EFFECTS OF AMPICLOX (AMPICILLIN/CLOXACILLIN), METRONIDAZOLE AND AMOXICILLIN ON THE PHARMACOKINETICS OF METFORMIN IN TYPE 2 DIABETIC PATIENTS

BY

GARBA, MUSA ABDULLAHI

DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

NOVEMBER, 2015

EFFECTS OF AMPICLOX (AMPICILLIN/CLOXACILLIN), METRONIDAZOLE AND AMOXICILLIN ON THE PHARMACOKINETICS OF METFORMIN IN TYPE 2 DIABETIC PATIENTS

BY

Garba, MUSA ABDULLAHI,
(B.Pharm.1986, M.Sc.Pharm.Chem.2008, A.B.U.)
Ph.D/Pharm-Sci/00617/2008-2009

BEING

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

IN PARTIAL FULFILLMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL CHEMISTRY

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

NOVEMBER, 2015.

DECLARATION

I declare that the work in the dissertation titled **"The Effects of Ampiclox (Ampicillin/Cloxacillin), Metronidazole and Amoxicillin on the Pharmacokinetics of Metformin in type 2 Diabetic Patients"** has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry under the supervision of Prof.M.Garba, Prof.M.T.Odunola and Prof.I.A.Yakasai. 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 at this or any other institution. The work of other investigators are acknowledged and referred to accordingly.

Garba, Musa Abdullahi

| --- | --- | --- |
| Name of Student | Signature | Date |

CERTIFICATION

This dissertation entitled **"The Effects of Ampiclox (Ampicillin/Cloxacillin), Metronidazole and Amoxicillin on The Pharmacokinetics of Metronidazole in Type 2 Diabetic Patients" by Garba, Musa Abdullahi** meets the regulations governing the award of the degree of Doctor of Philosophy in pharmaceutical Chemistry of Ahmadu Bello University Zaria and it is approved for its contribution to knowledge and literary presentation.

_____ _____ _____

Chairman Supervisory Committee. SignatureDate
Prof. M. Garba
B.Sc.Pharm.MSc,Ph.D (Pharm.Chem) ABU
Department of Pharmaceutical and Medicinal Chemistry
Faculty of Pharmaceutical Sciences
Ahmadu Bello University, Zaria.

_____ _____ _____

Member Supervisory Committee Signature Date
Prof. M.T. Odunola
B.Sc.Biochem (UNIMAID.),MSc,Ph.D (Pharm.Chem) ABU
Department of Pharmaceutical and Medicinal Chemistry
Faculty of Pharmaceutical Sciences
University of Ilorin.

_____ _____ _____

Member Supervisory Committee Signature Date
Prof. I.A. Yakassai
B.Pharm.,MSc,Ph.D (Pharm.Chem) ABU
Member Supervisory Committee
Department of Pharmaceutical and Medicinal Chemistry
Faculty of Pharmaceutical Sciences
Ahmadu Bello University, Zaria.

_____ _____ _____

Dr.A.Musa Signature Date
B.Pharm.,MSc,Ph.D (Pharm.Chem) ABU
Head of Department of Pharmaceutical and Medicinal Chemistry
Faculty of Pharmaceutical Sciences
Ahmadu Bello University, Zaria.

_____ _____ _____

Prof. Kabir Bala Signature Date
B.Sc,MSc,Ph.D (ABU)
Dean, School of postgraduate Studies
Ahmadu Bello University, Zaria.

ACKNOWLEDGEMENT

In the name of "ALLAH" the most gracious the most merciful. I want to thank Almighty "Allah Subhanahu Wata'ala" for sparing my life, giving me the opportunity and enablement in bringing this work to a successful conclusion. With special regards I want to acknowledge the efforts of numbers of individual who contributed alot for the actualization of this dream. My most sincere gratitude goes to my Supervisors,Professors M. Garba, (Mrs.) M.T. Bakare-Odunola and I.A. Yakassaiand for their kind excellent counseling, wisdom, tolerance and constant encouragement to the course of this work. I appreciate all the staff of the Faculty particularly Dr. Aminu Musa, Aliyu Musa(Head of Department), Musa Gombe,Pharm.Asmau Hamza and the present Dean and Deputy Dean Prof. U.U. Pateh and Isa Muhammed respectively for their support . I wish to express appreciation to the Chief Technologist Malam Iliya, present and past members of the Governing Council especially my able Chairperson Mrs Lauretia Mallam (former Hon. Minister of environment), entire Management and Academic Committee members of Shehu Idris College of Health Sciences and Technology, Makarfi, notably Mal. B.M. Ahmed (Registrar), Mall. Shehu A. Allahgaba (College Librarian) and Mal. Nuhu Gambo Kusfa (College Bursar) for their support and courage . I must acknowledge the contribution of Dr. Ado Zakari , his wife Dr. Maryam and Matron Jumai Wada of Gambo Sawaba memorial Hospital, Abdu Sule Kaura of Judiciary kaduna, and Habeeb Abdulkadir of Kaduna Polytechnic, Kaduna. I also wish to acknowledge the contribution of my daughter Saudatu a student of Faculty of Law and my obedient wives. I want to acknowledge the contributions of Mal. Sani (my collegue), Bashir Ibrahim (Directorconsultancy services), Shu'aibu Muhammad(Director of Academic planning) , Dr.Musa Muhammad ofNARICT

Zaria, Kabir Shehu of Kaduna Polytechnic, my staff especially Shamsuddeen,Mallam Yakubu P.A. to Bursar, Mustapha Abdullahi, Sani Sarki Maijaki, my able driver Malam Ameeru Ashiru and my messengersAdo and Mall. Mustapha) for their contributions in one way or the other towards the successful completion of this study.

Finally to my parents, I have to say (Oh Allah have mercy on them as they brought me up when I was an infant), “AL-HAMDU LILLAH.”

DEDICATION

I dedicate this dissertation to my parents, who nurtured my dream in actualizing this research, my family for their patient and support and “ummah” in general.

ABSTRACT

Infection is common in diabetes and in the course of treatment;ampiclox, metronidazole andamoxicillin may be co-administered with metformin for treatment. This study was therefore designed to evaluate the effects of co-administration of ampiclox, metronidazole and amoxicillin on the pharmacokinetics of metformin in type 2 diabetic patients.

Eighteen patients with age 25-55 years, weight range 50-70 kg, and height 1.5-1.75 m took part in the study. The study was divided into two phases with a washout period of seven days between the phases. In phase one,metformin alone was administered to all the subjects with 150 ml of water after an overnight fasting. In phase two, the subjects were divided into three groups, with six subjects ineach group. The first group received a single dose ofmetformin with ampiclox, thesecond group receivedmetformin co-administered with metronidazole and the third group received metformin with amoxicillin. Blood samples were collected at interval of 0, 0.5, 1.5, 3.0, 4.0, 6.0 and 8.0 hours and stored at

-4^{0}C before analysis. Plasma was obtained from the blood and the drug was extracted from the plasma using three times its volume of acetonitrile. The samples were analyzed for metformin using HPLC method on a reversed phase column C-8,4.6 x 150 nm, mobile phase acetonitrile/potassium dihydrogen orthophosphate (21:79), and aUV detector at 236 nm.

The absorption rate constant(K_a), mean peak plasma concentration (C_{max}), area under the curve (AUC), elimination half-life ($t_{1/2\beta}$) of metformin alone were 0.46±0.04 hr^{-1}, 1,140.43±0.52, 4,388.72 ±0. 71and 3.8±0.07 hr^{-1}respectively. When metformin was co-administered withampiclox,K_a increased to 0.58 ±0.04, C_{max} to 1.28 ±0.35 µg/ml, while

AUC and $t_{1/2\beta}$ increased to 5,179.71 ±0.02 and 6.2± 0.02 respectively. When metformin was co-administered with metronidazole,K_a increasedto 0.59 ±0.03 and $C_{max}$to 1.35±0.42 µg /ml, while AUCand $t_{1/2\beta}$ increased to 5,179.71 ±0.02 and 6.2± 0.02 hr^{-1} respectively. All these increases were found to be significant ($p < 0.05$). No significant ($p< 0.05$) changes were observed when metformin was co-administered with amoxicillin.

The findings indicated that ampiclox and metronidazole may have potentiating effects on the hypoglycemicaction of metformin. Consequentlydiabetic patients who require ampiclox and /or metronidazole in addition to metformin may need adjustment of dose regimen to avoid the risk of toxicity.

TABLE OF CONTENT

Content Page

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

NIDDM	=	Non-Insulin Dependent Diabetes Mellitus.
G.D.M	=	Gestational Diabetes Mellitus
MODY	=	Maturity Onset Diabetes Mellitus of the young.
ml	=	Milliliter.
ROS	=	Reactive Oxygen Species.
PPM	=	Parts per million.
HPLC	=	High performance Liquid Chromatography
U.V	=	Ultraviolet
HP	=	Hydrogen –ion Concentration.
S.E.M	=	Standard Error of the Mean.
N.R.C.F.N.B	=	National Research Council, Food and Nutrition Board
WHO	=	World Health Organization.
β	=	Beta.
IDDM	=	Insulin dependent Diabetes mellitus.
G T F	=	Glucose tolerance factor.
G.C.D.W.Q.	=	Guidance for Canadian drinking water Quality.
F.D.A	=	Food drug Administration.
µg	=	Microgram.
Min	=	minutes
ESSADI	=	Estimated Safe and adequate daily dietary intake.
F.N.B.N.A.S.	=	Food and Nutrition Board of United State National Academy of Science.

USA	=	United State of America
hr	=	Hour
P	=	Probability
°C	=	Degree Centigrade (Celsius)
∞	=	Infinity
λ_{max}	=	Lamda Max
AUC	=	Area Under the Curve
FBS	=	Fasting blood sugar
RBS	=	Random blood sugar
C.V	=	Coefficient of Variation.
B.P.	=	British Pharmacopea
PR	=	Percentage recovery
HPLC	=	High Performance Liquid Chromatography

CHAPTER ONE

1.0 INTRODUCTION

1.1 Diabetes Mellitus

Diabetes mellitus is regarded as a complex disease characterized by a grossly abnormal pattern of full usage or over utilization of glucose by the liver and underutilization by other organs (WHO, 2014). Diabetes is refers to the excessive urination and mellitus is from Latin meaning sweetened with honey, and also refer to the high level of glucose in the urine. Mellitus distinguishes the disease from diabetes insipidus which is caused by impaired renal reabsorption of water (Makaryus and McFarlane, 2013).

When blood glucose level falls below the renal threshold (10 mmol/l or 180mg/100ml) glycosuria ceases in diabetes as does the osmotic diuresis of water and electrolytes. Polyuria with dehydration and excessive thirst are thus alleviated. If the blood-glucose falls much below normal levels, appetite is stimulated. Also at metabolic level, in addition to enabling glucose to pass across cell membranes the transit of amino acids and potassium into the cell is enhanced. Insulin regulates carbohydrate utilization and energy production, and enhances protein synthesis (Wilcox, 2005).

Diabetes mellitus is classified by World Health Organization into four main groups: - Type 1, type 2, gestational diabetes and “other types” (Gardner and Shoback, 2011). The disease is characterized with polyuria, nocturia, decreased visual acuity, frequent thirst, dry mouth, volvular pruritis in women, severe skin infection, impotence and dark-spot due to skin necrosis. Gangrene of the feet and other extremities, coronary artery disease, retinal

diseases and parathesis due to neuropathy are complication of diabetes mellitus. Others include urinary tract infection due to favorable media, pulmonary or respiratory diseases due to depressed body immune system, and skin infections with Staphylococcus organisms as a result of skin abrasion (Walker *et al.,* 2014).

1.1.1 Type 1 – Diabetes mellitus

Type 1 – Diabetes mellitus- this is formally known as insulin dependent diabetes (IDDM), childhood diabetes, it is characterized by loss of insulin – producing β- cells of the islets of Langerhans of the pancreas leading to a deficiency of insulin (Shapiro, 2006). Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes comprise up to 10% of total cases in the North America and Europe, though this varies with geographical location (American Diabetes Association (ADA) (2005). This type of diabetes mellitus can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of cases of diabetes affecting children. The most common causes of β-cell loss leading to type 1diabetes is auto-immune destruction, accompanied by antibodies directed against insulin.

It was reported in 2006, that the principal treatment of type 1 diabetes is replacement of insulin. Without insulin, ketosis and diabetes ketoacidosis can develop and coma or death will result (Razari *et al.*, 2006).

1.1.2 Type 2 diabetes mellitus

Type 2 Diabetes mellitus was previously known as adult onset diabetes, maturity - onset diabetes or non-insulin dependent diabetes mellitus (NIDDM). This is because of the fact that, there is defective insulin secretion and insulin resistance or reduced insulin sensitivity (defective responsiveness of tissue to insulin), which almost certainly involves the insulin receptor in the cell membrane. In the early stage, the pronominal abnormality is reduced i.e. reduced insulin sensitivity characterized by elevated level of insulin in the blood. At this stage, hyperglycaemia can be reversed by a variety of measure and medications that improve insulin sensitively or reduce glucose production by the liver, but as the disease progress, the impairment of insulin secretion worsens, and therapeutic replacement of insulin often become necessary (Weiss and Sumpio, 2006). There are numerous theories as to the exact cause and mechanism for this resistance but central obesity (Fat concentrated around the waist in relation to abdominal organs) is known to predispose for insulin resistance, possibly due to its secretion of adipokins (a group of hormone) that impair glucose tolerance (Knowler *et al.,* 2002).

1.1.3 Gestational diabetes mellitus

Gestational diabetes mellitus is the type of diabetes that involves a combination of inadequate insulin secretion and responsiveness resembling type 2 diabetes in several respects. Studies have shown that it is normally developed during pregnancy and may improve or disappear after delivery although it may be transient or may damage the health of the mother or foetus. About 20 – 50% of women with gestational diabetes develop type 2 diabetes mellitus later in life (Stuebe *et al.,* 2005).

It was observed that gestational diabetes mellitus (GDM) occurs in about 2-5% of all pregnancies (Stuebe *et al.,* 2005). It is temporary and fully treatable but, if left untreated it may cause problems with pregnancy, such as microsomal (high birth weight), fetal malfunction and congenital heart diseases. It requires careful medical supervision during the pregnancy (Stuebe *et al.,* 2005). In large population, clinical investigation includes detecting sugar level of patients; and level above 7mml/l is a strong indication of the disease condition. Management plan for diabetic patients involves diet and oral hypoglycaemic agent, regular exercise, education of patients and insulin therapy (Fowler, 2007).

1.1.4 Incidence of diabetic mellitus

According to World Health Organization and the American Diabetic Association's criteria, an adult is considered diabetic if fasting blood sugar measured exceeds 8mmol/l on more than one occasion, or if on two occasions, the concentration of glucose exceeds 11mmol/l 30, 60, 90 minutes after ingesting about 75 g glucose and remains about this levels two hours after ingestion (ADA,2013).

Diabetes mellitus is one of the major causes of death in Europe and the United States (Fowler, 2007). From various studies, 5% of the general population of the USA will eventually develop the disease. The prevalence is similar to other countries. About 382,000,000 people of the World population have been reported to be afflicted with diabetes mellitus (Melmed *et al.,* 2012; Shi and Hu, 2014). In Zaria and environs, about one out of 50 cases in the Department of medicine Ahmadu Bello University Teaching Hospital, Zaria are diabetic. The rate of hospital visit among the affected group of the rural

dwellers is quite low as they often resort to traditional medication and only visit the hospital when their condition worsens. The demand for constant checkups also made some patients to abandon the therapy altogether among this group (DAN, 2011).

1.2 Statementofthe Research Problem.

Successful treatment of diabetes is usually measured by the degree to which various infections and complications of the disaster are prevented. It is on this basis that multiple drugs therapy in diabetics cannot be ruled out.

The prescription pattern in the diabetic clinic of Ahmadu Bello University Teaching Hospital Zaria,for infected diabetics patients show a combination of ampiclox or metronidazole or amoxicillin together with metformin. This combination may lead to interaction resulting in toxicity or therapeuticfailure. There are no documented records of prior studies of the effects of co-administration of ampiclox, metronidazole and/ or amoxicillin on the pharmacokinetics of metformin in type 2 diabetic patients attending the diabetic clinic of Ahmadu Bello University TeachingHospital, Zaria.

1.3Justification

The use of several drugs often is essential to obtain a therapeutic objective or to treat coexisting disease. Multiple drug therapy can give rise to drug interactions which can be of concern, because the outcome of concomitant drug administration could diminish therapeutic efficacy or increased toxicity of one or more of the administered drugs.Consequently there is theneed to evaluate the possible drug-drug interactions that are likely to occur in such cases.

1.4 Aim and Objectives

The aim of the study is to determine the effects of ampiclox, metronidazole and amoxicillinif any on the pharmacokinetics of metformin in the treatment of type 2 diabetic patients.

The objectives of the study are to:

- assess the quality of all the drugs used in the study.
- optimize and validate the adopted method of analysis.
- Generate pharmacokinetic profile of metformin in the type 2 diabetic patients to be used in this study.
- evaluate the effects of concomitant administration of ampiclox, metronidazole tablets and amoxicillin capsules on the pharmacokinetic profile of metformin.

1.5 Research Hypothesis

Ampiclox, metronidazole and amoxicillin will have significant effects on the pharmacokinetics of metformin in type 2 diabetic patients.

CHAPTER TWO

2.0 LITRATURE REVIEW

2.1 Pharmacokinetics

The overall biological response of an organism to a drug depends on two major phenomena: one termed the pharmacodynamics response is the action of the drug at specialized and specified sites in the body to give a pharmacological response. The other pharmacokinetic, is the action of the organism on the drug to impede its penetration or absorption, to dilute, distribute, transformed excrete the active principles, and thus diminish its availability at the site of action. The elucidation of the parameters which describes these procedures which modify the time –course of the active drug in the body is a function of pharmacokinetics (Rosenbaum, 2012).

Pharmacokinetics deals with changes of concentration of drug and or its metabolites in the human or animal body following administration. That is, the change of drug concentration in different body fluids and tissues in the dynamic system of liberation, absorption, distribution, metabolism and excretion (Sakai, 2008; Rosenbaum, 2012).

Pharmacokinetic knowledge provides the followings:

- Estimation of rates of absorption, metabolism and urinary excretion.
- Bioavailability – estimation of relative extent of absorption and relative rate of absorption following administration of two or more products or formulations
- Prediction of blood levels after multiple doses from blood levels measured after single dose.

- Determining quantitative effects of drugs in patients.
- Aid in elucidation of drug interactions.
- Aid in determining effect of plasma protein binding of drug distribution.

When applied to therapy, clinical pharmacokinetics are used for selecting and adjusting drug dosage schedules and to facilitate the interpretation of measured serum concentration of drugs (Khillon and Gill, 2006; Rosenbaum, 2012).

2.1.1 Pharmacokineticparameters

Some of the most useful pharmacokinetic parameters obtained following drug administration include: -

Absorption half-life (t½ abs), area under the curve (AUC), systemic and total body clearance (Cl), volume of distribution and rate constant, elimination half-life (t½ elim) and absorption/ elimination rate constant (DiPiro *et al.*, 2010; Rosenbaum, 2012). Others are lag time, Cmax, Tmax

2.1.1.1 *Area under the curve* (AUC)

This is the integral of drug blood level over time from zero to infinity and is a measure of quantity of drug absorbed (Bioavailability) in the body. Methods of calculating area under the curve are discussed by various authors (DiPiro et al., 2010; Rosenbaum, 2012)

The methods include planimetry, cut and weigh methods, trapezoidal rule and computer applications. Many authors have also used the areas under the curve analysis to develop a pharmacokinetic pathway to obtain the rate constant associated with the proposed

pharmacokinetic model which is important for calculating plasma clearance and apparent volume of distribution.

2.2.1.2 Apparent volume of distribution

This is defined as the volume in which the amount of drug in the body would need to be uniformly distributed to produce the observed plasma concentration, i.e. higher the volume of distribution the more extensively the drug distributed from the blood stream into the various tissues, organs and binding site of the body. The concept of volume of distribution is used to relate the plasma concentration of a drug to a dose administered (if no elimination has occurred or to the amount of drug in the body), the apparent volume of distribution does not relate to the physiological or anatomical space, but provided an estimateof body fluid compartment and its uptake by tissue.

Vd can be determined from the following relationship.

$$\mathrm{Vd} = \frac{\mathrm{FxD}}{\mathrm{AUC}}$$

Value F = Bioavailability

D = Dose

AUC = Area under the Curve

2.2.1.4 Elimination half-Life (Elim $t_{½\beta}$).

It is the time taken for half the amount of the drug present in the body to be eliminated either by excretion or metabolism or both. In practical terms, it is the time taken for the plasma concentration to reduce by 50% of its original value (Remington , 2005; DiPiro et al., 2010; Rosenbaum, 2012), and can be determined for both one and two compartment

model. This is supported mathematically by combining of function to give

$$\text{Elim } t_{½\beta} = \frac{0.693}{Ke}$$

Elimination half-life is a useful parameter in the determination of suitable dosage interval and the time required to attain steady state during intravenous or chronic therapy (Remington, 2005; DiPiro et al., 2010; Rosenbaum, 2012).

2.2.1.5 Absorption half-Life ($t_{½a}$)

This is the time taken for half the amount of the drug to be absorbed into the systemic circulations. In practical terms, it is the time taken for the plasma concentration to reach half concentration of the drug administered

Mathematically it can be calculation by equation as follows:-

$t_{½}abs = \frac{0.69}{K_a}$ Where K_a = Absorption rate constant

2.2.1.6 Relationship among Pharmacokinetics Parameters

Clearance (CI) and volume of distribution (V) are two major pharmacokinetic parameters that influence the shape of the plasma concentration-time profile of drug after different routes of administration. The CI and V of drugs represent distinct processes in the body (elimination and distribution, respectively) which are independent of each other. However, a third parameter, elimination half-life ($t_{1/2}$) (or rate constant, K) is a reflection of the extent of both distribution and elimination, thus depending on both CI and V. There is a mathematical relationship among these 3 parameter which allows estimation of the third parameter when the other two are known.

However, the use of equation without a complete understanding of the physiological relationship among those parameters and their interdependency may result in erroneous

estimation of CI or V when there is a change in the kinetics of the drug based on the assumption of one-component linear kinetics (Reza, 2004).

The physiological relationship among pharmacokinetics parameters i.e. clearance of drug in human is dependent on certain physiological parameters of the subject and physicochemical properties of the drug. For a particular drug, clearance varies among different subject because each subject may exhibit different physiological parameters. For example metabolic clearance of drugs by the liver is dependent on hepatic blood flow, degree of protein binding of the drug in blood and the intrinsic capability of the liver enzymes to metabolize the drugs. If one or more of these parameters are changed in a patient because of the disease states, interacting drugs or environmental factors, the clearance of the drug in the patient may change for instance; rifampicin induces the liver enzyme responsible for the metabolism of warfarin (O' Reilly, 2001). Therefore, in the presence of rifampicin, the hepatic clearance of warfarin is significantly increased.

The extent of distribution of drugs however, is independent of their clearance. Therefore, a change in the clearance of a drug does not necessarily alter the distribution. Indeed, an increase in the clearance of warfarin because of enzyme induction by rifampicin did not affect the volume of distribution of the drug. Similar to clearance, the distribution is dependent on certain physiological parameters of the patient and physicochemical properties of the drug, such as tissue perfusion, permeability of tissues to drug, plasma and tissue binding, and the volume of body water. Therefore, a change in any of these parameters resulting from interacting drugs, disease states, and/or age, may affect the volume of distribution of the drug independent of what may or may not happen to its

clearance. For example, the volume of distribution of gentamycin is reduced in patient with sepsis who are dehydrated (Lingvall *et al.,* 2005).

However, the renal function and the clearance of gentamycin may be normal in these patients. Therefore, clearance and volume of distribution are independent of each other and may change in the absence of a change in the other. In some situations, it is possible that both major kinetics parameters clearance and volume of distributionchanges simultaneously. However, the changes in volume (V) and clearance (CL) are independent of each other, and because of the underlying mechanism(s) that affects both processes.

In contrast to CL and V, which signify certain physiological processes, the elimination half-life (or rate constant) does not represent any independent processes by itself and is influenced by both the distribution and elimination processes. An increase in clearance (elimination efficiency) results in a reduction in the half-life (an increase in K). This is easy to understand because the more efficient the elimination pathway, the faster is the decline in the plasma concentrations. An increase in V, however, results in prolongation of half-life (a decrease in K). This is because an increased V results in a more extensive distribution into the tissues, where the drug is safe from elimination.

2.2CompartmentModels

Compartment models are used to describe and interprete a set of data obtained by experimentation. A model in pharmacokinetics is a hypothetical structure which can be used to characterize the behaviour and reproducibility and the fate of a drug in biological system when given by a certain route of administration and in a particular dosage form.

A compartment is an entity which can be described by a definite volume and a concentration of a drug contained in a volume. The compartments are classified into one or two or multi compartment (Gibaldi and Perrier, 1991).

Before any equation may be derived from these models, some basic assumptions have to be made. Firstly drugs enter the system only via the central compartment. Secondly, reversible transfer occurs between a central and peripheral compartment, and thirdly, the exit of drugs from all compartment in the system is described by first order elimination rate constant which has units of reciprocal time like min^{-1}or hr^{-1}(Paxton, 1989). All these assumptions are relatively valid for most drugs. The majority of the drugs enter the body via the blood stream and from the blood stream via the kidney.

There are few exceptions which are primarily eliminated by biotransformation, and if given in high doses results in saturation of the metabolic enzyme system. The elimination kinetics then becomes zero order which means that, rate constant are independent of drug concentration (Paxton, 1989).

2.2.1 Single or one compartment model

This is the simplest model which depicts the body as a single homogeneous unit in which when the drug enters the body it is distributed instantly between the blood and body fluid or tissues (that is, the exchange of drug between the plasma and tissue proceeds rapidly compared with the rate of elimination). In this compartment, the whole body is considered mathematically as a single compartment, this model is useful in the pharmacokinetics analysis of salivary and urinary excretion data for drugs which are rapidly distributed

between plasma and other body fluids and tissues upon entry in to the systemic circulation (Remington, 2005).

The assumption that the body behaves as a one compartment – model does not mean that the drug concentration in all body tissues at any given time are the same. However, from one compartment model, it is assumed that any changes that occur in the plasma quantitatively reflect changes occurring in tissues drug level: -

Assuming instantaneous distribution after I.V injection into this model, the concentration (Co) in the plasma immediately after injection is equal to the dose (D) divide by the volume of this compartment (Vd)

Central Compartment

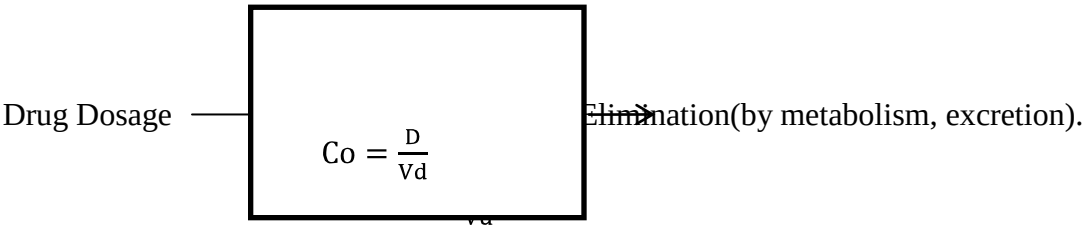


Figure 2.1: A single compartment model, rapid (iv) injection of drug in to a single compartment (Paxton, 1989).

The apparent volume of distribution (Vd) of the drug is not a true volume, but that volume into which all drug in the body will appear to be distributed to achieve a concentration same as that in the plasma (Sakai, 2008).

2.2.2 Two compartment model

The plasma concentration – time curve of a drug is often not a straight line on a semi-log plot (Paxton, 1989). If distribution of the drug is however so slow that it cannot be disregarded, a model must then be considered which contained a central compartment and at least one other peripheral compartment Figure 1.2 illustrate this compartment model.

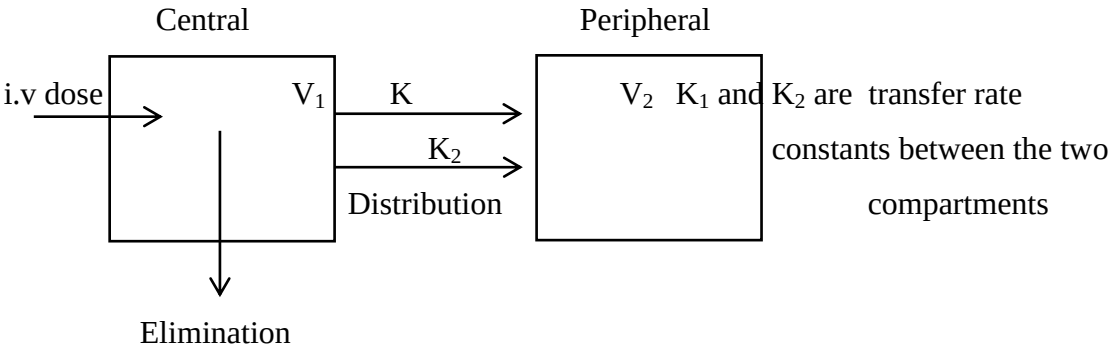


Figure 2.2 Showing two compartment model (Paxton, 1989).

Drugs distribute within a few minutes through this compartment and equilibrium between plasma and tissue is rapidly established. The peripheral compartments are then formed by less perfused tissues like skin, muscle, or adipose tissue in which drugs enter more slowly. The combined effect of two compartments give rise to a biphasic curve on i.v injection with two distinct linear portions when drawn on a semi-log, scale as shown in figure2.3

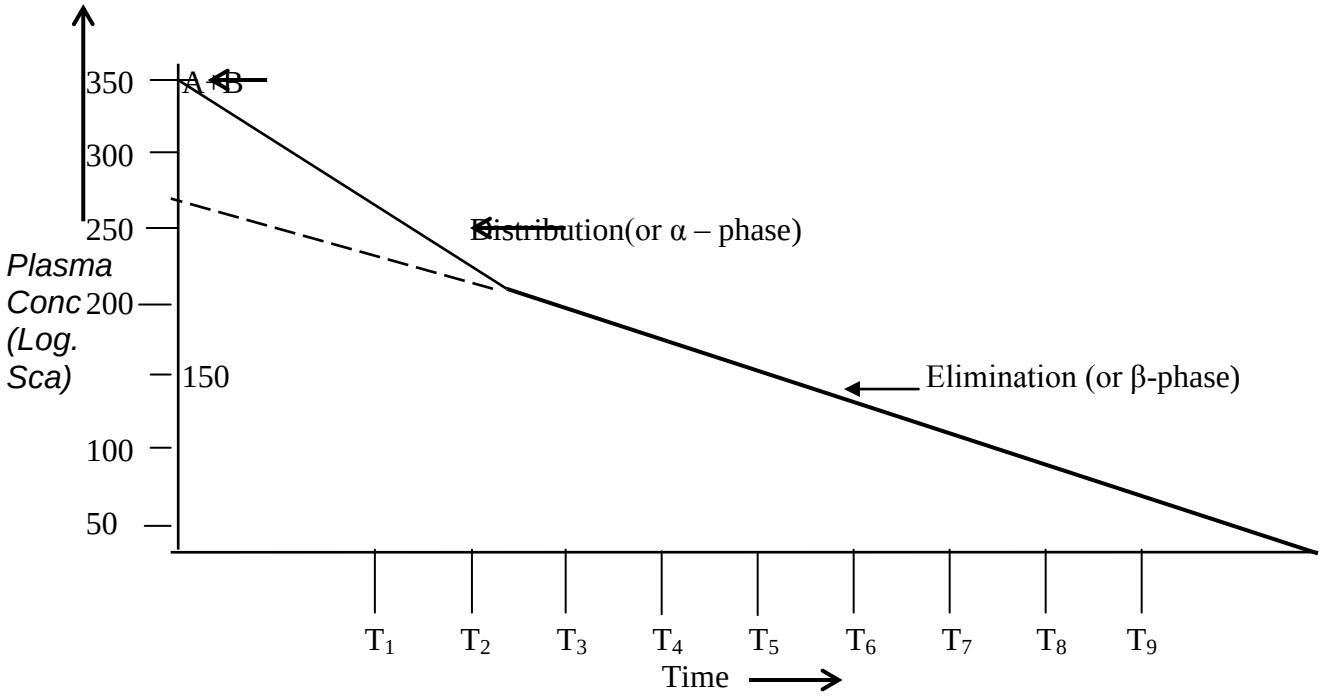


Figure 2.3 Showing plasma log-concentration Vs time curve for a two compartment model.

Although drug distribution is slow, it is usually much faster than elimination. Thus, the initial rapid fall in concentration (the α-phase) mainly represents the relatively rapid process of drug distribution from central to peripheral compartment. Once distribution is complete, the curve enters the relatively slow (β-phase) or elimination phase during which drug disappearance is determined mainly by irreversible elimination from the central compartment (Gibaldi and Perrier, 1991).

The two compartment model may be expanded to contain additional compartment which can be described mathematically as the sum of as many individual exponential function as

there are relevant compartment (Multiple Compartment models). In reality, a maximum of three compartments is allowed in assay techniques, (Tozer, 1979).

2.3 Elimination

The term elimination is used to express the removal of drug activity from the body. Drug effects are usually terminated by two processes of elimination, metabolism or excretion (Namara *et al*., 1979). Metabolism or drug biotransformation chiefly occurs in the liver, but can also occur in plasma, gastrointestinal tract, kidney and lungs. The biochemical mechanism which is complex, but common to all is an increase in the water solubility or polarity of the metabolic product as compared with the parent drug. This means there is a progressive decrease in lipid solubility which makes metabolites less able to enter cells and more readily excreted in the urine. Metabolism usually leads to deactivation and loss of pharmacological activity. However, an increase number of exceptions to this rule are becoming apparent (Vesta and Wood, 1987).The other process of elimination is excretion of the unchanged drug (and metabolite).This takes place mainly in the urine, although biliary excretion and from there into feaces may be important for some drugs (Paxton, 1989). Other minor routes of excretion include exhalation of some anaesthetics by the lungs (Rowland, 1988).

2.4 Bioavailability

It is important to distinguish between the rate and extent of drug absorption and the amount of drug that ultimately reaches the systemic circulation. The amount of drug that reaches the system circulations depend not only on the administered dose but also on the fraction of

the dose (f) that is absorbed and escape any first-pass elimination, the fraction is the drug bioavailability (Rowland, 1988).

Bioavailability is the term used to indicate measurement of the relative amount of an administered dose that reaches the systemic circulation. The absolute bioavailability of an oral dose by any other route cannot be determined unless an intravenous study with the same dose in the same subject is performed (Remington, 2005).

It is worthwhile noting here that a low oral bioavailability may not necessarily indicate poor absorption. Loss of drug to various degrees may occur due to rapid uptake and metabolism in the liver, as the drug is being transferred by the portal system from its absorption site in the gastrointestinal treat to the general circulation (Remington, 2005).

The so-called "first-pass elimination" during the first circulation through the organ is observed for a number of drugs in common use, such as propranolol and aloprenolol. Many other drugs such as morphine and lignocain have extensive first-pass elimination that they are rarely given orally (Paxton, 1989). According to this understanding of bioavailability, the absorption of an intravenously administered drug is rapid and complete (Paxton, 1989).

The therapeutic action of a drug is dependent on an adequate concentration of the drug reaching the site of action and this concentration is dependent on the passage of the drug across the biological membranes. The rate and extent of absorption of a drug by the body is influenced by a number of biological and physiological factors but it may be affected also by the rate at which the drug dissolves in the body fluid at or before the site of absorption, (Rowland, 1988).

Both the effective disposal of the drug particles in the body fluid and its solubility may affect the rate of dissolution. These factors may be dependent on the physiochemical characteristics of the drug itself and on other materials and manufacturing processes used in the preparation of the dosage form (Rowland, 1988).

Bioavailability or bioequivalence studies are usually based on measurement of the active drug moiety or and its metabolite(s), in biological fluid as a function of time . In vivo bioavailability testing in human usually dictates that the biological fluid sample be limited to blood, urine and saliva (Benet and Haener, 2002).

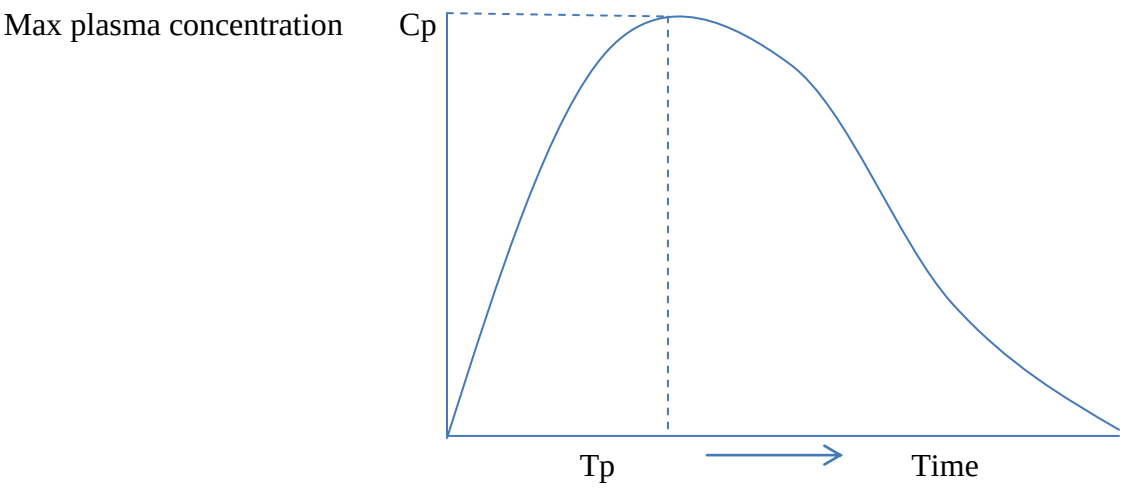


Figure 2.4: A plasma –time curve obtained after oral administration of a dosage.

The shape of the curve is dependent on number of factors: -

Elimination of the drug begins as soon as it appear in the blood and absorption distribution and elimination (metabolism) and will all modify the shape of the curve.

There are three important parameters that can be derived from the curve these are:-

- The peak plasma (C_{max})
- The time of the peak plasma curve (T_{max}) and
- Area under the plasma level versus time curve (AUC) (Khillon and Gill, 2006).

The first two parameter, C_{max} and T_{max} are related to one another and are used as a measure of the rate of bioavailability from the dosage form. The extent absorption is related to the third parameter i.e. AUC (Gibaldi and Perrier 1991; Sakai, 2008; Khillon and Gill, 2006). The determination of "absolute bioavailability" of a drug involves a comparison of AUC obtained following oral and intravenous administration. This is often not possible, and in such cases, relative bioavailability is determined by comparing the area under the curve of the test drug and a secondary reference standard such as an orally administered solution of the drug or dosage form, which has been accepted as a standard (Sakai, 2008; Khillon and Gill, 2006).

The plasma level of some drugs may be very low such that an easy method sensitive enough to measure these levels may not be available. Other drugs have long half life and the determination of the total area under the plasma concentration- time curve requires plasma samples to be obtained for excessively long period of time. This is some time desirable to determine availability based on steady state plasma drug level following multiple dosing (Sakai, 2008; Khillon and Gill, 2006). To measure bioavailability area under the curve is required. The most commonly employed methods for calculating AUC are the cut and weigh method, planimetry, trapezoidal rule and by computer operations (Bakare, 1991; Sakai, 2008; Khillon and Gill, 2006).

Important area of application of bioavailability include, determination of drug interaction, the effect of food on drug disposition following ingestionthat is drug-food interaction) and determination of those formulation factors that alters the bioavailability of an active ingredient in a drug product or products (Bakare, 1991)

2.4.1 Factors influencing drug bioavailability

The most widely used oral dosage form is compressed tablet which often present bioavailability problems due to the reduction in drug surface area in the formulation in addition to the excipients and tableting methodologiesused (Rowland, 1988).

Absorption may also be affected by same dosage forms from different manufacturers and also by different batches of a dosage form from a single source. Most common conventional oral dosage form in decreasing order of relative dissolution and absorption rates are solutions, suspensions, capsules, tablets, coated tablet and sustained release preparations (Muir *et al.*, 1997; Smith and Williams, 2006; Tomlin, 2010).

The crystal form in which drug is presented to the patients is also an important factor influencing bioavailability of drug. The crystal form of a drug may change because the substance exhibit polymorphism. Drug may interact reversely to form complexes with substances occurring in the body or with pharmacologically inactive compounds of the pharmaceutical dosage form.

Pharmaceutical factors are therefore extremely important in the control of drug availability. In addition to the drug substance, there are excipient, disintegrating agents, binders, diluents, lubricants and dyes, all of which may contribute to the performance of the dosage form as a drug delivery system. The active drug substance may comprise less than 10% of

the total tablet weight, under conditions of fast disintegration; the dissolution rate of the solid dosage form will limit the appearance of drug in the blood (Benet and Sheiner, 1985; Smith and Williams, 2006; Tomlin, 2010). Another important factor is the influence of food and specific dietary component (William *et al.,* 1996; Smith and Williams, 2006; Tomlin, 2010). The presence of food have shown to decrease, delay, increase or not to affect drug absorption (Burton *et al.,* 2006).The following factors have been found to be responsible for the observed effects. That is, the type of drug and dosage form, the nature and size of meals, fluid intake, the condition of the subject and the time sequence between dieting and drug administration (Bakare, 1991).

Drug-drug interactions could also affect bioavailability. Drug interaction could be direct as in the case of tetracycline chelating metallic ions or indirect as in the case of increase acetaminophen absorption through the stimulating effect of metochlopropamide on the stomach emptying rate (Ivan, 1994; Greenblatt *et al.,* 2002; McCabe*et al*., 2005; Esimone, 2011).

Volumes of co-administered fluid many have a considerable effect on drug absorption. Drug absorption might be expected to occur more rapidly from concentrated solution than diluted solutions; nevertheless, the reverse appears to be the case. The pharmaceutical actively of sodium phenobarbitone and also the circulating level of salicylates, were greater from dilute than from concentrated solution (McCabe *et al*., 2005).

Age, pregnancy, stress, disease state, cigarette smoking and exposure to chemicals such as insecticide also affect drug disposition. Gender is also a significant variable in some animals but appear to be less in humans (Greenblatt *et al.,* 2002)

2.5DrugDrug Interactions

Drug- drug interaction is a phenomenon which occurs when the effects of one drug are modified by the prior or concurrent administration of another drug. This may arise as a result of either alteration of absorption, distribution, biotransformation or excretion of one drug by the other or from combination of their actions or effects. This action can be synergistic (when drug's effect is increased) or a new effect can be produced that neither produces on its own (Haider, *et al.,*2007*).* However, interactions may also exist between drugs and food (drug – food interactions) as well as drug and medicinal plants or herbs (drug- herb interaction). It is therefore easy to see the importance of these pharmaceutical interactions in the practice of medicine. It was reported that, if a patient is taking two drugs and one of them increase the effect of the other, it is possible that overdose may occur. Interaction of two drugs may also increase the risk of side effect to occur. On the other hand, if the action of the drug is reduced it may cease to have any therapeutic use because of under dosage (Marine *et al*., 2005). Notwithstanding the above, on occasion, these interactions may be sought in order to obtain an improved therapeutic effect (McCabe *et al*., 2005; Esimone, 2011). It is also possible for interaction to occur outside an organism before administration of the drug has taken place. This could happen when two drugs are mixed for instance in a saline solution prior to intravenous injection.

Drug interactions that are of greatest concern are those that reduce the desired effects or increase the adverse effects of the drugs. Drugs that increase absorption or reduce the elimination or metabolism of the drugs, increase the concentration of other drugs in the body and lead to increase amount of drugs in the body and more side effects (Merle *et al.,* 2005).

2.5.1 Drug absorption interactions

Absorption of drugs from the gastrointestinal tract is the most part understandable in terms of simple diffusion across the gastro intestinal epithelium. The rate of such diffusion is proportional to the lipid solubility of the compound in question. The absorption is complete process that depends on many physiological factors such as pH, blood flow, gastric emptying time, solubility complexation. If the drug is a weak acid or bases, it is non-ionized form, it said to be more lipid soluble and the pH within the gastrointestinal tract become a major determinant. Alcohol soluble non- electrolyte is rapidly absorbed into the blood stream by diffusion across the gastric intestinal mucosa. On the other hand, weak base such as quinidine and ephedrine which are predominantly ionized at the pH of the gastric juice, are poorly absorbed through the gastric mucosa and are absorbed through the intestinal mucosa.

Weak acids such as salicylate and barbiturates which are predominantly non- ionized in acid gastric contents and are more readily absorbed from the stomach (Led Sack *et al.*, 1992; Kashuba and Bertino, 2006). Absorption interaction usually results into reduction in the rate of absorption and the total amount of drug absorbed so that the drug's effects are reduced or abolished.

2.5.2 Drug receptor interactions

The binding forces that holds a drug in combination with its receptor arise from the numerous bonds existing in the body system are as follows: - covalent, ionic, and vander-Waals forces. Conformational changes in the drug-receptor combination cause changes in the effect of drug other than the ones known. Structuralvariations in the drug molecule

cause marked changes in its potency as in the case with narcotic analgesics (Maehle *et al.*, 2002; Limbird, 2004).

2.5.3 Drug excretion interactions

Only the free fraction of a drug is dissolved in the blood plasma can be removed through the kidney. Therefore, drug that are tightly bound to proteins are not available for renal excretion as long as they are not metabolizedwhen they may be eliminated as metabolites. (Haider *et al.*, 2007).

Changes in renal function can modify a number of pharmacokinetic processes thereby leading to unanticipated drug interaction (Maehle *et al.*, 2002).Creatinine clearance is used as a measure of kidney functioning but it is only useful in cases where the drug is excreted in an unaltered form in the urine. The excretion of drugs from the kidney's nephrons has the same properties as that of any other organic solute: passive filtration, reabsorption and active secretion. In the latter phase the secretion of drugs is an active process that is subject to conditions relating to the saturability of the transported molecule and competition between substrates. Therefore these are key sites where interactions between drugs could occur. Filtration depends on a number of factors including the pH of the urine, it having been shown that the drugs that act as weak bases are increasingly excreted as the pH of the urine becomes more acidic, and the inverse is true for weak acids (Taro, 2004).

In the latter phase, the secretion of drugs is an active process that is subject to conditions relating to the saturability of the transported molecule and competition between substrate. Therefore, these are the key site where interactions between drugs could occur. Filtration depends on a number of factors including the pH of the urine, having been shown that the

drugs that act as weak base are increasingly excreted as the pH of the urine becomes more acidic and the inverse is true for weak acids.This mechanism is of great use when treating intoxications (by making the urine more acidic or more alkali) and it is also used by some drugs and herbal products to produce their interactive effect. (Barley *et al.*, 1998; Kashuba and Bertino, 2006).

2.5.4 Pharmacodynamic interactions

The changes in an organisms' response on administration of a drug is an important factor in pharmacodynamic interaction. These changes are extraordinarily different to classify given the wide variety of models of action that exist and the fact that many drugs can cause their effect through a number of different mechanisms. Pharmacodynamics interactions occur when one drug directly alters the molecular, cellular or physiological action of another. Example of pharmacodynamics interaction is as follows:-

The increased effect produced by the two drugs acting at different receptor site such as interaction often results in an effect which is greater than the sum of the component effect (i.e. potentiation)(Ameer and Waintraub,1997).

2.5.5 Clinical significance and importance of drug interaction

In the past, medical practitioners prescribed many drugs to a patient at a particular time and thus, new interactions between medications are increasingly reported (Middleton, 2006). Complexity of this type of prescription is likely to have had considerable effect in susceptible patients. In modern medicine, this problem is described as polypharmacy.

Consequently, it is no longer practical for physicians to rely on memory alone to avoid potential drug-drug interactions. Multiple drug regimens carry the risk of adverse

interactions. Precipitant drugs modify the object drugs absorption, distribution, metabolism excretion or actual clinical effect. From the result of various drugs interactions, certain lessons that are drawn can be summarized as follows:

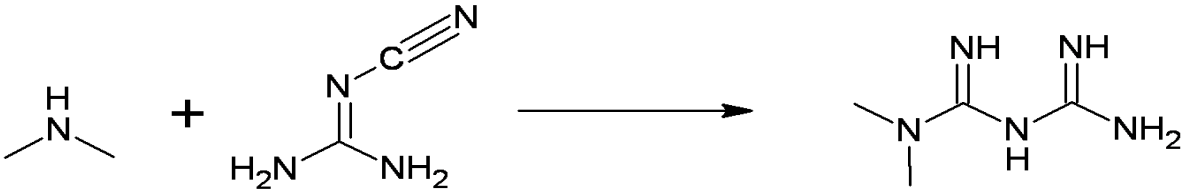
- Polypharmacy is wide spread in modern medical practice and there has been a large increase in the number of potentially significant drug interaction.
- Drug- drug interaction data obtained from healthy human volunteers will provide potentially useful information, which should be extrapolated with caution to disease individuals.

2.6Metformin

2.6.1. Chemistry of metformin

Metformin was first synthesized and found to reduce blood sugar in the 1920s; metformin was forgotten as research shifted to insulin and other antidiabetic drugs. Interest in metformin was rekindled in the late 1940s after several reports that it could reduce blood sugar levels in people, and in 1957, French physician Jean Sterne published the first clinical trial of metformin as a treatment for diabetes. It was introduced to the United Kingdom in 1958, Canada in 1972, and the United States in 1995. Metformin is now believed to be the most widely prescribed antidiabetic drug in the world; in the United States alone, more than 48 million prescriptions were filled in 2010 for its generic formulations (Bailey and Day, 2004).

The usual synthesis of metformin, originally described in 1922 and reproduced in multiple later patents and publications, involves the reaction of dimethylamine hydrochloride and 2-cyanoguanidine (dicyandiamide) with heating.



(1) (2) (3)

Metformin hydrochloride {N,N-dimethylbiguanide hydrochloride} is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5 \bullet HCl$ and a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. The pKa of metformin is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68. It has a melting point of 218-220^{0}C

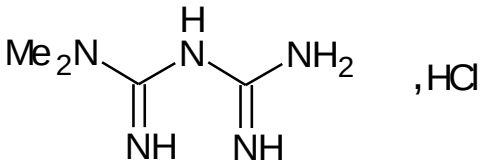


Figure 2.8: Structure of metformin

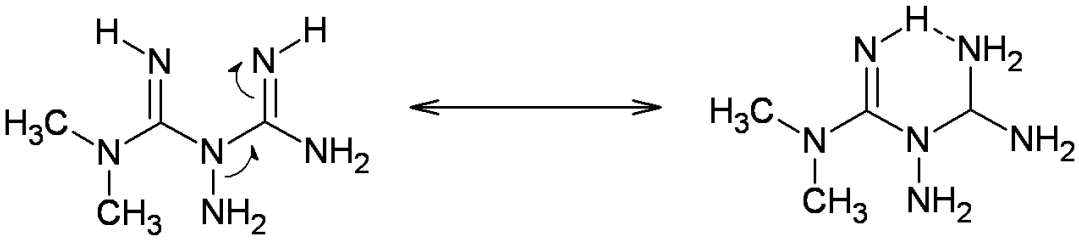


Figure 2.9 Tautomeric forms of metformin in acidic media

The structure of metformin was generally represented in a wrong tautomeric form for a number of years. This was corrected in 2005 (Bharatam *et al*., 2005). The energy difference between the correct tautomer and the generally represented tautomer is about 37 kJ/mol (9 kcal/mol). The drug is administered as metformin hydrochloride. The structure of metformin hydrochloride was also corrected recently (Patel *et al.,* 2009). According to these studies carried out by Petel in 2009, it was reported that metformin has different electronic structure compared to its protonated form. The neutral species is a simple conjugated system. Upon protonation, (i) the conjugation breaks down, (ii) the intramolecular hydrogen bond breaks down, (iii) the molecule becomes non-planar (iv) two

lone pairs get accumulated at the central nitrogen (v) dynamism increases in the system via C=N rotational process and via N-inversion process. Thus, electronically, metformin hydrochloride should be treated as a simple extension of metformin. The nucleophilicity of metformin hydrochloride is moderate and that is a desired property. Metformin is shown to belong to a new class of compounds called nitreones (Patel *et al.*, 2009).

2.6.2 Therapeutic effects of metformin.

Metformin have been most often prescribed for patients whose hyperglycemia is due to ineffective insulin action, i.e. insulin resistance syndrome. The dose of metformin is from 500mg to maximum of 2.55g daily with the lowest effective dose being recommended. Common schedule would be to begin with a single 500 mg given with breakfast for several days. If this is tolerated without gastrointestinal discomfort and hyperglycemia persists, a second 500 mg may be added with the evening meal. If further dose increase is required after one week, dosage should always be divided, since ingestion of more than 1000 mg at any time usually provokes significant gastrointestinal side effects (Patel *et al.*, 2009).

2.6.3 Mechanism of action of metformin

Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects and does not cause

hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease.

2.6.4 Adverse effects and toxicity of metformin

The most common adverse effect of metformin is gastrointestinal upset, including diarrhea, cramps, nausea, vomiting and increased flatulence; metformin is more commonly associated with gastrointestinal side effects than most other antidiabetic drugs (Bolen *et al*., 2007). It was reported in 2010 by Khurana *et al.*, that the most serious potential side effect of metformin use is lactic acidosis. This complication is very rare, and the vast majority of these cases seem to be related to comorbid conditions, such as impaired liver or kidney function, rather than to the metformin itself.

Metformin has also been reported to decrease the blood levels of thyroid-stimulating hormone in people with hypothyroidism and, in men testosterone (Vigersky *et al*., 2006). It should be noted that the clinical significance of these changes is still unknown (Shegem *et al*., 2002).

Metformin is contraindicated in people with any condition that could increase the risk of lactic acidosis, including kidney disorders (creatinine levels over 150 μmol/l (1.7 mg/dL) lung and liver disease (Jones *et al.,* 2003). It was reported in accordance to American prescribing information that, the heart failure, in particular, unstable or acute congestive heart failure, increases risk of lactic acidosis with metformin. A systematic review of controlled trials, however, suggested that metformin is the only antidiabetic drug not associated with any measurable harm in people with heart failure, and that it may reduce mortality in comparison with other antidiabetic agents (Eurich *et al*., 2007). The most

serious potential adverse effect of biguanide use is lactic acidosis, the incidence for which is 9 per 100,000 person-years (Stang *et al*., 1999).

When there is impaired renal function, however, clearance of metformin and lactate is reduced, leading to increased levels of both, and possibly causing lactic acidosis due to a buildup of lactic acid. Because metformin decreases liver uptake of lactate, any condition that may precipitate lactic acidosis is a contraindication to its use. Metformin has also been suggested to increase production of lactate in the small intestine; this could potentially contribute to lactic acidosis in those with risk factors (Garry *et al.,* 2011). However, the clinical significance of this is unknown, and the risk of metformin-associated lactic acidosis is most commonly attributed to decreased hepatic uptake rather than increased intestinal production.

2.6.5 Pharmacokinetics of metformin

The absolute bioavailability of metformin 500 mg tablet given under fasting conditions was approximately 50% to 60%. Studies using single oral doses of metformin 500 to 1500 mg, and 850 to 2550 mg, indicated that there was lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination (Stang *et al*., 1999).

Food decreases the extent of and slightly delays the absorption of metformin, as shown by approximately a 40% lower mean peak plasma concentration (Cmax), a 25% lower area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation of time to peak plasma concentration (Tmax) following administration of a single 850 mg

tablet of metformin with food, compared to the same tablet strength administered fasting. The clinical relevance of these decreases is unknown.

When metformin is administered orally with food C_{max} is achieved within seven hours and peak plasma level is reached at approximately 20% lower compared to when the same dose of metformin is administered alone.

Metformin is known to have oral bioavailability of 50-60% under fasting condition and it is said to be absorbed slowly from gastrointestinal tract. It is not metabolized,and iscleared from the body by tubular secretion and excreted unchanged in the urine (Heller, 2007). Metformin is reported to have acid dissociation constant of 2.8 – 11.5 and exist largely as hydrophilic cationic species at physiological pH value. (Jayasagar, *et al.*, 2002).

2.7 Ampicillin/Cloxacillin

2.7.1 Chemistry of ampicillin/cloxacillin

The history mpicillin/cloxacillin is as the history of the penicillins since they are mere analogues of the penicillins (Hills, 1987; Sneader, 2008). In 1929, Fleming published an account of his work, he described the antibacterial action of the substance which he named penicillin, mentioned its lack of toxicity for human leucocytes and wounds, and was impressed by the possibility that it could be used to suppress bacterial growth (Batchelor *et al.*, 1995; Sneader, 2008).

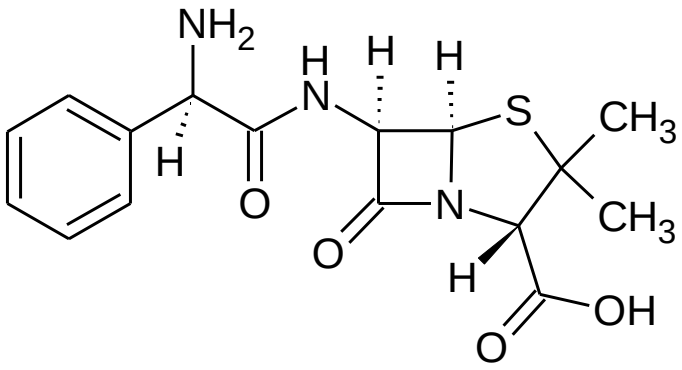


Figure 2.10: Structure of Ampicillin

Today, owing to its high level of safety, the penicillins are the most widely prescribed antibiotic in the world effective against both gram positive and gram negative cocci (Petersdorf and Turck, 1996; Sneader, 2008).

The limitation of the penicillins however is the fact that they are destroyed by β – lactamase enzyme; they are also acid labile, and resistant strains of bacteria have been found against this class of antibiotic (Sneader, 2008).

These limitations were the basis for various modifications of the penicillin. Penicillin are therefore, the derivative of 6-aminopenicillamic Acid (6-APA),the key to understanding the chemistry of the penicillins.Various side chain s may then be added to produce the other active compounds or analogues in use today (Olaniyi,1998).

The aim in ampicillin and cloxacillin combination therefore is to confer on ampicillin the ability to be penicillinase resistant while retaining its broad spectrum capabilities (Hills 1987)

Structural activity relationship of penicillin

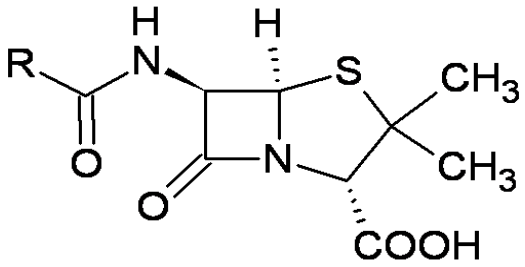
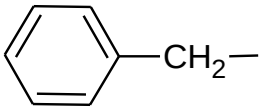
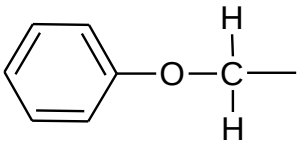
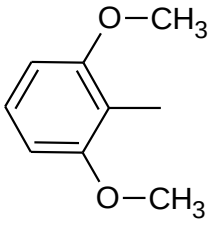
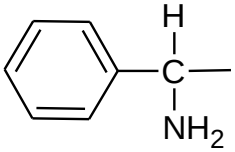
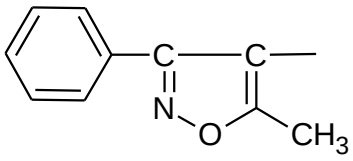


Figure 2.11 6- penicillanic Acid (6-APA)

R	Compounds	Remarks
	Benzylpenecillin (Penecillin G)	The enzyme β-lactamase splits the β -lactan ring producing inactive penecilloic acid, and amidase removes the side chain to reduce 6-APA.
	αPhenoxy-methyl(penicillin V)	This is acid stable and orally administered but are destroyed by β-lactamase
	2-6 dimethoxy phenyl penicillin (methicillin)	It is active against penecillinase producing staphylococci and unstable only given parenterally
	Aminobenzylpenicillin (ampicillin)	The addition of an α $-NH_2$ group wide spectrum of activity and covers many Gram negative organism
	3-chlorophenyl 5-methyl -4-isoxazoyl penicillin (cloxacillin)	An evolution of the penicillins with hindered compounds which are penecillinase-resistant and relatively acid stable

2.7.2 Therapeutic effects of ampicillin/cloxacillin

Penicillin sensitive organisms are Gram positive, penicillin insensitive organisms are Gram negative, notable exceptions being meningococci and gonococci. The introduction of the reactive group, NH_2 into the penicillin molecule leads to broadening of the antibacterial spectrum and the most useful compound to emerge is amino phenylacetamido-penicillin acid (ampicillin), (Hochadel, 2012). This compound is comparable to the tetracyclines and the chloramphenicol; it is active against non-penicillinase-producing staphylococci, and most streptococci; also, pneumococci, meningococci and gonococci, but ampicillin is less active against these cocci than penicillin G. It is highly active against *H. influenzae* and many strains of *E. coli*. It is inactive against pseudomonas and some strains of Proteus (Hills, 1987). It shows in vitro activity against Shigella and Salmonella, but its clinical usefulness in infections due to these organisms has been disappointing. Ampicillin is well absorbed when given by mouth, peak blood levels appear in 1-3 hours and detectable amounts persist for about 6 hours. Ampicillin has several disadvantages; it is destroyed by some penicillinases, resistance to coliform bacteria develop rapidly and hypersensitivity reactions to this drug may be severe (Stewart, 1980; Tindall *et al.*, 2014). Such disadvantages were the basis for the formulation of ampicillin/ cloxacillin combination resulting in a therapeutic advantage over the individual agent. In antibacterial spectrum, cloxacillin resembles penicillin G, covering organisms such as streptococci, pneumococci meningococci, gonococci and staphylococci. Cloxacillin is less active against those organisms for which penicillin G might be considered specific, such as pneumococci and haemolytic group A streptococci (Duguid, 1994). In chronic pulmonary infections,

cloxacillin may not always remove all staphylococci and the drug has little effect on nasal carriers of staphylocci.

2.7.3 Mechanism of action of ampicillin/cloxacillin

The possession of mucopeptide by bacterial cells appears to be unique factor distinguishing it from human cells. It is this difference which confers on penicillin its ability to be selectively toxic for bacterial cells (Park and Strominger, 1997; Eghianruwa, 2014).

When Fleming discovered penicillin, he also discovered an enzyme, lysozyme and recognized that lysis resulting in bacteria from the action of lysozyme was caused by the disruption of the bacterial cell wall. When the cell wall is impaired or destroyed, the organism is unable to withstand osmotic action if the surrounding medium is hypotonic in relation to the interior of the cell. It assumes spherical shape, enlarges and finally ruptures. These changes occurred in both Gram positive and Gram negative bacteria, indeed were most marked in Gram negative organisms which showed most resistance to lysis by penicillin (Hills, 1987; Lemke *et al.*, 2008). These observations on the morphological changes in bacteria subjected to the action of penicillin focused attention on the cell wall and interest was then centered on the biochemical and metabolic behavior of this structure. From biochemical point of view, Park and Strominger (1997) showed that penicillin prevented the uptake of glutamic acid in staphylococci but not in Gram negative coliform bacilli, and in 1997 Park and Johnson found that phosphates accumulated in staphylococci grown in low concentrations of penicillin. It was therefore proposed that uridine diphospho-acetyl muramyl-pentapeptide, the major accumulated peptide, was a cell wall precursor, and its accumulation resulted from inhibition of cell wall synthesis by penicillin (Hills,

1987; Lemke *et al.*, 2008). This hypothesis has been confirmed by isotopic measurements of cell wall synthesis in Gram positive and Gram negative bacteria (Park and Johnson, 1989).

Recent studies suggest that the rigidity of the cell wall is at least in part accounted for by cross-linking between the various mucopeptide structures, and it seems probable from recent work that a transpeptidation is inhibited by the action of penicillin (Gilmore *et al*., 2001; Duguid, 1994).

In conclusion, the action of these penicillin analogues may be summarized by the observed physical changes: swelling of the cell, inhibition of cell divisions, and production of spheroplasts. The biochemical factors include the accumulation of nucleotide precursors of both mucopeptide and teichoic acids, and in the breaking of the transpeptide bridge between two glycopeptide chains (Hills, 1987; Gilmore *et al*., 2001).

2.7.4 Adverse effects and toxicity of ampicillin/cloxacillin

Although all antibiotic have in common, that in low concentrations, they exert a bacteriostatic or bactericidal action on microorganisms or certain cell systems, they differ in many microbiological, pharmacokinetic and toxicological aspects (Hills, 1987).

The penicillins have a very low acute toxicity both in humans and in most animals. The lack of intrinsic toxicity of the penicillins to man and animals is explained by their peculiar mode of action. In bacteria, they interfere with the formation of cell walls, which are chemically unrelated to any substances to be found in the human and animal body (Duguid, 1994).

The penicillins and their analogues are notorious because of the frequency of hypersensitivity reactions they may provoke (Grammer and Greenberger, 2009). In the absence of allergy however, they belong to the safest drugs now available. This also applies to their use during pregnancy and early childhood.

Cloxacillin cause relatively few side reactions: hypersensitivity reactions, a history of any penicillin reaction in a patient is to be regarded as a contraindication to the use of these drugs since they show cross allergy. In the gastrointestinal tract, cloxacillin causes a minimum shift or disturbance of the enteral bacteria (Kasik and Thompson, 1989; Grammer and Greenberger, 2009).

After oral administration of ampicillin by 90% of patients with infectious mononucleosis (glandular fever, Pfeiffer's disease) they develop a rash (McKay *et al.*, 2010). Mononucleosis is often associated with sore throat and positive throat culture for haemolytic streptococci. In some individuals, ampicillin may cause depression of the bone narrow, which is at least in some patients, has been fatal. This rare form of bone marrow aplasia has also occasionally been observed with methicillin (Riel-Romero, 2006). Agranulocytosis during therapy with ampicillin occurred in at least two patients (Seth and Seth, 2009). Much about the cutaneous reactions following oral ampicillin has been reported; a distinction has to be made between true penicillin reactions, which are usually of the urticarial type, and the reactions specific for ampicillin and perhaps some other semi-synthetic penicillins that are maculo-papular in type. The latter start most commonly over the elbows and knees, and often spread symmetrically to most areas of the body. They usually, give rise to much less irritation than urticarial due to penicillin (Hills, 1987).

Ampicillin causes a far higher incidence of contact dermatitis than other penicillins (Samuels *et al.,* 1998). Patients receiving oral ampicillin may complain of a number of gastrointestinal tract reactions, such as loose stools, diarrhea, pruritis, nausea and vomiting. Abnormal serum transaminase levels were found after parenteral administration of ampicillin (Reisman and Arbesman, 1988). Ampicillin may promote the selective multiplication of resistant organisms such as Candida, Pseudomonas, Enterobacter, and certain group of Proteus. Especially in debilitated or malnourished patients, and those receiving corticosteroids, immunosuppressive agents, or irradiation are at risk of developing infections or superinfections (Hills,1987). Bacterial resistance to ampicillin/cloxacillin is usually of the extra chromosomal type.

2.7.5 Pharmacokinetics of ampicillin/cloxacillin

These penicillins are rapidly absorbed, diffuse easily into body cavities and joint spaces and are very quickly excreted by the renal tubules (Atkinson *et al*., 2012). Very little crosses from the blood into the cerebrospinal fluid (CSF) when the meninges are intact. The level of this drug in the bile is usually several times that of the plasma; if there is obstruction to the bile ducts, no penicillin may reach the bile. Because of very rapid renal excretion, high blood levels of penicillin can only be maintained by large dose at frequent intervals or by reducing the rapidity of renal excretion. A planned pharmacological approach by Beyer led to the discovery of carinamide and later, the more effective probenecid which depresses renal tubular excretion, but does not eliminate glomerular filtration of penicillin (Hills, 1987). Excretion of antibiotics other than penicillins, cephalosporins and amino salicylic acid is not affected. Penicillins may be found in inflammatory exudates (Pines *et al.,* 1986). Another variable in the availability of penicillin at any given site is the proportion bound in

plasma and tissues. The bond between penicillins and serum is reversible so that, an equilibrium is established between the protein and free antibiotic on one hand, and the protein – bound drug on the other (Kunin, 1996).

Ampicillin is well absorbed when given orally, peak blood levels appear in 1-3 hours and detectable amounts persist for about 6 hours (Petersdorf and Turck, 1996). It is excreted for the most part unchanged in the urine and reaches quite high levels (up to ten times the plasma level) in bile. Cloxacillin also is well absorbed when administered orally; peak levels appear in 2 hours and persist in blood for about 8 hours being higher than oxacillin and intramuscular injection provides double the plasma level of a comparable oral dose (Hills, 1987). The $t_{1/2}$ of ampicillin and cloxacillin is 75 minutes and 30 minutes respectively. While ampicillin is 20% bound to protein, cloxacillin is 95% protein bound (Hills, 1987).

2.8 Metronidazole

2.8.1 Chemistry of metronidazole

Metronidazole is an oral synthetic antiprotozoal and antibacterial agent, l-β-hydroxyethyl)-2-methyl-5-nitroimidazole, which has the following structural formula:

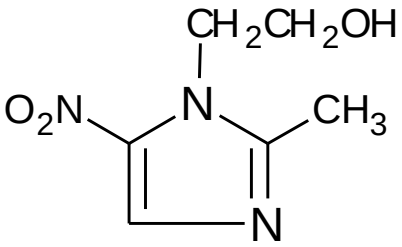


Figure 2.12: Structure of metronidazole

2.8.2 Therapeutic effects of metronidazole

Metronidazole is available in a 500mg/100ml intravenous solution, a 375mg oral capsule, a 250 mg and 500mg oral tablet, and a 750mg extended release oral tablet. Topically, metronidazole is available as a 0.75% and 1% cream, a 0.75% topical emulsion, gel/jelly, lotion and vaginal gel/jelly. The drug is contraindicated in patients with a metronidazole or nitroimidazole sensitivity, people who are sensitive to the parabens used in gel formulations, and in women in their first trimester of pregnancy (can be used cautiously in 2nd and 3rd trimester). Precaution should be used in patients that are receiving anti-coagulant therapy, that have concomitant blood dyscrasia, or a prior or current history of central nervous system disease (risk of seizure or peripheral neuropathy). Patients with severe hepatic disease or that might have enhanced elimination due to phenytoin or phenobarbital therapy should be closely monitored. A patient with concomitant alcohol

consumption could have a possible disulfiram reaction, which includes flushing, nausea, vomiting, and tachycardia (Torcher and Edward, 1994).

2.8.3 Mechanism ofaction ofmetronidazole

Metronidazole is cytotoxic to facultative anaerobic bacteria such as *Helicobacter pylori* and *Gardnerella vaginitis*, but the mechanism of this action is not well understood (Edwards, 1993). However, its activity against obligate anaerobes occurs through a four-step process:

Entry into the microorganism — metronidazole is a low molecular weight compound that diffuses across the cell membranes of anaerobic and aerobic microorganisms. However, antimicrobial activity is limited to anaerobes (Edwards, 1993). Reductive activation by intracellular transport proteins — metronidazole is reduced by the pyruvate:ferredoxin oxidoreductase system in the mitochondria of obligate anaerobes, which alters its chemical structure. Pyruvate: ferredoxin oxidoreductase normally generates ATP via oxidative decarboxylation of pyruvate. With metronidazole in the cellular environment, its nitro group acts as an electron sink, capturing electrons that would usually be transferred to hydrogen ions in this cycle. Reduction of metronidazole creates a concentration gradient that drives uptake of more drug, and promotes formation of intermediate compounds and free radicals that are toxic to the cell (Torcher and Edward, 1994).

Reduced intermediate particle interacts with intracellular targets — cytotoxic intermediate particles interact with host cell DNA, resulting in DNA strand breakage and fatal destabilization of the DNA helix. Breakdown of cytotoxic intermediate products — The toxic intermediate particles decay into inactive end products (Tocher and Edwards, 1994).

Metronidazole exerts rapid bactericidal effects against anaerobic bacteria; with a killing rate proportional to the drug concentration (Stratton *et al.,* 1991) dependent killing has also been observed with *Entamoeba histolytica* and *Trichomonas vaginalis.* Metronidazole kills *Bacteroides fragilis* and *Clostridium perfringens* more rapidly than clindamycin (Ralph and Kirby, 1995).

2.8.4 Pharmacokinetics of metronidazole

Disposition of metronidazole in the body is similar for both oral and intravenous dosage forms, with an average elimination half-life in healthy humans of eight hours. The major route of elimination of metronidazole and its metabolites is via the urine (60 to 80% of the dose), with fecal excretion accounting for 6 to 15% of the dose. The metabolites that appear in the urine result primarily from side-chain oxidation [l-β-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and 2-methyl-5-nitroimidazole-1 -yl-acetic acid] and glucuronide conjugation, with unchanged metronidazole accounting for approximately 20% of the total. Renal clearance of metronidazole is approximately 10 mL/min/1.73m^2 (Hellgren *et al*., 2003).

Metronidazole is the major component appearing in the plasma, with lesser quantities of the 2-hydroxymethyl metabolite also being present. Less than 20% of the circulating metronidazole is bound to plasma proteins. Both the parent compound and the metabolite possess *in vitro* bactericidal activity against most strains of anaerobic bacteria and *in vitro* trichomonacidal activity. Metronidazole appears in cerebrospinal fluid, saliva, and human milk in concentrations similar to those found in plasma. Bactericidal concentrations of

metronidazole have also been detected in pus from hepatic abscesses (Ravdin and Skilogiannis 1989; Hellgren *et al.*, 2003)..

Following oral administration, metronidazole is well absorbed, with peak plasma concentrations occurring between one and two hours after administration (Hellgren *et al.*, 2003).

Plasma concentrations of metronidazole are proportional to the administered dose. Oral administration of 250 mg, 500 mg, or 2,000 mg produced peak plasma concentrations of 6 mcg/ml, 12 mcg/ml, and 40 mcg/ml, respectively. Studies reveal no significant bioavailability differences between males and females; however, because of weight differences, the resulting plasma levels in males are generally lower.

Decreased renal function does not alter the single-dose pharmacokinetics of metronidazole. However, plasma clearance of metronidazole is decreased in patients with decreased liver function.

2.8.5 Adverse andtoxicity of metronidazole.

Mild side effects of metronidazole therapy may include nausea, headaches, loss of appetite, a metallic taste, and occasionally a rash. Metronidazole is generally well tolerated, so serious side effects are rare. If a patient experiences seizures and damage of nerves resulting in numbness and tingling of extremities, the drug should be discontinued. Metronidazole can increase the blood thinning effects of warfarin and increase the risk of bleeding. Cimetidine increases the blood level of metronidazole. Other probable drug-drug interactions include those that occur with amiodarone, amprenavir, busulfan,

carbamazepine, cholestyramine, cyclosporine, dihydroergotamine, disulfiram, ergoloid mesylates, ergonovine, ergotamine, fluorouracil, lithium, methylergonovine, milk thistle and tacrolimus. Metronidazole can be taken with or without food, and the patient should be sure to complete the course of treatment rather than discontinue upon improvement of symptoms. Metronidazole will continue to be a useful and effective antibiotic in the future. If resistance becomes more common, other 5-nitroimidazoles such as tinidazole will become more popular in clinical use (Hellgren *et al*., 2003).

2.9Amoxicillin

2.9.1 Chemistry of amoxicillin

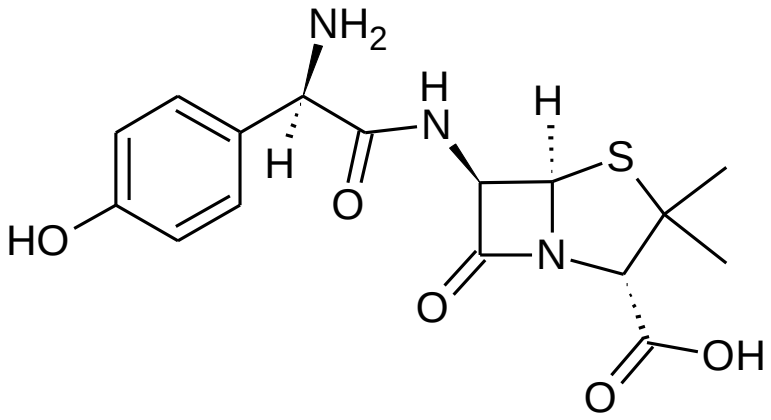


Figure 2.13: Structure of amoxicillin

Synonyms: AMX, *p-hydroxyampicillin*

Formula: $C_{16}H_{19}N_3O_5S$

Its IUPAC chemical name is (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid and its chemical formula is $C_{16}H_{19}N_3O_5S$, giving it a molecular mass of 365.4042 g/mol. It is a beta-lactam drug and is hence susceptible to degradation by -lactamase enzymes.

2.9.2 Therapeutic effects of amoxicillin

Amoxicillin (AMX), also spelled amoxycillin, and also called p-hydroxyampicillin, is an antibiotic drug that is very similar to ampicillin but which has enhanced stability towards gastric juices compared to other β -lactam antibiotics. It is effective against a wide range of Gram-positive bacteria but limited Gram-negative bacteria. It is used for bacterial strains that do not produce β -lactamase, an enzyme which would degrade the drug. The incidence

of β -lactamase-resistance in bacteria is increasing, leading to the increased use of clavulanic acid, a β -lactamase inhibitor, with amoxicillin and other β -lactam drugs. It is used to treat infections in the ears, nose and throat, the genitourinary tract, the skin and the respiratory tract. It is effective against *E. coli, S. pneumoniae, Staphylococcus spp., H. influenzae, P. mirabilis, E. faecalis, N. gonorrheae* and some strain (Wilson, 1995).

2.9.3 Mechanism of action of amoxicillin

Amoxicillin binds to the penicillin-binding protein 1A (PBP-1A) located inside the bacterial cell well. It inhibits the last stage of bacterial cell wall synthesis by acylating the penicillin-sensitive transpeptidase C-terminal domain thereby stopping the cross-linking of peptidoglycan strands. Autolytic enzymes in the bacteria then lyse the cell (Wilson, 1995).

2.9.4 Adverse effects and toxicity of amoxicillin

The penicillins are remarkably non-toxic. Most of serious adverse effects are due to hypersensitivity. Products of penicillin particularly peneciloic acid and product of alkaline hydrolysis bound to host protein. Allergic reaction includes anaphylactic shock (very rare-0,05% of recipient), urticarial, fever, joint swelling, intense pruritis and a variety of skin rashes and anaemia. Large doses of penicillins given orally may lead to gastrointestinal upset particularly , nausea, vomiting and diarrhea (Wilson, 1995).

2.9.5 Pharmacokinetics of amoxicillin

Amoxicillin is stable in the presence of gastric acid and is rapidly absorbed after oral administration. The effect of food on the absorption of amoxicillin have been partially investigated; 400-mg and 875-mg formulations have been studied only when administered at the start of a light meal. Orally administered doses of 250-mg and 500-mg amoxicillin

capsules result in average peak blood levels 1 to 2 hours after administration in the range of 3.5 mcg/mL to 5.0 mcg/mL and 5.5 mcg/mL to 7.5 mcg/mL respectively.

Orally administered doses of amoxicillin suspension, 125 mg/5 mL and 250 mg/5 mL, result in average peak blood levels 1 to 2 hours after administration in the range of 1.5 mcg/mL to 3.0 mcg/mL and 3.5 mcg/mL to 5.0 mcg/mL respectively (Spartt, 1994).

Amoxicillin diffuses readily into most body tissues and fluids, with the exception of brain and spinal fluid, except when meninges are inflamed. In blood serum, amoxicillin is approximately 20% protein-bound. Following a 1-gram dose and utilizing a special skin window technique to determine levels of the antibiotic, it was noted that therapeutic levels were found in the interstitial fluid. The half-life of amoxicillin is 61.3 minutes. Approximately 60% of an orally administered dose of amoxicillin is excreted in the urine within 6 to 8 hours. Detectable serum levels are observed up to 8 hours after an orally administered dose of amoxicillin. Since most of the amoxicillin is excreted unchanged in the urine, its excretion can be delayed by concurrent administration of probenecid.

2.10Methods of Pharmaceutical Analysis

The science of drug analysis is an extremely active one in terms of research on newer, more reliable, or more sensitive methods of analysis (Olaniyi and Ogunlana, 1995; Lee and Webb, 2003; Ahuja and Scypinski, 2011).

In the development and application of separation and analytical techniques to the determination of drugs present in biological fluids or tissues, it is essential to know the potential and limitations of the technique selected. The validity of the method depends on the assumption that a drug added to a tissue dispersion will behave identically with regard to separation as it would if present in the tissue of intact animals. The logical development of the methodology should therefore include:

- Determination of the characteristics (Linearity) of the instrumentation used for analysis of drug concentration by construction of an absolute concentration curve.

- Determination of a calibration curve for the total procedure by application to aqueous drug solutions to obtain ‘‘aqueous recovery’’ values.

- Calibration of the total procedure by application to biological samples to which known concentrations of the drug have been added, to obtain ‘‘tissue recovery’’ values.

- Determination of the specificity of the procedure with respect to interference from both endogenous compounds (tissue blank) and drugs metabolites. This usually involves application of techniques different from those used in

the separation procedure to establish that the desired compound has been separated and is being solely estimated by the analytical procedure under application (Trevor *et al.,* 1971).

The various analytical methods that have been developed for the quantitation of drugs from biological samples. - Titrimetry and Gravimetry, Chromatography, Spectropholomery (U.V/Visible, I.R, N.M.R), Mass spectroscopy, Atomic Absorption spectroscopy and flame photometry, Refractometry and polarimetry (Olaniyi and Ogunlana, 1989; Lee and Webb, 2003; Ahuja amd Scypinski, 2011).

Any of these methods could provide desired results thus; a method to be adopted may depend on the instrument available. For making a choice of an analytical method, the criteria to bear in mind are selectivity, sensitivity, reliability and convenience.

Similarly the understanding of physiochemical characteristic of a drug being studied is very vital too, these include solubility in variety of solvents, pK_a values of ionisable compounds (which will lead to solubility -pH relations) molecular weight and weather the drug exist in optically active form (Olaniyi and Ogunlana, 1995; Lee and Webb, 2003; Ahuja amd Scypinski, 2011).

2.10.1 High performance liquid chromatography

Chromatography is a separation techniques used in the analysis of component of a mixture. The chromatographic method of determination of metformin in human plasma particularly high pressure/performance liquid chromatography has the following advantages:

(a) It provides a specific, sensitive and precise method for analysis of complicated samples.

(b) There is ease of sample preparation and sample introduction.

(c) There is speed of analysis.

(d) The analysis by HPLC is specific, accurate and precise

The type of chromatography where a liquid (mobile phase) is forced through the column (stationary phase) under pressure is known as high performance liquid chromatography. When the stationary phase is solid, the mobile is a liquid – is called liquid –solid Chromatography (LSC), when the stationary phase is liquid the mode is a Liquid – is called Liquid- liquid Chromatography (LLC).

The real breakthrough in HPLC came with the introduction of packing materials in which a stationary phase was covalent link to the silica support bonded. This enables reproducible and durable reverse – phase column to be manufactured, the used method is to react the silica with organochlorosilane or similar reagent to make a stationary phase of any desire polarity.

$$\text{-SiOH} \ {}_{+}\text{Cl-SiR}_3 \longrightarrow \text{-SiO-SiR}_3$$

The most popular supports have been R is octadecyl or octyl. The former is suitable for all application where is important to note here any residual interaction with the silicon atoms which sterically shielded. Octyl phase are less lipophilic and retain drug molecule less strongly: separation can then be faster for equivalent length of column. Some packing materials which are commercially available under variety of names which include:

(1) Ubonokpak Phenyl

(2) Ultrasphere ODS C18

(3) Apex Octadelyl

(4) Novapak C18

(5) Spheresorb C8

(6) Hypersil

(7) Supelco C8

Although packing from different manufacturer appear to have the same description on paper. The different methods of manufacture and treatment, as well as purity of reagents can lead to different performance characteristics thus the transfer of methods from one laboratory to another may not be as straight forward as would appear on the surface and the analysis should be aware of the potential differences. Apart from robustness of the bonded stationary phase, the new columns were eminently suited to drug analysis in biological fluids. First, the drug is likely to be one of the most lipophilic components of the sample and hence is retained on the column longer than most endogenous compounds. This includes metabolites, which generally one more polar than the parent drug.

Secondly the reverse – phase mode means that, the mobile phase is aqueous which is appropriate medium for analyzing an aqueous sample.

Improvement in the performance of reserved- phase systems have been claimed by the use of radial compression chromatographic columns; these are intended to eliminate the channeling that can occur at periphery of the column by compressing the column inward,

and assays using such column have been described for Azathioprine, Lomefloxacin, and galocitabine (Olaniyi and Ogunlana,1995; Chamberlain, 1995).

2.10.1.1 *Mobile phases*

In HPLC it is now more common to settle on a single reverse – phase column and achieve the required separation by controlling the partition between the organic stationary phase and the mobile aqueous phase.

The simplest form of mobile phase is water such a simple system would be extremely limited however, the addition of water – miscible organic solvents such methanol, ethanol or acetonitrile, may be used to modify the polarity of the organic phase.

For drugs which are weak acid or weak base the partition can be adjusted by altering the pH of the aqueous phase with suitable buffer. In the simpler applications, small amount of acid (usually acetic or phosphoric, but some time nitric or sulphuric) are added to the aqueous mobile phase, this will cause increase retention of the organic acids as the ionization is suppressed and decreased retention of bases as the ionic form is preferred.

Nicholls *et al*. (1995) described the optimization of the separation of anthrocytidines and the metabolites using reversed- phase liquid chromatography, and the use of a retention index library has been initiated for screening by reversed – phased HPLC.

2.10.1.2 Detectors

In the early development of HPLC the two most popular detectors were the refractive index detector and the ultraviolet absorption.

The most popular detector by far- for drug analysis and other applications have been the U.V absorption detector although for very sensitive applications, the fluorescence detector is also very popular. U.V detectors are the workhouse of HPLC analysis for applications which are not very demanding. In its simplest form, the u.v detector consist of a single wavelength source (the 254nm mercury line) the detector monitors the effluent from the column and will produce a response for all compounds which have some absorption at this wavelength, it is very necessary that the compound should exhibit its maximum absorption at this at this wavelength, u.v. spectra display wide absorption bands and hence practice the use of fixed wavelength detectors is not a limited as first appear. The limit of sensitivity of detection with this detector depends on the extinction coefficient of the analyte at 254nm and also on the efficiency of the column. If the chromatographic peak is broad the measured drug is considerable diluted.

The limit of detection of particular component can be extended by using variable wavelength detector. This can be done by setting the wavelength of the absorption maximum of the drug. However it is often just as useful to choose a wavelength that minimizes interference, rather than minimizes response.

In screening methods, where drugs may have absorption maxima at widely different wavelength some of the sensitivity of the detector may be lost and detectors which can simultaneously monitor column effluent at several different wavelengths have proved particularly useful.

Naturally, the drug molecule need to have u.v. absorbing, group to be used with a u.v. detector unsaturated ketones, such as in the steroid sex hormones and cortisone provide

excellent absorbing species, but even saturated ketones can be detected with reasonable sensitivity.

2.10.2 Ultraviolet analysis

For detection of drugs in body fluids, ultraviolet (UV) analysis is used in the analysis of drug in body fluid. This technique is applicable to a wide variety of compounds that are capable of absorption of energy from radiations in the wavelength 200 – 350 μm. Absorption in this range is usually associated with the displacement of outer electrons within the molecules giving rise to a new energy state.The spectrum is usually characteristic for a given molecule and the extent of absorption at a given wavelength can be utilized for quantitative analysis.

The UV method is also of value in identification and estimation of purity of a compound. Both molar coefficient and wavelength at which maximum absorbance occurs are characteristics of the molecule.

Although a wide variety of solvents are permissible, it is important to check that the solvent does not have absorption bands in the same region as that selected for measurement of the compound.

Ultraviolet spectrometry is characterized by a high degree of precision and accuracy, but lacks sensitivity. A compound rarely has a sufficiently high molar extinction coefficient to permit quantitation of less than the μg per ml of solution (Trevor *et al.,* 1971).

2.10.2.1 *Factors governing absorption of radiation in the ultraviolet region*

Radiation in the U.V region is absorbed though excitation of the electrons involved in the bonds between the atoms making up the molecule so that the electron cloud holding the atoms together redistributes itself and the orbitals occupied by the bonding electrons no longer overlap. Short wavelength UV radiation <150 nm (>8.3 eV) can cause the strongest bonds in organic molecules to break. It is the weaker bond in the molecule that are of more interest to analysts because they can be excited by longer wavelength UV radiations >200 nm which is at a longer wavelength than the region in which air and common solvents absorb.

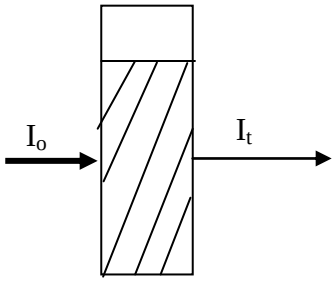
Considering a very simple organic molecule, ethylene, $CH_2=CH_2$, it contains two types of carbon-carbon bonds, a strong σ bond and a weaker π bond. The σ bond would become excited and break when exposed to radiation at 150nm. The weaker π bond requires less energetic radiation at 180nm to produce π* excited state. This excitation can occur without the molecule breaking since the σ orbitals remain unexcited by the longer wavelength radiation at 180nm. A single double bond is not useful as a *chromophore* for determining analytes by UV spectrophotometry since it is still in the region where air and solvents absorb.

Absorption takes place at longer wavelengths with greater intensity if more double bonds are present in a structure in conjugation. Such extended system of double bonds are known as '*chromophores'*. The most common chromophore found in drug molecules is a benzene ring.

Benzene has its absorption maxima at a much shorter wavelength than a linear triene such as hexatriene (absorption max. 275nm) and its strongest absorbance is at the wavelength of absorption of an isolated double bond at 180nm. It also has a strong absorption band at 204 nm.This is due to the symmetry of benzene; it is not possible to have an excited state involving all the bonds in benzene because this would mean that the dipole (polarisation of the chromophore), a two dimensional concept which is created in the excited state, would be symmetrical and thus would have to exist in thee dimensions rather than two. There is a weak absorption in the benzene spectrum close to the absorption max. for hexatriene and this can occur because vibration of the benzene ring in a particular direction can distort its symmetry and thus allow all thee double bonds to be involved in an excited state. If the symmetry of the benzene ring is lowered by substitution, the bands in the spectrum undergo a bathochromic shift (a shift to a longer wavelength). Substitution can either involved extension of the chromophore or attachment of an *auxochrome* (a group containing one or more lone pair of electrons) to the ring or both. The absorption spectrum of a drug molecule is due to the particular combination of auxochromes and chromophores present in its structure (Trevor *et al.,* 1971).

2.10.2.2*Beer-Lambert's Law*

Absorption of radiation by a solution containing a UV absorbing compound is depicted below



Sample

The measurement of light absorption by a solution of molecule is governed by the Beer-Lambert's law, which is written as follow:-

$$\text{Log} \quad \frac{I_o}{I_t} = A = \Sigma bc$$

Where I_o – is the intensity of the incident radiation.

I_t – is the intensity of the transmitted radiation.

A – is known as the absorbance and is a measure of the amount of light absorbing by the sample.

Σ – is a constant known as molar extinction coefficient and is the absorbance of a IM solution of the analyte.

b – is the path length of the cell in cm, usually I cm

c – is the concentration of the analyte in moles litre^{-1}.

The molar coefficient and wavelength at which maximum absorbance occurs and characteristics of the molecule, a calibration curve can be constructed at various concentration of the drug (Trevor *et al.*, 1971).

Even with the availability of more sensitive and sophisticated instruments for drug analysis nowadays, U.V –spectrophotometric technique still remain one of the most basic and fundamental technique used for of handling, maintenance and availability (Blanchard and Sawchuck, 1995).

2.10.2.3. Precision

This is an analytical control in which the degree of agreement among individual tests when the method is applied repeatedly. Precision is normally express as coefficient of variation (C.V), where a small CV indicates higher precision.

It is usually determined by assaying sufficient number of aliquots of a homogenous sample in order to estimates the mean (X) and standard deviation (S.D) , the C.V coefficient of variation, is then calculated using the formular

$$C.V = \frac{X}{S.D}$$

For good precision, the value of C.V should not be >15 %

2.10.3 Percentage eextraction recovery (PR)

The percentage extraction recovery of analytical method is to test the efficiency and acceptability of the extraction method to be employed, in the analysis and also for the

procedure to be reproducible. The percentage extraction recoveries were calculated using the following relationship:-

$$PR = \frac{\text{Amount of metformin recovered after plasma extraction}}{\text{Amount in the standard preparation solution of metformin}} \times \frac{100}{1}$$

2.10.4 Validation of analytical method

Validation of analytical method includes all the proceeding of a particular analytical method for the quantitative determination of the concentration of an analyte or series of analytes in biological materials for the intended application. The rationales of validation of analytical method include the following:-

- To establish confidence in the analytical data generated from the experiment.
- To insure that a selected analytical procedure will give reliable result and reproducible, so that it will therefore be necessary to validate a method according to the well-established criteria of precision, accuracy, sensitivity specificity and reproducibility.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1Materials

3.1.1 Test drugs

3.1.1.1 Metformin

Batch No. 10 3144BC

Manufacturing date: December, 2012

Expiry Date : December, 2016

Manufacturer : Merck Pharm Serono UK

3.1.1.2 Ampicillin/cloxacillin (Ampiclox)

Batch No. : AS5010019A

sManufacturing Date: June, 2011

Expiry Date: June, 2015

Manufacturer: Beecham Pharmaceuticals Brentford England

3.1.1.3 Metronidazole

Batch No. : M6257

Manufacturing Date: February, 2012

Expiry Date: February, 2016

Manufacturer: Emzor Pharmaceutical Ind.Ltd.Nig.

3.1.1.4 Amoxicillin

Batch No. AS4500087C

Manufacturing Date: March, 2011

Expiry Date: March, 2017

Manufacturer: Beecham Pharmaceuticals Brentford England

3.1.1.4 Reference compounds

Metformin standard powder (Merck pharm serono UK)

Sulfadoxine standard powder (Fidson Pharmaceutical. Nig.Ltd)

3.1.2 Glass wares

Beakers: 100 and 250ml (Pyrex, England).

Volumetric flask: 100 and 250 ml (Pyrex, England).

Samples bottles.

Conical flasks: 50,100 (Pyrex, England).

Measuring Cylinders 50,100ml and 1L (Pyrex, England).

Pipettes: 1 ml, 5ml, 10 ml (Pyrex, England).

Centrifuge tube (Pyrex, England)

Automatic water distiller Model NO SZ-1 (Gallenkamp, England).

3.1.3 Equipment

Centrifuge tube (Pyrex England).

Melting point apparatus-Eureka England.

Double beam UV. Spectrophotometer (Pyrex Unicam).

Refrigerator-premier Thermocool Nig.

Hot air oven –big size Gallenkamp, England.

High performance liquid chromatography (Agilent Technogies 1120 Compact LG).

Auto rotator mixer (-Stuart, England).

Electronic balance-(Metter Toledo EA204).

Disintegration rate study (Eureka, England).

Dissolution rate study apparatus (Eureka, England).

3.1.4 Reagents and standard samples

Methanol - Analar grade (BDH Chemicals Ltd., Poole, England).

Potassium dihydrogen orthophosphate- Baker Inc. USA.

Acetonitrile - Analar grade (BDH Chemicals Ltd., Poole, England)

Metformin standard- Fibson pharmaceutical. Nig. Ltd.

Sulfadoxine standard powder- Ranbaxy Nig. PLC.

Ortho-phosphoric acid – May and Baker Ltd., Dagenham, England.

3.2 Methodology

3.2.1 In-vitro studies of metformin

3.2.1.1Identification test of metformin tablets

(a). A quantity of the powdered tablets containing 20 mg of metformin hydrochloride was shaken with 20 ml of *absolute ethanol* , filtered and the filtrate was evaporated to dryness on a water bath .The residue was dried at 105°C for 1 hour.

3.2.1.2 Assay of metformin (B.P.2002)

Twenty metformin tablets were accurately weighed; the average weight determinedand the tablets were powdered. A quantity of thepowder containing metformin hydrochloride(0.1 g) was weighed; 70 ml of water was added and shaken for 15 minutes. The mixture was diluted to 100 ml with water and filtered. 10 ml of the resulting solution was diluted to 100 ml with waterand the resulting solution 10 ml was also diluted to 100 ml with water. Finally theabsorbance of the resulting solutionwasread at 232 nm. The total content of metformin in the sample was calculated taking 798 as the value of A (1%, 1 cm) at 232 nm (see appendix vi)

3.2.2 In-vitro studies of ampicillin/cloxacillin

3.2.2.1 Identification of ampicillin/cloxacillin in ampiclox capsules

The identification test was carried out by weighing 0.038g of the ampiclox powder, and 2mg of the powder was transferred into a test-tube. 2mg of chromotropic acid sodium salt and 2ml of sulphuric acid (96%w/w) were then added to the content of the test tube, and immersed in paraffin oil-bath at 150^{o}C. The solution was shaken and observed for colour change at an interval of 30 seconds.

Also, 20mg of powdered capsules was suspended in 1ml of water and a mixture of 6ml of water and 2ml of potassium curpritartarate solution was then added to the contents of the test tube.

In another qualitative test, 0.2g of the powdered capsule was dissolved in 2ml of water contained in a test-tube. No precipitate was observed by adding 2ml of 15% w/w solution of potassium carbonate even after being heated to boiling. The test continued by adding

4mlpotassium antimonate solution to the contents of the test-tube and then heated to boiling (B.P. 2002).

3.2.2.2 Chemical assay of ampiclox capsules (Ampicillin/Cloxallin)(B.P.2002).

(a) Ampicillin

The assay of ampicillin in ampiclox was carried by weighing 20 capsules and average weight was determined. Ampicloxpowder(0.15 g) was weighedand dissolved in sufficient water and made up to 500ml in a volumetric flask.The mixture wasshaken for 30 minutes and filtered. Thefiltrate (10 ml) was transferred to a 100 ml volumetric flask and 10 ml of boric buffer **p**H 9.0 and 1 ml acetic anhydride-dioxane solution were added. The resulting solution was allowed to stand for 5 minutes and finally the solution was made up to 100 ml by adding sufficient distilled water.

Two 2-ml aliquots of this solution were placed in two separate stoppered tubes; to one of the tube (A),10ml of imidazole-mercury reagent was added and mixed. The tube was tightly covered, immersed in a water bath at 60^0C for 25 minutes with occasional swirling. To the second tube (B), 10 ml of water was added and finally the absorbance of the two solutions A and B were measured at 325nm.

The content of ampicillin was calculated from the difference between the absorbance of solution A and B from the difference obtained by repeating the operation using 0.17 g of ampicillox trihydrate BPCRS in place of the substance being examined and from the declared content of ampicillin in ampiclox (see appendix VII).

(b). Cloxacillin

The chemical assay of cloxacillin in ampiclox was carried out by weighing the content of 20 capsules of ampiclox (0.25g) of the ampiclox powder was weighed and added to 70ml of water, followed by shaking for 15minutes and sufficient water was added to make up to 500ml.

The mixture was shaken for 30 minutes and filtered. 10ml of the filtrate was transferred to a 100 ml volumetric flask and 10ml of boric buffer pH 9.0 and 1 ml acetic anhydride-dioxane solution were added and allowed to stand 5 minutes and finally sufficient water was added to produce 100 ml.

Two 2-ml aliquots of this solution were placed in two separate stoppered tubes, to one of the tube (A), 10 ml of imidazole-mercury reagent was added and mixed. The tube was tightly covered, immersed in a water bath at 60^0C for 25 minutes with occasional swirling. To the second tube (B) 10 ml of water was added and finally the absorbance of the two solutions A and B were measured at 325nm.

The content of cloxacillin was calculated from the difference between the absorbances of solution A and B from the difference obtained by repeating the operation using 0.17 g of cloxacillin sodium BPCRS in place of the substance being examined and from the declared content of cloxacillin in ampiclox (see appendix VIII).

3.2.3 In-vitro studies of metronidazole

3.2.3.1 Identification test for metronidazole tablets (B.P. 2002)

A quantity of the powdered containing 10 mg of metronidazole was dissolvedin 1 ml of water and 0.25 ml of hydrochloric acid and heated in water bath with 10 mg of zinc powder

for 5 minutes, cooled in iceand 5ml of sodium nitrite solution was added and excess of Nitrite was removed with sulphuric acid, 0.5ml of the product wasadded to a mixture of 0.5 ml of 2-naphthol solution and 2 ml of 5M sodium hydroxide and observed for any colour change.

3.2.3.2 *Assay test of metronidazole tablets*(B.P 2002).

The assay of metronidazole was carried out by weighing 20 tablets of metronidazole noting the average weight and powdering them thereafter. A quantity of the powder containing (0.2 g) of metronidazole was transferred to sintered glass crucible and extracted with six (10 ml) quantities of hot acetone and cooled.To the combined extracts, 50 ml of acetic anhydride and 0.1 ml of 1 % solution of brilliant green in anhydrous acetic acid were added and titrated with 0.1 M perchloric acid to a yellow- green end point.

A repeat of the titration was carried out without powdered metronidazole tablets, the difference between the end point of the titration with and without powdered metronidazole tablets represent the amountofperchloric acid required. Each ml of 0.1M perchloric acid VS is equivalent to 17.12 mg of $C_6H_9N_3O_3$(see appendix IX).

3.2.4 In-vitro studies of amoxicillin

3.2.4.1 Identification test for amoxicillincapsules

Identification test for amoxicillin capsules was carried out by preparing 0.01 % w/v solution of amoxicillin powder in 0.067M mixed phosphate buffer pH 7.0 in a test tube (solution A). 0.5 ml of dilute penicillinase solution was added to 10 ml of the solution A and allowed to stand for 10 minutes at 30^0C (Solution B).

5 ml of each of solution A and B were mixed in separate test tube and 10 ml of acetate buffer pH 4.6 and 5 ml of 0.0005 M iodine were added to the content of each test tube,

(0.1 ml) of starch solution was then added to each of the test tube .The mixture produced with solution A was blue while B remained colorless (B.P 2002).

3.2.4.2 Assay of amoxicillin capsules (B.P.2002)

The assay of amoxicillin capsules was carried out by weighing 20 capsules of amoxicillin and the average weight was determined. The equivalent weight of 0.03444 g of amoxicillin was dissolved in sufficient water to produced 100 ml and shaken for 30 minutes and filtered.

2ml of the filtrate was transferred to a 100 ml volumetric flask and added 2ml of boric buffer pH 9.0 and 0.2 ml acetic anhydride-dioxane solution were and allowed to stand 5 minutes and finally sufficient water was added to produce 20 ml.

Two 2-ml quantities of this solution were placed in separate stoppered tubes. To one tube 2 ml of imidazole –mercury reagent was added and mixed then immersed in a water bath at 60^0C for exactly 25 minutes, with occasionally swirling .The tube was removed and rapidly cooled to 20^0C (solution A).

To the second tube (solution B), 2 ml of water was added and mixed and without delay the absorbance of A and B were taken at 325 nm.

The content of amoxicillin was calculated from the difference between the absorbance of solution A and B by repeating the operation using 0.17g of amoxicillin trihydrate BPCRS in place of the substance being examined and from the declared content of amoxicillin in amoxicillin trihydrate BPCRS (see appendix X).

3.3Preparation of Solution Metformin and Sulfadoxine(I.S)

3.3.1 Preparation of metformin

Stock solution of metformin was made by dissolving 0.1 g of metformin standard powder in 100 ml of methanol to give 1 mg/ml.

Serial dilutions of working concentrations of 0.030-4.0μg/ml were prepared from the stock solution using the formula $C_1 \times V_1 = C_2 \times V_2$.

Stock solution of Sulfadoxine (Internal standard) was also prepared in a similar manner. From the stock solution, 4.0 μg/ml was prepared using the formula $C_1 \times V_1 = C_2 \times V_2$

3.3.2 Preparation of buffer

0.01M of potassium dihydrogen orthophosphate phosphate was prepared by dissolving 1.36g of KH_2PO_4 crystals in 1L of distilled water and the pH adjusted to 2.5 using phosphoric acid.

3.3.3 Development of solvent system

The optimization of the solvent system was carried out inwhich acetonitrile and potassium dihydrogene orthophosphate were used as mobile phase. Several conditions for ratios of mobile phase, pH and flow rate were tried (trial and error) and the chromatograms were obtained. Solvent system used by Bhavesh et *al.,* 2007 was adopted, modified and validated to ascertain the reliability and reproducibility.

3.4 Extraction procedure

The processes of recovering the drug from the plasma samples were conducted as follows:-

I. 3.0 ml of the separated plasma was taken from each of the samples and placed in a cleaned and dried glass test tube. Acetonitrile was added to plasma samples in the ratio1:3 and mixed on a vortex mixer for 5 minutes to precipitate the proteins and liberated the drug bound to the proteins.

II. The mixture was spun in a Uniscope bench laboratory centrifuge for 10 minutes at the rate of 4000 revolutions per minute. The supernatant was collected with aid of 5 ml needle and syringe.

3.4.1 Precision and percentage extraction recovery of metformin

The precision of the analytical method was determined by assaying a number of aliquots homogenous samples in order to calculate statistical valid estimate of mean among the individual test applied. Repeatedly for at least five times for three concentration points with estimate of S.D and coefficient of variation (C.V) for both within day and between day precision.

Percentage extraction recovery was estimated using two different concentrations (0.2 and 0.8 µg/ml) and was calculated using the following relationship:-

$$PR = \frac{\text{Amount of metformin recovered after plasma extraction}}{\text{Amount in the standard preparation solution of metformin}} \times \frac{100}{1}$$

3.5 Calibration Curve

Calibration curve of metformin was prepared using blank plasma sample (3.0 ml) spiked with 1.0 ml each of different concentrations (0.03, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 3.0 and 4.0 µg/ml) of the standard metformin and 4.0µg/ml of sulphadoxine (I.S) in separate test-tubes. Acetonitrile (1.0 ml) was then added to the ten different test tubes and mixed on a vortex mixer for 5 minutes. The acetonitrile layer containing metformin were run into the HPLC machine. The peak area ratios of metformin to sulphadoxine from the chromatograms obtained were plotted against their corresponding concentration to give the calibration curve (see Table 3.1 and appendix XI).

Table 3.1: Metformin concentrations (μg/ml) and their respective peak area ratio in high performance liquid chromatograms

Conc. μg/ml	Peak area ratios
0.03	238.0
0.05	240.0
0.1	224.0
0.2	253.0
0.4	316.0
0.8	380.0
1.0	292.0
2.0	817.0
3.0	1307.0
4.0	1527.0

3.7Study Design

Eighteen type 2 diabetic patients participated in the study. The criteria for selecting the participants were based on the National Diabetes Data group's recommendation of 1989 and the selection was done by the practicing clinician, none of participants was below the age of 35 years. The age of the patients ranged from 35-55 years (mean ±S.D 45 ±6.4 years) weight ranged from 50-70 kg (mean ±S.D, 62.3 ±7.34 kg) with height range 1.5-1.75m (mean ±S.D, 1.65 0.1M) took part in the study.

The approval of the research was anoffshoot of the approval granted by the Ethical Committee of Ahmadu Bello University Teaching HospitalZaria, Nigeria (see appendix XII).

The study was divided into two phases with a washout period of seven days between the phases. In phase 1, metformin alone was administered to all the subjectswhile in phase 2, the subjects were divided into three groups, with six subjects in each groups for co-administration of metformin with the three interacting drugs

The protocol of the study was explained to each of the patients and informed consent were obtained. They were not allowed any drugs or alcohol two weeks prior to the study.

3.7.1 Phase 1

1 g of metformin alone was administered to each subject with 150 ml of water after an overnight fasting. 3ml of blood samples were then taken at time interval of 0, 0.5, 1.5, 3.0, 4.0, 6.0, 0.8 hours. The blood samples collected were centrifuged for ten minutes at 100 revolutions per minutes (rpm), and the plasma transferred into a bijoux bottle with a Pasteur pipette. These stoppered bottles were then stored in the freezer at -4^0C before analysis. This was followed by a wash out period of seven days. Glucose levels were determined for the samples taken at each sampling time.

3.7.2 Phase 2

Phase 2 involved regrouping the eighteen patients intothree groupsof six volunteers.The first group was co-administered 1 g metformin and 1 g ampiclox .

The second group was concomitantly administered 1g metformin with 400 mg metronidazole.

The third groups wereconcomitantly administered 1 g metformin and 1g amoxicillin. 3ml of blood samples were then taken at each sampling time. The blood samples collected foreach of the group were centrifuged for ten minutes at 100 revolutions per minutes (rpm),and the plasma transferred into a bijoux bottle with a Pasteur pipette. These stoppered bottles were then stored in the freezer at -4^0C before analysis. This was followed by wash out period of seven days. Glucose levels for each of the groups were determined for the samples taken ateach of the sampling time.

3.8 Analysis

Agilent Technologies 1120 compact model LC Series (USA) consist of pump type L-7100 with U.V absorbance detector (VWD) part NO-G1314 and with thermostat column at ambient temperature with auto sampler, and automatic injection system of 10-20µl volume with different column of each test drug, was used in the analysis using optimized condition.

3.8.1 High Performance Liquid Chromatography (HPLC) Variables and chromatographic conditions used for the determination of metformin and sulfadoxine (I.S).

Mobile phase	: -	Acetonitrile:	0.01M KH_2P04
	21	79	
Pressure	:	120-245 psi	
Column	:	Eclipse X BD C-8 4.6 x150mn	
Flow rate	: -	1.50 ml/min.	
Injection volume	: -	20 µl	
Wave length	: -	236 nm	
pH	:	5.4 (adjusted with phosphoric acid)	
Column Temperature	: -	ambient temperature	

CHROMATOGRAM

		Retention time
Metformin	:	1.06 min
Sulfadoxine (I.S)	:	2.25min

3.9 Laboratory Documentation

The results of various studies in the whole work were carefully recorded, figures and tables were drawn where appropriate and graphical representations, pharmacokinetics values were generated using computer method in determining the pharmacokinetic parameters (Joel*et al.*, 2012).

The following parameters were obtained from each plot:-

Lag time (time at which absorption commenced).

C_{max} (maximum concentration attained by the drug).

T_{max} (time taken to reach the maximum concentration)

$t_{1/2}$ (absorption half- life)

K_{α} (rate constant of absorption)

$t_{1/2\beta}$ (Elimination half-life)

K_{β} (Elimination rate constant)

AUC (Area under the curve)s

Other parameters were calculated (Paxton,1981) based on the derived parameters from the plots and these include

Volume of distribution (V_d)

$$Vd = \frac{FxD}{AUC}$$

F= fraction of the dose absorbed (assumed as 1)

D = dose of the drug administered

AUC = Area under the curve

K_{β} (Elimination rate constant)

The Area under the curve was by trapezoidal rule (Gibaldi and Perrier,1991).The AUC derived, is from 0-infinity ($AUC_{0-\infty}$), consisting of $AUC_{0—8}$ hours using trapezoidal rule and $AUC_{8---\infty}$ hr obtained by deviling the last concentration in the plasma level-time plot, by elimination rate constant of the slow phase.

Therefore $AUC_{0--\infty}$= AUC_{0---8} hrs + $AUC_{8---\infty}$ hrs.

Clearance (cl) $=K_{\beta} \times V_d$

Absorption half-life $=t_{½}abs = \frac{0.69}{K_a}$

Elimination half-life= Elim $t_{½\beta} = \frac{0.693}{Ke}$

(Paxton, 1989)

All the results were expressed as Mean ± standard error of mean (SEM). They were analyzed for statistical significance using student t-test for paired data. Statistical analysis was carried out. P Value less than 0.05($p<0.05$) were considered of statistical significance while $p>0.05$ were not considered to be statistically significant (Harper, 1984)

CHAPTER FOUR

4.0 RESULTS

4.1 In-vitro Studies

4.1.1 Identification test for metformin tablets

An orange red colour was produced which darkened on standing, indicated the presence of metformin hydrochloride. The sample passed the test.

4.1.2 Chemical assay for metformin tablets

The metformin tablets analyzed contained 99.40% of the active ingredients using A (1%, 1 cm) 798 value at 232nm. The sample complied with the official specification of drug content (95.0 -105%), B.P 2002. The sample passed the test.

4.1.3 Identification tests of ampiclox capsule (ampicillin/cloxacillin) (2002)

Chemical test with chromatopic acid sodium salt produced a purple colour after two minutes.

Chemical test with potassium cupritartarate solution produced an instant violet colour characteristic of ampicillin. A white dense precipitate was observed in the chemical test involving potassium carbonate and potassium antimonite solution.

4.1.4 Chemical assay of ampicillin/cloxacillin in ampiclox capsules

The ampiclox capsules assayed contained98.53% of ampicillin and 98.69 % of cloxacillin. The sample complied with the Official specification of 92.5 -107.5 % for each of the active ingredients (B.P.2002).The sample passed the test.

4.1.5 Identification test for metronidazole tablets

The melting point of the precipitate after washing with water and dried at about 105^0C was 150^0C. An orange to red colour produced indicated the presence of metronidazole in accordance to the specification of (B.P. 2002).The sample passed the test.

4.1.6 Chemical assay for metronidazole tablets

The percentage content was 99.44.The sample complied with the official specification of drug content 99.0-101.0 %(B.P.2002).The sample passed the test.

4.1.7 Identification test for amoxicillin

The mixture produced with solution A was blue while B remained colorless .The sample complied with the specification of (B.P 2002).

4.1.8 Chemical assay for amoxicillin.

The percentage content was 98.5.The sample complied with the official specification B.P 2002 which inferred that the sample has passed the test.

4.2 Solvent System and Optimization of Hplc Conditions

The solvent system combinations tried in six point variation in the development of mobile phases are as shown in Table 4.1. Acetonitrile: potassium dihydrogen orthophosphate (21:79) with pH 5.4 gave the best resolution with better peak symmetry and no baseline instability. The sampleswere ran using an Eclipse X DB C-8 column with dimension of 4.6 x 150 nmat temperature30 ^{0}C and flow rate of 1.5 ml /min. The samples were detected at wavelength 236 nm using a U.V detector. The retention times of metformin and sulfadoxine (I.S) recorded was 1.066 and 2.255 minutes respectively (see page 85).

When metformin powder was combined with the interacting drugs, to test whether or not there was going to be separation in an optimized conditions, the result showed changes in the retention time of metformin from 1.066 to 1.62; as a result of change in the flow rate from 1.5ml/min to 1.0 ml/min. (see page 85 b).

Table 4.1: Optimization of Solvent System for the Determination of Metformin in Biological Fluid.

Solvent Combination	pH	Flow rate (ml/min)	Retention time (min)
Acetonitrile: KH_2O_4			
21 : 79	5.4	1.5	1.066
25 : 75	5.2	2.0	1.54
30 : 70	5.0	1.0	1.32
50 : 50	5.5	1.4	1.07
40 : 60	4.9	2.5	1.50
20 : 80	4.5	1.6	1.00

4.3 Precision and Percentage Extraction Recovery

The precision of the analytical method was observed in both within- day and between days runs. The low values of coefficient of variation (C.V) indicate the reproducibility of the method, while the percentage recoveries found was between (96.52 and 98.43%). This indicated the efficiency of the extraction procedure employed (See appendix I).

4.4Calibration Curve

The calibration curve of the peak area ratios of metformin to sulphadoxine against their corresponding concentrations was found to be linear in the range of 0.03 – 4.0 µg/ml.The linear regression of equation from the plot is y = 343.94x + 161.11; where y is the peak area ratios, x is the concentration, 343.94 is the slope while 161.11 is the intercept and a correlation coefficient (r) of 0.983 (See appendix XI).

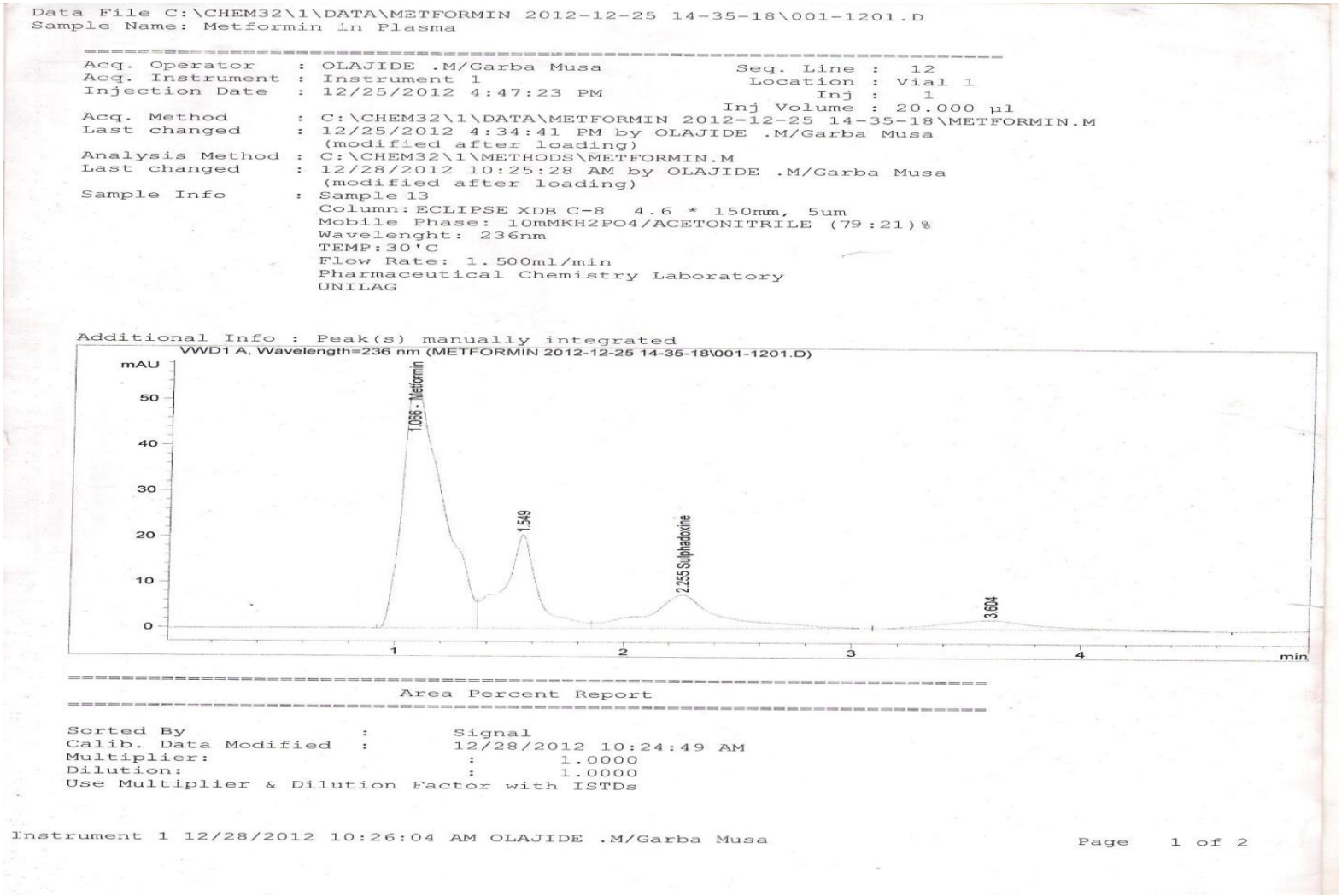


Figure 4.1High performance liquid chromatography of plasma sample of Type (2) Diabetic patients with Metformin tablet and sulfadoxine as Internal standard.

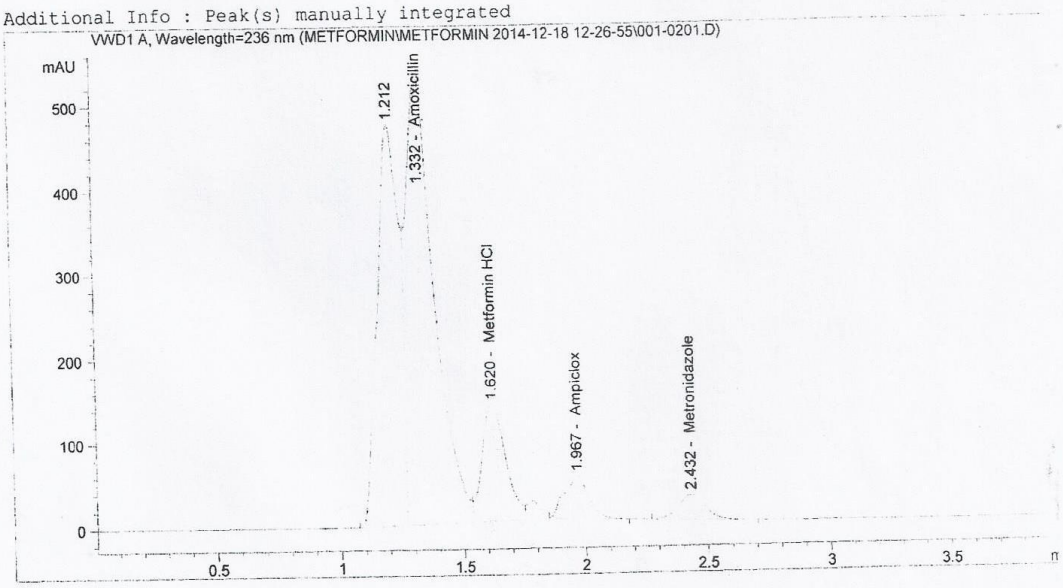
Data File C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 12-26-55\001-0201.D
Sample Name: Mixed Std

Acq. Operator : OLAJIDE M./BELLO S.
Acq. Instrument : Instrument 1
Injection Date : 12/18/2014 12:32:47 PM

Seq. Line : 2
Location : Vial 1
Inj : 1
Inj Volume : 20.000 µl

Acq. Method : C:\CHEM32\1\DATA\METFORMIN\METFORMIN 2014-12-18 12-26-55\METFORMIN.M
Last changed : 12/18/2014 12:31:35 PM by OLAJIDE M./BELLO S.
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\METFORMIN.M
Last changed : 12/18/2014 2:12:15 PM by OLAJIDE M./Musa Garba
(modified after loading)
Sample Info : Mixed Std Metformin HCl - 100Ug/mL; Ampiclox; Metronida
zole & Amoxicillin - 50Ug/mL
Column:Eclipse XDB C8 4.6 * 150mm, 5um
Mobile Phase: 25mMKH2PO4(pH5.4)/ACETONITRILE(79:21)%
Wavelenght: 236nm
TEMP:30'C
Flow Rate: 1.000ml/min
Pharmaceutical Chemistry Laboratory
UNILAG

Additional Info : Peak(s) manually integrated



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 12/18/2014 2:11:15 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

85b

Table 4.2: Mean Plasma concentrations of metformin (n=6) whenmetformin alone, and metformin co-administered with ampiclox,metronidazole and amoxicillin to type 2 diabetic patientsPlasma concentrations (µg/ml)

Time (hr)	Phase One	Phase Two		
	Metf.alone	Metf+Ampiclox	Metf. +Metro.	Metf. + Amoxicillin
0.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.5	0.208±0.18	0.526±0.19	0.435±0.18	0.232±0.03
1.5	0.833±0.16	0.943±0.15	1.090±0.19	0.821±0.08
3.0	1.140±0.20	1.280±0.31	1.347±0.39	1.104±0.07
4.0	0.601±0.38	0.919±0.17	0.0.654±0.09	0.574±0.18
6.0	0.343±0.16	0.377±0.18	0.454±0.90	0.325±0.05
8.0	0.234±0.22	0.201±0.12	0.2 35±0.18	0.218±0.02

Metf.= matformim Metro. = metronidazole

Comparison of plasma concentrations (µg/ml) of single dose of metformin alone and when co-administered with ampiclox, metronidazole, amoxicillin and comparison of postprandial mean glucose concentrations following oral administration of 1 gm metformin alone and when co-administered with 1 g ampiclox, 400mg metronidazole and 1g amoxicillin each are shown in Figures 4.2 – 4.8 respectively.

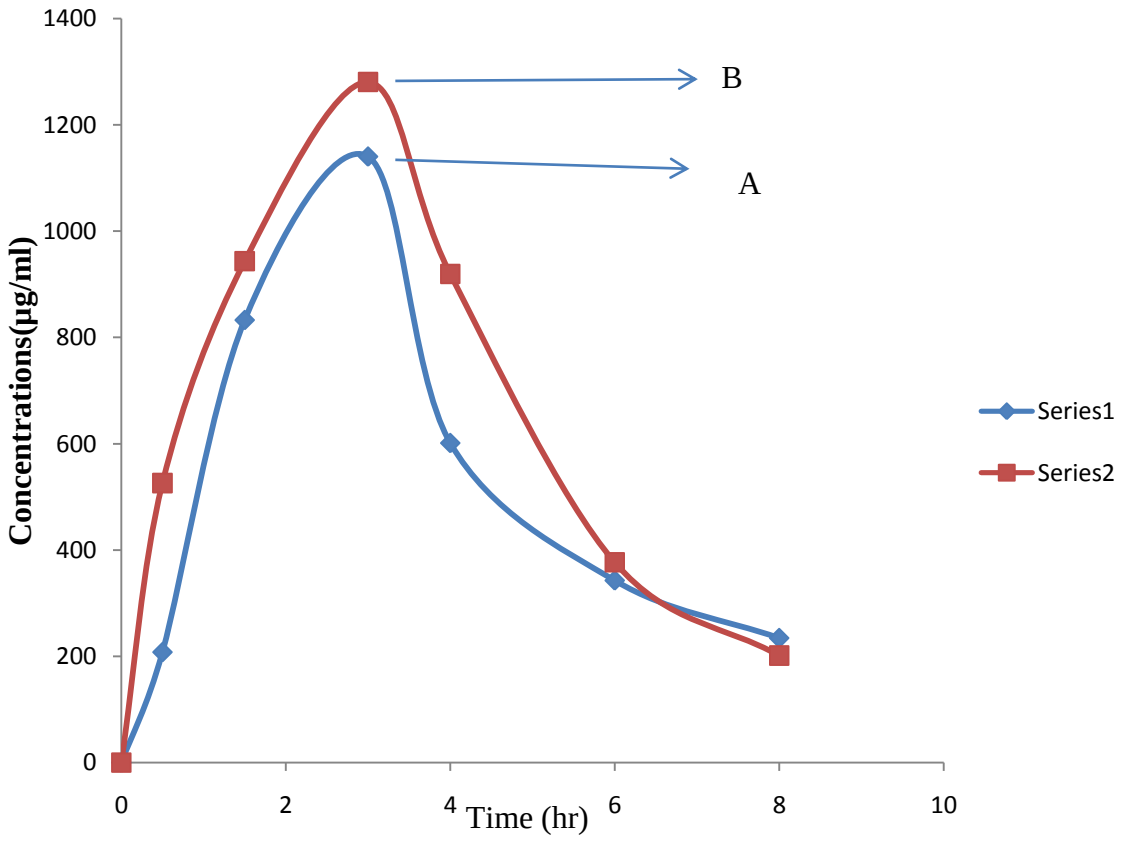


Figure 4.2: Comparison of plasma concentrations (µg/ml) of metformin alone (A) and co-administered with ampiclox (B).

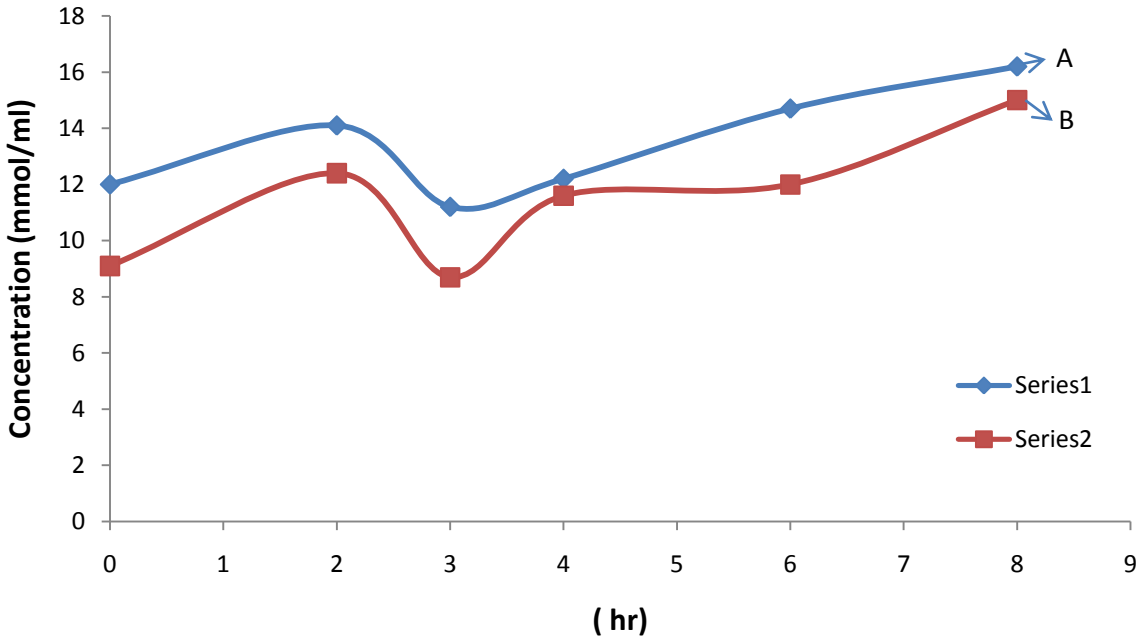


Figure 4.3: Comparison of mean glucose concentrations in six type 2 diabetic patients following oral administration of 1 g metformin (A) tablets and concomitantly with 1 g ampiclox capsule (B)

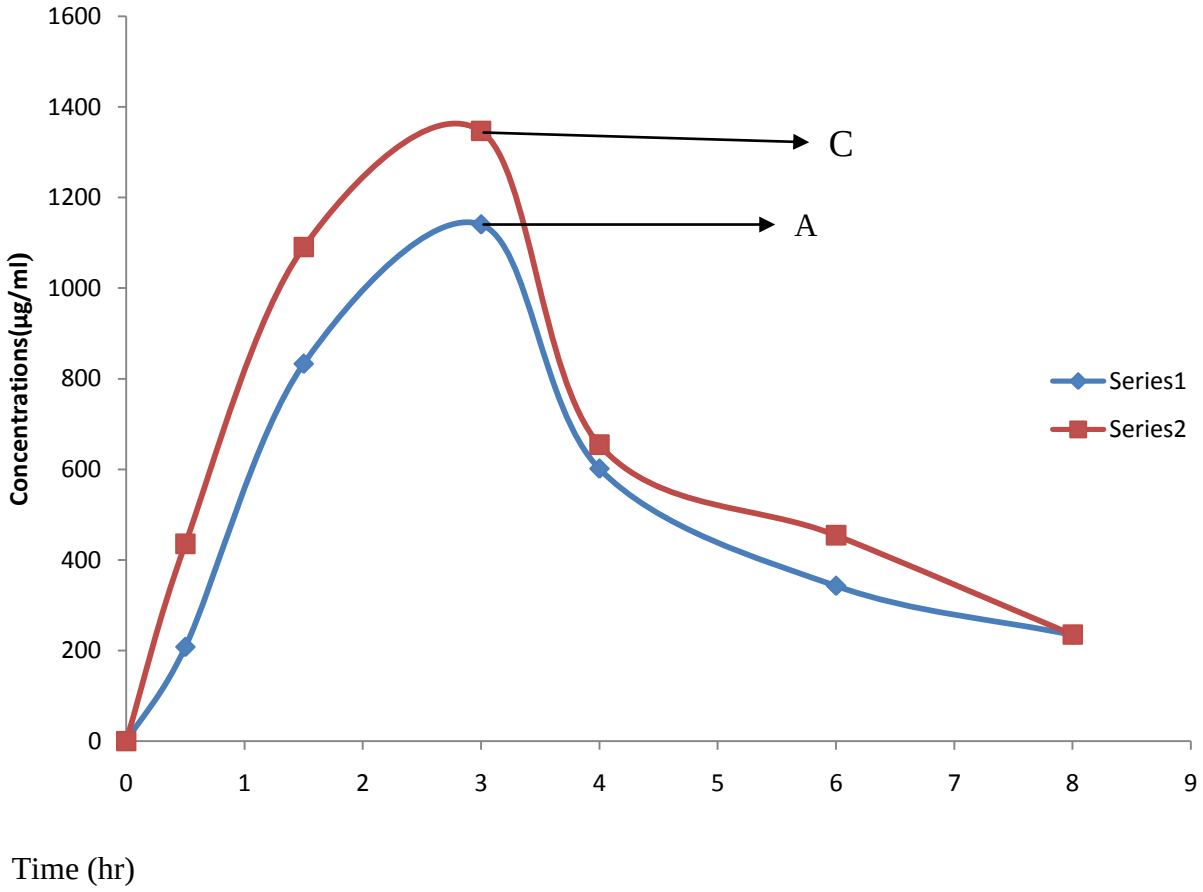


Figure 4.4: Comparison of plasma concentrations (µg/ml) of metformin alone (A) and co-administered with metronidazole (C)

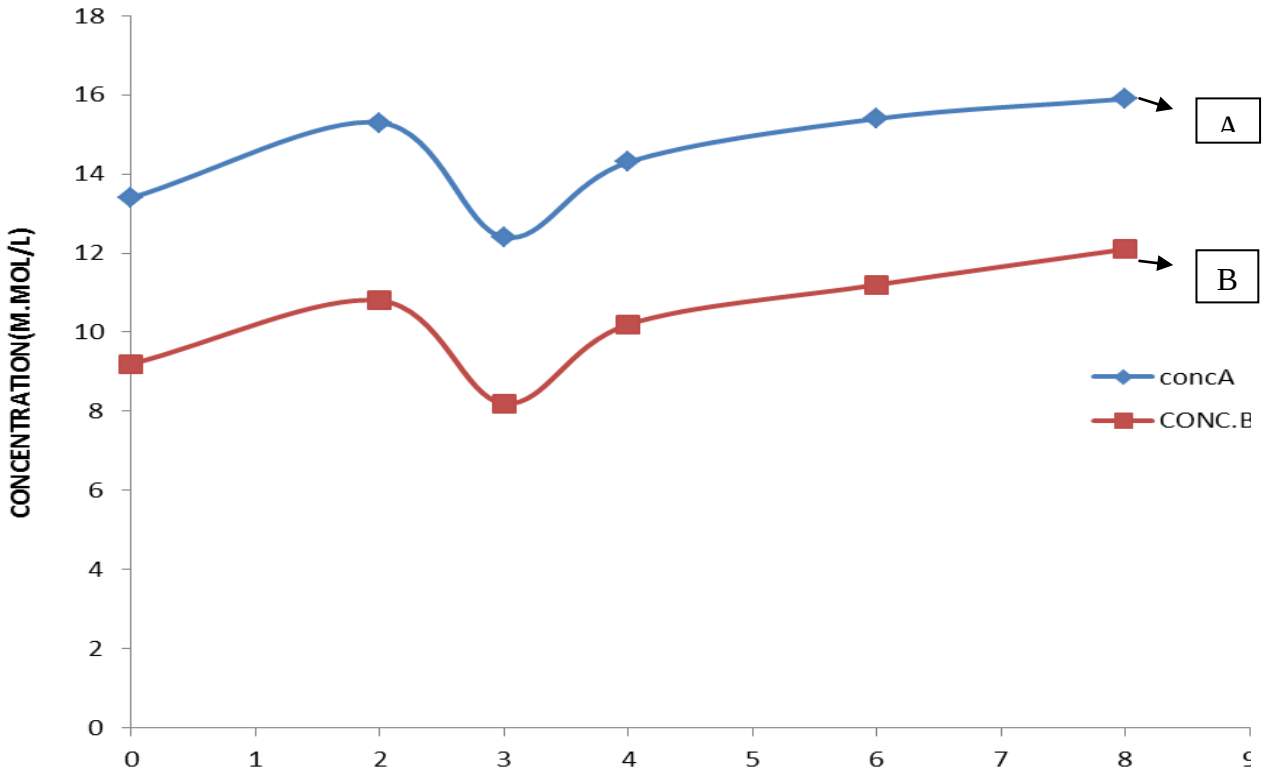


Figure 4.5: Comparison of mean glucose concentration s in six type 2 diabetic patients following oral administration of 1g metformin (A)tablets and concomitantly with 1 g metronidazole capsule (B)

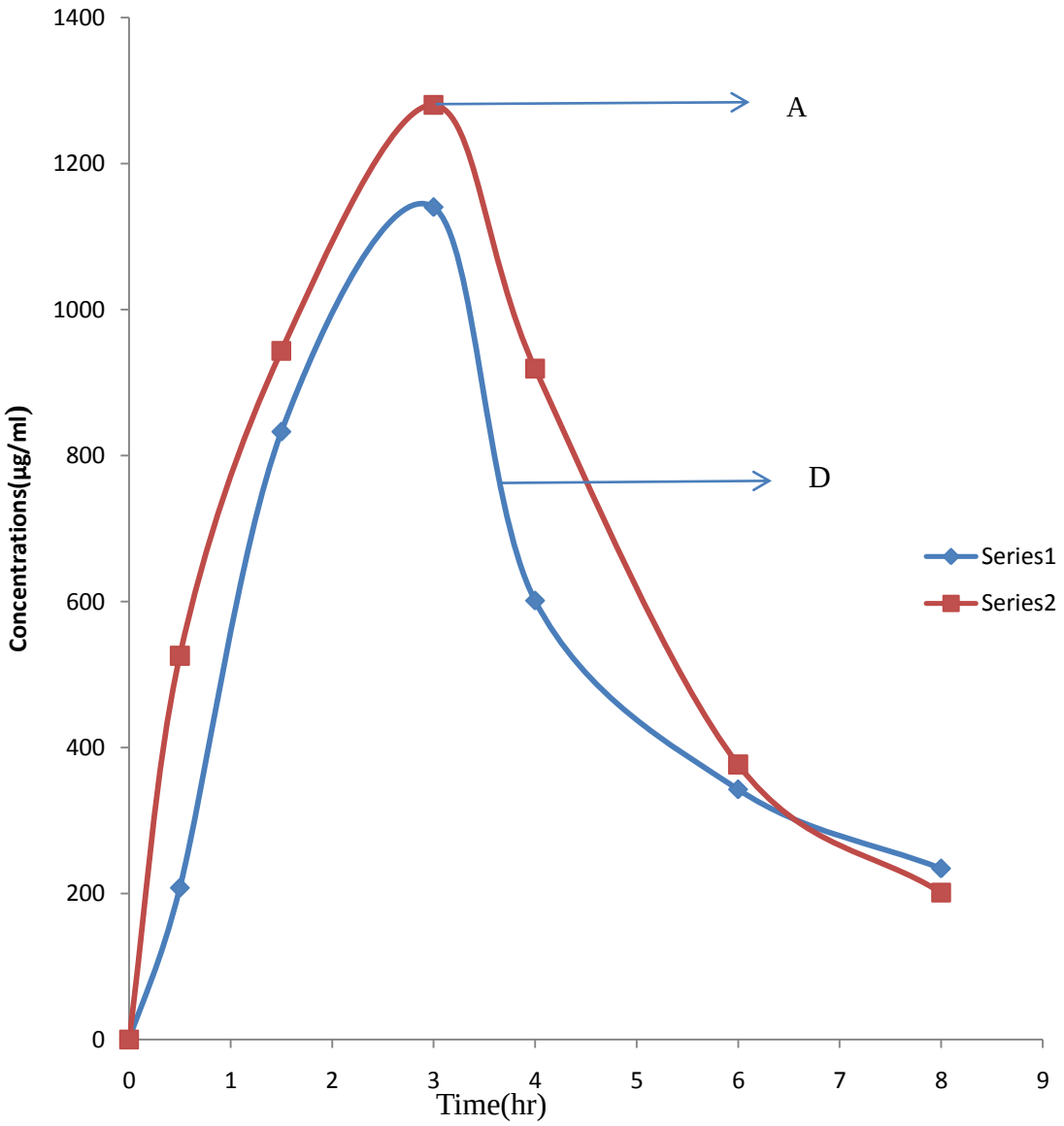


Figure 4.6: Comparison of plasma concentrations(µg/ml) of metformin alone (A) and co-administered with amoxicillin (D).

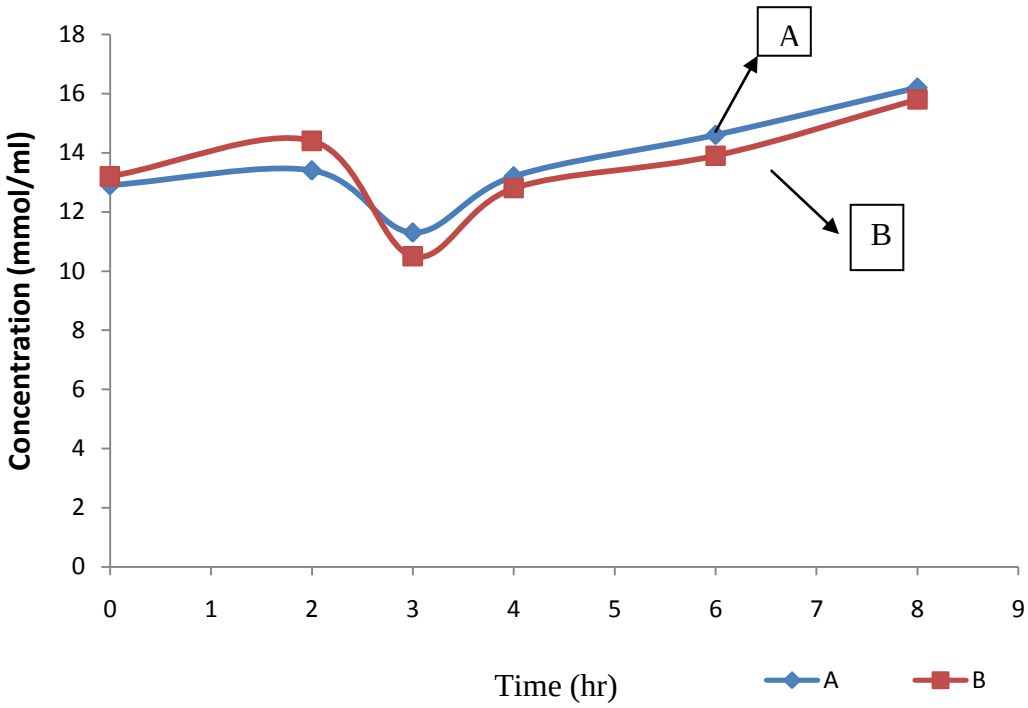


Figure 4.7: Comparison of Mean glucose concentrations in six type 2 diabetic patients following Oral administration of 1 g metformin (A) tablets and concomitantly with 1 g Amoxicillin capsule (B)

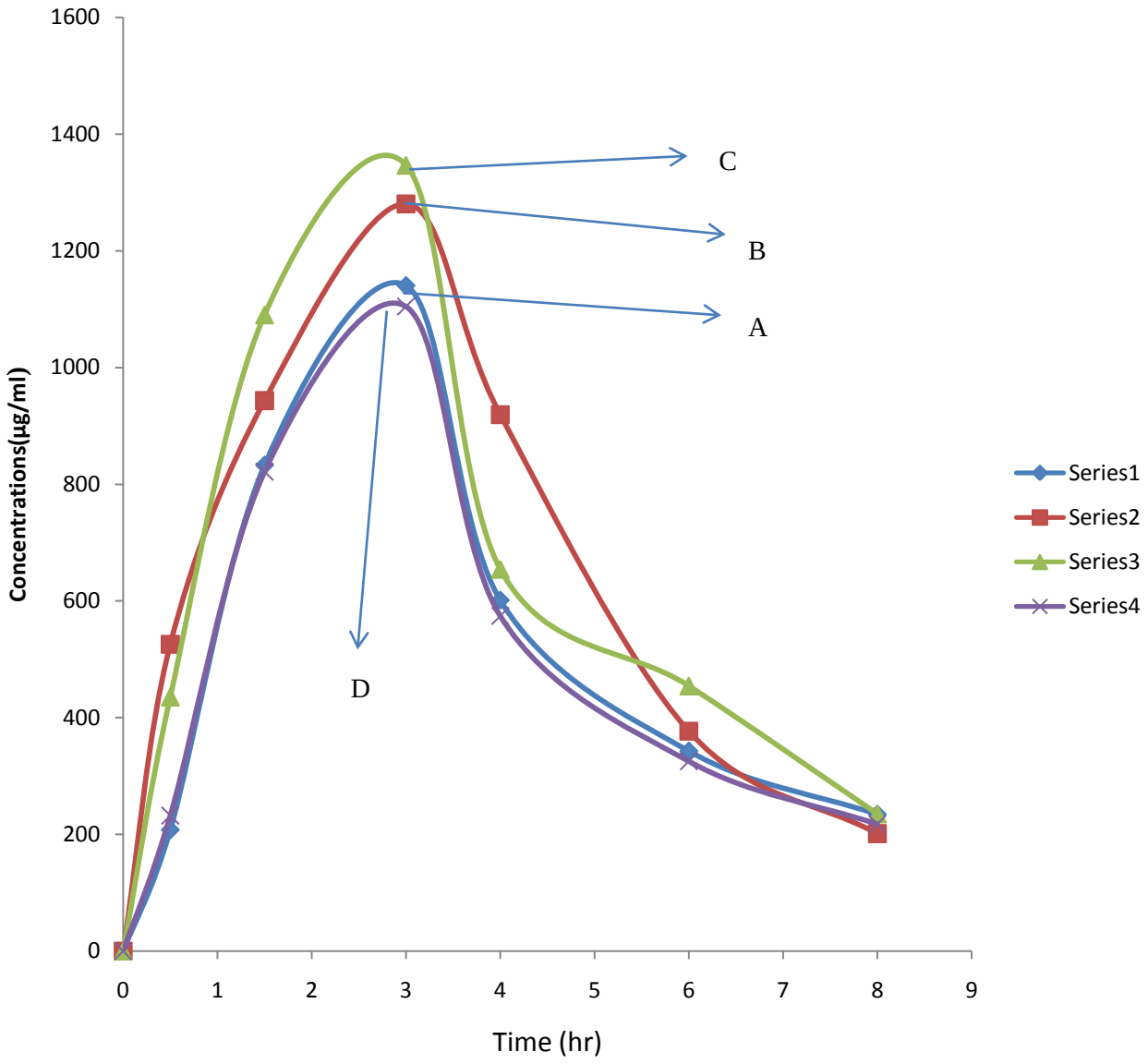


Figure 4.8: Comparison of plasma concentrations (µg/ml) of metformin alone (A) and co-administered with ampiclox (B), metronidazole (C) and amoxicillin (D)

Table 4.3: Pharmacokinetic parameters of Metformin alone andmetformin co-administered with ampiclox (Mean ±S.D,N=6)

	Metformin alone	Metformin + Ampiclox	Paired sample T-TEST
Lag time (hr)	0.12±0.02	0.15±0.02	NS
Absorption half-life ($t_{1/2\alpha}$(h)	1.5±0.03	1.2±0.05	S
Absorption rate constant K_a (hr^{-1})	0.46±0.03	0.58±0.04	S
Peack plasma Conc. C_{max} (µg/ml)	1.14±0.52	1.28±0.35	NS
Time to peak conc. T_{max}(min)	3.0±0.1	3.0±0.19	NS
Area under the curve AUC_{0-8}(hr µg/ml/hr)	4.39±0.71	5.18±1.02	NS
$AUC_{0-\infty}$ (hr µg/ml/hr)	7.57±0.51	8.66±0.17	NS
Volume of distribution Vd (ml)	337,852.19±0.27	303,061.43±0.40	NS
Clearance CL(ml/hr)	59,013.39±0.41	41,028.98±0.37	S
Elimination half-life $t_{1/2\beta}$(hr^{-1})	3.8±0.07	5.1± 0.09	S
Elimination rate constant Ke(hr-1)	0.18±0.12	0.14±0.01	S

$p < 0.05^{*}$ = Significant(s) $p > 0.05$ = Not significant (NS).

Table 4.4: Pharmacokinetic parameters of Metformin alone andmetformin co-administered with metronidazole ((Mean ±S.D, N=6)

	Metformin alone	Metformin+Metro.	Paired sample T- test value
Lag time (hr)	0.12±0.02	0.11±0.05	NS
Absorption half-life $t_{1/2\alpha}$(h)	1.5±0.03	1.2±0.05	S
Absorption rate constant K_a (hr^{-1})	0.46±0.04	0.59±0.03	S
Peack plasma Conc. C_{max} (µg/ml)	1.14±0.52	1.35±0.42	S
Time to peak conc. T_{max}(min)	3.0±0.19	3.0±0.19	NS
Area under the curve AUC_{0-8}(hr µg/ml/hr)	4.39±0.71	5.36±0.80	S
AUC_{0-8}(hr µg/ml/hr)	7.57±0.51	8.45±0.22	S
Volume of distribution Vd (ml)	337,852.19±0.87	313,061.43±0.02	NS
Clearance CL(ml/hr)	59,013.39±0.41	42,435.56±0.21	S
Elimination half-life $t_{1/2\beta}$(hr)	3.8±0.07	6.2± 0.02	S
Elimination rate constant Ke(hr-1)	0.18±0.12	0.11±0.02	S

$P<0.05^{*}$ =Significant(S) p>0.05 = Not significant (NS).

Table 4.5: Pharmacokinetic parameters of Metformin alone and metformin co-administered with amoxicillin (Mean ±S.D, N=6)

	Metformin alone	Metformin + Amoxicillin	Paired sample T- test value
Lag time (hr)	0.12±0.02	0.11±0.05	**NS**
Absorption half-life $t_{1/2\alpha}$(h)	1.5±0.02	0.75±0.02	S
Absorption rate constant K_a (hr^{-1})	0.46±0.04	0.19±0.01	S
Peack plasma Conc. C_{max} (µg/ml)	1.14±0.52	1,104.40±0.04	NS
Time to peak conc. T_{max}(min)	3.0±0.19	3.0±0.19	NS
Area under the curve AUC_{0-8}(hr µgml/hr)	4.39±0.71	4,250.25±0.45	NS
AUC_{0-8}(hrµg/ml/hr)	5.57±0.51	6,591.29±0.90	NS
Volume of distribution Vd (ml)	337,852.19±0.27	3497, 352.06±0.11	NS
Clearance CL(ml/hr)	59,013.39±0.41	62,196.88±0. 39	NS
Elimination half-life $t_{1/2\beta}$(hr)	3.80±0.07	3.70 ± 0.02	S
Elimination rate constant Ke(hr-1)	0.18 ± 0.12	0.19 ± 0.01	S

$p < 0.05^{*}$ = Significant(S), $p > 0.05$ = Not significant (NS).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Quality Control Test

Before the commencement of the in vivo study, metformin tablets, ampiclox capsules, metronidazole tablets and amoxicillin capsules were subjected to quality control tests (Identification and chemical assay tests).

The World Health Organization (WHO) have always maintained the rationale for such tests and the necessity for such tests was strongly emphasized after the Bangladesh epidemic a reported case of fatal renal failure caused by propylene glycol in paracetamol elixir. (Hannif *et al*, 1995).

The standard sample of metformin as well as the tablets of metformin, metronidazole and capsules of ampiclox and amoxicillin complied with the B.P. (2002) specification for quality control test such as identification and chemical assay test.

5.2 In-vivo Bioavailability Studies

5.2.1 Effect of ampiclox (ampicillin/cloxacillin) on pharmacokinetics of metformin

Following the concomitant administration of 1 g of metformin with 1 g ampiclox to type 2 diabetic patients, the influence of ampiclox capsules on the pharmacokinetics of metformin clearly revealedsignificant pharmacokineticchanges ($P < 0.05$) (see Table 4.9).

An increase in the absorption rate constant k_a from 0.46 ± 0.04 to 0.58 ± 0.04 $hr^{-1}$was observed, these changes were found to be significant($p < 0.05$, using student t-test for

paired data) differences in the C_{max} , AUC, $V_{d,}$ Cl, lag- time while other parameters were not significantly different in their values, ($p>0.05$).

There was an increase in peak plasma concentration (C_{max}) from 1.14±0.52 to 1.28 ±0.35µg/ml while area under the curve (AUC) increased from 4.39 ±0.71 to 5.18 ±0.02 µg/ml/hr they were statistically insignificant at ($P>0.05$), which is also in agreement with the finding of Paxton in 1989 that, the high AUC value of metformin in the presence of ampiclox capsules is most likely responsible for the decreased plasma glucose concentration following treatment with the two drugs. It could be due to the fact that both drugs bound to plasma protein and the same binding sites. Competition for binding sites when both drugs were administered concomitantly may result in displacement of metformin. This may be the most likely reason for high bioavailability of metformin observed which resulted in increased of AUC and C_{max}.

Both drugs are tightly bound to plasma protein, and there exist the possibility of common binding sites (Ptalsky, 1980, Hills, 1987). Competition for binding sites when both drugs are concurrently administered may lead to the displacement or metformin from its binding sites. Activity of any drug is usually a function of the fraction of the unbound drug, high level of metformin and thus, an enhanced activity in the presence of ampiclox capsules must have accounted for the significant decrease in plasma glucose level observed following their concomitant administration.

Greater percentage of metformin and ampiclox are excreted by the renal – tubular secretion mechanism (Burger and Mitchell 1985). Thus, there is a possibility that both drugs compete for the same renal – tubular secretion. When this happens, there is every tendency that

ampicillin/cloxacillin known to be rapidly eliminated from the system will be secreted first in preference to metformin when they are co-administered. By such mechanism, the clearance rate of metformin will be reduced as well as its volume of distribution as shown in Table 4.6. In this study, there was an insignificant decrease ($P > 0.05$) in the volume of distribution (Vd) of metformin from 337,852.19±0.27 to 303,061.43 ±0.40 ml in the presence of ampiclox capsules which agreed with the result of the study carried out byBakare *et al.*, (2001) on the influence of ampiclox on the pharmacokinetics of chlorpropamide in type 2 diabetic patients.

The observed significant decrease ($p<0.05$) in clearance from 59,013.39 ±0.41 to 41,028.98±0.37 ml/hr with a reduction in the volume of distribution when metformin 1 g was co-administered with 1 g ampiclox to type 2 diabetic patients, may be due to the decrease in elimination rate constant (Charles *et al.*,2009) .

The mean postprandial glucose level increased significantly ($p<0.05$) at 2 hours but reduced at 3 hours for both metformin alone and when co-administered with ampiclox but not prominent as when compared with when metformin co-administered with metronidazole (Figures 4.4 and 4.6), this could be as a result of potentiation effect observed when metformin was co-administered with the interacting drugs.

5.2.2 Effect of metronidazole on pharmacokinetics of metformin

Following the concomitant administration of a single dose of 1 g of metformin with 400 mg metronidazole to Type 2 diabetic patients, significant increase ($p<0.05$) in the absorption rate constant (k_a), peak plasma concentration (C_{max}), and area under the curve

(AUC) of metronidazole were observed (Table 4.10). The greater the amount of drug absorbed, the greater the AUC and the greater the bioavailability.

A decrease in elimination rate constant k_e (hr^{-1}) from 0.18±0.12 to 0.11±0.02 $hr^{-1}$was observed on co- administration of metformin with metronidazole to type 2 diabetic patients, with an increase in elimination half-life 3.8±0.07 to 6.2±0.02 hr and decrease clearance from 59,013.39 ±0.41 to 42,435.56 ±0.21 ml/hr which were significant ($p<0.05$).This is in agreement with the reportof the study carried out by Bakare-Odunola *et al.*,(2001), on Chlorpropamide and metronidazole in which there was significant increase renal tubular secretion of metformin by metronidazole. They indicated that both drugs may be competing for the same renal tubular secretion.

The mean postprandial glucose level increased significantly at 2 hours but reduced at 3 hours for both metformin alone and metformin co-administered with metronidazole, this may be due to the potentiation when metformin was co-administered with the interacting drug (Figure 4.6).

5.2.3 Effect of amoxicillin on pharmacokinetics of metformin

The result of concomitant administration of a single dose of 1 g of metformin with 1 g amoxicillin capsules to type 2 diabetic patients, revealed significant changes in the pharmacokinetic of amoxicillin.

Absorption rate constant decreased significantly($p<0.05$) from 0.46±0.04 hr^{-1}. A statistically significant decrease ($p<0.05$) in C_{max} of metformin from 1.14±0.52 to 1.10 ± 0.04 µg/ml with also decrease area under the curve from 4.39 ±0.71 to 4.25 ±0.45 µg/ml/hr when administered concomitantly with amoxicillin was recorded.

The significant ($p<0.05$) reduction in absorption rate constant (k_a)resulted in an insignificant increase ($p>0.05$) in volume of distribution 337,852.19±0.27 to 349,352.06 ±0.11ml. This is in agreement with the report of the study carried out by Samogyi *et al*. (1987) on metformin and cimetidine in which there was reduction renal tubular secretion of metformin by cimetidine.

The mean postprandial glucose level changes were not significant when metformin was co-administered with amoxicillin. This may be as a result of non- interaction when metformin was co-administered with amoxicillin.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

From the study, the quality of the drug products evaluated was conformed to have satisfied the requirements of the B.P (2002) with respect to identification and assay tests.

It has been established in the course of this study that ampiclox capsules, metronidazole tablets and amoxicillin capsules have influenced the Pharmacokinetics of metformin and strongly suggested pharmacokinetics interaction between the interacting drugs with metformin.

The method of high performance liquid chromatography, in the monitoring of metformin in the plasma was very effective and efficient.

The results of the findings indicated pharmacokinetics changes when metformin was administered alone and co-administered with ampiclox, metronidazole, and amoxicillin.

Potentiation effects on metformin was only was observed with concomitant administration of a single dose of 1 g metformin with 400 mg of metronidazole tablets and 1 g ampiclox capsules by increasing the peak plasma concentration of metformin.

Concomitant administration of 1 g metformin with 1 g of amoxicillin had no significant effect.

6.2 Recommendations

The findings indicated that diabetic Patients on metformin who require ampiclox or metronidazole needs adjustment of dose regiment to avoid the risk of toxicity or therapeutic failure.

On the other hand, the combination of metformin with amoxicillin capsule in management of Type 2 diabetic patients may be clinically safe.

References

ADA (American Diabetes Association) (2005).Total prevalence of diabetes and Pre-diabetes. *(http://www.diabetes.org.)*

ADA (American Diabetes Association) (2013). Nutritional recommendations and principles for individual with diabetes Mellilus. Diabetes care. 10:126 – 132.

Ahuja, S amd Scypinski, S (2011). Handbook of Modern Pharmaceutical Analysis. 2nd edn vol. 10. USA. Academic press. Pp228-415

Ameer, B. and Weintraub, R.A. (1997). Drug interaction with grape fruit juice. Clinical Pharmacokinetics. 3:103 – 21.

Atkinson, A.J., Juan, S., Lertora, J.J. and Markey, S.P. (2012). Principles of Clinical Pharmacology. USA, Elsevier. 3rd pp 402.

Bailey, C.J. and Day, C. (2004). Metformin: its botanical background. Practical Diabetes International. 21(3):115–7.

Bakare-Odunola, M.T., Garba, M., Enemali, S.I., Okeniyi, S.O. and Gebi, J. (2001). The influence of ampicillin/cloxacillin on the pharmacokinetics of chlorpropamide in Type 2 diabetic Patients. *West African Journal of pharmacology. Drug Research 17: (1and 2) 74-76.*

Barley, D.G., Malcolm, J., Arnold, O. and Spence, J.D. (1998). Grape fruits juice – drug interaction *British Journal of Clinical Pharmacology 46(2):101 – 10.*

Batchelor, E.P., Doyle, E.P., Naylor, J.H. and Robinson, G.N. (1995). Synthesis of penicillin: 6 – APA in penicillin Fermentations. Nature (London) 183,257.

Benet, L.Z. and Haener, B. (2002). Charges, in plasma protein binding have little clinical relevance. Clinical Pharmacology& Therapeutics. 71 : 115

Benet, L.Z. and Sheiner, L.B. (1985). Pharmacetics: In thermodyna9mics –named drug absortion, and elimination. In the pharmaceutical basis of therapeutics (7^{th} eds). Eds. Gilman, A. G Goodman, L. S, Rall, T. N, and Murad, F, PP. 1 – 9.

Bharatam, P.V., Patel, D.S. and Iqbal, P. (2005). Pharmacophoric Features of Biguanide Derivatives: An Electronic and Structural Analysis. *Journal of Medicinal Chemistry. 48(24):7615– 7622.*

Bhavesh, D., Chelan, G., Bhat, K.M. and Shivprakash (2007).Estimation and pharmacokinetics of metformin in human Volunteers. *Indian Journal of Pharmaceutical Education and Research 41(2).*

Blachard, J. and Sawchuck, R.J. (1995). Drug availability. An overview. In: Principles and perspective in drug bioavailability. Eds:-4001Base Sweezerland pp.1-2.

Bolen, S., Feldman, L. and Vassy, J. (2007). Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. Annals of Internal Medicine.147(6):386–99.

Burton, M.E., Shaw, L.M., Schentag, J.J. and Evans, W.W. (2006). Principles of Therapeutic Drug Monitoring. Baltimore, Lippincott Williams and Witkins. Pp243-247

Burger A.S.V and Mitchell J.F (eds).1985.General Pharmacology, kinetics and sulphonylureas.In: Gaddum pharmacology. Oxford University Press, London.PP17, 162-201.

Chamberlain, J. (1995). The analysis of drugs in Biological fluid. 2nd edition (CRC) PP.141.

Charles, B.G., Precechagoom, Y., Lee, T.C., Sheer, P.A., Flennady, V.J. and Debuse, N. (2009). Population pharmacokinetic of intravenous amoxicillin in very low birth weight infants. *Journal of Pharmaceutical Sciences. 86(22):1288-12292.*

DAN (Diabetes Association of Nigeeria). (2011). National Clinical Guideline for Diabetes Management in Nigeria. Federal Ministry of Health. pg 16.

DiPiro J.T., Spruill, W.J., Wade W.E., Blouin, R.A. and Pruemer, J.M. (2010). Concepts in Clinical Pharmacokinetics. 5th edn. ASHP. pp 19-29,59-75

Duguid, J.P. (1994). The Sensitivity of bacterial to the action of pencillin. Edinburgh *Medical Journal.*53, 401.

Edwards, D.I. (1993). Nitroimidazole drugs--action and resistance mechanisms. I. Mechanisms of action. *Journal of Antimicrobial Chemotherapy. 31:9.*

Eghianruwa, K. (2014). Essential Drug Data for Rational Therapy in Veterinary. UK. Author House. pp 26.

Esimone, C.O. (2011). Drug-drug and Herb-Drug Interactions-A Comment. Journal of Research in National Development. 9:1

Eurich, D.T., McAlister, F.A. and Blackburn, D.F. (2007). Benefits and harms of antidiabetic agents in patients with diabetes and heart failure. Systematic review. 335(7618):497.

Fowler,S M.J. (2007). Diabetes Treatment, Part 1: Diet and Exercise. *Clinical Diabetes.* 25(3):105-109

Gardner, D.G. and Shoback D. (2011). Greenspan's Basic and Clinical Endocrinology. chapter 17. 9th Edn. 2011. McGraw-Hill Medical: China.

Garry, G.G., Jeroen, P., Manit, A., Richard, O.D., Matthew, P.D., Janna, K.D., Timothy, J.F., Jerry, R.G., Louise, C.G., Carl, M.K., John, E.R., Peter, T. and Kenneth, M.W. (2011). Clinical Pharmacokinetics of Metformin. Clin Pharmacokinetic ; 50 (2): 81-98

Gibaldi, M. and Perrier (1991). Bioavailability.Pharmacokinetic.Vol.1.Ed: Swarbrick, J pp.129-173.

Gilmore, K.S., Sahm, D.F. and Gilmore, M.S. (2001). Methicillin Resistance in Staphylococcus aureus. In: Lewis, R.G., Salyers, A.A., Taber, H.Q. and Wax, R.G. (2001). Bacterial Resistance to Antimicrobials. New York. Marcel Dekker. pp

Grammer, L.C. and Greenberger, P.A. (2009). Patterson's Allergic Diseases. 7th edn. Philadelphia. Lippincott Williams and Wilins. Pp 300

Greenblatt, D.J., von Moltke, L.L., Harmatz, J.S. and Shader, R.I. (2002). Pharmacokinetics, Pharmacodynamics, And Drug Disposition. In: Davis, K.L., Charney, D., Coyle, J.T. and Nemeroff, C. Neuropsychopharmacology:The Fifth

Generation of Progress. Lippincott, Williams, & Wilkins. pp 402-521Harper W.M.(1984).

Graphs and statistical Analysis.In:Statistics (4th ed.).Macdonalds and

EvansCo.Ltd Pp. 30-85.

Hannif.M.Mobarak, M., and Ronan, A (1995).Fatal renalfailure causedby diethylene glycol in paracetamol Elixir,the Bangladesh epidemic.*Brit.Med.J*.311:88-91.

Haider, S.I., Johnnell, K., Thorslund, M., Fastbom, J. (2007). "Treinds in Poly – pharmacy and potential drug-drug interactions across educational groups in elderly patients in Sweden for the period 1992 – 200". International journal of clinical pharmacology and therapeutics.45(12):643 – 653.

Heller J.B (2007). Metformin overdose in dogs and cats vetrinary medicine 231-233.

Hellgren, U., Ericsson, O., AdenAbdi, Y. and Gustafsson L.L. (2003). Handbook of Drugs for Tropical Parasitic Infections. U.K. Taylor and Francis - pp 104.

Hills, S. (1987). Penicillin Antibiotics. In: *Antibiotics in Clinical use.* Oxford University Press, London. pp 2-4.

Hochadel, M. (2012). Mosby's Drug Reference for Health Professions. 3rd edn. Missouri, Elsevier Mosby. pp 101

Ivan, H.S. (1994). General considerations and outline survey of some basic interaction mechanism, In: Drug interaction (3rd ed.). American Pharmaceutical Association, N.W, Washington, D.C 20037, pp.1-3.

Jayasagar G,Kumar,Krishna Kumar,M,Chandra Sskar K,Madhusudan Rao Y(2002).Effect of Cephalexin on the pharmacokinetic of metformin in healthy human Volunteers Drug interact 19(1):41-48.

Joel, I.U, Atul, D. and Diane, T.L. (2012). PK Functions or Microsoft Excel. Department ofPharmacokinetics and Drug Metabolism Allergan, Irvine, CA 92606, U.S.A.

Jones, G., Macklin, J. and Alexander, W. (2003). Contraindications to the use of metformin. British Medical Journal. 326(7379):4–5.

Kashuba, A.D.M. and Bertino, J.S. (2006). Mechanisms of Drug Interactions I: Absorption, Metabolism, and Excretion. In: Infectious Disease: Drug Interactions in Infectious Diseases. 2nd edn, Edited by Piscitelli, S.C. and Rodvold, Edn. K.A. Totowa, Humana Press Inc. pp 13-30

Kasik, J.E. and Thompson, J.S. (1989). Allergic reactions to antibiotics. *Medical Clinics of North America.* 54, 59.

Khillon, S. and Gill, K. (2006). Basic pharmacokinetics. In: Dhillon, S. and Kostrzewski, A. Clinical Pharmacokinetics.London, Pharmaceutical press.pp 1-40

Khurana, R. and Malik, I.S. (2010). Metformin: safety in cardiac patients. Heart. 2010;96(2):99–102. Ledsack, M. and Rayerson,N.D. (1992).Effect of gastric acidity on enoxiacin absorption. Clinical pharmacology and therapeutics.52:252-6.

Knowler, W., Barrette-cornnor, E., Fowler, S., Hamman, R., Lichin, J., Walker, E., Nathan, D. (2002). Reduction in the incidence of type ii diabetes with life style intervention or metformin. *The New England Journal of Medicine.* 346(6): 393- 403.

Kunin, C.M. (1996). Clinical Pharmacology of the new penicillin. Effect of drugs which interfers with binding to serum proteins. *Clinical pharmacology and therapeutics.* 7, 180.

LeadSack,M ,Rayerson,N.D,(1992).Effectof gastric acidity on enoxiacin absoption .Clinical pharmacology and therapeutics.52:252-6.

Lee, D.C., and Webb, M. (2003). Pharmaceutical Analysis. Oxford, Blackwell Publishing ltd. pp48-60

Lemke, T.L., Williams, D.A., Roche, V.F. and Zito, S.W. (2008). Foye's Principles of Medicinal Chemistry.6th edn. Liipincott. Williams and Wilkins. pp 1054

Limbird, L.E. (2004). The reseptor Concept. A continuing evolution. Molecular Interventions. 4(6):326 –36

Lingvall, M., Reith, D. and R Broadbent, R. (2005). The effect of sepsis upon gentamicin pharmacokinetics in neonates. *British Journal of Clinical Pharmacology.* 59(1):54–61.

Maehle, A.H., Prull, C.R. and Halliwell, R.F. (2002). The emergence of the drug receptor theory. "Nature reviews. Drugs discovery 1(8): 637 – 41.

Makaryus, A.N. and McFarlane, S.I. (2013). Diabetes insipidus : Diagnosis and treatment of a complex disease. *Cleveland Clinic Journal of Medicine*. 73(1):65-71

Marine, S.F. Fernandez, A.M. and Ruiz, A. (2005). Fundamentos de Farmacología *Básicaly* Clinica .pp.232.

McCabe, B.J., Frankel, E.H. and Wolfe, J.J. (2005). Handbook of Food-Drug Interactions. London, CRC Press. Pp 252-260.

McKay, G.A., Reid, J.L. and Walters, M.R. (2010). Lecture Notes: Clinical Pharmacology and Therapeutics. 8th edn. US, Wiley-Blackwell. pp 132

Melmed, S., Polonsky, K.S., Larsen, P.R. and Kronenberg, H.M. (2012). Williams Textbook of Endocrinology, 12th Edn. Philadelphia: Elsevier/Saunders. pp. 1371–1435.

Merle, L., Laroche, M.L., Dantoine, I. And Charmes, J.P. (2005). Predicting and preventing Adverse Drug Reactions in the very old. *Drugs and aging*. 22(5):375-392.

Middleton, R.K. (2006). Drug Interactions. In: Helms, R.A., Quan, D.J., Herfindal, E.T. and Gourley, D.R. (2006). Textbook of Therapeutics: Drug and Disease Management. 8th edn. Lippincott Williams and Wilkins. pp47-68

Muir, N., Nicholas, J.D., Clifford, J.M., Stillings, M.R., and Hoare, R.C. (1997). The influence of the Dosage form on Aspirin kinetics implications of acute cardiovanenlar use. Current Medical Research Openion . 10: 42 – 553.

Namara, P.I, Levy, G, Gibaldi, M,(1979).Effect of plasma protein and drug binding on the time course of drug concentration in plasma.*Pharmacokinetic Biopharmaceutical 7:195-205.*

Nicholls, G., Clark, B.J. and Brown, J.E. (1995). Solid-phase extraction and optimized and separation of doxorubicin epirubicin and their metabolite using Reversed- phase HPLC. *Journal of Pharmaceutical and Biomedical Analysis,*1049

O'Reilly, R.A. (2001). Interactions of sodium warfarin and rifampin studies in man . *Annals of Medicine.* 81:237-40.

Olaniyi, A.A. and Ogunlana, E.O. (1995). Ultraviolet Spectroscopy. In: Pharmaceutical analysis and drug Quality Assurance. Shaneson C.I. Ltd pp.174-181.

Olaniyi A.A. (1998). Chemo therapeutic Agents: Drugs acting against infectious Diseases In: Essential Medical Chemistry Shaneson C.I., Ltd pp.428 – 435.

Park, J.P. and Strominger, J.W. (1997). Mode of action of penecillin: A biochemical basis for the mechanism of action of penicillin and for its selective toxicity. Science. 125, 99.

Park, T.J. and Johnson, M.J. (1989). Accumulation of labile phosphate in staph.aureus growth in the presence of penicillin. Biochem. 179,585.

Patel, D.S., Bharatam, P.V. and Novel N.L. (2009) 2 species with two lone pairs on nitrogen: systems isoelectronic to carbodicarbenes. Chemical Communications. (9):1064–6.

Paxton, W.J. (1989). Drug absorption and distribution. In: Pharmacokinetics in clinical practice1. *The New Zealand Medical Journal. 694: 304-306.*

Petersdorf, R.G and Turck.M. (1996). The new penicillins and their proper uses. Annint 64, 207. Pines, A, Reafat, H. Siddiqui, G.M and Greenfield, J.B.S. (1970). Treatment of severe pseudomonas infections of the bronchi. *British Medical Journal*, 1, 663.

Pines, A, Reafat, H. Siddiqui, G.M and Greenfield, J.B.S. (1986). Treatment of severe pseudomonas infections of the bronchi. *Brit. Med. J.1*, 663.

Ptalsky, K.M (1980).Disease-induced changes in the plasma binding of basic drugs. *Clin pharmacokin.*5;246-262.

Ralph, E.D. and Kirby, W.M. (1995)**.** Unique bactericidal action of metronidazole against Bacteroides fragilis and Clostridium perfrinens. Antimicrobial Agents and Chemotherapy. 8:409.

Ravdin, J.I. and Skilogiannis, J. (1989). In vitro susceptibilities of *Entamoeba histolytica* to azithromycin, CP-63,956, erythromycin, and metronidazole. Antimicrobial Agents and Chemotherapy. 33:960.

Razari, R., Yincham, F., Nikoo, A., Xuejun, L., Xiang, W., Jason, Y., Hubert, T., Lau, T., Sue, T., Sai, P., Maris, J.P., Driver, D., Sereze, M..W., Satter, H. and Micheal, D. (2006). R. TRPV1(+) Sensory Nervous control β-cells stress and islet inflammation in auto- immune diabetes. Retrived July, 2014 from http://mailman13.u.washington.edu/pipermail/phnutr-l/2006-ecember/018193.html

Reisman, R.E. and Arbesman, C.E. (1988). Systemic allergic reactions due to inhalation of penicillin. *The Journal of the American Medical Association.* 203, 986.

Remington, J.P. (2005). Remington: The science and practice of pharmacy. Edited by David B. Troy, and Paul Beringer. 21st edn. Lippincott Williams & Wilkins Pp1175-1178

Reza, M. (2004). The Relationship Among Pharmacokinetic Parameters: Effects of Altered Kinetics on the Drug Plasma Concentration-Time Profiles. *American Journal of pharmaceutical Education. 68(2):1-8.*

Riel-Romero, R.M.S. (2006). Acute transverse myelitis in a 7-month-old boy after diphtheria–tetanus–pertussis immunization. Spinal Cord. 44: 688–691

Rosenbaum, S.E. (2012). Basic Pharmacokinetics and Pharmacodynamics: An Integrated

Textbook and Computer Simulations.Basic Pharmacokinetics and Pharmacodynamics: An Integrated Textbook and Computer Simulations. John Wiley & Sons. chapter 1,2,5,6

Rowland, M. (1988). Drug administration and Regimen in: Clinical Pharmaco (2nded J). Eds. Melmen, K.I. and Morrelli it. I NV. Macmillan Publishing Co. PP. 25 – 70.

Sakai, J.B. (2008). Practical Pharmacology for the Pharmacy Technician. Lippencot. Williams and Witkins. pp 27-40

Samogyi, C., Stokely, J., Keal, P. and Bockner, F. (1987). Reduction of metformin renal tubular secretion by Cimetidine. *British Journal of Clinical Pharmacology.*23(5); 545-551.

Samuels, M.L., Howe, C.D. and Frei, E. (1998). Toxic epidermal necrolysis resembling erythema multiforme. Texas Medical Association. 64, 44.

Serah, W., Gojka R., Anders, G., Richard, S. and Hilary, K. (2004). Global Prevalence of Diabetes . *Diabetes Care* 27:1047–1053.

Seth, S.D. and Seth, V. (2009). Textbook Of Pharmacology. 3rd edn. India, Elsevier. pp x-70.

Shapiro, W. (2006). Intervention trials of the educational protocol for islet transplantation. *The New England Journal of Medicine.* 355; 1318-1330.

Shegem, N.S., Nasir, A.M., Jbour, A.K., Batieha, A.M., El-Khateeb, M.S. and Ajlouni, K.M. (2002). Effects of short term metformin administration on androgens in normal men. *Saudi Medical Journal. ;23(8):934–7.*

Shi, Y. and Hu, F.B. (2014). The global implications of diabetes and cancer. *The Lancet.* 383(9933):1947–1948

Smith, J. and Williams, H. (2006). Smith and Williams' Introduction to the Principles of Drug Design and Action. 4th edn. CRC Press. US. pp36-50

Sneader, W. (2008). Drug Discovery: A History. Chichester. John Wiley and Sons. – pp 321

Spartt, B.G. (1994). Resistance to antibiotic mediated by target alteration. Science. 264; 389

Stang, M., Wysowski, D.K. and Butler-Jones, D. (1999). "Incidence of lactic acidosis in metformin users". *Diabetes Care* 22 (6): 925–927.

Stewart, G.T. (1980). Penicillin Allergy.Clinical pharmacology and therapeutics. 11,307.

Stratton, C.W., Weeks, L.S. and Aldridge, K.E. (1991). Comparison of the bactericidal activity of clindamycin and metronidazole against cefoxitin-susceptible and cefoxitin-resistant isolates of the Bacteroides fragilis groupDiagnostic Microbiology and Infectious Disease. 14:377.

Stuebe, A.M., Edward, J.W., Willet, W.C., Manson, J.E. and Micheals, K.B. (2005). Duration of lactation and incidence of type of diabetes, *Journal of the American Medical Association.* 294(20): 2601-09.

Taro, D.S. (2004). Update : Drug interaction with grape fruit of juice. Drug link Vesta R.E, worod A. J. J. (1980). Influence of age and smoking on drug kinetics in man studies using model compound. *Clinical Pharmacokinetics*. 5: 309 – 319.

Tindall, W.N., Sedrak, M.M. and Boltri, J.M. (2014). Patient-Centered Pharmacology: Learning System for the Conscientious Prescribe. Philadelphia, F.A. Davis Company. pp 315

Tocher, J.H. and Edwards, D.I. (1994). Evidence for the direct interaction of reduced metronidazole derivatives with DNA bases. Biochemical Pharmacology. 48:1089.

Tozer, T.N (1979).Pharmacokinetics Principles relevant to bioavai`lability studies. In:

Principles and perspectives in drug bioavailability.(2nd Ed.) EdsBl anchard,J.L,sawchk,R.J and Brodie.B.S.Kargar A.G4011 Basle, Switzerland. Pp 120-154.

Tomlin, M. (2010). Pharmacology & Pharmacokinetics: A Basic Reader. London, Pringer-Verlag. pp16-1

Trevor, A., Rowland, M. and Way, E.L. (1971). Techniques for studying Drug Disposition in vivo. *In*: La Du, B.N., Mandel, H.G. and Way E.L. (Eds) *Fundamentals of drug metabolism and drug disposition.* The William and Wilkins Company. Baltimore, U.S.A., pp 369-398.

Vesta, R.E., Wood, A.J.J. (1987). Influence of age and smoking on drug kineticsin man studies using model compound. Clinical Pharmacokinetics. 5: 309 – 319.

Vigersky, R.A., Filmore-Nassar, A. and Glass, A.R. (2006). Thyrotropin suppression by metformin. *The Journal of Clinical Endocrinology and Metabolism.*006; 91(1):225–7.

Wagner, J.G.(1986). Bioavailability trials: A modern perspective. *Pharmacy*

International.pp 184-187.

Walker, B.R., Colledge, N.R. Ralston, S.H. and Penman, I.D. (2014). Davidson's Principles and Practice of Medicine. 22nd edn. Elsevier, Chapter 21.

Weiss, J. and Sumpio, B. (2006). Review of prevalence and outcome of vascular disease in patients with diabetes mellitus. *European Journal of Vascular and Endovascular Surgery*. 31(2):143-50.

WHO (World Health Organization) (2014). Diabetes Programme. Retrieved April, 2014 from http://www.who.int/diabetes/action_online/basics/en/.

Wilcox, G. (2005). Insulin and Insulin Resistance. *Clinical Biochemist Reviews.* 26(2):19-39.

William J. Spruill, William E. Wade, Joseph T. DiPiro, Robert A. Blouin, and Jane M. Pruemer (2014). Concepts in Clinical Pharmacokinetics, 6th Edition. 2014; Approx. 264 pages; softbound

William, S.L., Hill, D.P., Daris, J.A. and Lowenthal, D.T. (1996). The influence of food on the absorption and metabolisms of drug: an update. *The European Journal of Drug Metabolism and Pharmacokinetics*. 21: 201 – 11.s3e

Wilson, W.R. (1995). antibiotic treatment of adults with infective endocarditis due tostreptococci, enterococci, Staphylococci microorganisms. *The Journal of the American Medical Association.* 274(21):1706-1713.

Appendices

Appendix I:The precision of the analytical method

Sample	Concentration(µg/ml)	C.V %	N
Within day run			
Metformin	0.05	3.4	5
	1.00	2,8	5
	4.00	1.2	5
Between day run			
	0.05	4.2	5
	1.00	3.1	5
	4.00	2.3	5

Appendix II:Percent extraction recovery of metformin

Sample	Concentration (µg/ml)	Recovery % ± S.D	N
Metformin	0.20	96.52 ±6.7	5
	0.40	98.43 ±7.0	5

Appendix III: Plasma concentrations of metformin for type 2 diabetic patients.

Time (hr)	P1 (µg/ml)	P2 (µg/ml)	P3 (µg/ml)	P4 (µg/ml)	P5 (µg/ml)	P6 (µg/ml)	Mean ±S.D, (n=6)
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00
0.50	0.21	0.20	0.21	0.20	0.21	0.20	0.21 ±0.18
1.50	0.84	0.83	0.83	0.84	0.83	0.84	0.84 ± 0.16
3.00	1.14	1.14	1.13	1.15	1.14	1.14	1.14 ±0.20
4.00	0.61	0.60	0.60	0.60	0.61	0.60	0.60±0.38
6.00	0.34	0.34	0.34	0.34	0.34	0.34	0.34±0.16
8.00	0.23	0.24	0.23	0.24	0.23	0.23	0.23±0.22

P=Patients.

AppendixIV: Plasma concentration s of metformin co-administered with ampiclox totype 2 diabetic patients.

Time (hr)	P1 (µg/ml)	P2 (µg/ml)	P3 (µg/ml)	P4 (µg/ml)	P5 (µg/ml)	P6 (µg/ml)	Mean ±S.D, (n=6)
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00
0.5	0.52	0.53	0.52	0.53	0.52	0.53	0.53±0.19
1.5	0.60	0.70	0.67	0.72	0.54	0.83	0.71±0.16
3.00	1.28	1.27	1.29	1.28	1.28	1.28	1.28±0.31
4.00	0.92	0.92	0.92	0.92	0.92	0.92	0.92±0.17
6.00	0.38	0.38	0.38	0.37	0.38	0.38	0.38±0.18
8.00	0.20	0.97	0.20	0.21	0.20	0.21	0.20±0.12

Appendix V: Plasma concentration s of metformin co administered with metronidazole for type 2 diabetic patients.

Time (hr)	P1 (µg/ml)	P2 (µg/ml)	P3 (µg/ml)	P4 (µg/ml)	P5 (µg/ml)	P6 (µg/ml)	Mean ±S.D, (n=6)
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00±0.00
0.50	0.44	0.43	0.43	0.43	0.44	0.43	0.44±0.18
1.50	1.09	1.09	1.09	1.09	1.09	1.09	1.09±0.19
3.00	1.32	1.33	1.33	1.32	1.33	1.32	1.33±0.39
4.00	0.64	0.65	0.64	0.65	0.64	0.65	0.65±0.09
6.00	0.45	0.46	0.45	0.45	0.45	0.45	0.45±0.90
8.00	0.23	0.24	0.24	0.23	0.24	0.23	0.21±0.18

AppendixVI:

Weight of 20 tabs of metformin ~~10.5606 g~>

Average weight = $\frac{10.560}{20}$

= 0.528 g

0.5g of 1 tab = 0.528 g

0.10 g = x

X = $\frac{0.10x0.528}{0.50}$

= 0.106 g

0.106 g in 100 ml

= 0.106g/ml ⟶ C_1

V_1 = 10 ml

V_2 = 100 ml

First dilution C_2

$$\frac{0.106X10}{100} = C2$$

0.00106 g/ml

Actual concentration = 0.00106 g/ml

The value of A1%, 1cm at 232nm = 398

Theoretical concentration = $\frac{\text{Absorbance}}{\text{A1\%,1cm}}$

= $\frac{0.410}{398}$

= 0.001030151g/ml

% Content of metformin = $\frac{\text{Actual concentration}}{\text{Theoritical concentration}}$

$$\frac{0.001030151x100}{00106}$$

97.0%metformin

Appendix VII:

Weight of 20 ampiclox capsules = 11.4353 g

Average weight = 0.57165 g

Each capsule = 0.250 g

0.250 g = 0.57165 g

Equivalent weight of 0.17 g = x g.

$$X = \frac{0.17 \times 0.57165}{0.25}$$

0.388722g

0.388722g in 100 ml ⟶ 0.388722 g/ml C_1

V_1 =5 ml

V_2 = 20 ml

$$C_2 = \frac{0.388722 \times 5}{20}$$

0.071805g/ml.

Actual concentration = 0.071805g/ml.

Absorbance of 0.071805 g/ml of ampicillin powder in ampiclox capsule =0.034

Absorbance of 0.071805 g/ml of ampicillin standard powder = 0.033483

Absorbance of standard powder 0.033483 = 100% ampicillin content

Absorbance of ampicillin powder in ampiclox capsule 0.034 = x % amoxicillin content.

$$X = \frac{0.0335}{0.034} \times 100$$

98.53 % ampicillin

Appendix VIII:

Weight of 20 ampiclox capsules = 11.4353 g

Average weight = 0.57165 g

Each capsule of cloxacillin = 0.25 g

0.25 g = 0.57165 g

0.1 g = x g

= 0.22866 g

Equivalent weight of 0.1 g = 0.22866 g. in 500 ml

But in 100 ml = 0.045732 g

But in 100 ml = 0.045732 g ⟶ 0.045732 g /ml C_1

V_1 =5 ml

V_2 = 20 ml

$C_2 = \frac{0.045732}{20} \times 5$

0.011433g/ml.

Actual concentration = 0.011433g/ml

Absorbance of 0.011433g/ml cloxacillin powder in ampiclox capsule =0.1737

Absorbance of 0.011433g/ml cloxacillin standard powder = 0.

176

Absorbance of standard powder 0.1737 = 100% cloxacillin content

Absorbance of cloxacillin powder in ampiclox capsule 0.1737 = x % cloxacillin content.

$X = \frac{0.1737}{0.176} \times 100$

98.69% cloxacillin

Appendix IX:

Weight of 20 tablets of metronidazole =10.3977g

Average Weight = 0.519885 g

0.4 g of metronidazole =0.519885

0.2 g = x g

$X = \frac{0.2 \times 0.5188}{0.4}$

= 0.2599425 g

End point of titration with the powder = 13.8 ml

End point of titration without the powder =2.3 ml

Volume of perchloric acid = 13.8 – 2.3

11.5 ml

0.1 ml ⟶ 17.12 mg

11.5 ml ⟶ X mg

$X = \frac{17.12}{0.1} \times 11.5$

198.8mg

198.8 mg = 0.19688 g

% content of metronidazole = $\frac{\text{Actual concentration}}{\text{Theoritical cncentration}} \times 100$

$\frac{0.1988}{0.2} X100$

99.44 % metronidazole

Appendix X:

Weight of 20 Amoxicillin Capsules = 11.4482 g

Average weight = 0.57241 g

Each capsule contains 500 mg.

O.5 g ⟶ 0.57241 g

0.15 g ⟶ x

$$X = \frac{0.5724 \text{ X } 0.15}{0.5}$$

0.171723 g =500 ml

X g = 100 ml

$$\frac{0.171723 \text{ x}100}{500 \text{ ml}}$$

=0.03444 g.

0.0344 g dissolved in 100 ml

X g ,, 1 ml

X =0.00344g/ml ⟶ C_1

V_1 = 2 ml

V_2 = 20 ml

$$C_2 = \frac{0.00344 \text{ x } 2}{20}$$

0.00034 g/ml

Absorbance of 0.00034 g/ml of amoxicillin powder in a capsule =0.1737

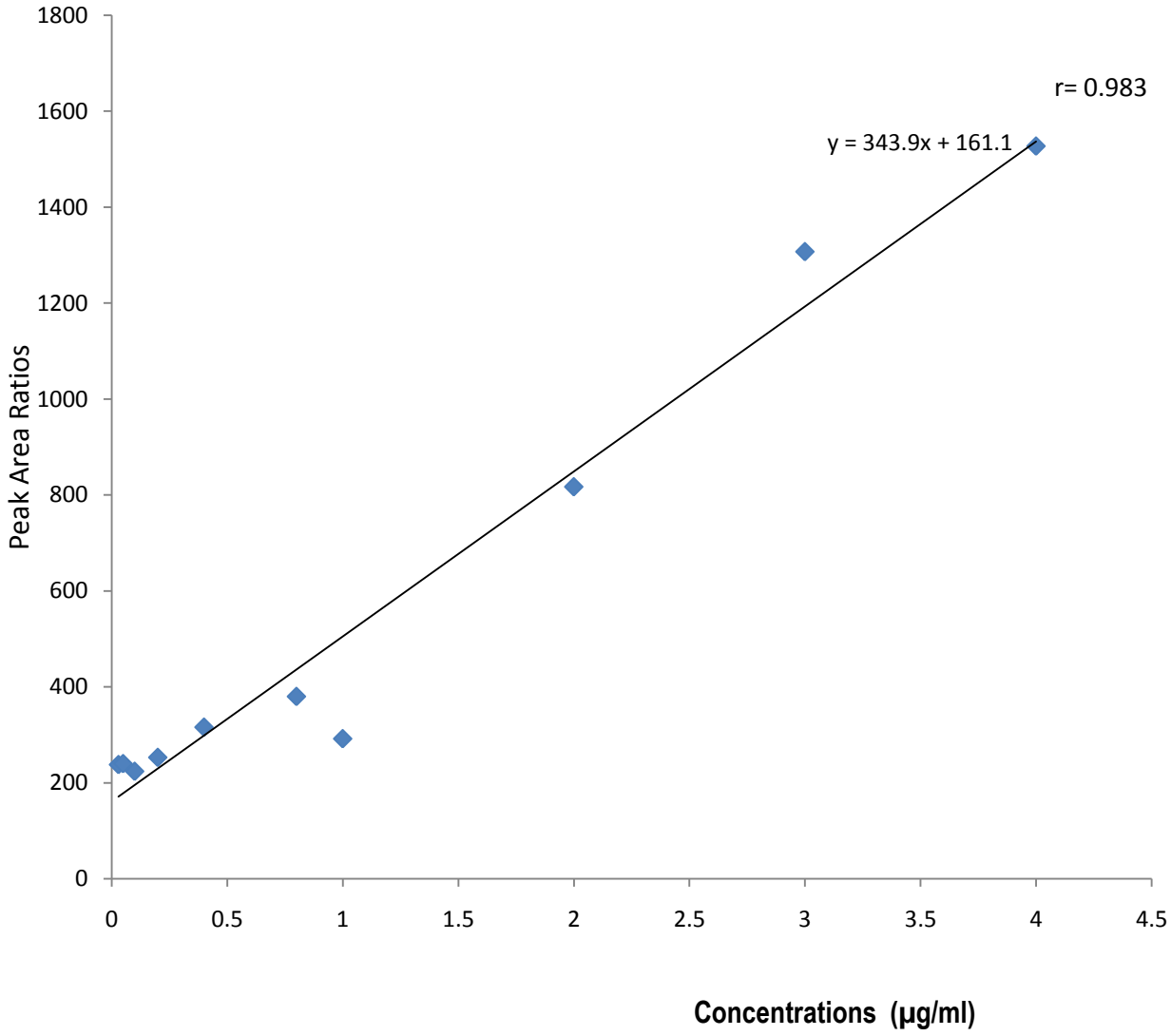
Absorbance of 0.0003 g/ml of amoxicillin standard powder = 0.176

Absorbance of standard powder 0.176 = 100% amoxicillin content

Absorbance of amoxicillin powder in a capsule 0.1737 = x % amoxicillin content.

$$X = \frac{0.1737}{0.176} \text{x } 100$$

98.5 %



Appendix XI: Calibration Curve of metformin

XII

FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY
Z A R I A

Our Ref: F-MED/COMM.19 Date: 3rd April, 1995

Dr. Moji T. Bakare,
Department of Pharmaceutical and
Medicinal Chemistry,
Faculty of Pharm. Sciences, A.B.U. Zaria.

Dear Sir/Madam,

DECISION OF THE MEDICAL ETHICAL COMMITTEE

I write to infrom you that at its meeting held on 25th Jan., 1995 the Medical Ethical Committee approved/~~disapproved~~ your research proposal titled: THE INFLUENCE OF AMPICLOX AND METRONIDAZOLE ON THE PHARMACOKINETICS OF CHLORPROPAMIDE AND GLIBENCLAMIDE IN YOUNG HEALTHY ADULTS AND TYPE II DIABETIC PATIENTS.

The following is the extract of the relevant minutes:

.....

.....

.....

.....

Project Number: ESC/95/00069

Yours faithfully,

Dr. H.A. Aikhionbare
Chairman,
Medical Ethical Committee.

N.B: Members of the Committee would like to have a progress report ~~on~~ your proposals in future.