablets to disnitegrate into a particles. For compressed uncoated tablets the testing fluid is water at 370C, but in some cases the monographs direct that simulated Gastric fluid be used. (Remington, 1975). The BP specification is that uncoated tablets should daistientwegitrhin 15 minutes and film coated in 30 minutes while USP specifies that uncoated and film coated tablets should disintegrate within 30 minutes. (Ngwuluekta,al., 2009).

* + 1. Dissolution rate

Dissolution testing is required for all solid oral dosafogrems 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- 1(510minutes) disintegration

for soluble or radi-olabelled drugs. Generally experiments are conducted atÛ37& 8 6 3 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 medPiu, m20(0B2).

* + 1. 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 referenceto the average content of the sample. The test is not required for multivitamin and trace element preparations and in other justified andriazuetdhocircumstances

(BP, 2002).

* + 1. Friability of tablets testing

Friability testing is use to evaluate the liatybiof 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. Hardness testing

Hardness testing assesses the abilfitytaoblets 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 o4f - 15kgF is the acceptable limit (Ogaeht,al.,2002).

* 1. 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. fiScaplelyc,i 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)a inpharmacokinetics 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 efficienycof 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 essesnimtiailallrytherapeutic 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 ebqiouivalence study is performed in an appropriate manner (WHO, 2006).

* 1. Bioequivalence and Bioavailability

Bioequivalence testing is considered as a surrogate for the chemical evaluation of the therapeutic performance of drug product (Awofisayo, 201B0iao)e. quivalence shows that two formulations of one drug are equivalent. Bioequivalence testing can be used f-odrrudgrug

interaction studies and food effect availability studies (Zh**e**tnagl,., 2012).

On the other hand bioavailability is the rate and exotef natbsorption of a drug from its dosage form as determined by its concentra-titoimne 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 thiseferrence 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 produacnt eosr tablished product) in

the formulation, utilizing the same route of administration (Olaniyi, 2005).

* 1. Comparative Bioavailability

Most bioavailability studies, whether for a new or generic product, possess a common theme. A test was conducted toeindtify 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 modi-fri **e**dlease formulation in comparison to a conventoi nal 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. 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 senqcuees, two treatments, two period, single dose and crossover study with a washout period of two weeks (Bheot iar,l., 2012). The subjects should abstain from food and drinks, which may interact with circulatory, gastrointestinal, hepatic or renal functione.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 aasswdeullring the

study (European Medicine Agency, 2010).

* 1. 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.odTeheofcfederal regulations explicitly states that the accuracy, sensitivity, specificity and reproducibilityt of tes

methods employed by the firm should be evaluated and documented (Chan, 2008). The

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

* + 1. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the values which is accepted either as a conventional truen oarccaepted 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 betwtheeenvalues 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 relativteansdard deviation (RSD). Acceptable limit is- 15% (Harvey,

2000).

* + 1. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurement obdtafirnoem multiple sampling of thesame 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 potsosiobbletain 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 maseurements (European medicine Agency, 1995).

* + 1. 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 specifica**l**y 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, resesrpveincgificity for

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

* + 1. Linearity

ICH defines linearity of an analytical method as the ability (within a given rangoeb)tatoin 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 apr**e**aaks,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 concentrationnda 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 ndilouftitohe 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 usedlctuolactae 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 determinatio2n) o(rf • 7 K

sum of squares, and y einrtcept should also be reported as required by ICH (Chan, 2008).

* + 1. 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 analytciceadluprerohas 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 cove0r-7120 % of the nominal concentration. For

dissolution testing, a normal range+i2s0 % 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 raneg 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. Robustness

Robustness of an analytical procedure is aasmuree 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 phasaend depends on the type of procedure under study. Experimental design (e.g., Fractional factorial design or plac-kBeutrman design) is common and useful to investigate multiple parameters simultaneously. The result will help to identify critical paramtheatet rws ill

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. 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 detedrmeiniteher 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 subiltea number of samples near or at the DL.

DL = /

S

: K H U H / L V W K H V W D Q G D U G G H Y

(Chan, 2008).

* + 1. Quantitation limit

The quantitation limit (QL) is a characteristics of quantitaatisvseay 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 trioa 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 = / S

: K H U H / V W D Q G D U G G H Y L D W L R

* 1. Application of Validated Method to Routine Drug Analysis

Assay of all samples of an analyte in a biological matrix should be completed withinmethe ti

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 eisftorruprocedures 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 alnysis 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 concentratieoninraandgdition 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 bsheoruel**d**efined 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. Statement of the Research Problem

Theconcern 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 (Nascimenetot ,al., 2011). Metronidazole insot 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 substandaredt (Musa,

al., 2011).

* 1. 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 news dtoruegnsure the quality of

drugs marketed (Nascimenteot, 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 prtosdcuacn be used interchangeably (Chandrasekaran,

et al.,2011).

* 1. Research Hypothesis
     1. Null hypothesis

There is nosignificantdifference inthe pharmaceutical, chemical and bio equivalenbceetws een Loxagyl® and other mul-tigeneric sources of mentriodazole tablets marketed in Zaria metropolis.

* + 1. Alternate hypothesis

There issignificant difference inthe pharmaceutical, chemical and bio equivalenbceetsween Loxagyl® and other mul-tigeneric 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 bioequicvealenas well as interchangeability of various brands of metronidazole tablets marketed in Zaria metropolis with reference to comparator products (Loxagyl).

CHAPTER 2 LITERATURE REVIEW

* 1. History of Metronidazole

Metronidazole is one of thexamples of drug developed against parasite, which has since gained broad use as an antibacterial agent. Briefly, at R-Ph**o**unl**e** nc in France, extracts of Streptomycespecies were screen for activity agaiTnrsict homonas vaginal,isa 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 metronidazroelde acupatient of both trichomonad vaginitisand bacterial gingivitis. However, it was not until the 1970s that metronidazole was popularized (Samuelson, 1999).

Also, the isolation of the antibiotic, azomycin-n(2itroimidazole) from a Streptomyces by Maedaand 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( -hydroxyethyl)-2-methyl-5-nitroimidazole, now called metronidazole

(FLAGYL®). It was observed that metronidazole has high actinvi-tvyitro andin-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 trichosmoniasi

(Phillips and Stanley, 2006)L.ater studies revealed that metrdoanzi ole 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 givnigtiist 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 adnudcepsrorelatively 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 (Saumelson, 1999).

* 1. Chemistry of Metronidazole

Metronidazole is (-2(2 methy-l5- nitromidazole-1-yl) ethanol) (BP, 1993; Kolawole, 2004 and Usman et al, 2011). However it can be named as-(2[-1hydroxyethyl)-2-methyl-5- nitroimidazole] (Alveset 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 Mustaphae,t al., 2006). It is a prototype of nitroimidazole class of antimicrobials (Ezzeldin and E-Nl ahhas, 2012). It is white or yellowish, odourless crystals or crystalline powder. It darkens on exposure to light. Its formula6His9NC3O3 with a molecular

mass of 171.2 and chemical formula is represented in the figure below

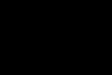


Figure 2.1: Chemical formula of metronidazole (Martindale, 1999)

* 1. Physicochemical Properties of Metronidazole
     1. Salt and esters form

The British pharmacopoeia, the European pharmacopoeia, the International pharmacopoeia, and the US pharmacopoeia hamveonographs 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).

* + 1. Solubility of metronidazole

Metronidazole’s solubility in water has been reported as 10mg/ml at Û20 & $ Q R W K 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 hanidt ,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).

* 1. 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 (Redigeut iaelr;i,2011).

* 1. Available Dosage Form Strengths

The WHO Model List of Essential Medicine mentione2d00 mg to 500 mg of metronidazole tablets dosage form, 200 mg/5ml metronidazole (as benzoate) oral susp5e0n0simong, 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 (Sono-fiaventis, 2012).

* 1. Analytical Methods for Analysis of Metronidazole from a Dosage Form Metronidazole can be determined with microbiological technsiq(uRealph, 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 mewtehroedstime 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 techniquHePs.LC 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 mtheods 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

aqueoustitration 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. UniteedsSptahtarmacopoeia describes HPLC and no-naqueous titration method for the assay of metronidazole only (Dineet sahl.,, 2003)

* 1. Indications

Metronidazole is used in the treatment of susceptible protozoal infections such as amoebiasis, balantidiasis, Blastcoystis 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 antimicrobianlds, eaither 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 (guine-waorn infection) and has given in the atrtme ent of perional crohins

disease and hepatic encephalopathy. It has also been tried as an adjunct to the radio therapy of malignant neoplasms (Martindale 1999).

* 1. Adverse Effects

The adverse effects of metronidazole are generally dose related. Thecommomston 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 antibioti-cassociated colitis associatedthwimetronidazole (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 epsrenting 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 thrombocoyptenia 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 iannodgecnaircc

in some animals (Martindale, 1999).

* 1. Interactions of Metronidazole with Some Drugs The following are some of the drug interactions of metronidazole.
     1. Alcohol

Metronidazole may provoke a disulfiram like reaction in some individuals whveenn gwi ith 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 recnegividisulfiram. 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).

* + 1. Busulfan

The use of busulfan with metronidazole significantly increased plasma concentrations of busulfan and the degree of associated toxicity, including elevations of liver function tes-ts, veno

occlusive disease and mucositis (Martindale, 1999).

* + 1. Antacid, kaolin- pectin or colestyramine

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

* + 1. 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,nda giardiasis or amoebiasis in children (Stockley’s drug interactions, 2005).

* + 1. Disulfiram

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

* + 1. Carbamazepine

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

* + 1. Phenytoin

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

* 1. Pharmacokinetics of Metronidazole

Metronidazole is readily and almost completely absorbed after oral dPoseeask. plasma

F R Q F H Q W U D W L R Q V R I D E R X W D

dose of 250 and 500 mg respectively. Some accumulation occurs and consequently there are higher concentrations when multiple doses are given. rApbtioson 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. Pea-kstsatteeady

S O D V P D F R Q

ZF LH WQ

KW U

WD WU RL RX JQ K

R FI

R QD

FE HR

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 concenatrtions 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 pesamkapla

concentrations of about 2 mg/ml after a dose of 500 mg. An-vinatgrianal 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 dbisu**t**rei d. 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 pltaacea**n** d rapidly enters the foetal circulation. No more

than 20% is bound to plasma proteins.

Metronidazole is metabolized in the liver by s-icdheain 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-m2ethyl-5-nitroinitrozole-1- acetic acid (the acid metabolite), which has virtually no antibacterial activity and is often ndoet tected in plasma, but is excreted in urine.

Small amounts of reduced metabolites, acetamide a-n(2d- Nhydroxyl ethyl) oxamic acid (HOA), have also been detected in urine and are probably formed by the intestinal flora (Martindale, 1999).

The eliminationhalf-life of metronidazole is about 8 hours that of the hydroxyl metabolite is slightly longer. The ha-llfife of metronidazole is reported to be longer in neonates and in patients with severe hepatic impairment; that of the hydroxyl metabolite is prolionnpgaetdients

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).

* 1. 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 monitoringe. trTahditional 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 plasmleavels because saliva lacks “the drama of blood” (Heotld,

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

CHAPTER 3 MATERIALS AND METHODS

* 1. Materials The following were the materials used for the study.
     1. Reagents and chemicals used

Analytical grade chemicals and reagents (BDH) were used.

* + 1. 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 DisintegratioTnime Test apparatus (Type ZT3-G, mbH, Germany), Euweka friabilator (Type T-3AR, GmbH, Germany)

Monsanto Hardness tester (Manesty Machines Liverpool, England), pH meter (Fisher Scientific, Singapore), Rotor mixer (Gallankamp, England), UV double beamtrospehcotometer M( NF, Helious Zeta,Thermo Scientific Englan)dand Water bath (model BJE 750A Gallenkamp, England)

* + 1. Samples

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

Different brands of metronidazole tablets collected from Zaria metropolis.

* 1. Method
     1. Sample collection

Different brands of metronidazole tablets were randomly purchased at different retail pharmacies and drug outletsSinabon 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.

* + 1. Assessment oifn-vitro parameters of the samples
       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).

* + - 1. 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.

* + - 1. Identification Test

A quantity of the powered tablectsontaining 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 exce**s** nitrite was removed with sulphamic acid. Then 0.5 ml-noaf p2hthol solution and 2 ml of

5 M sodium hydroxide were added. The formation of or-arnegdecolour indicates the presence of metronidazole. The procedure was repeated for all the samples (BP., 2002)

* + - 1. 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 rewheteigd. The loss in weight was noted and the percentage lost calculated (BP, 2002).

* + - 1. Disintegration Test

Six tablets were selected at random for each brand. The disintegration time for each brand was determined using Euweka disintegration apparatuast.eWr was used as the medium maintained

at 37 ± 1oC. 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 asethdisintegration time for a brand (BP, 2002).

* + - 1. 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 lauctiiodns. o

The medium was maintained at 37± 00C.5. 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 tlhuetiosnowas 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 condvetroteconcentration in % w/v from which percentage released was calculated using E 1 %, 1 cm of 380 (BPC, 1978).

* + - 1. Hardness Test

This is an unofficial method of ascertaining the quality of a tablet. Five tablets were selected randomly. A tablet was apcl ed 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 (Muh,aemt mal.e,d

2012).

* + - 1. 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 quiaenstiot f 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 enpdoint. 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 o6Hf 9CN3O3. The procedure was repeaet d for all the brands (BP, 2002).

* + - 1. 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 1o0f **0**m.1l 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 tom1l 0w0ith 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 2- **3**050 nm (BP, 1993).

* + 1. Analytical Method

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

* + 1. 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 in0tom1l 0cleaned 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 stockOso X W L R Q R I

through serial dilution method.

* + 1. 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 wadsiv**i**dnually 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 0M.1 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

* + 1. Construction of calibration curve

Metronidazole has a pkeaplasma 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

— J P O Z H U H S U H S D U H e G I U R

aid of chewing gum to stimulate salivation from healthy human volunteers. The saliva was centrifuged at 3 g for 10 minutes. Tshuepernatanwt as collected and 1 ml of thseupernatanwt as transferred to a test tube. 3 ml of buffer solution with pH 9 wadsedadfollowed by the spiking

1. I G L I I H U H Q W F R Q F H Q W U D W L R Q V

— J P O R I P H W U R Q L G D ] R O H

with a rotor mixer for 1 minute. The suotilons 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 metronidazeo)l using double beam UV spectrophotometer. The absorbance were

1. O R W W H G D J D L Q V W F R Q

2;FinteHrceQpt

W U D W L

R Q W K H Y H U W L F D O D [ L V & V

method of least square with the aid Microsoft Excel 2010, limit of detection, LOD and limit of

T X D Q W L W D W L R Q / 2 4

D QZ HG

U H/ 2 4G H W H U

is slope of the graph) respectively.

* + 1. Validation and optimization of the analytical method
       1. Linearity

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

* + - 1. Precision

Precision was determined by repeatability (i-ndtaray) and intermediate precision (in-tdeary).

5 H S H D W D E L O L W \ Z D V G H W H U P L Q H 3ml buffer solution with a pH 9. The absorbance was taikxetnimses at an interval of 1 hour in

the first day under the same experimental condition.-Idnateyrprecision was evaluated by taking

W K H D E V R U E D Q F H R I P O R I

times in three consecutivdeays.

* + - 1. Accuracy and Percentage Recovery

7 K L V Z D V G R Q H E \ V S L N L Q J corresponding to concentration of 80 %, 100 % and 120 % of a nominal concentration of 10

— J P O Ve inRto 1O mlXof sWupeLrnatRant Qportion oRf saIliva (GhPaen**t**Hea,l.,W2012U; Nascrimento,et al., 2011; Sundaraganapatehtyal, 2011 and Wrass-Seongoi,et al., 2010). 3 ml

R Q L G D

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.

* + 1. In -vivo application of the method
       1. Selection of subjects

Six healthy human volunteers were enrolledthfoer study. Their health status was confirmed by urinalysis. The subjects were n-somn okers, no-nalcohol 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 stud. yTheir mean age was 26.33±0.95 years and mean body mass indices was 22.08±1.11 kg/2mwhich were within the acceptable normal range of –1855 years (European Medicine Agency, 2010).

* + - 1. Study design

The study was single dose, randomized, crossovseigr ndestudy with a washout period of two weeks.

* + - 1. 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 administeredbowuitth a

200 ml of water. No food or drink allowed 3 hours after administration of the drugs.

* + - 1. 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 weenrterifcuged 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 freeze-r 4atOC prior analysis.

* + - 1. Sampling time

Blank saliva was colletecd 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.

* + 1. Extraction procedure

The extraction procedure was adopftreodm the work of Kolawole and Ameh (2004). The frozen samples were melted with the aid of water bath maintained at a temperatureOCo.f T3h7e 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.

* + 1. 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 meanmCax and the mean mTax 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 approiaptre.

CHAPTER 4 RESULT

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

* 1. The Labels of the Samples of Metronidazole (200 mg) Tablets Randomly Selected

as Samples

Table 41. : Labels of the Samples of Metronidazole (200 mg) Tablets Randomly Selected as Samples.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Brand | Country  Origin | of | Batch No. | NAFDAC No. | Manuf. Date | Expiry Date |
| MT01 | Nigeria |  | MA1048 | 04-0283 | 01/201 | 12/201 |
| MT02 | Nigeria |  | UGT0290 | 04-8426 | 12/2010 | 11/2013 |
| MT03 | Nigeria |  | 21590 | 04-4012 | 05/2011 | 05/2014 |
| MT04 | Nigeria |  | 1109 | 04-9936 | 03/201 | 03/201 |
| MT05 | Nigeria |  | 0015 | 04-7473 | 01/201 | 05/201 |
| MT06 | Nigeria |  | 14 | 04-1308 | 12/2010 | 05/2013 |
| MT07 | Nigeria |  | 0160 | 04-0386 | 06/201 | 02/201 |
| MT08 | Nigeria |  | 15331 | 04-0072 | 03/201 | 09/201 |
| MT09 | Nigeria |  | 524136 | 04-0275 | 03/2011 | 02/2013 |
| MT10 | Nigeria |  | 11015 | 04-0980 | 02/201 | 02/201 |
| MT11 | Nigeria |  | MT470 | 04-4283 | 04/2011 | 05/2014 |
| MT12 | Nigeria |  | 142 | 04-7612 | 05/2011 | 05/2014 |

* 1. 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 surfa | + | Dull | Smoot |
| MT04 | White | Circular, convex surface | - | Dull | Smooth |
| MT05 | Yellow | Circular, flat surface | - | Dull | Smooth |
| MT06 | Yellow | Circular, flat surfac | + | Dull | Rough |
| MT07 | White | Circular, convex surface | + | Shiny | Smooth |
| MT08 | Yellow | Circular, flat surface | + | Dull | Smooth |
| MT09 | Yellow | Circular, flat surfac | - | Dull | Smoot |
| MT10 | Yellow | Circular, flat surfac | + | Dull | Smoot |
| MT11 | Yellow | Circular, flat surface | + | Dull | Smooth |
| MT12 | Yellow | Circular, flat surfac | + | Dull | Rough |

* 1. 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.

* 1. 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) TabalegteDFoosrm

|  |  |  |  |
| --- | --- | --- | --- |
| Brand | n=20  Mean weight/mg(SEM) | No. of tabletsdeviated by | n=6   * 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 variatnio, no more | than 2 tablets deviate by• | % 3 |

* 1. Assay, Dissolution, Friability and Hardness Tests of Metronidazole Tablets Dosage Form Table 4.4:Result for Assay, Dissolution, Friability and CrunsghiStrength Tests of Metronidazole Tablets Dosage Form.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Brand | n=6  Assay (%) | n=6  Mean dissolution (%) | n=6  Friability (%) | n=5  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 (04. 0) |
| 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: -81515 %, IP: 90-110 %) Mean dissolution rate (BP not < 70% at 45 minutes), Mean friability (BP and U” SP

Mean hardness -140 Kg

* 1. Identification of Metronidazole Reference Standard Using UV Spectrophotometry According to BP 1993 Method

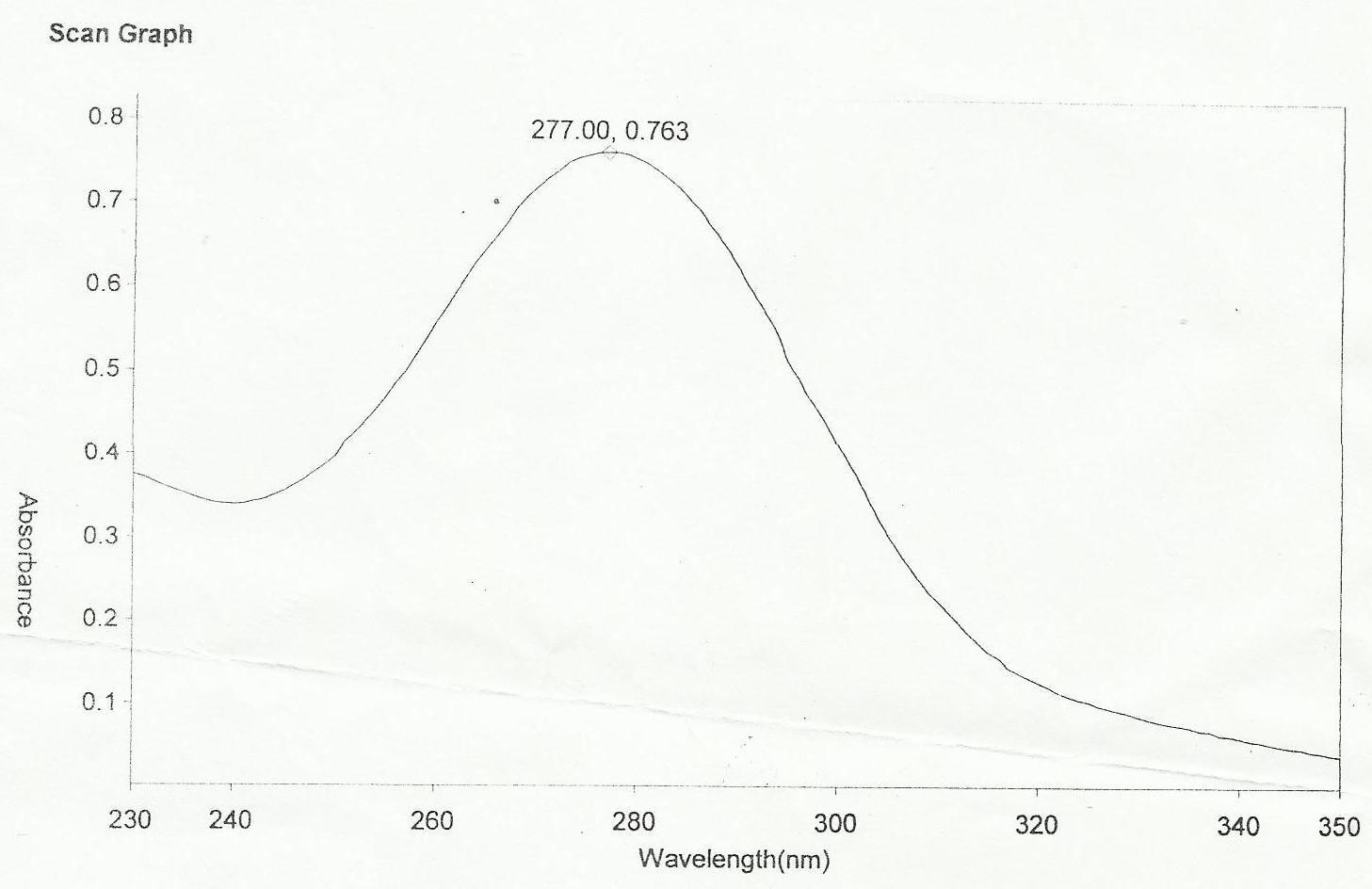


Figure 4.1: Absorbance of 0.02 %w/v Solution of Metronidazole Standard Powder in 0.1 M HCl scanned at 23-3050 nm.

* 1. Effect of PH on the Absorbanc H R I — J P O 6 W Reference Standard Spiked into Blank Saliva

Table 4.5: ( I I H F W R I S + R Q W K H $ E V R

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 | 0.050 |
| 6. | 9 | 0.050 |

* 1. Intra - and Inter-Day Precisions of the Method

Table 4.6: Absorbance for int-raand inte-r G D \ 3 U H F L V L R Q V R

Reference Standard at pH 9 spikedo ibnltank Saliva

|  |  |  |
| --- | --- | --- |
| s/n | Absorbance |  |
|  | Intra -day precision | Inter -day precision |
| 1. | 0.050 | 0.050 |
| 2. | 0.0509 | 0.0512 |
| 3. | 0.0507 | 0.0512 |
| 4. | 0.0513 | 0.0514 |
| 5. | 0.0510 | 0.0513 |
| 6. | 0.051 | 0.050 |
| Mean | 0.0510±0.000 | 0.0511±8.7×1-5 |
| SD | 2.5×104 | 2.13 ×10-4 |
| RSD (%) | 0.491 | 0.417 |

NOTE: Acceptable limit, RSD ”

* 1. 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 MetrolneidRazeoference Standard spiked into Blank Saliva

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| S/N | n = 3  Amount (µg/ml) | added | 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 valu | 98.30 | | | | 1.98 |

The mean value for pceerntage recovery was 98.03% (acceptable limit o–f 91802 %; Chan,

2008) and accuracy of the method was 1.98 %Er (acceptable ra-5ng%eE: 1r)

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

Absorbance (nm)



y = 0.0037x + 0.003 R² = 0.9970

~ R P l u o

0.07

0.06

0.05

0.04

0.03

0.02

0.01

0

0

2

4

6

8

} v

10 12

š Œ

14 16

š ] } v

v

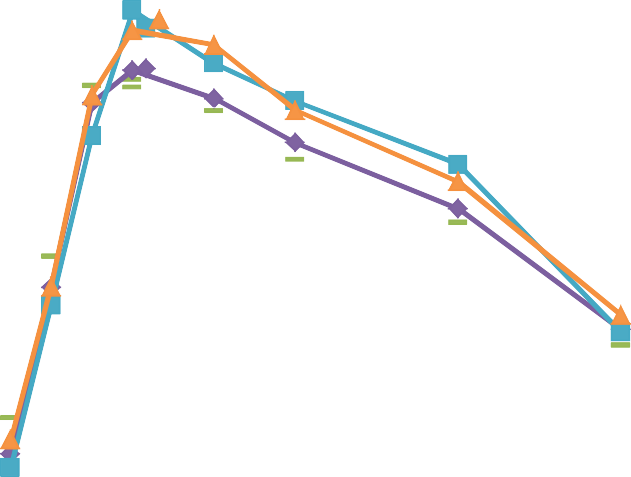
Figure 4.2: Calibration Curve foirn-vivo Availability Studies of the Samples of Metronidazole Tablets

* 1. Linearity of the Method Table 4.8: Result for Linearity of the Method

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S/N | Parameter |  |  |  |  | Value |
| 1. | 0 D [ L P X | Pmax | Z | D | Y | H277OnmH Q J W K |
| 2. | Calibration curve ran |  |  |  |  | 2.5- |
| 4. | Regression equation |  |  |  |  | Y = 3.66×10-3X + 3.02×10-3 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 5. Intercept on the vertical axis, C | | | 0.0030 |  | |
| 6. Correlation coefficient,2 | | | 0.997 |
| 7. Limit of detection, LOD | | |  | — J | P |
| 8. Limit of quantitation, LOQ | | |  | — J | P |
| 9. | 6 W D Q | G2 | 1.322 | | |

* 1. Concentration-Time Curve for Metronidazole from Saliva Samples



12

10

8

6

4

2

MT01 MT02 MT03 MT04 MT05 MT06 MT07 MT08 MT09 MT10 MT11

MT12

0

0

2

4

6

8

10

time (h)

v š Œ

š ] } v

~ R P l u o

Figure 4.3: Concentratio-tnime Curve for thein-vivo Administration of Various Brands of Metronidazole(200 mg) Tablets in six Healthy Human Volunteers

} v

* 1. 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 SEiMn brackets

Brand Cmax — J P TOmax h (SEM)6 ( AU0 C0 : K — J K P O 6 (

|  |  |  |  |
| --- | --- | --- | --- |
| 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) |

* 1. Bioequivalence Ratio of Reference and Test Table 4.10: Bioequivalence Ratio of Reference and Test

Brand Cmax (µg/ml) AUC0 : K — J K P O

|  |  |  |
| --- | --- | --- |
| 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 me | 0.96 | 0.96 |
| Remark | Bioequivalent | Bioequivalent |
| MT06 (Test) | 9.25 | 71.63 |
| Point estimae ratio of the mea | 1.03 | 0.96 |
| Remark | Bioequivalent | Bioequivalent |
| MT07 (Test) | 8.43 | 72.19 |
| Point estimate ratio of the me | 0.94 | 0.96 |
| Remark | Bioequivalent | Bioequivalent |

Acceptable range for poinetstimate ratio of the mean: 0-1.8.25

Continuation for tbale 4.10

Brand Cmax (µ/ml) AUC0 : K — J K P O

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

Acceptable range for point estimate ratio of the mean-1: .02.58

CHAPTER 5 DISCUSSION

All the samples of metronidazole tablets examined were registeredthwe iNthAFDAC 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 variationainoolergptic 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 breankgi 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 assaystedsotnweaaccording to BP 2009

and the content uniformity was found to be in the range of 9-21.4056.14 % (table 4.4). The values were in agreement with the BP (2002) specification o-f 18155 % for tablets dosage form. International Pharmacopoeia specifiiocnatfor tablet dosage form is 9-0110 % (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 MMTT0058, and MT12 was

an indication of deviation from the compendial specifications (table 4.3). The uniformity of weight might be as a result of n-ou**n**iformity of active ingredient and pressure different during the compression process (Mullaichareatn,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 (Ibeeztima, l., 2008).

Uniformity of weight doesesrves as a pointer for good manufacturing practice (GMP) as well as amount of the pharmaceutical ingredient (API) in a prodNugctw(uluka, 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 aintidoinc 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 importan-pt rioncess means of assessing whether the tablets being prodcued are firm enough to withstand breakage, chipping or crumbling and yet not so hard as to delay disintegration (Mbeatha, l., 2012). It is also influences friability. The greater the pressure applied, the harder the tablet (Mullaicheatraaml.**,** 2012). The acceptable

limit is 4 - 10 kgF (Mohammede, t 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 metdroaznoi le 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 (taeb4l .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 nagmcoapsule 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 oinf-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 mkaerd 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 (Esimetonale.,,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. Absorpotifo0n.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 Mishertaa, l (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 ran-ge of 2.5

12.5 µg/ml. precision of the methowdas done by assaying 6 determinations of 15 µg/ml solution of metronidazole (Wras-sSeangoi,et al., 2010) for both intra and int-edray precisions. Intraday

and inte-rday 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 -o2f <1.5

% (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 vf athlueepoercentage recovery

was 98.30 % (table 4.7) which falls within the acceptable limit o-1f 0928 % (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 -o5f %1 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 (Ulelat hal, 2009). Pahklaet al (2005,) in

their workshow 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 falvyouwraitbh conventional treatment (Pahkelat 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 parame,tesursch as the peak drug concentrationma(xC), the

time to reach this concentration (mTax), and the area under the drug concentra- **ti**mone 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 nmI.n-vivo study showed that the mean mCax for the various brands of

metronidazole tablets ranged 8- .1 — J P O 7 K H Y D

reported by other researchers on similar topic on healthy human volunteers revealed the

following: Usmanet al, (2007) reported Cmax of 7.00±0.43– “ st and — reference sample of 400mg metronidazole tablet respectively from saliva samples using UV

visible spectrophotometer. Kolawole and Ameh, (2004) reported-8.72 — J P

metronidazole tablet from saliva samples using UV visible spectr**o**mpheotet r. Usmanet al,

(2011) reported Cmax “ — J P O I R U using HPLC method. The point estimate ratio omfaCx for all the brands of metronidazole with

reference to brand MT01 as the comparator darunggerd 0.93- 1.05, which were within the bioequivalence acceptance limit of -01..825

The mean Tmax for the various samples of metronidazole tablets extrapolated from the concentratio-ntime curve were within the range of 1.8–32.83h. The values were in rhmaony

with the reported values of 0.2-54h for non-pregnant patients by Zenegt al, (2010) for 500mg of metronidazole from blood samples using HPLC method. However, Redeigt uairl,i (2011) reported Tmax of 0.25-4h for oral doses of 250, 500, 750 and 20m0g0of metronidazole tablets. Ezzeldine and E-Nl ahhas, (2012) reported mTax of 2.75-3 h from blood on healthy human volunteers using HPLC method. Kolawole and Ameh, 2004 reportemadx oTf 2.03±0.052- 2.77±0.96 h for 400 mg of metronidazole from salivna hoealthy human volunteers using metronidazole samples with UV visible spectrophotometer. Also, Usemt **a**ln, (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 salivsaamples on healthy human volunteers using UV visible spectrophotometer. While Usmaent al (2011) reported 1.774±0.06 h for 400 mg of metronidazole from blood samples using HPLC method

Furthermore the AUC0 :

shoWuld cover at least 80% of the AU0C :

(Eu’ ropean Medicine

Agency, 2010). The mean AU0C : for allKthe brands of the samples were within the range of

70.80 - — J K P O 7 K H 0 :

oYf ’

D O X H V

82.0 -

0 : , 80 %K—= 6J5.6–

P O /ml) by Ez$zeld8ine&and E-N—l ahhJas,

(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: Usmane

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| t al, (2011) repotred AUC 0 : | R’ I |  | 0 : , |  | K | “ |  |
| Z D V | 0 : K — J | K | PZ | OD |  | V Y | H |

patients from blood samples using HPLC method. Hougehttoanl, (1982) reported AUC0 : of ’

“ 0 :

, 80—%K wJas K P

—$ J8 &

K P O I U R

Usman et a,l 2007 reported AUC0 : of 69K.93±1.15 for reference drug and 65.50±0.89

— J K P O W H V W G U X J I U R P V D O L

reported AUC0 :

of 7’ 3.69 W R

AUC 0 :

, 80 wKas 58.95–— J K P O — J

the other hand, Emani et al, 2006 reported A0UC:

R’ I

AUC 0 :

, 80 %K was — J

— J AUKC 0 :

P OK

I R U

Z WD VH V W

reference drug. Turgut and Ozyazici, (2004) reported AUC of 1–01059 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 A0UC: for thKe all brands of metronaidzole

with respect to MT01 as the comparator product were ranged–01**.**9070. The range was within the acceptable limit of 0.–8 1.25 for bioequivalence studies.

CHAPTER 6

* 1. SUMMARY, CONCLUSION AND RECOMMENDATIONS
  2. 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 caceptable 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 abclceevpatalue

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 hardtneesst.s

1. 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.
2. All the samples of metronidazo2le00 mg tablets dosage form were bioequivalent with a point estimate ratios within 0-.18.25 for bioequivalence studies.
   1. Conclusion

It has been shown from the validation parameters of the method that it was robust and reproducible with high degree of caucracy 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 usedinterchangeably. This shows that there is no difference in the pharmaceutical, chemical and bio equivalences between Loxa®gaylnd other brands of metronidazole marketed in Zaria metropolis during the course of this research. However, there was variantiotnhe i

pharmaceutical parameters of the sampSleasm. ple MT05, MT08 and MT12 failed weight variation test. MT05, MT07, MT08 and MT11 failed friability test; while MT10 and MT11

failed the hardness te. sHt owever, statistical analysis of bioequivalence parearms esthows no significant statistical difference. Thus, the samples have comparable bioavailability.

* 1. Recommendation

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

chain.

1. 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.
2. There should also be a need for routine post market surveillance of pharmcaalscebuyti

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.LTahtien American Journal of Pharmacy;26(2), 266-269.

Apu, A.S., Khan, N.H., Karim, M., Bola, N.G., Sziruale, M.K. Jamaluddin, A.T.M., and

Rahman, Z. (2011). -Invitro Evaluation of the Pharmaceutical Equivalence of

Phenoxymethylphenicillin Tablet Formulations Available in BangladTehseh.Journal of Pharmacy Researc, h4(5), 1445-1447.

Awofisayo, SO. ., Awofisayo, O.A., Eyen, N., anUddoh, I.E. (2010 a). Comparative Assessment of the Quality Control Measurements of Multisource Ofloxacin Tablets Marketed in Nigeria. Journal of Dissolution Technologies1, 6(16), 20-31. Retrieved from [http://www.dissolutiontech.com](http://www.dissolutiontech.com/)

Awofisayo, S.O., Willie, E., andUmoh, E. (2010 b). Quality Control Evaluation of Multisource Arthemethe-rLumefantrine Tablets Prescribed for Uncomplicated Multi drug Resistant Malaria. The Indian Journal of Novel Drug Delive, r2y(4), 153-157.

Azam, G., andHaider, S.S. (200)8. Evaluation of Dissolution behaviour of Paracetamol SuspensionsT. he Dhaka University Journal of Pharmaceutical Scien1c(e1s)**,** 53-58.

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

Bano, R., Gauhar, S., Shyu-mNaqvl, S.B., andMahmood, I.S. (2011). Pharmaceutical Evaluation of different Brands of Levofloxacine Tablets (250 mg) Available in Local

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

Bhoir, S., Gaikwad, P., Bhagwat, A., anJdathar, S. (2012). Stea-dsytate Pharmacokinetics of Immediate-release and Controlle-rdelease Metronidazole Tabsle. tThe International Journal of Pharmacy and Pharmaceutical Scienc4e(3s),, 353-356.

British Pharmaceutical Codex (1978P).roduct Monograph: MetronidazoleH. er Majesty’s Stationary Office, University Press Cambridge.11 ed. pp.567

British Pharmacopoeia (1399). Product Monograph: MetronidazoleV. olume 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 EDG)o. odman

& Gilman's the Pharmacological basis of Therapeu(tipcps 4). United States: McGra-w

Hill Medical Publishing Division.

Centre for Drug Evaluation and Research (200G1)u. idance for bioavailability and Bioequivalence StudieMs.inistry of Health and Family Welfare, Government of India, New Delhi

Chan, C.C. (2008). Analytical Method Validation: Principles anadctPicres. InPharmaceutical Manufacturing Hand Book: Regulations and Qual(iptyp 727-742). John Willey and Sons, inc.

Chandrasekaran, A.R., Jia, C.Y., Theng, C.Su.,niMandy, T., Muralidharan, S., anDdhanaraj,

S.A. (2011). In-vitro Studies and Evaluationf oMetformin Marketed Table-tsMalaysia.

Journal of Applied Pharmaceutical Science1,(5), 214-217. Available at http://www.japsonline

Cudmore, S.L., Delgaty, K.L., Haywa-rMdcClelland, S.F., Petrin, D.P., anGdarber, G.E. (2004). Treatment of Infections Caused by Metronida-Rzoelseistant Trichomonas vaginalis. Clinical Microbiology Review, 17(4), 783-793. Available at <http://cmr.asm.org/content/17/4/783#-rliesft-1>

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

Esimonea, C.O., Okoye, BF..C., Onah, B.U., Nwrou, C.S., andOmeje, E.O. (2008). I-nvitro Bioequivalence Study of Nine Brands of Artesunate Tablets Marketed in NiTghereia.

Journal of Vector Borne Diseas4e5, (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., andEl-Nahhas, T.M (2012). New Analytical Method for the Determination of Metronidazole in Human Plasma: Application to Bioequivalence StuTdroyp. ical 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., anSdhivsharan(2012). Development and Validation of a RP-HPLC Method for Simultaneous Estimation of Metronidazole and Norfloxacin in Bulk and Tablet Dosage Form. ThIenternational Journal of Pharmacy and Pharmaceutical Science4s(,4), 241-245.

Harvey, D. (2000). Spectsrocopic Methods of Analysis. In Modern Analytical Chemistry (pp 409). United States: McGra-Hwill Higher Education.

Hold, K.M., Douwe de Boer, J. Z., anMdaes, 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., andBrown, S.A. (2008). In vitro Prediction of in vivo Bioavailabilityand Bioequivalence of Brands of Metronidazole Tablets in Eastern Nigerian Drug Market. TShceience Research and Essa3y(s1,1), 552-558. Available at<http://www.academicjournals.org/SRE>

International Phamr acopoeia (2003). Tests and General Requirements for Dosage Forms, Quality Specifications for Pharmaceutical Substances and TabWleotsrl.d Health Organization, Geneva, Vol. 5 PP. 14-1784.

Kishore, K.A., andAmareshwar, P. (2012). Quality Evaluation and Cpoarmative Study on Tablet Formulations of different Pharmaceutical Companies. JTohuernal of Current Chemical and Pharmaceutical Scien,c2e(s1), 24-32.

Kolawole, J.A., andAmeh, I.U. (2004). Chronopharmacokinetics of Metronidazole in Healthy Human Volunteesr. The Journal of Pharmacy and Bioresources, 1(1-)3, 42.9

Kumar, M.S., Gupta, B.K., andGhosal, S.K. (2000). Assessment of Bioavailability of

Experimental Control Release Microcapsules of NifedipiTnhee. Journal of Drug

Research7, (3), 175-180.

Mbah, C.C.,Emosairue, C.O. Builders, P.F.s,imI i, C.Y., andKunle, O.O. (2012). Effect of Process Parameters on the Properties of some Metronidazole Tablet and Capsule Formulations.TheAfrican Journal of Pharmacy and Pharmacolo, g6y(24), 1719-1725.

Available at<http://www.academicjournals.org/AJPP>

Mishra, A.K., Yadava, R., Mishra, A., Verma, A., anCdhattopadhyay, P. (2010). Development and Validation of UV Spectrophotometric Method for the Determination of Metronidazole in Tablet Formulation. ThIneternational Journal of Pharmacy Research and Developmen2t,(6), 1-5. Available at[www.ijprd.com](http://www.ijprd.com/)

Mohammed, F.A., Arunachalam, A., Venkatarami, .R, .PGallavi, V., Moulali, S.K., andRama

Raju, T.V.T. (2012). Formulation and Evaluation of Carbamazepine Extended Release Tablets USP 200mg. Thienternational Journal of Biology and Pharmacy Research; 3(1), 145-153.

Moses, P., Subramanian, LP.,alanichamy, S., Jeganath, S., aTnhdirupathi, A.T (2010).

Formulation and Evaluation of Ciproflouxacine controlled release Matrix Tablets.

Scholars Research Libra,ry 2(2), 237-243. Retrieved from

<http://www.scholarsresearchlibrary.comc/hairve.html>

Mullaicharam, A.R., Ahmed, J..,J and Halligudi, N. (2012). Evaluation of Pharmaceutical Equivalence of different Brands of Ranitidine Tablets from Multinational in O. man

International Journal of Nutrition, Pharmacology and Neurological Dise,a2se(1s), 40-

44. Available at[http://www.ijnpnd.com.](http://www.ijnpnd.com/)

Musa, H., Sule, Y.Z., anGd warzo, M.S. (2011). Assessment of Physicochemical Properties of Metronidazole Tablets Marketed in Zaria, Nigeria. TIhneternational Journal of Pharmacy and Pharmaceutical Scienc3e(s3,), 27-29.

Mustapha, K.B., Odunola, M.T., Garba, M., anOdbodozie, O. (2006). Rapid, C-oEsftfective Liquid Chromatograghic Method for the Determination of Metronidazole in Biological

Fluids. The African Journal of Biotechnolog,y 5(13), 1188-1190. Available

<http://www.academicjournals.org/AJB>

Nascimento,G.N.L., Rosa, D., Nishijo, H., anAdversi-Ferreira, T.A. (2011). Validation of a Spectrophotometric Method dtoetermine Ciprofibrate Content in Tablets. TBhreazilian Journal of Pharmaceutical Science4s7,(1), 23-29.

Ngwuluka, N. C,. Lawal, K., Olorunfemi, P.O., anOdchekpe, N.A. (2009). Po-smt arket In-vitro Bioequivalence Study of Six Brands of Ciprofloxacin Teatsb/lcaplets in Jos, Nigeria. The Scientific Research and Ess, a4y(4), 298-305. Accessed June 18, 2012 from <http://www.academicjournals.org/SRE>

Ogah, C.O., Falade, O.M., anEdrorini, O.C. (2002). Quality foChloroquine Phosphate and Paracetamol Tablets Preparations in Lagos, Nigeria. WTheest African Journal of pharmacy. 20(1), 58-63.

Olaniyi, A.A. (2005). Chemical and Physicochemical Methods. In Principles of Quality assurance and Pharmaceutical Analypspis 1(37-206). 5 Oleware Obasa street Ibadan: Mosuro publishers.

Omeje, E.O., Nwodo, N., anUd zochukwu, I. (2007). Quality Control Assessment and the Possibility of Interchange Ability Between Multisourced Norfloxacine Tablets Marketed in Nigeria.The Scienitfic Research and Essay2.(8), 348-352. Available online at<http://www.academicjournal.org/SRE>

Pahkal , E.R., Koppel, T., Saag, M., anPdahkla, R. (2005). Metronidazole Concentration in Plasma, Saliva andePriodontal Pockets in Patients with PeriodontiTtihs.e Journal of Clinical Periodonto,l 32(1), 163-166.

Phillips, M.A. and Stanley, S.L. (2006). Chemotherapy of Protozoal Infections: Amebiasis,

Giardiasis, Trichomoniasis, Trypanosomiasis, Leishmaniasisd, oatnher 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 Associationof Clinical Chemistry

Ralph, E.D., Clarke, J.T., Libke, .DR., Luthy, R.P., and Kirby, W.M.M (1974)

Pharmacokinetics of Metronidazole as determined by BioasTshaey. Antimicrobial Assay and Chemotheraph6y(,6), 601-696.

Rediguieri, C.F., Porta, V., Nunes.,GS., Nunes, TM. ., Junginger, H.E., Kopp, S. anBdarends,

D.M. (2011). Biowaiver manographs for immediate release Solid Oral Dosage Forms: Metronidazole. TheJournal of Pharmaceutical Scienc; e1s00(5), 1618-1627.

Remington’s Pharmaceutical Sciences (19T75h)e. Science of Pharmaceutical dosage forms (ed 15). Easton, Pennsylvania, U.S.A.: Mack Publishing Company.

Martindale, (1999). Monograph on Metronidazole.RInoyal Pharmaceutical Society of Great Britain (32 Ed)the Complete Drug Referenc(PeP 585-588). Great Britain, England: Pharmaceutical press.

Martindale(2007). Monograph on Metronidazole. InR: oyal Pharmaceutical Society of Great Britain, the Complete Drug Referen. ceCD ROM. Great Britain, England: 6Pharmaceutical Press.

Samuelson, J. (1999). Why Mroent idazole is active against both Bacteria and Parasites.

Antimicrobial Agents’ Chemotherapy, 43(7), 1533-1541. Retrieved from <http://aac.asm.org/content/43/7/1533#-lrisetf-1>

Sanof-i avenits (2012). Product monograph: Flagyl. Sano-afiventis(Submission No. 155394).

West Laval Quebec, Canada: Sa-naovfei ntis.

Schmauster, B. (2010). Analytical method development. (Training workshop Pharmaceutical development with focus on paediatric formulantsi)o. Beijing: World Health Organisation. Available at <http://www.who.int/prequal/trainingresources/pqores/workshop_2ch0i1n0a/english/23/0>

06-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.

Sundaraganaphayt, R., Jambulingam, M., Ananda, T.San.,dSubasini, U. (2011). Development and validation of UV Spectrophotometric Method for the Determination of Venlafaxine Hydrochloride in Bulk and Solid Dosage Forms. ThIneternational Journal of Pharmaceutical and Idnustrial Research1, (1), 28-31.

Thiessen, J.J. (2000B).ioavailability and Bioequivalenc. e(Doctorate Thesis, Leslie Dan Faculty

of Pharmacy, University of Taranto, Taranto, Ontario M5S2S2 Canada). Retrieved from [www.iuphar.org](http://www.iuphar.org/)

Turgut, E.H., andOzyazici, M. (2004). Bioavailability file: Metronidazole. ThFeABAD Journal of Pharmaceutical Scienc2e9, (1), 39-49.

Ullah, A., Azad, M.A., Sultana, R., Karb, iE.R., Mahbub-Latif, A.H.M., and Abul, H. (2009).

Pharmacokinetics Sudt y of Amoxicilline Capsules in Healthy Bangladeshi Subjects

using urinary excretion datTa.heDhaka University Journal of Pharmaceutical sciences, 8(1), 53-59.

Usman, M., Ashiq, ,I. Ashraf, M.O., Khokhar, M.I., andSaeed-ul-Hassan, S. (2011).

ComparativePharmacokinetics of Metronidazole in Healthy Volunteers and in Patients Suffering from AmoebiasisT. hePakistan Journal of Pharmacy2;4 (1 & 2), 41-46.

Usman, M.A., Sule, M.I., Ahmadu, A.A.a, nd Ojochenemi, D.A. (2007). Bioequivalence evaluation of two barnds of Metronidazole Tablets in Healthy Human VolunteTehrse.

Nigerian Journal of Pharmaceutical Science6s(2; ), 55-58.

World Health Organisation. (2011M). odel list of Essential Medicines(R. eport No. EDL17). Geneva: World Health Organisation. <http://www.who.int/medicines/publications/essentialmedicines/en/index.html>

World Health Organisation. (2011R).evision of Monograph on Tablets: Final text for Addition to the International Pharmacopoeia(.Report No. QAS/09. 324/final). Geneva: World Health Organisation. Retrieved frohmttp:/[/w](http://www.who.int/entity/../Tabs-GeneralMono-)w[w.who.int/entity/.**.**/Tabs-GeneralMono-](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 Interchange,ab(Rilietyport No.

QAS/04.093/Rev.4). Geneva:World Health Organiastion. Retrieved from http://www.int/medicines/areas/quality\_safety/quality\_asnscuer/amultisource/pharmaPr

oductsGuidelinesRegistraionRequirements/InterchageabilityTRS937Annex7.pdf

World Health Organization (2006). Additional guidance for organizations perform-invigvoin

bioequivalence studies. (Technical Report No. 937). GeneWvao:rld Health Organisation. Retrieved from <http://www.who.int/medicines/areas/quality_safety/quality_assuer/aAndcditionalGuidan>

ceOrganisation/performanceBioequivalence/StudiesTRS937

Wrasse-