# THE EFFECTS OF CHLOROQUINE, PARACETAMOL AND PROMETHAZINE ON THE PHARMACOKINETICS OF CHLORPROPAMIDE IN HUMAN VOLUNTEERS

*BY*

**UZAMA DANLAMI**

**MARCH 2008**

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**BY**

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**A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL CHEMISTRY**


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


**MARCH 2008**

## DECLARATION

I declare that the work in the dissertation entitled 'The Effects of chloroquine, paracetamol and promethazine on the pharmacokinetics of chlorpropamide in human volunteers' has been performed by me in the Department of pharmaceutical and medicinal chemistry under the supervision of **DR (MRS) MOJIRADE TAIBAT BAKARE – ODUNOLA and PROF. MAGAJI GARBA** both of the **Department of pharmaceutical and medicinal chemistry, A.B.U, Zaria, Nigeria.** The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

| _____ | _____ | _____ |
|---|---|---|
| Uzama Danlami | Signature | Date |

## CERTIFICATION

This dissertation entitled the effects of chloroquine, paracetamol and Promethazine on the pharmacokinetics of chlorpropamide in human volunteers by **UZAMA DANLAMI**, meets the regulation governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literally presentation.

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## DEDICATION

Dedicated to my dear parents, lovely wife and beloved children

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## PUBLICATIONS

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D. Uzama, M.T. Bakare – Odunola and M. Garba (2008). Comparative Study on the Precision of high Performance Liquid Chromatography (HPLC) and Spectrophotometry in the Determination of Chlorpropamide Contents in Diabinese Tablets, Biological and Environmental Science Journal for the Tropics vol. 5, no. 1, pp 132

D. Uzama, M.T. Bakare – Odunola, and M. Garba (2008). Assessment of Chemical Quality of some Brands of Chlorpropamide Tablets Obtained from Pharmacy Shops in Katsina, Nigeria, Biological and Environmental Science Journal for the Tropics vol. 5, no. 1, pp 138.

## ABSTRACT

The effect of chloroquine, paracetamol and Promethazine on the pharmacokinetic profile of chlorpropamide was investigated in human subjects.

The batches of chlorpropamide, chloroquine, paracetamol and Promethazine used were subjected to quality control studies using official methods. The drugs complied with the B.P (1993) requirements stated in their monographs.

A high performance liquid chromatography (HPLC) method was developed for the analysis of plasma chlorpropamide concentration. Pharmacokinetic parameters of chlorpropamide were derived with Winnonlin standard non-compartment software programme.

About 16%, 14% and 15% decrease in the mean maximum concentration ($C_{max}$) of chlorpropamide was observed following oral administration of chlorpropamide with chloroquine, paracetamol and Promethazine respectively.

About 44%, 11% and 30% decrease in the area under curve (*AUC*) of chlorpropamide was observed following concurrent administration of chlorpropamide with chloroquine, paracetamol and Promethazine respectively. The plasma clearance of chlorpropamide increased by 86% following chlorpropamide interaction with chloroquine.

The absorption half-life ($t_{½a}$) of chlorpropamide increased by 4%, 85% and 69% when concomitantly administered with chloroquine, paracetamol and Promethazine respectively.

The elimination constant ($K_{el}$) of chlorpropamide was found to have increased by 100% and decreased by 31% following concomitant administration of chlorpropamide with chloroquine and paracetamol respectively. The elimination half-life ($t_{½el}$) of chlorpropamide was found to have decreased by 47% and increased by 13% and 29% following concomitant administration to chlorpropamide with chloroquine, paracetamol and Promethazine respectively.

The study implied impaired absorption and elimination of chlorpropamide by chloroquine, paracetamol and Promethazine.

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## ABBREVIATIONS

| | | |
|---|---|---|
| $K_{el}$ | - | elimination rate constant |
| Cp | - | plasma concentration |
| Clr | - | clearance |
| Vd | - | volume of distribution |
| AUC | - | area under curve |
| HPLC | - | High performance Liquid Chlomatography |
| IDDM | - | insulin dependent diabetes mellitus |
| NIDDM | - | Non-insulin dependent diabetes mellitus |
| uv | - | Ultraviolet |
| WHO | - | World Health Organization |
| Cp | - | Plasma Concentration |
| BDH | - | British Drug House |
| GPR | - | General Purpose Reagent. |
| nm | - | nanometer |
| ml | - | Millitre |
| μg | - | microgramme |
| CV | - | Coefficient of variation |
| SEM | - | standard error of the mean |
| BP | - | British pharmacopoeia |
| min | - | Minute |

| | | |
|---|---|---|
| $^0C$ | - | degree centigrade (Celsius) |
| LSD | - | Least square difference |
| ANOVA | - | Analysis of variance |
| NSP | - | Non-starch polysacckaride |
| BNF | - | British National Formulary |
| MIM | - | Multilateral initiative in malaria |
| TDR | - | Research and Training in Tropical Diseases |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Pharmacokinetics

Pharmacokinetics is the study of the time course of drug and metabolite levels in different fluids, tissues, and excreta of the body, and of the mathematical relationships required to develop models to interpret such data (Gibaldi and Perrier, 2004). Neligan (2006) defined Pharmacokinetics as the study of what the body does to a drug.

Pharmacokinetics can also be defined as the study of the time course of drug movement in the body during absorption, distribution, metabolism and elimination (excretion and biotransformation) (Sadee and Beelen, 1980; Coker, 2001). Pharmacokinetic studies can be applied for the safe and effective therapeutic management of a patient. The pharmacokinetic profile of a drug strongly influences its delivery to biological targets, thereby affecting its efficacy and potential side effects (Van de water beemd and Gifford, 2003).

The Pharmacokinetics of a compound are typically understood, analyzed and interpreted in the context of their underlying absorption, distribution, metabolism and excretion (ADME) processes (Gram, 2003). However, there is no unique mathematical model for any of these processes, usually a number of different models with different underlying assumptions, parameterization and applicability are concurrent (Poulin and Theil, 2002). Absorption is the process by which a drug proceeds from the site of administration to the site of measurement within the body (Brodie, 2007).

The routes of drug administration are: oral, intravenous, buccal, sublingual, rectal, intramuscular, transdermal, subcutaneous, inhalational and topical (Neligan, 2006). Of all these routes, the usual way of administration is the oral route (Benet, 1998). The rate of absorption of a drug depends upon its dissolution characteristics, its ability to pass through biological membranes and upon a number of physiological variables (gastrointestinal) motility, presence of food in gastrointestinal tract etc. Apart from physiochemical properties of the drug (ionization constant, lipid-en water solubility, molecular radius, diffusion coefficient) also the pharmaceutical formulation may be of extreme importance in determining rate of absorption

(Bozler and Van Rossum, 2002). We have linear and non-linear absorptions. Linear absorption is the type of absorption whereby the free diffusion of a drug through biomembranes is the rate limiting step in absorption. The non-linear absorption is the type of absorption where some other steps become the rate limiting step. For instance, dissolution of drug in the gastrointestinal fluid, non-linear phenomenon in the absorption process will arise (Birkett, 2002). Some drugs require the presence of food in the gastrointestinal tract to be absorbed, others on the contrary are better absorbed when taken in an empty stomach (Health Encyclopedia, 2006). Distribution is the process of reversible transfer of a drug to and from the site of measurement (Birkett, 2002). Some of the factors that determine the distribution of a drug in the body are: blood flow; protein binding; and lipid solubility and the degree of ionization.

### 1.1.1 Blood flow

When a drug is introduced into the body, it enters the blood which carries it to the site of action.

The tissues with the highest blood flow receive the drug first, before those that have low blood flow.

### 1.1.2 Protein binding

Most drugs bind to proteins, either albumin or alpha-l-acid glycoprotein (AAG), to a greater or lesser extent (Tillement, 1997). Drugs in their free state travel throughout the body, in and out of tissues and have their biological effect. The highly bound drugs have a longer duration of action and a lower volume of distribution. For a drug that is highly protein bound, loads of it should be administered to get a therapeutic effect, as so much of it is bound to protein. The amount of free drug will be increased when another drug competes with a drug for the binding site on the protein. This is really important for drugs that are highly protein bound. For instance, if a drug is 97% bound to albumin and there is a 3% reduction in binding, then the free drug concentration doubles.

The binding affinity of a drug for plasma proteins and the number of available binding sites, are the two factors that determine the degree of plasma protein binding.

Plasma protein concentration is proportional to the number of binding sites. Drug molecules will equilibrate with tissue compartment if plasma protein concentration decreases. The free

drug concentration in the plasma (Cp free) and tissue (C+ free) as well as the drug concentration bound to pharmacological active sites in the tissue will increase by an insignificant amount if the drug's volume of distribution is relatively large.

### 1.1.3 Lipid solubility and the degree of ionization

Highly ionized drugs cannot cross lipid membranes and unionized drugs can cross freely. Morphine is highly ionized, fentanyl is the opposite. Consequently the latter has a faster onset of action. The degree of ionization depends on the pKa of the drug and the pH of the local environment.

Most drugs are either weak acids or weak bases. Acids are most highly ionized at a high pH (i.e in an alkaline environment). Bases are most highly ionized in an acidic environment (low pH). For a weak acid, the more acidic the environment, the less ionized the drug, and the more easily it crosses lipid membranes. Weak bases have the opposite effect (Neligan, 2006).

### 1.1.4 Volume of distribution (Vd)

Is the amount of drug in the body divided by the concentration in the blood (Gillian, 2001).

Volume of distribution can also be defined as the amount of drug in the body relative to the concentration of drug in the blood (or plasma) (Rowland and Tozer, 1980).

$$Vd = \frac{D}{Cp}$$

Where Vd is the apparent volume of distribution, D is the drug dose, and Cp is the plasma concentration of the drug.

Drugs that are highly lipid soluble, such as digoxin, have a very high volume of distribution (500 litres). Drugs which are lipid insoluble, such as neuromuscular blockers, remain in the blood, and have a low volume of distribution.

Plasma concentration (Cp) is the sum of drug that is bound to plasma protein plus drug that is unbound or free. The free or unbound drug is the pharmacologically active moiety, as it is in equilibrium with the receptor site.

Plasma drug concentration can be used to evaluate the adequacy or potential toxicity of a prescribed dosage regimen.

Pharmacokinetic calculations can be sued to modify dosing regimes so as to *max*imize the therapeutic response while minimizing the risk of toxicity (Mary and Lloyd, 1992).

Steady-state concentration is the drug concentration at which the body is in equilibrium (i.e the rates of drug absorption and elimination are equal). Elimination is the irreversible loss of drug from the site of measurement. A constant fraction of the drug in the body is eliminated per unit time. The rate of elimination is proportional to the amount of drug in the body.

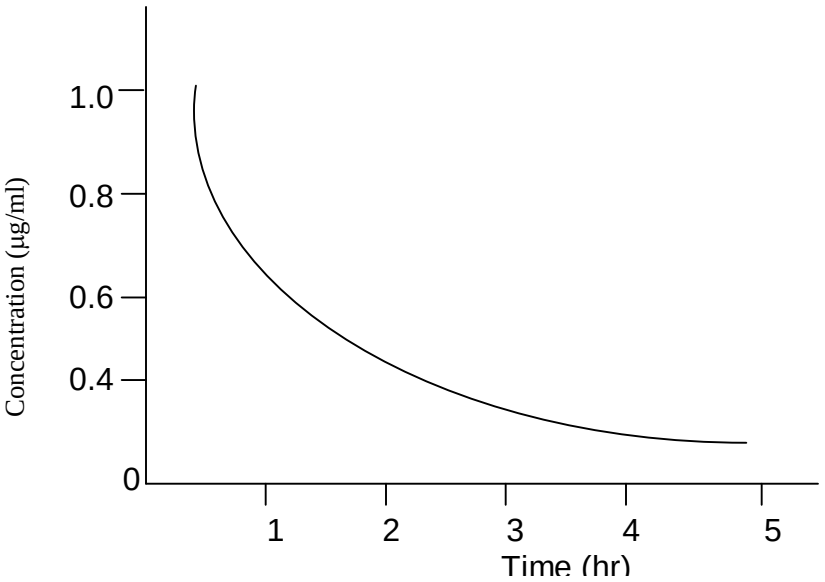



Fig 1: Concentration Vs time profile for plasma in a first order kinetics.

Source: Software supported modelling in pharmacokinetics by Regina, et al, 2006.

The fraction of the drug in the body eliminated per unit time is determined by the elimination constant ($K_{el}$). This is represented by the slope of the line of the log plasma concentration versus time (Regina, et al, 2006).

Rate of elimination = clearance x concentration in the blood.

Elimination rate constant ($k_{el}$) is a function of the drugs volume of distribution and its clearance. It can be calculated by dividing the clearance of the drug by its volume of distribution.

$$K_{el} = \frac{Clr}{Vd}$$

Where $K_{el}$ is the elimination constant, Clr is the clearance, while Vd is the volume of distribution. The elimination rate constant is utilized to predict how the plasma concentration varies with time, assuming no additional drug is added. For example, if $Cp_1$ is the initial plasma concentration and $Cp_2$ is the plasma concentration after an interval of decays, $Cp_2$ can be calculated using the elimination rate constant.

$$Cp_2 = Cp_1\, e^{-Kelt}$$

$e^{-Kelt}$ is the fraction of the initial plasma concentration which is remaining at the end of the time interval.

Most drugs are eliminated by renal excretion and metabolism. The clearance (Clr) of a drug is the volume of plasma from which the drug is completely removed per unit time. Clearance is a proportionality constant which makes the rate of drug elimination equal to the rate of drug administration at steady state (Mary and Lloyd, 1992).

Clearance = elimination constant x volume of distribution

Clr = $K_{el}$ x Vd

(Neligan, 2006; Regina, et al, 2006).

Increased clearance reduces the *AUC* (*AUC*: Area Under Curve) after single drug doses, and leads to a reduced mean steady state concentration during multiple dose therapy.

Decreased drug clearance will increase the *AUC* during single or multiple drug doses and lead to higher plasma concentration during chronic therapy. Total clearance is equal to the sum of the individual clearances for renal excretion and metabolism.

Total clearance = renal clearance + metabolic clearance

Excretion is the irreversible loss of the chemically unchanged drug.

Drug loss by the kidneys is determined by the next effects of glomerular filtration, tubular secretion and tubular reabsorption. Glomerular filtration is not greatly influenced by other drugs, although displacement from protein binding sites may lead to increased concentration of drug in glomerular fluid and enhanced renal elimination.

The pH of the tubular fluid is a determinant of drug's reabsorption. The clearance of acidic drugs is greater in an alkaline urine, while the clearance of basic drugs is more rapid in acidic urine (Solomon, 2000). Drug metabolism is the conversion of one chemical species to another. Metabolism terminates a drug's effects, as drug metabolites are usually devoid of pharmacological activity.

Metabolism of drugs can lead to the formation of polar (lipid-insoluble) derivatives which can undergo renal excretion. Many commonly used drugs are oxidized in the hepatic endoplasmic reticulum (Smith, 2001). Elimination half-life ($t_{1/2el}$) is the time taken for plasma concentration to reduce by 50%.

The half-life of a drug is dependent upon its volume of distribution and clearance. The relationship is as follows:

$K_{el}$ = the log of 2 divided bv the $t_{1/2el} = \frac{0.693}{t_{1/2el}}$

Likewise, Clr = $K_{el}$ x Vd

So, Clr = $\frac{0.693 \times Vd}{t_{1/2el}}$

And then, $t_{1/2el} = \frac{0.693 \times Vd}{Clr}$

The half-life of a drug is of importance during maintenance therapy, to estimate the dosing interval. It can be used to predict the time it will take for a patient on a constant dosage regimen to reach a steady state. It can also be used to predict the time it will take for all the drug to be eliminated from the body.

## 1.2 Drug bioavailability and its relationship to clinical effectiveness

Bioavailability is the fraction of the administered dose that reaches the systemic circulation (Gibaldi and Perrier, 2004). It can also be said to be the rate and extent to which the active drug ingredient is absorbed at the site of action.

Bioavailability is 100% for intravenous injection but varies for other routes of drug administration. For a drug administered orally, the bioavailability is the area under the curve of a plot of plasma concentration versus time, as shown in the diagram below (Fig. 2).

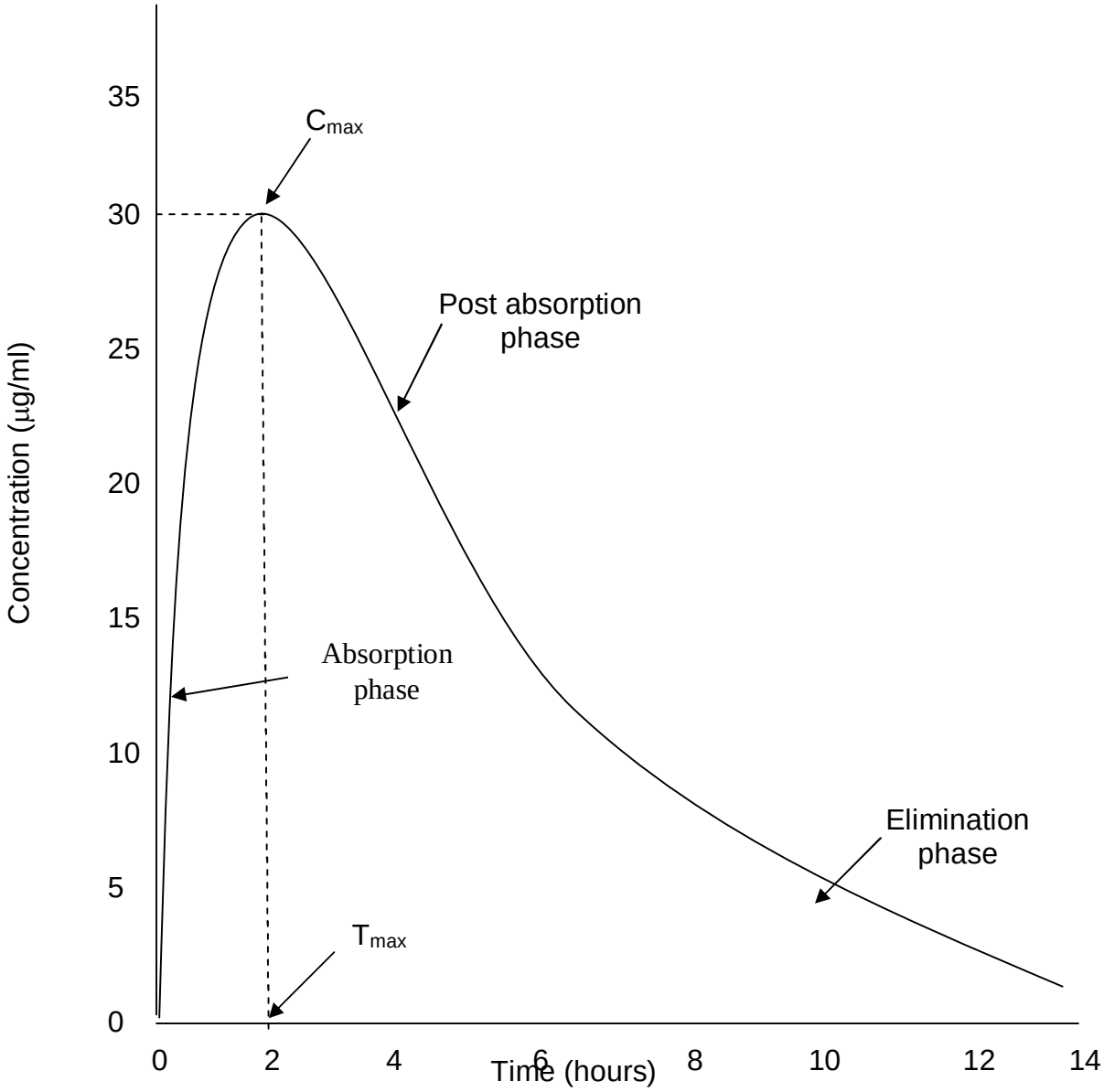



Fig. 2: A plot of plasma drug concentration vs time for an orally administered drug.

Bioavailability is normally estimated by the measurement of the active drug concentration in body fluids or rate of excretion (Sugiyama et al., 2004). One of the important objective methods for evaluating a drug preparation and

establishing its equivalence to a referenced drug is bioavailability. Bioavailability testing in humans is usually limited to blood, urine, and saliva. To carryout the bioavailability test, drug is administered to subjects who are in the fasting state, and are not permitted to eat food for several hours after receiving drug. Blood samples are collected and analyzed. The area under the plasma concentration versus time curve after oral administration is compared with that after intravenous administration by simply plotting arithmetically the plasma concentration curves of unchanged drug (Brodie et al., 2001).

### 1.2.1 Some factors that influence the bioavailabilitv of a drug

Several factors influence the bioavailability of a drug:

#### *1.2.1.1 Dosage form and route of administration*

An injection that is administered intravenously is readily available at the site of action. A tablet which is administered orally, has to pass through both the gastrointestinal wall and the liver, before reaching the systemic circulation. The

bioavailability of drugs administered by the intravenous route is higher than that of any other route (Wagner et al., 2004).

Changes in gastric emptying rate, by pathological, pharmacological or physical means, may change the time of onset, the intensity and the duration of effect, after single drug doses.

#### *1.2.1.2 Solubility*

A drug must be both lipid and water soluble to be absorbed from the gastrointestinal tract. If a drug is too water soluble, it cannot pass through the lipid membrane of the gastrointestinal tract and will not be absorbed following oral administration. Other drugs that are more lipid than water soluble may have administered or delayed absorption because they must first dissolve in the aqueous fluids of gastrointestinal tract before they can be absorbed. Drugs which have intermediate lipid water solubilities are more likely to be completely bioavailable by the oral route if properly formulated (Mary and Lloyd, 1992).

*1.2.1.3. Hepatic drug clearance*

This is important because drugs administered orally are delivered from the gut to the portal vein to the liver. The liver gobbles up a varying chunk of the administered drug (pre-systemic elimination) and less is available to the body for therapeutic effect (Neligan, 2006). If the blood flow increases, the liver has less chance of gobble up the drug, and the extraction ratio falls. This occurs when the intrinsic clearance is low.

*1.2.1.4 Drug binding to plasma proteins*

A number of drugs become bound to plasma proteins with varying degrees of firmness. When bound, these drugs have no direct pharmacologic action on cells, when released from the bound state, they exert their effects.

About 90% of some drugs are ordinarily bound to plasma protein, leaving only 10% of the total dose active (Solomon, 2000).

#### *1.2.1.5 Interactions (food-drug or drug-drug interactions)*

When a drug is administered orally or by any other route, it may interact with some common foods or drugs, which may inactivate it. This interaction affects the fraction of the drug that reaches the systemic circulation to perform the expected therapeutic benefit (Gillian, 2001).

### 1.2.2 Bioavailability relationship to clinical effectiveness

Bioavailability problems are problems concerning clinical effectiveness. There is a relationship between dose or drug concentration, and effect. This relationship may be relatively simple and easily identifiable, or complex and difficult to define. All other factors being constant, the intensity of drug action ordinarily increases with increasing doses in the therapeutic dose range. When a dose is not fully absorbed, its effect is decreased and the patient's condition may deteriorate. When a patient is treated with a drug product which is only partly absorbed, and he subsequently receives the same drug in a dosage form from which the drug is fully absorbed, then the effect of the drug will be greater than anticipated or desired, and serious adverse effects may occur.

Bioavailability and therapeutic effectives are related, but uniquely so for each individual drug and each drug effect (Brodie, et al, 2001).

The intensity of a pharmacologic effect is often linearly related to the logarithm of the amount of drug in the body in the central portion of the dose-response curve. The entire curve is usually s-shaped and can be seen in Fig. 3.

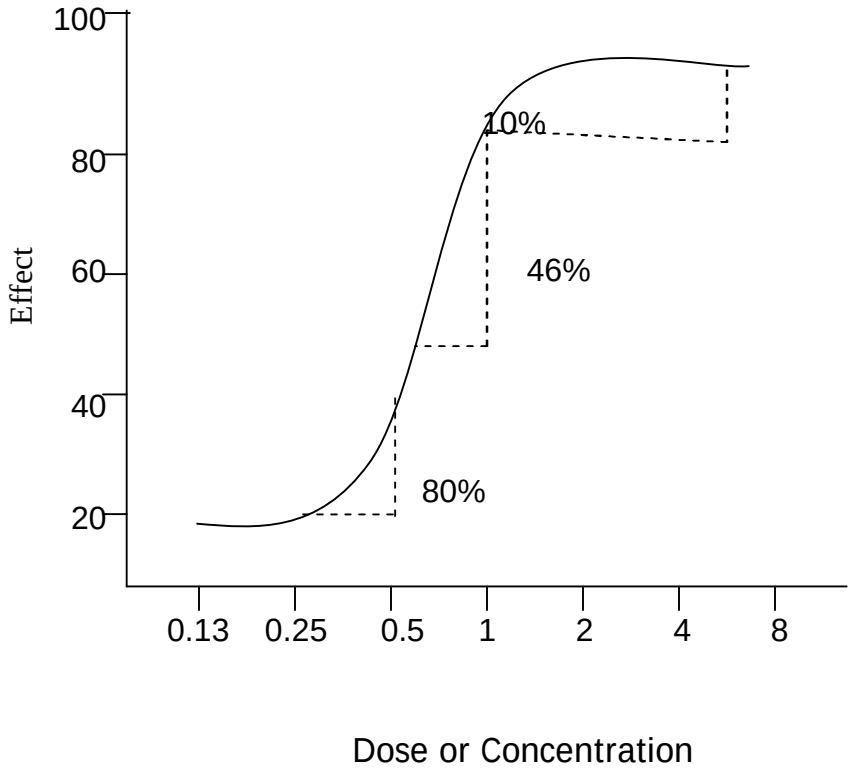



Fig. 3: Effect of bioavailability on pharmacologic effect

A constant fractional decrease in bioavailability will have a relatively much more pronounced effect on drug action if the dose is in the lower range of the dose-response curve. The usual therapeutic doses of most drugs are in the middle or lower range of the curve and any decrease in bioavailability may decrease the drug effect more than proportionately. On the other hand, reduced bioavailability will not be reflected proportionately in the diminution of the effect of those drugs

whose usual therapeutic dose is in the maximum effective dose range (Brodie et al., 2001).

However, incomplete bioavailability is not the only reason why the clinical efficacy of drugs may be impaired; differences in absorption rate can affect the metabolic fate and the overall effectiveness of a drug even though the extent of bioavailability is not decreased (Levy, 1998). The pharmaceutical formulation can profoundly affect the therapeutic efficacy of a drug even without changing the rate or extent of bioavailability. This is particularly shown very strikingly in the case of an aspirin preparation which contains sodium bicarbonate as a buffer. Though the bioavailability of this preparation did not differ significantly from that of another which did not contain bicarbonate, the steady state plasma salicylate levels were substantially different due to the change in urine pH produced by sodium bicarbonate (Levy and Leonards, 1991).

### 1.2.3 Drug level monitoring

This is the quantitative determination of drugs and their metabolites in biological specimens and the interpretation of

such data using principles of pharmacokinetics and pharmacodynamics (Evans et al., 1992).

Drug level monitoring incorporates the following fields: Drug metabolism, pharmacokinetics, pharmacodynamics, pathophysiology, drug analysis, clinical chemistry, clinical toxicology, clinical pharmacokinetics and therapeutic drug level monitoring.

Pharmacological researches often utilize drug level monitoring to study the mechanism of drug action, including the contribution of metabolites to the drug effect. Bioavailability studies depend on drug level monitoring (Sadee and Beelen, 1990).

### 1.2.4 Drug-drug interactions

Drug-drug interaction is the modification of the potency of one drug by another (or others) taken concurrently or sequentially (Smith, 2001). Drug-drug interaction also means the modification of the action of a drug by the action of another drug (Reigelman et al., 1990).When drugs are

administered at the same time; they may interact or exert their effects independently. The interaction may be potentiation or antagonism of one drug by another. Some drug interactions are harmful and some may have therapeutic benefits. Drugs may also interact with various foods. Drug-drug interaction may be classified into two, namely:

Pharmacokinetic interaction, and Pharmacodynamic interaction.

*1.2.4.1 Pharmacokinetic interactions*

Are interactions due to alteration in the absorption, distribution, and elimination of one drug by another (Wagner, et al, 2004). Pharmacokinetic interaction is the type of drug-drug interaction where the action of a drug alters the absorption, distribution, metabolism or excretion of another drug, thereby increasing or reducing the amount of drug available to produce its pharmacological effects (Neuvoneu et al., 2004).

The pharmacokinetic changes may or may not result in an altered pharmacological response or therapeutic outcome.

They are not easily predicted and many of them affect only a small proportion of patients taking the combination of drugs. Pharmacokinetic interactions occurring with one drug cannot be assumed to occur with the related drugs unless their pharmacokinetic properties are known to be similar.

Pharmacokinetic interactions are of several types:

i. Absorption interaction
ii. Distribution
iii. Metabolism alteration
iv. Renal excretion alteration

*Absorption alteration*

This occurs when the absorption of a drug is impaired by another. Drug interactions can alter the total amount of drug absorbed. Delayed drug absorption is rarely of clinical importance unless high peak plasma concentrations are required. For example when giving an analgesic, reduction in the total amount of drug absorbed may result in ineffective therapy. Metoclopramide increases the rate of gastric emptying and this hastens the absorption of analgesics in the treatment of acute attack of migraine, a beneficial effect.

The absorption of a drug in the gastrointestinal tract may be affected by any of the followings:

a) Drugs that alter gastrointestinal pH;

b) Binding resins such as cholestyramine;

c) Drugs with large surface area such as antacids, and

d) Drugs that affect the gastrointestinal mortality.

The absorption of a drug may be reduced to the extent of impairing the therapeutic response of such a drug. In some cases the reduction in the serum concentration of the object drug may be small. A reduction in the absorption rate of a drug does not alter the steady state serum concentration of that drug, so the drug response will be reduced. However, where a rapid drug response is required, a slowed rate of absorption could be clinically important.

*Distribution*

Most drugs are loosely bound to plasma proteins to a variable extent. Protein-binding sites are non-specific and one drug can displace another thereby increasing its proportion free to diffuse from plasma to its site of action. This only produces a detectable increase in effect if it is an extensively bound drug

(more than 90%) that is not widely distributed throughout the body.

Displacement interactions are usually not important unless the displaced drug is highly bound, has a limited distribution in the body, is slowly eliminated and has a low therapeutic index. Even when a displayed drug does possess these properties, the enhanced pharmacologic effect only occurs transiently (for a few days) because more unbound drug is available for elimination in the liver or kidney. Displacement from protein binding plays a part in the potentiation of warfarin by phenybutazone, sulphonamides, and tolbutamide, but the importance of these interactions is due mainly to the fact that warfarin metabolism is also inhibited. When these drugs are displaced, their clearance rate increases in proportion to the degree of displacement and so at steady state the total concentration of drug in the plasma falls to a new equilibrium value, and the unbound concentration is the same as it was before the precipitant drug was introduced, inspite of an increase in the unbound fraction (Winter, 1998).

*Metabolism*

Drug interactions involving metabolism occur when the metabolism of an object drug is either inhibited or increased by a precipitant drug. Alteration in the metabolism of one drug by another probably cause more clinically important drug interaction than any other type of pharmacokinetic interaction. There are two phases of drug metabolism.

Phase I metabolic reactions, for example, hydroxylation, deamination, dealkylation, sulphoxidation, are carried out by isoenzymes of the mixed-function oxidase system and are subject to interactions.

Phase II reactions are conjugations, for example, acetylation, methylation, glucuronidation, and sulphatation, which are not affected by interactions.

Many drugs are metabolized in the liver. Induction of the hepatic microsomal enzyme system by one drug and gradually increase the rate of metabolism of another, which results in lower plasma concentration and a reduced effect. On

withdrawal of the inducer, plasma concentrations increase and toxicity may occur. The onset and offset of enzyme induction is gradual, since onset depends on the accumulation of the inducing agent and the synthesis of new enzyme, while offset depends on elimination of enzyme inducing drug and decay of the increased enzyme stores. Thus, enzyme inducers with long half lives (e.g Phenobarbital) would be expected to have a longer onset and effect than inducers with shorter half-lives, such as rifampin (Rimactane). Barbiturates, dichlorophenazone, griseofulvin, most antiepileptics, and rifampicin are the most important enzyme inducers in man. Drugs affected include warfarin, and the oral contraceptives. When a drug inhibits the metabolism of another, there will be an increase in plasma drug concentration, which rapidly results in an increased effect with possible toxicity.

*Excretion interaction*

This involves the reduction of the excretion of one drug by another, leading to increased plasma levels and possible toxicity. Drugs are eliminated through the kidney by glomerular filtration and by active tubular secretion.

Competition occurs between those drugs which share active transport mechanism in the proximal tubule. Two or more drugs may be excreted by the kidneys by active tubular secretion. When a patient combines two drugs that are actively secreted by the same process, one or both drugs may interfere with the renal elimination of the other. For example, probenecid inhibits the tubular secretion of penicillin, increasing the blood concentration of penicillin and prolonging its therapeutic effects, a beneficial interaction. Amiobarone, quinidine and verapamil inhibit the tubular secretion of digoxin by inhibiting the transport of protein, glycoprotein, increasing plasma digoxin concentrations and causing toxicity. Salicylates inhibit the active secretion of methotrexate. Asprin may increase the toxicity of methotrexate (Miners et al., 1984).

*1.2.4.2 Pharmacodynamic interaction*

is the type of drug-drug interaction in which the effect of a drug is altered at its site of action. Pharmacodynamic interaction occurs when one drug directly alters the molecular, cellular or physiological actions of another (Wagner et al.,

2004). This interaction is usually predictable from the knowledge of the

pharmacology of the interacting drugs and, interactions demonstrated with one drug are likely to occur with related drugs. They occur to a greater or lesser extent in most patients who receive the interacting drugs.

Four varieties of pharmacodynamic interactions may be described:

i) Enhanced effects produced by two drugs acting at the same site. The magnitude of the observed interaction may be equal to, or greater than, the sum of their individual effects.

ii) The increased effects produced by two drugs acting at different receptor sites. Such an interaction often results in an effect which is greater than the sum of the component effects (i.e potentiation).

iii) Enhanced effects of a drug by one which is devoid of action itself. Interactions of this type are extremely unusual, but may account for the increased anticoagulant effect of warfarin during therapy with clofibrate or D-thyroxine.

iv) Antagonism of the effect of one drug by another.

This type of interaction can be caused by several underlying mechanism. The commonest variety is that produced by competition for receptor sites by two drugs both with affinity for the receptor but only one (the agonist) having receptor 'stimulant' activity (often called intrinsic activity). Examples of antagonists include antihistamine, muscle relaxants. (Herman, et al., 2004). Pharmacodynamic drug interactions occur commonly in clinical medicine, and when anticipated, usually do not cause serious clinical difficulties. The drug-drug interaction may be either direct or indirect.

The direct pharmacodynamic interaction is the type of interaction which occurs when two drugs either act on the same receptor site, synergism or antagonism, while indirect pharmacodynamic interaction is the type of drug-drug interaction in which the pharmacological, therapeutic or toxic effect of the precipitant drug in some ways alter the therapeutic or toxic effect of the object drug, but the two effects are not themselves related and do not themselves interact (Pronsky, 2006).

### *1.2.4.3 Undesirable drug interactions*

Serious and fatal consequences can result from the interactions of drugs with other drugs, with foods, and with substances. Active research in this area has increased the understanding of the ways in which interactions and interferences may occur.

*Interaction between drugs and parenteral fluids*

Some drugs that are designed to be administered intravenously cannot be safely mixed with some of the available intravenous infusion fluids. At times, the solubility of a drug in a particular fluid is low, so that when the drug is mixed with that fluid, crystals form. When the crystals are clearly evident, the infusion is usually stopped at once and little or not damage may result, but sometimes microcrystals form and are recognizable only as a faint cloudiness which is sometimes missed. Under such circumstances, the patient might receive a large number of microcrystals intravenously, many of them forming microscopic thrombi and in vital organs. The damage might not be evident for sometime and - its etiology might never be recognized.

Some drugs are more soluble in an alkaline medium, when added to an acid or neutral solution, they may precipitate. Other drugs are more soluble in acid solutions, and may precipitate when added to alkaline or neutral solution.

*Physical incompatibilities of two or more drugs mixed together in intravenous infusions*

It is a common practice in the hospital to add one or more drugs to intravenous infusions. There is the possibility of a harmful interaction between two of the drugs when they are placed in the solution. Sometimes this interaction causes the precipitation of one or more of the drugs. For example, mixing an alkaline drug such as sodium pentobarbital with an acid drug such as chlorpromazine hydrochloride, might cause precipitation of either or both of the drugs.

Drugs are formulated with various additives including preservatives, solublizers, and so forth. When two formulations are mixed together, the additives may interact causing precipitation, or the solublizers may become ineffective, causing precipitation of the drug (Pronsky, 2006).

*Inactivation of one drug by another*

Sometimes when two drugs are mixed together in the same solution, one of them will inactivate the other, thereby depriving the patient of the expected therapeutic benefit. The most striking example is the inactivation of tetracycline by the riboflavin in vitamin B mixtures. Unfortunately, there are no visible signs of this reaction, and the physician may believe that the patient is receiving potent tetracyclines. In reality, depending on concentrations, time of contact, temperature, and other factors, enough tetracycline may be inactivated by the riboflavin that the patient actually receives an ineffective medication.

*Drug interaction within the body*

Drugs administered to a patient may interact to produce a harmful effect. The interaction may occur within hours, days or weeks. Sometimes the interaction may take place only after one of the drugs has been taken for several weeks.

*Potentiation*

Potentiation occurs when one drug increases the effect of another drug to a greater degree than could be expected from simple addition of effects. Often, potentiating drugs are in the same general pharmacologic group as the drugs they potentiate.

For example, if two drugs with sedative properties are given together, the total sedation may be much greater than the expected sum of the effects of each drug separately. Unfortunately, this effect has resulted in accidental death for several people who took barbiturates and alcoholic drinks close together in time. In the past decades, potentiation was used by some physicians in an attempt to augment the effectiveness of their prescriptions.

The potentiation effect may apply to a basically therapeutic action of the drug, raising it to levels that are harmful or lethal. There are circumstances in which one drug may potentiate the toxic effects of another drug while exerting little potentiation on its therapeutic effects.

*Diminution of drug effects*

Diminution of drug effects is the reducing or eliminating the expected therapeutic benefit of a drug.

This can be disastrous to a patient.

The administration of two drugs with opposite actions may produce a diminution or elimination of the effectiveness of both.

For example, sodium bicarbonate makes the urine more alkaline while ammonium chloride makes it more acidic. Giving both together will reduce or eliminate their effects on the reaction of urine.

*Plasma protein binding*

One drug may reduce the degree of protein binding of another drug by competing for the binding site on the protein. A reduction of protein binding, therefore, means an increase in pharmacologic activity. This phenomenon can sometimes result in greatly magnified drug effects. For example, about

90% of some drugs is bound to plasma proteins leaving only 10% of the total dose active.

When another drug is given that competes for the binding site and displaces about half of the bound portion of the first drug, making it unbound and pharmacologically active, the active fraction of the first drug will therefore rise from 10% to 55% of the total dose, an increase that will probably cause severe toxic effect.

*Food and drug interaction*

Some common foods can interact with drugs to produce toxic and fatal effects.

For example, some patients receiving MAO inhibitors for the treatment of depression, and eating cheese developed transient episodes of extreme hypertension with unbearable headaches, and in a few cases, fatal subarachnoid hemorrhage.

It is now known that several foods can produce this kind of interactions.

*Effect of one drug on excretion of another drug*

One drug can increase the excretion rate of another drug, thereby, lowering its therapeutic effectiveness. Conversely, decreasing the excretion rate of a drug can increase its toxicity. It is better to increase the dosage of a drug in order to get a greater therapeutic effect than to decrease excretion. It is obvious that diuretics tend to increase the excretion of other drugs and consequently reduce effectiveness.

*Interference with absorption*

Some drugs may interfere with gastrointestinal absorption of other drugs, thereby reducing their effectiveness. An example is cholestyrammine. Some substances can enhance the absorption of drugs from the skin.

*1.2.4.4 Controlling adverse drug interactions*

Drug interactions may be harmless or potentially harmful. The severity of an interaction varies form one patient to another. Drugs with a small therapeutic ratio (e.g phenytoin) and those which require careful control of dosage (e.g anticoagulants, antihypertensives, and antidiabetics) are most often involved.

In some prevention regimens, drug interactions may even cause more harm than good. For example, one drug might reduce blood levels of another drug, leading to the development of drug resistance. In other words, drug interaction could result in the development of a disease unresponsive to standard treatment. The added toxicity of numerous combined therapies may also outweigh their potential benefit for preventing disease.

Patients at increased risk from drug interactions include the elderly and those with impaired renal or liver function.

The simple way of controlling or avoiding adverse drug interaction is to avoid combinations that are known to be dangerous. If that is not possible, the dosage of the object drug should be reduced in advance of starting the precipitant drug and the precipitant drug should be introduced slowly. When a theoretical interaction is anticipated on the basis of the known properties of two drugs, even if it has not been previously described, careful monitoring will help recognize adverse effects early.

It is important to discuss potential drug interactions with a health care provider and a pharmacist. Before starting a new therapy, you have to consider the potential drug interactions and side effects. Drug interactions can be managed by monitoring carefully, adjusting the doses, or discontinuing the therapy as need be (Smith, 2001).

## 1.3 Diabetes

Diabetes mellitus is a clinical syndrome characterized by hyperglycaemia due to deficiency in insulin. Lack of insulin affects the metabolism of carbohydrates, protein and fat, and causes a significant disturbance of water and electrolyte homeostasis (Frier et al, 1999).

There are two types of diabetes mellitus, namely:

Type 1 diabetes or insulin dependent diabetes mellitus (DDM), and Type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM).

Genetic factors account for about one-third of the susceptibility to type 1 diabetes, the inheritance of which is polygenic.

Genetic factors are more important in the aetiology of type 2 than type 1 diabetes. Some forms of type 2 diabetes which are as the result of single gene defects have been identified. However, these subtypes, such as the syndrome of maturity onset diabetes of the young (MODY) are uncommon and constitute less than 5% of all cases of type 2 diabetes. For majority of the cases of type 2 diabetes, several genes are involved. In this polygenic model, inheritance of abnormalities in individual genes would not be sufficient to cause type 2 diabetes directly (Frier et al., 1999). Epidemiological studies have shown that overeating when combined with obesity and underactivity, may lead to type 2 diabetes. Obesity acts as a diabetogenic factor in those genetically predisposed to develop type 2 diabetes. However, it should be noted that not all obese people arc diabetic (Fajan, 2000).

Insulin sensitivity is reduced during normal pregnancy through the action of placental hormones, and this affects glucose tolerance. This increases demand in women who are genetically predisposed to develop either of the primary types of diabetes, may not be met by the insulin-secreting cells of the pancreatic islets. The likelihood of developing permanent diabetes in obese women may be increased by repeated pregnancy.

Gestational diabetes is the type of hyperglycaemia which occurs for the first time during pregnancy. Studies have shown that about 80% of women with gestational diabetes eventually developed permanent diabetes (Boyrd, 2004).

Increased hepatic production of glucose and resistance of the action of insulin in muscle are invariable in both obese and non-obese patients with type 2 diabetes (Orchard, 1990). Some pathological changes are typical of type 2 diabetes, the most consistent of which is deposition of amyloid-This is accompanied by atrophy of the normal tissue, particularly islet epithelial cells. Deposition of amyloid is not a cause of

diabetes, but rather reflects a pathological process which is increased in type 2 diabetes. While beta cell numbers are reduced by 20 to 30% in type 2 diabetes, alpha cell mass is unchanged and glucagons secretion is increased, and this may contribute to the hyperglycaemia.

Insulin resistance tends to raise blood glucose and stimulate insulin secretion to prevent hyperglycaemia, Any further increase in fasting blood glucose level when the *max*imal insulin secretory capacity has been exceeded will cause a decline in insulin generation.

The mechanisms for beta cell decompensation include glucotoxicity, an intrinsic failure of insulin production, a switch to abnormal processing pathways producing biologically inactive products and chronic degranulation of the beta cell (Frier, 1999).

Some symptoms like thirst, polyuria, nocturia and rapid weight loss are prominent in type 1 diabetes, but are often absent in patients with type 2 diabetes.

Complaints such as chronic fatigue and malaise are often made by type 2 diabetic patients.

Uncontrolled diabetes is associated with an increased susceptibility to infection and patients may present with skin sepsis (boils) and genital candidiasis, and complain of pruritus vulvae or balanitis.

Patients with type 1 diabetes often have no physical signs attributable to diabetes, but weight loss is common. In the fulminating case with ketoacidosis, the striking features are those of salt and water depletion, with loss of skin turgor, furred tongue and cracked lips, tachycardia, hypotension and reduced intra-ocular pressure. Breathing may be deep and sighing, the breath is usually fetid and the sickly-sweet smell of acetone may be apparent. Emotional apathy, confusion or a reduced conscious level may be present.

For patients with type 2 diabetes, more than 70% are overweight, and about 50% are hypertensive. Hyperlipidaemia is also common, skin lesions such as xanthelasma and

eruptive xanthomata are relatively rare. The patients may complain of paraesthesiae, pain and muscle weakness in the legs with signs of peripheral neuropathy or foot unceration, or deterioration of vision from cataracts or retinopathy. Signs of macrovascular disease are common and may include diminished or impalpable pulses in the feet, bruits over the carotid or femoral arteries and ischaemic toes. Cutaneous features of diabetes include a dermopathy with trophic brownish scars on the skins and the much rarer nectobiosis lipoidica diabeticorum.

Diabetic patients can be treated via three methods, namely: Diet alone; Diet and an oral hypoglycaemic drug; and Diet and insulin.

The type of treatment needed is determined by the circulating plasma insulin concentration. In clinical practice, the age and weight of the patient at diagnosis are closely related to the plasma insulin and usually indicate the type of treatment required. In the treatment, the regimen adopted is chosen by therapeutic trial.

There is always a change of life style such as taking regular exercise, observing a healthy diet, reducing alcohol consumption and stopping smoking, so as to improve on the glycaemic control (Bressler and Johnson, 2002).

Dietary management is required in the treatment of all types of diabetic patients in order to achieve an overall therapeutic goal. All diabetic patients are required to consume a diet containing an appropriate energy content, as this greatly influences glycaemic control. An individual patient's daily energy requirement involves consideration of factors such as age, sex, actual weight in relation to desirable weight, activity and occupation.

A suitable diet for diabetic patients should contain about 50% of the daily caloric intake derived from carbohydrate, of which significant amounts should be in the form of non-starch polysaccharide (NSP) as dietary fibre. Fat intake should be restricted to about 35% of energy with less than 10% as saturated fat, less than 10% as poly unsaturated fat, and 15% as mono unsaturated fat. Patients should abstain from

alcohol, and reduce the intake of sodium in the form of salt. Low-calorie and sugar-free drinks are useful for patients with diabetes. The non-nutritive sweetners saccharim, aspartame, sucramate, and acesulphane K are the most widely used, and provide means for reducing energy intake without loss of palatibility.

Low-energy, weight-reducing diets and weight maintenance diets are used in the treatment of diabetes. Dietary prescription which induces a weekly weight loss of about 0.5kg is administered to the obesed.

Rapid weight reduction may provoke loss of lean body tissue, and in the elderly, care must be taken to avoid the omission of essential nutrients, vitamins and minerals. For weight maintenance, there should be high intake of carbohydrate and low intake in fat. For the insulin treated diabetes, a regular pattern of meals and snack is important to maintain a constant daily intake of carbohydrate. Carbohydrate should be the main art of the meal in conjunction with vegetables. The consumption of protein-containing foods should be limited.

For the effective reduction of hyperglycaemia in type 2 diabetic patients, various oral hypoglycaemic drugs are used. The sulphonylureas and biguanides have been the main drugs of treatment for many years, but novel agents such as the insulin-enhancing agents, the thiazolidinediones, meglitinides, and the alphaglucosidase inhibitors, which delay carbohydrate digestion and absorption of glucose, are now available.

Sulphonylureas are valuable in the treatment of non-obesed patients with type 2 diabetes who fail to respond to dietary measures alone. The first-generation sulphonylureas consist of chlorpropamide, tolazamide and toibutamide. The second-generation sulphonylureas consist of Glibendamide, glipizide, glimepiride and gliclazide (Gillian, 2001).

The Biguanides are less widely used than the sulphonylureas because of a higher incidence of side effects, particularly gastrointestinal symptoms (Adams, 2006). The alpha-glucosidase inhibitors delay carbohydrate absorption in the gut by selectively inhibiting disaccharides. Acarbose is currently available and is taken in a dose of 50 - 100mg with

each meal. The novel drugs, thiazolidinediones, work by enhancing the actions of endogenous insulin (Bluementhal, 2001). The first drug to be commercially available is troglitazone, which can be prescribed either alone or in combination with insulin. Insulin injections are used for the management of type 1 diabetes. Various insulin regimes are sued in the treatment of diabetes. The choice of regimen depends on the desired degree of glycaemic control, the patient's lifestyle and his or her ability to adjust the insulin dose. The ideal management for diabetes would allow the patient to lead a completely normal life, to remain not only symptom-free but in good health, to achieve a normal metabolic state and to escape the long-term complications of diabetes (Frier et al., 1999).

## 1.4 Malaria

This is a protozoal infection of the human blood caused by the plasmodium falciparum, plasmodium vivax, plasmodium ovale and plasmodium malariae. The malaria parasites are transmitted by the bites of anopheles mosquitoes (Francesca and Polina, 2006; Griffin et al.,1999).

It is predominantly a disease of hot and wet climates, and it is endemic or sporadic throughout most of the tropics and subtropics below an altitude of $1500m_7$ excluding the Mediterranean littoral, the Untied States of America and Australia.

Inspite of decades of efforts, malaria continues to rank as one of the foremost infectious global disease. It remains a major public health problem in tropical and sub-tropical areas of the world. The disease threatens two-fifth of the world's population globally, it is estimated that there are about 300 to 500 million cases of malaria per year (Ruzangi, 2000).

The exact frequency of malaria related mortality is virtually unknown, largely due to a general under reporting of death and inaccurate cause of death. However, it is estimated that 1.5 to 3 million death occur annually (Jane, 2004).

Severe anaemia exerts a heavy toll on African children in malaria endemic countries, a recent estimate suggesting that 1.4 to 5.7 million cases of anaemia each year kill 190,000 to

974,000 children under 5 years, with the highest mortality occurring in infants of less than 12 months (Jane, 2004). Following WHO sponsored campaigns of prevention and more effective treatment, the incidence of malaria was greatly reduced in 1950 to 1960 but since 1970 there has been a resurgence. In the 1980s plasmodium falciparum became resistant to chloroquine over a steadily increasing area. Most serious was the emergence of resistance in Africa, where it is now widespread (Frier, et al, 1999; Ruzangi, 2000).

Is it possible to imagine a world where mosquitoes do not carry the parasite that causes malaria? There are researches going on now to end the mosquitoes that carry malaria. This could be by genetically modifying the Anopheles mosquitoes. The research is being carried out by 275 researchers from 26 countries (Yega, 2006).

An important preventive tool may be lost if mosquitoes develop resistance to insecticide. The use of bednets treated with insecticides is an important strategy in the prevention of malaria attacks (Olumide, 2006). The burden of malaria falls

on the under privileged inhabitants of under developed countries being a disease directly related to poverty. Malaria morbidity and mortality were largely reduced by the use of antimalarial drugs.

The incubation period is often longer than the pre erythrocytic cycle and may be up to several weeks for p. falciparum or months for p. vivax (Hay, 1996). The illness starts with a period of several days of continued fever before the development of classical bouts of fever on alternate days. Fever starts with a rigor. The patient feels cold and the temperature rises to about 40°C. After half an hour to an hour, the hot or flush phase begins. It lasts several hours and gives way to profuse perspiration and gradual fall in temperature. The cycle is repeated 48 hours later. The spleen and liver gradually enlarge and become tender. P. malariae infection is usually associated with mild symptoms and bouts of fever every third day. It causes glomerulonephritis and nephritic syndrome in children (Rex et al., 1998).

P. falciparum infection is more dangerous than other forms of malaria. The onset is often insidious, with malaise, headache and vomiting. Cough and mild diarrhea are common. The fever has no particular pattern and does not usually rise quite as high as in the other forms. The cold, hot and sweating stages are seldom found. Jaundice is common due to haemolysis and hepatic dysfunction. The liver and spleen enlarge and become tender, anaemia develops rapidly. A patient with falciparum malaria, apparently not seriously ill, may develop serious complications. Children die rapidly without any special symptoms other than fever (Rinaldi, 1997). Repeated infections lead to anaemia, considerable enlargement of the spleen, which is in danger of rupture, and chronic ill health with bouts of fever. The growth and development of children may be retarded.

For the chemotherapy of the acute attack of P. vivax, p. ovale, and p. malariae infections, chloroquine is the drug of choice (Griffin et al, 1999). P. flaciparum is resistant to chloroquine in most parts of the world, and so falciparum malaria should not be treated with chloroquine, unless it is certain that the

infection was acquired in an area of chloroquine sensitivity. Infections with p. falciparum from a chloroquine-resistant area should be treated with quinine dihydrochloride or sulphate 600mg salt (10mg/kg) 8-hourly by mouth until better and the blood is free of parasite (usually 3-5 days). This regimen should be followed by a single dose of sulfadoxine 1.5g combined with pyrimethamine 75mg, i.e 3 tablets of
Fansidar. The dose is reduced to 12-hourly if quinine toxicity develops. For patients with complicated p. falciparum malaria, quinine is given as an intravenous infusion over 4 hours. The dose is 10mg/kg of quinine salt up to a maximum 700mg. The dose should be repeated at intervals of 8-12 hours until the patient can take drugs orally.

A drug combination of artesunate and amodiaquine is used for the treatment of malaria (Francesca and Polina, 2006).
Clinical attacks of malaria may be preventable by drugs such as proguanil which attack the pre-erythrocytic form or by drugs such as chloroquine or mefloquine after it has entered the erythrocyte.

It has been observed that the old drugs that had been in use for the treatment of malaria for the last 50 years are losing their effectiveness, and the World Health Organization now recommends new combination therapies that are more effective and reduce the ability of the malaria parasite to develop resistance to these medicines (Ogundahunsi, 2006). A single dose of sulphadoxine-pyrimethamine, administered to Tanzanian infants at 2, 3, and 9 months of age at the time of routine EPI vaccination, reduced episodes of malaria by 59% and episodes of anaemia by 50% (Schellenberg, 2002).

Another study in Tanzania showed that the use of pyrimethamine-dapsone combination for the chemoprophylaxis of malaria, reduced the episodes of malaria by 60% between the ages of 2 to 11 months, and also reduced the episodes of anaemia by 57%, during the first year of life (Menendez, 2002).

The use of an antimalaria drug, Lapdap (chlorproguanil-dapsone), has been developed, a drug which is taken once daily for three days to treat uncomplicated falciparum malaria

in children and adults (Jane, 2004). Two new medications for malaria have been introduced, which are: (i) Chlorproguanil hydrochloride-dapsone-artesunate (CDA), and (ii) artesunate/mefloquine (AS/MQ). These are fixed-dose combination medications. CDA is used in treating adults and children with p. falciparum malaria (Pagnoni, 2006). Artemisinin, a natural product from a traditional Chinese herbal remedy, has been recommended by WHO in all malaria treatments to help reduce drug resistance. It is available for the treatment of p. falciparum (Vennerstrom et al., 2006). Malaria cannot be prevented by chemoprophylaxis alone, there is the need to avoid anopheles mosquitoes, which bite at night. This can be achieved by wearing long sleeves and trousers while outside the house. Repellant creams and sprays can be applied. Also the risk can be reduced by the use of mosquito nets, screened windows, and burning of repellant coils.

## 1.5 Objective and scope of the study

Most often, two or more drugs are co-administered, and as a result, some of these drugs do interact. The interaction may be harmful or harmless, depending on the type of drugs involved.

Drug interaction could result to increased or decreased drug levels in the blood. It may also lead to a decrease in the metabolism and effectiveness of a drug. When drugs interact, there could be increased risk of side effects like liver toxicity, kidney toxicity, ocular toxicity, bone marrow toxicity, central nervous system toxicity, peripheral neuropathy, ancephalopathy, high blood sugar levels, low blood pressure, nausea and ; vomiting.

Some drug combinations may or may not interact. The severity of the interaction varies from one patient to another.

Gillian (2001) reported that chlorpropamide absorption increases when administered with antacids.

According to Pronsky (2006), the absorption of chlorpropamide increases when administered with magnesium supplements.

It was reported by Kristensen (1993), that chlorpropamide levels in blood was doubled by Bishydroxycoumarin, when the two drugs were concurrently administered.

Petit Pierre et al (1992) in their study observed that when chlorpropamide and allopurinol were administered concurrently, the half-life of chlorpropamide increased from 36h to 44h and 55h respectively.

According to the studies carried out by Fabre et al (1997), when 2g of chloramphenicol were administered together with chlorpropamide, the half-life of chlorpropamide was doubled.

The area under curve (*AUC*) of chlorpropamide increased by 28% when 100mg of fluconazole was administered with 20mg of chloropropamide (Johnson et al., 1990). The rate of absorption of chlorpropamide was increased by magnesium hydroxide (Kivisto and Neuvoneu, 1992). Rifabutin and Rifampin when administered concurrently with chlorpropamide individually, decreased its levels in blood. Chlorpropamide is extensively bound to plasma proteins (90%). For a drug that is highly protein bound, loads of it should be administered to get a therapeutic effect, as so much of it is bound to protein. The amount of free drug will be increased when another drug competes with the drug for the binding site on the protein. This is really important for drugs that are highly protein bound. For

instance, if a drug is 96% bound to albumin and there is a 4% reduction in binding, then the free drug concentration doubles. This interaction is serious as it could lead to toxicity of the cells in the body.

Since, chloroquine, paracetamol and promethazine may be co-administered together with chlorpropamide in diabetic patients having malaria, the present study is intended to evaluate the effects of chloroquine, paracetamol and promethazine on the pharmacokinetics of chlorpropamide in healthy volunteers.

Each healthy subject will undergo the following treatments:-

i) Administration of a single oral dose of chlorpropamide (250mg) tablet each in a fasting state with 200ml of water.

ii) The same healthy subjects will be divided into three groups.

The first group will be given a single oral dose of chloroquine (1000mg) and chlorpropamide (250mg). The second group will be given a single oral dose of paracetamol (1000mg) and chlorpropamide (250mg). The third group will be given a single oral dose of promethazine (25mg) and chlorpropamide (250mg).

The chlorpropamide levels in the plasma will be determined.

Ultraviolet spectrophotometer technique would be employed for the chemical assay and dissolution rate test (quality control) of the chlorpropamide tablets, while high performance liquid chromatographic technique would be employed for the determination of the chlorpropamide concentration in the plasma.

# CHAPTER TWO

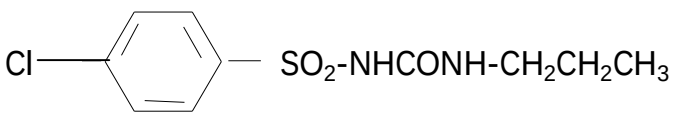
# LITERATURE REVIEW

## 2.1 Chemistry of chlorpropamide, chloroquine, paracetamol and promethazine

### *2.1.1 Chlorpropamide*

Chlorpropamide is a member of the first-generation sulphonylureas, which is used in the treatment of type 2 diabetes mellitus (Gillian, 2001; Ferner and Neil, 1999). The realization of the fact that the ideal treatment for diabetes is insulin, led many into researches. As a matter of fact, many chemical derivatives have been introduced, but later abandoned because of their toxicity. For example, guanidine derivatives, which their prolonged usage resulted into renal and hepatic damage (Olaniyi, 1989). There has been a steady flow of structural modifications of those chemical agents which have been found to be clinically useful for the treatment of diabetes, for example, carbutamide.

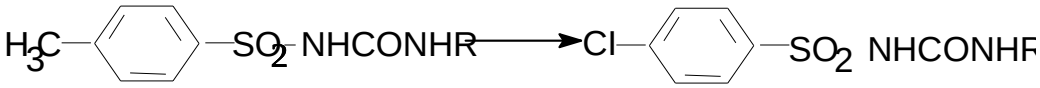
Chlorpropamide has a chemical structure.



and a chemical name:

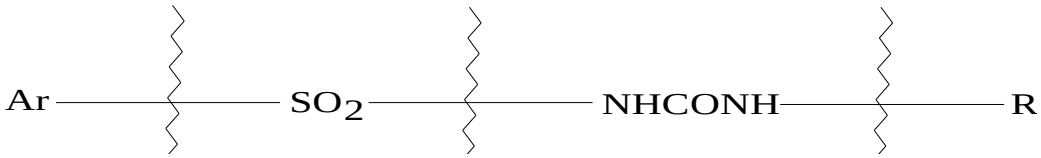
1- [(p-chlorophenyl) sulphonyl]-3-propylurea

The sulphonylurea, Tolbutamide, has a short duration of action, which is due to the oxidation of the vulnerable nuclear-substituted methyl group. When the methyl group is replaced with a chlorine atom, with the shortening of the alkyl chain, this gave rise to the drug, chlorpropamide, which has its biological half-life extended by six-fold.




Tolbutamide; R=C4H9 Chlorpropamide, R=C3H7

The structure of the drug consists of three portions:




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

The chlorine atom attached to the aryl moiety in the structure, makes it to be of increased potency and longer action than the other members of the group. The alkyl group confers lipophilic properties on the molecule. Chlorpropamide is a white crystalline powder, which is odourless. It has a melting point of 127 - 129°C. Less

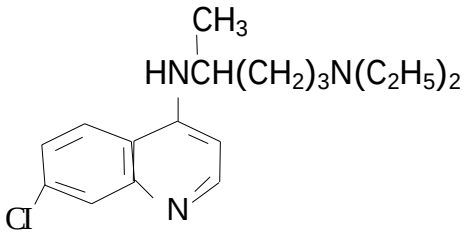
than Ig of chlorpropamide is soluble per litre of water at 14°C. About 50 to 100g of it is soluble per litre of 95% Ethanol at 14°C. About 100g or more of chlorpropamide is soluble per litre of acetone at 14°C. It is soluble in solutions of alkali hydroxides but slightly soluble in ether and benzene. Chlorpropamide has a pKa of 5.0 at 20 C. It is stable under normal laboratory conditions. Solutions of chlorpropamide in water, ethanol and acetone should be stable for up to 24 hours under normal laboratory conditions.

### *2.1.2 Chloroquine*

Chloroquine is a 4-aminoquinoline, which is used in the treatment of malaria.

There was a further search for synthetic antimalarial as a result of the success with the 8-aminoquinolines. A series of compounds with good antimalarial properties were found by changes in the basic side chain attached in position 4 of the quinoline nucleus, of which the 4-amino-7- chloroquinolines showed highest activity. These include chloroquine, sontoquine and amodiaquine. Chloroquine has been used as the most common antimalarial drug in all developing countries for many years (Rollo, 1980).

Chloroquine has a chemical structure:




and a chemical name:

7-Chloro-4-(4-dietylamino-1-methylbutylamino)quinoline,

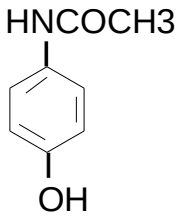
Chloroquine is a white, odourless powder. It discolours slowly on exposure to light. Chloroquine is soluble 1 in 4 of water, very slightly soluble in alcohol, and insoluble in chloroform and ether.

### *2.1.3 Paracetamol*

Paracetamol is a derivative of aniline which serves as an antipyretic and analgesic agent.

Acetanilide which earlier served as an antipyretic agent in 1886 was excessively toxic, so there was a search for less toxic compounds. Phenacetin was discovered in 1887. Owing to the toxic effects of phenacetin, such as haemolytic anaemia, methaemoglobinaemia and nephrotoxicity, it was replaced by paracetamol (Acetaminophen) in 1893 (Parkhouse, 1979).

Paracetamol has a chemical structure:




and a chemical name:

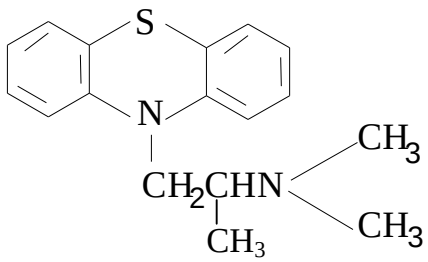
N-acetyl-p-aminophenol.

The aminobenzene structure of the drug is responsible for its antipyretic activity.

Paracetamol is a white odourless crystalline powder, which is soluble in water and alcohol. It has a melting point of 169° to 172°C.

### *2.1.4 Promethazine*

Promethazine is a phenothiazine derivative which acts as an antihistamine. The search for effective antimalarials among the phenothiazine derivatives during the World War II, led to the discovery of some active antihistamines. Further researches were conducted, as the ones initially discovered were very toxic. Promethazine was discovered to be seven times more potent than certain other antihistamines and has been in clinical practice for many years (Taylor et al., 1983). Promethazine has a chemical structure:



and a chemical name:

10-(2'-Dimethylaminopropyl) phenothiazine.

Promethazine is a white or faintly yellow odourless crystalline powder with a melting point of 220°C. It is soluble in water and alcohol but insoluble in ether and ethylacetate. The phenothiazines are used generally as salts (HCI) which are water soluble crystalline powders, the base being water insoluble.

Among the phenothiazine derivatives, the structural features responsible for high antipsychotic potency are: A chain of three atoms between the central ring and a terminal amino group; an electron attracting atom or group such as chlorine, methoxy or trifluoromethyl at C-2 position; and a tricyclic ring system with a six or seven membered central ring.

## 2.2 Pharmacokinetics of chlorpropamide, chloroquine, paracetamol and promethazine

### *2.2.1 Pharmacokinetics of chlorpropamide*

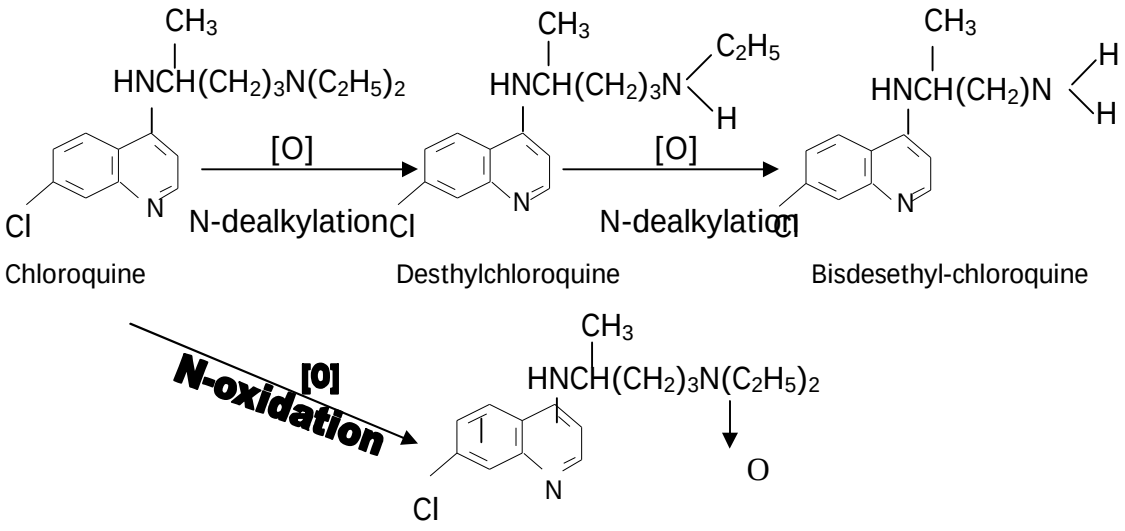
Chlorpropamide is rapidly absorbed from the gastrointestinal tract, and is extensively bound to plasma proteins (about 90%). It is readily detectable in the blood within one hour, reaching a maximum within two to four hours. It is metabolized in the liver and excreted in the urine as the unchanged drug (Martindale, 1989; Gillian, 2001), or as hydroxylated or hydrolysed metabolites. Some of the metabolites include: p-chlorobenzene sulphonylurea, p-chlorobenzene sulphonamide,2-hydroxychlorpropamide and 3-hydroxychlorpropamide (Brotherton et al., 1989 and Taylor, 1988).

Chlorpropamide has a biological half-life that averages about 36 hours, and within 96 hours, 80-90% of a single oral dose would have been excreted. In long-term use, absorption and excretion rates become stabilized, usually in about 5 to 7 days (Jackson, 1989), so preventing accumulation of the drug in the blood.

### *2.2.2 Pharmacokinetics of chloroquine*

The metabolic pathways of chloroquine include:

N-dealkylaiton (mono-and di-), C-hydrocylation and N-oxidation.

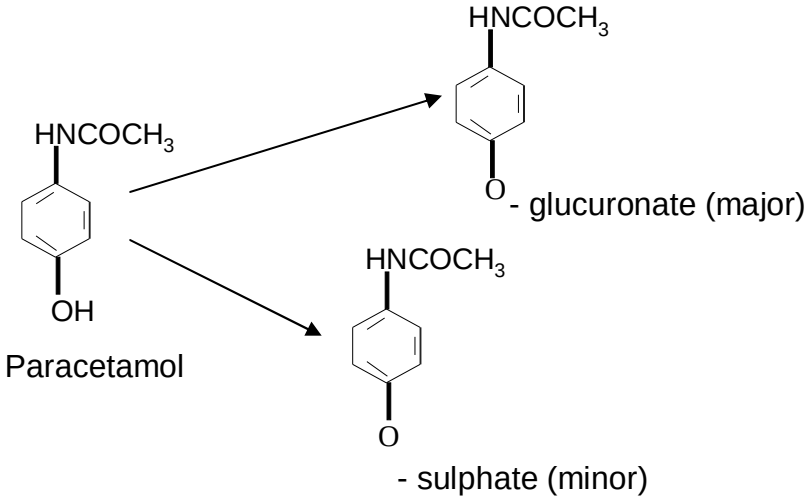



The major metabolites of chloroquine are: desethylchloroquine and bisdesethylchloroquine (Gunnar, et al., 1982).

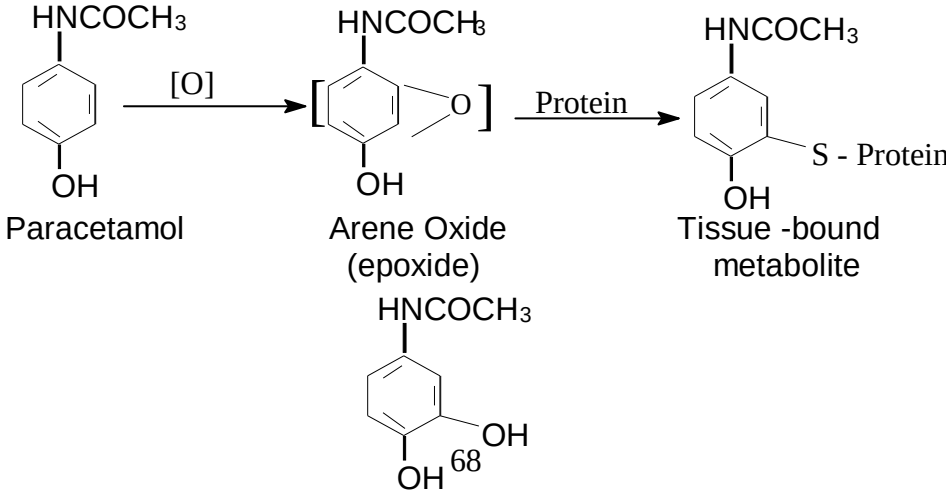
It is rapidly absorbed from the gastrointestinal tract and slowly excreted. The drug has a high volume of distribution with a bioavailablity of about 90 percent. It has a plasma half-life of about 3 to 7 hours and can be detected in the plasma with its metabolites for up to 56 days (walker et al., 1983). Chloroquine binds to serum proteins, RNA and DNA, and a considerable proportion of a dose is retained in the liver, spleen, lungs kidneys (Olaniyi, 1990).

### 2.2.3 *Pharmacokinetics of paracetamol*

The metabolic pathways of paracetamol include glucuronate and sulphate conjugations. The metabolism is primarily by hepatic microsomal enzymes.




The oxidation of paracetamol gives a reactive intermediate, arene oxide (an epoxide), which when bound to protein in the liver leads to hepatotoxicity. The formation of epoxides from polycyclic hydrocarbons is the cause of the well known carcinogenicity of the compounds and their reputation as dangerous environmental pollutants (Swinyard, 1990).

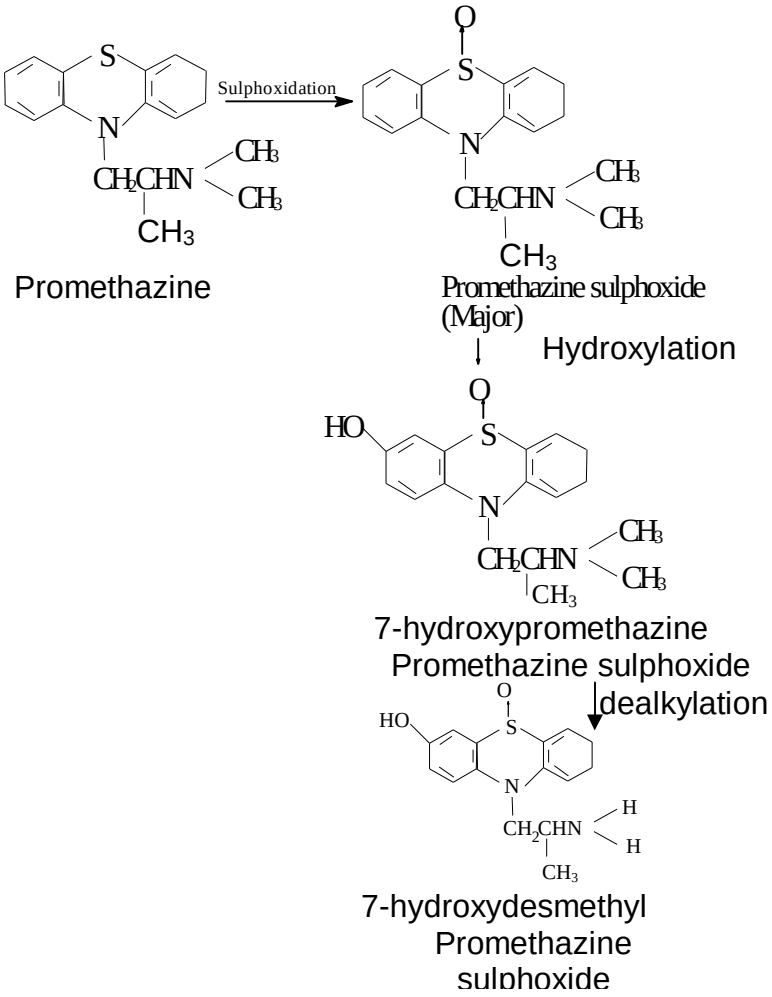


Paracetamol is readily absorbed in the gastrointestinal tract, and has a half-life of about 2 hours. It is uniformly distributed in the body and the plasma concentration reaches a peak within 45 minutes.

### 2.2.4 *Pharmacokinetics of promethazine*

Promethazine is metabolised in the liver and excreted in the urine as metabolites.

In the phenothiazine ring, there is sulphoxidation and hydroxylation in positions 3 and 7. There is also N-oxidation and demethylation (mono- and di-) of the side chains. The metabolites include: Promethazine sulphoxide, 7-hydropromethazine sulphoxide, 7-hydroxydesmethylpromethazine sulphoxide and conjugates, of which promethazine sulphoxide is the major metabolite.




Promethazine is readily absorbed in the gastrointestinal tract. It has a large volume of distribution.

A plasma concentration of 18ng/ml was attained within 3 hours, with 22ng/ml being the maximum plasma concentration. It has an elimination half life of 13 hours (Taylor et al., 1983).

## 2.3 Pharmacological action and mechanism of action of chlorpropamide, chloroquine, paracetamol and Promethazine

### 2.3.1 *Pharmacological action and mechanism of action of chlorpropamide*

chlorpropamide is one of the first category of oral drugs used to treat type 2 diabetes mellitus patient (Non-insulin dependent diabetes mellitus - NIDDM) or maturity onset diabetes (Frier et al., 1999).

It is administered orally, as it is safe, more acceptable, convenient and very effective in lowering blood glucose (Aguwa, 1986; Herman et al., 2004).

The initial dose for the middle-aged stable diabetic patient is 250mg daily. The elderly patients should be started on smaller amounts of chlorpropamide, in the range of 100 to 125mg daily. There is no fixed dosage regimen for the management of diabetes mellitus with chlorpropamide. It is adjusted according to response, with a maximum dose of 500mg daily, taken with breakfast. The patient's

blood glucose must be monitored periodically to determine the minimum effective dose for the patient (Frier et al., 1999).

The primary significant adverse effects of chlorpropamide are hypoglycemia and weight gain. Chlorpropamide may cause facial flushing after drinking alcohol. Other side effects are generally mild and infrequent, this include: gastrointestinal disturbances, headache, nausea, rashes, fever, jaundice, haemolytic anaemia and pancytopoenia (Coniff et al., 2005).

The proprietary name of chlorpropamide is diabinese. Chlorpropamide works by lowering blood sugar. It does this by stimulating the secretion of insulin from the pancreas, thereby increasing the body's sensitivity or response to insulin, and reducing the release of sugar from the liver (Miller and Moses, 2004; Ferner, 1988). Chlorpropamide may also affect glucose levels by several extrapancreatic mechanisms, such as increasing insulin receptor binding affinity, increasing insulin's effect by a past-receptor action, and decreasing hepatic insulin extraction (Skillman and Feldman, 2004; Miller and Moses, 2004).

### 2.3.2 *Pharmacological action and mechanism of action of chloroquine*

Chloroquine is used for the treatment of malaria. It is also used for the treatment of extra intestinal amoebiasis, juvenile rheumatoid arthritis, amoebic hepatitis and abscess, discoid and systemic lupus (Rollo, 1980). It is marketed as chloroquine sulphate (Nivaquine®) or chloroquine phosphate (Araten®, Resochin®, Pfizerquine®, Fevokine® and Avloclor® in the forms of tablets, syrups and injections.

Chloroquine can be administered orally and by injection. When initiating therapy with chloroquine, it is necessary to administer a loading dose that is twice the maintenance dose. This is done to saturate tissue binding and to reach effective blood levels. The usual course of treatment is 1000mg of the effective base followed by 500mg base in 6 hours then 500mg base twice daily for 2 more days (Griffin et al., 1999).

Half of the chloroquine in the plasma is protein bound, and there is the possibility of completion for binding sites on a protein molecule in the presence of another drug that competes for similar binding

sites on the protein. This may lead to a decrease in the amount of bound drug and increasing free drug concentration. This may produce higher blood and tissue levels of free drug and may result in increased clinical effect while the potent drugs, it may lead to serious effects.

After a single therapeutic dose of chloroquine, some of the side effects that may be observed include: abdominal discomfort, nausea, vomiting, headache, dizziness, blurring of vision, mental and physical weakness, fatigue and pruirities, cardiovascular collapse, dyskinesia, neuromuscular disorders, skin eruption and itching.

The mode of action of chloroquine appears to be a primary inhibition of DNA replication and a secondary inhibition of RNA transcription from DNA which mediated through the inhibition of the DNA and RNA polymerase reactions. Currently, chloroquine is believed to act by being schizontocide against the plasmodium parasites.

### 2.3.3 *Pharmacological action and mechanism of action of paracetamol*

Paracetamol is used as an analgesic and antipyretic. It can also be used as an anti-inflammatory and antirheumatic.

Paracetamol is mostly administered orally in the forms of tablets and syrups. The dosage is Ig (2 x 500mg tablets) every 8 hours.

The side effects of paracetamol include haematological reactions and skin rashes. The symptoms of paracetamol over-dosage include pallor, nausea, vomiting, anorexia, and abdominal pain. Liver damage may become apparent 12 to 48 hours after ingestion.

Some of the proprietary names of paracetamol include Panadol, Acetaminophen and Beserol.

Paracetamol acts peripherally by causing the inhibition and release of prostaglandin synthesis in the cells (Parkhouse, 1979).

### 2.3.4 *Pharmacological action and mechanism of action of promethazine*

Promethazine is used for the symptomatic relief of urticarial lesions, seasonal rhinitis, angioneuretic oedema, hay fever, sneezing, rhinnorrhoea and itching. It is also sued for allergic conditions,

parkinsonism, nausea, vomiting and sedation, antipsychotic, muscle relaxant, antiadrenergic, and anticholinergic.

It is administered orally and by injection. Promethazine is marketed in the forms of tablets (10mg, 25mg), elixir (5mg/5ml) and injection with a popular proprietary name as phenergan. A single therapeutic dose of promethazine HCI is 25mg (BNF, 1988).

The sedative effect of promethazine HCI may be potentiated by alcohol and certain other drugs which depress the central nervous system including tricyclic antidepressants (Macris and Levy, 1985). Some of the side effects include dizziness, drowsiness, hypotension, drymouth, muscle weakness, sedation, gastrointestinal disturbance such as nausea, vomiting, diarrhea or constipation, headache, blurred vision, tinnitus, depression, irritability, nightmares and anorexia. Paradoxical stimulation may be seen in some patients. Promethazine displays maximum antihistamine action, and it has a very powerful long-lasting antihistaminic activity.

It acts by occupying the receptor site to prevent or abolish the pharmacological effects of histamine in the body. This it does by competing with the histamine for the receptor site (Keeri-Szanto, 1994).

## 2.4 Drug-drug interaction studies

Oral hypoglycaemic drugs have the potential to interact with other medications and if the result is hypoglycaemia or hyperglycaemia the consequences can be serious. The interaction may be pharmacodynamic (another drug independently raises or lower blood glucose) or pharmacokinetics (another drug alters the absorption, metabolism or excretion of the hypoglycaemic drug).

Chlorpropamide has substantial renal excretion. It is excreted much more rapidly in alkaline urine, so its half-life and duration of action are reduced with excessive ingestion of alkalis. Antacids may increase the absorption of all the sulphonylureas and hence produce higher peak concentrations of the drugs and a risk of temporary hypoglycaemia (Gillian, 2001).

Sulphonylureas are highly protein bound drugs and may be displaced from blood protein binding sites by drugs such as the non-steroidal anti-inflammatory drugs. This can cause a short-term increase in free (unbound) sulphonylurea and hence temporary hypoglycaemia (Skillman and Feldman, 2004).

Magnesium supplements increase the absorption of Chlorpropamide (Pronsky, 2006).

The citrates of potassium, calcium and magnesium decrease the blood levels and therapeutic effects of Chlorpropamide (Adams, 2006). According to the study carried out by Neuvoneu et al (2004), on the effect of enzyme induction and pH on the pharmacokinetic of Chlorpropamide in healthy and epileptic patients; the half-life of Chlorpropamide was shorter in the patients (34.4 h) than in the healthy volunteers (50.2h). The clearance and volume of distribution of total chlorpropamide were higher in the patients (2.99m/$h^{-1}$ $kg^{-1}$ and 126ml $kg^{-1}$) respectively, than in the healthy volunteers (1.60ml$h^{-1}$ $kg^{-1}$ and 106ml $kg^{-1}$) respectively. The unbound fraction of chlorpropamide in serum was also higher in the patients (5.7%) than in the healthy subjects (4.4%). The renal clearance of chlorpropamide correlated well with urine pH and was almost one hundred-fold higher at pH 7 than at pH 5.

The absorption of chlorpropamide is higher when it is administered in fasting state than when it is concomitantly administered with food (Bakare, 1992). It was reported by Kivisto and Neuvonen (1992) that 850mg magnesium hydroxide increased the rate of absorption of 250mg of chlorpropamide in normal subjects, but the insulin and glucose response were unaffected.

Johnson et al (1990) observed that chlorpropamide and lovastatin serum levels were unchanged when lovastatin was used (20mg twice daily for 6 weeks) in a study on chlorpropamide. Aslam and Stockley (1999) also observed that the hypoglycaemic effects of chlorpropamide could be increased by the concurrent use of Karela (Momordica Charantia).

Petit Pierre et al (1992) in their work observed that the hypoglycemic effects of tolbutamide and chlorpropamide were increased by the concurrent use of chloramphenicol.

Petit Pierre et al (1992) also reported of six patients who were given chlorpropamide and allopurinol concurrently. The half-life of chlorpropamide in one patient with gout but normal renal function exceeded 200h (normal 36h) after taking allopurinol for 10 days. In two others the half-life was extended to 44 and 55h respectively. The other three patients were given allopurinol for only one or two days, and the half-life of chlorpropamide remained unaltered.

There are some drugs that potentiate the hypoglycemic action of chlorpropamide, such drugs include salicylates, probenecid, monoamine oxidase inhibitors, sulfonamides, coumarins, beta

adrenergic blocking agents, non steroidal anti inflammatory agents and chloramphenicol. When any of such drugs are administered with chlorpropamide to a patient, the patient should be observed for hypoglycemia. The patient should also be observed for loss of control when any of such drugs are withdrawn (Rx List, 2006).

Some drugs tend to produce excess of sugar in the blood (hyperglycaemia), such drugs include phenothiazines, phenytoin, thyroid products, estrogens, oral contraceptives, sympathomimetics, diuretics, isoniazid, nicotinic acid, calcium channel blocking drugs, corticosteroids and thiazides. When any of such drugs are administered with chlorpropamide to a patient, the patient should be closely observed for loss of control. The patient should also be observed closely for hypoglycemia when any of such drugs are withdrawn (RxList, 2006). Back et al., (1984) reported that when chloroquine and oral contraceptives (ethinyloestradiol + norethisterone) were concurrently administered, the chloroquine serum level remains unchanged and the efficacy of the drugs (chloroquine, ethinyloestradiol and norethisterone) was unaltered. McElnay, et al (1992) reported.that when six normal subjects were given 1g chloroquine with 1g magnesium trisilicate or 1g Kaolin

after an overnight fast, the magnesium trisilicate reduced the *AUC* (area under the curve) of the chloroquine by 18.2% and the Kaolin reduced the absorption of single 1g doses of oral ampicilin by about a third (from 29 to 19%) in seven normal subjects.

When chloroquine is taken together with antacid, the absorption of chloroquine is reduced (Bruce-Chwatt et al., 1986).

Kerri-Szanto (1974) reported that the analgesic requirement of more than 300 patients treated with a variety of narcotic analgesics (morphine, petithine, oxymorphone, hydromorphone, fentalyl, pentazocine) were reduced (28-44%) when they were given promethazine. 50mg/70kg body weight.

According to Macris and Levy (2005) the concurrent use of pentobarbitone, promethazine and hyoscine increases agitation, and it has been suggested that the triple combination should be avoided. Murphy et al (1990) reported that the toxicity of paracetamol was increased by isoniazid, so that the normal daily analgesic (4g) may not be save in some individuals.

The administration of paracetamol an hour after the ingestion of cimetidine, impaired the absorption of paracetamol (Garba et al., 1998).

Welting (1980) reported that the rate of paracetamol absorption was reduced by propantheline. 30mg propantheline IV delayed the peak serum levels of paracetamol (1.5g) in six convalescent patients from about 1h to 3h. Peak concentrations were lowered by about a third, but the total amount of paracetamol absorbed was unchanged (Nimmo, et al.,1993).

It was observed by Miners et al (1984) that the clearance of paracetamol was increased (46%) by phenytoin and carbamazepine. Nimmo et al (1993) reported that the rate of absorption of paracetamol was increased by metaclopramide.

Mitchell et al (1983) observed that the plasma clearance of paracetamol in seven women taking oral contraceptives was increased by 63%, from 287 to 470ml/min, following a single 1.5g dose and the elimination half-life decreased by 43%, from 2.40 to 1.67h, when compared with women not taking oral contraceptives.

### 2.4.1 *Controlling adverse drug interactions*

Drug interactions may be harmless or potentially harmful. The severity of an interaction varies from one patient to another. Drugs with a small therapeutic ratio (e.g phenytoin) and those which require careful control of dosage (e.g anticoagulants, antihypertensives, and antidiabetics) are most often involved.

In some prevention regimens, drug interaction may even cause more harm than good. For example, one drug might reduce blood levels of another drug, leading to the development of drug resistance. In other words, drug interaction could result in the development of a disease unresponsive to standard treatment. The added toxicity of numerous combined therapies may also outweigh their potential benefit for preventing disease.

Patients at increased risk from drug interactions include the elderly and those with impaired renal or liver function.

The simple way of controlling or avoiding adverse drug interactions is to avoid combinations that are known to be dangerous. If that is not possible, the dosage of the object drug should be reduced in advance of starting the precipitant drug and the precipitant drug

should be introduced slowly. When a theoretical interaction is anticipated on the basis of the known properties of two drugs, even if it has not been previously described, careful monitoring will help recognize adverse effects early. It is important to discuss potential drug interactions with a health care provider and a pharmacist. Before starting a new therapy, you have to consider the potential drug interactions and side effects. Drug interactions can be managed by monitoring carefully, adjusting the doses, or discontinuing the therapy as needed.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 *Drugs*

Chlorpropamide (Diabinese $^{R}$) tablets.

Manufactured by Neimeth International Pharm. Plc,

Plot 16, Akanni Doherty Layout, Oregun, Lagos,

Nigeria.

Lot No: 42417006

Manufacturing date 07-2006

Expiring date 07-2009

Paracetamol tablets.

Manufactured by Emzor Pharmaceutical industries Ltd,

Plot 3c Block A, Aswani Market Road,

Isolo, Lagos, Nigeria.

Batch No: 63906

Manufacturing date 08-2006

Expiring date 07-2009

Promethazine HCI tablets.

Manufactured by Vardhman Exports Ghatkopar,

(W), Mumbai -400086, A- 188, MIDC,

Kharine Nouvi Mumbai -400701.

Batch No: P - 19, M.L. No: KD - 2006

Manufacturing date 03-2006

Expiring date 04-2009

Chloroquine tablets.

Manufactured by Fidson Healthcare Ltd, Lynson chemical avenue,

KM. 38, Lagos - Abeokuta Expressway, Sanyo, Ota, Ogun State, Nigeria.

Batch No: TA 32041

Manufacturing date 05-2006

Expiring date 06-2009

Chlorpropamide Standard Powder.

Supplied by Neimeth International Pharm. Plc,

Plot 16, Akanni Doherty Layout, Oregun, Lagos,

Nigeria.

Batch No: 62017588 ,

Chloroquine Standard Powder.

Supplied by May & Baker Nigeria, Plc, 3/5

Sapara street, Industrial Estate, P.M.B. 21049,

Ikeja Lagos, Nigeria.

Batch No: 14689

Paracetamol Standard Powder.

Supplied by May & Baker Nigeria, Plc, 3/5

Sapara street, Industrial Estate, P.M.B. 21049,

Ikeja Lagos, Nigeria.

Batch No: 56712

Promethazine Standard Powder.

Supplied by May & Baker Nigeria, Plc, 3/5

Sapara street, Industrial Estate, P.M.B. 21049,

Ikeja Lagos, Nigeria.

Batch No: 476125

Tolbutamide Standard Powder.

Supplied by Cox Pharmaceuticals, Whiddon valley,

Barnstable, North Devon Ex 32 8NS, UK.

Batch No: 45670

### *3.1.2 Equipment*

High Performance Liquid Chromatography (HPLC) Model
CE 1200 with
CE1100 Liquid Chromatography pump CECIL 1100 series.
CE1200 High Performance variable wavelength detector, model CECIL 1100 series.

Ultrasphere ODS column, 4.6mm x 25cm, USA, Part no 235329.

235a Column Temperature controller WATLOW.
HPLC injector (25μl, 50μl, 100μl), Hamilton Reno Nevada, the measure of Excellence,
P.O. Box 10030, UK.
Epson FX - 880 Printer,
Model No.P9804, Seiko Epson Corp.

Spectrophotometer Model SU 1103 series, 320 - 1000nm,
SL Hillsdale Monhein, Germany.

Spectrophotometer Model UV 1100 series
Jouan centrifuge, model 11175600, series No. 03140214, K,
Vulvan Laboratory Pven, LIE Scientific, UK.

Blood bank refrigerator, LEG, REF 2140, SC24

Ringdrift Scientific Laboratory supplies, UK.

Refrigerator (Philips, England)

Bibby Rotary Evaporator, RE 100, made in UK by Bibby sterlin

Ltd, stone Stafford shiree, England, STI505A model RE 100B

Vortex mixer.

Vortex Genie - 2

Made in USA by Scientific industries, Inc. Bohemia,

N.V, 11716, USA. Model - G - 560E. serial No. 85376.

Ultrasonic bath, BAT 2013, U500D, digital

Ringdrift Scientific laboratory supplies, UK.

pH meter (digital), Philips, England.

Melting point Apparatus (UK)

Eureka dissolution Apparatus and accessories,

Eureka Appateban GMBH 6056, Heusenst- amm Kr.

Offenbuch - Main.

Distilling machine, Jencons Scientific Ltd, Cherry courtway Industrial Estate, stranbridge Road, Leighton, Buzzard.

Water bath, Giant, BAT 3540, RS14, W14, Ringdrift Scientific laboratory supplies, UK, Ringdrift services Ltd, 4/6 High street, long Eaton, Nottingham, NG101GH, UK.

Analytical balance, HM series, BAL 2384,
Ringdrift services, Ltd, UK.

Analytical balance, Type AE 240 mettler Instruments Ltd, Kingsmead House. Abbey Barn Raod, High Wycombe Buckinghamshire, England.

CECIL 9000 series Ultraviolet spectrophotometer, Aquarius double beam, England.

Genesis II FTIR™ Infrared, Mattson, England.

### 3.1.3 *Reagents*

| | | |
|---|---|---|
| Acetonitrile | - | Hipersolv' for HPLC, BDH chemicals Ltd, Poole, England. |
| Methanol | - | Hipersolv for HPLC, BDH chemicals Ltd, Poole, England. |
| Acetic acid | - | Analar grade, BDH chemicals Ltd, Poole, England. |
| Ethanol | - | Analar grade, BDH chemicals Ltd, Poole, England. |
| Choroform | - | Hipersolv', sigma chemicals. |
| Nitric acid | - | Analar grade, BDH chemicals Ltd, Poole, England. |
| Hydrochloric acid | - | Analar grade, BDH chemicals Ltd, Poole, England. |
| Diethylether | - | Analar grade, May & Baker Ltd, Dagenham |
| Acetone | - | Analar grade, May & Baker Ltd, Dagenham |
| Sulphuric acid | - | Analar grade, BDH chemicals Ltd, Poole, England |

Dichloromethane - Analar grade, BDH chemicals Ltd, Poole, England

Perchloric acid - Analar grade, sigma chemicals..

Phenol - Analar grade, Hopkin & Willian, Chawell Health, Essex England.

Benzoic acid - GPR, sigma chemicals.

Orthophosphoric acid - Analar grade, BDH chemicals Ltd, Poole, England.

Formic acid - Analar grade, sigma chemicals.

Ethylacetate - Analar grade, BDH chemicals Ltd, Poole, England.

Hexane - 'Hipersolv' for HPLC, BDH chemicals Ltd, Poole, England.

Sodium hydroxide pellets - Analar grade, BDH chemicals Ltd, Poole, England.

Sodium acetate - GPR, Sigma chemicals.

Sodium chloride - GPR, BDH chemicals Ltd, Poole, England

Sodium nitrate - GPR, BDH chemicals Ltd, Poole, England

Sodium carbonate - GPR, BDH chemicals Ltd, Poole, England.

Potassium dihydrogen orthophosphate - Analar grade, Sigma chemicals.

Barium chloride - GPR, BDH chemicals Ltd, Poole, England

Potassium dichromate - GPR, BDH chemicals Ltd, Poole, England.

Ammonium dihydrogen phosphate - GPR, BDH chemicals Ltd, Poole, England.

Potassium carbonate - GPR, BDH chemicals Ltd, Poole, England.

Silver nitrate - GPR, BDH chemicals Ltd, Poole, England.

Disodium hydrogen phosphate - GPR, BDH chemicals Ltd, Poole, England.

Sodium dihydrogen phosphate - GPR, sigma chemicals.

### 3.1.4 *Glasswares*

| | |
|---|---|
| Conical flasks | (250ml, 500ml, 1000ml) |
| Volumetric flasks | (10,50, 100,250,500, 1000ml) |
| Pipettes | (0.5ml, 1ml, 2ml, 5ml, 10ml) |
| Beakers | (50ml, 250ml, 500ml,) |
| Measuring cylinders | (10,250,500ml) |
| Extraction tubes | (10ml) |
| Screw -capped sample bottles | (20ml) |
| EDTA bottles | (5ml) |

| | |
|---|---|
| Cannular | (21g) |
| Heparinized sample bottles | (10ml) |
| Bijou bottles | (7ml with Al. cap) |
| Syringes and needles | (5ml) |
| Eppendorf pipette | (1ml) |
| Handy step dispenser tip filter | |
| Glass Pasteur pipettes | (Mexcel) |
| Disposable pipettor tips | (Plastibrand) |
| Filtration flask | (Buchner) |
| Borosilicate glass funnel | |
| Pipette filter | (Standard model) |

## 3.2 Methods

### 3.2.1 *Identification tests for chlorpropamlde. chloroquine. paracetamol and promethazine tablets.*

*3.2.1.1 Chlorpropamide*

An equivalent weight of 1g of Chlorpropamide from powdered chlorpropamide tablets was extracted with five 4ml of acetone. This was filtered and evaporated to dryness on a water bath. The residue was subjected to the following tests:

i. 0.1g of the chlorpropamide residue was boiled with 8ml of a 50% (w/w) solution of sulphuric acid under a reflux condenser for 30 minutes. The residue was cooled and filtered. The melting point of the residue was determined after it has been recrystallized with water and dried.

ii. The filtrate from test (i) above was made alkaline with 5M sodium hydroxide solution and then heated.

iii. 0.1g of the chlorpropamide residue was heated with 1g of anhydrous sodium carbonate for 10 minutes and cooled. This was later extracted with water and then filtered. The filtrate was acidified with 2M nitric acid and then silver nitrate solution was added to it.

*3.2.1.2 Chloroquine*

(i) An equivalent weight of 0.1 g of chloroquine from powdered chloroquine tablets was added to a mixture of 10ml of water and 1ml of 2M hydrochloric acid. This was shaked for 10 minutes and filtered. 1ml of barium chloride solution was added to 2ml of the filtrate.

(ii) The melting point of the residue was determined.

*3.2.1.3 Promethazine hydrochloride*

i. To an equivalent weight of 5mg of promethazine hydrochloride from powdered promethazine hydrochloride tablets was added 5ml of sulphuric acid. This was allowed to stand for 5 minutes.

ii. An equivalent weight of 0.2g of promethazine hydrochloride from powdered promethazine hydrochloride tablets was dissolved in 2ml of water. This was filtered and saturated with 2ml of potassium carbonate sesquihydrate. It was then extracted with two 10ml of ether. The extracts were evaporated to dryness. The residue was dissolved in 2ml of methanol, this was then poured into a solution of 0.4g of picric acid in 10ml of methanol at about 50°c. This was cooled and the side of the container was scratched to induce crystallization. It was allowed to stand for 4 hours and then filtered. The melting point of the crystals obtained was determined.

*3.2.1.4 Paracetamol*

i. 1ml of hydrochloric acid was boiled with an equivalent weight of 0.1 g of paracetamol from powdered

paracetamol tablets for 3 minutes. 10 ml of water was added and cooled. 0.05ml of 0.0167 potassium dichrome was added.

ii. The melting point of the extracted paracetamol from the powdered paracetamol tablets was determined.

### 3.2.2. *Chemical Assay of chlorpropamide, chloroquine, paracetamol, and promethazine tablets, and their reference standard powders*

*3.2.2.1 Paracetamol tablets*

Twenty tablets of paracetamol were weighed individually and powdered.

0.1259g of the powdered paracetamol tablets, equivalent to 120mg of paracetamol, was weighed and transferred into a 1000ml volumetric flask and 10ml of methanol was added. This was shaken for 5 minutes. The volume was made up to mark with freshly distilled water. A standard solution of paracetamol of the same concentration (12μg/ml) was prepared.

The absorbance of both solutions were concomitantly determined in the same medium, at a concentration of about 12 μg/ml in 1cm cells, at the wavelength of maximum absorbance at about 244nm with a suitable spectrophotometer, using freshly distilled water as the blank. The quality in mg, of $C_8H_9NO_2$ in the paracetamol taken was calculated using the formula 10C(Au/As), where C is the concentration in μg/ml of paracetamol in the standard solution, and Au and As are the absorbancs of the solution of paracetamol and the standard solution respectively.

*3.2.2.2 Chlorpropamide tablets*

Twenty tablets of Chlorpropamide were weighed individually and powdered.

An equivalent weight of 0.25g of chlorpropamide from powdered chlorpropamide tablets was transferred into a 50ml volumetric flask. To this was added 40ml of methanol and this was shaken for 20minutes. Sufficient methanol was added to make up to mark and this was well mixed and filtered. 5ml of the filtrate was pipetted into a 100ml volumetric flask and this was diluted to mark within 0.1 M hydrochloric acid. 10ml of

this solution was pipetted into hydrochloric acid. 10ml of this solution was pipetted into a 250ml volumetric flask, and this was diluted to mark with O.IM hydrochloric acid.

The absorbance of the resulting solution was measured at a maximum of 232nm. 0.25g of the reference standard of chlorpropamide powder was also diluted the same way as for the tablets. The absorbance of its resulting solution was measured. The absorbance of the reference standard and the test samples were compared by using the formula.

$$\frac{\text{Absorbance of test sample}}{\text{Absorption of reference sample}} \times 100$$

*3.2.2.3 Chloroqinue tablets*

Twenty tablets of chloroquine sulphate were weighed individually and powdered.

0.3696g of the powdered chloroquine sulphate tablets, equivalent to 0.25g of chloroquine, was weighed and transferred into a 1000ml volumetric flask. 5ml of freshly distilled water was added and shaken for 10 minutes. This was made up to mark with dilute hydrochloric acid and filtered.

10ml of the solution was pippetted into 100ml volumetric flask and diluted to mark with dilute hydrochloric acid to obtain a solution of 10μg/ml.

A standard solution of chloroquine reference powder of the same concentration was prepared. The absorbance of both solutions in 1cm cells, were concormitantly determined at the wavelength of maximum absorbance at about 343nm, using dilute hydrochloric acid as the blank.

The quality in mg of $C_{18}H_{26}$ $CIN_3$, $H_2SO_4.H_20$ in the portion of chloroquine sulphate taken was calculated using the formula 10C(Au/As),, where C is concentration in μg/ml of chloroquine sulphate in the standard solution, and Au and As are the absorbance of the solution of chloroquine sulphate and the standard solution, respectively.

*3.2.2.4 Promethazine tablets*

The procedure was carried out with the drug being protected from light.

Twenty tablets of promethazine hydrochloride were weighed individually and powdered. An equivalent weight of 25mg of

promethazine hydrochloride from powdered promethazine hydrochloride tablets was transferred into a 500ml volumetric flask. To this was added 10ml of 2M hydrochloric acid and shaken. 200ml of water was added and shaken for 15 minutes. Sufficient water was added to make up to mark. About 50ml of the mixture was centrifuged ,at 2000g for 10 minutes. To 5ml of the clear, supernatant liquid was added 10ml of 0.1 M hydrochloric acid in a 100ml volumetric flask. Sufficient water was added to make up to mark. The absorbance of the resulting solution was measured at a maximum of 249nm.

0.25g of the reference standard of promethazine powder was also diluted the same way as the tablets. The absorbance of its resulting solution was also measured. The absorbance of the reference standard and the test samples were compared by using the formula:

$$\frac{\text{Absorbance of test Sample}}{\text{Absorbance of reference sample.}} \times 100$$

### *3.2.3 Dissolution rate tests for chlorpropamide tablets*

1000ml of 0.68% (w/v) solution of potassium dihydrogen orthophosphate was used as the dissolution medium. The pH of the medium was adjusted to 6.8 by the addition of 1M sodium hydroxide. The dissolution medium was transferred into a vessel which was immersed in a water bath maintained at a constant temperature of 37°c. One tablet of chlorpropamide was introduced into the basket in a dissolution apparatus, and this was lowered into position.

The basket was rotated at 100 revolutions per minute. At 45minutes, 10ml of the sample was withdrawn from a point half way between the surface of the dissolution medium and the top of the rotating basket.

The collected sample was filtered and diluted, and its absorbance was measured at the maximum of 230nm. 250mg of the standard reference powder of chlorpropamide was diluted as was done for the solution of the tablets. The absorbance of the resulting solution was measured at the maximum of 230nm.

The absorbance of the reference powder was compared with the absorbance of the tablet and the percentage of the dissolved drug in 45minutes, was calculated using the formula given below:

$$\frac{\text{Absorbance of dissolved drug}}{\text{Absorbance of reference powder}} \times 100$$

### *3.2.4 Solvent development an optimization studies*

Some mobile phases were tried to develop the one that was used for this work.

The components of the mobile phase were premixed before use. For the separation of the drugs, different columns and solvents were varied. The columns tried were: Ultrasphere C18 columns (75cm x 4.6mm), ultraspehere ODS column (25cm x 4.6mm), and ultrasphere 3μm column (75cm x 4.6mm). The flow rate of the mobile phase was varied.

For the mobile phase acetonitrile-water, the content (%,v/v) of the components were varied and the effect of pH of the mobile phase on the suitable ratio of the solvent system was

studied, to ascertain the optimum composition. Chromatographic conditions include:

Flow rate = 1.0ml/min

Mobile phase = Acetonitrile-water

60 : 40

pH of the mobile phase = 6.7

Pressure = 410 bars

Sensitivity = 1

The Components of the mobile phase were varied in order to optimize the mobile phase by (i) increasing the Acetonitrile component; (ii) increasing the water component.

The resolutions of the peaks were calculated using the relationship below (Wilson and Walker, 2000).

$$\text{Peak resolution (Rs)} = \frac{2\Delta t}{W_A + W_B}$$

Where $\Delta t$ = The difference in seconds of peak separation at the up of the two peaks.

$W_A$ & $W_B$ = The width of peak A and B measured at the base of the peaks.

The pH of the mobile phase was optimized by varying pH of the mobile phase.

### 3.2.5 *Extraction recoveries*

1ml of 50 μg/ml of chlorpropamide was mixed with 1ml of 50μg/ml of the internal standard (Tolbutamide). 25μl of the mixture was injected on to the column.

1ml of blank plasma was pipetted into a 12ml capped centrifuge tube, and to this were added 1ml of 50μg/ml chlorpropmide, 1ml of 50μg/ml internal standard (Tolbutamide,) 50μl of copper (ii) tetraoxosulphate (vi) solution and 2.5ml of Acetonitrile. These were vortex mixed for 10 seconds and then centrifuged for 3 minutes at 2000g. 25μl of the clear supernatant upper layer was injected into the column.

The percentage of extraction recoveries of chlorpropamide was calculated by applying the formula given below:

$$\frac{\text{Peak height ratio of the extracted chlorpropamide}}{\text{Peak height ratio of the unextracted chlorpropamide}} \times 100$$

The extraction procedure described above was then repeated with a concentration range of 5-50μg/ml for chlorpropamide while that of tolbutamide (IS) remained constant (50μg/ml)

### 3.2.6 *Preparation of standard solutions*

*Stock solution of Tolbutamide*

100mg of Tolbutamide was weighed and dissolved in 100ml volumetric flask using acetonitrile to prepare 1mg/ml stock solution. Subsequent dilutions were made from this solution.

*Stock solution of Chlorpropamide*

50mg of chlorpropamide was weighed and dissolved in 50ml volumetric flask using acetonitrile to prepare 1mg/ml stock solution. Subsequent dilutions were made from this solution.

*Copper (ii) tetraoxosulphate (vi) solution (5%)*

50 mg of copper (ii) tetraoxosulphate (vi) was weighed and dissolved in 100ml volumetric flask with distilled water.

*Buffer solution for dissolution rate tests*

6.8g of potassium dihydrogen orthophosphate was weighed into a beaker and dissolved with 100 ml of distilled water. This was carefully transferred into a 1000ml volumetric flask with rinsings and then made up to mark.

The solution was warmed to 37°c and the pH was adjusted to pH 6.8 with 1M sodium hydroxide.

*Sodium hydroxide solution (1M)*

4g of sodium hydroxide pellets was weighed into a beaker and dissolved with 30ml of distilled water. This was carefully transferred into a 100ml volumetric flask, with rinsing and then made up to mark.

### 3.2.7 *The pharmacokinetic studies of chlorpropamide in healthy human subjects*

*3.2.7.1 Subjects*

Eighteen (18) healthy male adults human subjects volunteered for the study. The volunteers were of a height range of 1.20m to 1.85m, mean ±S.D 1.52 (± 0.04)m and a weight range of 60kg to 75kg, mean ± S.D. 68 (± 0.83)kg. They were of the age range of 22 to 37 years mean ± S.D 29 (± 0.83) years.

*3.2.7.2 Study design*

The protocol of the study was explained to the volunteers. No drugs, alcohol or kolanut was allowed two weeks before the

study. Each of the volunteers fasted overnight before the study, few minutes of relaxation was allowed on the day of the study. A cannula was then inserted in the anticubital vein for the earlier samplings. 5ml of blood was withdrawn from each volunteer. The eighteen (18) subjects were grouped into three of six subjects each. In the first phase (A) of the study, the volunteer were orally administered with one 250mg chlorpropamide tablet with 200ml of water. The volunteers were allowed to eat after three hours of the study.

The blood samples were collected from the volunteers at the following time interval:

0.5, 1,2, 4,6, 8, 12, 24, 36, 48, 72, 144, 168, hours. Plasma was obtained by centrifuging blood samples at 2000g for 10 minutes, after allowing them to stand for sometime.

The plasma was transferred into some bottles with Pasteur pipettes and stored in a deep freezer for analysis.

For the second phase (B) of the study, the first group were orally administered with one 250mg chlorpropamide tablet and 4 tablets of 250mg chloroquine.

The second group were administered with 250mg chlorpropamide tablet and 2 tablets of 500mg paracetamol. The third group was administered with one 250mg chlorpropamide tablet and one 25mg Promethazine tablet.

Blood samples were collected and treated as described above.

### *3.2.8 Plasma drug level analysis*

#### *3.2.8.1 Preparation of calibration curve*

1ml of blank plasma was pipetted into a 10ml capped centrifuge tube. To this were added 1ml of the required concentration of chlorpropamide (5μg/ml, 10μg/ml, 20μg/ml, 25μg/ml, 30μg/ml, 35μg/ml, 40μg/ml. 1ml of tolbutamide (50μg/ml), 50μl of copper (ii) tetraoxosulphate (vi) solution (5%) and 2.5ml and of acetonitrile. These were vortex mixed for 30 seconds and centrifuged for 3 minutes at 2000g. 25ul of the supernatant layer was injected on to the column.

A graph of peak height ratio against concentration was generated.

*3.2.8.2 Precision*

To 1ml of blank plasma each were added 1ml of different concentrations of chlorpropamide and 1ml of the internal standard, Tolbutamide (50μg/ml).

The drug was extracted by applying the extraction procedure. The coefficient of variation (C.V) at five different concentrations of chlorpropamide (2, 10, 20, 30 and 40 μg/ml) were studied, within-run and day-to-day run.

*3.2.9* ***Data analysis***

Following the single oral dose of chlorpropamide and co-administered drugs, the pharmacokinetic parameters were calculated using a non-compartmental open model (Winnonlin computer software). Results were expressed as standard error of mean (±SEM) and were analyzed for statistical significance by student's t-test for paired data. The difference among the data were compared by analysis of variance (ANOVA), and the significant points were further analyzed using the least square difference (LSD) where applicable.

# CHAPTER FOUR

# RESULTS

## 4.1 Identification tests for chlorpropamide, chloroquine, paracetamol and promethazine tablets

### *4.1.1 Chlorpropamide tablets*

The melting point of dried chlorpropamide, which was extracted from chlorpropamide tablets and recrystallized, was $143^0$C. The filtrate of drug obtained following treatment with 5M NaOH solution produced an ammonical odour. Extracted drug heated with anhydrous sodium carbonate produced a white precipitate on treatment with silver nitrate solution.

### 4.1.2 *Chloroquine tablets*

The filtrate of drug obtained following treatment with 2M hydrochloric acid produced a white precipitate on treatment with barium chloride solution.

### 4.1.3 *Paracetamol tablets*

The melting point of paracetamol extracted from paracetamol tablets was $169^0$C. No precipitate was produced when

powdered tablets of paracetamol was treated with hydrochloric, but when potassium dichromate solution was added, a violet colour was produced.

### *4.1.4 Promethazine tablets*

The melting point of Promethazine hydrochloride which was extracted from powdered tablets of Promethazine hydrochloride was $220^0$C.

A red colour was produced when the powdered tablets of Promethazine hydrochloride was treated with sulphuric acid.

## 4.2 Chemical assay of chlorpropamide, chloroquine, paracetamol and promethazine tablets, and their reference standard powders

### *4.2.1 Chlorpropamide tablets*

The chlorpropamide tablets contained 99.08 $\pm$ 0.002% of the active ingredient, while the chlorpropamide reference standard powder contained 100.20 $\pm$ 0.003%. This was in conformity with the B.P (1993) specification of drug content (92.50 – 107.50) %.

The results are shown in Table 1.

### *4.2.2 Chloroquine tablets*

The chloroquine tablet contained 100.00 $\pm$ 0.001% of the active ingredient, while the reference standard powder contained 103.50 $\pm$ 0.02%. This complied with the B.P. (1993) specification of drug content (92.50 – 107.50)%. The results are tabulated in Table 2.

### *4.2.3 Paracetamol tablets*

The paracetamol tablets contained 98.33 $\pm$ 0.003% of the active ingredients, while the reference standard power contained 100.00 $\pm$ 0.003%. The percentage of the active ingredient contained in the tablets and the reference standard powder was in conformity to the B.P (1993) specification of drug content (98 – 101)%. The results are shown in Table 3.

### *4.2.4 Promethazine tablets*

The Promethazine hydrochloride tablets contained 99.15% of the active ingredients; the reference standard powder contained 100.00 $\pm$ 0.001%. The percentage of the active ingredient complied with the B.P (1993) specification of drug content (90 – 110)%. The results are presented in Table 4.

Table 1: Quantitative Determination of chlorpropamide tablets and standard powder

| | **Drug contents (mg)** | **% ± S.E.M** |
|---|---|---|
| Chlorpropamide tablets | 247.70 | 99.08± 0.002 |
| Chlorpropamide standard powder | 250.50 | 100.20 ± 0.003 |

Table 2: Quantitative determination of chloroquine tablets and standard powder

| | Drug content (mg) | % ± S.E.M |
|---|---|---|
| Chloroquine Tablets | 250.00 | 100.00 ± 0.001 |
| Chloroquine standard powder | 258.75 | 103.50± 0.002 |

Table 3: Quantitative determination of Paracetamol tablets and standard powder

| | Drug content (mg) | % ± S.E.M |
|---|---|---|
| Paracetamol Tablets | 491.65 | 98.33 ± 0.003 |
| Paracetamol standard powder | 500.00 | 100.00± 0.003 |

Table 4: Quantitative determination of Promethazine tablets and standard powder

| | Drug content (mg) | % ± S.E.M |
|---|---|---|
| Promethazine Tablets | 24.79 | 99.15 ± 0.004 |
| Promethazine standard powder | 25.00 | 100.00± 0.004 |

## 4.3 Dissolution rate test of chlorpropamide

The mean percentage of the active ingredient of the chlorpropamide released in solution during the dissolution rate test was 79.70 ± 0.30% (Table 5). This was in conformity to the B.P (1993) requirement (not less than 70% per 45 minutes).

Table 5: Percentage of the active ingredient of chlorpropamide released in solution

| No of tablets | Percentage of dissolved drug in 45 minutes |
|---|---|
| I | 79.20 |
| II | 79.60 |
| III | 79.90 |
| IV | 80.10 |
| | Mean = 79.70 + 0.03 (n =4) |

## 4.4 Plasma drug level analysis

### *4.4.1 Optimization studies*

The separation of the drugs, chlorpropamide and tolbutamide, (IS) was achieved with ultrasphere ODS column (4.6mm x 25cm) and the mobile phase, Aceronitrite – Water (60:40), pH 6.7, at a flow rate of 1.0ml/min. The retention time of chlorpropamide was 2.7min and Tolbutamide was 4.2min.

The effects of varying the ratio of Acetonitrile and water on the resolution of chlorpropamide and tolbutamide are shown in table 6a, Figure 4a and table 6b and Figure 4b respectively. The influence of pH and the flow rate of the mobile phase on the resolution of chlorpropamide and tolbutamide are shown in Table 6c, Figure 4c and table 6d, figure 4d respectively.

There was no separation at pH below 6.7. The determined ratios for the resolution of chlorpropamide and tolbumide are 60% for Acetonitrile and 40% for water.

The flow rate suitable for the separation of the drugs was 1.0ml/min. At this flow rate the resolution was optimum.

Table 6a: Effect of Acetonitrile on the Resolution of chlorpropamide and Tolbutamide using solvent system Acetonitrile – Water with Beckman ultrasphere ODS Column (USA).

| Solvent Percentage | | |
|---|---|---|
| Acetonitrile | Water | Resolution (R) |
| 45 | 55 | 0.21 |
| 55 | 45 | 0.31 |
| 60 | 40 | 0.60 |
| 65 | 35 | 0.17 |
| 75 | 25 | 0.00 |
| 85 | 15 | 0.00 |

Table 6b: Effect of water on the Resolution of chlorpropamide and Tolbutamide using solvent system Acetonitrile – Water with Beckman ultrasphere ODS Column (USA).

| Solvent Percentage | | |
|---|---|---|
| Water | Acetonitrile | Resolution (R) |
| 45 | 55 | 0.40 |
| 55 | 45 | 0.00 |
| 60 | 40 | 0.20 |
| 65 | 35 | 0.00 |
| 75 | 25 | 0.00 |
| 85 | 15 | 0.30 |

Table 6c: Effect of pH on the Resolution of chlorpropamide and Tolbutamide using solvent system Acetonitrile – Water with Beckman ultrasphere ODS Column (USA).

| pH of Mobile phase | Resolution (R) |
|---|---|
| 2.7 | 0.0 |
| 4.5 | 0.0 |
| 5.5 | 0.0 |
| 6.5 | 0.0 |
| 6.7 | 0.6 |
| 6.9 | 0.3 |

Table 6d: Effect of flow rate on the Resolution of chlorpropamide and Tolbutamide using solvent system Acetonitrile – Water with Beckman ultrasphere ODS Column (USA).

| Flow rate (ml/min) | Resolution (R) |
|---|---|
| 0.1 | 0.2 |
| 0.5 | 0.4 |
| 1.0 | 0.7 |
| 1.5 | 0.5 |
| 2.0 | 0.2 |
| 2.5 | 0.0 |

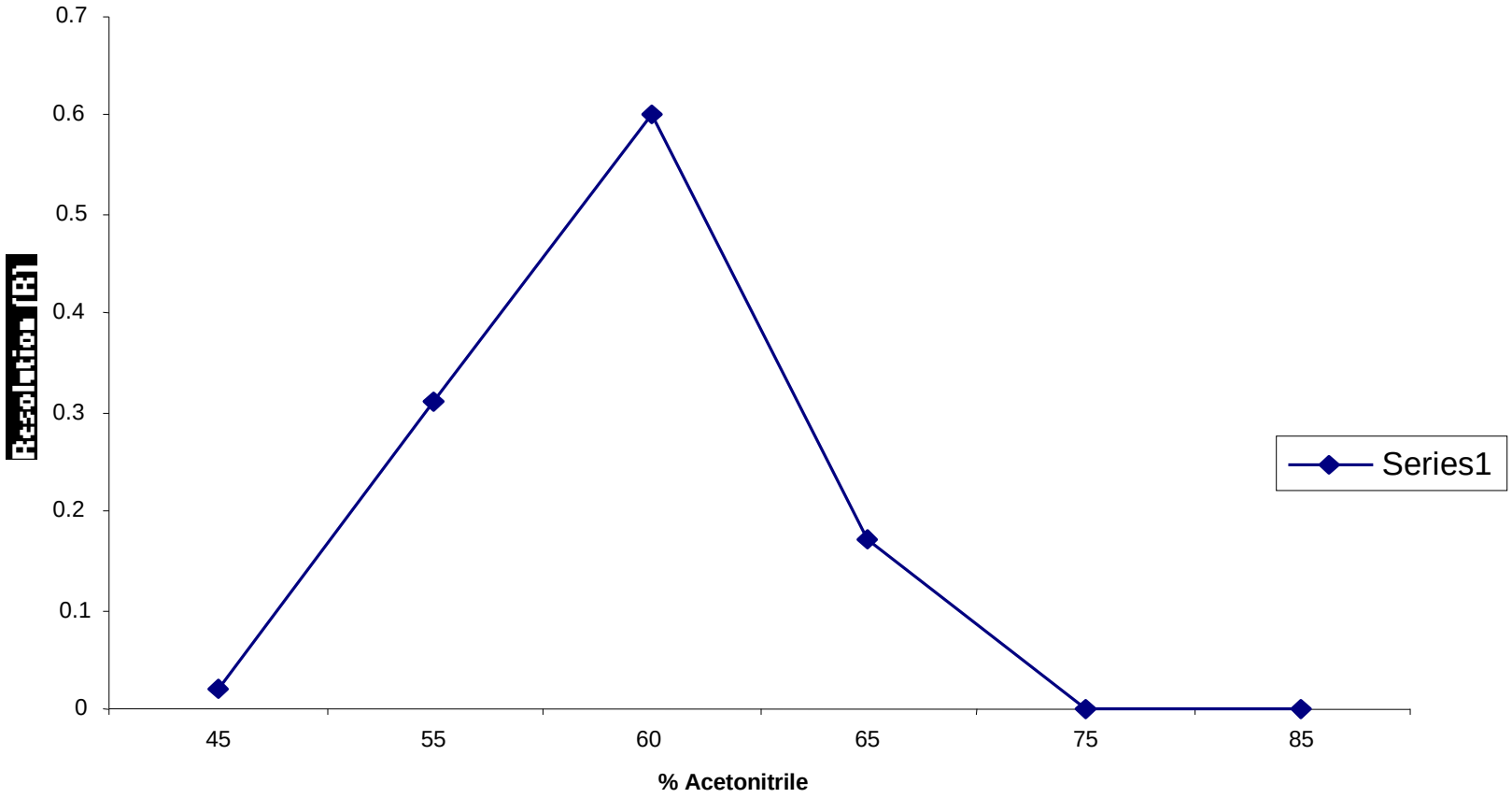



Fig. 4(a): Effect of Acetonitrile on the Resolution of chlorpropamide and Tolbutamide using solvent system Acetoritrile - Water with Beckman ultrasphere ODS column (USA)

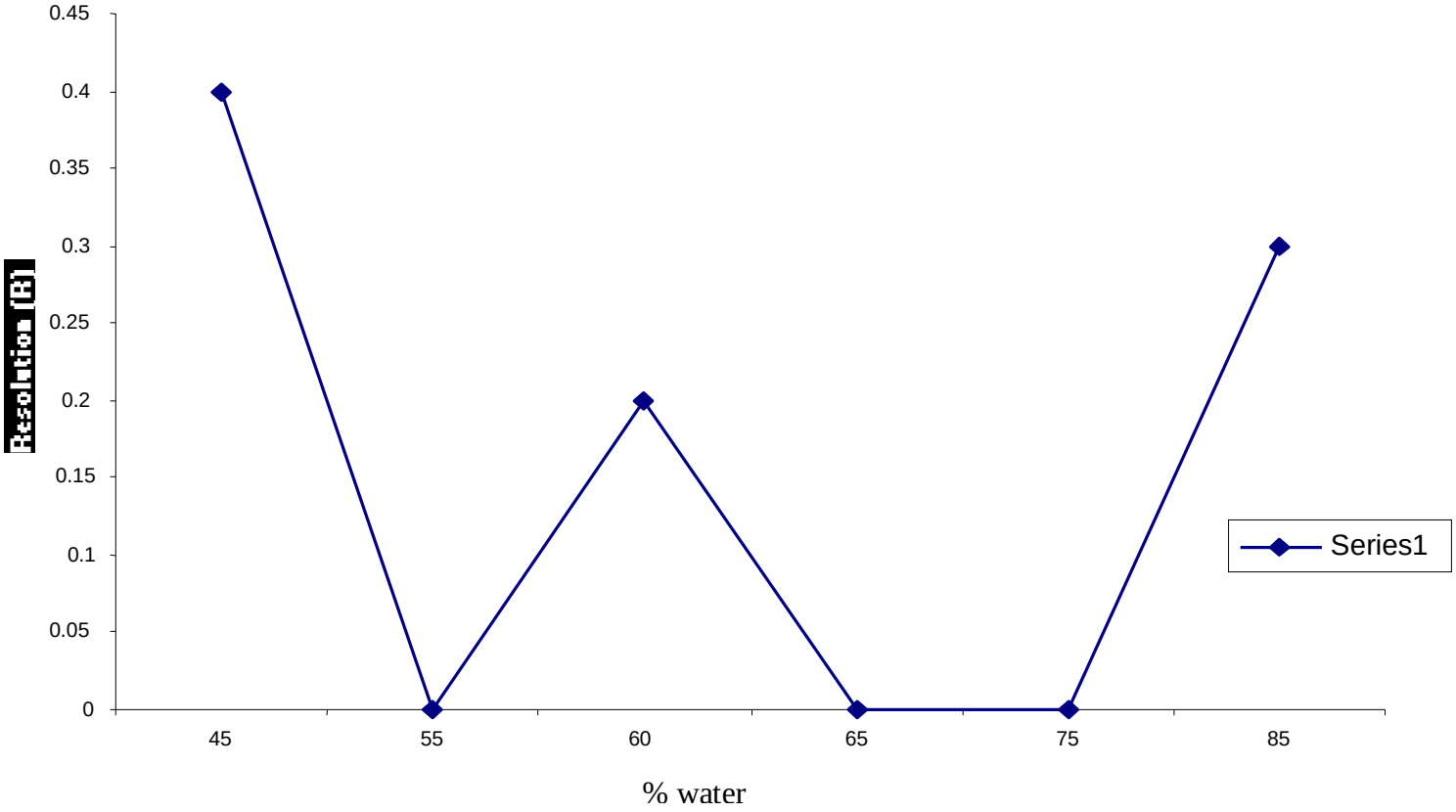



Fig. 4(b): Effect of water on the Resolution of chlorpropamide and Tolbutamide using solvent system with Beckman ultrasphere ODS column (USA)

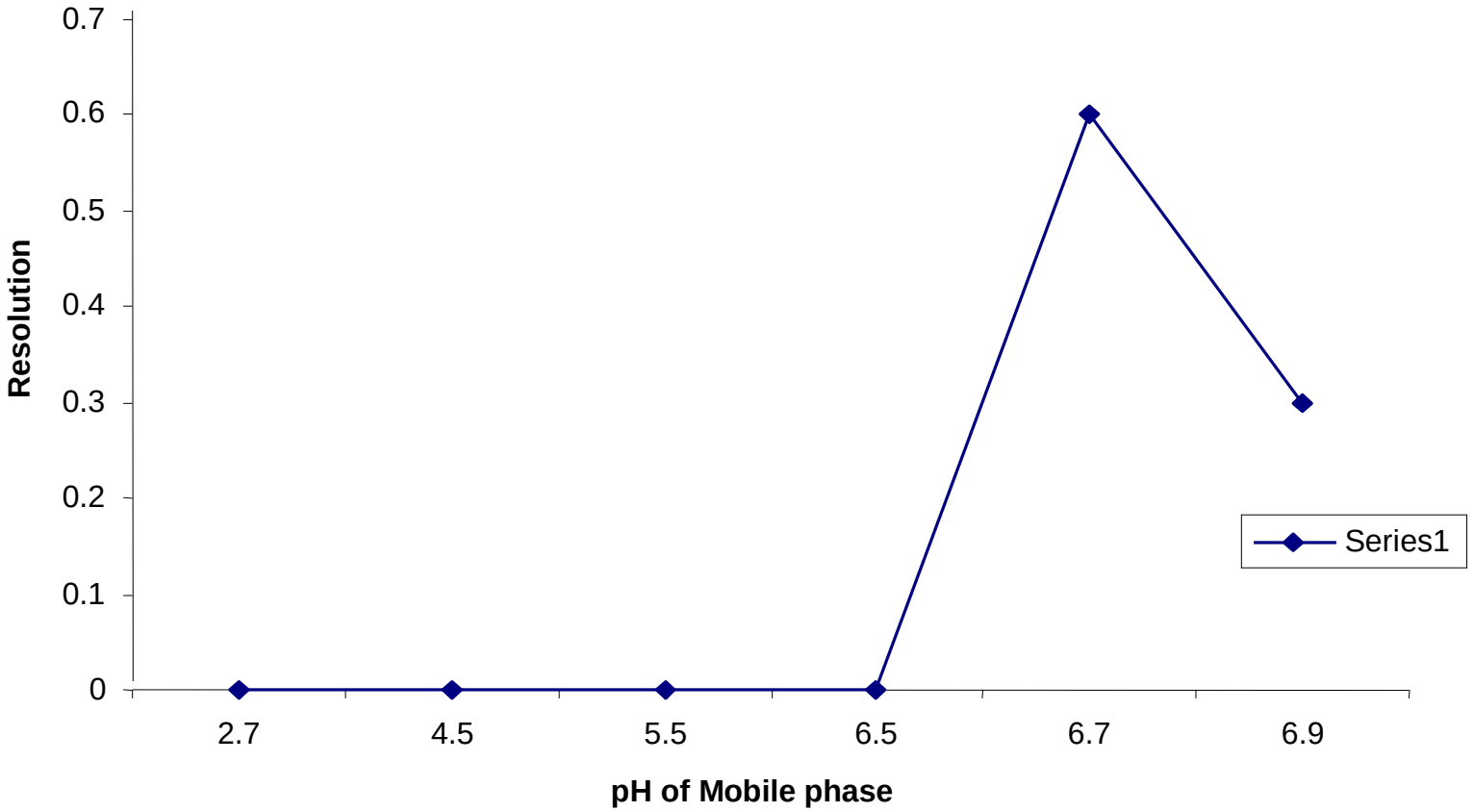



Fig. 4(c): Effect of pH on the Resolution of chlorpropamide and Tolbutamide using solvent system Acetonitrile- wa
Beckman ultrasphere ODS column (USA)

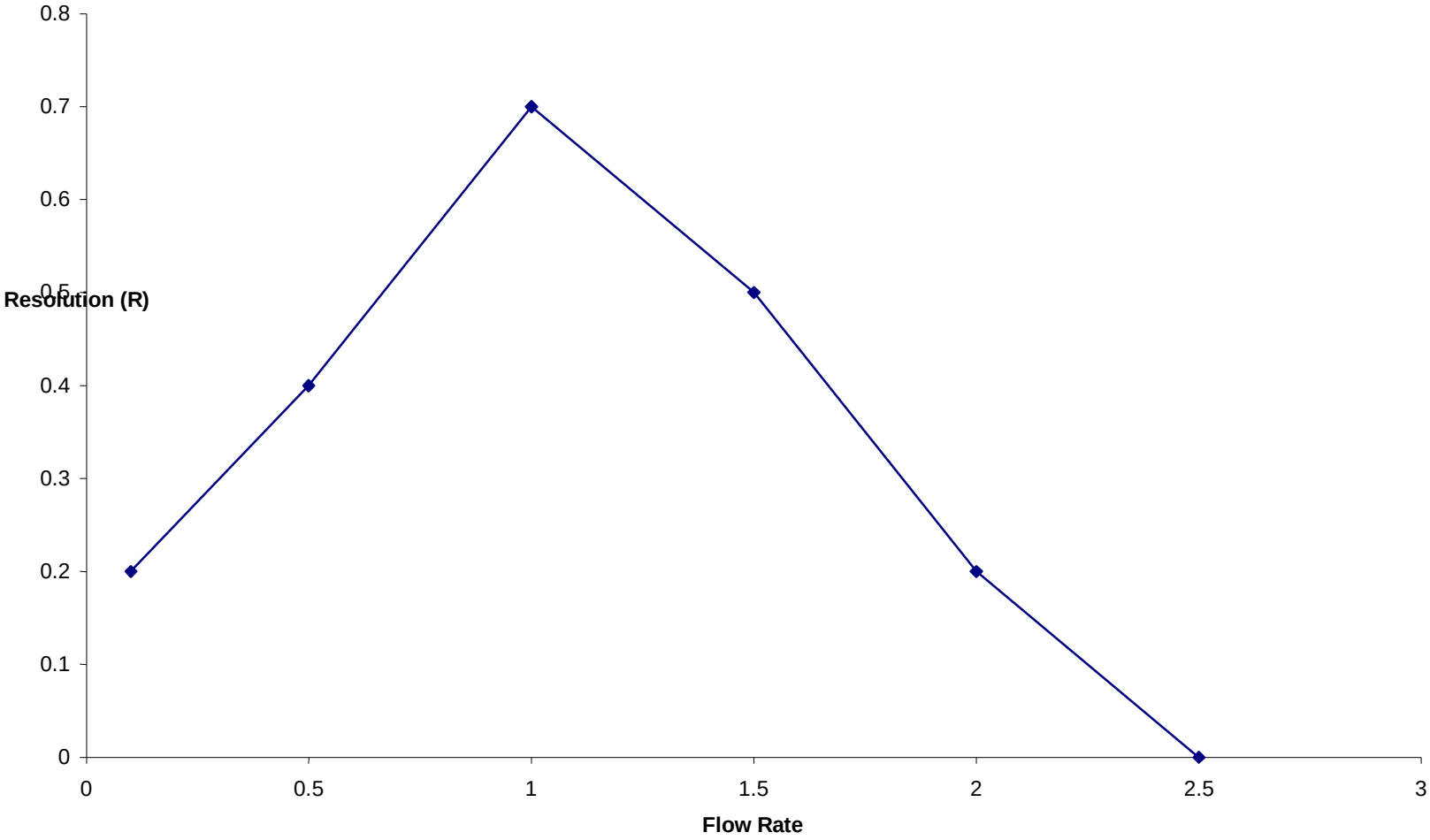



Fig. 4 (d): Effect of flow rate on the Resolution of Chlorpropamide and Tollbutamide using Solvent System Acetonitril Water with Beckman Ultrasphere ODS Column (USA)

### *4.4.2 Extraction from biological samples*

Extraction trial with various solvents for extraction identified acetonitrile as the most suitable solvent for the extraction of chlorpropamide and tolbutamide (internal standard) from human plasma. The percentage of chlorpropamide recovered from drug free human plasma is shown in Table 7. The mean percentage recovery obtained was 98.24 $\pm$ 1.20.

Table 7: The mean percentage extraction recoveries of chlorpropamide from plasma

| Concentration (μg/ml) | Mean % recovery $\pm$ S.E.M |
|---|---|
| 0.5 | 99.40 $\pm$ 1.76 |
| 5 | 96.20 $\pm$ 1.04 |
| 10 | 97.35 $\pm$ 0.07 |
| 15 | 99.80 $\pm$ 0.15 |
| 30 | 98.90 $\pm$ 2.05 |
| 40 | 97.80 $\pm$ 2.12 |
| | Mean = 98.24+ 1.20 |

### *4.4.3 Solvent system*

The solvent system identified for the analysis was Acetonitrite and water mixture in ratio 6:4. The calibration curve (Fig.7) for chlorpropamide in plasma was linear with a correlation coefficient of 0.999

The limit of detection was 1.45 x $10^{-4}$ μg/ml. The coefficiency of variation (C.V) values that established the precision of the method of analysis determined for 6 samples at 5 – concentration level for within – day and between – day assay are 1.66% and 2.24% respectively. The results are shown in table 8.

The chromatogram of chlorpropamide and tolbutamide extracted from blank plasma was indicated in fig 5(a). Fig 5(b) indicated the chromatogram of chlorpropamide when it was extracted alone from blank plasma.

The HPLC chromatogram of an extract of test sample obtained from human subjects 4hours after an oral administration of chloropropamide was indicated in fig. 6.

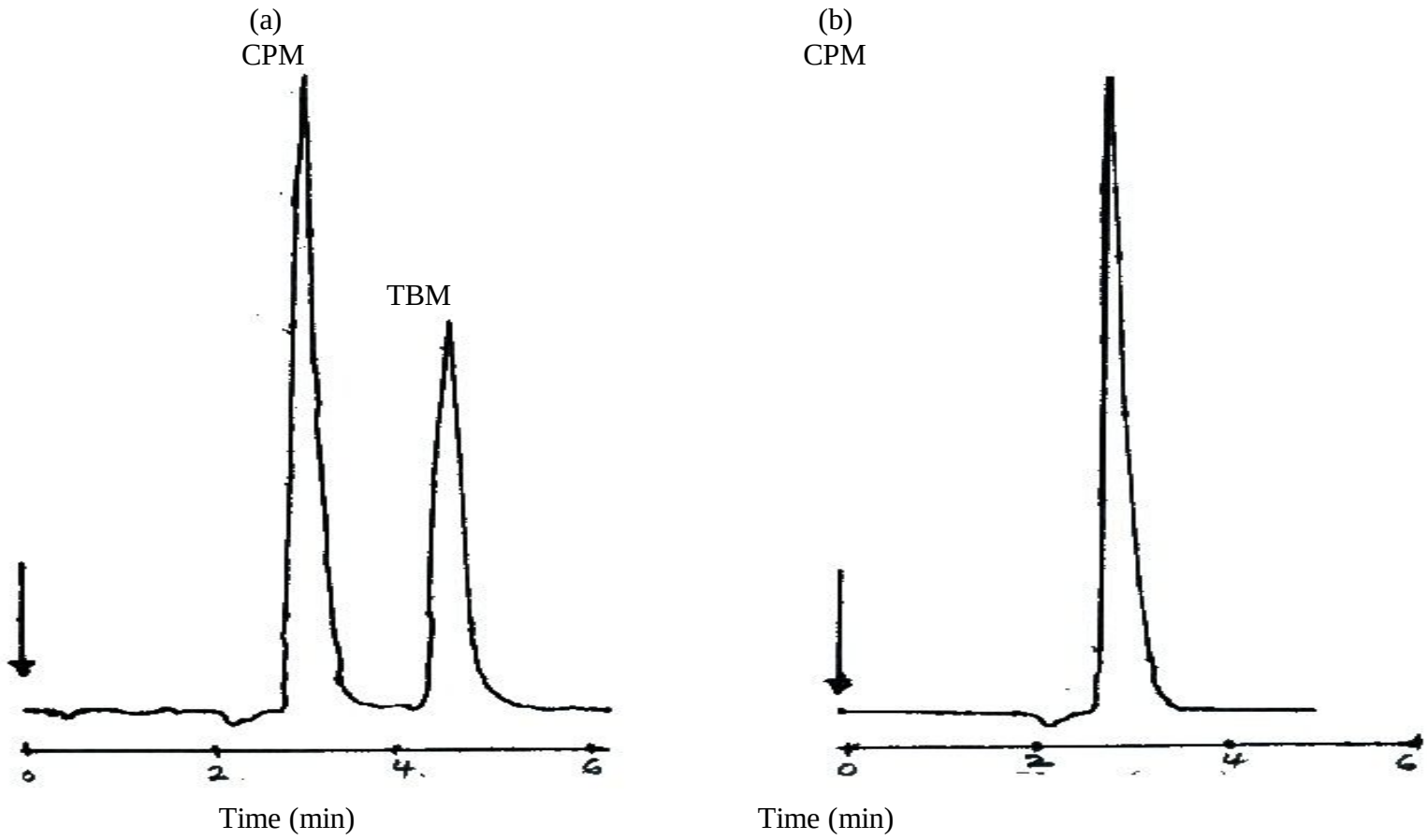



Fig. 5: (a)Chromatogram of chlorpropamide and Tolbutamide extracted from blank plasma

(b) Chromatogram of Chrorpropamide extracted from blank plasma

CPM = Chlorpropamide TBM = Tolbutamide

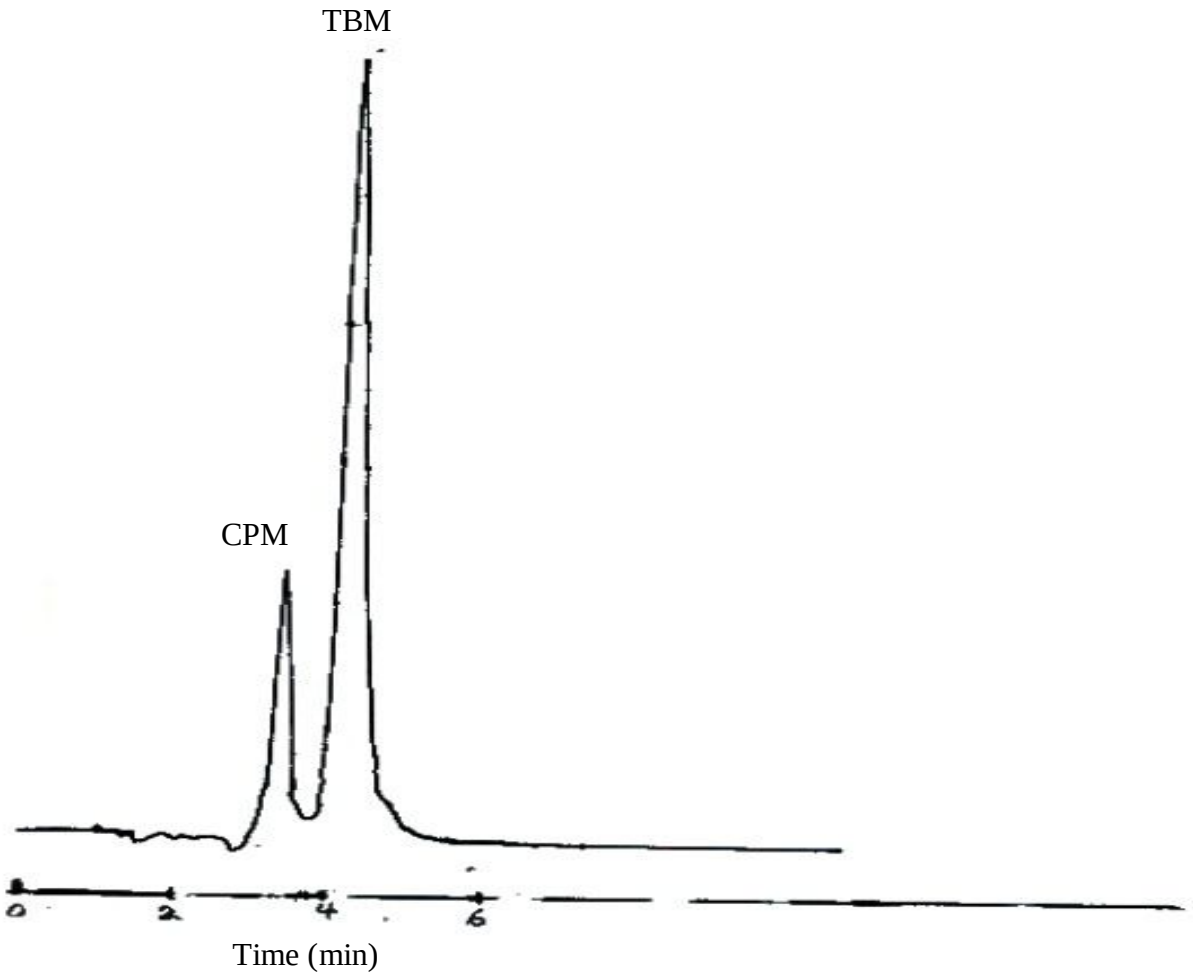



Fig. 6: High performance liquid chromatograms of an extract of test sample obtained from human subjects 4hours following oral administration of chlorpropamide.

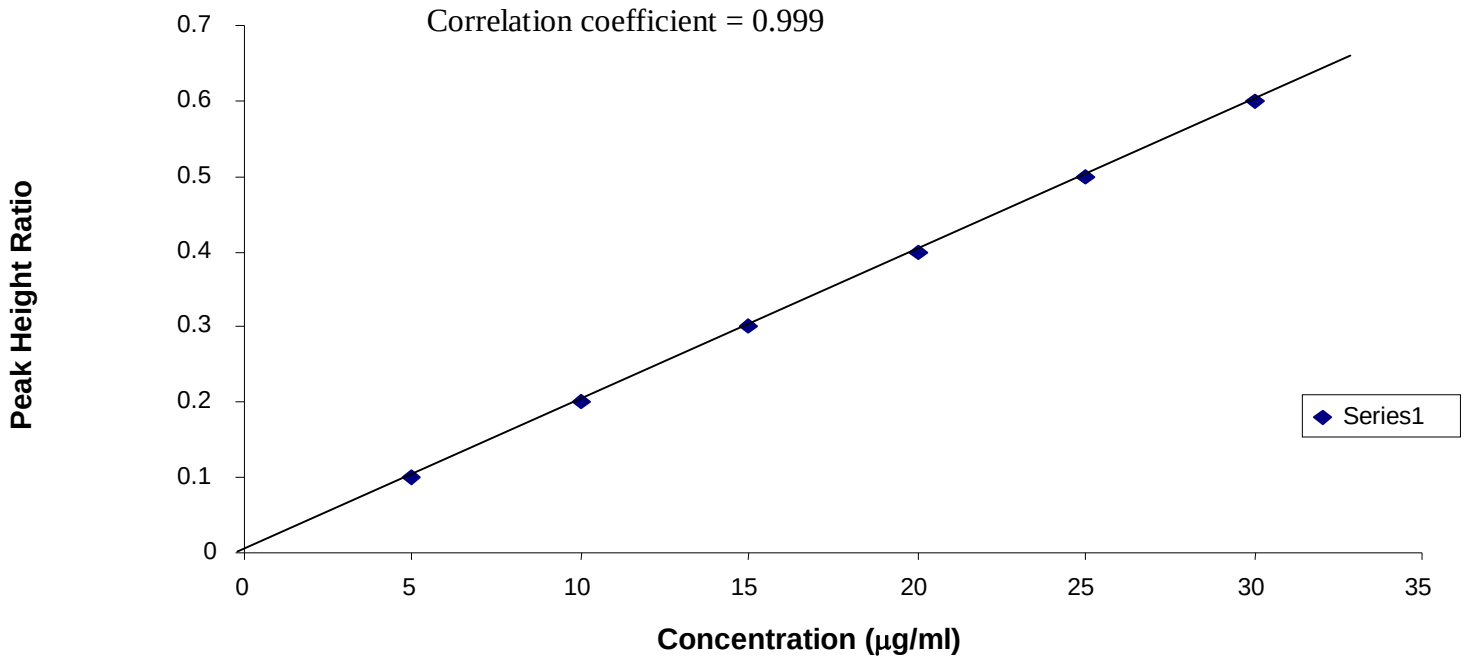



**Fig. 7: Calibration Curve for Chlorpropamide in plasma using HPLC**

Table 8: Precision of the method of analysis using HPLC method for within –day and day-to-day

| | Concentration (μg/ml) | C.V (%) | n |
|---|---|---|---|
| Within -day run | 2 | 2.5 | 6 |
| | 10 | 2.0 | 6 |
| | 20 | 1.5 | 6 |
| | 30 | 1.2 | 6 |
| | 40 | 1.1 | 6 |
| Day-to-day run | 2 | 2.7 | 6 |
| | 10 | 2.5 | 6 |
| | 20 | 2.4 | 6 |
| | 30 | 1.9 | 6 |
| | 40 | 1.7 | 6 |

### *4.4.4 Chlorpropamide and chloroquine interaction studies in human volunteers – group I*

After a single oral dose of chlorpropamide was administered to human volunteers, the mean maximum concentration ($C_{max}$) obtained was 27.75 $\pm$ 4.57 μg/ml, the mean time to maximum peak concentration ($t_{max}$) was 4.00 $\pm$ 0.00hr. The mean half-life of elimination $t_{½el}$ was 5.61 $\pm$ 0.69 while the mean half-life of absorption ($t_{½a}$) was 0.168 $\pm$ 0.05 $hr^{-1}$

A mean $AUC_{0-168}$ of 1616.05 $\pm$ 403.63 μg/ml/hr and a mean clearance (Clr) of 1.23$\pm$ 0.15 $^{L}/_{hr}$ were obtained. The mean volume of distribution and mean lag time were 10.44 $\pm$ 2.59 (L) and 0.41 $\pm$ 0.22hr respectively.

When chlorpropamide and chloroquine were concomitantly administered to the volunteers in this group, the mean maximum concentration ($C_{max}$) decreased to 23.33 $\pm$ 2.37 μg/ml, while the mean time to maximum peak concentration($t_{max}$) increased to 4.67 $\pm$ 0.42hr .

A decreased in the mean area under the curve, $AUC_{0-168}$, 906.29 ± 157.49 μg/ml/hr (P< 0.03), mean volume of distribution ($V_d$) 9.45 ± 0.93L, (P<0.90), mean absorption constant ($K_a$) 4.12 ± 0.33 $hr^{-1}$, (P<0.02); mean elimination half-life (t½el ) 2.97 ± 0.41hr, (P<0.01) were observed, while an increase was recorded in the mean clearance, Clr, 2.29 ± 0.17 $^L/_{hr}$, (P<0.01); mean elimination constant, $k_{el}$ 0.267 ± 0.05 $hr^{-1}$ (P<0.02); mean absorption half-life, $t_{½a}$, 0.174 ± 0.01hr (P< 0.04) and lag time, o.53 ± 0.13hr (P<0.53).

The mean area under the curve, $AUC_{0-168}$, decreased significantly (P<0.03) by44% (from 1616.05 ± 403.63 to 906.29 ± 157.49 μg/ml/hr).

The mean elimination half-life ($t_{el\ ½}$ ) decreased significantly (P< 0.01) by 47% (5.61± 0.69 to 2.97 ± 0.41hr).

The mean clearance (Clr) increased significantly (P<0.01) by about 86% (from 1.23 ± 0.15 to 2.29 ± 0.17 L/hr)

The mean elimination constant, $K_{el}$, increased significantly (P<0.02) by 100% (from 0.133 ± 0.01 to 0.267 ± 0.05 $h^{-1}$).

The mean absorption half-life, $t_{½a}$, increased by 4% ($P<0.04$). Table 9 presents the plasma concentration of chlorpropamide following oral administration of chlorpropamide tablet (A) and in combination with chloroquine tablets (B) to human volunteers. Mean pharmacokinetic parameters of chlorpropamide in the absence (A) and in the presence of chloroquine is presented in table 10. Figure 8 shows chlorpropamide plasma concentration-time curve following oral administration of chlorpropamide and chloroquine.

Table 9: Mean (±SEM) plasma concentration of chlorpropamide in 6 human subjects given chlorpropamide (A) with chloroquine (B)

| Time (hr) | Plasma concentration of chlorpropamide (μg/ml) | |
|---|---|---|
| | A | B |
| 0.5 | 13.58 ± 6. 90 | 7.75 ± 1.25 |
| 1 | 18.25 ± 8.26 | 10.75 ± 1.08 |
| 2 | 21.50 ± 7.87 | 14.67 ± 2.14 |
| 4 | 27.75 ± 11.19 | 21.33 ± 1.89 |
| 6 | 21.92 ± 10.69 | 21.92 ± 6.75 |
| 8 | 19.75 ± 10.89 | 18.50 ± 4.56 |
| 12 | 19.00 ± 10.78 | 13.75 ± 2.70 |
| 24 | 17.75 ± 10.50 | 10.00 ± 2.17 |
| 36 | 16.17 ± 10.43 | 8.08 ± 1.39 |
| 48 | 14.42 ± 8.38 | 6.25 ± 1.29 |
| 72 | 13.58 ± 8.27 | 4.92 ± 1.80 |
| 144 | 12.75 ± 7.72 | 4.00 ± 1.48 |
| 168 | 12.08 ± 7.77 | 2.75 ± 1.04 |

Table 10: Mean (± SEM) Pharmacokinetic Parameters of chlorpropamide following oral administration of chlorpropamide (A) with Chloroquine (B) in six volunteers

| **Mean Pharmacokinetic parameters (± S.E.M.)** | **A** | **B** | **Student's t-test for paired data** |
|---|---|---|---|
| Lag time (hr) | 0.41±0.22 | 0.53±0.13 | P<0.53 |
| Absorption half-life [$t_{1/2a}$(hr) | 0.168±0.05 | 0.174±0.01 | P<0.04 |
| Absorption rate constant [$k_a$ ($hr^{-1}$] | 5.80±1.37 | 4.12±0.33 | P<0.02 |
| Maximum conc. [$C_{max}$(µg/ml)] | 27.75±4.57 | 23.33±2.37 | P<0.02 |
| Time to peak conc. [$T_{max}$ {hr.)] | 4.00±0.00 | 4.67±0.42 | P<0.01 |
| AU $C_{0\text{-}168}$ [µg/ml.hr] | 1616.05±403.63 | 906.29±157.49 | P<0.03 |
| AU $C_{0\text{-}00}$ [µg/ml.hr]. | 1661.30±414.93 | 917.17±159.38 | P<0.03 |
| Clearance [CI (1/hr.)] | 1.23±0.15 | 2.29±0.17 | P<0.01 |
| Volume of distribution [$V_d$(1)] | 10.44±2.59 | 9.45±0.93 | P<0.90 |
| Elimination half-life [$t_{1/2el}$(hr). | 5.61±0.69 | 2.97±0.41 | P<0.01 |
| Elimination rate constant $K_{el}$ (hr.). | 0.133±0.01 | 0.267±0.05 | P<0.02 |

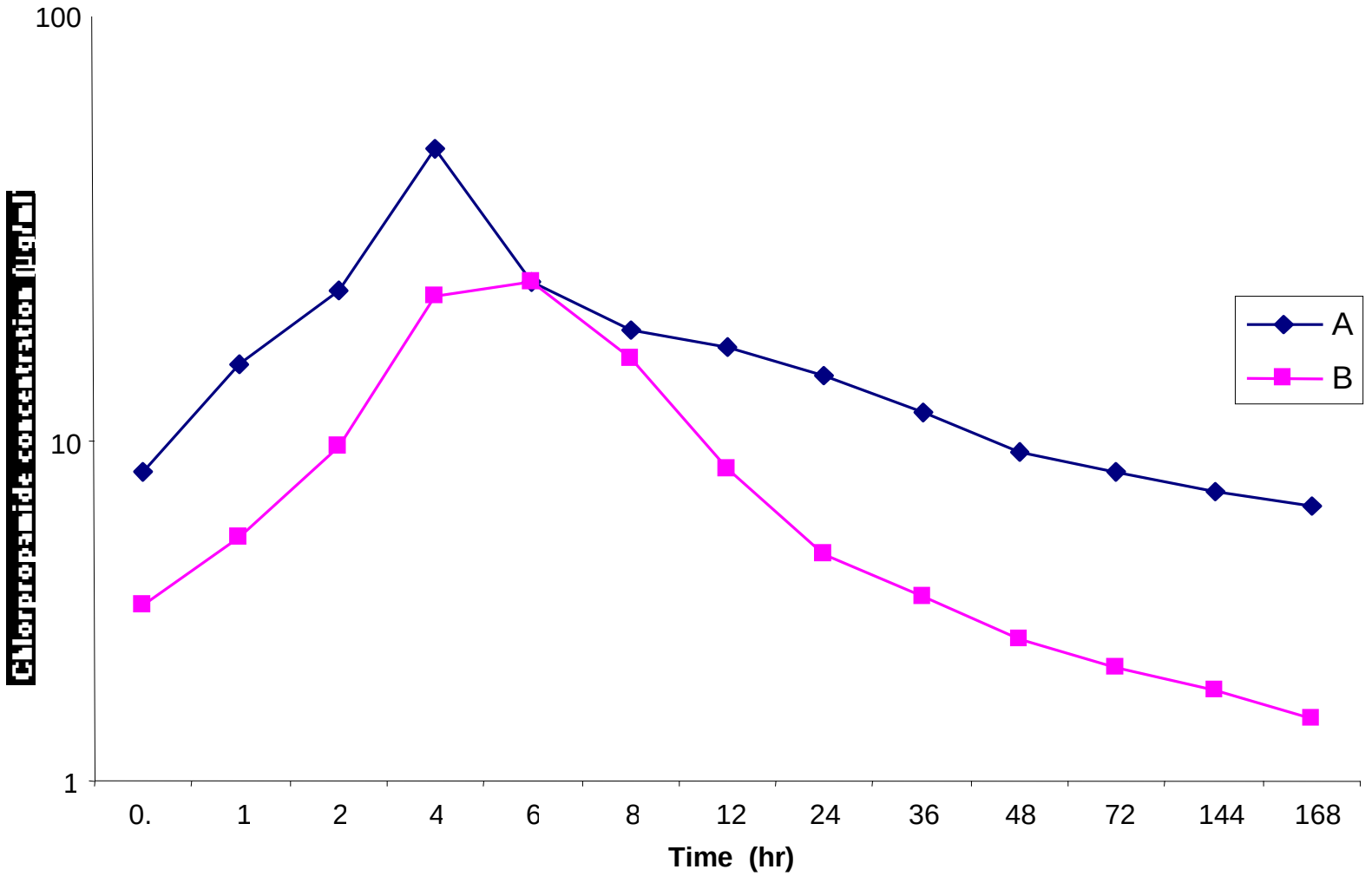



Fig. 8: Semi-log plot of chlorpropamide concentration against time following oral administration of chlorpropamide (A) and in combination with Chloroquine (B) in 6 human subjects

### 4.4.5 Chlorpropamide and paracetamol interaction studies in human volunteers – group II

The table for the mean pharmacokinetic parameter of chlorpropamide after a single oral dose of chlorpropamide with paracetamol shows that after the administration of chlorpropamide alone in the six human volunteers, the mean maximum concentration ($C_{max}$) derived was 31.50 $\pm$ 2.22 mg1ml at a mean time to maximum peak concentration ($t_{max}$) of 4.33 $\pm$ 0.33 hr.

The mean area under curve determined was 1836.92 $\pm$ 465.86 μg/ml/hr with a mean volume of distribution of 7.28 $\pm$ 0.66L and a mean clearance (Clr) of 1.75 $\pm$ 0.30l/hr.

The mean elimination constant obtained was 0.26 $\pm$ 0.65 $hr^{-1}$, while the mean absorption constant was 5.83 $\pm$ 0.92 $hr^{-1}$

The mean absorption and elimination half-lives were 0.14 $\pm$ 0.02 hr and 3.46 $\pm$ 0.85 hr respectively. A mean lag time of 0.44 $\pm$ 0.24hr was derived from the study of chlorpropamide alone.

After a single oral co-administration of chlorpropamide and paracetamol, the mean maximum concentration ($C_{max}$) decreased to 27.00 $\pm$ 1.64 m1ml ($P<0.02$), while the mean time to maximum peak concentration ($t_{max}$) increased to 6.00 $\pm$ 0.00 hr ($P <0.004$).

A decreases was observed in the $AUC_{0-168}$, from 1836.92 $\pm$ 465.86 to 1629.67 $\pm$ 108.79 μg/ml/hr. The decrease was 11% ($P<0.03$). A decrease was also recorded in the mean clearance, Clr (1.51 $\pm$ 0.06 $^{L}/_{hr}$, $P<0.01$); mean elimination constant, $k_{el}$, 0.18 $\pm$ 0.02$h^{-1}$ ($P<0.02$); and mean absorption constant, ka, (2.92 $\pm$ 0.43 $hr^{-1}$ $P<0.02$).

There was about 31% significant ($P< 0.02$) decreases in the mean elimination constant ($K_{el}$) where as the mean absorption constant, Ka, decreased by about 50% ($P< 0.02$). There was an increase in mean volume of distribution, from 7.28 $\pm$ 0.66 to 8.41 $\pm$ 0.52 ($P<0.90$).

The mean absorption half-life increased from 0.14 $\pm$ 0.02 to 0.26 $\pm$ 0.04hr. This increase was about 85% ($P<0.04$). The mean

elimination half-life (t½el) increased by 13% ($P<0.01$) from 3.46 ± 0.85hr to 3.91 ± 0.33hr.

Table 11 shows the result of the plasma concentration of chlorpropamide following oral administration of chlorpropamide (A) and paracetamol (B) inhuman subjects. The mean pharmacokinetic parameters of Chlorpropamide in the absence of paracetamol (A) and in the presence of paracetamol (B) obtained are presented in Table 12.

Figure 9 represents chlorpropamide plasma concentration time curve following oral administration of chlorpropamide and with paracetamol.

Table 11: Mean (± SEM) plasma concentration of chlorpropamide in 6 human subjects given chlorpropamide (A) and with paracetamol (B)

| Time (hr) | Plasma concentration of chlorpropamide (μglml) | |
|---|---|---|
| | A | B |
| 0.5 | 14.58 ± 7.79 | 6.83 ± 1.57 |
| 1 | 19.17 ± 7.92 | 10.17 ± 1.75 |
| 2 | 23.75 ± 8.13 | 14.50 ± 3.62 |
| 4 | 29.08 ± 7.67 | 19.25 ± 3.03 |
| 6 | 25.42 ± 7.45 | 27.00 ± 4.01 |
| 8 | 19.58 ± 6.44 | 23.75 ± 3.46 |
| 12 | 17.83 ± 6.68 | 21.17 ± 2.56 |
| 24 | 14.58 ± 3.98 | 14.83 ± 2.04 |
| 36 | 10.83 ± 4.75 | 11.67 ± 2.56 |
| 48 | 8.92 ± 3.73 | 10.25 ± 2.04 |
| 72 | 6.92 ± 2.78 | 8.67 ± 1.63 |
| 144 | 5.25 ± 2.19 | 6.17 ± 1.69 |
| 168 | 3.50 ± 1.55 | 4.58 ± 2.15 |

Table 12: Mean (± SEM) Pharmacokinetic Parameters of Chlorpropamide following oral administration of chlorpropamide (A) with Paracetamol (B) in six volunteers

| Mean Pharmacokinetic parameters (± S.E.M.) | A | B | Student's t-test for paired data |
|---|---|---|---|
| Lag time (hr) | 0.44±0.24 | 0.49±0.29 | P<0.01 |
| Absorption half-life [$t_{1/2a}$(hr) | 0.14±0.02 | 0.26±0.04 | P<0.01 |
| Absorption rate constant [ka (hr-1] | 5.83±0.92 | 2.92±0.43 | P<0.02 |
| Maximum conc. [$C_{max}$(µg/ml)] | 31.50±2.22 | 27.00±1.64 | P<0.02 |
| Time to peak conc. [$T_{max}$ [hr.)] | 4.33±0.33 | 6.00±0.00 | P<0.004 |
| AU $C_{0-168}$ [µg/ml.hr] | 1836.92±465.86 | 1629.67±108.79 | P<0.002 |
| AU $C_{0-00}$ [µg/ml.hr]. | 1884.68±477.97 | 1645.97±109.88 | P<0.002 |
| Clearance [CIr (l/hr.)] | 1.75±0.30 | 1.51±0.06 | P<0.03 |
| Volume of distribution [Vd(1)] | 7.28±0.66 | 8.41±0.52 | P<0.03 |
| Elimination half-life [$t_{1/2el}$(hr). | 3.46±0.85 | 3.91±0.33 | P<0.03 |
| Elimination rate constant $K_{el}$ (hr.). | 0.26±0.05 | 0.18±0.02 | P<0.02 |

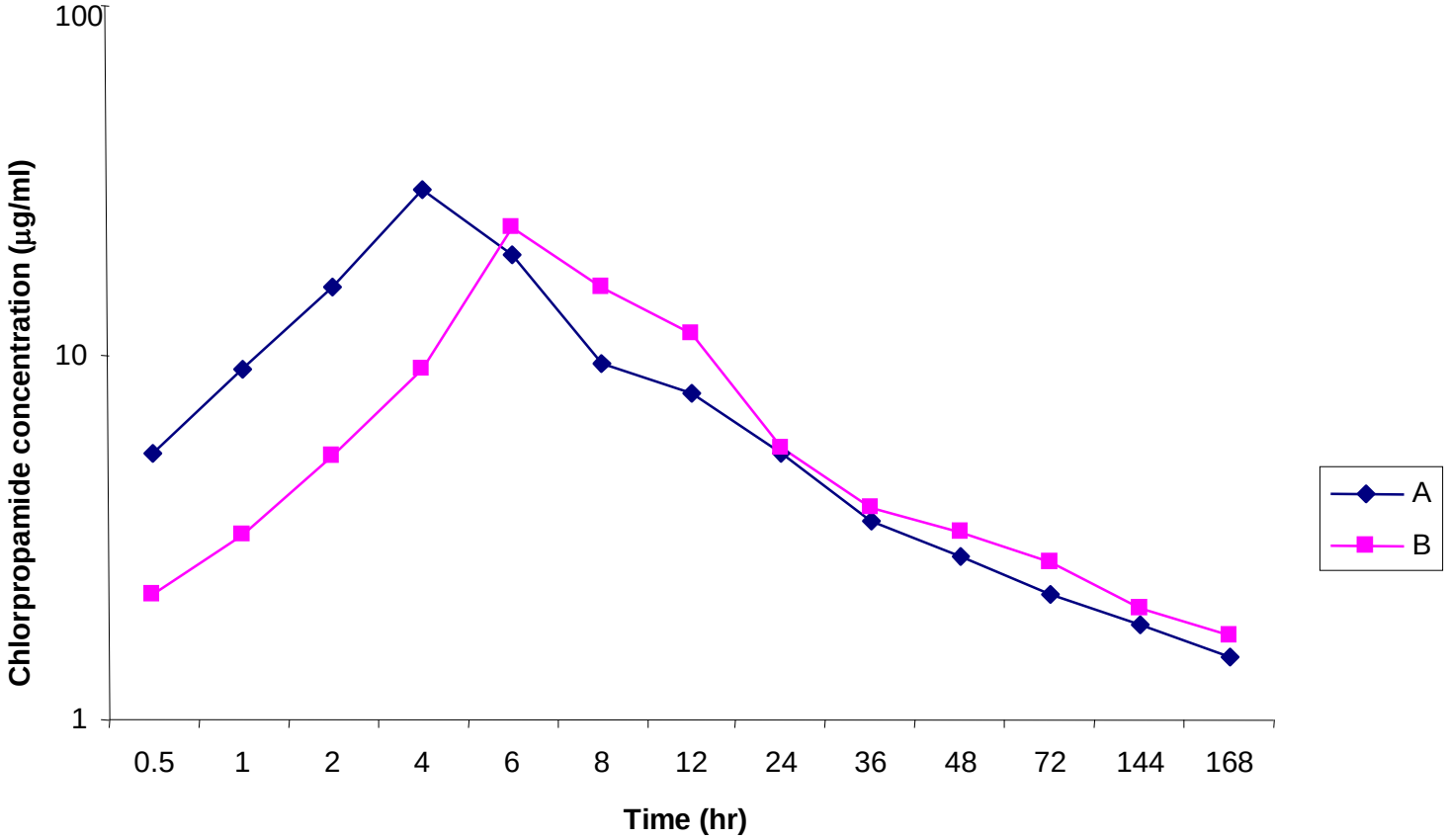



Fig. 9: Semi-log plot of chlorpropamide concentration against time following oral administration of chlorpropamide (A) and in paracetamol (B) in 6 human subjects.
combination with

### *4.4.6 Chlorpropamide and promethazine interaction studies in human volunteer – group III*

Following the oral administration of Chlorpropamide 250mg tablet to human volunteers, the mean maximum concentration ($C_{max}$) derived was 26.33 $\pm$ 1.38 μg/m1 at a mean time to maximum peak concentration ($t_{max}$) of 4.33 $\pm$ 0.33hr.

The mean volume of distribution, Vd and clearance, Clr obtained were 8.56 $\pm$ 0.40l and 1.75 $\pm$ 0.16 l/hr respectively.

The derived mean area under curve was 1716.13 $\pm$ 274.07 μg/ml/hr. The mean absorption and elimination half-lives were 0.13 $\pm$ 0.009hr and 3.5$\pm$ 0.30hr respectively. The mean elimination constant was 0.21 $\pm$ 0.05 $hr^{-1}$, whereas the mean absorption constant was 5.55 $\pm$ 0.47$hr^{-1}$. A mean lag time of 0.45 $\pm$ 0.30hr was recorded. Following the concomitant administration of a single dosage of chlorpropamide and Promethazine to the volunteers in this group, the mean maximum concentration ($C_{max}$) decreased to 22.50 $\pm$ 1.88 μglml ($P<0.02$), the decrease was about 15%.

The mean $t_{max}$ increased to 4.67 $\pm$ 0.42, this was statistically significant (P<0.004).

There was a decrease in the mean area under curve from 1716.13 $\pm$ 274.07 μg/ml/hr to 1203.75 $\pm$ 141.97 μg/ml/hr. The decrease was statistically significant (P< 0.002) with about 30% decrease.

An increase was recorded in the mean Vd, 10.17 $\pm$ 1.07L, mean absorption half-life, $t_{½a}$, 0.22 $\pm$ 0.04hr, and elimination half-life, $t_{½e}$, 4.51 $\pm$ 0.8hr.

The increase in the mean volume of distribution, Vd, is statistically significant (P <0.03) and it is about 19% increase.

The increase in the mean absorption and elimination half-lives are statistically significant and they are about 69% and 29% increase respectively.

There was a decrease in the mean clearance, Clr, from 1.75 $\pm$ 0.16 to 1.65 $\pm$ 0.12 (P<0.004). The decrease was not more than 6%.

There was also a decrease in the mean elimination constant ($k_{el}$) from 0.21 $\pm$ 0.05 to 0.17 $\pm$ 0.02 $hr^{-1}$ (P<0.02). There was an increase in the mean lag time which was statistically significant (P<0.01).

There was a decrease in the absorption constant ($K_a$) from 5.55 $\pm$ 0.47 to 3.68 $\pm$ 0.55$hr^{-1}$. The decrease in the absorption constant was about 34% and this was statistically significant ($P<0.02$).

Table 13 shows the plasma concentration of chlorpropamide following oral administration of chlorpropamide (A) and with Promethazine (B) in human subjects.

Table 14 presents the pharmacokinetic parameters of chlorpropamide when 250mg of chlorpropamide alone (A) and with Promethazine (B) was administered to the subjects In this group.

Figure 10 represents chlorpropamide plasma concentration time curve following oral administration of chlorpropamide (A) and with Promethazine (B).

The influence of chloroquine, paracetamol and Promethazine on the pharmacokinetics of chlorpropamide across the study in presented in table 15.

Table 13: Mean (± SEM) plasma concentration of chlorpropamide in 6 human subjects given chlorpropamide (A) and with Promethazine (B)

| Time (hr) | Plasma concentration of chlorpropamide (μg/ml) | |
|---|---|---|
| | A | B |
| 0.5 | 10.08 ± 4.50 | 7.67± 1.99 |
| 1 | 15.42 ± 4.43 | 10.50 ± 1.26 |
| 2 | 21.67 ± 3.17 | 14.58 ± 3.23 |
| 4 | 25.58 ± 4.04 | 21.33 ± 4.69 |
| 6 | 24.33 ± 3.84 | 20.08 ± 4.02 |
| 8 | 20.50 ± 3.02 | 15.17 ± 3.67 |
| 12 | 18.17 ± 2.42 | 12.75 ± 1.86 |
| 24 | 14.33 ± 3.50 | 10.58 ± 0.80 |
| 36 | 12.83 ± 3.09 | 9.08 ±0.97 |
| 48 | 11.00 ± 2.76 | 7.50 ± 1.22 |
| 72 | 9.67 ± 2.23 | 6.00 ± 1.58 |
| 144 | 7.92 ± 2.08 | 4.75 ± 1.75 |
| 168 | 6.50 ± 1.64 | 2.75 ± 0.88 |

Table 14: Mean (± SEM) Pharmacokinetic Parameters of Chlorpropamide following oral administration of chlorpropamide (A) with promethazine (B) in six volunteers

| **Mean Pharmacokinetic parameters (± S.E.M.)** | **A** | **B** | **Student's t-test for paired data** |
|---|---|---|---|
| Lag time (hr) | 0.45±0.30 | 0.53±0.41 | P<0.01 |
| Absorption half-life [$t_{1/2a}$(hr)] | 0.13±0.009 | 0.22±0.04 | P<0.001 |
| Absorption rate constant [ka (hr-1] | 5.55±0.47 | 3.68±0.55 | P<0.03 |
| Maximum conc. [$C_{max}$(µg/ml)] | 26.33±1.38 | 22.50±1.88 | P<0.03 |
| Time to peak conc. [$T_{max}$ {hr.)] | 4.33±0.33 | 4.67±0.42 | P<0.175 |
| AU $C_{0-168}$ [µg/ml.hr] | 1716.13±274.07 | 1203.75±141.97 | P<0.01 |
| AU $C_{0-00}$ [µg/ml.hr]. | 1759.03±280.92 | 1214.58±143.25 | P<0.01 |
| Clearance [CIr (l/hr.)] | 1.75±0.16 | 1.65±0.12 | P<0.004 |
| Volume of distribution [Vd(1)] | 8.56±0.40 | 10.17±1.07 | P<0.727 |
| Elimination half-life [$t_{1/2el}$(hr). | 3.5±0.3 | 4.51±0.82 | P<0.02 |
| Elimination rate constant $K_{el}$ (hr.). | 0.21±0.05 | 0.17±0.02 | P<0.071 |

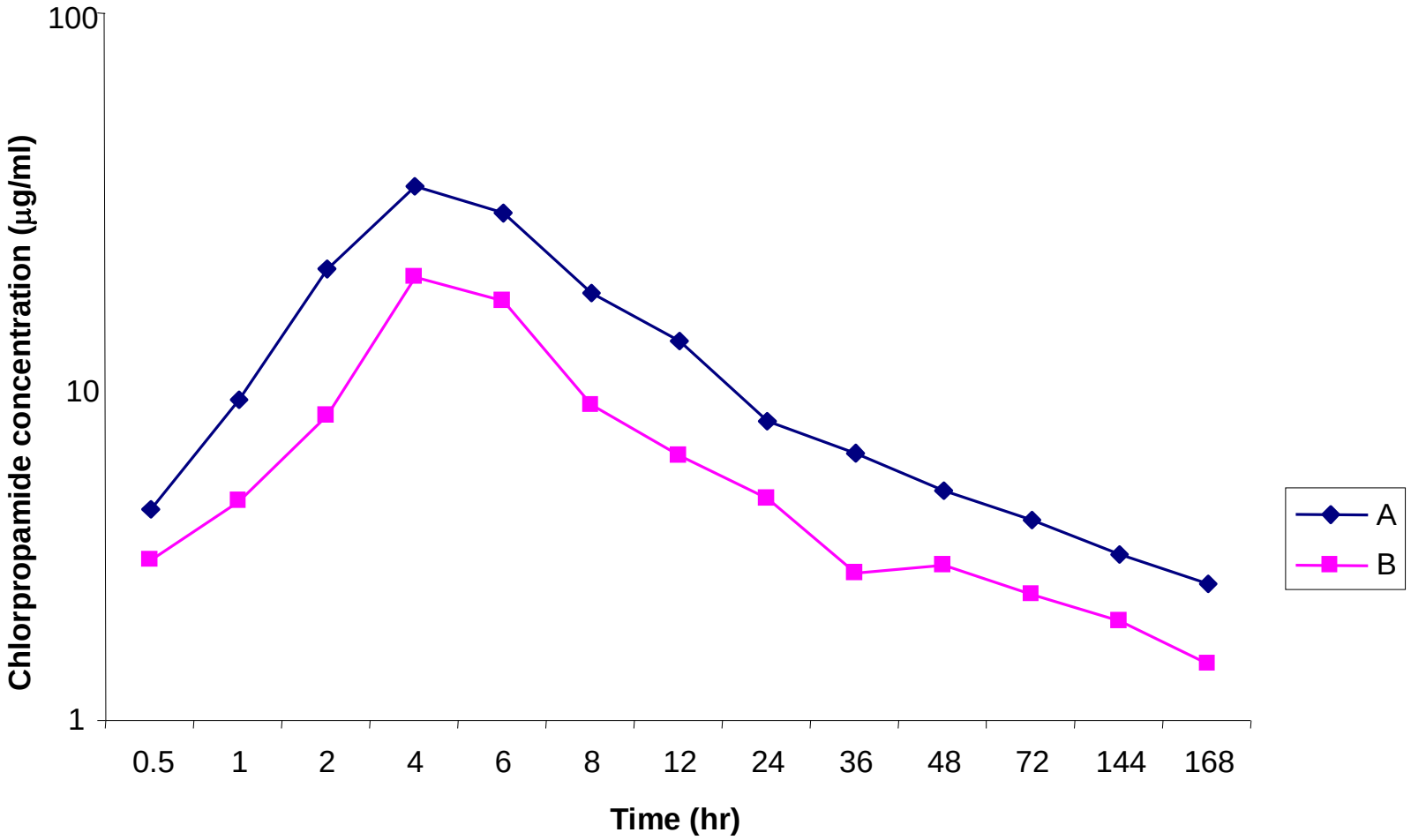



**Fig. 10: Semi-log plot of chlorpropamide concentration against time following oral administration of chlorpropamide (A) and in combination with promethazine (B) in 6 human subject.**

Table 15: Mean pharmacokinetic parameters of the three study groups compared.

| Mean pharmacokinetic parameters (± SEM) | Control (A) n=6 | Study groups with chloroquine (IB) paracetamol (IIB) and Promethazine (IIIB) | | |
|---|---|---|---|---|
| | | IB (n=6) | IIB (n=6) | III B (n=6) |
| Lag time (hr) | 0.51 ± 0.22 | 0.53 ± 0.13 | 0.49 ±0.29 | 0.53 ±0.01 |
| Absorption half life $t_{½a}$ (hr) | 0.14 ± 0.001 | 0.174 ± 0.01 | 0.26 ± 0.04 | 0.22 ±0.04 |
| Absorption rate constant $K_a$ (hr) | 5.73 ± 0.54 | 4.12 ± 0.33 | 2.92 ±0.43 | 3.68 ±0.55 |
| Maximum concentration $C_{max}$ (μglml) | 28.53 ± 1.73 | 23.33 ± 2.37 | 27.00± 1.64 | 22.50± 1.88 |
| Time to peak concentration $T_{max}$ (hr) | 4.22 ± 0.15 | 4.67 ± 0.42 | 6.00 ± 0.00 | 4.67 ± 0.40 |
| Area under curve $AUC_{0-168}$ (μglml.hr) | 1723.03± 212.35 | 906.29± 157.49 | 1629.67 ±108.79 | 1203.75 ±0.01 |
| Clearance Cl (l/hr) | 1.58 ± 0.13 | 2.29 ± 0.17 | 1.51 ± 0.06 | 1.65 ± 0.2 |
| Volume of distribution $V_d$ (l) | 8.76 ± 0.90 | 9.45 ± 0.93 | 8.41 ± 0.52 | 10.17± 1.07 |
| Elimination half life $t_{½\ el}$ (hr) | 4.19 ± 0.43 | 2.97 ± 0.41 | 3.91 ± 0.33 | 4.51 ± 0.82 |
| Elimination rate constant $K_{el}$ ($hr^{-1}$) | 0.199 ± 0.02 | 0.267 ± 0.05 | 0.18 ± 0.02 | 0.17 ± 0.02 |

# CHAPTER FIVE

# DISCUSSION

## 5.1 Quality control of chlorpropamide, chloroquine, paracetamol and promethazine tablets

The quality of the chlorpropamide tablet used for the study was assessed and confirmed as part of product quality assurance.

The drugs used in the study conformed to the specifications under drug monographs in B.P. 1993. Thus, the batch of chlorpropamide tablets, chloroquine tablets, paracetamol tablets and promethazine tablets that were used in the study was found to have satisfied the official requirements (BP, 1993).

### *5.1.1 Chlorpropamide tablets*

The filtrate of the recrystallized drug produced a characteristic odour of ammonia. The addition of NaOH to the filtrate of the recrystalized product resulted in a white precipitate (B.P 1993). The melting point of the recrystalized drug was 143$^{o}$C, and compared well with the B.P 1993 specification. Thus, the

drug product complied with the identification test for chlorpropamide.

The content of the active ingredient from the drug product assay yielded 99.08 $\pm$ 0.002 and 100.20 $\pm$ 0.003% for the tablet and reference standard respectively. Thus, the product complied with official specification (B.P, 1993) of drug content (92.5% - 107.5%). Within 45 minutes, the dissolution rate test for chlorpropamide gave the average amount of drug content released into solution as 79.70$\pm$ 0.03% and this complied with official specification (B.P. 1993) of not less than 70% in 45 minutes

### *5.1.2 Chloroquine tablets*

The melting point of the recrystalized drug was 207$^0$C, which compared well with the BP. 1993 specification.

The white precipitate produced when the filtrate from the powdered tablets of chloroquine was treated with barium chloride solution was in compliance with the specifications of B.P 1993 for its identification. The percentage content of the active ingredient content in the tablets and reference standard

powder are 100.00 ± 0.001% and 103.50 ± 0.002% respectively, are in conformity with the B.P 1993 specifications (92.50 – 107.50)%.

### *5.1.3 Paracetamol tablets*

The melting point of the extracted paracetamol was $169^0$C, and this was in conformity with the B.P. 1993 specification. A violet colour was produced when powdered tablets of paracetamol which was treated with hydrochloric acid and boiled, was reacted with potassium dichromate solution, but no precipitate was produced when it was dissolved in water. This was in conformity with the specifications of B.P 1993 for identification

The content of the active ingredient from drug product assay yielded 98.33±0.003% and 100.00±0.003 for the tablet and reference standard respectively. Thus, the product complied with official specification (B.P, 1993) of drug content (98 – 101)%.

### *5.1.4 Promethazine tablets*

The red colour produced when the powdered tablets of promethazine was treated with sulphuric acid was in compliance with the specifications of B.P 1993 for its identification. The melting point of promethazine which was extracted from the powdered tablets was 160$^{o}$C, which complied with the official specifications of B.P 1993.

The percentage content of the active ingredient contained in the tablets and reference standard powder are 99.15 $\pm$ 0.004 and 100.00 $\pm$ 0.001 respectively are in conformity with the B.P 1993 specifications (90 – 110)%.

## 5.2 Plasma drug level analysis

Accurate plasma levels of chlorpropamide were determined by HPLC, following the development of an appropriate extraction procedure and optimized chromatographic conditions. The best extraction solvent identified was acetonitrile. Extraction with other solvents resulted in a low percentage recovery and with interfering peaks. The extraction with acetonitrile became the solvent of choice for extraction in a single step extraction

procedure. This result is in agreement with that of Roger et al (2002)

Following complete optimization, good peak resolutions (Fig 5 and 6) were clearly obtained with mean retention times of 2.7 minutes and 4.2 minutes for chlorpropamide and the internal standard tolbutamide respectively.

The calibration curve (fig 7) generated for the determination of plasma concentration of chlorpropamide was linear with a correlation coefficient of 0.999. The accuracy of the assay method determined was 98.24 $\pm$ 1.20%. The sensitivity of the assay method determined was $1.45 \times 10^{-4}$ μg/ml. the coefficient of variation (CV) of the method of analysis for within-day and between-day assay were 1.66% and 2.24% respectively.

Thus, the assay method was founds to be accurate, precise and reproducible.

## 5.3 Pharmacokinetics of chlorpropamide when co-administered with chloroquine

When chlorpropamide was given alone (IA), peak concentration ($C_{max}$), area under curve (*AUC*), the apparent volume of distribution (Vd), and the half-life of absorption ($t_{½a}$) and other pharmacokinetic parameters were in agreement with the reports of previous workers (Odunola et al, 1994). Following concurrent administration of chlorpropamide and chloroquine in the crossover study, the ka decreased significantly ($P<0.02$) by 29%. The $t_{½a}$ increased significantly ($P<0.04$) by 8%. $C_{max}$ and *AUC* of chlorpropamide were significantly reduced ($P<0.02$ and $P<0.03$) respectively. 16% decrease occurred in the $C_{max}$ while the *AUC* decreased by 44%. There was a significant increase ($P<0.01$) in the $t_{max}$ by17%

The significant reduction in the *AUC* could be due to the reduction in $K_a$, since there is a limit to which the $K_a$ can be reduced without affecting the *AUC*. The delayed $K_a$ could also have led to delayed $t_{max}$ and $C_{max}$

The significant increase in $t_{½a}$ and $t_{max}$ indicated impaired absorption of chlorpropamide by chloroquine. The decrease in

the $C_{max}$ of chlorpropamide may be attributed to the interaction via liver cytochromes 3A4, 2C8, 2C9 or CYP450 isoenzymes. Chlorpropamide being a sulphonylurea is metabolized by CYP450 isoenzyme. Specific family and subfamily of the CYP450 isoenzyme of chlorpropamide has not been elucidated (Oette et al, 2005). The presence of chloroquine may induce the liver enzyme activity including CYP450 isoenzyme system. Increased CYP450 enzyme activity might have increased the metabolism which may lead to increased plasma clearance ($P<0.01$), 86% increase and reduced $t_{½el}$ ($P<0.01$) 47% of chlorpropamide.

The resultant effect as observed is significant ($P<0.02$) increase in $K_{el}$ by 100% reduction in $C_{max}$ and *AUC*. According to Miners et al (1984) chloroquine induces the tubular secretion of chlorpropamide.

One drug can increase the excretion rate of another drug thereby lowering its therapeutic effectiveness. Conversely, decreasing the excretion rate of drug can increase its toxicity (Grahame, 2004).

## 5.4 Pharmacokinetics of chlorpropamide when co-administered with paracetamol

Following co-administration of chlorpropamide and paracetamol the $K_a$ decreased significantly ($P<0.002$) by 50%. The $t_{½a}$ increased significantly ($P<0.01$) by 85%. $C_{max}$ and *AUC* of chlorpropamide were significantly reduced ($P<0.02$) by 14% and 11% respectively.

The induction of the hepatic microsomal enzyme system by paracetamol may increase the rate of metabolism of chlorpropamide, which might have resulted in the impaired absorption of chlorpropamide.

The plasma clearance decreased significantly ($P<0.03$) by 14%. Also the Vd increased significantly ($P<0.03$) by 16%. There was also a significant reduction ($P<0.02$) of the $K_{el}$ by 31%

The significant increase in the apparent volume of distribution might have resulted in the significant reduction in plasma clearance and rate of elimination ($K_{el}$) of chlorpropamide. The resultant effect was a significant increase in $t_{½\ el}$.

## 5.5 Pharmacokinetics of chlorpropamide when co-administered with promethazine

Following the concomitant administration of chlorpropamide and promethazine in the study, $C_{max}$ and *AUC* of chlorpropamide were significantly reduced ($P<0.03$ and $P<0.01$) respectively. The $C_{max}$ decreased by 15% while the *AUC* by 30%. The $t_{½a}$ increased significantly ($P<0.01$) by 69% also the $K_a$ decreased significantly ($P<0.03$) by 34%. The impairment of absorption in chlorpropamide on co-administration of promethazine could also be explained as described for paracetamol and chloroquine.

However the co-administration of promethazine with chlorpropamide significantly ($P<0.04$) reduced the plasma clearance, resulting in significantly ($P<0.02$) increased $t_{½el}$. There was a significant decrease ($P<0.04$) of the plasma clearance by 6%.

# CHAPTER SIX

# SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

## 6.1 SUMMARY

An investigation was carried out on the effects of chloroquine, paracetamol and promethazine on the Pharmacokinetic parameters of chlorpropamide in human subjects.

The quality control studies revealed that the drugs used in this study were of high quality as they complied with B.P (1993) requirements stated in their monographs.

A high performance liquid chromatography (HPLC) method was developed for the analysis of plasma chlorpropamide concentration.

About 16%, 14% and 15% decrease in the mean maximum concentration ($C_{max}$) of chlorpropamide was observed following oral administration of chlorpropamide with chloroquine, paracetamol and promethazine respectively.

About 44%, 11% and 30% decrease in the area under curve (AUC) of chlorpropamide was observed following concurrent administration of chlorpropamide with chloroquine, paracetamol and promethazine respectively. The absorption half-life ($t_{½\ a}$) of chlorpropamide increased by 4%, 85% and 69% when concomitantly administered with chloroquine, paracetamol and promethazine respectively.

The elimination half-life ($t_{½\ el}$) of chlorpropamide was found to have decreased by 47% and increased by 13% and 29% following

The co-administration of chlorpropamide with chloroquine, paracetamol or promethazine, impaired the absorption and elimination of chlorpropamide.

## 6.2 Conclusions

The results of identification tests and chemical assay carried out on the tablets and standard reference powder of chlorpropamide, chloroquine, paracetamol and promethazine showed that the batches of the drugs used in this study satisfied the B.P (1993) official requirements.

Majority of the significant interactions with sulfonylureas are due to the induction or inhibition of cytochrome P450 enzymes in the liver (Gillian, 2001).

The influence of co-administration of chlorpropamide together with paracetamol or promethazine follows a similar pattern. The absorption parameters Ka, $t_{½a}$, $C_{max}$, $t_{max}$ and *AUC* were significantly altered. The elimination phase was also impaired.

The $t_{½el}$ and plasma clearance were increased significantly. However, the co-administration of chloroquine with chlorpropamide as compared with chlorpropamide alone, produced similar influence on absorption phase, but the plasma clearance and $t_{½el}$ were significantly reduced. In conclusion the co-administratiion of chloroquine or paracetamol or promethazine together with chlorpropamide impaired the absorption and the elimination of chlorpropamide in healthy human subjects. Effect of such significant interactions on absorption phase may be lowered therapeutic effectiveness, and the decreased rate of elimination can increase the toxicity of drugs like chlorpropamide having long half-life.

## 6.3 Recommendations

Chlorpropamide level monitoring or close observation of diabetic patients on anti-malarial treatment with the studied drugs is recommended.

There is the need to alter the study protocol in order to reduce the observed interactions.

The study can be conducted in type II diabetic patients. Such study should determine the glycaemic response.

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# APPENDICES

Appendix 1: Plasma concentration of chlorpropamide for individual subject in various study groups

Group I

| Time (hr) | Plasma Concentration of Chrorpropamide (μglml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| | A | B | A | B | A | B | A | B | A | B | A | B |
| 0.5 | 20.00 | 8.00 | 19.00 | 6.50 | 18.00 | 6.00 | 14.50 | 7.00 | 5.00 | 9.00 | 5.00 | 8.00 |
| 1 | 21.00 | 10.00 | 23.50 | 9.50 | 20.50 | 10.00 | 28.00 | 12.00 | 10.00 | 12.00 | 6.50 | 11.00 |
| 2. | 24.00 | 15.00 | 27.50 | 10.50 | 22.50 | 15.00 | 30.00 | 16.50 | 16.50 | 16.00 | 8.50 | 15.00 |
| 4. | 32.50 | 20.00 | 28.50 | 25.00 | 34.00 | 21.50 | 41.50 | 20.00 | 20.00 | 20.50 | 10.00 | 21.00 |
| 6. | 26.50 | 17.50 | 25.50 | 35.00 | 16.00 | 21.00 | 39.00 | 18.50 | 17.50 | 22.50 | 7.50 | 17.00 |
| 8 | 25.50 | 16.50 | 20.50 | 27.00 | 15.00 | 17.50 | 38.00 | 15.00 | 14.00 | 20.00 | 6.00 | 14.00 |
| 12 | 25.00 | 10.50 | 19.50 | 18.00 | 14.50 | 14.50 | 37.50 | 13.00 | 13.00 | 15.00 | 5.50 | 11.50 |
| 24 | 22.50 | 9.50 | 18.50 | 10.00 | 14.00 | 9.50 | 36.00 | 11.00 | 12.50 | 13.50 | 4.00 | 7.00 |
| 36 | 21.50 | 8.50 | 18.00 | 7.00 | 13.50 | 8.50 | 35.00 | 8.50 | 8.00 | 10.00 | 3.50 | 6.00 |
| 48 | 18.50 | 6.50 | 17.00 | 5.00 | 12.50 | 5.50 | 29.00 | 7.50 | 7.50 | 8.00 | 3.50 | 5.00 |
| 72 | 16.50 | 5.50 | 16.50 | 3.00 | 10.00 | 4.50 | 27.00 | 7.00 | 7.00 | 7.00 | 3.00 | 3.00 |
| 144 | 15.50 | 4.00 | 15.00 | 2.50 | 9.00 | 3.50 | 25.00 | 6.00 | 6.50 | 5.50 | 3.00 | 2.00 |
| 168 | 15.00 | 2.00 | 14.00 | 1.00 | 8.00 | 2.50 | 20.00 | 4.50 | 6.00 | 3.50 | 2.50 | |

Appendix II: Plasma concentration of chlorpropamide for individual subject in various study groups

Group II

| Time (hr) | Plasma Concentration of Chrorpropamide (μglml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| | A | B | A | B | A | B | A | B | A | B | A | B |
| 0.5 | 5.50 | 9.50 | 26.50 | 5.00 | 18.00 | 7.00 | 16.00 | 6.00 | 15.00 | 6.00 | 6.50 | 7.50 |
| 1 | 9.50 | 12.50 | 30.00 | 7.50 | 21.00 | 11.00 | 24.00 | 10.00 | 20.00 | 9.00 | 10.50 | 11.00 |
| 2. | 14.00 | 15.00 | 35.00 | 10.00 | 28.00 | 19.00 | 25.00 | 18.00 | 25.00 | 11.00 | 15.00 | 14.00 |
| 4. | 18.00 | 22.50 | 40.00 | 15.00 | 31.50 | 21.00 | 32.00 | 20.00 | 30.00 | 16.00 | 23.00 | 21.00 |
| 6. | 32.50 | 25.00 | 33.00 | 24.00 | 24.50 | 32.50 | 26.00 | 31.50 | 24.00 | 26.00 | 12.50 | 23.00 |
| 8 | 20.00 | 21.00 | 30.00 | 23.00 | 21.50 | 30.00 | 15.00 | 28.00 | 20.00 | 23.00 | 11.00 | 20.00 |
| 12 | 19.00 | 19.00 | 29.00 | 21.00 | 19.00 | 25.00 | 12.00 | 23.00 | 18.00 | 21.00 | 10.00 | 18.00 |
| 24 | 17.00 | 14.00 | 20.00 | 14.00 | 17.00 | 18.00 | 10.00 | 16.00 | 16.50 | 15.00 | 9.00 | 12.00 |
| 36 | 16.50 | 11.50 | 4.00 | 9.00 | 13.50 | 15.50 | 8.00 | 14.00 | 14.50 | 10.00 | 8.80 | 10.00 |
| 48 | 13.50 | 11.00 | 3.50 | 8.50 | 10.00 | 12.00 | 6.00 | 12.00 | 12.00 | 8.00 | 8.50 | 9.00 |
| 72 | 9.00 | 10.00 | 3.20 | 8.00 | 8.00 | 10.00 | 4.00 | 10.00 | 10.50 | 6.00 | 5.50 | 8.00 |
| 144 | 6.50 | 9.00 | 3.00 | 6.50 | 7.00 | 6.50 | 3.00 | 5.00 | 8.00 | 4.00 | 4.00 | 6.00 |
| 168 | 5.00 | 8.00 | 2.00 | 6.00 | 4.00 | 4.50 | 2.00 | 3.00 | 5.50 | 2.50 | 2.50 | 4.00 |

Appendix III: Plasma concentration of chlorpropamide for individual subject in various study groups

Group III

| Time (hr) | Plasma Concentration of Chrorpropamide (μglml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| | A | B | A | B | A | B | A | B | A | B | A | B |
| 0.5 | 7.00 | 9.00 | 15.00 | 5.50 | 16.00 | 8.50 | 7.50 | 8.00 | 10.00 | 5.00 | 5.00 | 10.00 |
| 1 | 12.00 | 10.50 | 18.00 | 9.50 | 20.00 | 9.00 | 12.50 | 12.00 | 20.00 | 10.00 | 10.00 | 12.00 |
| 2. | 17.50 | 12.50 | 20.00 | 13.00 | 22.50 | 11.50 | 26.50 | 20.50 | 23.50 | 15.50 | 20.00 | 14.50 |
| 4. | 23.00 | 30.00 | 21.00 | 19.00 | 26.50 | 16.00 | 30.00 | 21.50 | 20.50 | 20.50 | 22.50 | 21.50 |
| 6. | 21.00 | 24.00 | 25.50 | 24.50 | 21.00 | 14.00 | 29.00 | 18.00 | 28.50 | 21.50 | 21.00 | 18.50 |
| 8 | 19.00 | 11.50 | 23.50 | 21.00 | 17.00 | 11.00 | 23.50 | 16.50 | 22.50 | 15.00 | 17.50 | 16.00 |
| 12 | 18.00 | 10.50 | 20.50 | 15.00 | 15.50 | 10.80 | 20.00 | 12.00 | 20.00 | 13.50 | 15.00 | 14.50 |
| 24 | 8.00 | 10.00 | 18.00 | 10.50 | 14.00 | 10.50 | 17.00 | 10.00 | 15.00 | 11.00 | 14.00 | 13.00 |
| 36 | 7.50 | 9.00 | 15.50 | 10.00 | 13.50 | 10.20 | 15.50 | 8.50 | 14.00 | 7.50 | 11.00 | 10.00 |
| 48 | 6.50 | 8.00 | 13.00 | 9.00 | 10.00 | 10.00 | 14.50 | 6.00 | 11.50 | 6.00 | 10.50 | 8.50 |
| 72 | 5.50 | 7.00 | 10.50 | 7.50 | 9.00 | 9.80 | 12.50 | 4.00 | 11.00 | 4.50 | 9.00 | 5.50 |
| 144 | 4.00 | 6.00 | 8.50 | 6.50 | 6.50 | 9.50 | 11.00 | 3.00 | 9.00 | 3.50 | 7.50 | 3.50 |
| 168 | 2.50 | 3.00 | 5.00 | 3.50 | 5.50 | 8.80 | 9.00 | 2.00 | 7.50 | 2.50 | 6.50 | |

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## Appendix IV :Pharmacokinetic parameters for individual subjects in all study groups

| Pharmacokinetic parameters | Group I | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| | A | B | A | B | A | B | A | B | A | B | A | B |
| lag time (hr) | 0.42 | 0.54 | 0.43 | 0.55 | 0.44 | 0.56 | 0.39 | 0.51 | 0.40 | 0.50 | 0.38 | 0.52 |
| Absorption half-life t½ a(hr) | 0.38 | 0.22 | 0.14 | 0.20 | 0.08 | 0.14 | 0.06 | 0.13 | 0.14 | 0.18 | 0.21 | 0.17 |
| Absorption rate constant Ka ($hr^{-1}$) | 1.83 | 3.17 | 5.00 | 3.3 | 8.75 | 4.80 | 10.75 | 5.25 | 5.13 | 3.86 | 3.33 | 4.18 |
| Maximum concentration $C_{max}$ (μg/ml) | 32.50 | 20.00 | 28.50 | 35.00 | 34.00 | 21.50 | 41.50 | 20.00 | 20.00 | 22.50 | 10.00 | 21.00 |
| Time to peak conc. $T_{max}$ (hr) | 4.00 | 4.00 | 4.00 | 6.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 6.00 | 4.00 | 4.00 |
| $AUC_{0-168}$ μg/ml.hr | 3038.50 | 500.00 | 1719.75 | 527.50 | 573.25 | 1012.00 | 2417.75 | 1314.25 | 1359.25 | 1374.50 | 587.79 | 709.50 |
| $AUC_{0-00}$ μg/ml.hr | 1671.60 | 927.00 | 1681.00 | 937.34 | 1691.00 | 947.00 | 1651.60 | 907.17 | 1641.30 | 897.17 | 1631.30 | 887.34 |
| Clearance Cl(Uhr) | 1.24 | 2.92 | 0.96 | 2.63 | 0.85 | 1.98 | 1.07 | 1.81 | 1.40 | 2.13 | 1.86 | 2.29 |
| Volume of distribution Vd (L) | 7.35 | 10.87 | 8.33 | 5.10 | 6.94 | 10.00 | 5.68 | 11.36 | 11.63 | 8.93 | 22.72 | 10.2 |
| Elimination halflife t½ el (hr) | 4.10 | 2.58 | 6.03 | 1.35 | 5.63 | 3.50 | 3.67 | 4.36 | 5.78 | 2.90 | 8.45 | 3.15 |
| Elimination rate constant Kel ($hr^{-1}$) | 0.17 | 0.27 | 0.12 | 0.52 | 0.12 | 0.20 | o.19 | 0.16 | 0.12 | 0.24 | 0.08 | 0.22 |

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## Appendix V: Pharmacokinetic parameters for individual subjects in all study groups

| Pharmacokinetic parameters | Group II | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| | A | B | A | B | A | B | A | B | A | B | A | B |
| lag time (hr) | 0.45 | 0.15 | 0.46 | 0.50 | 0.47 | 0.48 | 0.43 | 0.52 | 0.42 | 0.46 | 0.41 | 0.47 |
| Absorption half-life t½ a(hr) | 0.22 | 0.16 | 0.11 | 0.39 | 0.12 | 0.28 | 0.07 | 0.28 | 0.11 | 0.31 | 0.18 | 0.17 |
| Absorption rate constant Ka ($hr^{-1}$) | 3.10 | 4.33 | 6.40 | 1.79 | 5.69 | 2.50 | 9.50 | 2.50 | 6.40 | 2.22 | 3.92 | 4.17 |
| Maximum concentration $C_{max}$ (μg/ml) | 32.50 | 25.00 | 40.00 | 24.00 | 31.50 | 32.50 | 32.00 | 31.50 | 30.00 | 26.00 | 23.00 | 23.00 |
| Time to peak conc. $T_{max}$ (hr) | 6.00 | 6.00 | 4.00 | 6.00 | 4.00 | 6.00 | 4.00 | 6.00 | 4.00 | 6.00 | 4.00 | 6.00 |
| $AUC_{0-168}$ μg/ml.hr | 1795.25 | 1861.75 | 771.50 | 1540.00 | 2430.00 | 1934.00 | 1040.00 | 1743.00 | 3820.50 | 1224.50 | 1164.25 | 1473.75 |
| $AUC_{0-00}$ μg/ml.hr | 1894.00 | 1656.94 | 1905.36 | 1675.97 | 1914.68 | 1615.00 | 1874.68 | 1665.00 | 1864.00 | 1635.97 | 1855.36 | 1626.94 |
| Clearance Cl(Uhr) | 1.14 | 1.34 | 1.78 | 1.41 | 1.98 | 1.55 | 3.01 | 1.50 | 1.63 | 1.74 | 0.98 | 1.49 |
| Volume of distribution Vd (L) | 6.94 | 9.26 | 5.43 | 9.62 | 7.14 | 6.94 | 6.41 | 6.94 | 7.58 | 8.06 | 10.20 | 9.62 |
| Elimination halflife t ½ el (hr) | 4.23 | 4.78 | 2.12 | 4.71 | 2.49 | 3.09 | 1.48 | 3.21 | 3.22 | 3.21 | 7.22 | 4.47 |
| Elimination rate constant Kel ($hr^{-1}$) | 0.16 | 0.15 | 0.33 | 0.15 | 0.28 | 0.22 | 0.47 | 0.22 | 0.22 | 0.22 | 0.10 | 0.16 |

## Appendix VI: Pharmacokinetic parameters for individual subjects in all study groups

| Pharmacokinetic parameters | GroupIII | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| | A | B | A | B | A | B | A | B | A | B | A | B |
| lag time (hr) | 0.46 | 0.54 | 0.44 | 0.52 | 0.48 | 0.56 | 0.42 | 0.50 | 0.43 | 0.51 | 0.47 | 0.55 |
| Absorption half-life $t_{½}$ a(hr) | 0.13 | 0.39 | 0.15 | 0.22 | 0.15 | 0.22 | 0.09 | 0.12 | 0.14 | 0.16 | 0.12 | 0.17 |
| Absorption rate constant Ka ($hr^{-1}$) | 5.30 | 1.78 | 4.67 | 3.11 | 4.75 | 3.10 | 7.75 | 5.75 | 5.03 | 4.33 | 5.75 | 4.00 |
| Maximum concentration $C_{max}$ (μg/ml) | 23.00 | 30.00 | 25.50 | 24.50 | 26.50 | 16.00 | 30.00 | 21.50 | 30.50 | 21.50 | 22.50 | 21.50 |
| Time to peak conc. $T_{max}$ (hr) | 4.00 | 4.00 | 6.00 | 6.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 6.00 | 4.00 | 4.00 |
| $AUC_{0-168}$ μg/ml.hr | 555.75 | 1493.25 | 1790.00 | 14975 | 1663.75 | 1492.75 | 2615.50 | 1003.25 | 2010.75 | 656.75 | 1661.00 | 1083.75 |
| $AUC_{0-00}$ μg/ml.hr | 1770.09 | 1214.50 | 1778.00 | 1223.00 | 1789.00 | 1230.75 | 1749.03 | 1196.75 | 1737.00 | 1180.00 | 1731.06 | 1177.50 |
| Clearance Cl(Uhr) | 2.48 | 1.61 | 1.48 | 1.56 | 1.86 | 1.24 | 1.44 | 1.79 | 1.69 | 2.11 | 1.57 | 1.57 |
| Volume of distribution Vd (L) | 8.77 | 7.58 | 8.62 | 8.62 | 8.62 | 15.15 | 7.81 | 10.42 | 7.35 | 9.26 | 10.20 | 10.00 |
| Elimination halflife $t_{½}$ el (hr) | 2.45 | 3.27 | 4.03 | 3.83 | 3.21 | 8.45 | 3.77 | 4.03 | 3.01 | 3.04 | 4.50 | 4.44 |
| Elimination rate constant Kel ($hr^{-1}$) | 0.28 | 0.21 | 0.17 | 0.18 | 0.22 | 0.08 | 0.18 | 0.17 | 0.23 | 0.23 | 0.15 | 0.16 |

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