EFFECTS OF ENVIRONMENTAL EXPOSURE ON THE PHARMACOKINETICS OF CIPROFLOXACIN TABLET IN HEALTHY HUMAN VOLUNTEERS

BY

ILIYA BUBA

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

AUGUST, 2016

EFFECTS OF ENVIROMENTAL EXPOSURE ON THE PHARMACOKINETICS OF CIPROFLOXACIN TABLET IN HEALTHY HUMAN VOLUNTEERS

By

Iliya BUBA, B.Pharm (ABU) 1994
(P13PHPG8007)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A
MASTER OF SCIENCE DEGREE IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
FACULTY OF PHARMACEUTICAL SCIENCES
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

AUGUST, 2016

DECLARATION

I declare that the work in this dissertation entitled 'Effects of environmental exposure on the pharmacokinetics of ciprofloxacin tablet in healthy human volunteers' has been carried out by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

BUBA Iliya _____ _____

Signature Date

CERTIFICATION

This dissertation titled EFFECTS OF ENVIRONMENTAL EXPOSURE ON THE PHARMACOKINETICS OF CIPROFLOXACIN TABLET IN HEALTHY HUMAN VOLUNTEERS by ILIYA BUBA meets the regulations governing the award of the degree of Master of Science degree in Pharmacology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. A.U Zezi

Signature _____ Date _____

Chairman, Supervisory Committee

Dr J. Ya'u

Signature _____ Date _____

Member, Supervisory Committee

Dr M.A Usman

Signature _____ Date _____

Member, Supervisory Committee

Prof. N.M Danjuma

Signature _____ Date _____

Head of Department

Prof. K. Bala

Signature _____ Date _____

Dean, School of Postgraduate Studies

ACKNOWLEDGEMENT

I wish to first of all appreciate God Almighty for giving me the grace and ability to accomplish this project.

I am highly indebted to my team of supervisors: Prof. A. U. Zezi, Dr. J. Ya'u and Dr M.A Usman for their time, valuable contributions, constructive criticism, encouragement and understanding to ensure the successful completion of this project.

I wish to acknowledge and appreciate the Dean of the Faculty of Pharmaceutical Sciences, Prof. U. Pateh for the support, care and encouragement throughout the period of this study.

I wish to sincerely appreciate the Head of Department of Pharmacology and Therapeutics, and the Head of Department of Pharmaceutical and Medicinal Chemistry for their encouragement, cooperation and understanding throughout the course of this work.

I wish to appreciate the Chief Medical Director Dr. A. Saidu and the entire management team of Federal Teaching Hospital, Gombe for the sponsorship, support and encouragement.

I wish to also appreciate and thank the Director of Pharmaceutical Services Pharm. Marafa A. P and the entire staff of the Pharmacy Department of Federal Teaching Hospital, Gombe for their love, co-operation and encouragement throughout the period of my study.

My warmest regards goes to my wife and my children for their prayers, patience and perseverance and all the inconvenience they had to pass through during the period of my study. I love you all.

I wish to thank all my lecturers, technologists and all the staff of the Department of Pharmacology and Therapeutics, Pharmaceutical and Medicinal Chemistry and Pharmaceutics and Pharmaceutical microbiology for their contributions to ensure the success of this work. I wish to particularly appreciate Mal. Umar U. U. of the Department of Pharmaceutical and Medicinal Chemistry for his time, perseverance and assistance. Also, I wish to thank my nephew Mr G. J. Atuman for the type settings, drawing and the referencing.

I also wish to thank and appreciate Dr, O. Kehinde, Mal. M. Mahdi and Mal. S. Auwal for their useful advice and guidance throughout the period of this work.

Finally, I wish to thank all my classmates, course mates and friends particularly Pharm. Rege, Mike, Mustapha (Class representative) and many others who made my study period and stay in Zaria interesting and exciting. I love you all.

DEDICATION

I wish to dedicate this dissertation to my late dad Mal. Buba Umaru and late mom Xwari Buba for their foresight and sacrifice to ensure I got a university degree.

ABSTRACT

Ciprofloxacin is a flouroquinololone antibiotic that is commonly prescribed empirically in Nigeria due to its broad spectrum of activity. However, the drug product is illegally handled by drug hawkers that allow the drug to be exposed to some environmental forces which may affect the drug quality and its pharmacokinetic profiles. This study is aimed at assessing pharmacokinetic profiles of exposed samples of ciprofloxacin marketed by hawkers in the three senatorial areas of Gombe State. Sample A represents non-exposed and control while samples B, C and D represent exposure to different environmental conditions in Gombe state for three months. The *in vitro* quality control of the drug sample was carried out using 2002 and 2009 B.P standards. The parameters determined were identification, assay, disintegration, dissolution and friability test. The method used was adopted and validated by U.V spectrophotometry and a wavelength (λmax) of 271 nm was measured which served as our working wavelength. The validation parameters used were: Precision (within day and between days), percentage extraction recovery and linearity. The linearity of the calibration curve was determined. In the pharmacokinetics studies, six apparently healthy volunteers were enrolled and were administered with 500 mg of ciprofloxacin each with non-exposed and the exposed samples of the ciprofloxacin and saliva samples were collected before and after administration with wash out period of one week intervals between studies. Pharmacokinetic parameters generated were: C_{max}, T_{max}, AUC_0-∞, lag time, $t_{1/2\alpha}$, $t_{1/2\beta}$, $K\alpha$, $K\beta$, Vd, Cl and were compared at $P \leq 0.05$ between the sample A and samples B, C, and D respectively. The results indicated that all the samples showed positive to identification test. Friability and disintegration values were within the acceptable limits ($\leq 1\%$ and ≤ 30 min respectively). The dissolution and assay parameters of the exposed sample D were 66% and 82.9% which were less than the accepted limits of $\geq 70\%$ and

95-105% respectively indicating low quality compared to others. The within day and between day precision were 1.1 and 1.5 % RSD respectively and both were within the acceptable limit of ≤ 2%. The percentage extraction recovery was 98.8% which was within the acceptable range of 95-105%. The calibration curve that was constructed was found to be linear within 1-6 μg/ml with a correlation coefficient of 0.998. When sample A was compared with each of sample B, C, and D, there was no significant difference except between sample A and D which showed a significant ($p \leq 0.05$) change in elimination half life ($t_{1/2\beta}$), 3.03 h for A and 1.63 h for D; and elimination rate constant ($K\beta$), 0.28 h^{-1} for A, and 0.638 h^{-1} for D. Elimination half life and elimination rate constant are parameters that determine how drugs are removed from the body. Shorter half life shown for sample D means the exposed drug will be easily removed from the body. This may give a sub-therapeutic drug level and loss of antibacterial activity. It can be concluded that the environmental conditions of the senatorial area where ciprofloxacin tablet sample D was exposed affected its assay, dissolution, elimination half life and elimination rate constant profiles thereby affecting its quality and pharmacokinetics.

TABLE OF CONTENTS

Title page……..……..i

Declaration ii

Certification.....iii

Acknowledgement.....iv

Dedication v

Abstract vi

Table of contents……...…viii

List of tables xii

List of figures xiii

Abbreviations…….xiv

List of appendices.....xv

CHAPTER ONE

INTRODUCTION.....1

1.1 Background 1

1.2 Statement of Research Problem 4

1.3 Justification 4

1.4 Aim of the Study 5

1.5 Specific objectives of the study.....5

1.6 Research Hypothesis 6

CHAPTER TWO

LITERATURE REVIEW.....7

2.1 Introduction 7

2.2 Order of Kinetics.....8

2.2.1 Linear pharmacokinetics 8

2.2.2 Non-linear pharmacokinetics 9

2.3 Pharmacokinetic Modeling 9
2.3.1 Compartmental modeling 10
2.3.2 Non compartmental modeling 12
2.3.3 Physiological modeling 12
2.4 Oral Drug Absorption 12
2.4.1 Physiological factors 13
2.4.2 Physical factors affecting oral drug absorption 15
2.5 Ciprofloxacin 18
2.5.1 Description 18
2.5.2 Mechanism of action 19
2.5.3 Development of resistance 20
2.5.4 Antibacterial activity 21
2.5.5 Pharmacokinetics 21
2.5.6 Drug interactions 28
2.5.7 Clinical uses 29
2.5.8 Adverse drug reactions 30
2.6 Saliva as an analytical tool 30
2.6.1 Collection and analysis of saliva 32
2.6.2 Mechanism of drug transfer to saliva 33
2.7 Effects of Environmental Exposure on the Stability and Quality of Pharmaceutical Products 35
2.7.1 Heat (temperature) 35
2.7.2 Humidity (moisture) 36
2.7.3 Light 36
2.7.4 Air (Oxygen) 37
2.8 Stability Testing of Pharmaceutical Products 39
2.9 Application of Ultraviolet-visible (UV/Vis) Spectroscopy 40

2.9.1 Beer Lamber's Law 40
2.10 In-Vitro Quality Control of Tablet (Evaluation Tests) 42
2.10.1 General appearance 43
2.10.2 Tablet hardness (crushing strengths) test 43
2.10.3 Friability tests 44
2.10.4 Disintegration tests 44
2.10.5 Dissolution test 44
2.10.6 Percentage content 45
2.11 Analytical Method Development and Validation 45
2.11.1 Types of analytical procedures to be validated 46

CHAPTER THREE

MATERIALS AND METHODS 51

3.1 Materials 51
3.2 Methods 52
3.2.1 Study area 52
3.2.2 Sampling method 53
3.2.3 Protocol/ Study design 54
3.2.4 Extraction method and Saliva drug concentration analysis 55
3.2.5 Generation of pharmacokinetic parameters 56
3.2.6 Data analysis 57
3.2.7 Analytical method Development 57
3.2.8 Validation of the adopted analytical method 58
3.2.9 In-vitro quality control tests 60

CHAPTER FOUR

RESULTS 64
4.1 In vivo Pharmacokinetic Results 64

4.1.1 Mean saliva concentration-time profiles of ciprofloxacin tablet samples A, B, C and D 64

4.1.2 Mean pharmacokinetic parameters of ciprofloxacin tablets. 65

4.2 Method Development and Validation of the Analytical Methods 71

4.2.1 Development of the analytical methods 71

4.2.2 Validation of the analytical method 74

4.3 In-Vitro Quality Control 77

4.3.1 Package labeling of the ciprofloxacin tablet 77

4.3.2 Identification test for ciprofloxacin hydrochloride powder and tablet samples A, B, C and D 77

4.3.3 Percentage friability test for ciprofloxacin tablet 78

4.3.4 Disintegration time (Min) for ciprofloxacin tablet 79

4.3.5 Dissolution test for ciprofloxacin tablet 80

4.3.6 Assay of the non-exposed and the exposed samples of ciprofloxacin tablet 81

CHAPTER FIVE

DISCUSSION 82

CHAPTER SIX

CONCLUSION, SUMMARY AND RECOMMENDATIONS 90

6.1 Summary 90

6.2 Conclusion 91

6.3 Recommendation 91

6.4 Contributions to knowledge 91

REFFERENCE 92

LIST OF TABLES

Table 2.1 Potential adverse effects of instability in pharmaceutical products 39

Table 4.1 Mean pharmacokinetic parameters of Sample A, B, C, and D of ciprofloxacin tablet……..……...69

Table 4.2 Solubility of ciprofloxacin standard powder in various solvents 72

Table 4.3 Within-days and between-day precision of 4 µg/ml ciprofloxacin hydrochloride solution…….…….……74

Table 4.4 Accuracy and percentage recovery of standard Ciprofloxacin hydrochloride powder spiked in blank saliva…..….….……75

Table 4.5 Package labeling of the ciprofloxacin tablet 77

Table 4.6 Percentage friability test for ciprofloxacin tablet.....78

Table 4.7 Mean disintegration time for the non-exposed and exposed samples (Min) of ciprofloxacin tablet……...……...……...79

Table 4.8 Dissolution rate of ciprofloxacin tablet in 0.1N HCl at 45 min 80

Table 4.9 Assay of the non-exposed and the exposed sample of ciprofloxacin tablet...81

LIST OF FIGURES

Figure 2.1 One-compartment open model.....11

Figure 2.2 Two compartmental open model 12

Figure 2.3 Step by step processes of drug absorption 15

Figure 2.4 Model to describe the dissolution of drug from a spherical particle 18

Figure 2.5 Chemical structure of Ciprofloxacin.....19

Figure 2.6 An incident light passing through a solution of organic compound.....41

Figure 3.1 Sample administration and collection protocol.....55

Figure 4.1 Super-imposed mean salivary concentration-time graphs of all the ciproprofloxacin samples (A, B, C and D)…….……...68

Figure 4.2 Semi-log plot of mean concentration-time profile of non-exposed ciprofloxacin tablet (sample A)…….…….……...69

Figure 4.3 Spectrum of 4 µg/ml solution of ciprofloxacin hydrochloride standard powder at 200-400 nm UV/VIS range.....73

Figure 4.4 Calibration curve of ciprofloxacin hydrochloride.....76

ABBREVIATIONS

ACRONYMS	DEFINITIONS
ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredients
AUC	Area under the concentration-time curve
BP	British Pharmacopoeia
BCS	Biopharmaceutics classification system
BMI	Body mass index
Cmax	Maximum plasma concentration
DNA	Deoxy ribonucleic acid
FDA	United States Food and Drug Administration
GMP	Good manufacturing practice
GIT	Gastro-intestinal tract
ICH	International conference on harmonization
LGA	Local Government Area
MIC	Minimum inhibitory concentration
NAFDAC	National agency for food and drug administration and control
NIMET	Nigeria meteorological agency
Rpm	Revolution per minute
SAR	Structure and activity relationships
SEM	Standard error of the mean
UV/VIS	Ultraviolet visible radiation
USP	United State Pharmacopoeia
λ_{max}	Maximum wavelength

LIST OF APPENDICES

Appendix I Friability and Disintegration test I

Appendix II Dissolution and Assay test II

Appendix III Precision III

Appendix IV Saliva Concentration-time profile of drug sample A for volunteer 2 and 6 IV

Appendix V Saliva Concentration-time profile of drug sample D for Volunteer 5and 6 V

Appendix VI Concentration-time curve of drug sample A for volunteer 2 and 6..... VI

Appendix VII Concentration-time curves of drug sample D for volunteer 5 and 6..... VII

Appendix VIII Equations for % Friability, Beer Lambert's Law, % Extraction recovery……..…….……..VIII

Appendix IX Equations for calculating pharmacokinetic parameters……..……IX

Appendix X Equations for Area Under the concentration-time curve (AUC)…...…….X

Appendix XI Gombe State Meteorological Data (2015)……...…XI

Appendix XII Biodata of subjects used for the study……..……XII

Appendix XIII Henderson-Dasselbalch equations…….….XIII

Appendix XIV Arrhenius equations……..... XIV

Appendix XV Concentration and absorbance for calibration curve of the ciprofloxacin hydrochloride……...…XV

Appendix XVI Summary of the validation parameters…….…..XVI

Appendix XVII Mean (± SEM) saliva concentration –time profile of ciprofloxacin tablet samples A, B, C and D (µg/ml)……. ..XVII

Appendix XVIII Mean concentration-time curve of non-exposed ciprofloxacin tablet (sample A)…….....XVIII

Appendix XIX Mean concentration-time curve of ciprofloxacin tablet exposed to Gombe North (sample B)…..……..…..…..……..XIX

Appendix XX Mean concentration-time curve of exposed ciprofloxacin tablet to Gombe central (Sample C)……..……..…..…XX

Appendix XXI Mean concentration-time curve of exposed ciprofloxacin tablet to Gombe south (Sample D)…..……...….XXI

Appendix XXII Consent form……..……..…XXII

CHAPTER ONE

INTRODUCTION

1.1 Background

The safety and efficacy of drug products can be guaranteed when their quality is reliable and reproducible from batch to batch. In order to maintain quality, drug manufacturers are expected to test their products during and after manufacturing and at various intervals during the shelf life of the product. The quality of medicines is an integral part of access in light of ensuring that the pharmaceutical products are fit for their intended use, comply with the requirement of the marketing authorization and do not expose consumers to risks (Jackson *et al.*, 2011). A drug product is the finished dosage form (e.g tablet, capsule, injectable) that contains the active pharmaceutical ingredients (API) which in most cases is in association with other inert ingredients also called excipients. (Shargel *et al.*, 2010).

For a product to be of good quality it has to conform to standard requirements as presented in official (national and international) monographs. The monograph contains laid down procedures for the production of specific items and they also contain details on expected quality of such items. For instance, tablets of the same batch should have the following physico-chemical properties: uniform weight, maximum weight variation not to exceed ±5%, uniform and consistent active ingredient, withstand handling stresses but not too hard for disintegration in the gut and release not less than 70% of the active ingredient into dissolution medium within 45 min (Yabo, 1996; Ogar *et al*., 2015). In practice even when all batches of tablet product conform to these standards,

they might not be kept in a good storage condition from the point of manufacture to the point of dispensing to patients. During transit between the two points, drugs are exposed to different environmental conditions. Drug products (e.g tablets) when exposed to environmental forces like light, heat, humidity and stress, their physicochemical properties change (Bajaj *et al.*, 2012). Physicochemical properties determine stability of the drug, its solubility, membrane permeability, and drug affinity to different tissue components (Hedaya, 2007). These changes may ultimately affect the pharmacokinetic profile of the drug product (Yabo, 1996). This is particularly critical with antibiotics whose bactericidal activity is concentration-dependent like ciprofloxacin (Jacobs, 2001).

When pharmaceutical dosage forms are administered, the active ingredient must reach its site of action before the pharmacological effect of the drug is exerted. If the drug is not introduced directly into the intravascular system, then it must be absorbed. This process of absorption is then followed by distribution through the blood plasma and different body fluids, to various tissues and organs of the body, including the site of drug action. The drug is finally distributed to organs that eliminate them through the process of metabolism and excretion which also determine its fate and duration of pharmacological activity (Ogunbona *et al.*, 2014). The study and characterization of the time course of drug absorption, distribution, metabolism and excretion, and with the relationship of these processes to the intensity and time course of therapeutic and adverse effects of drugs is called pharmacokinetics (Gibaldi and Levy, 1976).

Pharmacokinetic processes involve the transfer of drug across biologic membranes through various mechanisms such as: passive diffusion, active transport, pore transport, facilitated diffusion and transport by vesicles. Therefore, each pharmacokinetic process

may be associated with one or more parameters that are dependent on the drug, drug product and the patient.

Ciprofloxacin is a flouroquinolones which act by inhibiting bacterial DNA synthesis. It also exhibits a rapid onset of action, and lacks cross-reactivity with penicillin, cephalosporins and the aminoglycosides (Uduma *et al.*, 2011). Two mechanisms have been proposed for its action. The first is through the inhibition of bacterial topoisomerase II (DNA gyrase) and the second is by inhibiting topoisomerase IV (Katzung *et al.*, 2009). Ciprofloxacin displays *in vitro* activity against most gram-negative and many gram positive pathogenic bacteria, many of which are resistant to a wide range of antibiotics and this is of considerable potential clinical significance (Vance-Bryan *et al.*, 1990). Various side effects have been observed due to therapy with ciprofloxacin such as effects on the central nervous system and the gastro-intestinal tract as well as photosensitivity reactions of the skin. (Tiefenbacher *et al*., 1994). Absorption of ciprofloxacin after oral administration is rapid with absolute bioavailability of approximately 70%. Ciprofloxacin has shown good penetration and accumulation in tissues with a wide distribution throughout the body. Four metabolites of ciprofloxacin have been identified in the body fluid as desethylene-ciprofloxacin, sulpho-ciprofloxacin, oxo-ciprofloxacin and formayl ciprofloxacin. The terminal disposition half-life is about 3 to 4 hr. Glomerular filtration and tubular secretion account for approximately 66% of the total serum clearance. (Khan *et al.*, 2009).

1.2 Statement of Research Problem

In Nigeria, there are several brands of ciprofloxacin tablet available in the market and due to its brought spectrum of activity, it is prescribed empirically by physicians for the treatment of infectious diseases (Adegbolagun *et al.,* 2007; Ngwuluka *et al.,* 2009). Massive importation of the drug into the country that already has a chaotic drug distribution system has led to the drug being handled by non-professionals (Ogar *et al*., 2015). As such, the drug is not properly stored and is exposed to all kind of environmental forces like sunlight, heat, humidity and microbial contamination (Ehikwe *et al.,* 2015). Exposure to environmental factors may lead to deterioration of the Active Pharmaceutical Ingredient and the physicochemical properties of the dosage form (Bajaj *et al.,* 2012). These changes may ultimately affect the pharmacokinetic profile of the drug product (Yabo, 1996). Ciprofloxacin has a concentration-dependent pattern of bacterial killing and hence its efficacy will be affected which could further lead to a wide spread development of drug resistance. It could even lead to the formation of toxic principles (Bajaj *et al.*, 2012).

Currently there are fewer studies on the effects of environmental factors on ciprofloxacin tablet pharmacokinetics in Nigeria.

1.3 Justification

Drug distribution network in some West African countries including Nigeria consists of chaotic open markets which act as major sources for procurement which includes: medicine stores, pharmacy outlets, private and public hospitals, wholesalers/retailers and local pharmaceutical manufacturers, agents or representatives of foreign suppliers (Ogar *et al*., 2015). The result of this chaotic drug distribution makes drug monitoring very difficult. In addition, it gives room to drug hawking in buses, kiosks, by illiterate

vendors whose aims are solely profit oriented. The medicines are left under conditions that may facilitate their deterioration (Ehikwe *et al.*, 2015; Ogar *et al*., 2015). In Gombe state, drug hawking is a common practice. The state is located in tropical region which is characterized by high temperature, adequate sunlight with sometimes high relative humidity and the climatic conditions in the three senatorial zones of the state differ slightly. Exposure of ciprofloxacin tablet to these environmental forces may change its quality, efficacy, safety and ultimately clinical outcomes. This study was to investigate the effects of environmental exposure on the pharmacokinetic profiles of ciprofloxacin tablet. Information obtained from this study can be applied by all stakeholders in the pharmaceutical industry particularly the drug manufacturers, health workers, drug regulatory agencies and other policy makers in Nigeria.

1.4 Aim of the Study

The aim of this study was to determine the effect of environmental exposure on the pharmacokinetics of ciprofloxacin tablet in healthy human volunteers.

1.5 Specific objectives of the study

I. To carry out quality control assessment of the non-exposed (reference) and exposed ciprofloxacin tablet using Pharmacopoeia standards (B.P 2002, 2009).

II. To adopt and validate a UV spectrophotometric method for analysis of ciprofloxacin in biological samples (saliva).

III. To generate the pharmacokinetic profiles of the non-exposed (reference) with the exposed ciprofloxacin tablet samples from saliva concentrations.

IV. To compare the pharmacokinetic profiles of the non-exposed (reference) with the exposed ciprofloxacin tablet samples from saliva concentrations

1.6 Research Hypothesis

Environmental exposure will significantly affect the pharmacokinetic profiles of ciprofloxacin tablet.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The term pharmacokinetic was first introduced by F.H. Dost in 1953 in his book *Der blutspiegel* (Wagner, 1981). Although previously some of the subject matter were published before the word was coined, the term pharmacokinetics was later defined in a number of ways (Wagner, 1981). Literally, the word means the application of kinetics to pharmakon, the Greek word for drugs and poisons. Kinetics is that branch of knowledge which involves the change of one or more variables as a function of time. The purpose of pharmacokinetics is to study the time course of drug and metabolites concentrations or amount in biological fluids, tissues and excreta, and also of pharmacological response, and to construct suitable models to interpret such data. In pharmacokinetics, the data are analyzed using a mathematical representation of a part or the whole of an organism. Broadly then, the purpose of pharmacokinetics is to reduce data to a number of meaningful parameter values, and to use the reduced data to predict either the results of future experiments or the results of a host of studies which would be too costly and time-consuming to complete (Wagner, 1981). Gibaldi and Levy in 1976 defined pharmacokinetics as follows: "Pharmacokinetics is concerned with the study and characterization of the time course of drug absorption, distribution, metabolism and excretion, and with the relationship of these processes to the intensity and time course of therapeutic and adverse effects of drugs. It involves the application of mathematical and biochemical techniques in a physiologic and pharmacologic context (Wagner, 1981).

It is important to study pharmacokinetic processes because the onset, intensity and duration of drug action depend on them. Faster drug absorption leads to faster onset of drug effect which is critical in treatment of acute condition and in emergency situations. Also, not all the dose of drug administered through extravascular routes reaches systemic circulation. Extent of drug absorption determines the amount of drug in the body and its intensity of action. Studying the drug distribution is necessary because the drug has to be taken to the site of action to elicit its effect. Also studying the rate of drug elimination is important so as to know the frequency of drug administration. Drugs that are eliminated faster are administered more frequently so as to maintain an effective drug concentrations at all times during multiple drug administrations. Studying the organs responsible for drug elimination is also critical because patients with organ dysfunctions require dosage adjustments. As a result, it is critical to know the factors that can affect the pharmacokinetic process of each drug (Hedaya, 2007).

2.2 Order of Kinetics

2.2.1 Linear pharmacokinetics

Linear Pharmacokinetics is also known as dose-independent or concentration independent pharmacokinetics. Parameters such as half-life, total body clearance, and volume of distribution are constant and do not depend on the drug concentration or the amount of the drug in the body. It is also called first order processes. Therefore, when there is a change in drug dose it results in a proportionate change in the drug concentration-time profile in the body (Dhillon and Gill, 2006; Hedaya, 2007).

2.2.2 Non-linear pharmacokinetics

Non-linear Pharmacokinetics is also known as capacity-limited, dose dependent or saturation pharmacokinetics (Shargel *et al.*, 2010). This is because the pharmacokinetics parameters such as half-life, total body clearance and possibly volume of distribution are dependent on the drug concentration and the drug amount in the body. Here, at least one of the pharmacokinetics processes that affect the concentration-time profile inside the body is saturable and hence does not follow first order kinetics (Dhillon and Gill, 2006). When there is a change in drug dose it results in disproportionate increase or decrease in the concentration time-profile in the body (Hedaya, 2007). Thus, in non-linear pharmacokinetics, the AUC and the amount of drug excreted not proportional to the dose. Also, the elimination half-life may increase at high doses and the ratio of metabolites formed changes with increased dose. It is also known as zero order kinetics (Shargel *et al.*, 2010).

2.3 Pharmacokinetic Modeling

Pharmacokinetics data analysis is aimed at estimating the pharmacokinetic parameters that determine the rate of drug absorption, distribution and elimination. In order to achieve that, an assumption has to be made of a specific pharmacokinetics model (Hedaya, 2007). A model is a mathematical description of a biologic system and is used to express quantitative relationship (Shargel *et al.*, 2010). Pharmacokinetics models therefore allow quantitative (mathematical) description of the rate of drug absorption, distribution and elimination after administration. With modeling, it is possible to make prediction of the drug pharmacokinetic behaviour after administration of different dosing regimens and also understand pharmacokinetic behaviour caused by physiological and pathological changes (Hedaya, 2007).

2.3.1 Compartmental modeling

In this type of modeling, the body is divided into one or more compartments (Hedaya, 2007). A compartment is a group of tissues with similar blood flow and drug affinity. A compartment is not a real physiologic or anatomic region (Shargel *et al.*, 2010). This model describes the distribution of the drug between the compartments and elimination from one or more of the compartments. Different models have been established and which differ from one another in the number of compartments and the arrangement of the compartments relative to one another. Thus, there are: one-compartment, two-compartment and three-compartment models (Arthur and Atkinson, 2015; Dhillon and Gill, 2006).

2.3.1.1 One-compartment open model

This model assumes that following drug administration, the drug is instantaneously and homogeneously distributed-throughout all the tissues and body fluids for which the drug has affinity. After that, equilibrium is established between the drug in the blood and central compartment and other peripheral compartments in the body (Dhillon and Gill, 2006). Thus, the concentration of the drug in the blood or plasma is expected to be in equilibrium with concentration at the site of action of the drug. Also, it is expected that a correlation should exist between pharmacological effect and the drug plasma level especially the free drug concentration (Hedaya, 2007). This model can be illustrated in the figure 2.1.

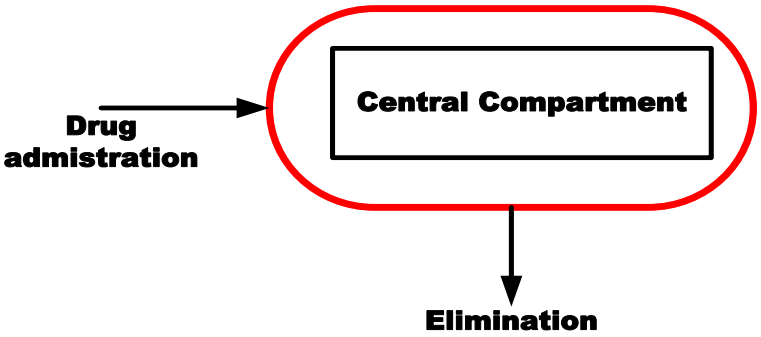


Figure 2.1: One-compartment open model (Ogunbona *et al*., 2014)

2.3.1.2 Two-compartment open model

In this model as against the one-compartment model, the distribution process requires longer time for pseudo equilibrium to be established between the drug in blood and drug in other body fluids and tissues. In other words, some drugs behave differently even when administered by a bolus dose. They do not distribute instantaneously and hence their concentration-time curve on a semi logarithm paper does not decline linearly as in one-compartment model (Dhillon and Gill, 2006). As such, an additional compartment is added to the central compartment called peripheral or tissue compartment in order to account for the drug disposition pattern of such drug (Ogunbona *et al.*, 2014). The central compartment is comprised of the blood, extracellular fluids, and highly perfused tissues like heart, brain, hepatic-portal system, kidney, endocrines glands, muscles, and bone narrow. While the second compartment (tissue or peripheral compartment) is made up of the slowly perfused tissues like the bone, ligaments, tendons, skin, adipose tissues, cartilage, teeth and hair (Ogunbona *et al.*, 2014). The two-compartment model can be illustrated in figure 2.2.

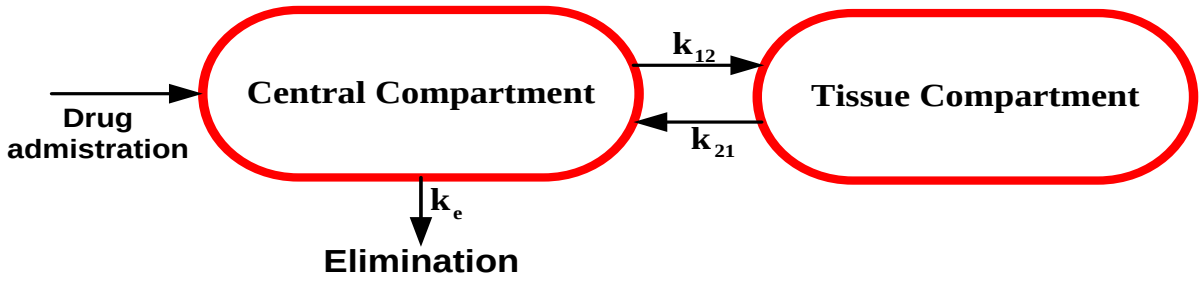


Figure 1.2: Two compartmental open model (Ogunbona *et al.*, 2014)

2.3.2 Non compartmental modeling

This model does not assume any specific model, but it uses the total body clearance, mean residence time, and the volume of distribution to describe the rate of drug disposition (Hedaya, 2007).

2.3.3 Physiological modeling

In physiological modeling, the body is divided into a series of organs or tissue spaces, and the model describes the uptake and disposition of the drug in each of these organs. Building the model depends on knowledge of the organ size, the organ blood flow, the drug uptake to each organ, and the drug elimination from different organs (Arthur and Atkinson, 2015). This model is useful because it can predict the difference in the drug pharmacokinetics in different species. It can also be used to predict the change in drug pharmacokinetics caused by physiological and pathological changes (Hedaya, 2007).

2.4 Oral Drug Absorption

The process by which a drug moves from its site of administration to the extracellular compartment of the body is known as drug absorption (Hedaya, 2007). There are different factors that affect the passage of drug from the oral site of administration to systematic circulation. These factors are as follows:

2.4.1 Physiological factors

2.4.1.1 Nature of the gastrointestinal (GIT) membrane

The biological membrane of the gastrointestinal tract (GIT) consist of a phospholipid bilayer with inter dispersed carbohydrates and integral protein groups. Also present in the membrane are small aqueous pores or channels (Hedaya, 2007). Drug molecules can cross these GIT membrane barriers through different mechanisms: passive diffusion, carrier-mediated transport, paracellular (pore) transport and transport by vesicles (Martinez and Amidon, 2002) .

2.4.1.2 Gastrointestinal physiology

The GIT segments differ from each other with respect to the anatomical structure, transit time, secretions and pH (Hedaya, 2007). When drugs are administered orally, they pass through the gastro-intestinal tract (GTI) but the three major segments of the GIT where absorption of the drug occurs includes: stomach, small intestine (duodenum, jejunum and ileum) and the large intestine (Ogunbona *et al.*, 2014). Their physiological environment and membrane surface areas available for drug absorption are different.

The stomach has a limited absorptive surface area due to lack of villi, but due to the acidic pH of gastric fluid, weak acidic drugs are well absorbed. However, the low gastric pH can cause decomposition of many acid labile drugs. Also, the gastric emptying rate determines the rate at which drug reaches the small intestine which is where most of the drugs are absorbed (Mudie *et al.*, 2010).

The small intestine is the most important site for drug absorption particularly at the duodenum. This is because the pH of the small intestine ranges from 6.0 to 6.5 in the duodenum and from 7.8 to 8.0 in the rest of small intestine. This provides a conducive environment for absorption of drugs that are not ionized in the pH (weak base). Also, this is due to the large surface area of the small intestine because of the presence of villi

and microvilli. The proximal segment of the small intestine has more surface area per unit length, more carriers-mediated transport system and aqueous pore size compared to the distal segment of small intestine. The large intestine on the other hand, does not have a large surface area as the small intestine. However, due to its long residence time, residual drug absorption due occur leading to prolonged drug absorption (Hedaya, 2007; Mudie *et al.*, 2010 ; Ayman andVarma, 2012).

Gastric emptying and gastrointestinal motility also affect oral absorption of drugs. The duration it takes from the time drug arrived at the stomach to the time it be emptied into the small intestine is called gastric emptying time (Mudie *et al.*, 2010). While the gastrointestinal motility affects the movement of drug and other substance through the GIT tract. Since the small intestine is known to be the largest site of drug absorption, therefore the rate and extent of drug absorption can be affected by change in gastric emptying time (Ogunbona *et al.*, 2014).

The level of blood flow affects the rate of removal of the drug from systemic circulation. The bioavailability of the duodenum region for instance is highly perfused with a network of capillaries and lymphatic vessels. This helps in maintaining concentration gradient between the GIT and the systematic circulation that facilitates drug absorption by passive diffusion process (Ogunbona *et al.*, 2014).

Generally speaking, food has an effect on the rate and extent (or amount) of drug absorption. How food affect drug absorption depends on the characteristics of the drug, the site of drug absorption and the type of food consumed (Hedaya, 2007). Food can also increase gastric acidity (pH) and which may increase or decrease the dissolution of the drug depending on the physic- chemical properties of the drug. Food can stimulate bile secretions which may increase solubility of lipid soluble drugs. Drug when

administered immediately before or after food, its rate of absorption is reduced due to delay in gastric emptying rate (Martinez and Amidon, 2002) .

Many diseases of the GIT modify oral drug absorption process. Gastric emptying time and gastric motility can be affected significantly by gastroenteritis and diarrhea. This can cause alteration in the absorption of prolonged released formulations. Some drug's main action or side effects may also affect drug absorption. Example anticholinergic effects or side effects of tricyclic antidepressants or phenothiazines can significantly delay the rate of drug absorption (Hedaya, 2007).

2.4.2 Physical factors affecting oral drug absorption

When drug in a solid dosage form is administered orally, it has to disintegrate first before it is dissolved and finally absorbed. This process can be simply illustrated in figure 2.3.

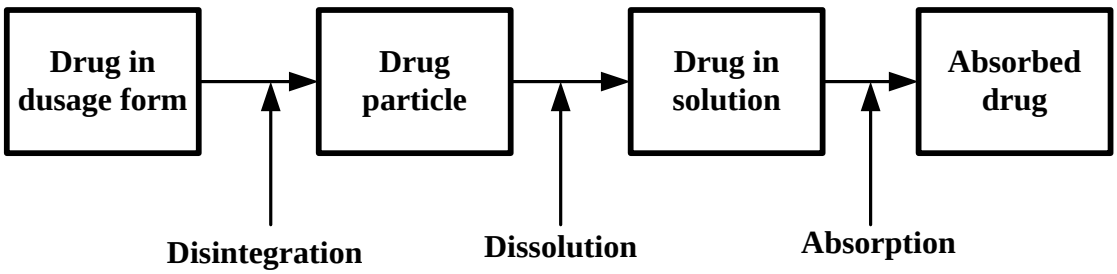


Figure 2.3 Step by step processes of drug absorption (Hedaya, 2007)

Based on the processes of absorption shown above, it follows that drug absorption process is dependent on the physicochemical properties of the drug, the solubility of the drug and the dosage form characteristics (Hedaya, 2007). In other words, the overall process consists of disintegration, dissolution and then permeation across the

membrane. Hence, the overall absorption process is determined by the slowest process called the rate limiting step. Rate of dissolution is usually slow for drugs with poor aqueous solubility and so it is the rate limiting step for the entire absorption processes of such drugs. For drugs that are highly aqueous soluble, it is the rate of permeation across the biological membrane that is slowest and hence the rate limiting step (Martinez and Amidon, 2002; Mudie *et al.*, 2010).

2.4.2.1 Drug physicochemical properties.

Drug lipid solubility: Drug has to be lipid soluble (or lipophilic) for it to cross the biological membrane which is also lipoid in nature. Partition coefficient determines the lipid solubility of a drug. Drugs also have to be hydrophilic for dissolution to occur. As such, drugs have to possess a balance between hydrophilicity to dissolve and lipophilicity to permeate the biological membrane (Hedaya, 2007).

pH partition theory: This is another factor that can control drug absorption. It is meant to describe the relationship among the drug pKa, the pH at the absorption site, and the lipid solubility of the drug. The pH of the GIT varies from 1.0 to 3.0 in the stomach and from 6.0 to 7.0 in the small intestine. Most drugs are either weak acids or weak bases as such; different segments of the GIT will have different percentage of the ionized and unionized drug. The unionize form of weak acid is more lipophilic and is absorbed more in the stomach than the ionized drug. However in the basic medium of the small intestine, weak acid drugs are more ionized, more hydrophilic and less absorbed (Hedaya, 2007). The relationship between pKa, pH and the percentage of the ionized and unionized drug is described by the Henderson-Hasselbalch equations (Appendix XIII) (Mudie *et al.*, 2010; Ayman and Varma, 2012).

2.4.2.2 Dissolution of the drug

Drugs in solid dosage forms cannot be absorbed unless they all dissolved. Different models have been proposed to describe the dissolution rate of any solid compound. The most popular model is the one described by Noyes-Whitney equation (Mudie *et al.*, 2010). This model assumed that the drug occur as a solid particles with total surface area equal to S and covered by an unstirred (stagnant) layer of the solvent. The thickness of the unstirred layer is h, while the diffusion coefficient of the drug in the stagnant layer equal D. The concentration of the drug on the surface of the solid particle represents the saturation solubility of the drug Cs. while the concentration of the drug in the bulk solvent is represented by C. They proposed that the dissolution process begin when the drug start dissolving into the solvent on the surface of the particles. The dissolved drug, then diffuse across the un-stirred layer into the bulk solution. This diffusion process is powered by the concentration gradient ($C_s - C$). As more drugs are dissolved from the surface of the particles, the more concentration of the drug in the bulk medium increases (Mudie *et al.*, 2010; Ayman andVarma, 2012).

The overall process can be described by the Noyes-Whitney equation: $\frac{dc}{dt} = \frac{DS}{h}(C_s - C)$

The model to describe the dissolution of drug from a spherical particle can be shown in figure 2.4.

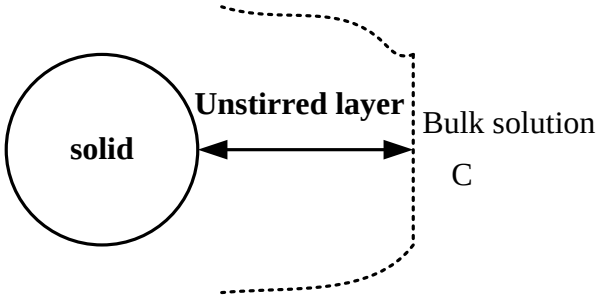


Figure 2.4 Model to describe the dissolution of drug from a spherical particle (Hedaya, 2007)

Thus, dissolution rate can then be said to be determined by four parameters stated in the equation above. These parameters are: surface area, diffusion coefficient, thickness of the unstirred layer and solubility of the drug.

2.4.2.3 Dosage form characteristics

Drugs administered in solid dosage forms like tablets and capsules will have to disintegrate to granules or small particles first before they can be dissolved and then absorbed. Disintegration rate of tablets is dependent on the tablet additives and the manufacturing procedures. Consequently, tablets are usually subjected to disintegration and dissolution tests as part of quality control after manufacturing (Hedaya, 2007).

2.5 Ciprofloxacin

Ciprofloxacin is a quinolone-carboxylic acid derivative with an extensive antibacterial spectrum of activity. It is a synthetic antibiotic of the second generation fluoroquinolone antibacterial (Uduma *et al.*, 2011).

2.5.1 Description

Ciprofloxacin is a 1-cyclopropyl-6-flouro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3 quinoline carboxylic acid. It is a faintly yellowish to light yellow crystalline substance. Ciprofloxacin hydrochloride is the monohydrochloride monohydrate salt of

ciprofloxacin. It is a faintly yellowish to light yellow crystalline substance with a molecular weight of 385.8 g/mol. Its empirical formula is $C_{17}H_{18}FN_3O_3HCl{\bullet}H_3O$ (Ali, 2014). Its melting process with decomposition is around 270°C to 385°C (Khan *et al.*, 2009).

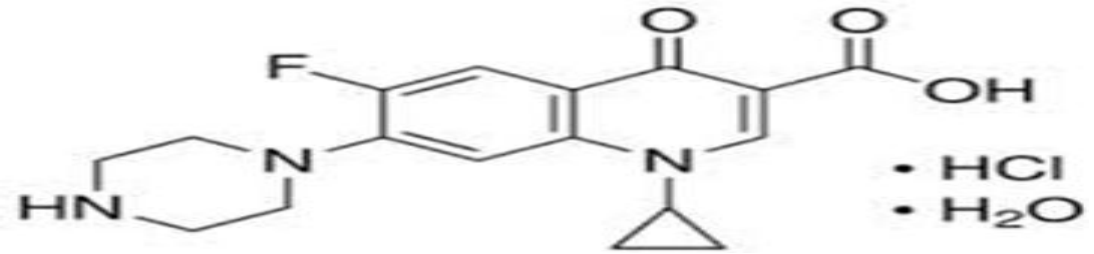


Figure 2.2 Chemical structure of Ciprofloxacin (Ali *et al.*, 2011)

2.5.2 Mechanism of action

Ciprofloxacin is a flouroquinolones antibiotic which acts by inhibiting bacterial DNA synthesis. Fluoroquinolones inhibit the replication and transcription of bacterial DNA, which eventually culminate in cell death. They either inhibit the activity of DNA gyrase, an essential adenosine triphosphate-hydrolyzing topoisomerase II enzyme or/and prevent the detachment of gyrase from DNA. The topoisomerases exert their bactericidal activity by interacting with the DNA. During the processes of replication and transcription, enzymes called helicases unwind/uncoil the DNA double helix leading to excess supercoiling of the remaining DNA double helix. A tension is created in this remaining double helix which must be relived in order to continue the process. The topoisomerase II enzyme allows the relaxation of supercoiled DNA by breaking both strands of DNA chain, crossing them over, and then resealing them. Bacterial gyrase is different enough from mammalian topoisomerase so that quinolones and fluoroquinolones show about 1000 fold selectivity towards bacteria over the corresponding enzyme in humans. Fluoroquinolones have also been found to inhibit

the *in vitro* activities of topoisomerase IV, having structure similar to DNA gyrase. This enzyme has an important role in partitioning of chromosomal DNA during bacterial cell division and may be the primary target of fluoroquinolone activity in gram positive bacteria. This mechanism is consistent with apoptosis rather than necrosis (Sharma *et al.*, 2009).

2.5.3 Development of resistance

Gram positive and gram negative bacteria have been reported to be resistant to quinolones. This resistance appears to be the result of one of the two fundamental processes: alterations in the quinolone enzymatic targets (DNA gyrase), decreased outer membrane permeability or the development of efflux mechanisms. First, by spontaneous mutations at various locations on the gyrase enzymes subunit A, which lower the affinity of the drug at the gyrase DNA complex. Mutations of subunit A are found in both gram negative and gram positive strains and involve amino acid alterations. These alterations are clustered between amino acid 67 and 106 in the amino terminus of subunit A, which is near the active binding site of the enzyme. For example, the substitution of leucine or tryptophan at the place of serine 83 is the most commonly observed alteration and causes a largest increase in resistance. Similar alterations have been seen in topoisomerase IV. Combination of both alterations results in fluoroquinolone resistance in *S. pneumonae.* Second mechanism that entails resistance to the fluoroquinolones is slow to appear, but when it appears it is mainly due to the efflux mechanism, which pumps the drug back to the cell. This is due to the mutation in the genes that code for porins, which are membrane proteins by which quinolones enter gram negative cells. These mutations raise tolerance limit of antibiotics to four folds and result in either reduced production of outer membrane proteins or stimulated cell efflux system, which lead to active drug expulsion. This type

of resistance has been described in both *E. coli* and *P. aeruginosa.* Similar evidence of enhanced quinolone efflux has been found in *S. aureus,* which lacks an outer cell membrane (Sharma *et al.*, 2009). The accumulation of several bacterial mutations (DNA gyrase and bacterial permeability) has been associated with the development of very high minimum inhibitory concentrations to ciprofloxacin in isolates of *Staphylococcus aureus, Enterobacteriaceae species and P. aeruginosa* (Soni, 2012).

2.5.4 Antibacterial activity

Ciprofloxacin is a broad spectrum antibiotic that is more sensitive to gram negative bacteria, and less effective against gram positive bacteria, including *Staphylococcus* aureus, *Streptococcus pneumoniae*, and *Enterococcus faecalis*. The drug has been shown to be active against most strains of the following micro-organisms both *in vitro* and in clinical infections: aerobic gram-positive micro-organisms: *Enterococcus faecalis, Streptococcus pneumonia, Streptococcus pyrogenes, Staphylococcus saprophyticus, Staphylococcus aureus; a*erobic gram negative micro-organisms*: Enterobacter cloacae, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia, Neisseria gonorrhoeae, Shigella sonnei* (Uduma *et al.*, 2011).

Ciprofloxacin is active against both methicillin-susceptible and resistant *Staphylococcus aureus*. *In vitro* testing has shown that this drug is rapidly bactericidal against both resting and actively growing cultures. Because its spectrum includes the most common nosocomially acquired pathogens, this agent appears promising for the empiric therapy of nosocomially acquired sepsis (Gonzalez *et al.*, 1985).

2.5.5 Pharmacokinetics

Absorption of ciprofloxacin after oral administration is rapid and can be satisfactorily described as a zero-order process; peak serum ciprofloxacin concentrations (C_{max}) are reached in approximately 1 to 2 hr. Concomitant administration of food does not cause

clinically significant impairment of absorption and may be helpful in minimizing gastric distress caused by the drug. Absolute bioavailability of ciprofloxacin is approximately 70%. A linear relationship between serum ciprofloxacin concentrations and the dose administered either orally or intravenously has been reported (Khan *et al.*, 2009). Serum concentrations increase proportionately with doses up to 100 mg (Uduma *et al.*, 2011).

The absorption of ciprofloxacin from different regions of the human gastrointestinal tract was investigated in four healthy males by Harder *et al*., (1990) using a remote-controlled drug delivery device (hf-capsule). Significant differences in AUC were observed between the control study group (oral administration of ciprofloxacin solution without the hf-capsule = 100%) and after release of ciprofloxacin in the jejunum (geometric mean: 37%), the ileum (mean: 23%), the ascending colon (mean: 7%) and the descending colon (mean: 5%), whereas time to reach maximum serum concentration (tmax) showed no difference for any of the absorption sites. Ciprofloxacin release in the stomach resulted in the greatest AUC (mean: 140%). Thus, the study concluded that the main absorption site of ciprofloxacin is the upper gastrointestinal tract, up to the jejunum.

In a study conducted in Nigeria on healthy male volunteers by Chukwuani *et al*., (2000), the peak plasma concentration was found to be 1.52 ± 0.94 mg/L; the AUC was 9.87 ± 4.10 mg. h/L and absolute bioavailability of 0.79 ± 0.49 was found after 250mg dose oral administration. When the bioavailability was corrected for clearance and elimination half life, the values reduced to 0.37 ± 0.17 and 0.31 ± 0.18 respectively. The corrected bioavailability in the study was found to be quite low which called for the reappraisal for bioavailability of ciprofloxacin in black Africans. Also in another

study by Drusano *et al*, 1986 on 12 healthy male volunteers, ciprofloxacin was found to be rapidly absorbed with absolute bioavailability of 69 ± 7 % and peak serum concentration reached in 0.71 ± 0.15 h and elimination half life of 4 .11 ± 0.74 h.

A study done by Davis *et al.,* (1986) on the pharmacokinetics of three oral formulations of ciprofloxacin on 18 healthy volunteers, they found ciprofloxacin well absorbed in all the formulations and concentrations was detected in both the serum and saliva. The mean peak serum concentration after 500mg oral dose was between 2.83 and 3.23 µg/ml and the mean saliva concentration was 25.5% of the simultaneous concentration in the serum. The reason for the low saliva level was not clear according to the study since ciprofloxacin has plasma protein binding affinity of only 20%. Also, the result showed the ratio of saliva to serum concentration varying widely within and between subjects but was greater early in the dosing interval. The possibility of un-rinsed drug left after drug administration was also ruled out since the saliva was collected directly from the parotid gland using suction device. The same study also found a statistically significant relationship between ciprofloxacin concentration in the saliva and pH.

Mallah *et al.,* (2015) studied the differences in pharmacokinetic parameters of two different tablet doses of Ciprofloxacin (750 and 1000) mg administrated to 28 healthy male volunteers in cross over design. The study revealed that the maximum absorption time (t max) was equal for both doses (3.0hr).The maximum concentrations (Cmax) were (1.93 and 2.45) µg/ml for the (750 and 1000) mg doses, respectively. Area under the curve (AUC) for 750 and 1000 mg doses were found to be (15.30 and 19.12 µg. h/ml) respectively. Variation was significantly found in the two ciprofloxacin doses (p

≤ 0.05) and the major pharmacokinetic parameters, namely C_{max} and AUC_{0-24} confirm a relative trend of bioavailability and absorption of Ciprofloxacin in human plasma.

The effects of milk and yogurt on the bioavailability of ciprofloxacin were studied by Neuvonen *et al.,* (1991) in seven healthy volunteers in a randomized crossover trial. After an overnight fast, 500 mg ciprofloxacin was given with 300 ml water, milk, or yogurt. Plasma ciprofloxacin concentrations were significantly ($p \leq 0.05$) lower during the milk and yogurt phases from 1/2 to 10 hour; at 1/2 hour the concentration was reduced by 70% by milk and by 92% by yogurt. Milk reduced the peak plasma concentration by 36% ($p \leq 0.05$) and yogurt by 47% ($p \leq 0.05$). The extent of bioavailability, measured as the total area under the plasma concentration-time curve and 24-hour urinary excretion of ciprofloxacin, was reduced by 30% to 36% by milk and yogurt ($p \leq 0.05$). The study concluded that the absorption of ciprofloxacin can be reduced by concomitant ingestion of milk or yogurt. To avoid therapeutic failures in infections where the causative organism is only moderately susceptible, ingestion of large amounts of dairy products in liquid form with ciprofloxacin is not recommended.

As zwitterions, ciprofloxacin has shown good penetration and accumulation in tissues with a wide distribution throughout the body. Concentrations of fluoroquinolones in tissues were found to be higher than that in plasma. The apparent volume of distribution (Vd) is 2 to 3 L/kg, after a single oral dose of 400 to 500 mg serum levels were found to be 1 to 3 μg /ml. Concentrations in urine range from 100 to 650 μg /ml, which far exceeds the minimum inhibitory concentration (MIC) required for most urinary bacterial pathogens (Khan *et al*., 2009). Oral ciprofloxacin can serve as an alternative to parenteral route in serious bacterial infections only if it can reliably be

bioavailable as well as be well distributed to the tissue compartment. Many studies have found ciprofloxacin to have a large volume of distribution exceeding 2.0 L/Kg (Drusano *et a*l., 1986; David *et al.,* 1999). The large volume of distribution implies a wide distribution out of the central compartment into the tissues. The availability of ciprofloxacin at the interstitial target site is considered an important determinant for the effectiveness of antimicrobial therapy and the clinical outcome of an infection (Brunner *et al.,* 2002).

In a study by Brunner *et al*, (2002) on target site concentrations of ciprofloxacin after single intravenous and oral doses, the concentrations in saliva and capillary blood were found to be similar to the corresponding total levels in the plasma. When 500mg oral dose was administered to 8 healthy male volunteers, peak saliva concentration of 0.95 mg/L was reached at 2.17 h with AUC of 2.95 mg. h/L, elimination half life of 2.85 h and a clearance of 169 L/h.

Four metabolites of ciprofloxacin have been identified in body fluid as des-ethylene ciprofloxacin, sulpho-ciprofloxacin, oxo-ciprofloxacin and formayl-ciprofloxacin (Khan *et al.,* 2009). Most alterations occurred in the piperazine ring and have included: formation of a 5' carbonyl group, and opening of the piperazine ring to form a 7-ethylenediamine derivative. Most of the other metabolites of fluoroquinolones have less antibacterial activity than their parent compounds. The metabolites constituted from 15 to 30% of the drug recoverable in the urine. For ciprofloxacin, the ratio of drug metabolites to total unchanged drug plus metabolites in urine increased by 44% when the drug was given orally rather than intravenously, suggesting some degree of first-pass hepatic metabolism when ciprofloxacin is given by the oral route (Gonzalez *et al.,*

1985). Ciprofloxacin is mainly metabolized in the liver. Factors that affect metabolism of ciprofloxacin and other gyrase inhibitors include smoking, gender, genetically determined metabolic competency, and dosing schedule. The extent of metabolism can be estimated by determination of the renal and nonrenal clearance of drug and by measurement of excretion of drug labeled with ^{14}C. The principal metabolic pathways of gyrase inhibitors like ciprofloxacin are piperazine ring-based reactions (formation of oxo-compounds, N-oxides, demethylation products where applicable, or ring cleavage with or without subsequent metabolic conversions) and acyl-glucoronidation at the carboxy group of the nucleus (Sorgel, 1989). Clinical experience has shown incidence of undesirable adverse effects. Several examples in the literature data indicate that free radical formation might play a role in the mechanism of some of these adverse effects, including phototoxicity and cartilage defects (Gurby *et al.*, 2001). Ciprofloxacin is known to decrease CYP3A- and CYP1A-mediated biotransformation by competitive inhibition and therefore have the potential to cause drug interactions with agents metabolized by these enzymes (Mcmellan *et al.*, 1996).

Non-renal clearance accounts for approximately 33% of the elimination of ciprofloxacin; fecal recovery of ciprofloxacin accounts for approximately 15% of an intravenous dose. Non-renal elimination includes metabolic degradation, biliary excretion and trans-luminal secretion across the enteric mucosa (Khan *et al.*, 2009).

The excretion of ciprofloxacin via the human parotid gland was investigated by Adler and Maier, (1989). Two (2) hours after an intravenous bolus injection (200 mg ciprofloxacin) mean serum concentrations of 0.43 mg/l were achieved, and these decreased within 6 h after injection to 0.17 mg/l. The concentrations of the drug in parotid saliva showed a close positive correlation to the serum levels ($r = 0.96$), but

they were significantly lower than serum levels. They decreased from 0.1 mg/l two (2) hours after injection to 0.04 mg/l after 6 h.

The biliary excretion and metabolism of ciprofloxacin was also studied in 25 hospitalized patients by Parry *et al*., (1988). An intravenous dose of 200 mg of ciprofloxacin given 2.5 to 3.0 h prior to cholecystectomy resulted in concentrations in common duct bile, gallbladder bile, and gallbladder wall of 5.69 ± 4.8, 5.43 ± 3.34, and 2.52 ± 1.30 μ/ml, respectively, all at least fourfold greater than simultaneous concentrations in serum. Ciprofloxacin concentrations in common duct bile exceeded peak concentrations in serum in all but two patients with common duct obstruction.

The excretion of ciprofloxacin in the small bowel was studied in 40 patients undergoing bowel surgery by Ramon *et al.*, (2001). Ciprofloxacin (200 mg) was administered iv, and intestinal samples were collected over a 120-min period. In ileal loops ciprofloxacin concentrations reached a peak of 4.0 mg/L, whereas in caecal fluid samples, concentrations were <0.16 mg/L. Ciprofloxacin administered directly into the ileal and caecal loops did not result in measurable blood levels for 2 h. The results confirm that ciprofloxacin is selectively excreted into the small bowel.

The terminal disposition half-life ($t_{1/2}$) of ciprofloxacin is about 3 to 4 hours (Khan *et al.*, 2009). Pharmacokinetic studies after multiple intravenous doses of ciprofloxacin have not reported significant differences in terminal disposition half-lives or systemic clearances between the first and the last dose (Vance-Bryan *et al.*, 1990).

In a study in 18 healthy human volunteers on the pharmacokinetics of three oral formulations of ciprofloxacin by Davis *et al*., (1985), the mean renal clearance of

ciprofloxacin were 372 ml/min and accounted for at least 50% of the total clearance. Fraction of the administered dose of ciprofloxacin recovered unchanged from the urine within 24 h were 44.4, 48.6, and 55.8% after dosing with the 250-mg tablets, 500-mg tablets, or solution, respectively.

The pharmacokinetics of ciprofloxacin in the elderly is significantly different from those observed in the young: the elderly have a reduced renal clearance, a significantly greater area under the concentration-time curve (AUC), a larger peak plasma concentration (C_{max}), and a prolonged half life ($t_{1/2}$). Several investigators have suggested that dosage intervals shorter than 12 hr be avoided in the elderly. In patients with varying degrees of renal dysfunction, the pharmacokinetics of ciprofloxacin is significantly altered. The half-life in end-stage renal disease is approximately twice that of healthy controls (8 hr). The observed AUC and C_{max} may also be elevated in these patients. A wide variability in the half-life of ciprofloxacin has been observed among patients with severe renal failure, resulting in the recommendation that changes be made in the daily dose rather than the dosing interval in order to achieve drug concentrations comparable with those observed in normal renal function. The clinical impact of chronic ambulatory peritoneal dialysis or haemodialysis over a 4 hr period on the pharmacokinetics of ciprofloxacin is not significant, and therefore dosage supplementation after or during these procedures is not necessary. Liver dysfunction also appears to exert little effect on ciprofloxacin pharmacokinetics and dosage adjustments are not recommended (Vance-Bryan *et al.*, 1990).

2.5.6 Drug interactions

The concomitant oral administration of magnesium-, aluminium-, or calcium-containing antacids, sucralfate, iron preparations and multivitamins containing zinc

significantly reduces the absorption of ciprofloxacin. Ciprofloxacin reduces the metabolism of theophylline by approximately 15 to 30%; caution is therefore necessary when using any fluoroquinolone in combination with xanthine compounds. Case reports have documented increases in prothrombin times among patients receiving warfarin and ciprofloxacin concomitantly (Vance-Bryan *et al.*, 1990).

In a study by Zhu *et al*., (1999), to investigate the possibility of a drug-drug interaction between ciprofloxacin and fennel (*Foeniculum vulgare*) in a rat model, a significant interactions between the aqueous Fennel extract and ciprofloxacin was observed. Absorption, distribution and elimination of ciprofloxacin were all affected. They suggested that these changes might be because of the formation of a more lipophilic ciprofloxacin chelate in the presence of relatively large amounts of metal cations. If, therefore, the two therapeutic agents are used concurrently, an adequate dosing interval is needed to ensure the efficacy of ciprofloxacin.

2.5.7 Clinical uses

Ciprofloxacin is a second generation fluoroquinolones with potent bactericidal, broad spectrum activity against many clinically important pathogens which are responsible for variety of infections including urinary tract infections (UTI), gastrointestinal infections, respiratory tract infections (RTI), sexually transmitted diseases (STD) and skin infections. It is use against urinary tract infections and is also clinically useful against prostatitis, infections of skin and bones and penicillin resistant sexually transmitted diseases. Also sometimes employed against bacterial enteric infections and prophylaxis in the immune-compromised neutropenic host (Sharma *et al.*, 2009).

2.5.8 Adverse drug reactions

Adverse reactions associated with ciprofloxacin administration are generally mild to moderate and usually do not result in termination of therapy; the worldwide incidence is in the range of 4 to 8%, which is similar to that reported for other fluoroquinolones. The most commonly reported adverse reactions involve either the gastrointestinal tract (nausea, vomiting and diarrhoea), metabolic or nutritional disorders, or the central nervous system (Vance-Bryan *et al.*, 1990). Other adverse reactions include abdominal pain, rash, headache, and restlessness. Rare allergic reactions such as hives and anaphylaxis have been described. Serious adverse effects include drug-induced psychosis, immunogenic hypersensitivity reaction, peripheral neuropathy, raised intracranial pressure, seizure, tendinitis, traumatic, or non traumatic rupture of tendon. Acute renal failure has also been described, mostly in cases related to overdose, but sometimes at ciprofloxacin dosages within therapeutic schedules. Because of its potency, broad-spectrum activity and general safety, ciprofloxacin is usually reserved as a drug of last resort to treat antibiotic-resistant infections (Olivera *et al*., 2010).

2.6 Saliva as an analytical tool

Saliva is a complex fluid produced by a number of specialized glands which discharge into the oral cavity of the glands of mammalian vertebrates. Most of the saliva is produced by the major salivary glands (parotid, submandibular, and sublingual), but a small contribution is made by the numerous small labial, buccal, and palatal glands which line the mouth. Saliva, like other body fluids, is a dilute aqueous fluid containing both electrolytes and protein with an osmolality less than or equal to that of plasma. Also present in saliva is a certain amount of cell debris arising from the epithelial cells of the mouth together with food residues. Circadian variations have been shown in

unstimulated and stimulated saliva for flow-rate, pH and some salivary constituents. The total volume of saliva produced each day in adults is 500 to 1500 ml (Karin *et al*., 1999). There is evidence that many organic compounds enter saliva by a passive diffusion process, where lipid solubility and the degree of ionization-dependent on plasma and salivary pH are important factors. In addition, it has been shown for some drugs that their concentration in saliva equals the free or protein-unbound concentration in plasma, which is an advantage because drug plasma concentrations generally represent both bound and unbound drug. Lithium is a well-known example of a drug that is actively secreted in saliva, but active transport mechanisms have also been proposed for organic drugs since discrepancies and time dependencies in the saliva/plasma concentration ratios have been reported, especially in single dose studies e.g. theophylline (Karin *et al.,* 1999). In drug analysis, researches involving the use of saliva sampling as non-invasive qualitative and quantitative techniques have become increasingly important. Being readily accessible and collectible, saliva may show many advantages over 'classical' biological fluids such as blood and urine (Karin *et al.,* 1999). Saliva can be collected by non-invasive techniques, generally following the stimulation of salivary flow by chewing on some semi-solid material or by spraying citric acid on the tongue. If saliva is to be used in therapeutic monitoring then the saliva to plasma concentration ratio should be constant over a wide plasma concentration range. For the anticonvulsant drugs Phenytoin, primidone, ethosuximide and carbamazepine, the existence of a consistent correlation between their concentrations in saliva and plasma has been established under steady state conditions. Saliva concentrations appear to be the same as the protein unbound concentrations in plasma, and the S/P ratio is not influenced by concurrent therapy with other anticonvulsants (Danhof and Breimah, 1978).

2.6.1 Collection and analysis of saliva

A mixture of fluids from the various glands in the mouth is variously referred to as whole saliva, mixed saliva, or oral fluids. Whole saliva may also contain other materials that are in the mouth such as shed mucosal cells or food residues. Salivary composition and flow can be affected by many factors, including oral diseases (Dennis *et al.,* 2004). A variety of methods are available for collecting saliva. Some involve stimulating saliva production, while others target collection of unstimulated (also referred to as non-stimulated) saliva. Unstimulated saliva can be collected by the draining method, which is performed by allowing saliva to drip from the mouth into a collection container. Several techniques may be used to collect stimulated saliva. The simplest involves tongue, cheek, or lip movements without the use of an external stimulus. Chewing paraffin wax, Parafilm®, teflon, rubber bands, gum base, or chewing gum are usually referred to as mechanical methods of stimulating saliva production. A lemon drop or citric acid can be placed in the mouth to provide a gustatory stimulus for saliva production. Following stimulation by one or more of these methods, saliva can be spit, suctioned, or swabbed from the mouth. Some collection techniques combine stimulation and collection of the saliva using absorbent materials such as cotton balls or cotton rolls. After the absorbent material becomes saturated with saliva, it is removed from the mouth and the saliva is extracted by centrifugation or by applying pressure to the material (Dennis *et al.,* 2004).

There are several potential problems associated with stimulating saliva production. Parafilm has been shown to absorb some drugs and, therefore, give erroneous results when saliva is tested for drugs or drug metabolites. Also, paraffin contains compounds that may affect chromatographic analyses and drug testing accuracy. Some salivary stimulants may change the salivary composition and, therefore, affect the saliva-drug

concentration. For example, citric acid may change saliva pH and consequently alter drug concentrations in the saliva. Citric acid and cotton have also been shown to alter immunoassay drug test results (Dennis *et al.,* 2004). However, there are several advantages of stimulating salivary flow. Large volumes can be obtained within a very short period of time. Also, the pH of stimulated saliva mostly lies within a narrow range around the value of 7.4, whereas the pH of unstimulated saliva shows a larger variability, which may be of importance for the salivary secretion of weak acids and basic drugs (Karin *et al*., 1999). Also, the variability in saliva/plasma concentration ratios of some drugs is narrowed, and less specimens are too viscous or discolored to allow drug analysis. Thorough rinsing of the mouth is required prior to saliva sampling as remnants of orally administered medicines may contaminate saliva specimens and give spuriously high values. Deviation from a simple but strict methodology accounts for some of the discrepancies found in the literature (Gorodisc her and Koren, 1992).

Several devices are commercially available for collecting saliva. Some devices are based on the collection techniques just discussed. Devices such as: Oral Diffusion Sink®, Proflow Sialometer TM, Orasure® and Salivette TM. They have been advocated for saliva collection when testing for ethanol, steroids, and many other drugs (Dennis *et al.,* 2004).

2.6.2 Mechanism of drug transfer to saliva

Salivary glands have a high blood flow. The direction of the blood flow is countercurrent to the direction of the salivary flow. Salivary secretion is a reflex response controlled by both parasympathetic and sympathetic secretomotor nerves. Stimulation of sympathetic fibers to all glands causes vasoconstriction. In man, stimulation of the sympathetic trunk in the neck or injection of epinephrine causes

secretion by the submaxillary but not by the parotid glands. Parasympathomimetic drugs cause high saliva flow rates and enlargement of the tight junctions of the secretory end pieces (Karin *et al*., 1999).

In order for any drug circulating in plasma to be discharged into the salivary duct, it must pass through the capillary wall, the basement membrane and the membrane of the glandular epithelial cells. The rate-determining step for this transportation is the passage of the drug through the lipophilic layer of the epithelial membrane. Physicochemical principles dictate that for such a passage to occur, drug must show a degree of lipophilicity. However, saliva is not a simple ultrafiltrate of plasma, as has sometimes been suggested, but rather a complex fluid formed by different mechanisms: by a passive diffusion process, by an active process against a concentration gradient, by ultrafiltration through pores in the membrane, or by pinocytosis (Karin *et al*., 1999). Secretory and reabsorptive processes which take place in the ductal system of the salivary glands, and the rate of flow of the secretion play major roles in the determination of the concentration of solutes in saliva. Drug passage into saliva follows the general principles of movement of drugs across biologic membranes. Only the unbound fraction of the drug in plasma is available for diffusion into saliva and a relationship exists between saliva pH and the saliva/plasma concentration ratio of many polar drugs (Gorodischer and Koren, 1992).

The flouroquinolones antibiotics including ciprofloxacin have very large volumes of distribution and high tissue/serum ratios, apparent even after a single dose. They have homogenate tissue/serum ratios. The quinolone distribution ratios are in excess of 1:1 at most tissue sites (David *et al.*, 1999). It is known that antibiotics leave the vasculature

and enter extracellular fluids (ECF) via passive diffusion through the spaces between vascular endothelial cells. At steady state, the free concentration of drug in serum is equal to the free concentration of drug in ECF, provided that passive diffusion is the only operational mechanism (David *et al*., 1999). There are extravascular sites in which antibiotic concentrations cannot be predicted by use of passive diffusion principles alone. Some of these, such as urine or bile, are sites of active transport. Billiary secretion appears active for quinolones antibiotics. Protein binding appears to affect such pharmacokinetic parameters as distribution volume and renal elimination (David *et al.*, 1999).

2.7 Effects of Environmental Exposure on the Stability and Quality of Pharmaceutical Products

It is a known fact that stability poses serious problems for many manufactured product like pharmaceutical products that are in global markets. This is particularly critical for those distributed in regions with adverse climatic conditions (Olaniyi, 2000). Pharmaceutical products when exposed to environmental factors like heat, light, humility, air undergo number of chemical or degradation reactions. These reactions may include: Oxidation, reduction, hydrolysis or racemerization (Bajaj *et al.*, 2012). Environmental forces cause degradation of pharmaceutical product through the following mechanisms:

2.7.1 Heat (temperature)

Ambient temperature plays an important role in determining the stability of drug products especially those used in the tropical region where temperature fluctuation is common. Increase in temperature accelerate chemical reaction and cause an appreciable increase in decomposition rate (Olaniyi, 2000). The relationship between storage

temperature and degradation rate is determined using the Arrhenius equation (Appendix XIV) (Bajaj *et al.,* 2012).

High temperature can also lead to recemisation of an optically active drug product. This change can cause the formation of an inactive racemic mixture with serious loss of therapeutic activity (Olaniyi, 2000). Study by Bushra *et al,* in 2013 found a percentage of drug degradation of ciprofloxacin hydrochloride to be 8.21% when subjected to a thermal condition of 60 ^{0}C for 24 hr.

2.7.2 Humidity (moisture)

Presence of water affects not only decomposition rate but also the kinetics of decomposition. High humidity accelerates decomposition through hydrolysis. Hydrolytic reactions are catalyzed by H^{+} and $0H^{-}$ ions depending on the pH of the environment. At higher pH, OH^{-} catalysis predominates but at lower pH, H^{+} ions dominate. Compounds containing ester, amide, glycoside or lactam linkages are prone to hydrolysis. Drugs can be stabilized against hydrolysis by adjusting the pH of the medium to value where that gives lowest rate constant and also, use of moisture resistance packaging and good storage condition (Olaniyi, 2000).

In a forced degradation study of ciprofloxacin hydrochloride by Bushra *et al*, 2013 using HPLC, the percentage drug degradation was found to be 19.24% and 23.63% in acidic and alkaline medium respectively. In the acidic medium the drug was dissolved in 0.1N HCl under thermal condition of 70 ^{0}C for 4 hr. While in the alkaline medium it was dissolved in 0.1N Na0H at 70 ^{0}C for 4 hr.

2.7.3 Light

Pharmaceutical compounds are known to undergo decomposition reaction when they absorb electromagnetic radiation in the ultraviolet-visible region (UV/VIS) region.

This is due to their complex chemical structures. When exposed to light at a given wavelength especially at shorter wavelength, derivative reactions such as, oxidation-reduction, ring rearrangement or modification and polymerization can occur (Olaniyi, 2000). If the molecules absorbing the radiation participate in the main reaction, it is called photochemical reaction but if it is not then it is called photosensitizers. Ciprofloxacin absorbs radiation energy in the ultraviolet range of the electromagnetic spectrum. Like other flouroquinolones, it has been found to exert phytotoxic or photoallergic effects *in vivo or in vitro* through an unknown mechanism. This could be induced by the substances themselves or by derivatives formed as a consequence of UV irradiation (Tiefenbacher *et al.*, 1994).

Loss of antibacterial activity of ciprofloxacin due to photodegradation after UV exposure has been observed by Phillips *et al.* (1990). The photoproducts formed outside the organism under natural light conditions might not only be related to the loss of antibacterial activity but also be responsible for occurrence of side effects. Therefore, to prevent progressive photodegradation, quinolones should be protected from natural and artificial light. These can be achieved by storing in amber glass bottles (Tiefenbacher *et al.*, 1994). A forced degradation study of ciprofloxacin hydrochloride was carried out by Bushra *et al*,(2013) using UV radiation at 254 nm for 5 days. The result indicated a percentage degradation of 28.12%.

2.7.4 Air (Oxygen)

Instability of drugs due to exposure to air is common especially due to the abundance of oxygen in the atmosphere (20%). This instability could be due to oxidative degradation since most drugs exist in the reduced form in which they tend to be converted to a more oxidized state. Light sensitive formulations when exposed to oxygen in the presence of light or heat could undergo oxidation reaction. This reaction

could occur spontaneously under normal conditions in the presence of free radicals hence it is also called autoxidation. Drugs carrying any functional groups like: olefinics, phenolics, amines and many antibiotics could decompose in the presence of oxygen, and light or heat. This type of reaction could lead to changes in colour, smell or consistency of the product. Thus for tablet formulations they can be protected by packaging them in a hermetic strip and stored at reduced temperatures and excluded from light (Olaniyi, 2000; Bajaj *et al.*, 2012).

Factors influencing the stability of pharmaceutical products therefore includes: stability of the active ingredients, interaction between the active ingredients and excipients, manufacturing container or closure system used for packaging. Also, light, heat and moisture conditions encountered during shipment, storage and handling are important. In addition, degradation reactions like oxidation, reduction, hydrolysis or racemization play a vital role in stability of pharmaceutical product. These reactions depend on other factors like concentration of reactants, pH, radiation, catalysts, raw materials used and the length of time between manufacture and usage of the product (Bajaj *et al.*, 2012). Potential adverse effects of instability in pharmaceutical products are summarized in the table 2.1.

Table 2.1 Potential adverse effects of instability in pharmaceutical products (Bajaj *et al.*, 2012)

S/NO	Adverse effects	Explanation/reason	Example	Stability parameter tested
1	Loss of Active ingredient	Degradation of API in product resulting in less than 90% drug as claimed on label-unacceptable quality	Nitroglycerine tablets	Time elapsed before the drug content no longer exceeds 90%
2	Alteration in Bioavailability	Change in rate and extent of absorption on storage	-	Dissolution or release studies
3	Loss of pharmaceutical elegance and patient acceptability	Speckling caused by the interaction of the drug containing amine group with a minor component in the lactose resulting in the formation of a chromophore.	Slight yellow or brown speckling on the surface of tablet containing spray-dried lactose	Visual examination
4	Formation of toxic degradation products	Degradation of the drug component	Formation of epianhydro tetracycline from tetracycline, proteins drugs	Amount of degradation producing shelf.

2.8 Stability Testing of Pharmaceutical Products

Stability of a pharmaceutical product may be defined as the capacity of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications. Also, it can be defined as the extent to which a product retains, within the specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its packaging. As such, stability study or testing is used to determine the quality of the drug substance or a formulated product which is utilized

for prediction of its shelf life, determine proper storage conditions and suggest labeling instructions (Bajaj *et al.*, 2012).

2.9 Application of Ultraviolet-visible (UV/Vis) Spectroscopy

Spectroscopy is the study of the interactions of electromagnetic radiation with matter. UV/visible radiation falls within the electromagnetic spectrum region of 200-700 nm wavelengths. Most drugs molecules absorb radiation in the ultraviolet region of the spectrum, although some are colored and thus absorb radiation in the visible region (Watson, 2012). UV/VIS spectroscopy is widely applied in pharmaceutical analysis as a robust method for quantification of drugs in formulation where there is no interference from excipients. It is employed to determine the release of drugs from formulations with time as in dissolution testing. Reaction kinetics of drug degradation can also be monitored using the UV spectrum. UV/VIS spectroscopy is also often used in a number of pharmacopeia identity checks. The advantages of using UV/VIS spectroscopy is that it is easy to use, cheap and robust method that gives good precision for quantitative measurements of drugs in formulations. It is also used to determine some of the physicochemical properties of drugs. However, its limitation is that it is moderately selective and has no real application to analysis of mixtures (Watson, 2012).

2.9.1 Beer Lamber's Law

When a light of intensity (I_o) is directed through a solution of organic compound, some of the light is absorbed while a part of it is not absorbed but transmitted. Absorption of part of the incident light causes a decrease in its intensity (Figure 2.6). Thus, the intensity of the transmitted light (I_t) is less than that of the incident light (I_o). The strength of absorption of the light is called absorbance (A).

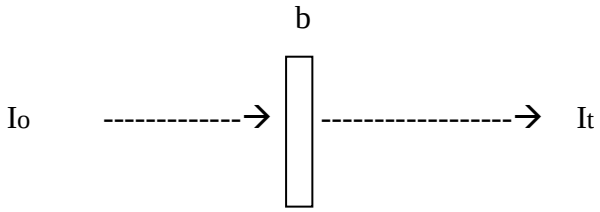


Figure 2.3 An incident light passing through a solution of organic compound (Watson, 2012)

The two laws made by Lambert and Beer explain the concept of absorption of light by molecules and form the principles for its application in quantitative analysis of drugs.

Lamber's law relates the total absorption to the optical path length (b).

$$\text{Absorbance}\left(\text{A}\right) = \log_{10}\left(\frac{\text{l}_\text{o}}{\text{l}_\text{t}}\right) = \text{kb}$$

Where, l_o is the incident light, l_t is the transmitted light, b is the thickness of the absorbing layer (path length) and K is the proportionality constant. Here, concentration is being kept constant.

Beer's law relates absorption to the concentration for the absorbing solute (c) in solution.

$$\log_{10}\left(\frac{\text{l}_\text{o}}{\text{l}_\text{t}}\right) = \text{kc}$$

here, the path length (b) is being kept constant

The combination of the two laws gives rise to the popular Beer-Lambert's law. This can be expressed mathematically as follows:

$$\log_{10}\left(\frac{I_o}{I_t}\right) = A = \varepsilon bc$$

Where, A is known as the absorbance and is a measure of the amount of light absorbed by the sample. ε is a constant known as the molar extinction coefficient which is the absorbance of a 1M solution of the analyte. b is the path length in centimeters (cm) usually 1 cm and C is the concentration of the analyte in moles litre^{-1}.

In pharmaceutical products, concentration and amount are usually expressed in grams or milligrams rather than moles.

Thus, Beer-Lambert's equation is written in the following form:

$$A = A(1\%, 1cm)bc$$

Where A is the measured absorbance, A (1%, 1cm) is the absorbance of a 1% w/v (1g/100ml) solution in a 1cm cell. C is the concentration of the sample in g/100ml.

Since b = 1 cm, the formula can be written as:

$$C = \frac{A}{A(1\%, 1cm)}$$

C gives the concentration, of the analyte in g/100 ml. BP monographs usually give a standard A (1%, 1cm) value for a 'drug which is to be used in its quantization (Watson, 2012).

2.10 *In-Vitro* Quality Control of Tablet (Evaluation Tests)

In order to ensure the safety and efficacy, pharmaceutical products are expected to be tested during and after the manufacturing and at various intervals during the shelf life of the product (Jackson *et al.*, 2011). The assessments involved the use of both

qualitative and quantitative methods of evaluation. The qualitative methods of evaluation includes tablet description i.e. colour size and shape, which are carried out by visual observation as well as thin layer chromatography (TLC), while quantitative evaluations are: uniformity of weight, friability, hardness, disintegration and dissolution tests as well as chemical content determination (Adegbolagun *et al.*, 2007). Uniformity of weight, assay, disintegration and dissolution are compendial standards to assess the quality of tablets while hardness and friability are referred to as non-compendial standards although friability is now included in the United States Pharmacopeia - USP, 1995 (Ngwuluka *et al.*, 2009). The monographs contain details on expected quality of such items. These tests are carried out in accordance with the official books like: British Pharmacopoiea (B.P) and United State Pharmacopoiea (U.S.P). They are as follows:

2.10.1 General appearance

The physical appearance of tablet determines their acceptability and hence compliance by patients to medication. Physical characteristics such as: size, shape, thickness and other organoleptic properties like color and odor helps in their identification and assessing stability changes.

2.10.2 Tablet hardness (crushing strengths) test

Crushing strengths shows the ability of tablets to withstand pressure or stress during handling, packaging, and transportation. It is the property of a tablet that is measured to assess its resistance to permanent deformation (Jackson *et al.*, 2011). Also, the mechanical strengths of a tablet determine the disintegration time and the rate of dissolution. Tablet hardness can also influence friability (Uduma *et al.*, 2011). Differences in hardness are usually encountered when different compression pressures are employed (B.P, 2005).

2.10.3 Friability tests

Friability is the mechanical property of a tablet which measures its ability to withstand abrasion during handling, transportation etc (Jackson *et al.*, 2011). United States Pharmacopeia and other reference books gave an allowable limit of ≤ 1% (USP, 2005).

2.10.4 Disintegration tests

The BP (2009) specification is that uncoated tablets should disintegrate within 15min and film coated tablets within 30 min. The USP (2005) specifies that uncoated and film coated tablets should disintegrate within 30 min (Uduma *et al.*, 2011). The disintegration test measures the time required for a tablet to disintegrate into particles when in contact with gastrointestinal fluids. The type and amount of excipients used in tablet formulation as well as the manufacturing process are all known to affect both the disintegration and dissolution parameters (Mu'az *et al.*, 2009). Disintegration could be directly related to dissolution and subsequent bioavailability of a drug (Uduma *et al.*, 2011).

2.10.5 Dissolution test

Dissolution testing is a requirement for all solid oral dosage forms and is used in all phases of development for product release and stability testing. It is a key analytical test used for detecting physical changes in active pharmaceutical ingredients (API) and in formulated product. Dissolution testing has also been employed as a quality control (QC) procedure, in research and development (R&D) to detect the influence of critical manufacturing variables and in comparative studies for *in vitro-in vivo* correlation (Zhang and Yu, 2004). *In-vitro* dissolution testing may be a useful tool to forecast the *in vivo* performance of drug products and potentially reduce the number of bioavailability/bioequivalence studies required. The choice of dissolution technique to be use is determined by the dosage form characteristics and the intended route of

administration. For solid dosage forms, industry standard dissolution testing methodologies are the United States Pharmacolpoeia (USP) Aparatus 1 (basket) and the USP Apparatus 2 (paddle). Immediate release, modified-release and extended release tablets bath with USP 2 paddles. Floating capsules and tablets generally use USP 1 baskets (USP, 2005). The development of a dissolution procedure involves selecting the dissolution media, apparatus type and hydrodynamics (agitation rate) appropriate for the product. Dissolution is the process of extracting the active pharmaceutical ingredient (API) out of the dosage form solid-state matrix into solution within the gastrointestinal tract (Wang *et al.,* 2009). The release of active pharmaceutical ingredient from drug product, the dissolution of the drug under physiological conditions and the permeability across the gastrointestinal tract determines the drug absorption (Ngwuluka *et al.*, 2009). It is the pharmacokinetic data that supplements and provide additional information regarding API absorption rate. The B.P. specifies that not less than 70% w/v labeled content should dissolve at 45 min (Adegbolagun *et al.*, 2007).

2.10.6 Percentage content

The USP specification is that the content of ciprofloxacin hydrochloride should not be less than 90% and not more than 110% while BP specifies that the content should not be less than 95% and not more than 105% (Ngwuluka *et al.*, 2009).

2.11 Analytical Method Development and Validation

Analytical method development and validation play an important role in drug discovery, development, and manufacture of Pharmaceuticals and estimation of small molecules. The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this

goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Analytical method development is also required by most regulations and quality standards that import laboratories (Kotte *et al.*, 2012; Sharma, 2012).

Analytical methods need to be validated, verified or revalidated in the following instances: before initial use in routine testing, when transferred to another laboratory and whenever the conditions or method parameters for which the method has been validated change (for example, an instrument with different characteristics or samples with a different matrix) and the change is outside the original scope of the method (Kotte *et al.*, 2012; Sharma, 2012). All analytical procedures should contain methods for identification, separation and quantification of the chemical components of natural and synthetic components. The choice of the analytical methodology depends on factors such as chemical properties of the analyte and its concentration, sample matrix, the rapidity and cost of the analysis, type of measurement (i.e quantitative or qualitative) and the number of samples (Kotte *et al.*, 2012). It is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

2.11.1 Types of analytical procedures to be validated

Analytical procedures need to be validated and the various types that are recommended include: regulatory analytical procedures, alternative analytical procedures and stability indicating assay (Sharma, 2012). According to the international conference on harmonization (ICH, 1995) guidelines, there are four most common types of analytical procedures: identification tests, quantitative tests for impurities' content, limit tests for the control impurities and quantitative test of the active moiety in sample of drug

substances or drug product or other selected components in the drug product. Other analytical procedures that were not addressed in the initial text document and which are equally important are: dissolution testing for drug product and particle size determination for drug substances.

Identification tests are intended to ensure that analyte is present in the sample. This is normally achieved by comparing the property of the sample (e.g spectrum, chemical reactivity) with a reference standard. Testing for impurities can be either a quantitative test or limit test for the impurity in a sample. Both are intended to reflect the purity characteristics of the sample. Assay procedures are intended to measure the presented analyte in a given sample. It therefore represents a quantitative measurement of the major components in the drug substance (Garcia *et al.*, 2011).

Type and degree of validation depends on the nature of the test. Different test methods will require different validation parameters. The various validation parameters are: accuracy and percentage extraction recovery, precision (repeatability and reproducibility), linearity and range, limit of defection (LOD)/limit of quantization (LOQ), selectivity/specificity, robustness/ruggedness, stability and system suitability studies.

2.11.1.1 Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. It may be determined by any of the following ways:

analyzing a sample of known concentration and comparing the measured value to the "true" value. Here, a well characterized sample (e.g reference standard) must be used.

Spiked-placebo (product matrix) recovery method: in this method, a known amount of pure active constituent is added to formulation blank, the resulting mixture is assayed, and results obtained are compared with the expected results. Formulation blank is a sample that contains all other ingredients except the active ingredients (Ravichandran *et al.*, 2010).

Standard addition method: in this method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed. The difference between the results of the two assays is compared with the expected answer. In both methods (i.e spike placebo recovery and standard addition method), recovery is defined as the ratio of the observed result to the expected result expressed as a percentage. Accuracy of a method may vary across the range of possible assay values therefore must be determined at several different fortification levels. It should cover at least 3 concentrations (80, 100, and 120%) in the expected range (Ravichandran *et al.*, 2010). The international conference on harmonization (ICH, 1995) documents recommends that accuracy should be assessed using three concentrations levels and three replicated determination for each concentration.

2.11.1.2 Precision

This is defined as the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It is usually expressed as the standard deviation or the relative standard deviation (RSD). It is also called coefficient of variation. Precision is a measure of degree of reproducibility or the repeatability of the analytical method under normal operating circumstances. Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over a short period of

time. While reproducibility involves precision study at different occasions, different laboratories and different batch of reagents, different analysts and different equipments (Ravichandran *et al.*, 2010).

2.11.1.3 Linearity (calibration curve)

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. The linear range of detectability that obeys Beer's law is dependent on the compound analysed and the detector used. The working sample concentrations and samples tested for accuracy should be within the linear range. For the method to be considered linear, the r value (correlation coefficient) should be close to $\pm$ 1 (Ravichandran *et al.*, 2010).

2.11.1.4 Range

The range of an analytical method is the interval between the upper and lower levels of the analyte that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. For example, the minimum specified ranges for the assay of an active substance or a finished product is normally from 80 to 120% of the test concentration (Ravichandran *et al.*, 2010).

2.11.1.5 Limit of detection and limit of quantitation

Limit of detection (LOD) of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantities. It is a limit that specifies whether or not an analyte is above or below certain value (Ravichandran *et al.*, 2010). The limit of quantization (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable accuracy and precision under the stated operational conditions of the method. It is a parameter of quantitative assays for low levels of

compounds in sample matrices such as impurities in bulk-drugs and degradation products in finished pharmaceuticals (Ravichandran *et al.*, 2010).

2.11.1.6 Selectivity and specificity

Selectivity of a method refers to the extent to which it can determine particular analyte in a complex mixture without interference from other components in the mixture (Ravichandran *et al.*, 2010). In other words, it is the ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample matrix (Kotte *et al.*, 2012). Specificity, on the other hand is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. These components might include: impurities, degradants, matrix etc (Kotte *et al.*, 2012).

2.11.1.7 Robustness

The robustness of an analytical method is a measure of the its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength, temperature, and determine the effect if any in the results of the method. If measurement are susceptive to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure (Ravichandran *et al.*, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Pharmaceutical grade ciprofloxacin standard powder made by May and Baker (M&B-UK) was used for the method development and validation. Ciprofloxacin tablet (500 mg) made in India, registered with NAFDAC and with long shelf life (expiry date of 01/05/2018) was purchased directly from Evans Pharmaceutical company.

The following solvents of analytical grade were used: 0.1M HCI (M&B–UK), 0.1M NaOH, Ethanol, Methanol, Chloroform, Acetone (Sigma-Germany), and Distilled water. Also, Phosphate buffer (pH 4) was used.

The apparatus used in this study includes: UV spectrophotometer (Thermo-Scientific Helios Zeta UV-Vis, ser. no; UV2-164917) with1cm path length, Analytical weighing balance (DENVER-ISTRUMENT (Apx-200), Friabilator, Disintegration apparatus (Erweka), and Dissolution apparatus (USP apparatus-basket method).

3.2 Methods

3.2.1 Study area

The study was conducted in Gombe State. Gombe State is located in the North Eastern part of Nigeria. It shares borders with all the 5 states of the North eastern region. Gombe State was created in 1996 and has an estimated population of 2.6 million in 2010 with a population density of 128 persons per sq. km (Abdulkadir *et al*., 2013). It is divided into 3 senatorial zones: Gombe North, Gombe Central and Gombe South. The state has eleven (11) Local Government Areas (LGAs). Gombe North comprises of 5 LGAs: Gombe, Kwami, Dukku, Funakaye, and Nafada. Gombe Central zone comprises of 2 LGAs: Akko and Yamaltu-Deba. While Gombe South comprises of 4 LGAs: Billiri, Kaltungo, Balanga, and Shongom. The state experiences two seasons: wet and dry seasons. The rainfall intensity is high between July and August. The dry season is between November and April with the coldest month experienced between the month of December and January. The study was conducted between the months of March and May, 2015. This period is usually the hottest season experience in the state. The eco-climatic characteristics of the three zones differ slightly (Abdulkadir *et al*., 2013). Gombe North zone has a very high acridity index, the moisture quality index is deficient and hydrologic growing season is very short due to very late real monsoon onset (rainfall) which ceases early. The Central zone is characterized by high acridity index with deficient moisture quality index, short hydrologic growing season, late real monsoon onset which ceases late. While the Southern zone experience high acridity index, adequate moisture quality index, long hydrologic growing season, late real monsoon onset which ceases late (Abdulkadir *et al.*, 2013). The three (3) senatorial zones served as our environmental exposure sites.

3.2.2 Sampling method

A purposeful sampling method was employed in selecting drug sample and the exposure sites. The ciprofloxacin firm coated tablet (Rapiflox®) that comes in transparent sachet form with long expiry date was purchased directly from the company. Product of the same expiring date and same batch number was used. The brand of ciprofloxacin tablet (Rapidflox®) was chosen because it is a common brand found in Gombe state drug markets. It is also handled by drug hawkers and probably because it is cheaper than other available brands. Also, because Rapidflox® brand that is available in transparent sachet form are also available in Gombe state drug market. One town was selected to represent Gombe North, Gombe Central, and Gombe South that served as our exposure site. The towns selected have big drug markets and strategically located at the heart of the zones they are located.

One drug hawker was selected from Dukku, Kumo, and Billiri markets representing each zone and was given some sachets of the sample drug which were kept inside their baskets containing other drugs. They were asked not to sell them but to be kept for a period of 3 months (March to May, 2015). After 3 month, the drugs were retrieved and kept in a controlled storage condition pending administration to the subjects. Temperature of the exposure sites were recorded throughout the study period. The quality of the non-exposed drug purchased directly from the manufacturer was tested and kept in a controlled storage condition which served as our reference drug sample (Sample A). The exposed samples to Gombe North, Gombe Central and Gombe South were labeled sample B, C and D respectively.

3.2.3 Protocol/ Study design

Six (6) apparently healthy male volunteers aged between 25-40 years and weight 50-70 Kg were recruited based on some inclusive and exclusive criteria. Only non-smokers and non-alcoholics were enrolled into the study. Those enrolled were asked to stay off all medications during the study period. Children and overweight subjects were excluded from this study. All subjects on medications at least two (2) weeks to the study were excluded. Also, all non-consented subjects were excluded. All subjects were informed about the aim and risks of the study and a written informed consent was sought (Appendix XXII).

The six volunteers were asked to fast overnight. After raising their mouth, 5 ml of the blank saliva was collected from each of them before drug administration. One tablet of the non-exposed ciprofloxacin tablet 500 mg (sample A) was given to each of them to take with 200 ml of water. 5 ml of the saliva sample was later collected at 15 min, 30 min, then 1, 2, 3, 4, 5, 6 and 8 hr after administration respectively. The saliva samples were collected in a sample bottles followed by extraction and analysis as described in (3.2.4) below. The same procedure was repeated using the same subjects for the exposed samples collected from the 3 exposure zones of the state. A washout period of 1week was observed between each drug administration. The design of the study is illustrated in figure 3.1.

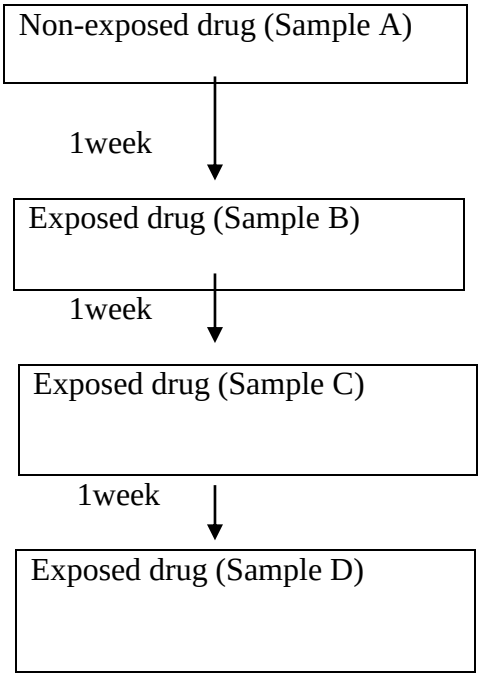


Figure 3.1 Sample administration and collection protocol

3.2.4 Extraction method and Saliva drug concentration analysis

Method of Sharma *et al*, (2010) was modified. Two milliliters (2 ml) of the saliva sample was collected, mixed with 2 ml of extracting solvent (distilled water) plus 1 ml of buffer. The resultant solution was mixed for 2 min then centrifuged at 3000 rpm for 10 min. Finally, 2 ml of the supernatant was collected into sample bottles and stored at -4^{o}C for analysis. The blank solution was prepared by mixing 2 ml of blank saliva, 2 ml of the extracting solvent and 1ml of buffer for 2 min then centrifuging for 10 min at 3000 rpm. The supernatant is used as a blank solution during analysis.

The extracted saliva samples were analysed using a double beam ultraviolet-visible (UV/VIS) spectrophotometer. The machine was fixed at 271 nm working wavelength and then blanked using the blank solution. The saliva sample is then introduced into the

1 cm cuvette then placed into the sample chamber and closed. All the saliva samples were scanned to determine their absorbance. The measured absorbance were all converted to concentrations with the aid of the calibration curve.

3.2.5 Generation of pharmacokinetic parameters

The pharmacokinetic parameters were generated using both trapezoidal rule and the residual method.

Residual method: after all the absorbance were converted to concentrations using the calibration curve, the saliva ciprofloxacin concentrations (μg/ml) for each volunteer was plotted against time (hr) on a 3-cycle semi-log graph. All the concentration-time graphs for the non-exposed ciprofloxacin sample A and the three exposed samples B, C and D were plotted. The elimination phase of the curve was extrapolated to the y-axis with the aid of a ruler to represent the extrapolated line or elimination line. Extrapolated concentrations were determined from the elimination line. The absorption phases of the curves represent the observed salivary concentrations. The residual concentrations were determined by subtracting the observed concentrations from their corresponding extrapolated concentrations at some randomly selected time intervals. The residual concentrations were then plotted also on the same graph to give the residual line. The elimination half life was determined from the extrapolated line and the elimination rate constant was calculated using the formula: $K\beta = 0.693/t_{1/2\beta}$. Similarly, absorption half life was determined from the residual line and absorption rate constant was calculated using the formula: $K\alpha = 0.693/t_{1/2\alpha}$. Lag time was determined from the corresponding time (hr) where the residual line intercepted the extrapolated line.

Trapezoidal rule method: in this method, the concentration-time data were used to determine the remaining pharmacokinetic parameters as follows: AUC_{0-8} (from 0 to 8 hr) and $AUC_{0-\infty}$ (from 0 to infinity) were calculated using the triangular-trapezoidal formula (Appendix X). Volume of distribution (Vd) and Clearance were also determined using formula (Appendix IX). The peak concentration (C_{max}) and the time to reach the maximum concentration (T_{max}) for each volunteer and for each samples of the drug were also determined using non-compartmental model (directly from the concentration-time profiles).

3.2.6 Data analysis

The mean values of all the pharmacokinetic parameters generated from the six volunteers in all the four sample groups A, B, C and D were calculated from the results for further statistical analysis.

All the mean pharmacokinetic parameter values obtained were compared using One-way ANOVA where ($P \leq 0.05$) was considered significant between the non-exposed sample and each of the exposed samples followed by a Post Hoc test. Where appropriate, some values were represented in percentages (%). Statistical software SPSS version 20 was used for the statistical analysis.

3.2.7 Analytical method Development

Method of Gogulamudi and Sujana, (2012) was adopted, modified and validated in accordance with the international conference on harmonization guidelines (ICH, 1995). The following procedures were carried out:

3.2.7.1 Solubility test of the standard ciprofloxacin powder in different solvents

Ciprofloxacin standard powder was weighted and added into volumetric flasks containing the following solvents each: water, n-hexane, ethanol, methanol, and chloroform. They were well shaken and were observed to see which solvent would give the best solubility.

3.2.7.2 Preparation of stock solution

10 µg/ml stock solution was prepared using the solvent of choice (distilled water). From the stock solution 4.0 µg/ml was prepared using serial dilution with distilled water.

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

The analytical method was developed by scan using UV spectrophotometer. Scanning at wavelength of 200-400 nm was done to select the analytical wavelength. The maximum wavelength (λ_{max}) obtained was used as the working wavelength.

3.2.8 Validation of the adopted analytical method

The adopted method was validated based on the international conference on harmonization (ICH, 1995) guidelines where precision, linearity, % extraction recovery tests were carried out.

3.2.8.1 Precision test

It is an agreement between replicated results (n = 6). The precision of the method was tested in terms of repeatability. The test was done by determination of 6 replicates of a fixed concentration of the drug (4 µg/ml) within the working wavelength in which their absorbance were measured. The test for both within day and between days were done where the results based on B.P 2009 were expressed as relative standard deviation (RSD) ≤ 2%. For the within day, 5 ml of 4 µg/ml solution of the drug was measured and absorbance was determined every 1 hr at 271 nm six times. For the between day

precision test, the absorbance of the same 4 µg/ml solution was measured six (6) times within two days to obtained at least 6 absorbance.

3.2.8.2 Construction of calibration curve

Blank saliva (15 ml) was collected from a healthy volunteer and centrifuged. A stock solution of 10 µg/ml of standard ciprofloxacin was prepared using distilled water as solvent. Out of the stock solution, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml were withdrawn and transferred into six different test tubes that are labeled. 2 ml of the blank saliva was measured and transferred each into six labeled test tubes containing the drug solution. Distilled water was added to each of the test tubes to make up to 10 ml giving 1, 2, 3, 4, 5 and 6 µg/ml ciprofloxacin solution respectively. Another 2 ml was withdrawn from each labeled test tubes and transferred into the centrifuge tubes which are also labeled 1, 2, 3, 4, 5 and 6 µg/ml respectively. 2 ml of the extracting solvent (distilled water) was added to each and finally 1ml of the buffer (pH 4) was also added to each and mixed for 2 min and centrifuged at 3000 rpm for 10 min. The supernatant layer was decanted and absorbance was determined at 271 nm wavelength. The absorbance were plotted against concentrations using Microsoft excel.

3.2.8.3 Accuracy and percentage (%) extraction recovery

It is the extraction efficiency of an analytical process reported as a percentage of the known amount of analyte. 2 ml blank saliva was collected prior to drug administration and then vigorously mixed with 2 ml of extracting solvent (distilled water) and 1 ml of buffer (pH 4) solution. This was then centrifuged at 300 rpm for 10 min and the upper layer formed served as blank solution. One male volunteer was given one tablet of the non-exposed ciprofloxacin tablet after an overnight fasting. Six hours (6 hr) after drug administration, 2 ml of saliva was collected. 2 ml of the extracting solvent was also added to the saliva sample then 1 ml buffer and were vigorously mixed for 2 min. The

whole mixture was centrifuged at 3000 rpm for 10 min, the supernatant layer was decanted and absorbance measured at 271 nm against the previously prepared blank. Also, 6 μg/ml, 7 μg/ml, and 10 μg/ml of ciprofloxacin solution were prepared using 0.1N HCl as solvent. Then 2 ml of the 6 μg/ml solution was measured and spiked in 2 ml of blank saliva and 1ml buffer. The mixture was also mixed for 2 min and centrifuged for 10 min at 3000 rpm. The upper layer was collected and absorbance measured at 271 nm using 0.1N HCl spiked with saliva as blank.

The % extraction recovery thus was determined by using the formula:

$$\%\,\text{Extraction recovery} = \frac{\text{concentration in saliva after 6hrs}}{\text{concentration in 0.1N HCL spike in blank saliva}} \times 100$$

The % extraction recovery of the sample was compared with standard acceptable value of (95-105%) according to the international conference on harmonization (ICH, 1995) guidelines.

3.2.9 In-vitro quality control tests

In vitro quality control test of the ciprofloxacin standard powder used this study was carried out to ensure that the standard powder is pure and free of impurities for it to be applied in the method development and construction of the calibration curve in accordance with the ICH,1995 guidelines. The qualities of the ciprofloxacin tablet samples were assessed before and after exposure to allow for comparison between the non-exposed samples (reference) and the exposed (test samples). BP (2002, 2009) and USP (2005) methods were used to carry out the following tests:

3.2.9.1 Identification test

B.P 2002 method was adopted where chloride test was employed as a confirmatory test for both the standard powder and the tablet samples. 2M nitric acid and 5% $AgNO_3$ solutions were freshly prepared.

Standard powder: the ciprofloxacin powder (0.1g) was weighed and dissolved in 10 ml of distilled water. 2 ml of the prepared ciprofloxacin solution was measured and mixed with 5 ml of 2M nitric acid and 1 ml of 5% $AgNO_3$ solution. The solution was left for 5 min to observe for appearance any color or precipitate.

Ciprofloxacin tablet: ten (10) tablets of the ciprofloxacin tablet from each exposure site were crushed and powdered separately. Test equivalent of powdered ciprofloxacin tablet (i.e exposed and non-exposed) was weighed and dissolved in 10 ml of distilled water. To 2 ml of the solution, 5 ml of 2 M nitric acid was added then 1ml 5% $AgNO_3$ solution. The solution was left to stand for 5 min to observe for appearance of any white precipitate.

3.2.9.2 Friability test

TA-3R Friabilator (Germany) was used to carry out the friability test according to B.P 2009 specifications. Ten (10) tablets were weighed from each of the samples to determine their initial weight (WA). After placing the tablets in a friabilator, the machine was set to rotate at 25 rpm for 4 min. At the end of the run, the tablets were dusted and re-weighed (WB). Percentage friability was calculated from the equation:

$$F = \frac{(WA - WB)}{WA} \times 100$$

3.2.9.3. Assay of the ciprofloxacin tablet

Ten (10) tablets of the ciprofloxacin 500 mg from each of the samples were powdered and weighed. An equivalent of 0.300 g of the drug was weight and dissolved in 80 ml of anhydrous acetic acid then titrated with 0.1M perchloric acid. Crystal violet solution (0.5%) was used as an indicator and the bluish-green colour formed serves as the end point. 1 ml of 0.1M perchloric acid is equivalent to 0.03314 g ciprofloxacin (B.P 2002). Blank titrations were carried out using 15 ml glacial acetic acid and titre values were adjusted by deducting the blanks determined from the assay.

3.2.9.4 Disintegration test

ZT3-model (Germany) disintegration test apparatus was used based on the British Pharmacopoeia, 2009 method. The disintegration medium is 0.1N HCI, was maintained at 37 ± 0.5°C. Six (6) tablets from each of the sampled area were used for the test. One tablet was placed into each of the six holes of the chambers and the machine was put on and timed with the aid of a stop watch. The disintegration time is the mean time needed for each of the tablet to break into particles, small enough to pass through the screen into the disintegration medium until no particle remain in the basket.

3.2.9.5 Dissolution test

USP, 2005 method was adopted. One liter (1000 ml) of the dissolution medium (0.1N HCl) was prepared and poured into the dissolution container and temperature of the medium was maintained at 37 ± 0.5 ^{o}C by heating the water bath with electric heater. Erweka D (Germany) model was used and the paddle was made to rotate at 50 rpm. A stop watch was used for the timing. After 45 min, ten milliliters (10 ml) of the dissolution medium was withdrawn using syringe and filtered using Whatman filter paper 125 mm (England). Concentration of the released ciprofloxacin was determined by measuring the absorbance of the filtrate using UV-1800 Shimazu spectrophotometer

against a blank (0.1N HCl) at 271 nm wavelength. A dilution factor of 1:100 was used to dilute the filtrate before measurement. The test was repeated three times for each of the samples.

CHAPTER FOUR

RESULTS

4.1 *In vivo* Pharmacokinetic Results

4.1.1 Mean saliva concentration-time profiles of ciprofloxacin tablet samples A, B, C and D

The mean saliva concentration-time profiles of ciprofloxacin after administration of 500 mg dose to each volunteer for the non-exposed sample (A) and all the three exposed samples (B, C, and D) are presented (Appendix XVII) and their respective saliva concentration-time curves shown in figure 4.1

The non-exposed ciprofloxacin tablet (Sample A) reached a peak saliva concentration of 10.12 ± 3.94 μg/ml at 6 hr as illustrated in figure 4.1 and Appendix XVII. Sample B is the exposed ciprofloxacin tablet to Gombe north which reached its peak saliva concentration of 11.59 ± 2.75 μg/ml at 6 hr. While samples C and D (ciprofloxacin tablets exposed to Gombe central and Gombe south respectively) reached their peak saliva concentrations of 8.83 ± 1.84 μg/ml for sample C and 7.10 ± 2.46 μg/ml for sample D at 7 hr each. Figure 4.1 shows super-imposed concentration-time curves of all the ciprofloxacin samples. The result shows that the saliva concentration of non-exposed ciprofloxacin tablet (Sample A) was higher than for the two exposed samples C and D but is lower than the exposed sample B. It can also be seen from the graph that the time to reach peak salivary concentrations for sample C and D were the same but shifted to the right and higher than for the non-exposed sample A.

Figure 4.2 show a semilog mean saliva concentration-time graph of non-exposed ciprofloxacin indicating first order kinetics for both the absorption and elimination phase.

4.1.2 Mean pharmacokinetic parameters of ciprofloxacin tablets.

The mean salivary pharmacokinetic parameters for the non-exposed ciprofloxacin tablet (sample A) and the exposed samples B, C and D were studied in 6 healthy volunteers in fasting state and are shown in table 4.1. The parameters were compared at $P \leq 0.05$ between the non-exposed (sample A) and the exposed samples B, C, and D respectively. The results indicated that there was no significant difference except between sample A and sample D (table 4.1). The results only showed a significant difference of change in elimination half life (3.03 hr for sample A and 2.10 hr for sample D) and elimination rate constant (0.28 hr^{-1} for A and 0.35 hr^{-1} for D). Lag time of non-exposed sample A (2.33 hr) when compared with the exposed samples B, C and D showed that sample A stayed longer before absorption than sample B (1.67 hr) and C (1.87 hr) but was absorbed faster than sample D (2.45 hr) but the difference was not statistically significant. The results for absorption rate constant showed that when samples A (non-exposed) was compared with exposed samples B, C and D, the absorption rate constant for A (0.46 hr^{-1}) is less than for the exposed samples B (0.57 hr^{-1}), C (0.67 hr^{-1}) and D (1.09 hr^{-1}) which are also in increasing order although there was no statistical difference ($p \leq 0.05$). Results for absorption half life indicated that when sample A (non-exposed) was compared with the rest of the exposed samples B, C and D, the absorption half life of sample A was higher and in decreasing order (1.77 hr for A, 1.30 hr for B, 1.28 hr for C and 1.10 hr for D) respectively. The difference was also not statistically significant ($p \leq 0.05$).

The non-exposed ciprofloxacin (sample A) gave a peak saliva concentration (Cmax) of 12.65 μg/ml which is higher than for exposed samples C (9.34 μg/ml) and D (9.31 μg/ml) but lower than the exposed sample B (12.60 μg/ml). The peak saliva

concentration (Cmax) is a measure of bioavailability. The higher the Cmax, means the higher the bioavailability. Statistical difference ($p \leq 0.05$) was not detected from this comparison.

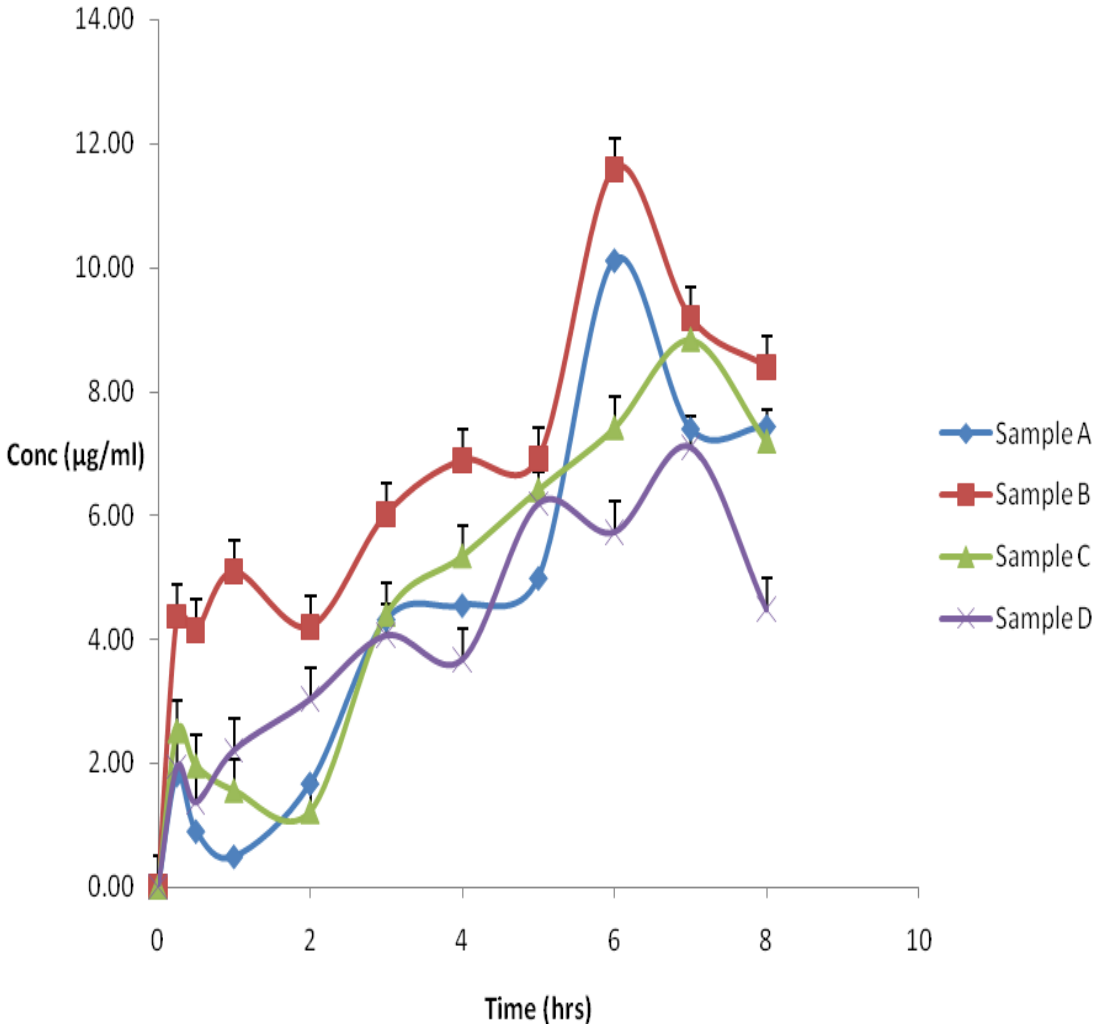
Results showed that the non-exposed drug sample A reached peak saliva concentration (Tmax) of 6.3 hr which is late when compared to drug sample C (5.50 hr) and sample D (5.50 hr) but earlier when compared to drug sample B (6.5 hr). There was no statistical difference ($p \leq 0.05$) between the non-exposed and the exposed samples. Area under the concentration-time curve (AUC_{0-8}) for the non-exposed sample A when compared with the rest of the exposed samples B, C and D showed that the AUC_{0-8} for sample A (71.29 μg/ml. hr) was less than sample B (81.11 μg/ml. hr) and sample C (79.31 μg/ml. hr) but higher than for sample D (47.07 μg/ml. hr).

Results for the elimination rate constant showed a significant difference when compared ($P \leq 0.05$) between the non-exposed ciprofloxacin tablet samples A (0.28 hr^{-1}) which was lower than the exposed sample D (0.64 hr^{-1}). Although the value for sample A was lower than for sample B (0.35 hr^{-1}) and higher than sample C (0.20 hr^{-1}), but the difference was not statistically significant ($p \leq 0.05$). When the elimination half life of non-exposed sample A is compared with the exposed samples B, C and D, there was a significant decrease in elimination half life of sample D (1.6 hr) when compared to sample A (3.03 hr). However, a decrease in elimination half life of sample B (2.10 hr) and increased in elimination half life of sample C (3.70 hr) was observed when compared to sample A but the difference was not statistically significant ($p \leq 0.05$).

The volume of distribution of the non-exposed sample A is 37.20 L which was higher than the exposed samples B (21.17 L) and sample D (32.45 L) when compared but

lower than for sample C (47.30 L). There was no significant difference between sample A and the rest of the samples when compared at $p \leq 0.05$.

Total clearance for non-exposed ciprofloxacin tablet sample A was 8.67 L/hr which was lower than for drug sample C (8.96 L/hr) and sample D (17.94 L/hr) but lower than value of sample B (7.22 L/hr). Significant difference was not detected also in this comparison.



Sample A is the non-exposed (reference) ciprofloxacin tablet while Samples B, C, and D are the samples of ciprofloxacin tablet exposed to Gombe north, Gombe central and Gombe South respectively.

Figure 4.1 Super-imposed mean salivary concentration-time graphs of all the ciprofloxacin samples (A, B, C and D).

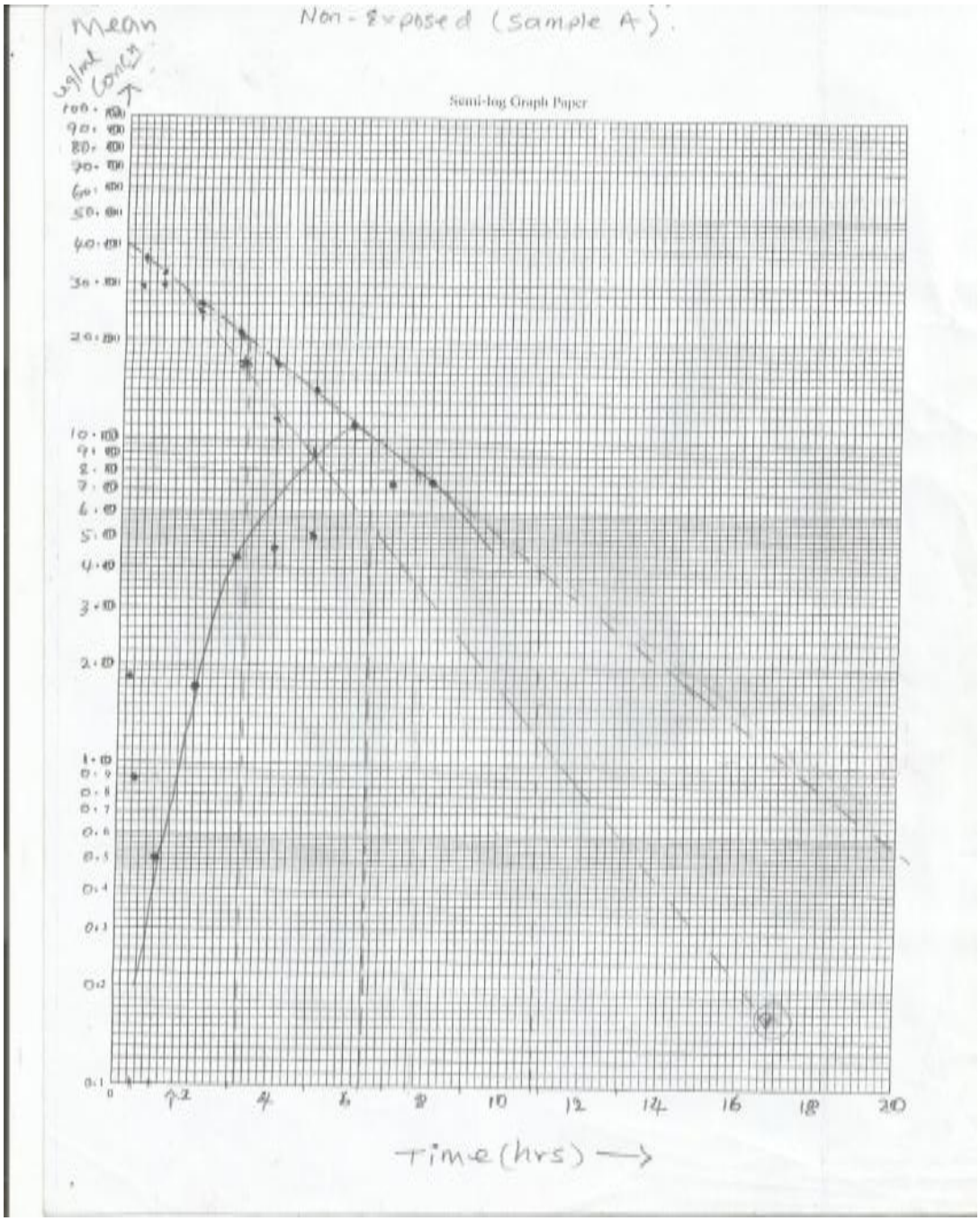


Figure 4.2 Semi-log plot of mean concentration-time profile of non-exposed ciprofloxacin tablet (sample A)

Table 4.1 Mean pharmacokinetic parameters of Sample A, B, C, and D of ciprofloxacin tablets.

Parameters	Sample A (Non-exposed)	Sample B	Sample C	Sample D
Lagtime (hr)	2.33 ± 0.49	1.67 ± 0.61	1.87 ± 0.42	2.45 ± 0.94
$T_{1/2\alpha}$ (hr)	1.77 ± 0.32	1.30 ± 0.12	1.28 ± 0.26	1.10 ± 0.25
$K\alpha$ (hr^{-1})	0.46 ± 0.07	0.57 ± 0.07	0.67 ± 0.15	1.09 ± 0.48
T_{max} (hr)	6.30 ± 0.33	6.50 ± 0.34	5.50 ± 0.76	5.50 ± 0.62
C_{max} (µg/ml)	12.60 ± 3.39	14.85 ± 2.14	9.34 ± 1.30	9.31 ± 2.01
$AUC_{0—8}$ (µg/ml.hr)	37.92 ± 8.72	55.47 ± 8.91	41.76 ± 9.28	34.68 ± 10.48
$AUC_{8-\infty}$ (µg/ml.hr)	33.38 ± 8.42	25.64 ± 5.68	37.56 ± 7.93	12.39 ± 4.99
$AUC_{0-\infty}$ (µg/ml.hr)	71.29 ± 15.14	81.11± 13.79	79.31 ± 16.38	47.07 ± 15.21
Vd (L)	37.20 ± 9.59	21.17 ± 3.56	47.30 ± 16.11	32.45 ± 7.40
$K\beta$ (hr^{-1})	0.28 ± 0.07	0.35 ± 0.04	0.20 ± 0.03	0.64 ± 0.23*
$T_{1/2\beta}$ (hr)	3.03 ± 0.52	2.10 ± 0.23	3.70 ± 0.40	1.63 ± 0.35*
Cl (L hr^{-1})	8.67± 1.61	7.22 ± 1.30	8.96 ± 3.03	17.94 ± 5.15

All mean parameter values ± SEM. n=6 and * signify a significant difference between the Non-exposed (reference) and the exposed samples at $p \leq 0.05$ using One-way ANOVA with Post-Hocs (Dunnett's) test. A=Sample A (non exposed), B=Sample B exposed to Gombe North, C= Sample C exposed to Gombe central, D=Sample D exposed to Gombe South.

4.2 Method Development and Validation of the Analytical Methods

U.V spectrophotometric method for the analysis of ciprofloxacin tablet was adopted from Gogulamudi and Sujana, (2012). It was modified and validated in line with the guidelines given by the international conference on harmonization (ICH, 1995).

4.2.1 Development of the analytical methods

Solubility of the standard ciprofloxacin powder was tested in both aqueous and non-aqueous solvents. Distilled water was found to be most suitable and served as working solvent. Ciprofloxacin was found to be slightly soluble in chloroform, methanol and ethanol but insoluble in acetone as shown in table 4.2. An analytical wavelength of 271 nm was obtained when a 4 µg/ml of ciprofloxacin hydrochloride was scanned through a workable UV wavelength region of 200-400 nm. The UV spectrum shows a plot of the absorbance verses wavelength (nm) where the peaks indicate the presence of the analyte. Peak absorbance of about 0.300 at 271 nm wavelength as shown in the figure 4.3 indicated that ciprofloxacin can be detected within that region and the concentration of ciprofloxacin used is within the detectable limit of the instrument.

Table 4.2 Solubility of ciprofloxacin standard powder in various solvents

Solvent	Solubility (mg/ml)	Inference
Water	20	Highly soluble
Chloroform	/	Slightly soluble
Methanol	/	Slightly soluble
Ethanol	/	Slightly soluble
Acetone	/	Insoluble

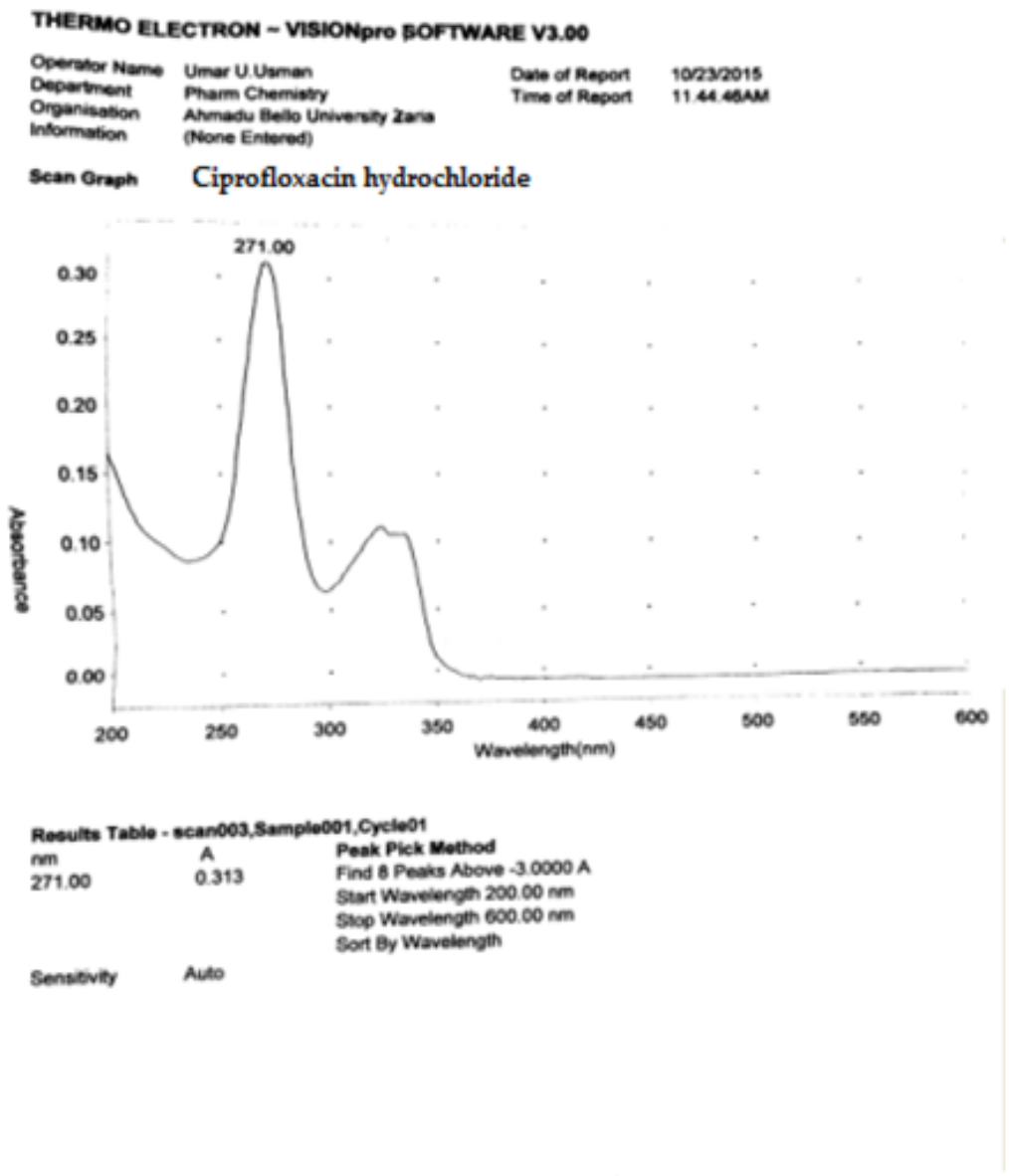


Figure 4.3 Spectrum of 4 µg/ml solution of ciprofloxacin hydrochloride standard powder at 200-400 nm UV/VIS range

4.2.2 Validation of the analytical method

4.2.2.1 Precision test

The international conference on the harmonization (ICH, 1995) guidelines specifies an acceptable limit of ≤ 2%. The within-day precision is 1.1% while the between-day precision is scored 1.5%. The developed method is more precise in the within-day than the between day although they are all within the acceptable limit of ≤ 2% as shown in table 4.3.

Table 2.3 Within-days and between-day precision of 4 µg/ml ciprofloxacin hydrochloride solution

Concentration (µg/ml)	Within-day	Between-day
4	0.618	0.618
4	0.619	0.619
4	0.616	0.598
4	0.631	0.603
4	0.629	0.612
4	0.623	0.611
Mean	0.623	0.610
RSD (%)	1.1	1.5

Precision test is expressed as percentage relative standard deviation (%RSD). n=6.

4.2.2.2 Accuracy and percentage recovery of standard ciprofloxacin Hydrochloride powder spiked in blank saliva.

The mean percentage extraction recovery values of 94, 98.8 and 103% were obtained. Percentage extraction recovery of 98.8% was within the acceptable range but 94 and 103% were outside but very close to the acceptable range (Table 4.4). The ICH 1995 guidelines expressed accuracy as percentage recovery and 98-102% is the acceptable limit.

Table 4.4 Accuracy and percentage recovery of standard ciprofloxacin hydrochloride powder spiked in blank saliva

S/No	Amount added (µg/ml) n=3	Amount recovered (µg/ml) n=3	Percentage recovery (%)
1	6	6.18	103.0
2	7	6.58	94.0
3	10	9.88	98.8

4.2.2.3 Calibration curve

Linear salivary calibration curve for standard ciprofloxacin powder over the concentration range of 1-6 µg/ml (using the modified extraction method of Sharma *et al.*, (2010) with distilled water and UV spectrophotometric detection at 271 nm) is shown in figure 4.4. A correlation coefficient (r) of 0.988 and linear equation y=0.079x - 0.052 were obtained indicating that the curve is linear. This shows that there is a good correlation between response of the detection system (U.V spectrophotometer) and the concentration of the ciprofloxacin concentrations in the saliva.

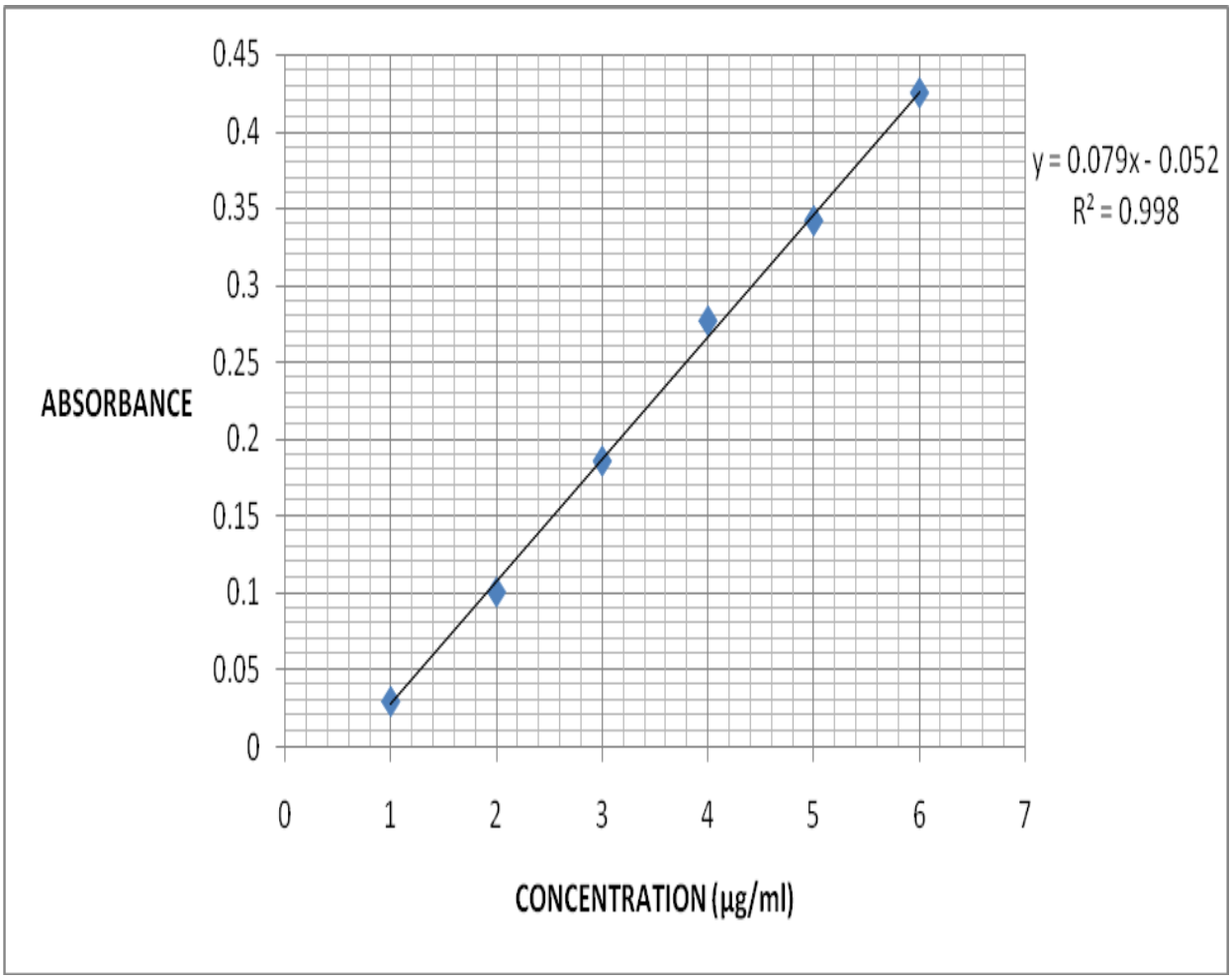


Figure 4.4 Calibration curve of ciprofloxacin hydrochloride

4.3 In-Vitro Quality Control

4.3.1 Package labeling of the ciprofloxacin tablet

The label information of the sample ciprofloxacin tablet collected directly from pharmaceutical company (EVANS) includes: the batch number, NAFDAC registration number, manufacturing date, expiring date and country of origin are shown in table 4.5. It showed that the drug was legally imported from India and is within its shelve life.

Table 4.5 Package labeling of the ciprofloxacin tablet

Brand Name	NAFDAC No	Mfd date	Exp date	Batch No	Country of origin
Rapidflox®	04-3221	02/05/14	01/05/18	E 413	India

Mfd = manufacture, Exp =expire, NAFDAC=National agency for food and drug administration and control

4.3.2 Identification test for ciprofloxacin hydrochloride powder and tablet samples A, B, C and D

A chloride test using B.P 2002 was adopted for both the ciprofloxacin hydrochloride standard powder and the tablet samples A, B, C and D. They all gave a white precipitate which confirmed the presence of ciprofloxacin hydrochloride. The identification test for the standard powder was done to guarantee its use in preparing the calibration curve and in method development. While test for the tablet samples were carried out for quality control purpose and to allow for comparison so as to assess the effects of exposure on their quality.

4.3.3 Percentage friability test for ciprofloxacin tablet

The non-exposed drug sample (A) and all the exposed samples (B, C, D) passed the friability test with values less than 1% according to the B.P 2009 specification. However, samples exposed to Gombe North and Central had the highest values of 0.46 each, followed by Gombe South (0.16) then the non-exposed sample A (0.00) which had the lowest friability value (Table 4.6).

Table 4.6 Percentage friability test for ciprofloxacin tablet samples

Samples	%friability	Remarks
A	0.00	Passed*
B	0.46	Passed
C	0.46	Passed
D	0.16	Passed

Sample A is the non-exposed ciprofloxacin tablet, sample B, C and D are the ciprofloxacin tablets exposed to Gombe north, Gombe central and Gombe south respectively. n=6.

* Samples that passed the friability test with score less than 1% according to B.P 2009 specification

4.3.4 Disintegration time (Min) for ciprofloxacin tablet

B.P 2009 specifies a maximum of 30 min as the acceptable limit for the disintegration time of a firm coated tablet when placed in a medium of 0.1N HCl at 37^{o} C. All the ciprofloxacin tablet sample drugs passed the test with disintegration time of 2.94 ± 0.30 for sample A, 4.03 ± 0.59 for sample B, 3.88 ± 0.83 for sample C and 4.21 ± 0.76 min for sample D which are less than 30 min. The disintegration time for the non-exposed sample A is shorter than the disintegration times for samples B, C and D but not statistically different when compared at $p \leq 0.05$ as shown in table 4.7.

Table 4.7 Mean disintegration time for the non-exposed and exposed samples (Min) of ciprofloxacin tablet

Samples	Mean disintegration time (Min)	Remarks
A	2.94 ± 0.30	Passed
B	4.03 ± 0.59	Passed
C	3.88 ± 0.83	Passed
D	4.21 ± 0.76	Passed

Sample A is the non-exposed ciprofloxacin tablet, sample B, C and D are the ciprofloxacin tablets exposed to Gombe North, Gombe central and Gombe south respectively. n=6. Parameter values are mean ± SEM. SEM is standard error of the mean. Passed= samples with disintegration time of ≤ 30 min.

4.3.5 Dissolution test for ciprofloxacin tablet

The B.P 2009 specifies that at least 70% of the drug be released in the dissolution medium at 45mins. The non-exposed ciprofloxacin tablet (98% for sample A) and two of the exposed samples to Gombe North (124% for sample B) and Gombe Central (96% for sample C) passed the test while the one exposed to Gombe South (66% for sample D) failed. The exposed samples C and D gave lower % drug released compare to the non-exposed sample A with the exception of sample B as shown in the table 4.8.:

Table 4.8 Dissolution rate of ciprofloxacin tablet in 0.1N HCl at 45 min

Samples	**Sample A**	**Sample B**	**Sample C**	**Sample D**
Drug released (%)	98	124	96	66
Remarks	Passed	Passed	Passed	Failed

Sample A is the non-exposed ciprofloxacin tablet, sample B, C and D are the ciprofloxacin tablets exposed to Gombe North, Gombe central and Gombe south respectively. The B.P 2009 specifies that at least 70% of the drug be released in the dissolution medium at 45mins. Passed = samples with % drug released ≥ 70%. Failed = samples with % drug released ≤ 70%.

4.3.6 Assay of the non-exposed and the exposed samples of ciprofloxacin tablet

The B.P 2002 specification for the acceptable limit of percentage content of ciprofloxacin tablet is 95-105%. The non-exposed (sample A) and the exposed (sample B) ciprofloxacin tablet passed the test with assay values of 98.3% and 97.2% respectively while the remaining exposed samples C and D failed the test with values 93.9% and 82.9% respectively as shown in table 4.9. The non-exposed sample A recorded the highest assay value while the exposed sample D has the lowest.

Table 4.9 Assay of the non-exposed and the exposed samples of ciprofloxacin tablet

Samples	% Content	Remarks
A	98.3	Passed*
B	97.2	Passed
C	93.9	Failed‡
D	82.9	Failed

Sample A is the non-exposed ciprofloxacin tablet, sample B, C and D are the ciprofloxacin tablets exposed to Gombe north, Gombe central and Gombe south respectively. *Samples that scored between 95-105% according to BP 2002 specifications. ‡Samples that scored less than 95% according to the BP 2002 specifications

CHAPTER FIVE

DISCUSSION

The ciprofloxacin tablet sample was collected directly from a pharmaceutical company. Its package label information was examined and found to have a NAFDAC registration number, manufacturing date, batch number, expiry date and the name of its country of origin. This indicates that the drug product was legally imported and registered with the Nigerian drug regulatory body: National Agency for Food and Drug Administration and control (NAFDAC).

The *in-vivo* study was aimed at assessing the effects of environmental exposure on the pharmacokinetic profiles of ciprofloxacin tablet in healthy volunteers. The results of the salivary concentration-time profiles indicated that ciprofloxacin was well absorbed. The peak and the trough salivary concentrations for the non-exposed sample A were: 10.12 $\pm$ 3.94 and 7.40 $\pm$ 1.65 µg/ml respectively. While for the exposed samples were: 11.59 $\pm$ 2.75 and 8.40 $\pm$ 2.03 µg/ml for sample B, 8.83 $\pm$ 1.84 and 7.19 $\pm$ 1.46 µg/ml for sample C, and 7.10 $\pm$ 2.46, 4.48 $\pm$ 0.86 µg/ml for sample D respectively. This study showed that there is to some extent changes in the levels of ciprofloxacin in the saliva as a result of the environmental exposure.

When the mean pharmacokinetic parameters of ciprofloxacin sample A (non-exposed) was compared with each of the samples B, C and D, there was no significant difference except between sample A and sample D. The result only showed a significant difference ($P \leq 0.05$) of change in elimination half life ($t_{½\beta}$) and elimination rate

constant (Kβ). The elimination half life of sample A was found to be 3.03 hr which was significantly longer than $t_{½\beta}$ of sample D (1.63 hr). This means that ciprofloxacin sample D has a shorter half life due to the effect of environmental exposure. This could be due to the effect of exposure on the chemical structure of the ciprofloxacin that makes it more susceptible to metabolism by liver enzymes and also more easily excreted by the kidney. It could also be due to the very low percentage content of drug sample D compared to sample A. Shorter half life means the drug will be quickly removed from the body. This is evident from the elimination rate constant of drug sample D (0.64 hr^{-1}) which is higher than the Kβ of sample A (0.28 hr^{-1}). This means more of the drug sample D was removed from the body than sample A. Elimination half life is the time taken for half of the amount or concentration of drug to be removed from body. On the other hand, elimination rate constant is the proportionality constant that relates the amount or concentration of drug in the body to its rate of elimination. That means, the higher the elimination rate constant, the shorter the elimination half life ($t_{½\beta}$). The study therefore, showed an inverse relationship between Kβ and $t_{½\beta}$ and also proved that the elimination of the exposed and non exposed ciprofloxacin followed first order kinetics. The clinical implications of this result can be seen when parameters that determine bioavailability and antibacterial activity such as C_{max} and $AUC_{0-\infty}$ of the exposed samples were compared to that of the non-exposed sample A. Sample D had low Cmax (9.31 µg/ml) and $AUC_{0-\infty}$ (34.68 µg/ml.hr) when compared to the non-exposed sample A (12.60 µg/ml and 71.29 µg/ml. hr) respectively. The difference although was not statistically significant at ($P \leq 0.05$), but the exposed sample D may have a reduced therapeutic efficacy. This might result into both bacteriological and clinical failure and probable development of drug resistance. Although the reduction in the C_{max} and $AUC_{0-\infty}$ of exposed sample D was not statistically significant, but that did

not mean clinical insignificance. Achieving bacteriological and clinical efficacy in antibacterial chemotherapy according to some authors requires favorable pharmacokinetic-pharmacodynamic (PK/PKD) parameters (AUC/MIC ratio of $\geq$ 25-30 and C_{max}/MIC ratio of $\geq$ 10-12) for immune-competent patients (Craig, 2001; Jacobs, 2001). In order to achieve any of these clinical goals, high plasma and tissue (saliva) concentrations have to be maintained. Ciprofloxacin has a concentration dependent pattern of antibacterial activity which means the higher the concentration the more the bacterial killing (Jacobs, 2001). This shows that reduced elimination half life and increased elimination rate constant of ciprofloxacin due to exposure to environmental forces could affect the concentration time profiles and hence therapeutic efficacy of ciprofloxacin tablet.

Changes in some pharmacokinetic parameters were recorded due to the effects of environmental exposure although they were not statistically significant. Volume of distribution of the non-exposed ciprofloxacin sample A was found to be fairly in close agreement with the exposed samples C and D but larger than the exposed sample B although, the differences were not found to be statistically significant. The discrepancies between sample A and B could be due to the manner sample B was stored indicating less exposure. When there is less exposure there will be high concentration in the central compartment and hence low Vd. When the clearance of the non-exposed sample A was compared to the other samples B, C and D, there was no statistical difference found. However, while exposed sample B and C have close agreement, exposed sample D had higher value. The high clearance value of sample D is a reflection of the significantly shorter half life and larger elimination rate constant when compared to sample A. High clearance of sample D means the drug is removed

more quickly from the body.. The lag-time of sample A (non-exposed) when compared with the exposed samples, the value of sample A closely agree with sample D but was longer than values of exposed sample B and D.

There was no statistical difference found between absorption half life ($t_{½\acute{a}}$) and absorption rate constant (K_{α}) of non-exposed sample A and the exposed samples. However sample D recorded the highest K_{α} and the shortest $t_{½\alpha}$ indicating fast absorption. Absorption half life ($t_{½\alpha}$), absorption rate constant (K_{α}) and time to reach maximum concentration (T_{max}) are the pharmacokinetic parameters that determine the rate of absorption. The factors that influence these pharmacokinetic parameters of absorption are the physico-chemical properties of the drug such as solubility, partition coefficient, friability, disintegration, dissolution and other physiological factors like gastro intestinal tract (GIT) pH, intestinal motility and gastric emptying time.

When non exposed sample A ciprofloxacin tablet was compared with the exposed samples, no correlation was noticed between friability, disintegration and the *in vivo* parameters. However, when assay and dissolution test results were compared with the absorption rate constant and absorption half life, a correlation was observed. The lower the percentage content and dissolution rate, the shorter the absorption half life and the higher the absorption rate constant. This means that absorption of the ciprofloxacin is faster at lower concentration in the gut and slower at higher dose. The exposed drug has reduced concentration in the gut due to environmental degradation (quantitative change). This study therefore showed that the rate and extent of absorption of ciprofloxacin tablet may be affected by its percentage content or dissolution rate. Hence, *in vitro* quality control parameters could correlate with *in vivo* pharmacokinetic parameters.

U.V spectrophotometric method for the analysis of ciprofloxacin tablet was adopted and validated in line with the guideline given by the International Conference on Harmonization (ICH, 1995). When 4 μg/ml of the ciprofloxacin solution was prepared and scanned through a UV/VIS region of 200-400 nm wavelengths, a maximum absorbance was obtained at 271 nm. The linearity of the method was tested to ensure compliance with Beer Lambert's law. This was done through construction of a calibration curve by plotting the absorbance against concentration. A concentration range of 1-6 ug/ml was chosen and a linear curve was obtained with a correlation coefficient of 0.998 indicating compliance with Beer-Lambert's law.

The method adopted was validated through tests for precision, linearity, and % extraction recovery. Validation is a process of establishing documented evidence which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and equality characteristics (Gogulamudi and Sujana, 2012). The precision of the method was demonstrated by within (intra-day) and between (inter-day) studies. The result showed a percentage relative standard deviation (% RSD) for the within day and between day studies as 1.1% and 1.5% respectively. The values are within the specified limit of RSD $\leq$ 2% as specified by International Conference on Harmonization (ICH, 1995) guidelines. This result confirmed the repeatability of the adopted method. The percentage extraction recovery was found to be almost within the accepted limit of 98-102% according to the ICH 1995 guidelines. The values obtained were 94%, 98.8% and 103%. Percentage extraction recovery is a validation test for the extraction method used in this study and hence it also validated the developed method. It is a measure of the extraction efficiency of the analytical process reported as a percentage of the known

amount of analyte. The extraction efficiency of the extraction method can generally said to be satisfactory.

In vitro quality control tests were done which comprises of identification test, friability test, disintegration time test, assay and dissolution tests. Results from the *in vitro* study showed that the non-exposed drug (sample A) and all the three exposed samples B, C and D passed both the friability test and the disintegration test. The percentage friability values for all the samples were 0.00, 0.46, 0.46, and 0.16 % respectively which are less than 1% indicating that all the samples passed the compendial specification. Friability is used to assess or measure tablets' resistance to abrasion. It ensures that the tablets remain intact from the point of manufacturing, storage, transport and dispensing to patient. Although all the samples passed the friability test, this study showed that the friability value of the exposed samples are slightly higher than for the non-exposed. The change in the friability values may be due to the effects of the environmental factors on the excipients (Bajaj *et al*., 2012). The non-exposed drug sample A and the exposed samples B, C and D disintegrated in less than 30 min thus meeting the required standard as specified in B.P 2009. Although sample A with a disintegration time of 2.94 $\pm$ 0.39 min differ slightly with the rest of the exposed samples, but the difference is not significant statistically. Disintegration times of sample B, C and D were 4.03 $\pm$ 0.59, 3.88 $\pm$ 0.83, and 4.21 $\pm$ 0.76 min respectively. The disintegration rate is an important factor in determining the drug dissolution and drug absorption. When tablets are administered, they disintegrate into granules and small fragments resulting into increase in their effective surface area. The test therefore determined the ability of the tablet to disintegrate within a reasonable period in the gastrointestinal tract (GIT). Thus, increase in disintegration time may delay the rate of dissolution and hence rate of

absorption. This may affect the therapeutic efficacy of the drug product (Udoma *et al*., 2011). This may be particularly critical in the treatment of acute infections. The slight increase in the disintegration time may be due to the effect of the exposure on the excipients like the binders and the disintegrants as reported by Bajaj *et al*, (2012).

The *in vitro* dissolution test results indicates that the non-exposed sample A and the exposed drug samples B and C passed the dissolution test with percentage content released of 98%, 124% and 96% respectively. The B.P 2009 specification is that at least 70% of the drug content should be released in the dissolution medium (0.1N HCl) at 45 min. Sample D can be said to have failed because the percentage drug content has reduced far below the minimum required value of 70%. This reduction in the percentage content released could be due to the effect of the exposure of the samples to severe environmental factors like heat, light and humidity in the Gombe south zone. This could mean poor storage condition. The idea of an *in-vitro* dissolution testing is to determine the rate and extent of dissolution of the drug from its dosage form under certain controlled conditions (Hedaya, 2007). Since this drug samples were sourced from the same batch, it follows that the differences in the dissolution rate between the non-exposed and the exposed sample D could be due to the effect of the physical factors like heat, light and relative humidity on the exposed drug sample. The *in-vitro* dissolution testing is a valuable test for the quality of the solid dosage form. For immediate release dosage form, a good correlation usually exists between *in-vitro* dissolution and *in-vivo* absorption when the dissolution rate of the drug is the rate limiting step for drug absorption. The assay results gave percentage contents of non-exposed drug sample A and the exposed sample B as 98.3% and 97.2% respectively. This indicates that sample A and B have passed the assay test specified by B.P 2002.

The acceptable limit is 95-105% according to the BP 2002 specifications. However, exposed samples C and D failed with percentage contents of 93.9% and 82.9% respectively. The low percentage content of sample C and D could be due to degradation caused by exposure to heat, light and relative humidity.

The *in vitro* study therefore showed that the non-exposed sample A drug which is our reference drug complied with all the compendial specification in B.P and U.S.P. This study agree with study conducted by Ngwuluka *et al.* (2009) where all the six brands of ciprofloxacin tablets marketed in Nigeria passed all the required tests. The assay result showed that the amount of drug in the non-exposed sample was in agreement with the labeled claims of the formulation.

CHAPTER SIX

CONCLUSION, SUMMARY AND RECOMMENDATIONS

6.1 Summary

The quality control of ciprofloxacin tablet sample was carried out using 2002, 2009 B.P and USP 2005 standards. The parameters determined were identification, assay, disintegration, dissolution and friability tests. The results indicated that both the exposed and the non-exposed drug samples showed positive to identification and passed both friability and disintegration tests. While the dissolution and the assay parameters of the exposed sample D was less than the accepted limit of ≥ 70% and 95-105% respectively indicating less quality compared to others. The method that was applied was adopted and validated by U.V/VIS spectrophotometry. The parameters for both method adopted (i.e solubility and λmax) were determined and validated. The validation parameters used were: precision (within day and between day) measured as percentage relative standard deviation (% RSD), linearity, and percentage extraction recovery.

In the *in vivo* studies, the following pharmacokinetic parameters were generated: C_{max}, T_{max}, $AUC_{0-\infty}$, lag time, $t_{1/2\alpha}$, $t_{1/2\beta}$, $K\alpha$, $K\beta$, Vd, Cl. They were compared at $P \leq 0.05$ between the non exposed (sample A) and the exposed samples B, C, and D respectively. When sample A (non-exposed) was compared with each of the samples B, C, and D, there was no significant difference except between sample A and D which showed a significant change in elimination half life and elimination rate constant. This may be due to effect of the exposure in the zone. Elimination half life and elimination rate constant are parameters that determine how drugs are removed from the body.

High elimination rate constant means the exposed drug will have shorter half life. This may give a sub-therapeutic drug level and loss of antibacterial activity.

6.2 Conclusion

It can be concluded from this study that exposure to environmental factors in Gombe South significantly affected the elimination half life and elimination rate constant of ciprofloxacin tablet and which could have a significant clinical implications.

6.3 Recommendation

It is recommended that Pharmacokinetic studies of this kind should be extended to other antibiotics in Gombe state.

6.4 Contributions to knowledge

The study was able to adopt, validate and applied an analytical method for *in vivo* quality assessment of ciprofloxacin. The study established an *in vitro* quality control parameters of ciprofloxacin tablet exposed to environmental factors in three zones of Gombe state. The study also generated and compared the pharmacokinetic profiles of ciprofloxacin between the non-exposed and the exposed samples and found a significant change in two of the parameters of the exposed sample in zone D.

REFFERENCE

Abdulkadir, A., Usman M, Shaba A, and Saidu S. (2013). An appraisal of the of eco-climatic characteristics in Northern Nigeria. *African Journal of Environmental Science and Technology, 7*(8), 748-457.

Adegbolagun, O., Olalade O, and Osumah S. (2007). Comparative evaluation of the biopharmaceutical and chemical equivalence of some commercially available brands of ciprofloxacin hydrochloride tablets. *Tropical journal of pharmaceutical research, 6*(3), 737-745.

Adler, D and Maier, H, (1989). Gyrase inhibitor ciprofloxacin in human parotid saliva. *Journal of Clinical Chemistry and Clinical Biochemistry, Zeitschrift fur Klinische Chemie und Klinische Biochemie*. 27(4):232-233] (PMID:2738528)

Ali, K. F. (2014). Estimation and eveluation of the effect of pH on ciprofloxacin in drug formulations. *Journal of Chemical and Pharmaceutical Resaerch, 6(4),* 910-916.

Ali, S.A., Mmuo C.C, Abdulraheem R.O, Abdulkareem S.S, Alemika E.T, Sani, M.A and Ilyas, M. A. (2011). High Performance Liquid Chromatography (HPLC) Method Development and Validation Indicating Assay for Ciprofloxacin Hydrochloride. *Journal of Applied Pharmaceutical Science*. 01 (08): 239-243

Arthur, J., and Atkinson J. (2015). Physiological spaces and multi compartmental Pharmacokinetic models. *Transl Clin Pharmacolol, 23*(2), 1-4.

Ayman, E., and Varma M. (2012). *Oral Absorption, Intestinal Metabolism and Human Oral Bioavailability*: Dr. James Paxton. ISBN: 978-953-51-0099-7, InTech, pp 1-21. Available from: http://www.intechopen.com/books/topics-on-drug-metabolism/oral-absorption-intestinalmetabolism-and-human-oral-bioavailability-

Bajaj, S., Singla, D, and Sakhuja, N. (2012). Stability testing of pharmaceutical products. *Journal of applied pharmaceutical science, 2*(03), 129-138.

Bushra, M. U., Huda M. N, Mostafa M, Sultan M. Z, and Rahman A. (2013). Study of forced degradation of ciprofloxacin HCl indicating stability using RP-HPLC method. *Der Pharma Chemica, 5*(6), 1-6.

British Pharmacopoeia (2002). Medicinal and Pharmaceutical Substances, (pp. 1301), London: The stationery office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA)

British Pharmacopoeia (2009). Medicinal and Pharmaceutical Substances, (pp. 1381-85, 8351-8353), London: The stationery office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA)

Brunner, M, Sta H, Hollenstein U, Mo¨ller J, Zeitlinger M, Mu¨ller M, Schrolnberger C, Eichler HG. (2002). Target Site Concentrations of Ciprofloxacin after Single Intravenous and Oral Doses. *Antimicrobial agents and chemotherapy*, 46 (12): 3724–3730. DOI: 10.1128/AAC.46.12.3724–3730.2002

Craig, W. A. (2001). Does the Dose Matter? *clinical infectious Diseases*, *33*(3), 233-237.

Danhof., M. and Breimer, D.D. (1978). Therapeutic Drug Monitoring in Saliva. *Clin. Pharmacokinet*. 3 (1): 39-57. doi: 10.2165/00003088-197803010-00003

David E. N, Goodwin S.D, Peloquin C.A, Rotella D.I, and Schentag J.J (1991). Antibiotic Tissue Penetration and Its Relevance: Impact of Tissue Penetration on Infection Response. *Antimicrobial agents and chemotherapy*, (35) 10: 1953-1959

Davis, RL, Koup JR, Williams-Warren, J, Weber, A, and Smith, AL.(1985). Pharmacokinetics of three oral formulations of ciprofloxacin. *Antimicrob. Agents Chemother*. *28(1) 74-77*. doi: 10.1128/AAC.28.1.74

Dennis, J. C., Day J, Baudys J. (2004). Evaluation of Saliva/Oral Fluid as an Alternate Drug Testing Specimen . NIJ Report 605-03. University of Utah, Center for Human Toxicology (CHT) Salt Lake City, UT 84112–9457 and Alim A. Fatah National Institute of Standards and Technology Gaithersburg, MD 20899

Dhillon, S., and Gill K. (2006). Basic pharmacokinetics. *Clinical pharmacokinetics*, 1-44.

Drusano, G.L., Standiford H.C, Plaisance K, Forrest A, Leslie J, And Caldwell J. (1986). Absolute Oral Bioavailability of Ciprofloxacin. *Antimicrobial Agents And Chemotherapy*, 30 (3): 444-446

Ehikwe, A. E., Eze F. J, and Odigbo B. E. (2015). Social marketing communications for check-mating sales of fake and adulterated drugs in Nigeria. *British Journal of Marketing Studies*, *3*(7), 30-49.

Eliopoulos, G. M., G. A, and M. R. C. Jr. (1984). In viro a activity of ciprofloxacin, a new carboxyquinoline antimicrobial agent. *Antimicrobial agents and chemotherapy*, *25*(3), 331-335.

Garcia, P. L., Buffoni E, Gomes F. P, and Quero J. L. V. (2011). *Analytical Method Validation, Wide Spectra of Quality Control, Isin Akyar (Ed.), ISBN: 978-953-307-683-6*.

Gibaldi, M., and Levy G. (1976). Pharmacokinetics in clinical practice: 2. Applications. *Jama*, *235*(18), 1987-1992.

Gogulamudi, L., and Sujana K. (2012). Development and validation of UV spectroscopic method for determination of tramadol hydrochloride in bulk and formulation. *International Journal of Pharmacy and Pharmaceutical Sciences, 4*(5), 1-5.

Gonzalez, M., Moranchel A, Duran S, Pichardo A, Magana J, Painter B, Forrest A, and Drusano G. (1985). Multiple-dose pharmacokinetics of ciprofloxacin administered intravenously to normal volunteers. *Antimicrobial agents and chemotherapy*, *28*(2), 235-239.

Gorodischer, R and Koren G (1992). Salivary excretion of drugs in children: theoretical and practical issues in therapeutic drug monitoring. *Developmental Pharmacology and Therapeutics* 19(4):161-177. (PMID:1343619)

Gurbay A, Gonthier B, Daveloose D, Favier A, Hincal F. (2001). Microsomal metabolism of ciprofloxacin generates free radicals. *Free radical biology and medicine*. 30 (10): 1118–1121

Harder, S., Fuhr U, Beermann D, Stalb AH (1990). Ciprofloxacin absorption in different regions of the human gastrointestinal tract. Investigations with the hf-capsule. *Britsh journal of clinical pharmacology*. (30)1: 35–39. doi: 10.1111/j.1365-2125.1990.tb03740.x

Hedaya, M. A. (2007). *Basic Pharmacokinetics*. Boca Raton, London, New York: CRC Press Taylor & Francis Group. Pp 1-5, 37-85, 225-237.

ICH Topic Q 2A (1995). Validation of analytical methods, Defination and Terminology. European Agency for the evaluation of Medicinal product and human medicine Evaluation. Ref. CPMP/ICH/381/95. Retrieved from hhp://www. fda.gov/guidances/ucm073381. pdf. Retrieved on 9/4/15.

Karin, M., Höld, B.S, Douwe de Boer; Zuidema J, Robert A.A. Maes, G. (1999). Saliva as an analytical tool in Toxicology. Utrecht Institute of Pharmaceutical Sciences (UIPS), Utrecht University, Department of Analysis and Toxicology, Sorbonnelaan 16, 3584 CA Utrecht, the Netherlands

Jackson, C., E., Akpabio, C., Ugwu, M., Etim, M, and Udofia, M. (2011). Quality control and in vitro bioequivalence studies on four brands of ciprofloxacin tablets commonly sold in Uyo Metropolis Nigeria. *J. Chem. Pharm. Res*, *3*(3), 734-741.

Jacobs, M. (2001). Optimisation of antimicrobial therapy using pharmacokinetic and pharmacodynamic parameters. *Clinical microbiology and Infection*, *7*(11), 589-596.

Katzung, B., Masters S, and Trevor A. (2009). Basic and Clinical Pharmacology. MaGraw-Hill Companies. *Inc., New York*, 773-778.

Khan, M. K., Khan M. F, Khan H and Mustafa G. (2009). Bioavailability of ciprofloxacin tablets in humans and its correlation with the dissolution rates. *Pak. J. Pharm. Sci, 22*(3), 329-334.

Kotte, S. C. B., Dubey P, and Murali P. (2012). Core components of analytical method validation for small molecules: an overview. *International Research Journal of Pharmacy*, 1-11.

Mallah E, Arafat B, Al khawaja B, **,** Abu Dayyih W, Abu Awad A, , Hroub A,Hamad M and Arafat T. (2015). Relative Bioavailability of Ciprofloxacin Doses (750 and 1000) mg in Healthy Male Volunteers by Using HPLC Method. Annex Publishers | www.annexpublishers.com. 1 (1): 1-6

Martinez, M. N., and Amidon G. L. (2002). A mechanisti approach to understanding the factors affecting drug absorption: A review of foundamentals. *J Clin Pharmacol*. doi: 42:620-643

McLellan, RA., Drobitch, R.K, Monshouwer, M and Renton K.W. (1996). Fluoroquinolone antibiotics inhibit cytochrome P450-mediated microsomal drug metabolism in rat and human. . *Drug Metab. Dispos.* 24**:**1134–1138.

Mu'az, J., Gazali L, Sadiq G, and Tom G. (2009). Comparative in vitro evaluation of the pharmaceutical and chemical equivalence of multi-source generic ciprofloxacin hydrochloride tablets around Maiduguri metropolitan area. *Nigerian Journal of Pharmaceutical Sciences, 8*(2).1-6

Mudie, D. M., A. G. L, and A. G. E. (2010). Physiological parametres for oral delivery and i*n vitro* testing. *Mol Pharm* 4,7 (5): 1388-1405. doi: 10.102/mp 100149j.

Neuvonen, P J., Kivisto K T, Lehto P. (1991). Interference of dairy products with the absorption of ciprofloxacin. *Clin. Pharmacol Ther*. 50(5 Pt 1):498-502

Ngwuluka, N., Lawal K, Olorunfemi P, and Ochekpe N. (2009). Post-market in vitro bioequivalence study of six brands of ciprofloxacin tablets/caplets in Jos, Nigeria. *Scientific Research and Essays, 4*(4), 298-305.

Ogar, J.N., Asira A.E, Eyimba M. (2015). NAFDAC and Health care in Nigeria: A philosophical probe. *World Journal of public health, Water and food* 1 (1): 1-12

Ogunbona, F.A., Onyeji, C.O, Bolaji O.O, and Adedoyin, A. (2014). *Pharmacokinetics: Principles and applications*. University of Ibadan, Ibadan, Nigeria: Ibadan University Press. pp 1-45, 183-223

Olaniyi, A. A. (2000). *Principles of drug quality assurance and pharmaceutical analysis*. 5 Oluware Obasa Street, Ibadan, Nigeria: Mosuro. pp 3-17, 89-111, 209-222

Olivera M.E., Barends, D.M, Manzo R.H, Junginger H.E, Midha K.K, Shah V.P, Stavchansky S, Dressman .B, Barends D.M. (2011). Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Ciprofloxacin Hydrochloride. *Journal of Pharmaceutical Sciences* 100 (1):1-12

Parry, MF., Smego, DA and Digiovanni, MA. (1988). Hepatobiliary kinetics and excretion of ciprofloxacin. *Antimicrob. Agents Chemother. 32(7): 982-98.* doi: 10.1128/AAC.32.7.982

Phillips, G., Johnson B, and Ferguson J. (1990). The loss of antibiotic activity of ciprofloxacin by photodegradation. *Journal of Antimicrobial Chemotherapy, 26*(6), 783-789.

Ramon, J., Ben-Haim, M, Shabtai, M, Rubinstein, E. (2001). Transepithelial Intestinal Excretion of Ciprofloxacin in Humans. *Clin Infect Dis. 32 (5): 822-823. doi: 10.1086/319206*

Ravichandran, V., Shalini S, Sundram K, and Harish R. (2010). Validation of analytical methods–strategies & importance. *International Journal of Pharmacy and Pharmaceutical Sciences, 2*(3), 18-22.

Shargel, L., Mutnick A. H, Souney, P. F, and Swanson, L. N. (2010). *Comprehensive pharmacy review*. 351 West Camden Street Baltimore, MD 21201, USA: Lippincott Williams and Wilkins. . pp 1-5

Sharma, P. C., Jain A, and Jain S. (2009). Fluoroquinolone antibacterials: a review on chemistry, microbiology and therapeutic prospects. *Acta Pol Pharm, 66*(6), 587-604.

Sharma A., Arora S, Grewal P, Chand P, Dhilon V, Malik K, Goel A, (2010) Comparative bioequivalence study of some brands of ofloxacin by urine and analysis in india. *International journal of drug development and research,* 2 (3): 534-546

Sharma, R. S. A. (2012). Validation of analytical procedures: a comparison of ICH vs Pharmacopoeia (USP) Vs FDA. *International Research Journal of Pharmacy, 3*(6):1-4

Soni, K. (2012). Fluoroquinolones: chemistry & action—a review. *Indo Glob J Pharm Sci, 2*, 43-53.

Sorgel, F.(1989). Metabolism of Gyrase Inhibitors *Clin Infect Dis. 11 (5): S1119-S1129*

Syeda, A. Q., Zehra A, Naqvi B, Shah S, and Bushra R. (2010). Resistance pattern of ciprofloxacin against different pathogens. *Oman Med J, 25*(4), 294-298.

Tiefenbacher, E. M., Haen E, Przybilla B, and Kurz H. (1994). Photodegradation of some quinolones used as antimicrobial therapeutics. *Journal of pharmaceutical sciences, 83*(4), 463-467.

Uduma, O. E., Agboke A. A, Amadi R. C, Okorie O, and Opurum C. C. (2011). Bioequivalence studies on some selected brands of ciprofloxacin hydrochloride tablets in the Nigerian market with ciproflox® as innovator brand. *Journal of applied pharmaceutical science, 1*(06), 80-84.

United State Pharmacopoeia (2005). National Formulary USP 26/NF21(pp1882-1883). United states: Pharmacopooeia Convention Inc.

Vance-Bryan, K., Guay D. R, and Rotschafer J. C. (1990). Clinical pharmacokinetics of ciprofloxacin. *Clinical pharmacokinetics, 19*(6), 434-461.

Wagner, J. G. (1981). History of pharmacokinetics. *Pharmacology & therapeutics, 12*(3), 537-562.

Wang, Q., Fotaki N, and Mao Y. (2009). Biorelevant dissolution: methodology and application in drug development. *Dissolution Technol, 16*(3), 6-12.

Watson, D. G. (2012). *Pharmaceutical analysis* (Third ed.). Edinburgh, London: Churchill Livingstone ELSEVIER. pp 1-27, 90-114

Yabo, M. M. (1996). *Quality assurance testing and pharmacokinetics of chloramphenicol capsules in healthy volunteers.*Unpublished Msc dissertation, ABU, Zaria, Zaria, Nigeria. pp 1-2

Zhang, H., and Yu L. (2004). Dissolution testing for solid oral drug products: theoretical considerations. *American Pharmaceutical Review, 7*, 26-31.

Zhu M, Wong, PYK, R.C.U. (1999). Effect of Oral Administration of Fennel (*Foeniculum vulgare*) on Ciprofloxacin Absorption and Disposition in the Rat. *Journal of Pharmacy and Pharmacology* 51 (12): 1391–1396 doi: 10.1211/0022357991777218

APPENDIX 1

Friability (%)

BRANDS	A	B	C	D
W1 (g)	6.39	6.41	6.42	6.41
W2 (g)	6.39	6.38	6.39	6.40
Difference	0.00	0.03	0.03	0.01
% Friability	0.00	0.46	0.46	0.16

W1=Initial weight, W2=Final weight

Disintegration time (Min)

BRANDS	A	B	C	D
	2.20	2.40	2.30	2.34
	2.25	3.22	2.34	2.48
	2.57	3.44	2.50	3.56
	3.00	3.53	3.23	4.47
	3.56	5.45	6.44	5.14
	4.05	6.13	6.49	7.28

APPENDIX II

Dissolution at 45 min (% Drug released)

Brands	A	B	C	D
% Released	98	124	96	66

Assay

Brands	Strength (g)	Equivalent of 0.3g to dissolve (g)	Final volume (mls)	% Content
A	0.5	0.295	8.90	98.3
B	0.5	0.288	8.7	97.2
C	0.5	0.282	8.50	93.89
D	0.5	0.268	8.1	82.9

APPENDIX III

Precision

Within-day	Between-day
0.618	0.618
0.619	0.619
0.616	0.598
0.631	0.603
0.629	0.612
0.623	0.611

APPENDIX IV

Saliva Concentration-time profile of drug sample A for volunteer 2

Time (hr)	Concentration (μg/ml)
0	0.00
0.25	2.48
0.5	1.61
1	0.52
2	0.39
3	1.35
4	2.08
5	5.28
6	4.70
7	7.10
8	4.80

Saliva Concentration-time profile of drug sample A for volunteer 6

Time (hr)	**Concentration (μg/ml)**
0	0.00
0.25	0.00
0.5	0.00
1	0.00
2	0.81
3	5.38
4	5.48
5	10.37
6	28.22
7	3.03
8	6.89

APPENDIX V

Saliva Concentration-time profile of drug sample D for volunteer 5

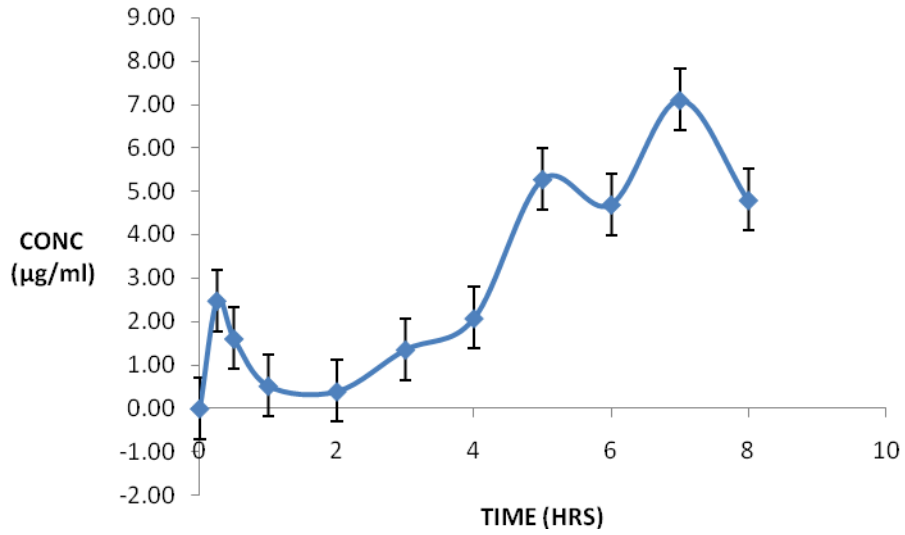
Time (hrs)	Concentration (μg/ml)
0	0.00
0.25	0.56
0.5	0.00
1	0.00
2	0.00
3	5.97
4	5.82
5	10.10
6	9.99
7	4.32
8	6.00

Saliva Concentration-time profile of drug sample D for volunteer 6

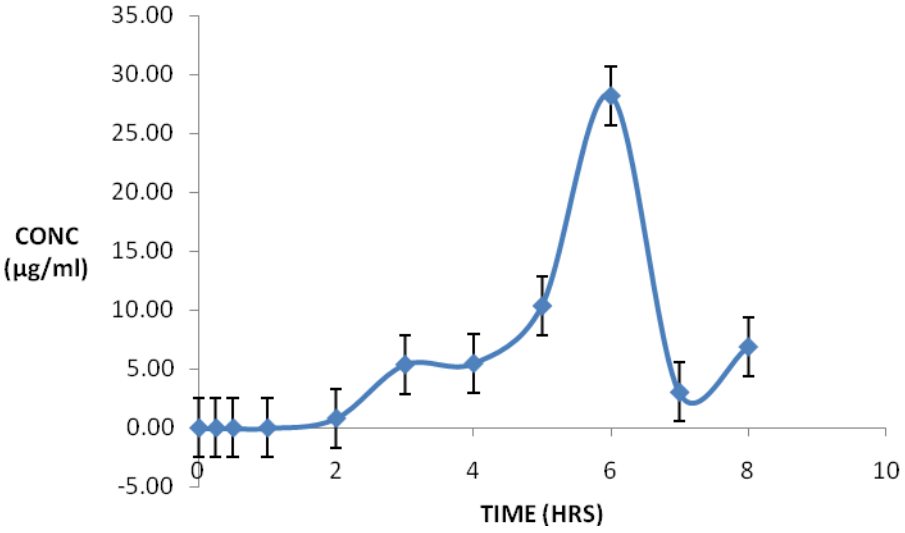
Time (hrs)	Concentration (μg/ml)
0	0.00
0.25	0.00
0.5	0.00
1	0.00
2	0.00
3	0.51
4	0.00
5	5.08
6	5.33
7	2.00
8	2.20

Appendix VI

Concentration-time curve of drug sample A for volunteer 2

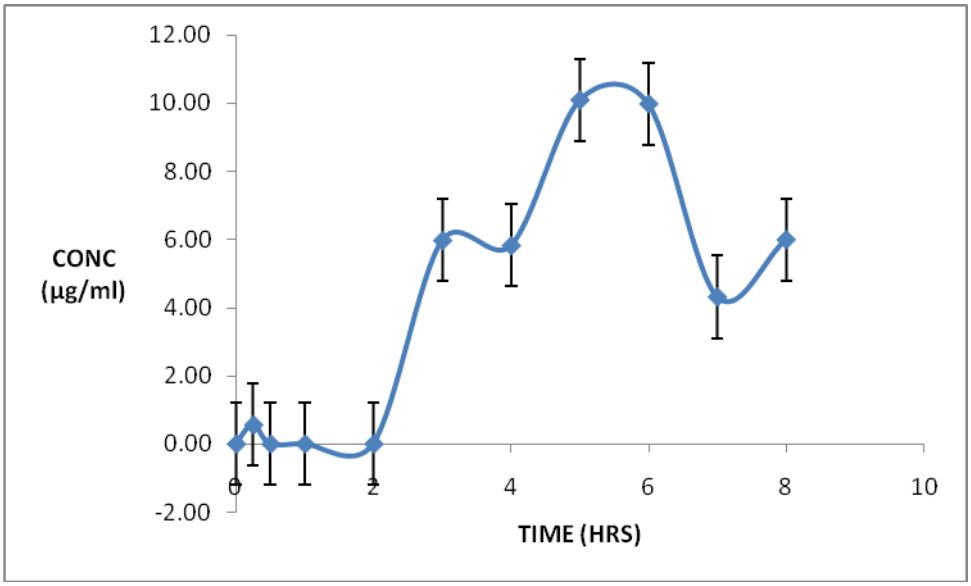


Concentration–time curve of drug sample A for volunteer 6

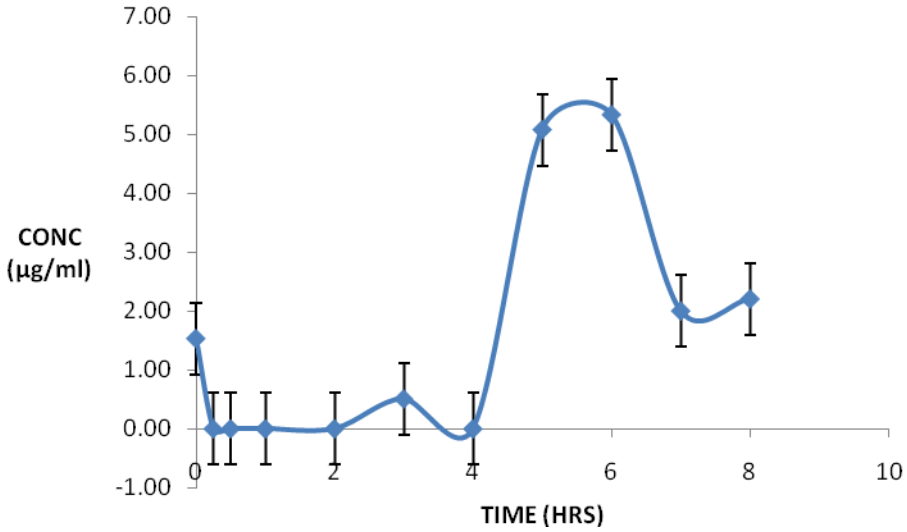


APPENDIX VII

Concentration-time curves of drug sample D for volunteer 5



Concentration-time curves of drug sample D for volunteer 6



APPENDIX VIII

Equations for % Friability, Beer Lambert's Law, % Extraction recovery

% Friability

$$\%\,\text{frability} = \frac{W_1 - W_2}{W_1} \times 100$$

Where, W_1 is the initial weight of (10) tablets

W_2 is the final weight of (10) tablets

$$\%\,\text{Extraction recovery} = \frac{\text{concentration in saliva after 6hrs}}{\text{concentration in 0.1N HCL spike in blank saliva}} \times 100$$

Beer - Lambert's Law

$$\log_{10}\left(\frac{l_o}{l_t}\right) = A = \varepsilon bc$$

where, A is the Absorbance

ε is a constant known as molar extinction coefficient

b is the path lenght in centimeters (cm)

c is the concentration of the analyte in g / 100ml

since b = 1cm,

$$\text{therefore, } c = \frac{A}{A(1\%, 1\text{cm})}$$

where, $A(1\%, 1\text{cm})$ is the absorbance of a 1%w / v solution in a 1cm cell.

Percentage Relative Standard Deviation $(\%\text{RSD})$

$$\%\,\text{RSD} = \frac{\text{S.D}}{\bar{x}} \times 100$$

where, S.D is the standard deviation

$\bar{x}$ is the mean

Appendix IX

Equations for calculating pharmacokinetic parameters

Pharmacokinetic Parameters

Absorbtion half life and elimination half life

$$t_{\frac{1}{2}\beta} = \frac{0.693}{k_{\beta}}$$

$$t_{\frac{1}{2}\alpha} = \frac{0.693}{k_{\alpha}}$$

where, $t_{\frac{1}{2}\alpha}$ is the absorbtion half life (hr)

$t_{\frac{1}{2}\beta}$ is the elimination half life (hr)

k_{α} is the absorbtion rate constant (hr^{-1})

k_{β} is the elimination rate constant (hr^{-1})

Total clearance (CL_T)

$$CL_T = \frac{F \times Dose}{AUC_{0-\infty}}$$

where, F is the bioavailability

$AUC_{0-\infty}$ is the total area under the concentration - time curve

Volume of distribution (V_d)

$$V_d = \frac{CL_T}{k_{\beta}}$$

APPENDIX X

: Equations for Area Under the concentration-time curve (AUC)

$$\text{Area under the concentration - time curve}\left(\text{AUC}\right)$$

$$\left(\text{Trapezoidal rule}\right)$$

$$\text{Area of the trapezoid} = \left(\frac{C_n + C_{n+1}}{2}\right)\left(t_{n+1} - t_n\right)$$

$$AUC_{t-\infty} = \frac{C_{last}}{k_\beta}$$

$$AUC_{0-\infty} = AUC_{0-t} + AUC_{t-\infty}$$

where, C_{last} is last measured concentration

AUC_{0-t} is the sum of the area of all the trapezoids

$AUC_{t-\infty}$ is the area of the tail

$AUC_{0-\infty}$ is the total AUC

APPENDIX XI

Gombe State Meteorological Data (2015)

Months	Temperature (^{o}C)	Relative Humidity (%)	Rain (mm)
January	30.7	15	0.00
February	37.1	17	0.00
March	37.6	21	8.40
April	37.9	20	TR
May	37.8	54	68.20
June	32.4	71	111.10

TR=Trace

Source: Nigerian Meteorological Agency (NIMET), Gombe state office

APPENDIX XII

Biodata of subjects used for the study

S/No	Code Name	Age (Years)	Weight (Kg)	Sex	Remark
1	AA	36	70	M	Healthy
2	AB	30	67	M	/
3	AC	27	57	M	/
4	AD	35	70	M	/
5	AE	40	65	M	/
6	AF	38	68	M	/

APPENDIX XIII

Henderson-Hasselbalch equations

For weak acids

$$PK_a - PH = \log \frac{(\text{un-ionized})}{(\text{ionized})} = \log \frac{(HA)}{(A^-)}$$

For weak bases

$$PK_a - PH = \log \frac{(\text{ionized})}{(\text{un-ionized})} = \log \frac{(HB^+)}{(B)}$$

Where : PH = PH of the GIT medium

PKa = P^H partition coefficient

(B) and (HA) = Concentration of the Unionized drugs

(HB^+) and (A^-) = Concentration of the ionized drugs

For example, if the P^H of the medium is equal to the PKa of the drug, 50% of the drug will be present in the ionized form and 50% in the unionized form

APPENDIX XIV

Arrhenius equations

$$K = Ae^{-Ea/RT}$$

Or,

$$\log k = \log A - \frac{E_a}{2.303RT}$$

Where K is the specific rate constant (degradation rate constant)

T is the absolute temperature (K)

E_a is the energy of activation (kJ/mol)

R is the universal gas constant (8.31×10^{-3} kJ/mol)

A is the frequency factors

APPENDIX XV

Concentration and absorbance for calibration curve of ciprofloxacin Hydrochloride

Concentration (µg/ml)	Absorbance
1	0.030
2	0.101
3	0.186
4	0.277
5	0.342
6	0.425

APPENDIX XVI

Table 4.10 Summary of the validation parameters

S/No	Parameters	Results obtained
1.	λmax	271nm
2.	Regression equation	y = 0.079x - 0.052
3.	Correlation coefficient	0.998
4.	Intercept	-0.052
5.	Range	1-6µg/ml
6.	Precision:	
	Within-day (%RSD)	1.1
	Between-day (%RSD)	1.5
7.	% Extraction recovery	98.8

%RSD is percentage relative standard deviation. λmax is maximum absorbance.

APPENDIX XVII

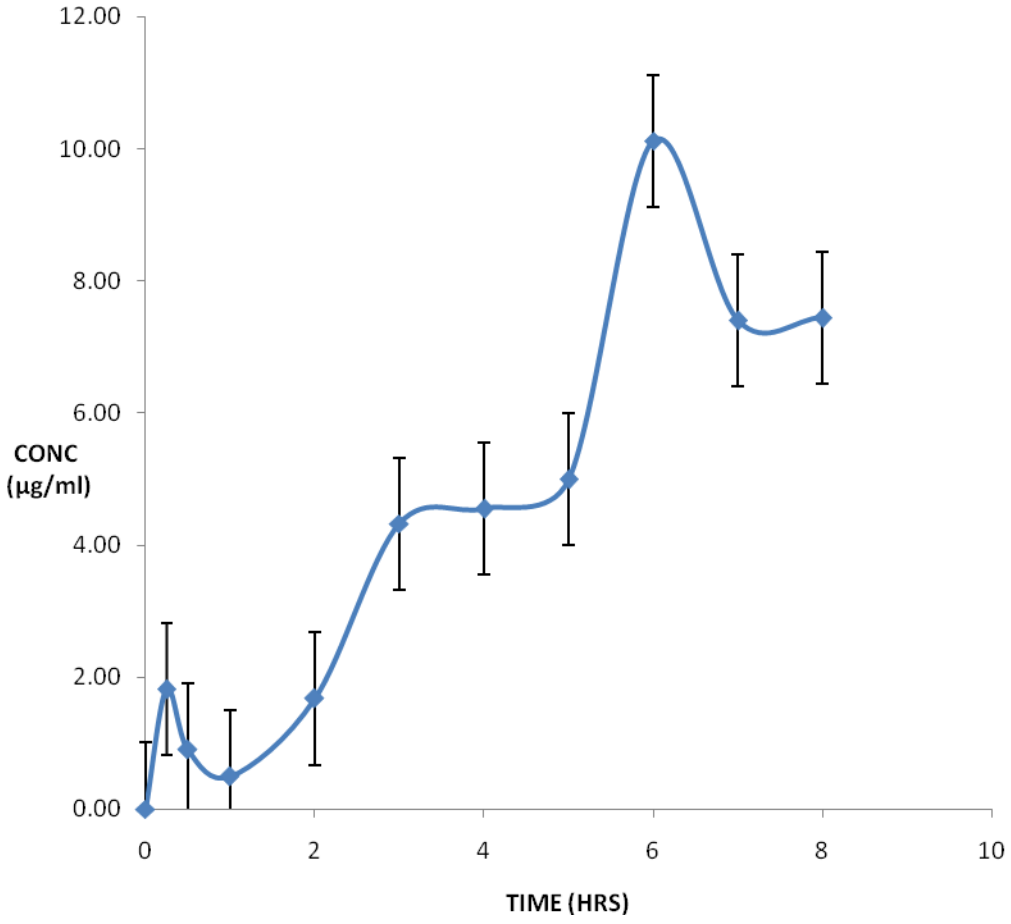
Mean (±SEM) saliva concentration-time profile of ciprofloxacin tablet samples A,B, C and D (µg/ml)

Time (hr)	Saliva concentrations (µg/ml) A	B	C	D
0.25	1.81 ± 0.86	4.37 ± 1.27	2.52±0.76	1.94±0.52
0.50	0.90 ± 0.42	4.14 ± 0.76	1.96±0.70	1.37±0.00
1	0.49 ± 0.37	5.09 ± 1.24	1.56±0.29	2.21±0.61
2	1.67 ± 0.90	4.20 ± 0.92	3.22±0.66	3.03±1.18
3	4.32 ± 1.81	6.02 ± 1.10	4.40±0.83	4.06±1.39
4	4.55 ± 1.83	6.89 ± 2.04	5.34±1.14	3.67±1.08
5	4.99 ± 1.58	6.92 ± 1.56	6.41±1.08	6.19±1.57
6	10.12 ± 3.94	11.59 ± 2.75	7.41±2.03	5.73±1.56
7	7.40 ± 1.65	9.19 ± 1.92	8.83±1.84	7.10±2.46
8	7.44 ± 1.34	8.40± 2.03	7.19±1.46	4.48±0.86

n=6. SEM is standard error of the mean. Sample A is the non-exposed ciprofloxacin tablet, samples B, C, D are samples exposed to Gombe North, Gombe Central and Gombe South respectively.

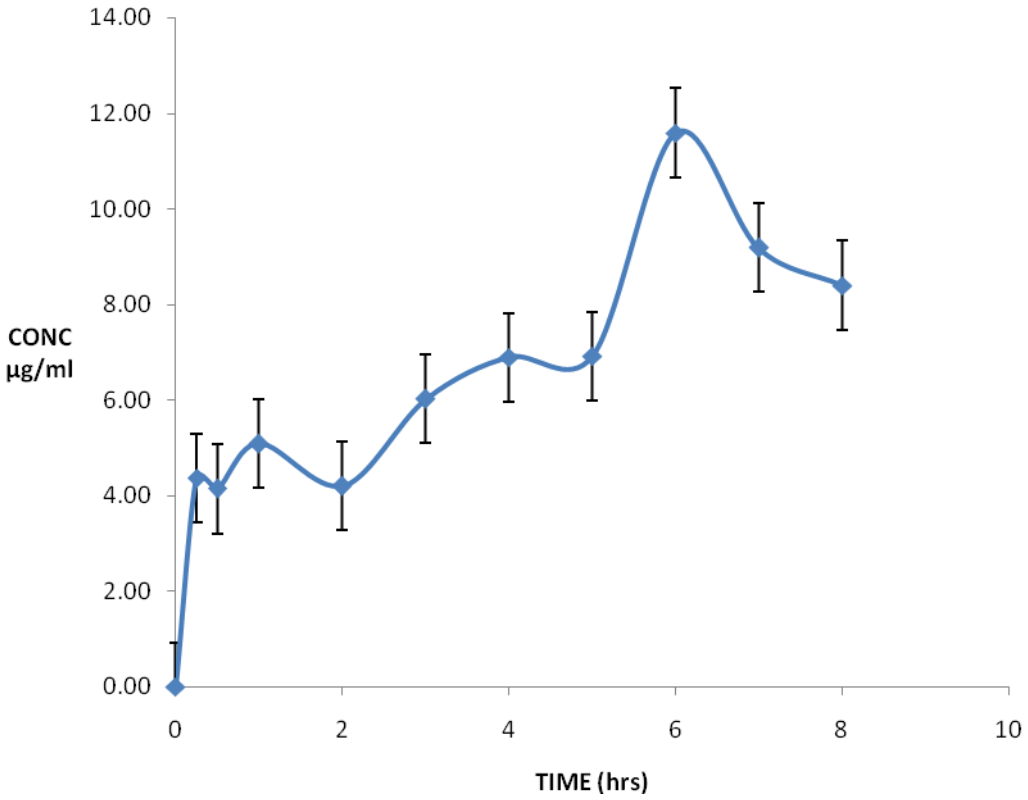
APPENDIX XVIII

Mean concentration-time curve of non-exposed ciprofloxacin tablet (sample A). Values are mean ± SEM



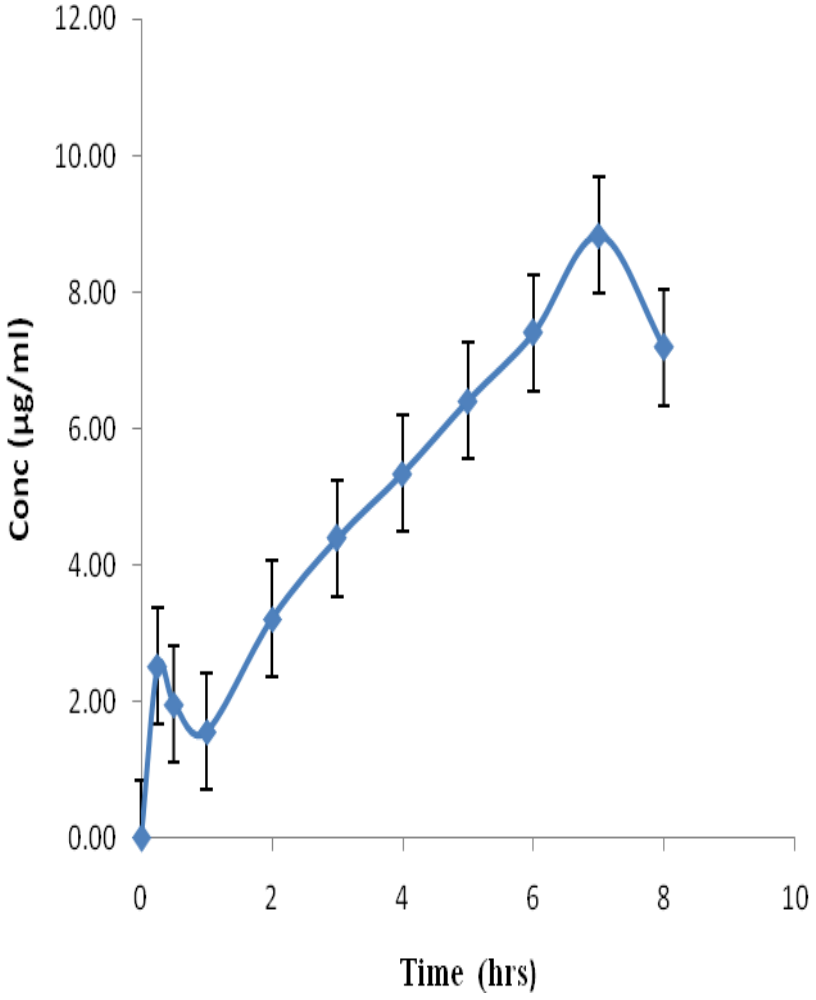
APPENDIX XIX

Mean concentration-time curve of ciprofloxacin tablet exposed to Gombe North (sample B). Values are mean ± SEM



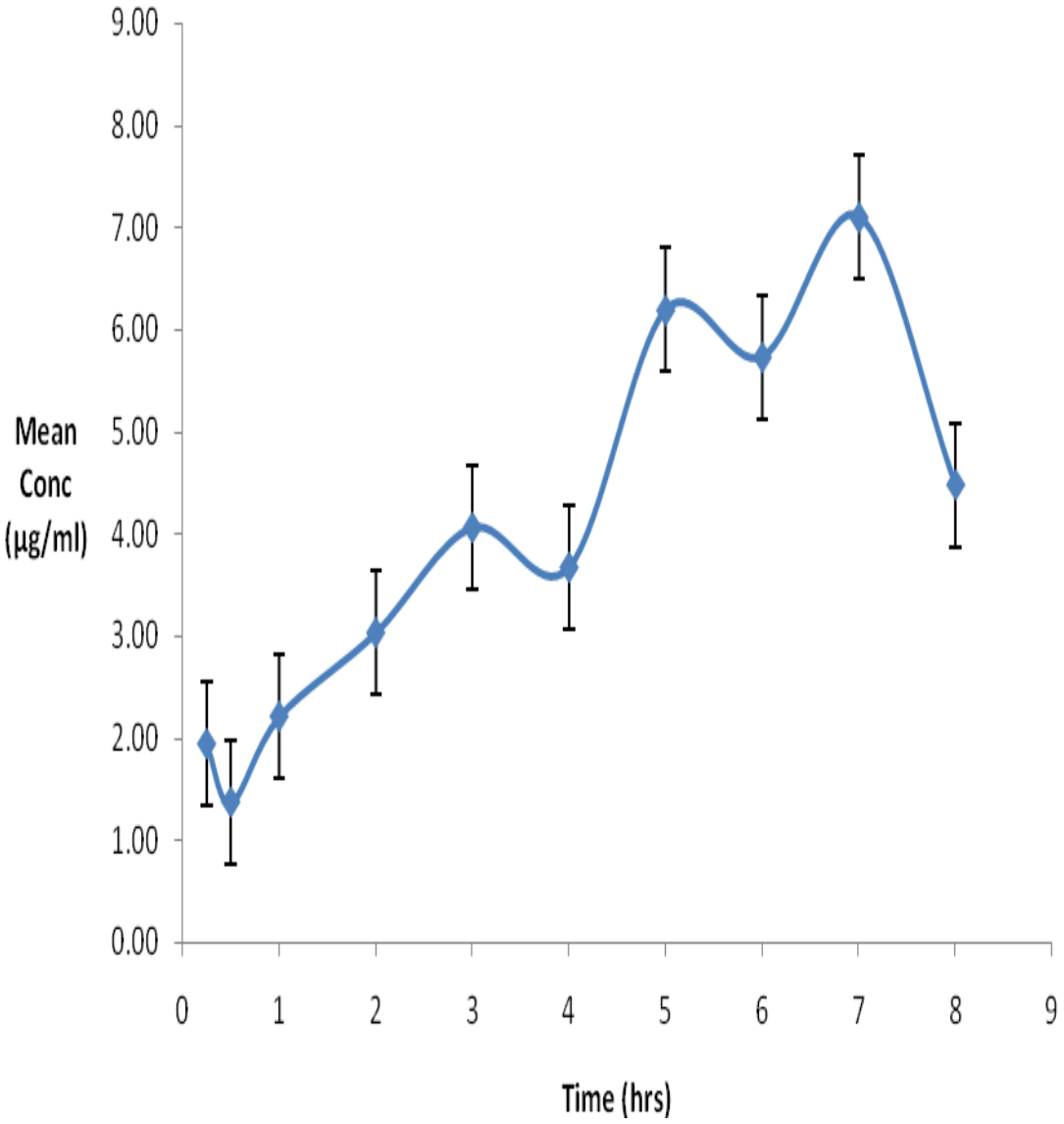
APPENDIX XX

Mean concentration-time curve of exposed ciprofloxacin tablet to Gombe central (Sample C). Values are mean ± SEM



APPENDIX XXI

Mean concentration-time curve of exposed ciprofloxacin tablet to Gombe south (Sample D). Values are mean ± SEM



APPENDIX XXII

CONSENT FORM

Department of Pharmacology and Therapeutics
Faculty of Pharmaceutical Sciences
Ahmadu Bello University, Zaria
Nigeria

Dissertation topic:
Effects of environmental exposure on the Pharmacokinetics of ciprofloxacin tablet in healthy human volunteers

We wish to request you to participate in this research study to investigate whether environmental exposure on ciprofloxacin tablet can affect its absorption, distribution and elimination in human body. Data collected from you will help improve treatment outcomes in our clinics/hospitals.

If you accept to participate, in a day you will be given one tablet of the non-exposed drug to take with water after fasting overnight and your saliva will be collected for a period of 8 hours. The same procedure will be repeated but with the exposed drug samples weekly for three times. Participation in this study is voluntary and you may decide to withdraw any time you are sick or any time you wish.

All information collected from you will be strictly confidential. There will be no unauthorized disclosure under any circumstances.

Thanks for participating.

Contact:

Name of investigator……Phone No:……Sign……

Subject's Consent

Signing this consent form indicate acceptance to participate in this study.

Name…… Sign/Thumb print…….. Date……. ……

Phone number:…….