THE INFLUENCE OF METRONIDAZOLE ON THE PHARMACOKINETIC PROFILE OF CHLORPROPAMIDE IN TYPE II DIABETIC PATIENTS

BY

KUDIRAT BELLO

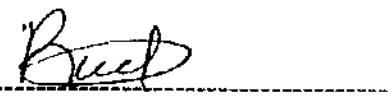
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DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the supervision of Dr. Garba Magaji the Head of Department of Pharmaceutical and Medicinal Chemistry A.B.U., Zaria, Dr (Mrs) Mojirade T. Bakare - Odulola of Department of Pharmaceutical and Medicinal Chemistry A.B.U., Zaria and Dr. (Mrs) Oby Obodozie Head of Department of Medicinal Chemistry and Quality Control NIPRD, Idu -Abuja.

It has not been presented in any previous application for higher degree. The works of other investigators are referred to and acknowledged accordingly.



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CERTIFICATION

This thesis entitled THE INFLUENCE OF METRONIDAZOLE ON THE PHARMACOKINETICS PROFILE OF *CHLORPROPAMIDE IN TYPE II DIABETIC PATIENTS* by *KUDIRAT BELLO* meets the regulations governing the award of the Masters Degree of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

Dedicated to Almighty Allah

For His Protection and Guidance

And

My daughter (Thairat Opeyemi Mustapha)

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Following the successful completion of a research work, certain people obviously deserve special thanks. I shall therefore use this forum to express my profound gratitude and deep appreciation to my supervisors, Dr. Garba Magaji, Dr. (Mrs) Mojirade T. Bakare - Odulola and Dr. (Mrs) Oby Obodozie their criticisms, valuable suggestions, guidance, keen interest and understanding throughout the course of this study cannot be forgotten.

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ABSTRACT

The influence of metronidazole on the pharmacokinetic profile of chlorpropamide in Type II diabetic patients was examined. The quality of chlorpropamide and metronidazole tablets used in the study were pre -determined using official methods. The tablets complied with the BP (1988) requirements stated under the monograph for chlorpropamide and metronidazole tablets.

Plasma levels of chlorpropamide were determined using colorimctric method (Martin et al, 1973). In addition, glucose levels were measured by standard glucose oxidase method (Martinek, 1964). Decreased levels of chlorpropamide were observed in concomitant administration of 250mg chlorpropamide with two 200mg metronidazole tablets(C) compared with administration of 250mg chlorpropamide tablet (control) (B). Insignificantly higher (pre and postprandial) levels of plasma glucose were observed in concomitant administration of metronidazole with chlorpropamide compared with administration of 250mg chlorpropamide alone.

The pharmacokinetic parameters were calculated using semi - logarimathmic plots of concentration- time data for chlorpropamide. The area under the curve was calculated by trapezoidal rule. Administration of metronidazole concomitantly with chlorpropamide significantly affected some pharmacokinetic parameters of chlorpropamide such as ka, Cmax, Vd, AUG, and ti/2Ct. The elimination parameters, Lag time, and tmax as calculated in this study were not significantly affected.

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GLOSSARY OF TERMS

BP	=	British Pharmacopoeia
°C	=	Degree centrigrade (Celsius)
g	=	Gramm
L	=	Litre
µg	=	Microgramme
mL	=	Millilitre
m.Mol	=	Millimole
MIN.	=	Minute
h.	=	Hour
HCl	=	Hydrochloric acid
HNO_3	=	Nitric acid
NaOH	=	Sodium hydroxide
USP	=	United States Pharmacopoeia
pH	=	Hydrogen - ion concentration
UV	=	Ultraviolet
CONC.	=	Concentration
SD	=	Standard Deviation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Pharmacokinetics

Pharmacokinetics is the study of the rate processes associated with Absorption, Distribution, Metabolism, and Excretion of a drug (ADME). That is quantitating drug and / or metabolite(s) levels in the body fluids, tissues and excreta at any point in time from the time of administration until elimination from the body is complete (Kaplan and Jack, 1980). Consequently, the major objective of every pharmacokinetic study can be defined as the quantitation of a drug following its administration till it is completely eliminated from the body.

The attempts to define the time course of drugs in the body have produced three major philosophical approaches to Pharmacokinetics. These are the classical compartment model approach, the physiological model approach and the compartment independent approach. The compartment model approach is the conventional method of characterising the Pharmacokinetics of a drug, with this approach the body is considered as consisting of one or more compartments with no real anatomical or physiological reality. Another characteristics of this approach is the assumption that transfer among compartments attains a fast equilibrium, such that sampling a central compartment (usually blood) is representative of the overall of the drug in the body, and drugs elimination from this

central compartments generally obeying a first order linear kinetics.

The compartment approach represents a recent development in the pharmacokinetic characteristic of drugs. It is based purely on the mathematical description of biological fluids concentration - time profiles for a drug and/or its metabolites. There are different types of compartment models. The one compartment model, the simplest model depicts the body as a single unit. The model is particularly useful for the pharmacokinetic analysis of blood, plasma, or serum concentrations, salivary and urinary excretion data for drugs which rapidly distribute between plasma and other body fluids and tissues upon entry into the systemic circulation. To assume the body behaves as a one compartment model does not necessarily mean that the drug concentrations in all body tissues at any given time are the same. However, a one compartment model, (Fig. la) does assume that any changes that occur in the plasma quantitatively reflect changes occurring in tissue drug levels

Drug in Dosage form at Administration site	→	Body Volume Concentration	→	Drug in urine Faces, Expired Air, sweat, milk
	Input		Output	

Figure. 1a: Concepts of open one - compartment model (Ritchie, 1980).

Absorption is the process by which a drug proceeds from the site of application to the site of measurement within the body. There are several sites at which drugs are commonly administered. These sites may be classified as either extravascular or intra vascular. Intra

vascular administration refers to the placement of a drug directly into the blood, either intra-arterially or intravenously. Extravascular modes of administration include the oral, buccal, sublingual, intramuscular, subcutaneous and rectal routes. To enter the blood, drug administered extra vascularly must be absorbed. No absorption step is required when drug is administered intra vascularly (Rowland and Tozer, 1980a).

Distribution is the process of reversible transfer of a drug to and from the site of measurement once absorbed, a drug is distributed to the various tissues of the body. The rate and extent of distribution are determined by how well each tissue is perfused with blood, the binding of drug to plasma proteins and to tissue compartments and the permeability of tissue membranes of the drug (Rowland and Tozer, 1988a).

Elimination is the irreversible loss of drug from the site of measurement. Elimination occurs by two processes, metabolism and excretion. Metabolism is the conversion of one of chemical species to another. The two important organs of elimination are kidney and liver. The kidney is the primary site for excretion of the chemically unaltered or unchanged drug. The liver is usual organ of drug metabolism; however, the kidney and other organs can also play an important metabolic role for certain drugs (Rowland and Tozer, 1980b).

Half life is one of the most useful pharmacokinetic parameter obtained following drug administration. The half-life of a drug is the time required to reduce the post-absorption

concentration of a drug in the blood stream by fifty percent. It is calculated by dividing 0.693 by the calculated slope of each exponential phase of Multi phasic blood level curve (Wagner, 1980), in first order kinetic process.

The area under the curve of blood versus time is another pharmacokinetic parameter frequently use. The method of calculating theses parameters are discussed by various authors (Koup and Gibaldi, 1980; Wagner, 1980).

The volume of distribution is used to relate the plasma concentration to the amount of drug in the body during the elimination phase. It is calculated by dividing the total amount of drug in the body by the plasma concentration. Total body clearance is a parameter used to quantify drug elimination. Experimentally, total body clearance is calculated by dividing the systemically available dose of a drug by the area under the plasma concentration - time curve produced by that dose. In terms of Pharmacokinetics, total body clearance is the product of rate constant and volume of distribution. It is the sum of individual clearances of the drug by the various organs and tissues of the body for a drug that is eliminate solely by renal excretion and hepatic clearance (Rowland and Tozer, 1980b). There are many ways of calculating volume of distribution (Vd.) depending on the pharmacokinetic profile of the drugs (Gibaldi and Perrier, 1975b).

Two compartment model when plasma drug levels are measured at early time periods after intravenous administration, an initial distributive phase often can be described kinetically. The distributive phase often may last from a few to several hours. The drug entering the body (Input) does not instantly distribute (equilibrate) between the blood and those other body fluids or tissues which it eventually reaches (Fig. 1b).

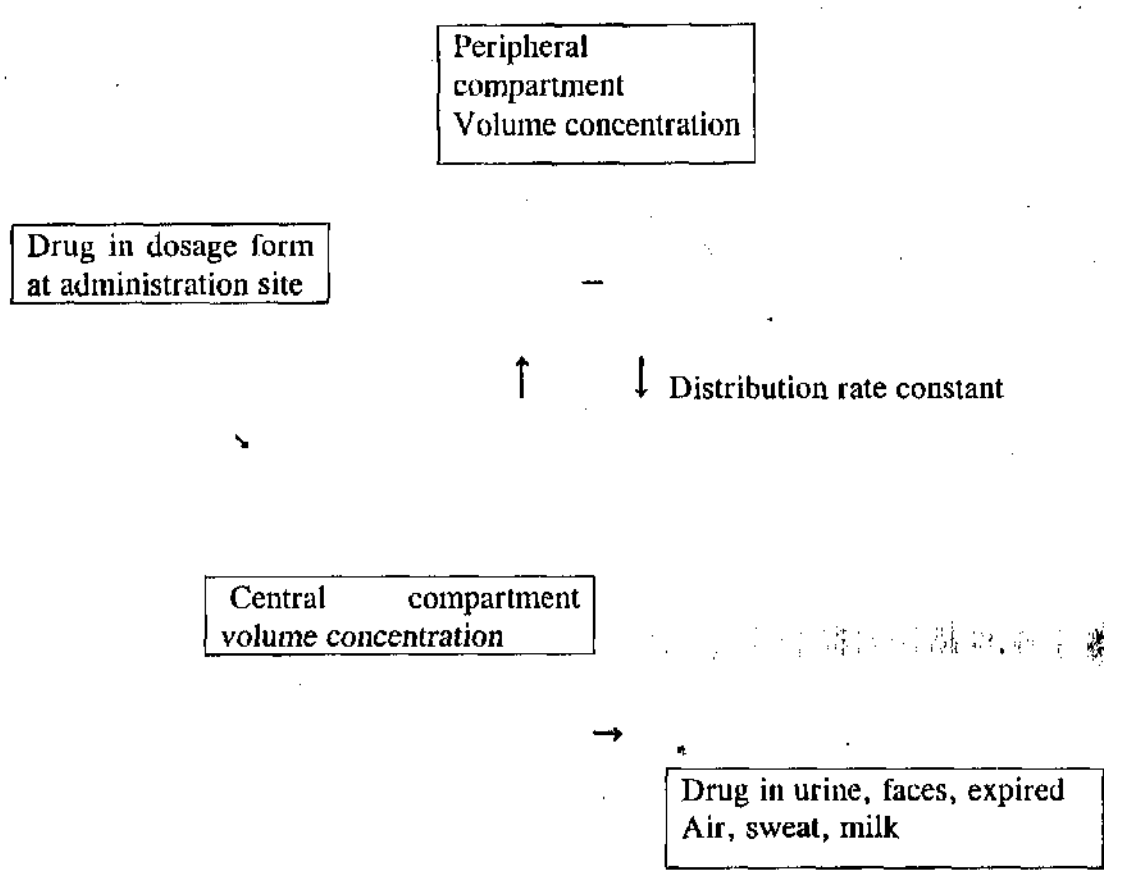


Figure 1b: Concept of Two-compartment model (Ritchie, 1980).

The distribution of the drug in blood and other soft tissues on the other hand occurs at different rates. Eventually a steady state will be reached which terminates the "distribution" phase (Ritchie, 1980).

Three compartment model is a design or modification model for oral dosing in which an additional compartment is incorporated to represent the volume (Va) from which absorption occurs at a first - order rate Figure Ic. In this model, it is assumed that the entire dose is rapidly introduced into the site of absorption from which it is absorbed into the central compartment.

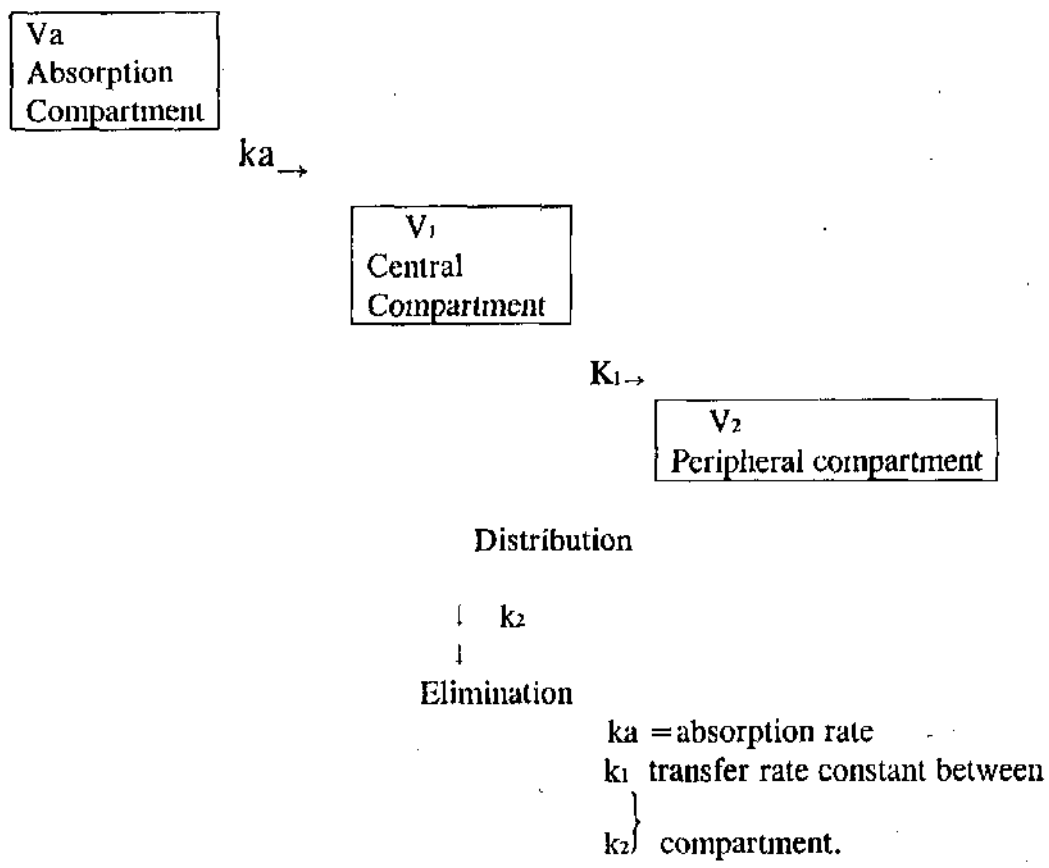


Figure 1c:A three compartment model (Lecture note).

1.2 Bioavailability

Bioavailability is defined as the rate and extent to which the active drug ingredient or the therapeutic moiety is absorbed from a drug product and become available to the site of action. Which is normally estimated by it concentration in body fluids, rate of excretion or acute pharmacological effect (Wagner, 1980).

The absorption of an intravenous administered drug is rapid and complete according to the above definition of Bioavailability. However, for reasons of convenience most drugs are administered orally. Bioavailability studies are usually based on measurement of the active drug moiety and / or its metabolite(s) in biological fluids as a function of time. Bioavailability testing in humans normally dictates that the biological fluid sample be limited to blood, urine or saliva. Drug is administered to subjects who are normally in the fasting state (approximately 12 hours) and who are not permitted food for several hours after receiving drug. Blood sample are then collected after drug administration at a frequency sufficient to permit an estimate of peak plasma or serum concentration of active drug and the total area under the plasma level versus time curve. In most studies where oral dosage forms, are compared the sampling time after each drug administration will be identical.

A typical plasma concentration versus time curve that may be obtained after oral administration of a solid dosage form is shown in Figure 1d. Inspection of Figure 1d

permits one to observe the major Bioavailability parameters peak plasma concentration (Cmax), time to achieve peak plasma concentration (tmax), and area under the plasma level versus time curve (AUC). The first two parameters that are Cmax and time are related to and used as measures of the rate of availability from the dosage form. The extent of absorption from the dosage form is related to the third parameter, AUC. The absolute Bioavailability of drug determination normally involves a comparison of AUC obtained, following oral and intravenous administration, (for which instantaneous and complete Bioavailability is assumed) of the drug. For practical reasons, relative Bioavailability is determined by comparing the AUC's of the test drug and a secondary reference standard such as an orally administered solution of the drug or a dosage form which has been accepted as a standard (Gibaldi and Perrier, 1975a; Blanched and Sawchuk, 1979).

The circumstances that arise that make it quite difficult to determine the availability of a drug following administration of a single dose are many.

The plasma levels of certain drugs may be very low such that an assay method sensitive enough to measure these levels may not be available. Other drugs have very long half-lives and the determination of the total area under a plasma concentration time curve requires plasma samples to be obtained for excessively long periods of time. Thus, it is sometimes desirable to determine availability based on steady-state plasma drug levels following multiple dosing (Gibaldi and Perrier, 1975b).

Determination of AUC is required for the estimation of availability. The most commonly

employed method for calculating AUC's are the "cut and weight' method employment of planimeter and application of Trapezoidal rule (Gibaldi and perrier, 1975c).

Important areas of application of Bioavailability are:

(a) Determination of formulation factors, that alter the Bioavailability of an active ingredient in a drug product or products.

(b) Determination of the effect of food, on drug dispersion, following ingestion (that is drug - food interaction).

(c) Determination of generic bioequivalence or bioinequivalence of two or more drug product(s) or formulation.

(c) Determination of drug-drug interaction

(e) Assessment of the magnitude and variability of the first pass effect with specific drugs after oral administration, such as rectal, nasal or buccal administration, avoid this effect.

(f) Determination of age - related effects and disease-conditions, on the disposition of administered drug.

1.3 Factors Influencing Drug Bioavailability

To be bioavailable, a drug must be at the absorption site in an absorbable form. Many factors involved in the manufactured dosage form cause marked changes in the rates of disintegration and dispersion of the granules and these process govern the rate at which surfaces become available for dissolution. Therefore, pharmaceutical factors are extremely important in the control of drug availability. In addition to the drug substance there are

excipients, 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. The Noyes-Whitney equation which characterises the dissolution rate of a drug in solid dosage form given by:

$$\frac{dw}{dt} = ks\ (C_s - C) \quad \text{----------(1)}$$

Where dw/dt is dissolution rate, k is a rate constant, S is surface area of dissolving solid, C is concentration of drug in dissolution medium and Cs is concentration of a saturated drug solution. The dissolution rate may therefore be controlled by manipulation of the surface area of the drug particles or solubility (Gillies *et al*, 1986).

Dosage form properties often account for some modification in the pharmacological activity of therapeutic agents by altering their intrinsic absorption pattern.

The schematic diagram in Figure 2 shows drug concentration in the blood as a function of time after oral administration of fixed amount of drug in three different dosage forms designated I, II, and III. The properties of the dosage forms in the hypothetical example produce a change in the overall absorption rate that is reflected in the blood level time curve. Activity occurs when the blood concentration reaches the minimum effective drug level (Fig 3). The onset of pharmacological action is significantly earlier with formulation

I than with formulation 11 and is never attained with formulation III (Blanchard and Sawchuk, 1979). The duration of clinical effect is often reflected by the length of time that the blood level of drug is equal to or higher than the minimum effective level. From the example in Figure 2, it can be seen that the duration of effect obtained from formulation I was significantly longer than that obtained from after equal dose of formulation II. It can be seen from the general example that dosage form factors have a significantly effect on the onset, duration and intensity of pharmacological action of a particular active component.

A number of processes determining Bioavailability are summarised in Table 1.

Table 1: Processes determining Bioavailability

Dosage form factors	Physiological factors
(1) Physical properties of drug	(1) Effect of luminal fluids are pH, mucus,
Lipid solubility	presence of bile salts,
	complexing components
Water solubility	(2) G.I. transit time: Gastric empting, food,
Pka	Bed rest and exercise, Entrohepatic cycling,
	Drugs
(2) Properties of dosage form	(3) Absorption site, surface area, Local blood
	flow
Disintegration time	(4) Metabolism of drug by gut wall, liver, skin
Dissolution rate	bronchia mucosa, and all factors affecting this
Manufacturing variables e.g compression	5) Pharmacogenetic factors determination
pressure, Lubricant concentration,	of hepatic metabolism
ageing of product	(6)Disease states e.g Malabsorption, thyrotoxic
	(7) Gut Flor

(Gillies *et al*, 1986).

Poole (1979) and Wade (1976) discussed extensively on the various formulations and dosage forms related factors such as particle size, crystal form, salt, complex, PH and solubility characteristics. It has been shown by Poole (1979) that Bioavailability of some drugs have been improved by particle size reduction.

Another factor influencing the Bioavailability of drugs is crystal form. The crystal form of a drug substance may change because the substance exhibits polymorphism. At least one third of all organic compounds have been reported to exhibit polymorphous. The substance can also occur in non-crystalline (amorphous) forms apart from their polymorphic existence. The energy required for a molecule of a drug to escape from a crystal in which it is located is greater than that required to escape from an amorphous powder.

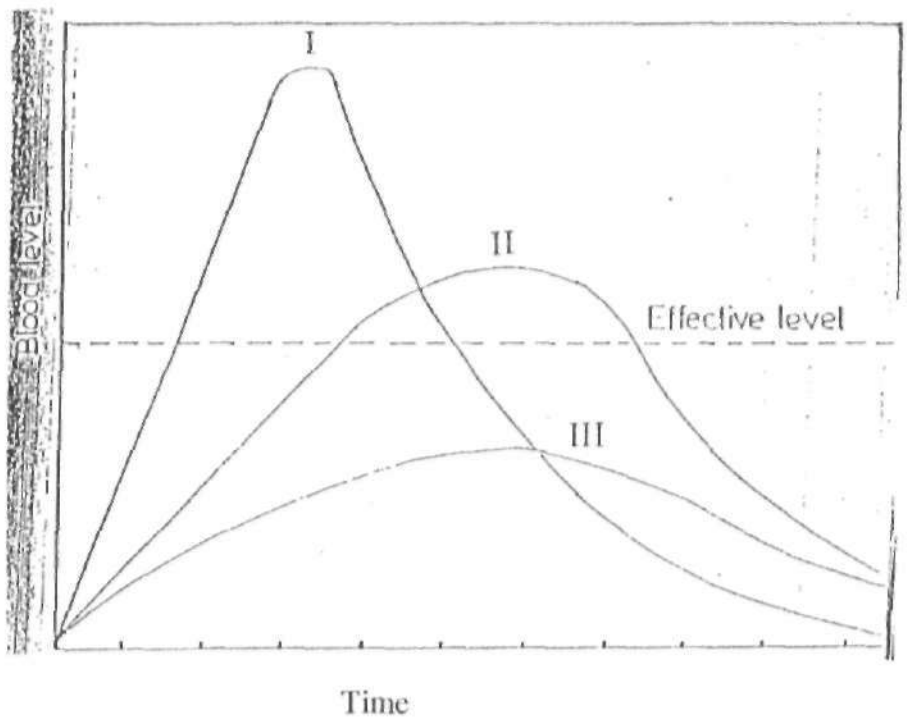


Figure 2: Blood level-time curves showing possible of dosage form on blood levels after trial administration of equal amount of active agent (Poole, 1979).

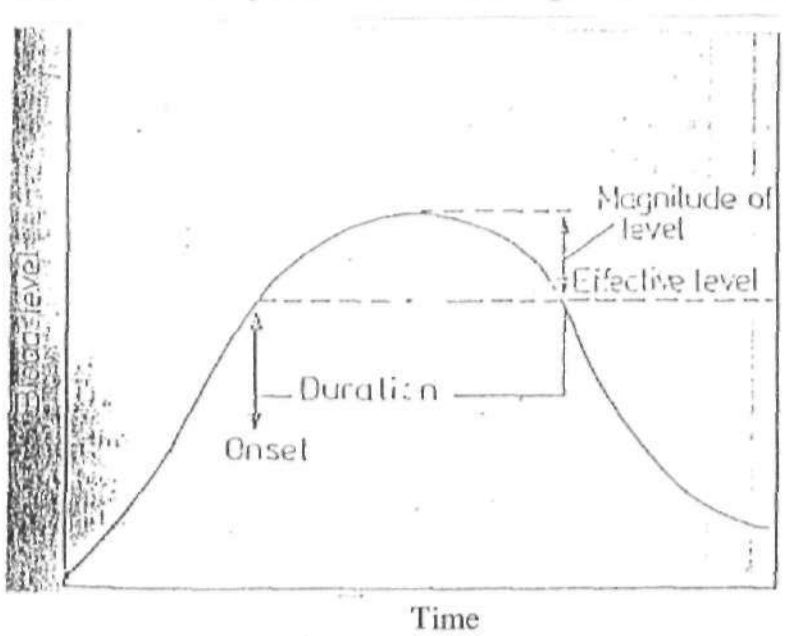


Figure 3: Relationship of blood level - time curve and pharmacologic effect (Poole, 1979).

Consequently, the amorphous form of a compound is always more soluble than the corresponding crystalline form and may exhibit correspondingly different therapeutic properties (Poole, 1979). Examples of drugs in which this has been observed *in vitro* are chloramphenicol and novobiocin. (Poole, 1979).

The following factors have been found to determine the observed effects, 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 dating and drug administration. Another important factor that affects Bioavailability of drug is the influence of food and specific dietary components.

Winstanley and Orme (1989), Haye's and Borzelleca (1985) extensively reviewed the influence of food and specific dietary components on the drug absorption. The reviews reflect the complex and unpredictable nature of drug food interactions and the difficulty of establishing patterns that may be used as guidelines for optimum drug therapy in relation to food. The presence of food has been shown to decrease, delay, increase or not to affect drug absorption. Such interaction may be indirect as with the increase rate of acetaminophen absorption due to the stimulating effect of metoclopromide on the stomach empting rate or direct, as in chelation of tetracycline by polyvalent metal. However, propantheline reduced the rate of acetaminophen asbroption by delaying stomach emptying rate (Welling, 1980).

The following factors have been found to determine the observed effects, 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 dating and drug administration.

Though, drugs Bioavailability studies are routinely carried out in healthy human subjects drugs are administered in practice to patients who, may be suffering a variety of illnesses. Diseases affecting the liver, cardiovascular system and gastrointestinal tract may alter circulating drug or metabolite level after oral dosing. Surgical removal of part of gastrointestinal tract reduced the absorption of most compounds, suggesting that patients who have undergone such procedure may need to be monitored carefully when taking drugs whose absorption efficiency is critical (Bakare, 1992).

1.4 Difference in Drug Bioavailability and their clinical implication.

The drug absorption has been shown to be potentially influence by a number of factors. The change in clinical response that may accompany altered drug Bioavailability is a function both of the drug mode of action (Pharmacodynamic) and also the extent of Bioavailability variance (Pharmacokinetics). Due to many factors contributing to nature of assessing clinical response it may be difficult to identify a clinical change with altered drug bioavalability.

However, several reports have documented actual changes in clinical responses that have been associated with changes in bioavaialbility of some drugs. Most of these drugs have well defined blood or tissue level for therapeutic effect, step dose response relationships

or narrow therapeutic indices or have suitable absorption or elimination mechanisms. (Azarnoff and Huffman, 1976).

It is appropriate to divide Bioavailability changes into three major groups, whether the changes in the Bioavailability of drug is caused by drug interaction, a pathological condition and product substitution or any other factor. The groups include those which have definitely caused changes in clinical response, those which have been shown to cause clinical changes but are highly likely to do so, and those which could bring about clinical changes in extreme situations.

(a) Bioavailability changes which have caused changes in clinical response

Instance where marked clinical changes have associated with impaired drug Bioavailability, due to formulation changes have been described for hydrochlorothriazide (Tannenbaum et al. 1968), chlorothiazide and spironolactone (Shaldon et al 1963), phenytoin (Eadie et al, 1968), Tyrer et al, 1970).

(b) Bioavailability changes which are likely to have clinical effects

If a drug meets any of the aforementioned criteria it is likely that a change in drug criteria, it is likely that a change in drug Bioavailability will have a measurable clinical effect. Included amongst these drugs are the anticongulatants, antihypertensive agents, the cardiac glycosides, anticonvulsants, antineoplastic agents, and inorganic ions such as lithium.

However majority of cases, levels of these compounds in the body greatly exceed the minimum required for activity, and small changes in Bioavailability are unlikely to be of clinical significance.

(c) Boavailability changes which may have clinical effects

A clinical change will occur for all drugs, provided that the change in Bioavailability is sufficiently large. Aspirin is considered to have a wide therapeutic index, but zero absorption of a particular formulation of this compound (Mirrison and Campbell, 1960) is clearly significant. The clinical effect may be altered by changes in the rate and extent of drug absorption. Changes in absorption rate are more important for drugs with short biological and pharmacological duration, but the effect tends to be attenuated with repeated dosing (Bakare, 1992).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1a Diabetic mellitus

Diabetes mellitus is a metabolic disease characterised by hyperglycaemia and eventually glycosuria. The disease is cause by an absolute or relatively lack of insulin that resulted into the inability of the tissues to carry out normal metabolism of carbohydrate, fat and protein. An absolute lack of insulin is found in some Diabetes patients who developed the disorder early in life (Juvenile or growth onset). Relative insulin deficiency is usually found in those Diabetics who developed the disease later in life (adult onset or maturity onset) (Aguwa, 1986).

2.1b Incidence of diabetes mellitus

Diabetes mellitus is one of the major causes of death in the United States. Various studies indicated that up to 5% of the general population of the United States might eventually develop this disease (Wilkerson and Krall, 1947). The prevalence is probably similar in other countries though, Juvenile diabetes mellitus is unusual in Africa and in the East, especially in Japan. Throughout the world 30,000,000 people have diabetes mellitus (Ritchie, 1990).

The world Health Organisation and the American Diabetes Association have suggested criteria for the diagnosis of the disease. An adult is considered diabetic if the fasting blood sugar measured in various plasma exceeds 8mol/L (140mg/100ml) on more than one occasion. An adult is also considered diabetics even if the fasting blood sugar is less than 8mmol/L. If on two occasions the concentration of glucose in venous plasma exceed 11nmmol/L (200mg/100ml) 30,60, or 90 minutes, after ingesting 75g of glucose and remains above this levels two hours after ingesting.

In children a diagnosis of diabetes mellitus is made if the child has symptoms of the disease together with a fasting blood sugar exceeding 11mmol/L, 30,60 or 90 minutes after ingesting 1.75g/kg of glucose and is still above this level two hours after ingestion of the glucose. In pregnancy a diagnosis of diabetes mellitus is made if the concentration of glucose in venous plasma is excessive on at least two occasions when measured 1, 2, and 3 hours after the ingestion of 100g of glucose. The concentration should not exceed 11mmol/L (200mg/100ml) 1 hour after taking the glucose, 9mmol/L (165mg/100ml) 3 hours afterwards (Ritchie, 1990).

Diabetes mellitus is basically classified into Insulin Dependent Diabetes Mellitus (IDDM), knows as Type I Diabetes, and Non-Insulin Dependent Diabetes Mellitus, (NIDDM) that is referred to as Type II Diabetes. And other types of diabetes includes Diabetes insipidus which is caused by renal reabsorption of water (Stryer, 1988b) and J, K and Z-types diabetes (Wealtherall, 1987). The type I is early age onset with weight loss while the type

II is characterised by relatively later onset, insensitive to insulin and partial insulin deficiency (Taylor and Agius, 1988).

Ordinarily, the level of glucose in the blood varies only within narrow limits, it rarely rises above 120mg/100ml or falls below 60 mg/100ml. Due to reliability of some tissues wholly on glucose for their metabolism, it is very essential to maintain the normal glucose level (especially brain). A decrease in blood glucose below a level of about 30 mg/100ml resulted into serious disturbance of metal function that may lead to coma. Meanwhile a rise in blood glucose produced osmotic diuresis and dehydration. There is usually a mechanism by which the level of glucose is maintained within the normal range both during starvation and after eating a carbohydrate meal, (Oakley *et al*, 1975).

Blood sugar increases after carbohydrate containing meal due to the breakdown of starch to glucose. The glucose is then absorbed and carried in the portal vein to the liver. The glucose is stored in the liver as the polysaccharide glycogen. Excessive glucose is synthesized into glycogen and in time of shortage of carbohydrate, glycogen is converted to glucose and thereby released into the blood stream. The pathway involved in glycogen breakdown is different from the pathway of glycogen synthesis, different enzymes being involved.

2.2 Biochemical basis of diabetes

The name diabetes came from the Greek word for a siphon and refers to the polyuria common in both Diabetes Mellitus and in diabetes insipidus. Mellitus is derived from Latin and means sweetened with honey, referring to the sweet taste of glucosuric urine (Ritchie, 1990).

The lack of insulin associated with diabetic mellitus results in deficient tissue utilization of carbohydrate necessitating increased catabolism of proteins and fats to supply the energy needs of the body. The increased catabolism of fats leads to Ketosis, while the hyperglycaemia cause excessive loss of glucose through urine (glucosuria). Thus, arise the cardinal metabolic manifestation of Diabetes, hyperglycaemia, ketosis and glucosuria, sometimes terminating in death (Reaven and Salans, 1964).

In addition to derangement, diabetes mellitus is also associated with wide spread alteration in the blood vessels kidneys, eyes, peripheral nerves and heart. Most of these vascular changes take the form of an accelerated aging process including agugmented arteriosclerosis and degenerative changes in the small blood vessels (Reavan and Salans, 1964) at two key stages in the process. Phosphorylase, which produced glucose-1-phosphate from glycogen by adding on phosphate group and glucose-6-phosphatase that hydrolyses glucose-6-phosphate to give free glucose. Both enzymes are influenced activity of hormones. For example, glucagon and adrenaline raise blood glucose by activating phosphorylase that depletes liver glycogen. This activation of phosphorylase involves the

intervention of the intracellular messenger substance. CyclicAmp (Adenosinemonophosphate). The conversion of glucose to glycogen is shown in Figure 4. Glucose, fructose and a number of other sugars less commonly present in foods stuff are converted into glucose or glycogen in the liver (Oakley et al, 1975).

Proteins are similarly broken down as complex carbohydrate into amino acids. Amino acids are absorbed into the portal blood stream and carried to the liver. These may be used in the synthesis of protein in muscle or kidney. Certain amino acids can be metabolised to glucose. This process is essential in starvation, which if prolonged, may lead to severe loss of body protein by its conversion into glucose in order to maintain the blood sugar level, (Oakley et al, 1975).

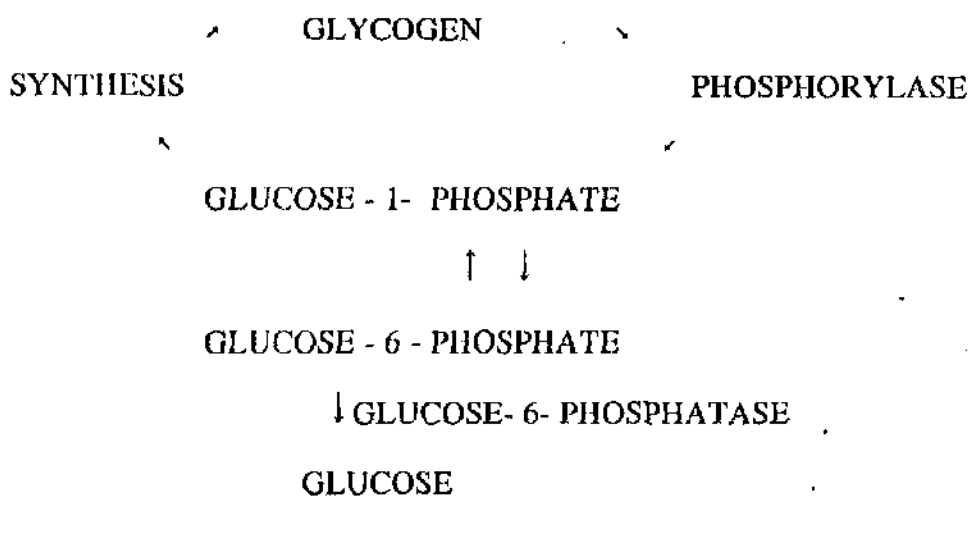


Figure 4:The low blood sugar level leads to decrease secretion insulin and increased secretion of glucagon.

Gluconeogenesis

Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors (Stryer, 1988c). The process is important for the maintenance of blood sugar in periods of starvation. It is greatly accelerated in diabetes where protein breakdown contributed substantially to the rise in blood sugar (Oakley, *et al,* 1975).

The process is important also during the periods of intense exercise (Stryer, 1988c). Pyruvate is converted to glucose in gluconeogenic pathways chiefly as pyruvate, Oxaloacetate and dihydroxy acetone phosphate (Figure 5.).

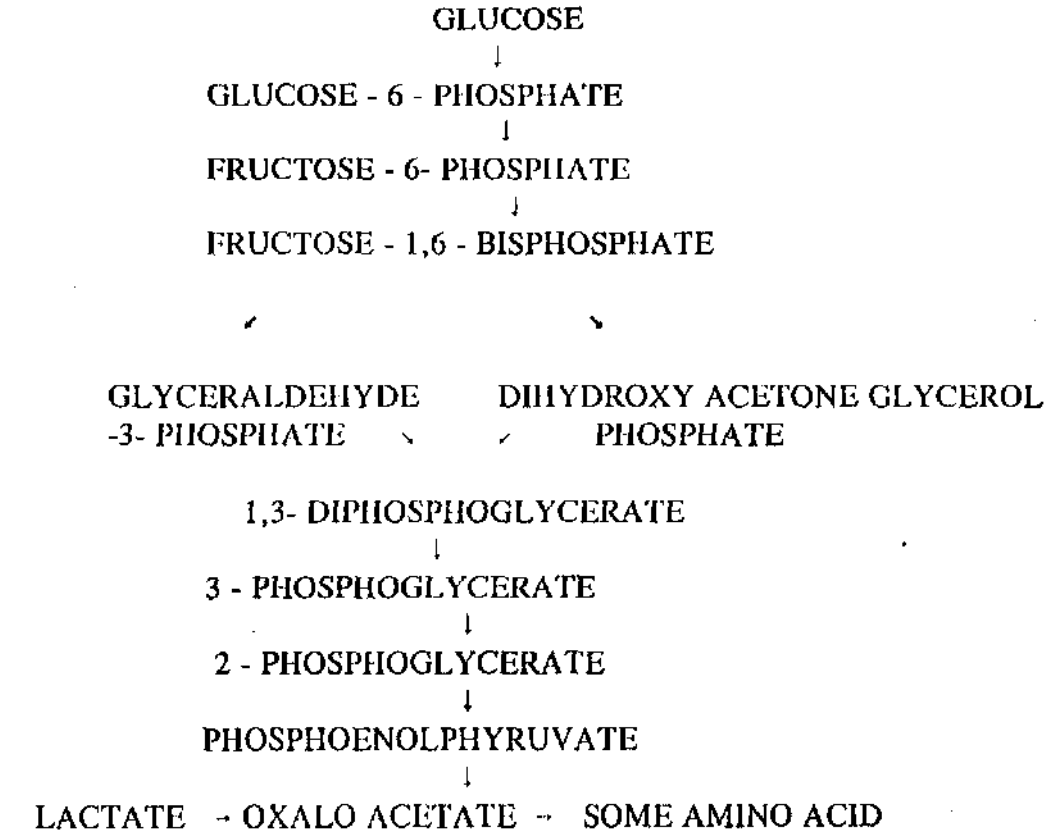


Figure 5: Gluconeogenic Pathway (Stryer, 1988c).

The major non-carbohydrate precursors are lactate, amino acids and glycerol. The enzymes of gluconeogenesis are located in cytosol. The major site of gluconeogenesis is the liver and also occur in the cortex of kidney, very little gluconeogenesis takes place in the brain, skeletal muscle or heart muscle. The gluconeogenesis in the liver and kidney help to maintain the glucose level in the blood so that brain and muscle can extract sufficient glucose from it to meet their metabolic activities (Stryer, 1988c).

Hyperlipidaemia in diabetes mellitus

Hyperlipidaemia - The entity is associated with increase in blood levels of lipids and lipid levels are elevated in Diabetes Mellitus (Marcus and Milton, 1978). Circulating levels of lipoprotein are often elevated along with glucose in Diabetes Mellitus. Chylomicrons which carry ingested fat and very low density lipoproteins (VLDL) which contain triglycerides converted from ingested carbohydrate, are transported in plasma, to fat depots. These are cleared by insulin dependent lipoprotein lipase when insulin levels are low (insulinpenic diabetic). In Type I or Type II diabetes defective glucose removal is associated with impaired removal of VLDL and Chylomicrons. Replacement of deficient insulin or restriction of caloric intake to restore effectiveness of endogenous insulin in obese diabetic patient facilitates clearance of these lipoproteins.

Fat Metabolism

Fat is stored in adipose tissue cell subcutaneously in the omentum and around the viscera.

Fat consists of a combination of fatty acids with glycerol. The first step in fat breakdown is the hydrolysis of neutral fatty acids and glycerol. This process is controlled by a number of hormones such as growth hormone, glucagon and adrenocorticoid hormone (ACTH). In adipose tissue free fatty acids are produced by the action of lipase and transported to other sites assess where they are further metabolised. Fat provides store for oxidisable material available in conditions of prolonged carbohydrate shortage, thus fat stored are readily broken down in starvation (Oakley et al, 1975).

Hypoglycaemic agents

These are drugs used in the treatment of diabetes mellitus, a hereditary disorder characterised by a relative or absolute deficiency of insulin. Three major methods adapted in the management of this disorder are oral hypoglycaemic agents, Dietary control and Insulin Therapy. Insulin is by no means an ideal treatment for Diabetics, the realisation of this fact has led to a great deal of research to develop more effective ways of treating the disease. For the management of Type II diabetics who do not respond adequately to restricted food intake and dietary advice, tablets are usually given.

Two basic groups of oral hypoglycaemic agents are:

Sulphonylurea(s), Examples are, Chlorpropamide (Diabinase ^{R}), Glibenclamide (Daonil^{R}), Tolbutamide (Orinase), and Tolazamide (Tohnase)

Biguanide(s), Examples are, Metformin (Glucophage), Phenformin (meltrol), and Buformin (Butformin).

2.3 History and chemistry of sulphonylureas

The sulphonylureas act to ameliorate hyperglycaemia in some patients with non-insulin Dependent Diabetes Mellitus (Type II), initially by increasing insulin secretion and secondarily by potentiating insulin. Chlorpropamide one of the sulphonylureas has the greatest usefulness in the treatment of Type II Diabetes mellitus subjects.

Two guanidine derivatives, sythalins A and B were introduced into diabetes therapy in the 1920's. Though, they were less toxic than insulin. It was shown that prolonged use of these synthetic derivations could result in renal and hepatic damage, consequensely their use was discontinued in early 1930's (Olaniyi, 1989).

Structure activity relation of sulphonylureas

The general structure of sulphonylureas consist of three portion

Ar ---------SO_2---------- NHCONH----------R

Aryl	Urea moiety	Alky group
(1)	(2)	(3)

Variation of the aryl moiety (1) proved most useful. The para NH_2 group was shown to be

unnecessary and replaced by a methyl group to give the drug "Tolbutamide" while being an effective hypoglycaemic agent, tolbutamide was rapidly metabolised by oxidation of the p-methyl group to a carboxyl function and excreted. Replacement of the methyl group by halogen (chlorine) gave a compound with increased potency and longer action. From a series of chloroaryl compound prepared, the 3-propyl derivative was the most effective and was given the generic name of chlorpropamide. Modification of the urea moiety led to loss of activity. Whilst variation in the alkyl side chain showed that 3 or 4- carbon atoms gave optimum activity. The alkyl group confer lipophilic properties on the molecule. Later development included the introduction of acetohexamide and tolazamide.

Physical Chemistry of Chlorpropamide

Chlorpropamide is a white odourless or almost odourless, crystalline powder.

It is practically insoluble in water; soluble 1 in 12 of chloroform; slightly soluble in ether; soluble in solutions of alkali hydroxides.

2.4 Absorption and metabolism of chlorpropamide

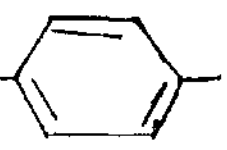
Brothertion et al (1969) using chromatographic techniques identified two metabolites (p-chlorobenzene- sulphonylureas and p-chlorobenzene sulphonamide) in the urine of diabetic patients. The proportion of the drug metabolised varied in different individuals on long term therapy. However, Chlorpropamide was thought for long to be almost totally excreted unchanged in the urine (Balant, 1981).

Taylor (1972), identified two additional metabolites, 2-hydroxy-chlorpropamide and 3-hydroxy chlorpropamide from the urine of four diabetic patients receiving 250- 400mg of chlorpropamide daily. He reported that the unchanged drug accounted for 95%. The alkyl group confers lipophilic properties on the molecule. Later development included the introduction of acetohexamide and tolazamide. The structures of chlorpropamide and tolbutamide are shown in Figure 6a (PP. 29)

a .  SO_2 – NHCONH – $(CH_2)_2$ – CH_3

Chlorpropamide (Diabinese ^{R})

1- [(p-chlorophenyl) (Sulphonyl)] -3-propylurea

b. CH_3  SO_2 – NHCONH – $(CH_2)_3$ – CH_3

Tolbutamide (Orinase^R)

1-Butyl-3 (p-toly-sulphonyl) urea

Figure 6a. Structure of chlorpropamide and Tolbutamide

2.5 Therapeutic significance of chlorpropamide

The development of sulphonylureas had a tremendous impact on diabetes treatment and research. Hypoglycaemic agent are orally effective in reducing blood glucose levels and also safe, while being more convenient and more acceptable than insulin to many patients. These agents are not prescribed for Type I Diabetics, pregnant women, those who have renal disease or those who hyperglycaemia can be controlled by diet. (Aguwa, 1986). Jackson (1969) reported that, it may take as long as 96 hours for 80-90 percent of chlorpropamide to be excreted in the urine and complete elimination of the drug takes 3-7 days.

Chlorpropamide is readily absorbed from the gastrointestinal tract and is extensively bound to plasma protein. The half-life in plasma is about 35 hours. It is partly metabolised in the liver to metabolites with some hypoglycaemic activity. The metabolites and unchanged drug are excreted in the urine (Martindale, 1989).

2.6 Mechanism of action of oral hypoglycaemic agent

The mode of action of the synthetic hypoglycaemic agents is not fully understood and most experimental work has yielded negative results. It has been shown that they do not possess direct insulin like action nor do they act by interference with glucagon secretion. They do

not inhibit hormonal antagonists of insulin. They do however require the presence of insulin containing pancreatin tissue for activity. This has led to the theory that their main action is to cause the release of "bound" or complexes" insulin both in plasma and in the tissue (Olaniyi, 1989).

Lebovitz et al, (1971) stressed the importance of stimulation of insulin secretion and or beta - cell hyper plasma (beta-cytotropic action). Others have shown that the chronic antidiabetic activity of sulphonylureas does not result in increased in insulin secretion. This suggests that one or more extra pancreatic actions are involved in the anti-diabetic effects of those agents (Lebovitz *et al* 1971). A direct drug effect in tissue insulin sensitivity has been claimed (Kolterman *et al*, 1984; Ward *et al*, 1985). All sulphonylureas used to treat Type II diabetes cause the bet - cell to secrete insulin. Probably by binding to the cell surface membranes, lowering the out flow of calcium, calcium binds to the protein calmodulin, which mediates microfilament concentration and so facilitates the secretion of insulin granules. *In vitro* experiment, the insulin releasing effects depend on the concentration of drug. There are 1,000 fold differences in potency in these in vitro studies between tolbutamide and Glibenclamide, but there is no evidence that efficacy differs among the clinically useful sulphonylureas in the systems used (Ferner, 1988).

Sulphonylureas may have extra pancreatic effects in addition to their effects on secretion of insulin. There is evidence for (and sometimes against) action on insulin receptor numbers or affinity. A body of work now favours the ideal that sulphonylureas affect

insulin action at a post receptor site, but do not consistently increase the number or affinity of receptors (Ferner, 1988).

The observation of increase plasma insulin to C-peptide ratio has led to the suggestion that decreased insulin extraction is a direct drug effect (Scheen *et al*, 1984). Tolbutamide reduced insulin extraction in the perfused rat liver (Marshall *et al*, 1970).

2.7 Adverse effect and toxicity of hypoglycaemic agent

In the early months and years of use of oral hypoglycaemic agents, much was said about the side effects. Allergic skin responses, gastrointestinal disturbances, blood dyscrasia and jaundice of cholestatic type were reported. However, patient carefully selected for oral agents, treatment, such untoward and toxic effects are uncommon and when they occur they are mild. But an exception is digestive disturbance such as anorexia, nausea, vomiting or diarrhea, which may be encountered with phenformin if doses given are unwisely large. Participants in university group diabetes programme (UGDP) in 1970 reported results that long time treatment of diabetes with tolbutamide increased the risk of death from cardiovascular causes and the similar results was presented by the same (UGDP) for phenformin. These leads to many discussions and disagreements certain clinicians discontinued the use of oral hypoglycaemic agents entirely.

Early studies carried out in an attempt to evaluate the long-time effects of oral agents, did not reveal any significant differences of cardiovascular disease, as a cause of death in such patient when compared with those who have been treated by means of diet alone or diet

plus insulin. Consequently the use of oral agents as before have continued in carefully selected patients. In recent years, there have been relatively few reports of short-term side effects or toxicity due to sulphonylureas(s), (Howard and Conn, 1975).

Chlorpropamide has a hyponatreamia side effect and likely to cause severe and prolonged hypoglycaemia in older patient or those with cardiovascular or renal disease. Chlorpropamide has occasionally been implicated in bone-marrow depression. A transient fall in white cell count has been noted. Also severe leucopenia, pancytopenia and fetal bone marrow aplasia with agranulocytosis (Jackson, 1969).

Preparation and dosage of chlorpropamide

Chlorpropamide (Diabinese) is marketed in the form of 100mg and 250mg tablet. 200mg daily dose will maintain a plasma level of about 10μg per 100ml while minimum and maximum effective concentration are achieved with 100μg and 500μg per 100ml respectively. Higher doses than this are dangerous because of cumulative elevation of the drug blood. In fact, the lower the dose, the safer the drug, so that a starting dose of 250mg or less is recommended. The danger of severe, hypoglycaemia from higher doses is greatest in elderly and mild diabetics (Jackson, 1969).

2.8 Drug interaction with chlorpropamide

Intolerance to alcohol or a disulfiram like facial flushing effect has been reported most frequently with Chlorpropamide. Alcohol itself a potential hypoglycaemic agent under

conditions of prolonged fasting or malnutrition may also increase the effect of chlorpropamide or insulin and may lead to a clinically significant hyperlacticacidaemia in phenformin treated diabetics. A number of drugs have been reported to increase effect of chlorpropamide but in practice such interaction are rarely common, (Graemes, 1987). It was proposed that probencid may inhibit the renal tubular secretion of chlorpropamide, (Petitpierre, 1970). Chlorpropamide and to a lesser extent, other sulphonylureas may provoke an "Antabuse reaction" to ethanol with flushing headache. Also ethanol may inhibit the antidiuretic effect of Chlorpropamide used to treat diabetes insipidus, (Yamamoto, 1976).

It was also proposed that allopurinol and its metabolites might compete with Chlorpropamide for renal tubular secretion but this suggestion has not been tested, (Petitpierre, 1972). Petitpierre (1970) proposed that clofibrate may prolong chlorpropamide half-life. Preliminary evidence indicates, that the half- life of chlorpropamide is also prolonged by concomitant administration of chloramphenicol, (Petitpierre, 1970). Thiazides tend to elevate blood glucose in diabetic and thus may antagonize the hypoglycaemic effect of antidiabetic drugs, (Hickman, 1968).

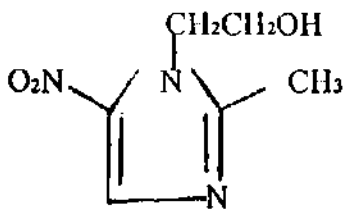
It was also noted in some patients that combined therapy with chlorpropamide and a thiazide diuretic may result in hyponatremia of a degree not seen with the use of either agent alone, (Fichman, 1971). The mean blood sugar concentration rose significantly when trichormethiazide or chlorthiazide was administered to patients on chlorpropamide,

(Kansal, 1969). In vitro studies have shown that sodium salicylates displaced chlorpropamide from plasma protein binding, thus temporarily increasing unbounded (active) sulphonylurea concentration, (Wishinsky, 1962). Since salicylilic acid, phenylbutazone, ethionamide and amino salicylic acid lowered blood sugar concentration they were associated with severe hypoglycaemic reactions in patients being treated with chlorpropamide, (Schulz, 1968). Mechanisms through which clofibrate may enhance the effects of oral hypoglycaemic drugs include displacement of sulphonylureas from plasma protein binding, decreased insulin resistance, (Ferrari, 1976), and competition between clofibrate and chlorpropamide for renal tubular secretion (Petitpierre *et al*, 1972). Large doses of ammonium chloride acidify the urine thus decreasing the ionization of chlorpropamide and decreasing its urinary excretion, (Neuvonen, 1983). Administration of sulfamethazine appeared to result in severe hypoglycaemia in a patient maintained on chlorpropamide, (Dall, 1967).

There is an increase in dose requirements of chlorpropamide in a patient taking rifampicin, (Self and Morris, 1980). Severe hypoglycaemia occurred in a patient taking chlorpropamide and phentofomin when given sulphafurazole. The ability of various anticoagulants to displace chlorpropamide from human serum albumin in vitro at pH 7.4 was in the following decreasing order, ethylbiscoumacetate, anisindione, nicoumalone, and phenprocoumon, (Judis, 1973).

2.9 Structure activity relation of Metronidazole

The chemical formula is 1- (β hydroxy ethyl) 2- methyl-5- metronidazole with the following structural formula.



Metronidazole (Flagyl^{R})

It is also referred to as 2- methyl-5- nitro imidazole - l-ethanol

Butler et al (1967), in an extensive structure-activity study, observed that some 2-alkyl derivatives of 1-(2-cyanoethyl)-5-nitro imidazole are about 5 times as active as metronidazole in T. foetus infections in mice. Ismailor *et al* (1966) reported high trichomonacidal activity for 4-chloro-1-methyl-5-nitro imidazole in experimental T. vaginalis infections.

Physical Chemistry of metronidazole

Metronidazole is a white or pale yellow crystalline powder, odourless or almost odourless. It is sparingly soluble in water, slightly soluble in ethanol (~ 750g/l) TS. Chloroform R. and ether R.

2.10 Absorption and metabolism metronidazole

An oral imidazole drug, metronidazole (Flagyl) has become available in the United States for the systemic treatment of Trichomonas infections (AMA, 1963). The drug has been

used since 1959 in France, England and Canada. Metronidazole was subsequently found to be effective against amoebic liver abscess and amoebic dysentery. Metronidazole is highly effective when given orally in the treatment of infections caused by *Trichomonas Vaginalis* in both male and female. Metronidazole appears to be specific for *Trichomonas*. It is inactive against *Candida Albicans* and other agents that cause V*aginitis*. The activation of latent *Candida* infections either during or following treatment with metronidazole was reported (Blasingame, 1966). Metronidazole is metabolised in the liver by side-chain oxidation and glucuronide formation. The principal oxidation metabolites are 1- (2-hydroxy ethyl)-2- hydroxy methyl-5-nitroimidazole (the "hydroxy" metabolite), which has antibacterial activity and is detected in plasma, but is excreted in urine. Small amounts of reduced metabolites, acetamide and N-(2-hydroxy ethyl) oxamic acid (HOA), have also been detected in urine and are probably formed by the intestinal flora.. In man and dog 60% to 70% of a dose of metronidazole is found to be excreted unchanged in the urine (Ings, 1966).

The plasma elimination half-life of metronidazole is about 8 hours, that of the hydroxy metabolite is slightly longer. The half-life of metronidazole was reported to be longer in neonate and patients with renal failure. The majority of a dose of metronidazole excreted in the urine mainly as metabolites, a small amount appears in the faeces. Depending on the assay method used, up to 80% of dose has been recovered in the urine within 48 hours.

2.11 Therapeutic significance of metronidazole

Metronidazole is readily absorbed following administration by mouth and Bioavailability approaches 100%. Peak plasma concentrations of approximately 5 and 10mg per ml are achieved an average of one hour after single doses of 250mg and 500mg respectively. Absorption may be delayed, but is not reduced overall by administration with food. Metronidazole benzoate is also given by mouth and is hydrolysed in the gastrointestinal tract to release metronidazole, which is then absorbed (Martindale, 1989).

Metronidazole is widely distributed. It appears in most body tissues and fluids including bile, bone, breast milk, cerebral abscesses, saliva, seminal fluid, and vaginal secretions, and achieves concentrations similar those in plasma. It also crosses the placenta and rapidly enters the foetal circulation. No more than 20 per cent is bound to plasma protein (Martindale, 1989).

Metronidazole is a 5-nitroimidazole derivative with activity against anaerobic protozoa and anaerobic bacteria. It also has a radio sensitising effects on hypoxic tumour cells. Its mechanism of action is thought to involve interference with DNA by a metabolite in which the nitro group of metronidazole has been reduced. Metronidazole is active against several protozoa including *Balantidium Coli, Blastocysts Hominid, Entamoeba histolytica, Giardia Intestinalis (Giardia Lamblia), and Trichomonas Vaginalis*. Most obligate anaerobic bacteria, including *Bacteroids* and *Clostridium* species are sensitive in vitro to metronidazole. It is bactericidal. It also has activity against facultative anaerobic *Garelnerella vaginalis* and *Campy lobacter* species. Resistance to metronidazole appears

to be rare therefore it is used in the treatment and prophylaxis of anaerobic bacterial infections. The specific bacterial infections treated with metronidazole include bacterial ***Vaginosis***, acute necrotising ulcerative, (Vincent's infection) and pseudo membranous colitis and has been given in inflammatory disease and as an adjunct to the radiotherapy of malignant neoplasms (Martindale, 1989).

2.12 Mechanism of action of metronidazole

The mechanism of metronidazole cytotoxic and mutagenic action on microorganism is not well understood. However, the observed cytoxicity has been attributed by some work, to the derivatives arising from the reduction of the nitro group for example, formation of hydroxylamine derivative or amines (Olaniyi, 1989).

2.13 Adverse effect and toxicity of metronidazole

Considering the relatively mild infection for which it is used, the drug possesses an uncomfortably narrow margin of safety. The recommended therapeutic dosage is 250mg orally three times daily or approximately 10mg/kg of body weight (Joseph, 1971). The adverse effect of metronidazole is generally dose-related. The most common are gastrointestinal disturbances, especially nausea and an up pleasant metallic taste. Nausea is sometime accompanied by headache, anorexia, and vomiting. Diarrhoea, dry mouth, a furred tongue glossitis and stomatitis may also occur. There have been rare report of pseudo membranous clotitis associated with metronidazole (Thomson, 1981). Peripheral neuropathy usually presenting as tingling in the extremists and epileptic form seizure are

serious adverse effects on the nervous system that have been associated especially with high doses, of metronidazole or prolonged treatment. Weakness, dizziness ataxia, drowsiness, insomnia and change in mood or mental state such as depression or confusion have also been reported (Dutty, 1988).

Temporary moderate leucopenia may occur in some patients receiving metronidazole. Skin rashes and pruritus occur occasionally and anaphylaxis has been reported rarely. Other side effects include urethral discomfort and darkening of the urine. Raised in liver enzyme value have occasionally been reported. Thrombophlebitis may follow the intravenous administration of metronidazole. The carcinogenic effect of the drug on bacteria and rodents has made some authors express the suspicion that the drug could be a possible human carcinogenic and mutagenic agent (Olaniyi, 1989).

Preparation and dosage of metronidazole

Metronidazole (Flagyl) is marketed in the form of 200, 250, and 400mg tablet (BP, 1988). The recommended dose is 2g in a single dose administration of 400mg, 3 times daily for 5 days. (Powell *et al*, 1969). The usual oral dosage for adults is 400mg three times daily for 7 days (Alan, 1986).

2.14 Drug interaction with metronidazole

When given in conjunction with alcohol, metronidazole may provoke a disulfiram-like

reaction in some individuals; reactions have occurred after the administration of pharmaceutical preparations formulated with alcohol, including injections (Drug Interact. News, 1987) as well as after drinking alcohol.

The concomitant use of metronidazole and disulfiram resulted into acute psychoses or confusion (Rothstein and Clancy; 1969). Although metronidazole had no effect on the elimination of antipyrine in healthy subjects (Staiger, 1984) and by inference has little effect on hepatic drug- metabolising enzymes, it enhance the anticoagulant effect of warfarin and may impair the clearance of phenytoin. There is some evidence that phenytoin might accelerate the metabolism of metronidazole (Wheeler, 1978).

Concomitant administration of metronidazole and phenobarbitone was reported to decrease the plasma concentration of metronidazole with a consequent reduction in the effectiveness of metronidazole (Mead, 1982). Cimetidine has increased the plasma concentration and might increase the risk of neurological side effect (Gugler and Jensen, 1983). Metronidazole may interfere with the measurement of liver enzymes in blood to produce abnormally low result (Rissing, 1978) and may interfere with the hexokinase method of measuring blood- glucose concentrations. It has also been reported to interfere with assays of blood concentrations of procainamide (Gannon and Phillips, 1982). Metronidazole has anti treponema activity and may mask the immunological response seen in untreated early syphilis (Pattman and Sprott 1985).

Metronidazole was considered to be unsafe in some patients with acute porphyria because it has been shown to be porphyrinogenic in animal and in vitro systems (Moore and Mccoll, 1987).

2.15 Ultraviolet (UV) and visible spectroscopy

Ultraviolet and visible absorption bands are due to electronic transitions in the region of 200 - 780nm. In case of organic molecule, the electronic transition could be ascribed to a Π or n electron transition from the ground state to an excited state(δ*, Π*, n*). Since the electron is involved firmly in the construction of a single bond, its transition required much more energy (usually in far UV) than the n- electron (nonbounding electrons) or less tightly bonded Π electrons.

There are four types of absorption bands that occur due to the electronic transition of molecule.

R - Bands: n → n*, in compound with C= O or NO_2 groups Emax < 100

K - Bands: Π → Π*, in conjugated systems Emax > 10,000

B- Bands: (Benzoid bands): due to aromatic and hetero aromatic systems max > 2000

E- Band (Ethylenic bands): In aromatic system

E max 2000 to 14,000 (Allan Misher, 1990).

When monochromatic radiation passes through a cell that contains a solution of an absorbing substance, the effects occurring are reflection at cell surfaces, absorption by the solute and transmission of unabsorbed radiation. The intensity of the total incident light is then given by

I = I reflected + I absorbed +I transmitted ----------(1)

Ireflected can be compensated for by means of a control cell containing solvent only, so that under the experimental conditions the intensity of the incident light can be represented by: I_o.

Where I_o = Iabsorbed + Itransmitted---------- (2) (Williams, 1970).

Beer's law: If incident light with wavelength D and intensity I_o impinges on a solution with concetration and path length, L of 1-cm, the radiation energy of the light is diminished in an exponential fashion. Thus, if a given concentration of a substance absorbs 50% of incident radiation, doubling the concentration will not absorb 100% but rather 75% of the light. The thickness of the sample or path length has a similar effect on the absorption. Mathematically, the radiation concentration and radiation - path length relation can be expressed by

$dI/dc = K_1 I$ and $dI/dc = K_2 I$ ----- ----------(3)

Integration of equation 3 gives

$$\int_{I}^{Io\ dI}/I \quad = -K_1 \int_{o}^{C} dC \text{ and } \int_{o}^{I} dI/I = -K_2 \int dI \quad \text{----------(4)}$$

Evaluation of the integrals between limits combining the two formulae and incorporating the value 2.303 (for transforming the natural log into a log of base 10). In the constant provides the more familar equation used in spectrometry.

$$\text{Log}\left(\text{Io}/I\right) = \text{ECL} \text{----------(5)}$$

Where I_o is the intensity of incident energy, I is the intensity of the emergent energy, C is the concentration, L is the thickness of the absorber (in cm) and E is the molar absorptivity (formerly expressed as molar extinction coefficient) for concentration in mole /L.

If the concentration is expressed in grams/L, absorptivity is designated by "a" instead of.

The term Log Io/I or log I/T is referred to as absorbance A (formerly stated as optical density or entiction). T is transmittance or I/Io. $\sum^{1\%}_{1cm}$ which is encountered less frequently in the literature represent a concentration of 1% w/v and a 1 cm cell thickness and is used primarily in the investigation of those substances of unknown or undetermined molecular weight (usually impure natural products) (Remington's, 1990).

Recently, Giuseppe and co-workers made use of both spectrophotometric and fluorometric method for the first time under non isothermal condition to carry out the study on the hydrolysis of Aspirin and the results obtained are identical to those obtained under the

same conditions but using traditional constant - temperature kinetic runs. This provided the possibility of reducing the amounts of times and chemical usually spent in collecting kinetic data in mechanistic studies in solution by an order of magnitude, (Giuseppe, 1996).

Ngo and Kinget (1996) measured the concentrations of artemisinin according to the method described by Zhao and Zeng, (1985) in which artemsinin is transformed into a UV absorbing compound with an absorbance maximum at 290nm, by heating the solution with sodium hydroxide. The sensitivity was 0.03mg/ml and interference of the sample was corrected with that obtained for pure artemisinin.

Some studies were carried out by Haijian, *et al* (1996), on the relationship between the three crystalline hydrates; the pentahydrate, heptahydrate, and decahydrate of a bivalent metal salt, nedocromil Magnesium, (Drug used in the treatment of reversible obstructive airways diseases, such as asthma). Through their characterization by differential scanning colorimetry(DSC). This was use to determine the differential scanning curves (Haijian *et al*, 1996).

Tominaga (1996), successfully quantify by conventional colorimetry, the amount of ∝-globin mRNA, on the plate within a linear range of 100pg to 10ng. It was stated that the method is more sensitive than conventional blotting and that the method may be applied to the measurements of specific mRNAs in various test materials. (Tominage, 1996).

Before now, theophylline concentrations in serum were measured by HPLC (Jusko and PoliszcZUK, 1976) in most patients and volunteers and by spectrophotometric assay in the pediatric patients (Ellis *et al*, 1976) and in some normal subjects (Koysooko *et al*, 1974). Good agreement was found between the two assay methods. (Jusko and PolisZCZuk, 1976).

2.16 Objective and scope of the study

Chlorpropamide is one of the oral hypoglycaemic agents used to control Type II diabetics. (Arrigoni *et al*, 1972). Chlorpropamide was shown to interact with phenothiazine. It has been proposed that allopurinol and its metabolites compete with chlorpropamidefor renal tubular secretion (Petitpierre, 1972). Concomitant adminstration of chlorpropamide and chloramphenicol was reported to prolong the half-life of chlorpropamide (Petitpierre, 1970). Oral contraceptives have been noted to impair glucose tolerance (Spellacy, 1969) and Dextrothyroxine administration may result in increased glucose concentration due to intrinsic metabolic effect, although an effect on the action of antidiabetic drugs can not be excluded at this time.

Infection is very common in diabetics. Some of the infections proven and suggested to be associated with diabetics are urinary tract infections (especially in female). Mucocutaneous candidiasis, *Gram - negative pneumonia*, Foot-ulcerelate infections, *tuberculosis influence* etc. Fungal infections are also commonly associated with poorly controlled diabetics. (Weatherall, 1987). *Candida* skin and *Staphyloccal* infection are frequently early signs of Type II diabetics (Hancox, 1987). Uncontrolled diabetes significantly alters the efficiency of body defence mechanisms. Granulocyte activity and its phagocytic ability are diminished, and there may be interference of bactericidal action of the blood. Vascular insufficiency and neuropathy contribute to infections in the lower extremities Mucro mycosis and non-clostridia gas infections occur in higher frequency in the diabetic patients, (Howard, 1975).

Infection can also profoundly affect the diabetic patients such that previous control of the diabetes is lost, insulin may become necessary for non-insulin treated patients, and ketoacidosis may be precipitated (Hancox, 1987). Since successful treatment of diabetes is measured by the degree to which the various complications of the disorder are prevented, multiple drug therapy can not be ruled out. Thus, the treatment of infection in diabetes with standard measures should be prompt and thoroughly adequate. (Howard, 1975). However, it will be important to establish the influence of metronidazole that has antiameobic, antibacterial and antifungal activities on the pharmacokinetics of chlorpropamide. Also, most of drug interactions are not only undesirable but can be fatal. It will be good to increase the knowledge in this area of research to prevent unwanted toxicity or interference with therapeutic control.

The present study is to obtain further knowledge of the pharmacokinetic behaviour of the combination effects of metronidazole and chlorpropamide tablets in the blood of the diabetic patients (Type II), following the single dose administration. Since it was noted that some of diabetic patients were given the metronidazole tablets together with the chlorpropamide tablets whenever there is an evidence of infections. The study will also assess the clinical significance of such interactions from the pharmacodynamic and the statistical analysis.

CHAPTER THREE

Materials and Methods

3.1 Materials

3.1.1 Test drugs

Chlorpropamide (Diabinese (R)) tablets.

Lot No.: 62417003.

Date of Manufacturing: October 1996

Expiry Date: October 1999

Manufacturer: PFIZER PRODUCTS PLC. Plot 16, Akanni Doherty Layout, Oregun, Lagos, Nigeria.

Metronidazole Tablets

Manufacturer: National Institute for Pharmaceutical Research and Development, P.M. B. 21, Idu -Abuja, Nigeria.

Batch No.: MZ022

Date of Manufacture: March 1996

Expiry Date: February 1999

3.1.2 Reference compounds

Chlorpropamide standard powder (PFIZER INTERNATIONAL, NEW YORK, U.S.A).

Metronidazole Standard Powder (May and Baker Dagenhyam, England).

3.1.3. Reagents

- Acetone - Analar grade (May and Baker Ltd. England
- Acetic Acid (Glacial) - Analar grade (BDH Chemicals Ltd., Poole, England.
- Methanol- Analar grade (BDH Chemicals Ltd., Poole, England
- Hydrochloric Acid - Analar Grade (BDH Chemicals Ltd, Poole, England.
- Sodium hydroxide pellets - (BDH Chemicals Ltd., Poole England)
- Sodium Nitrate - May and Baker Ltd., Dagenham, England
- Sodium Carbonate anhydrous (GPR, Hopkin and William, Chadwell Health, Essex, England)
- Brilliant green - B.P.C (Imperial Chemical (Pharmaceutical Ltd. Manchester made in England.
- Potassium dihydrogen phosphate.
- Sulphuric Acid - Analar grade (BDH Chemicals Ltd., Poole, England)
- Perchloric Acid - Analar grade (BDH Chemicals Ltd., Poole, England).
- Iso-Amyl alcohol - Analar grade (BDH Chemicals Ltd.,Poole, England).
- Pentyl acetate - Analar grade (BDH Chemicals Ltd.,Poole, England).
- Heparin – Sigma Chemical Co.
- Hexane -Analar grade (BDH Chemicals Ltd., Poole, England.
- 1-Fluoro-2, 4-dinitrobenzene - Analar grade (BDH Chemicals Ltd., Poole, England

- Glucose powder - Analar grade (BDH Chemicals Ltd., Poole, England).
- Phenol - Analar grade (BDH Chemicals Ltd., Poole, England).
- Sodium acetate anhydrous (G.P.R., Hopkin and William, Chedwell, Health, Essex England
- 4-Amino Phenazine - Analar grade (BDH Chemicals Ltd., Poole, England).
- Sodium Chloride - Analar grade (BDH Chemicals Ltd., Poole, England).
- Acetic acid - Analar grade (BDH Chemicals Ltd., Poole, England).

3.1.4 EQUIPMENT

Dissolution Apparatus and Accessories (Erweka Appalau G.M.B.H. 6056, Housenstamm kr. Offenbuch. Main)

KENT pH Metre model 7060 with a combination pH Electrode Model EL 887 (Electronic, Instruments, LTD., Chertsey, Surrey, England).

Centrifuge, ECONOSPIN Model 120010 (SORVAIL instruments, Republic Of Germany, Biotechnology System Division, USA).

MAINS STUART Auto Vortex Mixer (Gallenkamp, England).

Mettler Balance Model AE 240 (Mettler Instruments LTD, Buckinghamshire).

Sp8-100 Ultraviolet Spectrophotometer (PYE- UNICAM LTD, YORK Street, Cambridge, England CBI 2px).

3.2 Methods

3.2.1 Identification of chlorpropamide tablets

1.919g quantity of the powder tablets equivalent to 1g of chlorpropamide was extracted with five quantities each of 4 ml of acetone. Filtered and evaporated carefully to dryness on water bath.

The residue was used to carry out the following tests:

0.1g of the residue was boiled with 8ml of a 50% w/w solution of sulphuric acid under a reflux condenser for 30 minutes, cooled and filtered, the filtrate was reserved. The melting point of the precipitate was taken.

KASHIM IBRA

ii The filtrate reserved above was made alkaline with 5ml sodium hydroxide and heated.

iii 0.1g of the residue was heated with 1g of anhydrous sodium carbonate at a dull red heat for ten minutes, cooled. The residue was extracted with water and filtered. The filtrate was acidified with 2ml nitric acid and silver nitrate solution was added. A white precipitate was produced.

iv U.V. Spectrum of the sample was run together with that of reference sample supplied by PFIZER International.

3.2.1 Chemical assay of chlorpropamide tablets

Twenty chlorpropamide tablets were accurately weighed and the average weight determined. They were powdered and a quantity (0.479g) of the powder equivalent to 250 mg of chlorpropamide was weighed and added to 40ml of methanol in a 50ml volumetric flask, and sonicated for 20 minutes ultrasonic bath. Sufficient methanol was added to produce 50ml, mixed and filtered. 5ml of the filtrate diluted to 100ml with 0.1M hydrochloric acid. 10ml of the resulting solution was diluted to 250 ml with 0.1M HCl acid and mixed, and the absorbance of the resulting solution was measured at the maximum at 232nm. The percentage content of chlorpropamide was calculated taken 598 as the value of A (1%, 1 cm) at 232nm (BP, 1988).

Absorbance

Weight of the x A (1% 1 cm) x Dilution factor drug in gm

$$A\ (1\%, 1cm) = A/CD$$

3.2.2c Dissolution rate tests of chlorpropamide tablets.

One litre of physiological buffer solution, pH 6.8 as the dissolution medium was introduced into the vessel and immersed in a constant temperature water bath maintained at 37 ± 0.5°C. One Chlorpropamide tablet was placed in a dry basket in an assembled dissolution apparatus and the basket was lowered into position. The dissolution apparatus was operated and the basket containing the chlorpropamide tablet was rotated at 100 revolutions per minute. 10ml of the sample was withdrawn at 45 minutes from a point half -way between the surface of the medium and the top of the rotating basket, not less than 10mm from the wall of the vessel. The sample was filtered at 37°C and the amount of active ingredient present in the sample was determined by measuring the absorbance of the diluted sample at 230nm. The total content of chlorpropamide in the medium was calculated by taking 469 as the value of A (1%, cm) at the maximum at 230nm, complete operation was repeated four time (BP, 1988).

Preparation PH 6.8 Buffer.

6.8g of potassium dihydrogen orthophosphate was accurately weighed and dissolved in 1000 ml of water, sufficient distilled water was added to produce 1000ml. It was warmed to 37°C and the pH adjusted to pH 6.8 with 1 M sodium hydroxide (BP, 1988).

3.2.2d Dissolution rate tests of metronidazole tablets.

One litre of 0.1M hydrochloric acid, as the dissolution medium, was introduced into the vessel and immersed in a constant temperature water bath, maintained at 37 ± 0.5°C One

Where A (1%, 1cm) = the absorbance of a 1cm layer of a 1% w/w solution (1g in 100 ml) of the absorbing solute. It is also called the specific absorbance

C= concentration of the absorbing solute expressed as % w/w and

d = the thickness of the absorbing layer in centimetre

3.2.2a Identification test of metronidazole tablets:

A quantity of the powdered tablet (0.311g) containing 0.2g of metronidazole) was shaken with 4 ml of 0.5m sulphuric acid and filtered. 10ml of trinitrophenol solution was added to the filtrate and allowed to stand. The precipitate after washing with water and dried at 105°C, the melting point was determined.

3.2.2b Chemical assay of metronidazole tablets.

Twenty metronidazole tablets were accurately weighed and the average weight determined. They were powdered and a quantity (0.3111g) of the powdered equivalent to 0.2g of metronidazole was weighed and transfered into a sintered - glass crucible and extracted with six 10ml of quantities of hot acetone. Cooled and 50ml of acetic anhydride was added to the combined extracts and 0.1ml of a 1% w/v solution of brilliant green in anhydrous glacial acetic acid and titrated with 0.1M perchloric acid VS to a yellowish - green end-point. The operation was repeated without the powdered tablets. The difference between the titrations represents the amount of perchloric acid required. Each ml of 0.1M perchloric acid VS is equivalent to 0.01712g of $C_6 H_9 N_9 O_3$.

3.2.2c Dissolution rate tests of chlorpropamide tablets.

One litre of physiological buffer solution, pH 6.8 as the dissolution medium was introduced into the vessel and immersed in a constant temperature water bath maintained at 37 ± 0.5°C. One Chlorpropamide tablet was placed in a dry basket in an assembled dissolution apparatus and the basket was lowered into position. The dissolution apparatus was operated and the basket containing the chlorpropamide tablet was rotated at 100 revolutions per minute. 10ml of the sample was withdrawn at 45 minutes from a point half -way between the surface of the medium and the top of the rotating basket, not less than 10mm from the wall of the vessel. The sample was filtered at 37°C and the amount of active ingredient present in the sample was determined by measuring the absorbance of the diluted sample at 230nm. The total content of chlorpropamide in the medium was calculated by taking 469 as the value of A (1%, cm) at the maximum at 230nm, complete operation was repeated four time (BP, 1988).

Preparation PH 6.8 Buffer.

6.8g of potassium dihydrogen orthophosphate was accurately weighed and dissolved in 1000 ml of water, sufficient distilled water was added to produce 1000ml. It was warmed to 37°C and the pH adjusted to pH 6.8 with 1 M sodium hydroxide (BP, 1988).

3.2.2d Dissolution rate tests of metronidazole tablets.

One litre of 0.1M hydrochloric acid, as the dissolution medium, was introduced into the vessel and immersed in a constant temperature water bath, maintained at 37 ± 0.5°C One

metronidazole tablets was placed in a dry basket in an assembled dissolution apparatus and the basket was lowered into position. The dissolution apparatus was operated and the basket containing metronidazole tablet was rotated at 100 revolutions per minute. 10 ml of the sample was withdrawn at 60 minutes from a point half- way between the surface of the medium and top of the rotating basket, not less than 10 mm from the wall of the vessel. The simple was filtered at 37^0C and the amount of ingredient present was determined by measuring the absorbance of the diluted sample at 277nm. The total content was calculated by using the reference standard responses. Complete operations was repeated four times (USPXXII, 1990).

Study design.

Each patient received the following two treatments at two weeks intervals using cross over design. (i) One 250mg Chlorpropamide tablet administered in a fasting state; (ii) One 250 mg chlorpropamide tablet with two 200 mg Metronidazole tablets concomitantly administered orally also in a fasting state. 200ml of water are taken with each treatment. The protocol of the study was explained to each of the patients and an informed consent was obtained. No drugs including alcohol was allowed one month before the study and each patient fasted overnight before taking part. On the day of each test, prior to oral ingestion of the 250mg Chlorpropamide tablet, each subject attended the trial room and was allowed 5 minutes relaxation on a couch. A cannula was placed in an antecubital vein and 5 ml of blood was taken in a heparinized tube prior to dosing.

For the two treatments, the tablet(s) was/were swallowed on an empty stomach at 8.00

a.m. and the patient fasted for the next 4 hours after drug administration. The patient was then given cooked "Jollof" beans (350gm) before further samples. Further samples were at times 0.5, 1, 2, 4, 6, 8, 24, 48, 72, 144 and 168 hours. The blood samples collected were centrifuged at 2000rpm for 10 minutes. Using Pasteur pipette, the plasma was transferred into bijoux bottle, stoppered and stored in the freezer at - 20^{0}C until analysed. Their glucose levels were assessed at 0, 0.5, 1, 2, 4, 6, and 8hour.

Subject

Six type II diabetic patients (3 males and 3 females) freshly diagnosed, participated in the study. The diagnostic criteria were based on the National Diabetes Data Group's recommendation in 1979. Their age ranged from 28 - 53 years, mean ± SD 39 ± 8.20, height ranged from 1.54 to 1.88m, mean ± SD 1.68± 0.13, and their weight range was 52 to 70kg mean ± SD 60.83 ± 7.73.

3.2.3 Drug levels analysis.

Principle: The chlorpropamide drug was extracted from acidified plasma with pentyl acetate. At an elevated temperature the drug degraded to amine derivative and reacted with Fluoro dinitrobenzene. The resulting coloured complex was measured spectrophotometrically.

Procedure: 0.5ml plasma was added 1ml of phosphate buffer and 10ml of 0.5 per cent isoamyl alcohol in hexane. After mixing thoroughly for 5 minutes, it was centrifuged at 2000g for 10minutes for phase separation, 7ml of the hexane layer was then added to lml

of sodium hydroxide in another test tube, mixed and centrifuged at 2000g for 5 minutes. The hexane layer was then discarded and 1ml of 2N hydrochloric acid and 3mlpentyl acetate was added to the basic layer. This was mixed for 2 minutes and centrifuged at 2000g for 5minutes. 2ml of the pentyl acetate layer was then pipetted into another test tube containing 1ml of 0.05 percent fluoro dinitrobenzene in pentylacetate, stoppered lightly and heated for 1 hour at 125°C on a constant temperature oil bath. After cooling, the absorbance was read at 420nm against pentyl acetate (Matin and Rowland, 1973). Calculation based on the response factor derived from a standard curve.

Preparation of standard solutions

Stock solutions of chlorpropamide were prepared by dissolving 10mg of the reference sample in 10ml volumetric flask with methanol. Working solutions of appropriate concentrations were prepared every week by diluting the stock solution with 0.02mol/ml hydrochloric acid to make concentrations 5, 10, 25, 40, and 50 μg/ml of the chlorpropamide standard solution. Plasma calibration curve was prepared by spiking blank (0.5ml) plasma samples with 1ml of the above chlorpropamide standard solutions.

Precision

The precision of the assay was determined by analysing drug - free human plasma spiked with known amounts of chlorpropamide, the coefficient of variation (CV) at three different concentrations of plasma for five samples at each level were studied (within - assay and day to day assay).

3.2.4 Plasma glucose analysis

Principle:

Glucose oxidase is a specific enzyme that promotes the oxidation of glucose to acid with the production of an equivalent amount of hydrogen peroxide. In the presence of a second enzyme - peroxidase, oxygen from peroxide can be transferred to a suitable acceptor with the production of colour complex compound. The intensity of which is proportional to the concentration of glucose in sample (Martinek, 1964).

Glucose

Glucose oxidase Gluconolactone $H_2O \rightarrow O_2$ Gluconic acid + $2H_2O$.

H_2O_2 + Oxygen acceptor peroxidase colour complex + H_2O

Fig.7: Summary of the enzyme methods for glucose estimation.

Reagents and Preparation

(1) Phenol: 21.25mol/ml phenol in 0.15M sodium chloride solution (1.94g colourless phenol crystals in 1 litre of 0.9% sodium chloride).

(2) Colour Reagent

15ml Rercozyme (glucose oxidase), 15mg peroxidase, 300mg of 4- amino phenazone were mixed and made up to volume with sodium acetate acetic acid buffer (12.88g of sodium acetate anhydrous dissolved in 200ml distilled water, 320ml of glacial acetic acid was added) in 1000ml volumetric flask. Solution was stable for 8 weeks at 4C.

(3) Stock glucose solution:

18.02g glucose dissolved in 1- Litre of benzoic acid saturated with distilled water.

(4) Standard glucose working solution.

1.0ml of stock glucose solution diluted to 10ml with benzoic acid benzoic acid saturated with distilled water.

Procedure

Three tubes were labelled as test, standard and blank. The phenol reagent, plasma, standard glucose, distilled water and colour reagent was mixed as shown in the table below.

Reagent	Test (ml)	Standard (ml)	Blank (ml)
Phenol reagent	2.50	2.50	2.50
Plasma	0.05	-	-
Standard (10mmol/l)	-	0.05	-
Distilled water	-	-	0.05
Colour reagent	2.50	2.50	2.50

The tubes were incubated at 37°C for 20 minutes and cooled at room temperature for 45 min. The absorbance was measured at 520nm using agent blank. The amount of glucose in the sample was calculated using the following formulae.

$$\text{Glucose mmol/L} = \frac{\text{Absorbance of test - Blank}}{\text{Absorbance of standard -Blank}} \times \frac{10}{1}$$

3.2.6 Data analysis

After the single oral dose of chlorpropamide, the pharmacokinetic parameters were

calculated using the semi-logarithmic plots of concentration - time data for chlorpropamide. The area under the curve was calculated by trapezoidal rule (Rowland and Tozer, 1980). However, for the calculation of the $AUC_{0\rightarrow\infty}$ the last segment ($AUC_{168h\rightarrow\infty}$) was calculated by dividing the last blood concentration in the blood level - time plot, by the elimination rate constant of the slow phase (β). Therefore, $AUC_{0\rightarrow\infty}$was given by:

$$AUC_{0\rightarrow\infty} = AUC_{0\rightarrow 168h} + AUC_{168h\rightarrow\infty} \quad \text{----------(a)}$$

$$\text{Volume of distribution (Vd)} = F.D/AUC.\ k_e \text{----------(b)}$$

Where F is fraction of dose absorbed (assumed to be 1), k_e is rate of elimination and AUC is area under the curve.

$$\text{Clearance} = k_e.\ Vd \quad \text{----------(c)}$$

$$\text{Rate of absorption } (k_a) = 0.693 / t_{1/2}\alpha \text{----------(d)}$$

$$\text{Rate of elimination } (k_e) = 0.693 / t_{1/2}\beta \text{----------(e)}$$

Results are expressed as Mean±S.D. They were analysed for statistical significance by student t- test for paired data. All statistical analysis was carried out with '' PRISM Graph pad '' computer programm.

CHAPTER FOUR

4.0 RESULTS.

4.1.1 Assay of chlorpropamide tablets.

4.1.1a Identification test of chlorpropamide tablets

The melting point of recrystallized product of the extracted chlorpropamide was 143°c.

The filtrate obtained was treated with 5M NaOH solution produced an ammoniacal odour.

The extracted drug heated with anhydrous sodium carbonate and treated with $AgNO_3$ produced white precipitate. The solution of the sample obtained in the assay exhibited absorption at 232nm (Figure 7, PP. 66).

4.1.1b Chemical of chlorpropamide tablets

The chlorpropamide tablets contained 99.25 percent of the active ingredient using A (1%, 1cm) 598 value at 232nm (BP, 1988). Thus sample complied with the BP specification of drug content (92.5 - 107.5)%. The drug content values and the percentage composition are shown in Table 1a (PP. 67).

4.1.1c Dissolution Rate test of chlorpropamide tablets

The percentage of the active ingredient released in solution during the dissolution

test are shown in Table 1b (PP. 67). The mean amount released was 78.93±0.112%. The result complied with the official BP, (1988) requirement of not less than 70% in 45 minutes.

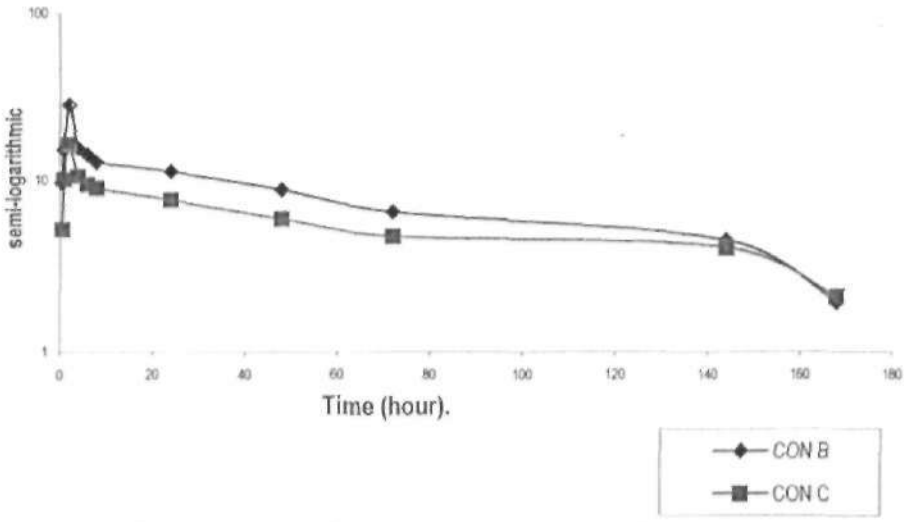


Figure 6b. Mean semi-logarithmic plots of concentration - time data for Chlorpropamide in six diabetic patients following oral adminstration of 250mg Chlorpropamide tablet (B) and concomitantly with two 200mg Metronidazole tablets (C).

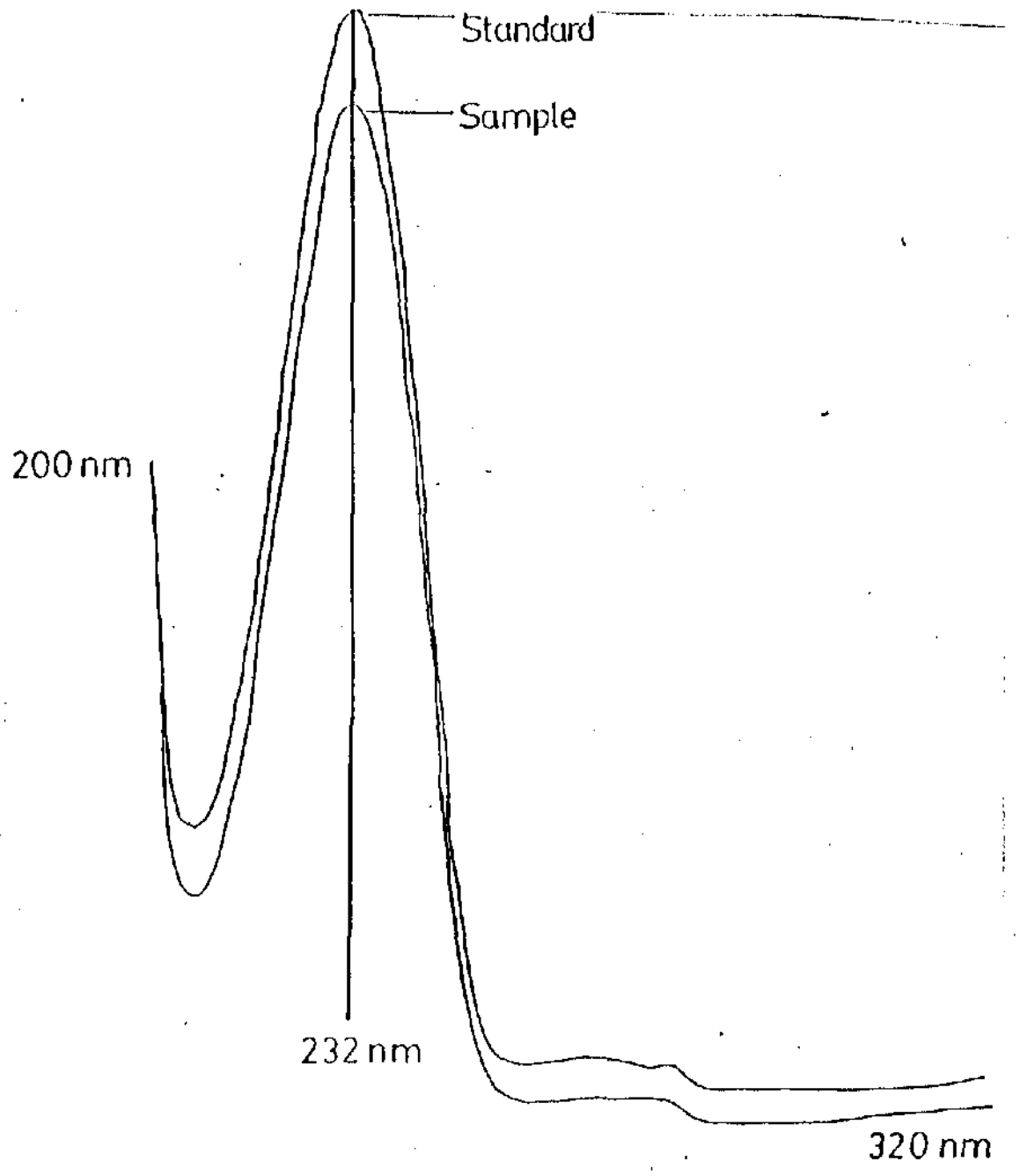


Figure 7. Absorbption spectra for chlorpropamide standard powder and tablet.

Table 1a: Assay of chlorpropamide tablets

Quantitative determination of chlorpropamide tablets using BP (1988) method.

Chlorpropamide powder	Drug content (mg)	% Content
Sample 1	247.50	99.00
2	246.25	98.50
3	249.30	99.70
4	249.50	98.80
Mean ± SD	248.14 ±1.55	99.00±0.51

Table 1b Dissolution of chlorpropamide Tablets in phosphate buffer pH 6.8 maintained at 37±0.50°C using USPXVIII Rotating basket method with a stirring rate of 100r.p.m

Number of Tablets	Percentage Dissolved
1	79.06
2	78.80
3	78.98
4	78.93
Mean ± SD	78.94 ± 0.11

SD = Standard Deviation

4.2.2 Assay of metronidazole tablets.

4.2.2a Identification of metronidazole tablets.

The melting point of crystallized product of the extracted metronidazole was 150°C.

4.2.2b Chemical Assay of Metronidazole tablets

The metronidazole tablets and reference sample contained 99.5 and 100 percent of the active ingredients respectively. Thus the sample and the reference standard complied with the BP, (1988) specification of drug content (95.0 - 105.0)%. The content values and the percentage composition are shown in Table 2a (PP. 69).

4.2.2c Dissolution Rate Tests of Metronidazole tablets

The percentage of the active ingredient released in solution during the dissolution test are shown in Table 2b (PP. 69). The mean amount released was 87.35 ±0.466 percent. The result complied with the official U.S.P.XXII, (1990) requirement of not less than 85% in 60 minutes.

Table 2a: Assay of metronidazole tablet and standard

Quantitative determination of metronidazole tablets and standard using BP (1988) method

Metronidazole	Drug content (mg)	% Content
Standard powder	200.00	100.00
Tablet powder 1	196.30	98.44
Tablet powder 2	200.30	100.10
Tablet powder 3	200.30	100.10
Mean ± SD	198.86 ± 2.48	99.55 ± 0.96

Table 2b: Dissolution of metronidazole tablets in 0.1M hydrochloric acid maintained at 37 ±0.5^{0}C using USP XVIII rating basket method with stirring rate of 100 rpm

Number of tablets	Percentage dissolved
1	87.88
2	86.86
3	87.58
4	87.07
Mean ± SD	87.35 ± 0.47

SD= Standard Deviation

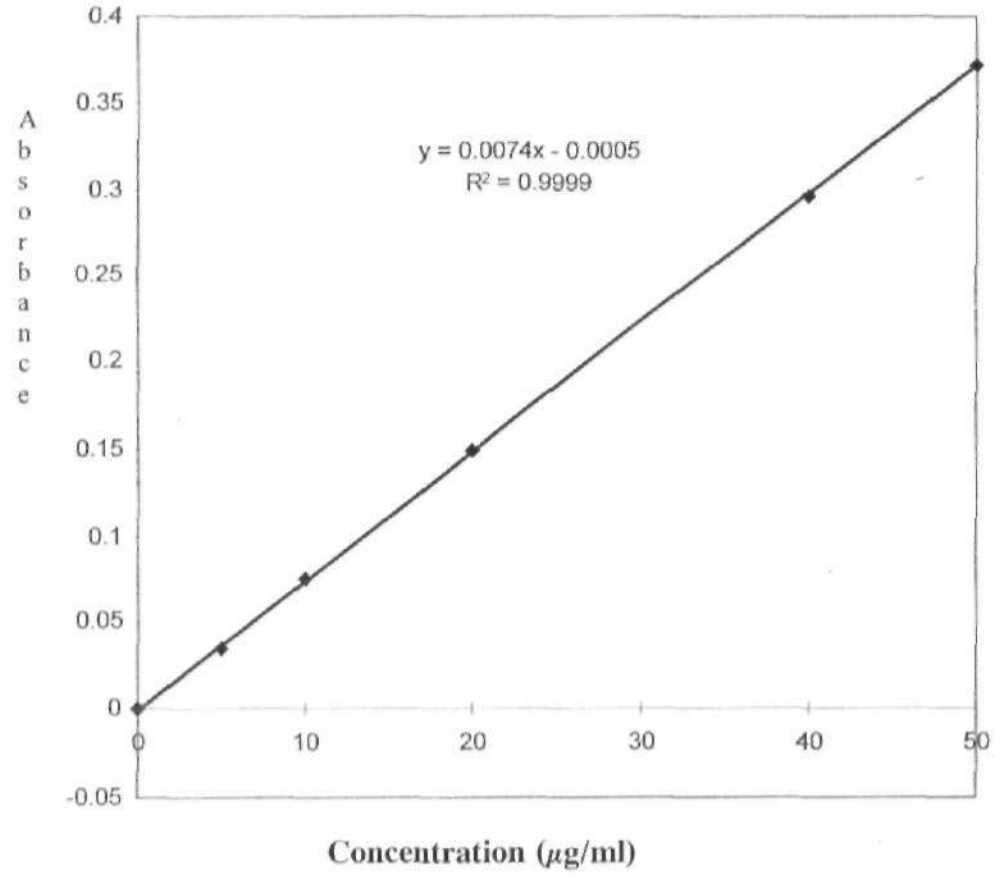


Figure 8. Standard curve for the assay of chlorpropamide, obtained from spiked plasma.

Table 3a: Mean (±SD) plasma concentration of chlorpropamide in six diabetic patients following oral administration of 250mg chlorpropamide tablet (B) and concomitantly with two 200mg metronidazole tablets (C).

Time (h)	Concentration (μg/ml) ± SD	
	B	C
0.5	9.7 ± 1.8	5.08 ±0.56
1	15.25±2.32	10.13±1.46
2	28.44±2.32	16.43±4.32
4	15.63±3.01	10.54 ±0.93
6	14.29± 2.80	9.43± 1.01
8	12.84± 2.32	8.95±1.06
24	11.24± 2.13	7.56±1.97
48	8.75 ±2.25	5.86±2.13
72	6.46±0.90	4.63±1.26
144	4.38±0.20	3.98±1.51
168	1.95±0.49	2.09±0.98

SD= Standard Deviation

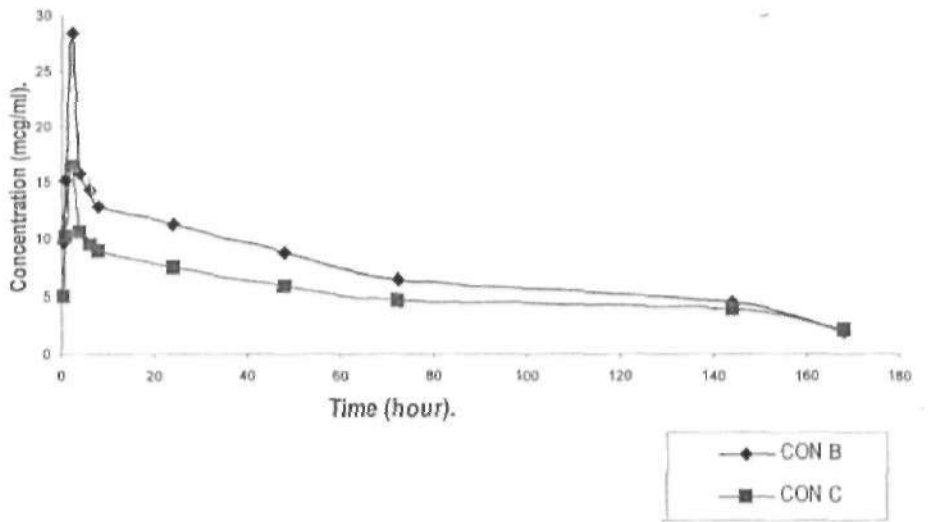


Figure 9. Mean plasma concentration of Chlorpropamide in six diabetic patients following oral adminstration of 250mg Chlorpropamide tablet (B) and concomitantly with two 200mg Metronidazole tablets (C).

Table 3b: Mean (± SD). Pharmacokinetic parameters derived from plasma concentration of chlorpropamide in six diabetic patients following oral administration of 250mg chlorpropamide (B) and concomitantly with two 200mg metronidazole tablets (C).

Mean parameter ± SD	B	C	T - Test (paired)
Lag-time(h)	0.23± 0.08	0.23±08	NS
Absorption half-life $t_{½} \propto$ (h)	0.52±0.20	0.73±0.22	P<0.05
Absorption rate constant k_{ab} (h^{-1})	1.54±0.67	1.06±0.40	P<0.05
Maximum concentration (Cmax) (μg/ml)	28.75±0.40	15.53±0.59	P<0.05
Time to peak concentration (tmax) (h)	2.54±0.40	2.40±0.20	NS
Area under the curve (AUC) $AUC_{o\rightarrow 168h}$ (μg/ml* h)	1208.59±149.32	875.45±258.66	P<0.05
$AUC_{o\rightarrow\infty}$(μg/ml* h)	1336.33±105.39	1043.96±337.19	P<0.05
Volume of distribution Vd(L)	13.37±3.85	22.78±0.13	P<0.05
Clearance CL (L/h)	0.21±0.027	0.32±0.13	NS
Elimination half-life t½β(h)	43.17±6.05	51.50±16.47	NS
Elimination rate constant ke(h^{-1})	0.016±0.003	0.015±0.005	NS

SD = Standard Deviation
NS= Not significant at 95% confidence level (P>0.05)

Table 4a: **Mean (±SD) Plasma Glucose Concentrations in Six Diabetic Patients Following Oral Administration Of 250mg Chlorpropamide Tablet (B) And Concomitantly With Two 200mg Metronidazole Tablets (C).**

Time (h)	Plasma Glucose B	Mmol/ml (±SD) C	T - Test (paired)
0	19.72±4.51	20.00±6.36	NS
0.5	17.35±3.03	18.15±5.74	NS
1	17.20±3.96	17.38±7.21	NS
2	16.55±2.53	17.05±6.12	NS
4	14.38±3.16	14.95±5.21	NS
6	16.43±5.02	16.73±4.65	NS
8	18.40±5.00	19.78±3.8	NS

SD= Standard Deviation

NS = Not significant At 95% confidence, level ($P>0.05$)

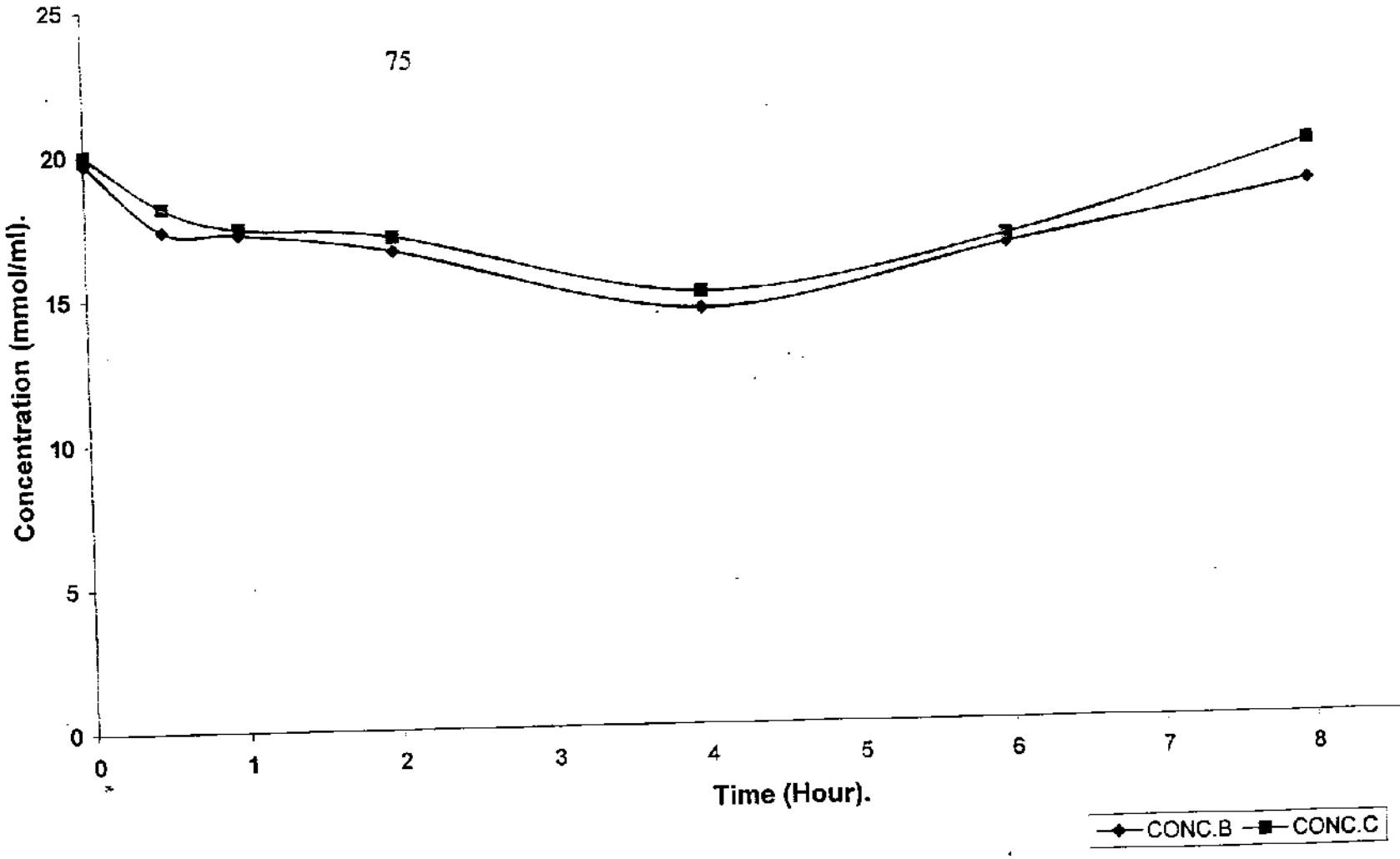


Figure 10. Mean glucose concentration in six diabetic patients following oral administration of 250mg chlorpropamide tablet (B) and concomitantly with two 200mg metronidazole tablets (C).

4.3 Drug levels analysis.

Effects of metronidazole tablet on chlorpropamide pharmacokinetic parameters and glucose levels.

The results of the effects of each mode of treatment on the plasma glucose levels in Diabetic patients is shown in Figure 10 (PP. 75) and Table 4a (PP. 74). Following administration of a single 250mg Chlorpropamide tablet and concomitantly with two 200mg Metronidazole tablets, the glucose level decreased and fell below the preprandial levels in the two cases. This occurred in the 4th hour and the glucose level further increases but had not reached the preprandial value at the 8th hour. The plasma glucose were not significantly affected by the metronidazole tablets at any sampling points.

Following oral administration of 250mg chlorpropamide and concomitantly with two 200mg metronidazole tablets, volume of distribution (Vd), Cmax, Vd, $t_{½}^{\alpha}$, ka, and AUC_{0-168h} were significantly altered. The tmax and the parameters of elimination calculated were not significantly affected. The different pharmacokinetic parameters are shown in Table 3a (PP.71) and plasma concentration time data are graphically presented in Figure 9 (PP.72). The statistical observation B and C when are paired, are also shown in Tables 3b (PP. 73) and 4a (PP.74).

Table 4b: Within - day and day to day precision of plasma chlorpropamide determination.

Biological fluid Plasma	Concentration (μg/ml)	%coefficient of variance(CV)	N
Day to Day	5	4.40	5
	10	2.25	5
	20	1.50	5
Within - run	5	4.60	5
	10	2.40	5
	20	1.99	5

CHAPTER FIVE

5.0 DISCUSION

5.2 Quality control of test drugs

5.2.1 Quality Control of Chlorpropamide Tablets

The melting point of the recrystallized product that was 143°C is similar to the BP value of 142°C. The ammoniacal odour produced by the filtrate of the recrystallize product, and the white precipitate produced by the filtrate on adding NaOH are in agreement with the BP (1988) specifications on identification of chlorpropamide. The 232nm, absorption maximum exhibited by the solution of the sample also agrees with the BP (1988), specification 232nm. Thus the tablets complied with the identification tests described for chlorpropamide in the BP (1988).

The percentage of the active ingredient in the tablets (99.25%) indicated that the batch of chlorpropamide tablets used in this study complied with the official BP (1988) specification of drug content (92.5-107.5%). The average amount of the active ingredient in solution (78.93±0.112%) indicated that the batch of chlorpropamide tablets used in this study meets the official BP (1988) requirement for dissolution rate test (not less than 70%) in 45 minutes.

5.2.2 Quality Control of Metronidazole

The melting point of the recrystallized product which was 151°C is close to the BP (1988) value 150 °C. Thus the tablets compiled with the identification tests described for metronidazole in the BP (1988).

The percentage of the active ingredient in the tablets (99-55%) and 100% of the standard indicated that the batch of metronidazole tablets and standard used for this study complied with the official BP (1988) specification of drug content (95.0-105.0%. The average amount of the active ingredient in solution (87.33±0.3466) indicated that the batch of metronidazole tablets used for this study complied with official specification in U.S.P. XXII (1990) for dissolution rate not less than 85%.

5.2.3 Dissolution Rate Tests.

The result of dissolution tests for both Chlorpropamide and Metronidazole was in accordance with the official specifications. For this study all batches of the drugs used passed the dissolution rate test. Therefore a strong correlation between dissolution of the drug and bioavailability of the drug is expected.

5.3 Analysis of pharmacokinetics parameters

From the pharmacokinetics parameter obtained (Table 3b), there were significant decreased in the Cmax, k_a, and $AUC_{0\rightarrow\alpha}$, also significant increased in $t_{1/2\alpha}$, and VD . The decrease in Cmax for concomitant administration © is proportional to the

decrease in AUC in comparison to the drug level data from the control samples (B).

The difference in the AUC can be explained by considering the fact that tmax is dependent on K_a while AUC is proportional to the amount or extent of drug absorption. The lower the total amount of drug absorbed, the lower is the bioavailability (Abdel - Monem and Henkel, 1978). There is a limit to how slow absorption may be without reducing the bioavailability. In some pharmacokinetics parameters observed, significant reduction could be due to physiological or direct chemical interaction between the two drugs.

The nature of drug-drug and drug-metabolite interactions is a factor to be considered in case of bioavailability differences. Such interaction can be physiological or chemical. In the case of physiological interaction drug protein binding is a common culprit. Chlorpropamide has high affinity for plasma protein (>90%) whereas metronidazole has relatively low affinity (10%) (Shargel, and Andrew 1992). However, the displacement effect is not likely to occure in this case, since the test drugs has different levels of affinity for plasma protein - binding therefore, there might not be much competition for the protein -binding site, and thus no case of displacement. Also the pharmacokinetic parameters obtained from this study shows that there was an increased in volume of distribution (VD) in concomitant administration of chlorpropamide with metronidazole tablets. As discussed in the literature (Shargel and Andrew 1992), "a high degree of tissue binding results in lower plasma drug levels and larger apparent volume of distribution".

In term of clinical interaction, since the blood levels of metabolites of chlorpropamide was not monitored, one can not categorically state that metronidazole may have induced faster breakdown of chlorpropamide in the blood, leading to lower plasma concentration. However, if there was such induction of metabolism the elimination rate (ke) and biological half- life ($t_{1/2}$) of chlorpropamide will be significantly different from those of concomitant administration of chlorpropamide and metronidazole. However, this was not the case, it is possible that the decrease in bioavailability was induced by the effect of metronidazole on the absorption phase of chlorpropamide. This is due to the fact that both ka, $t_{1/2}$ α, were significantly different for chlorpropamide alone and concomitant administration of chlorpropamide and metronidazole. Therefore, metronidazole may competitively delay the absorption of chlorpropamide at any of the processes involved in drug absorption.

5.3.3 Clinical significance

In this study, there was a slight increase in the blood glucose level at each sampling time under the concomitant administration of metronidazole and chlorpropamide (C), when compared with the control situation (B), however, this observation was not statistically significant. The therapeutic effect of chlorpropamide when administered alone manifested as a 16.99% decrease in fasting glucose level while that of concomitant administration with metronidazole manifested as a 14.21%

decrease. Thus, there is only a 2.78% increase in blood glucose levels, between the control and test studies. This implies that the 38% difference in relative bioavailability as well as the 46% decrease in Cmax resulted in 4.8% increase in blood glucose levels between pre and post prandial sampling in each study case (B or C). However the postprandial increment was below preprandial levels.

Despite this absence of statistical or clinical significance, there was a tendency towards an increased post prandial plasma glucose levels. This effect according to Sartor, *et al* (1974) may be due to a stronger action of the drug on insulin release or a more optimal timing between induction of insulin release. The blood glucose does not evoked increment of β-sugar level by the reduction of chlorpropamide level in the blood. The implication of this is that since there were statistically significant differences in Cmax, AUC, VD, k_a and $t_{1/2}$ without a corresponding significant decrease in clinical effect, the effect of metronidazole may not have been sufficient to affect the therapeutic efficacy of chlorpropamide. Also, the use of blood glucose level as a pharmacodynamic monitor may be inappropriate in this situation and this may necessitate the need for a surrogate effect such as frequency of micturition, hunger threshold and thirst to be monitored. The individual variability drug responsiveness can result from genetic differences in rates of drug metabolism or difference in the pharmacodynamic that is the relationship between the drug and the receptor (due to differences in receptor sensitivity to the drug).

Several factors could explain observed significant alteration in both pharmacodynamic and pharmacokinetics parameters. The difference in pharmacokinetics parameters obtained could be due to the effect of age on the gastrointestinal tract (GI tract), most of the diabetic patient that participated in the study had their age within the upper limit of the used age ranged. According to Sklar (1978), the GI tract is the most common site of distress in the aged. Considering the individual variations in the calculated individual pharmacokinetics parameters (see appendix b) there were differences as well as similarities. For example the K_e and Lag-time were similar whereas $t_{½}$ β, VD and AUC were different. However, the parameters derived for each subject were within the range of previously reported values. (Taylor, 1972; Sartor *et al*, 1980).

CONCLUSIONS / FURTHER RESEARCH WORK

All the drugs used in this study met the offial requirement for identification, assay and dissolution rate tests. Significant differences were observed in the absorption phase parameters ($t_{1/2\alpha}$, ka, Vd, and AUC) when the patients were given chlorpropamide alone compared to when chlorpropamide and metronidazole were concomitantly administered. The blood glucose levels were lower for chlorpropamide alone treatment compared to chlorpropamide and metronidazole were concomitantly administered.

In view of the above observations, this study could be extended to ascertain which of the absorption processes in the gastrointestinal absorption of chlorpropamide has been affected by the presence of metronidazole, and possibly, through which mechanism. It may also be necessary to monitor a secondary effect of the lowering of blood glucose by chlorpropamide, so as to get a better picture of the therapeutic significance of the differences in bioavailability that occurred on concomitant administration of chlorpropamide and metronidazole. Thus, steady state study can be used rather than the single dose study.

Lastly, there may be need to extend the study to sampling other biological fluids like saliva, in a comparative analysis of the pharmacokinetics of chlorpropamide and metronidazole, with a view to developing an equally sensitive assay method for these drugs in saliva. If the measurement of systemic availability and valid pharmacokinetics profile

of these drugs can be achieved by sampling saliva, than it eliminates the discomfort, possible hazard and necessary attendance of medical staff required for repeated venepuntures.

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APPENDICES

APPENDIX 1a

Chemical assay of chlorpropamide tablets

Chlorpropamide	1	2	3	4
Absorbance (232nm)	0.592	0.589	0.596	0.597

46998
RM
666-ME

APPENDIX 1b

Chemical assay of metronidazole tablets

Metronidazole	1	2	3
Volume of perchloric acid used	11.50	11.70	11.70

0.1M 1ml of perchloric acid = 0.01712g of $C_6H_9N_3O_3$

APPENDIX 1c

Dissolution test of chlorpropamide tables in phosphate buffer pH6.8 Maintained at 37± 50c using USP XVIII rotating basket method. Absorbance measure at 232nm

No. of tablet	1	2	3	4
Absorbance	0.925	0.924	0.926	0.927
Time (min)	45	45	45	45

APPENDIX 1d

Dissolution test of metronidazole tablets in 0.1M hydrochloric acid maintained at 37 ± 0.5^{0}C using USP XVIII rotating basket method stirring rate of 100 r.p.m

No. of Tablet	1	2	3	4
Absorbance of sample	0.863	0.853	0.860	0.855
Absorbance of standard	0.982	0.982	0.982	0.982
Time (min)	60	60	60	60

APPENDIX 2a

Plasma concentration of chlorpropamide in six diabetic patient following oral administration of 250 mg chlorpropamide tablet and concomitantly with two 200mg metronidazole tablets

CONCENTRATION (μg/ml)

Patients	I		II		III		IV		V		VI	
Time(h)	B	C	B	C	B	C	B	C	B	C	B	C
0.5	8.00	5.25	8.50	4.50	12.50	4.50	10.00	5.00	11.00	6.00	8.20	5.25
1	11.50	8.50	15.50	8.50	18.50	10.00	14.50	12.00	16.50	11.50	15.50	10.75
2	24.50	15.00	29.30	14.80	30.25	15.75	28.50	15.75	30.10	15.75	28.00	14.50
4	13.00	10.00	12.00	10.00	20.00	11.50	15.00	11.00	18.00	11.50	15.75	11.00
6	12.50	9.500	11.00	8.40	18.00	11.00	14.50	8.75	16.25	10.00	14.50	9.25
8	11.5	8.50	8.80	7.20	15.00	10.00	13.50	9.25	14.50	10.00	13.75	8.75
24	10.00	4.50	7.70	6.30	13.50	10.00	11.50	7.50	13.00	9.00	11.75	8.20
48	5.50	2.50	7.00	4.90	11.50	8.50	8.75	5.25	10.75	7.50	9.00	6.50
72	5.25	1.50	6.00	4.30	6.00	6.50	6.50	4.50	7.50	6.00	7.50	5.00
144	4.50	1.25	4.30	3.60	4.00	5.75	4.50	4.25	4.00	4.50	4.50	4.50
168	2.50	1.00	2.50	1.30	1.25	2.25	1.75	1.50	1.75	3.25	2.00	3.40

APPENDIX 2b

Pharmacokinetic parameters of chlorpropamide in plasma of individual diabetic patients following the oral administration of 250mg chloropropamide tablet and concomitantly with two 200mg metronidazole tablet.

Patient	No. 1		II		III		IV		V		VI	
Parameters	B	C	B	C	B	C	B	C	B	C	B	C
Lag-time(h)	0.20	0.25	0.10	0.10	0.25	0.20	0.20	0.20	0.30	0.30	0.20	0.30
T½∝(h)	0.30	0.40	0.72	0.85	070.	0.90	0.70	0.90	0.30	0.50	0.40	0.80
Ka (h-1)	2.31	1.73	0.92	0.82	0.99	0.77	0.99	0.77	2.31	1.39	1.73	0.87
(max(mcg/ml)	25.00	15.00	29.00	15.00	30.50	16.00	28.50	16.00	30.5	16.20	28.50	15.00
Tmax(h)	3.00	2.50	2.00	2.00	2.25	2.50	3.00	2.50	2.50	2.50	2.50	2.50
AUC_{0-168h}												
(mcg/h/ml)	1033.88	441	1016.05	775.43	1341.62	1173.75	1228.12	833.86	1353.03	1071.25	1278.85	957.41
$AUC_{0-\infty}$ (mcg/h/ml)	1212.45	499.82	1208.30	868.28	1404.16	1276.02	1331.06	938.04	1455.97	1351.42	1405.43	1332.18
Vd(L)	17.241	33.35	18.93	23.03	9.41	9.68	11.97	20.82	10.38	20.12	12.37	29.67
Cl(h)	0.24	0.567	0.246	0.322	0.186	0.123	0.204	0.299	0.185	0.233	0.197	0.261
Ke(h^{-1})	0.014	0.017	0.013	0.014	0.019	0.022	0.017	0.014	0.018	0.012	0.016	0.009
T½ β(h)	50.00	40.00	50.00	50.00	35.00	32.00	41.00	48.00	39.00	60.00	44.00	79.00

APPENDIX 3a

Plasma glucose levels (mmol/l) in six diabetic patients following oral administration of 250mg chlorpropamide tablet (b) and concomitantly with two 200mg metronidazole tablets (c)

Plasma Glucose mmol/l)

Patients	I		II		III		IV		V		VI	
Time(h)	B	C	B	C	B	C	B	C	B	C	B	C
0	15.6	17.6	24.9	30.2	19.5	19.0	16.8	12.5	25.6	24.5	15.9	1
0.5	12.9	16.6	22.0	28.8	18.0	18.5	15.6	11.8	18.3	18.0	17.3	1
1	14.1	15.9	22.4	28.3	13.9	18.0	15.1	7.5	22.1	21.5	15.6	1
2	14.4	15.6	16.1	23.4	15.3	12.6	12.7	9.8	19.8	19.0	12.7	1
4	10.7	13.7	15.1	23.4	15.3	12.6	12.7	9.8	19.0	19.0	12.7	1
6	14.6	16.8	22.4	24.4	17.3	16.2	13.4	12.0	21.6	20.0	9.3	1
8	16.6	21.7	23.9	25.1	16.8	16.5	18.3	16.4	24.0	22.5	10.8	1

APPENDIX 4a

Chlorpropamide standard curve obtained from spiked plasma

Concentration (mcg/ml)	0	5	10	20	40	50
Absorbance	0.000	0.035	0.073	0.149	0.296	0.372