

**COMPARISON OF THE EFFECTS OF CIMETIDINE AND DIAZEPAM
ON THE PHARMACOKINETICS OF PARACETAMOL IN
HEALTHY HUMAN VOLUNTEERS**

BY

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DECLARATION

I hereby certify that the work reported in this thesis under the supervision of Dr. Garba M. and Dr. (Mrs) Odunola, M.T. in the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, was carried out by me and that it has not been submitted elsewhere for the purpose of a degree.

The works of other investigators were duly acknowledged and referred to accordingly.

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CERTIFICATION

This thesis entitled “*Comparison of the effects of cimetidine and diazepam on the pharmacokinetics of paracetamol in health human volunteers*” by MUAZU, HARUNA-RASHEED meets the regulation governing the award of the Degree of Master of Science in Pharmaceutical Chemistry of Ahmadu Bello University and it is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my wife Hauwa'u and children; Muhammad, Abdussalam and Abdulkarim for their patience and perseverance through what seemed to be an unending PG Programme.

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ABSTRACT

The influence of Cimetidine (400 mg) and Diazepam (5 mg) on the pharmacokinetics of oral paracetamol (1000 mg) was studied and compared in eight (8) healthy human under two (2) protocols; concomitant and delayed administration of paracetamol. Subjects received one treatment at each of the five sessions with a 2 weeks wash out period in-between. Paracetamol alone was used as control. This was compared with concomitant administration with Cimetidine and then delayed (1 hour later) administration of paracetamol after Cimetidine. Diazepam was used in phase IV and V under the same protocol of concomitant administration with paracetamol and delayed administration of paracetamol 1 hour of diazepam. Ultraviolet spectrophotometer was used to determine the saliva concentration of paracetamol. Pharmacokinetic parameters (K_{ab} , $t_{1/2el}$, $t_{1/2(ab)}$, K_{el} , K_{ab} , lag time) were determined by plotting paracetamol concentrations against time on a logarithmic scale while other parameters were calculated.

There was no significant effect ($p > 0.05$) on the parameters on concomitant administration for both drugs (cimetidine and diazepam) when compared to the control. However, on delayed administration of paracetamol 1 hour after administration of diazepam, T_{max} and lag time were greatly reduced 31.11 % and 50 % respectively $P < 0.05$. $T_{1/2(ab)}$ and K_{el} were significantly increased 48.42 % and 54.57 % $P < 0.05$ respectively. The volume of distribution (V_d) and clearance (Cl) were not significantly affected $P > 0.05$.

Delayed administration of paracetamol 1 hour after cimetidine affected all the parameters C_{max} and K_{ab} were significantly reduced $P < 0.05$ by 30.02 % and

45.06 % respectively. While T_{\max} , $t_{1/2(ab)}$ and lag time were significant increased ($p < 0.05$) by 47.97 %, 42.32 % and 60.13 % respectively.

Clearance (Cl) and K_{el} were reduced significantly ($P < 0.05$) by 20.75 % and 42,76 % respectively. $T_{1/2(el)}$ and $AUC_{0-\infty}$ were increased by 39.20 % and 29.08 % respectively. The above findings show that concomitant administration of paracetamol + diazepam and of paracetamol + cimetidine does not cause any effect on the kinetics of paracetamol. On the other hand, delayed administrations of paracetamol one hour after cimetidine show decrease in absorption and delayed elimination probably due to delay in gastric emptying and inhibition of metabolism respectively.

Administration of paracetamol one hour after diazepam ingestion shows increase in absorption and change in other pharmacokinetic parameters due to enhanced gastric emptying and amplitude of contraction.

TABLE OF CONTENTS

Content	Page
Title page	i
Declaration	ii
Certification	iii
Dedication	iv
Acknowledgement	v
Abstract	vi
Table of contents	viii
List of Tables	xiii
List of Figures	xiv
Chapter 1: Introduction	1
1.1 General	1
1.1.1 Types of drug interaction	3
1.1.1.1 Physicochemical interaction	4
1.1.1.2 Pharmacokinetic drug interaction	5
1.1.1.3 Drug absorption interaction	5
1.1.2 Mechanism of drug absorption interaction	6
1.1.3 Drug distribution interactions	12
1.1.4 Drug metabolism interaction	15
1.1.5 Drug excretion interaction	17
1.2 Pharmacodynamic interaction	18
1.2.1 Principles of pharmacokinetics	19

1.2.2	Importance of the study of pharmacokinetics of drugs	22
1.3	Single compartment model	24
1.4	Two compartment model	27
1.5	Three compartments model	28
1.6	Pharmacokinetic Parameters	29
1.7	Method of residuals in pharmacokinetics	32
1.8	Spectrophotometric assay of drugs in biological fluids	33
1.8.1	Ultraviolet/visible absorption spectrophotometry	34
1.9	Analytical method development	36
1.9.1	Percentage extraction recovery	37
Chapter 2: Literature Review		38
2.1	Paracetamol	38
2.1.1	General	38
2.1.2	Chemistry of paracetamol	39
2.1.3	Absorption	39
2.1.4	Distribution	41
2.1.5	Structure activity relationship	42
2.1.6	Protein Binding	43
2.1.7	Metabolism	44
2.1.8	Excretion	46
2.1.9	Toxicity	46
2.1.9.1	Management of poisoning	48
2.1.10	Pharmacodynamics	49

2.1.11 Dosage	50
2.1.12 Salivary concentrations of paracetamol and assay methods	50
2.1.13 Paracetamol drug interactions	53
2.2 Cimetidine	54
2.2.1 Introduction and chemistry	54
2.2.2 Physicochemical properties	55
2.2.3 Assay methods in biological fluids	56
2.2.4 Pharmacokinetics	57
2.2.5 Pharmacological properties and indications	59
2.2.6 Administration and dosage	60
2.2.7 Adverse effects	60
2.2.8 Cimetidine drug interactions	61
2.3 Diazepam	65
2.3.1 Introduction and chemistry	65
2.3.2 Pharmacokinetics	66
2.3.3 Pharmacodynamic	67
2.3.4 Toxicity and adverse effects	68
2.3.5 Drug Interactions	69
2.4 Aims and objectives of the study	72
Chapter 3: Materials and Methods	73
3.1 Materials	73
3.1.1 Glassware	73
3.1.2 Equipment	74
3.1.3 Chemicals and standard samples	75

3.1.4	Tablet samples	76
3.2	Methods	77
3.2.1	Preparation of reagents, solutions and disintegration/dissolution medium	77
3.2.2	Quality Control	79
3.2.3	Preparation and validation of calibration curve	85
3.2.4	In vivo pharmacokinetic studies	89
3.3	Analytical method	93
	Chapter 4: Results	94
4.1	Quality control assessment	94
4.2	Construction and validation of calibration curve	95
4.2.1	Calibration curve	95
4.2.2	Validation of calibration curve	96
4.3	Validation of analytic method	97
4.4	Percentage extraction recovery	97
4.6	In-vivo pharmacokinetic studies	98
4.7	Pharmacokinetic parameters	100
	Chapter 5: Discussion and Conclusion	107
5.1	Quality control assessments	107
5.2	Validation of analytical methods	108
5.3	Percentage extraction recovery	109
5.4	Construction and validation of calibration curve	109
5.4.1	Validation of the calibration curve	111
5.5	In-vivo Pharmacokinetic studies	111

5.5.1	Single dose salivary pharmacokinetics of paracetamol	111
5.5.2	Influence of diazepam in pharmacokinetics of paracetamol	113
5.5.3	Influence of cimetidine on the salivary pharmacokinetics of paracetamol	114
5.6	Conclusion	116
	References	117
	Appendices	128

LIST OF TABLES

Table 3.1	Volumes of stock solution of paracetamol spiked to saliva samples and the concentrations obtained for the construction of the calibration curve	88
Table 3.2	Volumes of paracetamol stock solution spiked to saliva samples and the concentrations obtained for the validation of the calibration curve	89
Table 4.1	Quality control assessment results for paracetamol, cimetidine and Diazepam	94
Table 4.2	Calibration data for spiked saliva samples containing paracetamol	95
Table 4.3	Results obtained from the validation of the calibration curve	96
Table 4.4	Data obtained for the construction of the calibration curve	97
Table 4.5	Results for the validation of analytic method	97
Table 4.6	Results for the percentage extraction	97
Table 4.7	Mean salivary concentration of paracetamol \pm SD for paracetamol alone and with cimetidine phase II and III ($\mu\text{g/ml}$)	98
Table 4.8	Mean salivary concentration of paracetamol \pm SD for paracetamol alone and with diazepam phase IV and V ($\mu\text{g/ml}$)	99
Table 4.9	Mean value \pm SEM of pharmacokinetic parameters of paracetamol given alone and paracetamol + cimetidine concomitant (Phase II)	100
Table 4.10	Mean value \pm SEM of pharmacokinetic parameters of paracetamol given alone and paracetamol + cimetidine delay (Phase III)	101
Table 4.11	Mean value \pm SEM of pharmacokinetic parameters of paracetamol given alone and paracetamol + diazepam concomitant (Phase IV)	102
Table 4.12	Mean value \pm SEM of pharmacokinetic parameters of paracetamol given alone and paracetamol + diazepam delay (Phase V)	103

LIST OF FIGURES

Fig 1.1	Steps involved in the distribution and elimination of drugs in the body.	20
Fig 1.2	Characteristics of a single compartment model	25
Fig 1.3	Plasma concentration-time plot on a linear arithmetic scale following rapid IV injection	26
Fig 1.4	Plasma log concentration – time curve for a two compartment model	28
Fig 4.1	Mean Salivary concentration-time profile for paracetamol alone and for the influence of cimetidine under concomitant and delayed administration	104
Fig 4.2	Mean Salivary concentration-time profile for paracetamol alone and for the influence of diazepam under concomitant and delayed administration	105

Chapter 1

INTRODUCTION

1.1 General

Drug interaction is said to occur when the effect of an administered drug is altered by a prior or concurrent administration of other chemical substances in the form of another drug, food substances and their additives, alcohol, insecticides, tobacco etc (Griffin and D, arcy 1979).

Environmental factors such as smoking, atmospheric pollution or even the hardness of the water supply have also been reported to influence drug metabolism and also consequently drug interaction.

The use of herbal remedies in conjunction with conventional drug is on the increase possibly because of the erroneous belief that they are devoid of side effects when infact their use is surrounded by lack of purity, dose standardization and complete ignorance of their pharmacology and toxicology (Duker, 1973).

Clinical consequences of drug interactions will depend on drug administration factors and the patient's genetic and pathophysiological factors which may alter drug metabolism, elimination and response. Knowledge of these will help avoid or minimize incidence of adverse reactions.

Studies in drug interactions are of utmost importance considering the fact that most patients require the use of more than one drug in any given ailment. Cluff and Stewart (1971) discovered that the average patients admitted to a medical ward of their hospital received 14 drugs. In east and north-east Scotland, patients

were prescribed an average of five drugs during a hospital admission. These are only average figures and in contrast Cluff and Steward (1972) reported that one patient received a total of 36 different drugs during one hospital admission.

In Nigeria little information is available on the multiple prescriptions of drugs outside hospitals but it can be rightly assumed that every patient has at least two prescribers – his own doctor and himself while many have additional prescribers in their friends and other doctors.

Apart from the average 2.3 drugs per prescription from clinics about 50 % of these patients use over the counter drugs concurrently (Graham, 1977). In a study of a group of 60 patients using prescribed drugs, 43 % took drugs from more than one prescriber and aside from the 3.2 drugs (average) that were prescribed, an additional 2.9 drugs were used by patients within the month prior to their hospital visit (Steward and Cluff, 1971).

These figures coupled with the increased frequency of multiple drug prescribing in many countries (Crooks, 1974) leads to the conclusion that polypharmacy is widespread in modern medical practice. Over 900 possible interactions are indexed in a book on drug interaction (Stockley, 1973). The work of Hurwitz (1969) has confirmed that the rise in drug interaction is a result of polypharmacy. This work showed that the incidence of adverse drug reactions is directly proportional to the number of drugs prescribed during a hospital admission.

Intensive hospital monitoring systems have detected a rise in adverse drug reactions due to interaction and these vary from between 6.9 percent (Boston Collaborative drug Surveillance Programme, 1972) to 22 percent (Borda, Slone and Jick, 1978).

In Nigeria where it is easy to obtain almost all drugs without necessarily having a prescription, a significant percentage of the Nigerian population stand the risk of being exposed to potentially dangerous interactions. In the United States for examples an estimated 200,000 deaths per annum occur as a result of adverse drug interactions (Chyka, 2000). Another study by Kennedy *and co-workers* (2000) showed that about 50 % of the 1225 surgical patients were taking medicines not related to surgery. On the average these patients received nine different drugs with high possibility of interaction.

Studies of drug interactions have become important in order to enhance pharmacological effects or reduce side effects and avoid dangerous interactions (Jaiji *et al*, 1979).

1.1.1 *Types of drug interaction*

In the past, it was generally considered that drug interactions as a phenomena takes place at certain receptor sites. Recent findings have established that a number of drug interactions may occur outside the receptor sites. Interactions can occur both in vitro as in mixing of certain drugs with intravenous fluids or can occur in tablets or capsules when one excepiant in the formulation alters the

subsequent bioavailability of the active ingredient. This type of interaction is often referred to as physicochemical interaction.

On the other hand, the interaction can occur in-vivo in which case it can either alter the absorption, distribution or metabolism of a drug or completely alters the effect at the receptor sites.

Broadly speaking interaction could be either pharmacokinetic or pharmacodynamic.

Interactions could occur in the intestine before absorption where food or other drugs could modify the absorption characteristics of another drug. Competition at binding sites or antagonism at receptor sites could be another form of interaction.

Inhibition or induction of metabolic enzymes could modify the degradation of certain drugs especially those associated with the liver microsomes. Alteration of excretory processes of drugs in kidney tubules could either prolong or shorten the duration of action of certain drugs, thereby leading to among other things prolonged action and consequently of side effects. In fact there is no phase from formulation to elimination where drug interactions are not likely to occur (Griffin and D'arcy 1974).

1.1.1.1 *Physicochemical interaction*

This occurs when the active ingredient in a formulation reacts with one or more of the excipients. Intravenous infusions mixed with other drugs before administration gives a classical example of this type of interaction. This is

mainly due to incompatibility between the drugs added and components of the infusion fluid (Kramer, Ingloff and Cluxton, 1971).

The mechanisms for such interactions are varied. For example mixing of promethazine with chloroquine in the same syringe provides an instant colour change of the otherwise colourless liquids suggesting a possible chemical reaction. Another example is the instant formation of a precipitate when giving thiopentone sodium and vancuronium through the same giving set.

Studies by Trisel *et al* (1994) have clearly demonstrated numerous incompatibilities and drugs should never be mixed in this fashion unless the absence of reaction has clearly been established.

Physicochemical interactions could occur unintentionally in vitro between drugs and other components of pharmaceutical products including packaging materials during their preparation, storage or administration.

1.1.1.2 *Pharmacokinetic drug interaction*

This can occur during absorption, distribution or elimination. However in some cases interaction can occur simultaneously in the different phases as the drug passes through each stage.

1.1.1.3 *Drug absorption interaction*

Absorption interactions as a result of influence of some drugs on gastrointestinal function by affecting regional blood flow is common but has received relatively

little attention and rarely considered of clinical significance in pharmacokinetics (Klaus and Jouni, 2001).

Reduction in the total amount of drugs absorbed and rate of absorption generally reduce or abolish the effect of drugs.

The effect of reduction in rate of drug absorption and the reduction or abolition of amount absorbed has practical significance since the consequences may be quite different. Classical example where the rate of absorption of a long acting drug like warfarin would probably have little or no effect while a change in the total amount absorbed will have severe consequences. In contrast, a slowed down absorption of a drug with a short half life like procainamide may lead to non attainment of therapeutic plasma concentrations (Morselli, Cohen and Garattin, 1974).

Physiological and physicochemical factors play a key role in the absorption of drugs from the gastrointestinal tract (Lerine, 1970).

1.1.2 Mechanism of drug absorption interaction

Absorption of oral drugs is necessary for drugs to reach the circulatory system. The mucosal epithelium which forms a continuous cellular barrier from the buccal cavity to the anus along the gastrointestinal tract is the absorbing system. For drugs, the most important process of absorption is that of passive diffusion (Binns, 1971). This is so because it is not saturable, no energy is required and the

transfer is directly proportional to the concentration gradient and to the lipid water partition coefficient of the drug.

Below are some of the possible mechanisms of drug absorption interactions as provided by Morselli *et al*, 1974.

- pH effect on dissolution and ionization
- Changes in gastric emptying and GI motility
- Formation of complexes ion pairs and chelates
- Interference with active transport
- Distribution of lipid micelles
- Changes in portal blood flow
- Toxic effect on GI mucosa
- Changes in volume, composition and viscosity of secretions
- Effects on mucosal and bacterial drug metabolism
- Changes in membrane permeability

Most drugs are weak acids or bases that are present in solution as both the non ionized and ionized species. The non ionized molecules are usually lipid soluble and can diffuse across the cell membrane. In contrast, the ionized molecules are usually unable to penetrate the lipid membrane because of their low lipid solubility.

Therefore, the transmembrane distribution of a weak electrolyte usually is determined by its pka and the pH gradient across the membrane. It is assumed

that the gastric mucosal membrane behaves as a simple lipid barrier that is permeable only to the lipid-soluble non ionized form of the acid.

Many variables in addition to physicochemical factors that affect transport across membranes influence the absorption of drug. Absorption regardless of site is dependent on drug solubility. Drug in aqueous medium are more readily absorbed than those given in oily solution, suspension or solid form because they mix more readily with the aqueous phase at the absorptive site. Local conditions at the site of absorption alter solubility particularly in the GI tract.

It is often stated that the absorption of weak acids is reduced if they are given with alkali since less drug will be available in the unionized lipid soluble diffusible state. A few exceptions though occur as in case of Aspirin which is absorbed more rapidly from buffered alkaline solutions than from unbuffered solution at pH 2.8 (Cooke and Hunt, 1970). This is because of greater dissolution rate and aqueous solubility of aspirin in alkaline solution and rapid gastric emptying caused by an increase in the pH of the gastric contents. Contrary to the pH-partition theory, aspirin is absorbed much more slowly from the stomach than from the small intestines (Siurala *et al*, 1969).

In view of the fact that the rate of absorption of orally administered drugs is directly proportional to the rate at which drugs pass from the stomach to the intestine, drugs that affect GI motility may increase or decrease the rate of absorption of certain drugs. Drugs like atropine which reduce GI motility may decrease rate of dissolution of drugs in the GIT and consequently absorption. The

resultant prolonged contact with the absorption surfaces may lead to increase in plasma concentration.

Drug interactions during the absorptive phase result in either or both of the following potentially clinically significant effects (Barr, 1969).

- (1) Alteration in the rate of absorption
- (2) Alteration in the amount of drug absorbed

Even though a drug may eventually be completely absorbed, the decrease in rate of absorption may be so slow that

- (i) Effective serum levels may never be reached
- (ii) Onset of action may be greatly delayed and this could have severe consequence in instances where rapid onset is required to alleviate symptoms or
- (iii) The slow rate of absorption may act to sustain the release of the drug thereby unduly prolonging an effect.

Multiple dose regimens administered to achieve a constant serum level such as sedative, tranquilizers and antibiotics are hardly affected by drug interactions resulting in decreased rate of absorption. This is because the average steady-state serum levels in a multi-dose regimen is not usually affected by the relative rate of absorption but by the fraction of the drug absorbed (Evaluation of drug interactions 1976).

Drugs may interact either with food items or other drugs in the gastrointestinal tract to form complexes, ion pairs or chelates which may be absorbed more

rapidly or more slowly than the parent drug. Example is the inhibition of the absorption of tetracyclines by the formation of insoluble chelates with metals such as Calcium or Iron (Neuvonen *et al*, 1970) which are present in certain food items like milk and cheese products while dicoumarol absorption is increased by the formation of a more soluble chelates with magnesium hydroxide (Ambre and Fischer, 1973) so also is the enhancement of the absorption of quaternary ammonium antiarrhythmic agent, by ion-pair formation with salicylate or trichloroacetate (Gibaldi and Grundhofer, 1973). Dietary sources of L-Dopa and phenylalanine may competitively reduce the absorption of purines and pyrimidines which are absorbed by the small intestines active transport mechanism (Bianchine *et al*, 1971).

Phenobarbital (pka 7.6) and sodium sulphadiazine (pka 6.5), quinine (pka 8.5) which are high soluble and rapidly absorbed, their passage from the stomach to the intestine is the rate determining step in their absorption. Therefore antacids such as aluminum hydroxide that delay gastric emptying will delay their absorption.

The habit of administering drugs at meal times has become common practice without the knowledge of what effect food will have on the absorption of such drugs. Depending on the drug, foods can either increase or decrease the amount of drug absorbed. Example of drugs whose absorption is decreased when taken with food includes penicillins, tetracyclines, erythromycin, Levodopa, phenytoin and digoxin. Drugs whose absorption is increased when taken with food include

spironolactone (Melander *et al*, 1997), griseofulvin (Crouse, 1961) and itraconazole (Kastrup, 1999).

The stomach is not an important site for drug absorption. Drugs are probably absorbed more rapidly from the upper small intestine than from the stomach because of the much greater surface area of the intestine (Lerine 1970). The rate of gastric emptying may therefore limit the rate of drug absorption and is particularly important in the context of interaction since it can be influenced by many drugs (Morsech *et al*, 1974).

Drugs that might influence gastric emptying (Morcelli *et al*, 1974)

Atropine and anticholinergics

Antihistamines

Tricyclic antidepressants

Phenothiazines

Sympathomimetics

Narcotic analgesics

Nitrates

Isoniazid

Chloroquine

Metochlorpromide

Anticholinesterases

Caffeine

Prostaglandins

Antacids

Antihypertensives

Food generally retards the absorption of drugs largely because of the inhibitory effect of food on gastric emptying. However absorption is more rapid and an attendant increase in toxicity if the same dose of drug is given in a dilute solutions rather than the concentrated solutions and this is in part attributed to rapid gastric emptying (Borowit *et al*, 1971). Using paracetamol (Pka 9.5) weak acid which is largely un-ionized in both gastric and intestinal fluids it now established by Heading *et al* (1973) that the rate of absorption in man is directly related to gastric emptying rate.

1.1.3 Drug distribution interactions

After a drug is absorbed (oral) or injected into the blood stream, it may be distributed into intestinal or cellular fluids. Distribution can be said to be the act of apportioning or spreading out of a drug in an orderly manner once it reaches the blood circulation.

Patterns of drug distribution reflect certain physiological factors and physicochemical properties of the drug. An initial phase of distribution may be distinguished that reflects cardiac output and regional blood flow. The heart, liver, kidney, brain and other well-perfused organs receive most of the drug during the first few minutes after absorption.

Partition coefficient of drug, regional blood flow, binding to plasma proteins and tissue macromolecules and active transport are all factors that affect drug distribution (Rawlins, 1977).

Delivery of drugs to muscles, skin, most viscera and fat is slower and these tissues may require several minutes or hours before steady state is attained. A second phase of drug distribution may therefore be distinguished, this is also limited by blood flow and it involves a far larger fraction of the body of the body mass that does the first phase.

Based on this, drug that affect blood supply to these organs can significantly affect the pharmacokinetics of the other drugs.

Practically all drugs are bound to plasma components, red blood cells, and plasma proteins. Displacement interactions occur when drugs that compete for binding sites are concomitantly administered.

Albumin interacts with a variety of drugs than do other plasma proteins probably because its single polypeptide chain is spread out giving the molecule a relatively large surface area. This exposes its most reactive groups to the reactive groups in drug molecules. It also differs from other plasma proteins in that it contains more hydroxyl amino acids relative to its carboxy amino acid content thus leading numerous unbond cationic N-groups which can then bind anionic drug molecules (Gourley, 1971).

Since only unbound drugs are pharmacologically active, any increase in the concentration of free drugs increase its pharmacological activity. The displacement of drug from plasma protein by many anaesthetics in vitro does not appear to have any significance clinical consequence (Wood, 1991, Grandison *et al*, 2000). Phenylbutazone (though not used any more in clinical practice) displaces warfarin from protein binding sites thereby increasing the concentration of free warfarin resulting in increased anticoagulant activity with a possibility of haemorrhage.

Factors that limit the access of free drugs to both receptor sites and to biotransformation enzymes include binding of drugs to non-receptor site macromolecules. This may result in a diminished incidence of side effects and prolonged duration of action (Brodie, 1965).

For some acidic drugs, the limited carrying capacity of the protein is because they are attached to only one or two sites on the protein (Brodie, 1965) as such they appear to compete for the same number of non specific protein binding sites. Hence competition between co-administered drugs for binding sites in the body can result in displacement of one drug by another resulting in increase in concentration of the free and active fraction of the drug. This displacement or redistribution causes enhanced clinical effects and toxicity seen when certain drugs interact in man (Wardell, 1974).

For an interaction due to displacement to be of clinical significance, the drug should have extensive binding (> 90 %) and small distribution volume, the

increased free drug levels should not result in a significantly enhanced clearance from the body through increased delivery to the metabolism and excretory sites. There must be a few plasma protein binding sites which are largely saturated with drug and the levels of drug reached before displacement should be close to those causing toxicity. These conditions are likely to be met at the steady state situation where more surplus binding sites are approaching saturation than after a single dose (Bridges, 1977).

1.1.4 *Drug metabolism interaction*

The lipophilic nature of drugs that promote their passage through biological membrane and subsequent access to their site of action hinder their elimination from the body. Hence they have to be bio-transformed through either phase I or phase II metabolism. Renal excretion of unchanged drugs plays only a modest role in the overall elimination of most therapeutic agents since lipophilic compounds filtered through the glomerulus are largely reabsorbed through the tubular membranes. Biotransformation therefore leads to formation of more polar in-active metabolites that are readily excreted from the body. However sometimes active metabolites are produced.

Biotransformation reactions are classified either as phase I or II. Phase I usually involves addition of a functional group while phase II involve biosynthetic reactions (conjugation) in which the drug molecule or its metabolite is attached to a water soluble molecule such as glucuronic acid, acetyl group, glutathione, methyl group or glucosamine phase I reactions involve oxidation reduction and hydrolysis.

Oxidation is the most important Phase I reaction and usually catalysed by an enzymes known as cytochrome p450. Most metabolic drug interactions involve either the induction or inhibition of the cytochrome P 450 (CYP 450) enzymes (Levy *et al.*, 2000)

Oxidation reactions mostly involve aromatic and aliphatic hydroxylation, oxide formation, desulphurization, deamination, deacylation and sulphoxidation-Reduction reactions include azoreduction, aldehyde reduction and nitro reduction while hydrolytic reactions include de-etherification and determination.

Enzymes involved in oxidation and reductive reaction are most found in liver microsomes while hydrolytic enzymes are located in the plasma, liver microsomes and many other tissues.

The cytochrome P450 group of enzymes are haeme containing membrane proteins localized in the smooth endoplasmic reticulum of numerous tissues. They are characterized by a maximum absorption wavelength of 450 nm in the reduced state in the presence of carbon monoxide. Oxidative reactions catalysed by the microsomal monooxygenase system require the CYP 450 hemoprotein.

Analysis of their amino acid sequences shows that they are divided into families, sub families and specific enzymes. Those belonging to family CYP 1, CYP 2 and CYP 3 are involved mainly in the metabolism of drugs and other xenobiotics where as those belonging to the families CYP 4, CYP 5 and CYP 7 have endogeneous functions (Levy, 2000)

Quite a lot of chemicals are implicated in either the induction or inhibition of the CYP 450. Inducers include Phenobarbital, chlorinated hydrocarbons, insecticides, cigarette smoke etc. (Corney and Burne, 1972) while those causing inhibition include among others phenylbutazone, imidazoles (Powell and Donn, 1983).

Age, sex, strain and species affect the activity of the CYP 450 dependent system. Recent studies have shown that dietary supplements and natural products can modify the pharmacokinetics of drugs. The plant *Hyperuricum perforatum*, for example is widely used as an antidepressant in the United States has been shown to be a potent inhibitor of CYP3 A4 and can have potentially hazardous drug interactions when used with the substrates for CYP 3 A4 (Fugh-Berman, 2000).

1.1.5 Drug excretion interaction

Drugs are eliminated from the body either unchanged or as metabolites. The kidney is the most important organ for elimination of drug and their metabolites, other minor of elimination routes include feces, breast milk and lungs.

Drug excretion is slowed in patient with impaired renal function and as such dosage adjustments become necessary in such situations. Dettli, Spring and Ryter, 1971 have described the various methods for reducing the usual dose of drug eliminated from the body by the urinary excretion of unmetabolized drug to correct for the delayed excretion by extrarenal renal excretion rate fall and the major pathway of elimination may shift from renal to extra renal.

Excretion of drugs by kidneys involves three major processes viz:- passive glomerular filtration active tubular secretion and may lead to a major drug interaction involving the kidney for substance whose major route of eliminate is via this pathway.

In general, acidic or basic drugs may be expected to show the phenomenon of pH- dependent excreted if the unionized fraction is lipid soluble and if the pka is within a favorable range of 7.5 - 10.5 for weak bases and 3.0 - 7.5 for weak acids. Weak acids are excreted at a higher clearance in highly alkaline urine and weak bases in acid urine glomerular filtration rate of drugs is hardly affected by other drug except in disease states. However pH greatly modifies the tubular re-absorption. In an alkaline medium basic drugs like amphetamine will remain unionized and so will be reabsorbed thereby prolonging the effect of the drug. The opposite applies to acidic drug like nalidixic acid.

1.2 Pharmacodynamic interaction

This involves mainly interaction at receptor sites. There are numerous examples of drugs that interact at a common receptor site or that have additive or inhibitory due to actions at different sites in an organ.

Many drugs exert their pharmacological activity by binding to specific receptor sites. Other drugs can occupy these receptors without producing any response thereby preventing or reversing the effect. Example is the reversal of the antihypertensive effects of guanethidine by amphetamine, phenothiazines as effective α – adrenergic agonist, antihistamine and TCA's as potent agonists at

muscarinic receptors. Pharmacodynamic interactions could lead to either potentiation of action or antagonism. These can be grouped into four viz:

- i. Potentiation produced by two drugs acting at the same site e.g. observed in patients receiving streptomycin (which has weak depolarizing properties) in the presence of a depolarizing muscle relaxant (Toivakka and Hokkanen, 1965). This interaction is predictable though the extent depends on relative potencies and dosages of the two drugs,
- ii. Potentiation by two drugs acting at different receptors sites. Classical example is the combination of 2 different types of antihypertensives.
- iii. Potentiation by one drug which on its own has no effect. This is very unusual, example is the increased anticoagulant activity of warfarin during therapy with clofibrate or D-thyroxine (Schrogié and Solomon, 1967).
- iv. Antagonism of the effect of one drug by another. This can be caused by several underlying mechanisms the commonest being the effect produced by competition for receptor sites by two drugs both with affinity for the receptor but only one having the intrinsic activity. Example includes alpha and beta adrenoceptor blocking drugs, antihistamines and naloxone (Graham-Simth, 1975).

1.2.1 Principles of pharmacokinetics

The onset, duration and intensity of action of a drug after administration are controlled by the rate at which the drug reaches its site of action and by the concentration of the drug at the receptor. The physiological disposition of a drug

is controlled by the three major processes of absorption, distribution (into and within tissues) and elimination (metabolism and excretion).

A mathematical consideration of these three rate processes which relates the dose given, the concentration in the blood and the pharmacological response is known as pharmacokinetics (Olaniyi A.A., 2000).

It is concerned with quantitatively accounting for the whereabouts of a drug after it has been introduced into the body. It involves the study of these rates of absorption from the various loci of administration, distribution within the body, metabolic transformation and of elimination (Van der Kleijin and Vree, 1979).

The complexity of the drug molecule requires a careful study of its behaviour because not doing so could lead to therapeutic failure or even high incidence of adverse reactions. For most drugs, the extent and duration of action depends on how much of the drug gets to the receptor sites and for how long it stays there (Paxton, 1981).

The transfer of a drug from its absorption site to the blood and the various steps involved in the distribution and elimination of the drug in the body may be depicted as follows.

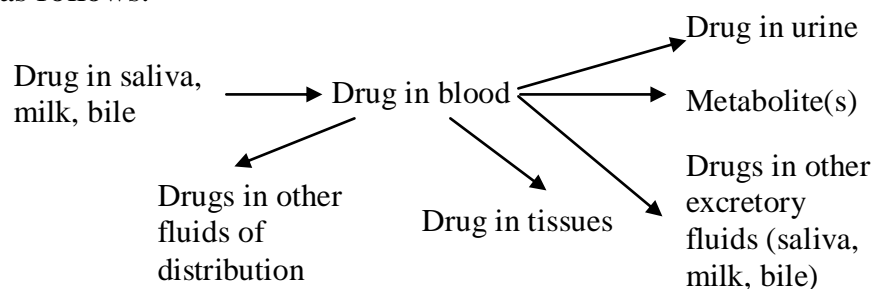
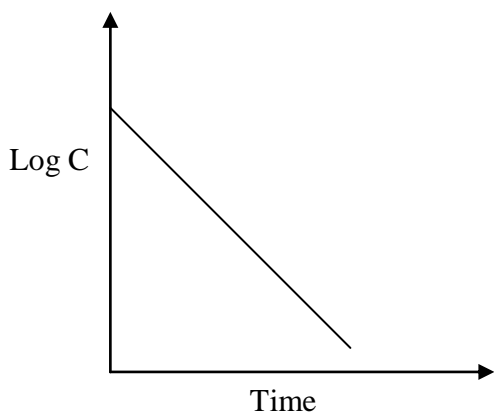


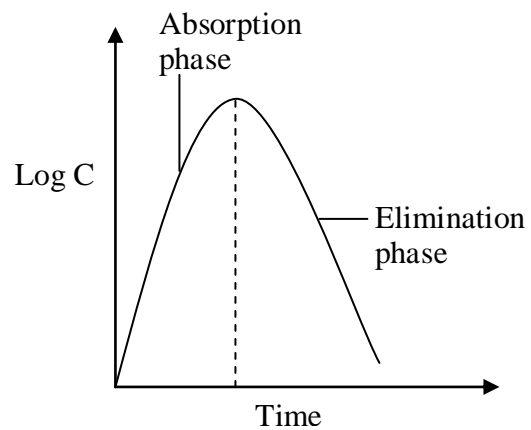
Fig 1.1 Steps involved in the distribution and elimination of drugs in the body.

It has been established that the intensity of the pharmacologic effects of many drugs is related to their concentration and/or to the concentrations of this metabolite (s) in plasma (Levy, 1976).

The primary standard of a pharmacokinetic study is based on the data obtained following intravenous administration of drug in which the entire dose administered is placed directly into the blood stream. The pharmacokinetic parameters obtained are not affected by all the potential rate-limiting and metabolic factors associated with drug absorption following other routes. (Paxton, 1981). Most drugs given by intravenous injection or by mouth will give blood (or plasma) concentration –time curves as shown below.



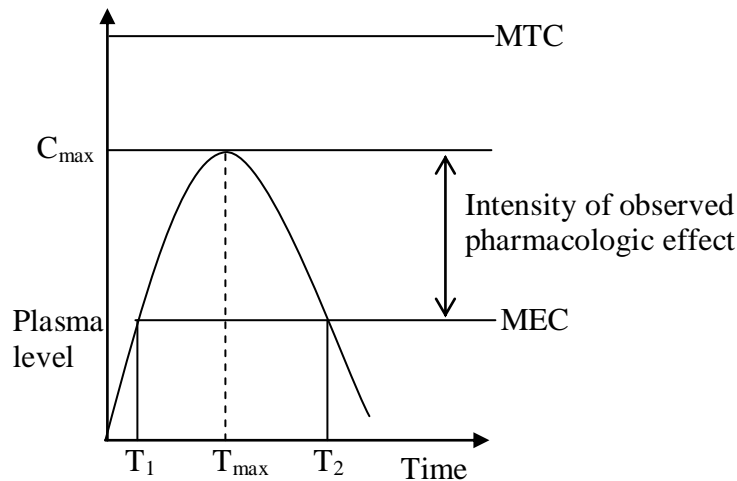
Typical semi-log plasma conc. Vs time curve for a drug administrated by iv injection



Typical semi-log plasma plot of plasma concentration vs time for drugs administrated orally

After oral administration, plasma-concentration initially increases while the drug is being absorbed, gets to a maximum and then decreases when elimination becomes the major process.

The figure below gives a plasma level-time curve showing peak time and peak concentration for a drug MEC is the minimum effective concentration of the drug just needed for therapeutic action while MTC is the minimum concentration of drug needed for toxic effect of the drug to manifest.



MTC – Minimum toxic conc

MEC – Minimum effective conc.

T_{max} – Time of peak plasma conc

T_1 – Time of onset of action

T_2 – Time of termination of action

T_1T_2 – Duration of action

1.2.2 Importance of the study of pharmacokinetics of drugs

This has become significantly relevant for the following reasons:

- i. It is important in the design of new drugs through assessment of the fate (absorption, distribution, metabolism and excretion) of drugs in the body.

- ii. It helps in the prediction of plasma, tissue and urine drug levels with any dosage regimen.
- iii. It provides a way of estimation of possible accumulation of drug/or metabolites in the body.
- iv. Helps in developing rational or optimal therapeutic dosage regimen (appropriate dose, dose interval and mode of administration for each patient since drug disposition differs with individuals.
- v. Provides explanation of drug – drug interaction
- vi. It describes how changes in physiology or disease of patient affect fate of drugs in the body.
- vii. It helps in evaluation of the difference in the rate or extent of availability of drugs between formulations.

Practical clinical pharmacokinetics involves the quantification of drug in readily accessible body fluids followed by attempts to describe the biological events in mathematical terms. The attempts to define this time-course of events in the body have produced three philosophical approaches to practical pharmacokinetics.

- i. Compartment model approach
- ii. Physiological model approach
- iii. Non-compartment approach

Compartment models are used to describe the behaviour of drugs in biological systems and could be classified into one, two or multicompartment models (Paxton, 1981).

In using the compartment model approach the following assumption must be made

- i. Drug enters the system only through the central compartment
- ii. There should be reversible transfer between central and peripheral compartments
- iii. Transfer among compartments and drug elimination from compartments all obey first order kinetics.

1.3 Single compartment model

This is the simplest model and assumes that after the drug is administered and absorbed, it is instantly and homogeneously distributed through out the fluids and tissues of the body. The exchange of drug between plasma and tissue proceeds more rapidly than the rate of elimination. This model is often referred to as one-compartment open model. The “open” indicates that input and output are unidirectional and that the one compartment (i.e. the body) is not within a confined space and hence does not come into chemical equilibrium with its environment.

This model is useful for the pharmacokinetics analysis of blood plasma or serum concentration, salivary and urinary excretion data for drugs which are rapidly distributed between plasma and other body fluids and tissues upon entry into the systemic circulation (Paxton, 1981).

The characteristics of a single compartment model may be illustrated in the figure below.

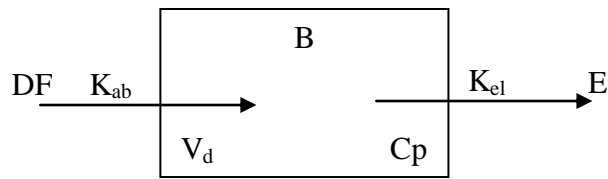


Figure 1.2 Characteristics of a single compartment model

- D - Dose of drug administered
- F - Bioavailability
- K_{ab} - Absorption rate constant
- B - Body (blood, body fluids and tissue)
- V_d - Volume of distribution
- C_p - Concentration of drug in plasma
- K_{el} - Elimination rate constant

Assuming intravenous injection into this model the concentration C_p , in the plasma immediately after injection is equal to the dose D divided by the volume of this compartment V_d .

$$C_p = \frac{D}{V_d}$$

This apparent volume of distribution V_d is a hypothetical volume into which all the drug in the body would appear to be distributed to achieve a concentration the same as that in the plasma (Paxon, 1981).

Elimination follows a first order kinetics which means that a constant fraction of drug present is eliminated for unit time. The plot of plasma drug concentration against time with each unit representing the time for one half of the drug to be eliminated ($t_{1/2}$) results in an exponential curve shown in figure below.

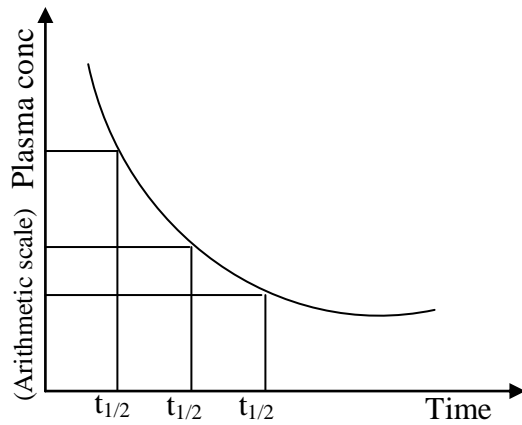
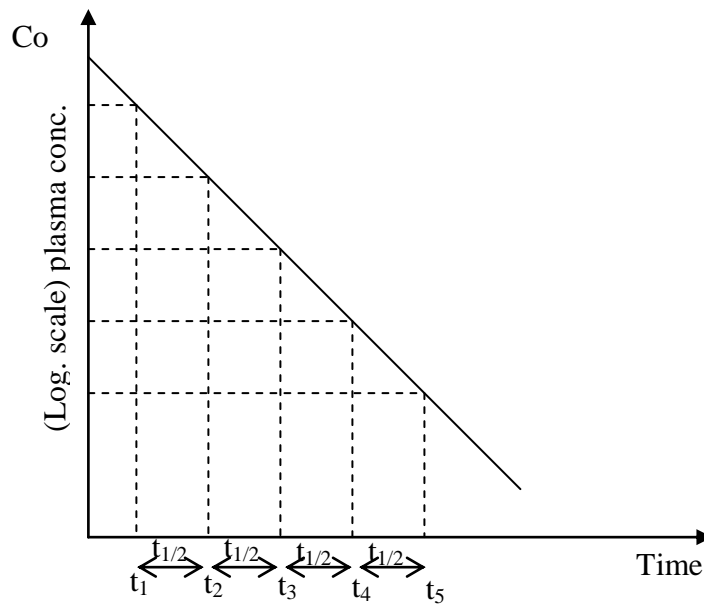


Figure 1.3 Plasma concentration-time plot on a linear arithmetic scale following rapid IV injection

Replotting this curve on a logarithm scale gives a straight line curve

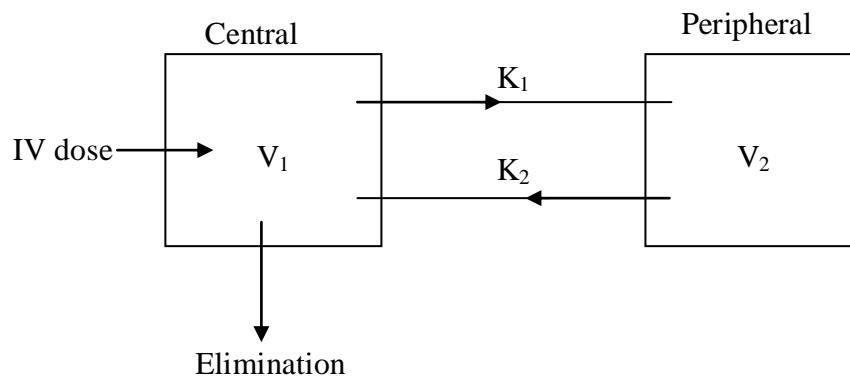


Plasma log. conc. – time curve for one compartment model

C_0 represents the concentration at time zero.

1.4 Two compartment model

If the distribution of a drug is slow, a model must then be considered which contains a central compartment and at least one other peripheral compartment.



K_1 and K_2 are the transfer rate constants between the compartments.

Two compartment model

Despite these compartments not having any physical or anatomical reality, for many drugs, the central compartment corresponds to the blood or plasma volume together with the extracellular fluid of highly perfused tissues such as heart, liver, kidney and endocrine glands.

The combined effect of two compartments gives rise to a biphasic curve on iv administration with two distinct linear portions when drawn on a semi log scale.

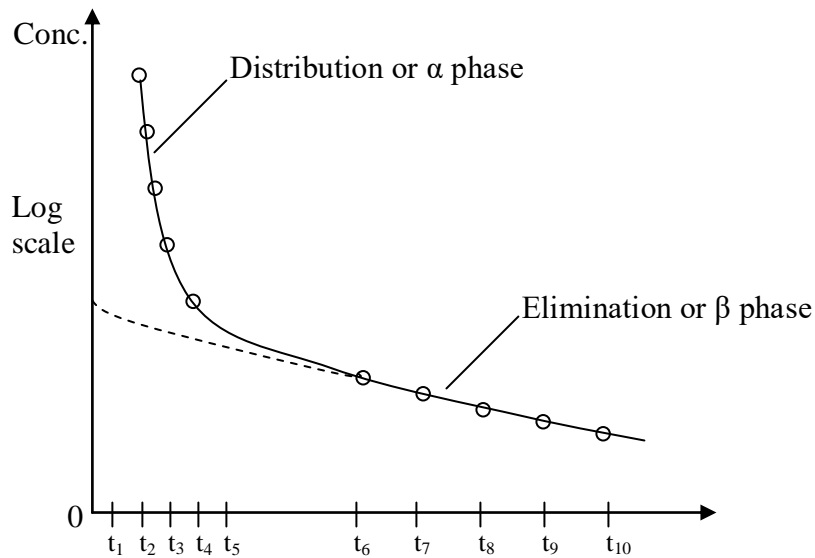
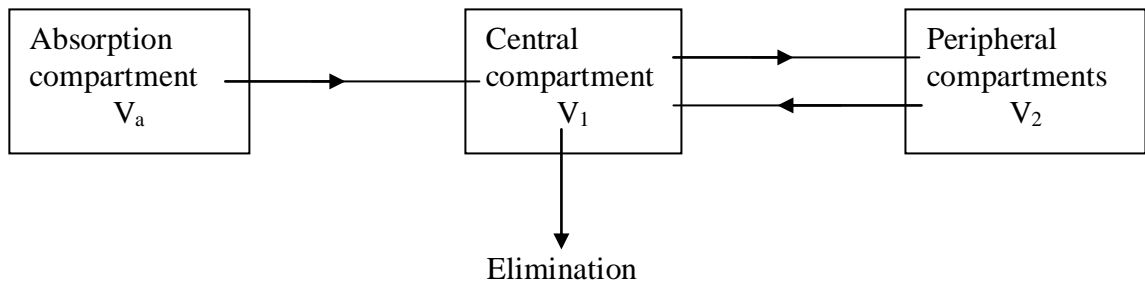


Fig. 1.4 Plasma log concentration – time curve for a two compartment model

The two compartment model may be expanded to contain additional compartment which can be described mathematically as the sum of as many individual exponential functions as there are relevant compartments. In reality, only 3 compartments is allowed in assay techniques (Paxton, 1981).

1.5 Three compartments model

In this model it is assumed that the entire dose of the drug is rapidly introduced into the site of absorption from which it is absorbed into the central compartment. It incorporates a third compartment to represent the volume V_a , absorption occurs at a first order rate.



Where K_a = absorption rate constant

K_1 and K_2 = transfer rate constant between the 2 compartments

Three compartments model

1.6 Pharmacokinetic parameters

The following have been identified as some of the most important pharmacokinetic parameters.

i. Clearance (Cl)

The desire to maintain a steady-state concentration within a known therapeutic range makes clearance the most important concept to be considered especially when a rational regimen for long term drug administration is to be designed (Goodman and Gilman, 1996).

It is the volume of plasma from which a drug is totally and irreversibly removed per unit time (Kamaludeen, A.B., 1996). The elimination of most drugs follows a first order kinetics i.e. a constant fraction of the drug is eliminated per unit time. If the mechanism for elimination becomes saturated, the kinetics becomes zero order i.e. a constant amount of drug is eliminated per unit time.

Mathematically,

$$Cl = \frac{\text{rate of elimination}}{C}$$

C = concentration, Cl – clearance

Clearance has dimensions of volume per unit time and expresses the efficiency with which a drug is eliminated and is made up of the sum of all the clearances due to individual organs. Where only one organ is involved e.g., the kidney then the plasma clearance becomes equal to the renal clearance.

$$Cl_{\text{renal}} + Cl_{\text{hepatic}} + Cl_{\text{other routes}} = Cl_{\text{system}}$$

Factors such as organ blood flow, ability of the organ to extract the drug from the blood and the extent of binding of the drug to plasma proteins are usually the physiological determinants of extent of clearance by any particular organ.

ii. Area under the curve (AUC)

Area under the plasma concentration curve from time zero to infinity is a measure of the extent of drug absorbed into the systemic circulation i.e. measure of bioavailability (Hinna, 1998).

Method usually employed to determine AUC include the use of planimeter, the cut and weigh method or the trapezoidal triangular method.

The most commonly employed is the trapezoidal method which involves dividing the plasma concentration-time curve into sections that approximate a

series of trapezoids with a triangle at each end. The individual areas of the trapezoids and triangle are summed to obtain the AUC (Notari, 1975)

iii. Apparent volume of distribution (V_d)

This is defined as the volume in which the amount of drug in the body would need to be uniformly distributed to produce the observed plasma concentration (Kamaludeen, A.A., 1996).

This volume does not necessarily refer to an identifiable physiological volume but merely to the fluid volume that would be required to contain all of the drug in the body at the same concentration as in the blood plasma.

$$V_d = \frac{\text{Amount of drug in body}}{C}$$

V_d can be determined from the following mathematical expression

$$V_d = \frac{FD}{AUC}$$

Where

$$F = \text{Bioavailability}$$

$$D = \text{Dose}$$

$$AUC = \text{Area under the curve}$$

The volume of distribution for a given drug can change as a function of the patient's age, gender, disease state and body composition (Goodman and Gilman, 1996).

iv. Elimination half life

This is the time required to change the amount of drug in the drug by one-half during elimination (Holford and Benet, 1995).

$$t_{1/2} = \frac{0.693}{K_{el}}$$

Where K_{el} is the slope of the first order plot based on the equation for a one compartment model or the final slope of the bisphasic plot on the equation for a two compartment model.

Elimination half-life is a useful parameter in the determination of suitable dosage intervals and the time required to attain steady state during intravenous or repetitive oral therapy (Paxton, 1981).

1.7 Method of residuals in pharmacokinetics

Residual method is also known as feathering and it refers to a graphical method for separation of exponent. Such as separating absorption rate constant (K_{abs} or α -slope) from the elimination rate constants (K_{el} or β -slope). Thus it can be applied to drugs that exhibit one-compartment kinetics and for which the absorption and elimination are first order (Tozer, 1979). Under these conditions, the rate of change of drug in the body is simply the difference between the rate in and the rate out.

For this graphical approach, the drug concentration data are plotted on a log scale versus time. In one compartment open model, the mono-exponential elimination slope is back extrapolated to the ordinate. The difference i.e. the residuals

between the measured plasma concentrations and the corresponding values on the back extrapolated line at each sampling time point at the absorption phase are calculated. These residuals are then plotted semi-logarithmically versus time on the same graph. The slope of this plot yields an estimated absorption rate constant (K_{ab}). This method of residuals is probably a reasonable means of graphically approximating the absorption rate constant if the data are well described by the difference between exponential terms and if the distribution of the drug in the body is truly unicompartamental (Tozer, 1979). The lag time can also be determined from this method and is determined from the point of interception of the line of residual and the back extrapolation.

1.8 Spectrophotometric assay of drugs in biological fluids

The interaction of radiation with organic molecules leads to the absorption of suitable wavelength of the radiation according to Planck's law. Such interaction of energy with organic molecules leads to changes in the electronic, vibrational or rotational energy inherent in the molecules all of which constitute what is known as the internal energy of a molecule (Olaniyi, A.A., 2000).

Various regions in the electromagnetic spectrum have applications in pharmaceutical analysis. The techniques frequently employed here include ultraviolet, infrared, atomic absorption and emission spectroscopy.

The spectral range for these measurements can for convenience be divided into UV (190 – 380 nm) visible (380 – 780 nm), near infrared (780 – 3000 nm) and the infrared (4000 – 250 cm).

1.8.1 *Ultraviolet/visible absorption spectrophotometry*

This is the commonest physicochemical method and with the most application is analysis of drugs in biological samples. This provides a characteristic spectrum that provides information on the identity or structure of the analyte. The quantitative information depends on the extent of absorption.

The Beer-Lambert law relates the total absorption to the optical path length

$$\text{Absorption (A)} = \log_{10} \frac{I_0}{I} = KI$$

I = Incident light

L = path length

K = Proportionality constant

The characteristic single spectrum which gives a sharp peak is achieved at a particular wavelength called maxima. But compounds with the same functional group will have a similar absorption spectra, the technique is therefore not suitable for identifying unknown compound of a sample. Metabolites with the same functional group can not be analysed by this technique.

The use of appropriate solvents at a particular pH ensures only parent drug is extracted and this further ensures specificity of absorption at a particular wavelength.

Drugs can be determined either as intact compounds or as a derivative formed by a chemical reaction with suitable reagents (Smith and Stewart, 1981). An

advantage of chemical derivation as an aid to UV analysis is that the sensitivity of the procedure is increased significantly.

One of the main reasons for its popularity is that the sensitivity of the method is in the range of 1 – 10 µg/ml which is comparable to the concentration levels of many drug substances in biological samples (Smith and Stewards, 1981).

This method has been employed extensively by scholars, some of which include Mustapha *et al* (1996) in the study of the effect of *Tamarindus indica* on the bioavailability of aspirin in healthy human volunteers. Also Garba *et al* (1999) employed the UV method to study the influence of cimetidine on the pharmacokinetics of paracetamol in healthy human volunteers.

In a study to determine paracetamol in urine, the UV spectra of investigated samples were recorded over the wavelength range 220 – 400 nm (step 0.21 nm, scan speed 60 nm/min) and second order derivatives spectra were calculated. Second order derivative spectra of different blank urine samples displayed the presence of a zero crossing point at 245 – 247 nm denoted Z_c . The zero order absorption of paracetamol in water displays maximum absorption at 243 nm, while in second order derivatives spectra a minimum peak at 246 nm was observed. Therefore the application of zero-crossing technique to the second derivative UV absorption spectrum should be useful for the determination of paracetamol using DZC. The obtained results were in accordance with published data on cumulative urinary excretion after oral administration of paracetamol

obtained applying different spectrophotometric methods of determination (Jelena *et al*, 2003).

1.9 Analytical method development

The chemical characteristics of the drug to be analyzed have to be known first to be able to decide on which method is best to adopt. NMR, mass spectra and UV spectra are all vital tools for both qualitative and quantitative analysis.

Another very important consideration which may well dictate the method to be adopted is the sensitivity required i.e. the lowest concentration which could be measured after optimization of analytical conditions. De Silva (1995) has developed concentration ranges for which various techniques are applicable.

Analytical method validation includes all the procedures required to demonstrate that a particular method for the qualitative determination of the concentration of the analyte (or series of analytes) in a particular biological matrix is reliable for the intended application.

Some frequently employed techniques include:

- 1 Chemical methods e.g. GC, HPLC, Mass spectrometry
- 2 Biological methods e.g. those based on immunoassay procedure and microbiological methods.

The objectives of analytical method validation are:

- (i) To build confidence in analytical data generated continually.
- (ii) To ensure that a selected analytical procedure will give productive and reliable results that is adequate for the intended purpose. It is therefore

necessary to validate a method according to well established criteria of precision, accuracy, sensitivity specificity and reproducibility

- (iii) To promote smooth transfer between laboratory and inter-laboratory collaborators (Olaniyi A.A., 2000).

1.9.1 *Percentage extraction recovery*

The percentage extraction recoveries of analytical method give the efficiency of the extraction procedures to be employed in the analysis. It also gives assurance on the reproductivity of the extraction method employed.

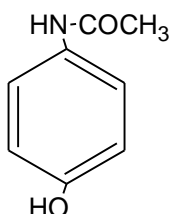
$$\% \text{ recovery} = \frac{\text{amount in concn. recovered from sample}}{\text{amount in concn. recovered after extraction in 0.1N HCl}} \times 100$$

2.1 Paracetamol**2.1.1 General**

Paracetamol also called acetaminophen was first introduced around 1950 after it was discovered that it is the active metabolite of acetanilide which had to be withdrawn because of high toxicity. Because it is well tolerated, it lacks many of the side effects of aspirin and is available without prescription. Its introduction in America in 1950 as a compound analgesic containing paracetamol 125 mg, caffeine 30 mg and aspirin 230 mg leads to its withdrawal because of suspicion that it could cause agranulocytosis. In 1963, paracetamol was added to the British pharmacopoeia (B.P) and its popularity as an over the counter (OTC) analgesic increased rapidly. The complete ban on the use of phenacetin as an analgesic ingredient in medicines further increased the popularity of paracetamol as the most acceptable alternative to aspirin as an antipyretic/analgesic. However, unlike aspirin its anti inflammatory activity is weak and so cannot be used in treating such conditions. In normal doses, it is remarkably free from adverse effects, and interactions with other drugs even in patient with established liver disease. However acute over dosage cause fatal hepatic damage and the number of self poisonings and suicide with paracetamol has grown alarmingly in the recent past (Godman and Gilman, 1996). The association of aspirin with Reyes syndrome and peptic ulcer has further boosted the popularity of paracetamol. It is well tolerated by patients with peptic ulcer (Thomas, 1993)

2.1.2 Chemistry of paracetamol

Paracetamol is 4-acetamidophenol (acetaminophen) or N-acetyl -p-amino phenol 4 hydroxy acetanilide and N-(4-Hydroxyphenyl) acetamide. It has the following chemical structure and formula



A saturated aqueous solution has a pH of about 6 and is stable with a half life of over 20 years. But stability decreases in acid or alkaline conditions, the drug being slowly broken down into acetic acid and P- aminophenol (Koshy and Lach, 1961).

2.1.3 Absorption

Paracetamol is rapidly and almost completely absorbed from the GI tract. Peak plasma concentrations are reached with 30-60 minutes (Prescott *et al.*, 1971). This could be decreased by co-administration of an effervescent buffer (Hinna, 1998).

Being a weak acid with PKa 9.5 it remains largely unionized both in the stomach and the intestines and so should be well absorbed from both sites (Schanker *et al.*, 1957).

Most of it is absorbed from the small intestine therefore the rate of absorption is likely to depend on gastric emptying rate (Sample, 1973, Weikel, 1958).

The peak plasma concentration after oral administration of 1.5 g paracetamol to 14 patients varied from 7.4 to 37.0 µg/ml and the time taken to reach these levels ranged from 30 to 180 minutes (Heading *et al.*, 1973). They suggested that their variation might be due to individual difference in the rate of gastric emptying.

The presence of food in the stomach reduces the rate of absorption of paracetamol (Heading *et al.*, 1973) probably due to inhibitory effect of food on gastric emptying rate (Welling, 1977).

Concomitant administration with food produced little effect on extent of absorption. Blood concentrations and urinary excretion data have shown that subjects who ingested 1 g paracetamol after over night fasting absorb the analgesic up to 5 times more rapidly. Subjects who ingested 1 g paracetamol immediately before sleep at night excreted over a period of six hours up to 36% less than when they took the same amount at 8.30 am (McGilveray and Matlock, 1972) absorption continued

Neonatal gastric pH is almost 7 and although this favours absorption of paracetamol the stomach is not an optimal entry point to the circulation. Paracetamol is therefore ideally absorbed from the small intestine. Slow absorption of paracetamol in infants less than three months old has been demonstrated (Anderson *et al.*, 2000).

The pharmacokinetics of paracetamol was also dose dependent during a single dose bioavailability study. Type of formulation also affects the availability

paracetamol preparation ,for example it was found that the absolute availability of an elixir was significantly greater than of tablets- 87% and 79% respectively (The pharmaceutical codex 1994).

In a study, it was established that paracetamol absorption can be used as marker for gastric emptying due to good correlation between scintigraphy and the paracetamol absorption technique (Willems *et al.*, 2000)

Alterations in gastric pH have no appreciable effect on paracetamol absorption (Pottage, *et al.*, 1972). Although paracetamol is rapidly absorbed unchanged from the GI tract, it is incompletely available to the systemic circulation after oral administration, available proportion being lost through first pass metabolism (Chion 1975, Perucca and Richens, 1979). It was suggested that the difference in the contribution of the first pass effect for rectal and orally administered paracetamol would not be of clinical significance (Chion, 1975).

2.14 Distribution

It is relatively uniformly distributed through out most body fluids (Flower *et al.*, 1985 and Goodman and Gilman 1996). With the exception of fat in dogs (Guilt *et al.*, 1963) paracetamol is well distributed evenly in all tissues.

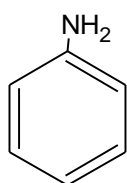
Paracetamol penetrates the brain of mice, rats, cats, dogs and monkeys to a greater extent than aspirin possibly because it is a non ionized, lipid soluble substance with a low degree of protein binding (Davson *et al.*, 1967).

Paracetamol has been found in human saliva in concentrations which correlate well with plasma levels (Glynn and Bastain, 1973).

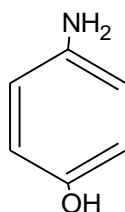
It is also present in breast milk though probably without any clinical significance (Findlay *et al.*, 1981 and Wallin, 1972)

Structure activity relationship

The antipyretic activity of the compound resides in the amino benzene structure. The introduction of other radicals into the hydroxyl group of paracetamol and into the free amino group of aniline reduces the toxicity without loss of antipyretic activity

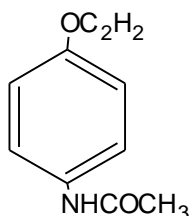


Aniline

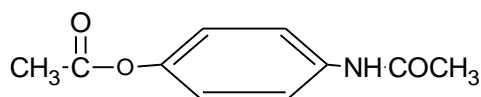


Para-aminophenol

Acetaminophen, phenacetin both of which are phenolic alkyl ether give best results (Goodman and Gilman 1996)



Phenacetin

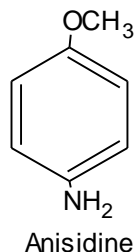


P-acetoxy acetanilide

The salicyl ester exhibits diminished toxicity and an increased in antipyretic activity

The weak anti-inflammatory activity of paracetamol may be attributed to the fact that acetaminophen is only a weak inhibitor of cyclooxygenase in the presence of the high concentrations of peroxides that are found in inflammatory lesions (Marshall, *et al.*, 1987; Hanel and Lands, 1982). Further, paracetamol does not inhibit neutrophil activation as do other NSAIDs (Abramson and Weissmann, 1989).

Esterification of the phenolic hydroxyl group produces stronger analgesics but increases toxicity e.g. anisidine



Acylation of the amino group with a free-OH group produced the best P-amino phenol analgesics e.g. paracetamol (Willette, 1982)

2.1.6 Protein Binding

Paracetamol exhibits negligible protein binding in humans (Gazzard *et al.*, 1973) and is highly lipid soluble. At a concentration of 280 µg/ml about 15-21% is protein bound corresponding to the levels observed after human overdose. (Gazzard *et al.*, 1973)

The sulphate and glucuronide conjugates of paracetamol do not bind to plasma protein even in the relatively high concentration found in anephric patients. (Lowenthal *et al.*, 1976)

With the exception of the pituitary, there is no marked localization of the drug in any organ in contrast to the salicylates, however, a considerable greater portion of acetaminophen enters the brain and presumably other tissues and much less remains in the blood. In animals, its concentration in the brain is equal to that in the blood 30 minutes after administration though it disappears more rapidly from the brain than does aspirin perhaps resulting in briefer analgesia and antipyretic activity than aspirin (Davison *et al.*, 1961)

2.1.7 Metabolism

Paracetamol is primarily metabolized by the liver. The pathway is a cytochrome P450 dependent N-hydroxylation process (Thorgeirson and Wirth, 1977). 90-100% of the drug may be recovered in urine within the first day after therapeutic dose primarily after hepatic conjugation with glucuronic acid (about 60%) sulphuric acid (about 35%). Small amounts of hydroxylated and deacetylated metabolites have also been detected (Goodman and Gilman 1996) less than 5% is excreted unchanged.

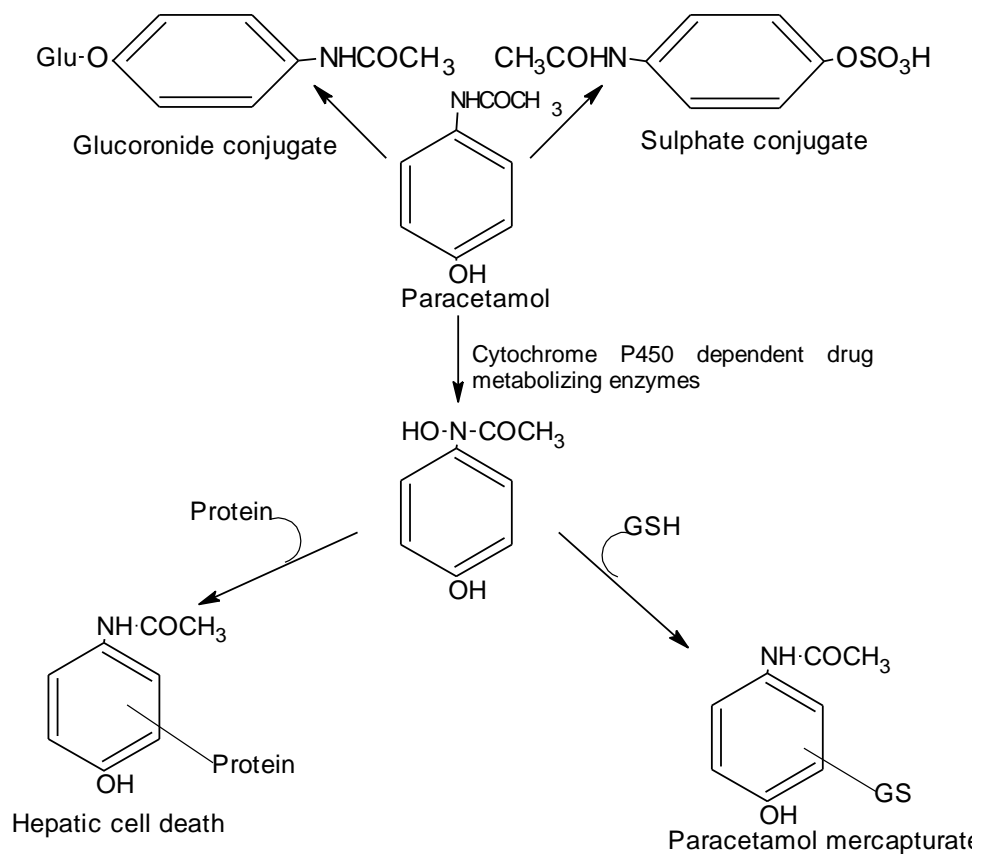
The manner in which the liver metabolizes paracetamol changes with age. There is a reversal of the usual adult ratio of 2:1 with respect to glucuronidation versus sulphation of paracetamol in young children. This pattern however reverts to adult pattern at the age of 12 years (Miller *et al.*, 1993).

When paracetamol intake far exceeds therapeutic doses, the glucuronidation and sulphation pathways are saturated and the cytochrome P 450 dependent pathway becomes increasingly important. Little or no hepatotoxicity occurs as long as

glutathione is available for conjugation however, with time, hepatic glutathione is depleted faster than it can be regenerated and accumulation of a reactive and toxic metabolite occurs. In the absence of intracellular nucleophiles such as glutathione, this reactive metabolite (thought to be an -N-hydroxylated product or N-acetylated iminoquinone) reacts with nucleophilic groups on cellular macromolecules such as protein resulting in hepatotoxicity (Correia, 1995).

In newborn infants, glucuronide formation of paracetamol may be delayed because the enzymes synthesizing system are not fully developed there by leading to enhanced toxicity (Vest and Streiff, 1959).

Acetaminophen is metabolized via a variety of pathways as shown in the figure.



Pathway of paracetamol metabolism

GSH = reduced glutathione (adapted from Correia, 1995)

Food and water deprivation in calves impairs the formation of major metabolites (glucoronide and sulphate) of paracetamol (Janus *et al.*, 2003)

The first metabolite to be recognized in the urine was the sulphate (Morner, 1989) and at a time this was thought to be the main product of conjugate (Greenberg and Laster, 1946).

2.1.8 Excretion

Paracetamol is excreted in the urine primarily as inactive glucuronate and sulphate conjugates. After oral administration in normal doses, the urinary excretion is as follows: - unchanged drug 5%, glucoronide conjugate 55%, sulphate conjugate 30% and mercapturic acid conjugate 8% (Thomas 1993)

2.1.9 Toxicity

Acute overdosing with paracetamol, whether accidental or deliberate is relatively common. The consequences can be extremely serious because of the narrow margin between therapeutic and toxic doses, ingestion of as little as 10 – 15g of paracetamol by adults may lead to severe hepatocellular necrosis (Martindale: The Extra Pharmacopoeia). The easy availability of paracetamol in shops and pharmacies without prescription has led to its being kept in many homes and it is therefore not surprising that it is often involved in episodes of accidental or deliberate self poisoning. In the United States, the American association of poison control centres reported an increase in episodes requiring treatment with the antidote N-acetyl cysteine from 3087 in 1984 to 4972 in 1989 and over the same period, paracetamol associated death increased from 23 to 57 (Thomas, 1993).

Symptoms that may occur within 24 hrs of overdose include nausea, vomiting, lethargy and sweating. Abdominal pain may be the first indication of liver damage which is not usually apparent for 24 to 48 hours and is generally at a maximum 72-96 hrs after ingestion. Hepatic failure, encephalopathy, coma and death may occur.

Normal side effects include hypoglycaemia, jaundice and haematologic disorders like neutropaenia, leucopenia, pancytopenia. Allergic reactions such as skin eruptions, urticarial and erythematous skin reactions may occur (Goodman and Gilman, 1996).

Toxicity following over dosage with paracetamol has been attributed to the production of a minor but highly reactive metabolite, N-acetyl-p-benzoquinoneimine (NABQI) by mixed function oxidase enzymes in the liver and kidney. The amount of NABQI produced after normal doses of paracetamol is usually completely detoxified by conjugation with glutathione and excreted as mercaptopurine and cysteine conjugates. However following paracetamol overdose tissue stores of glutathione become depleted allowing NABQI to accumulate and bind to sulfhydryl group within hepatocytes causing cell damage. The process may be reversible at various points in time when varying degree of cell damage has progressed and therefore may not involve irreversible covalent binding of hepatic macromolecular protein (Dwight, 2000).

Paracetamol (5 mmol/kg i.p.) caused liver damage in rats as indicated by increased plasma aspartate aminotransferase (AST, alanine amino transferase)

(ALT) and glutamate dehydrogenase (GDH) activities. No change in bilirubin or creatinine was noted (Flutter *et al*, 2001)

2.1.9.1 *Management of poisoning.*

An accurate account of the product taken and the amount involved is important for the early management of paracetamol poisoning. It involves skilled and prompt hospital management of the patient. Time of ingestion is important for the subsequent interpretation of plasma paracetamol levels (Thomas, 1993).

Fluid and electrolyte therapy may be required in the early stages of poisoning if nausea and vomiting has been a feature (Graff and Ariett,).

Activated charcoal will reduce paracetamol absorption significantly but should not be employed if oral acetylcysteine is contemplated since 1986 charcoal may prevent its absorption (Rumack and Perterson, 1978). Starting treatment after 15 hours was considered of no benefit. However late management has now been shown to be safe (Parker *et al*, 1990) and beneficial result may be obtained beyond 15 hours.

Substances capable of replenishing depleted stores of glutathione such as acetylcysteine or methionine are thus used as antidotes in paracetamol overdose. Acetylcysteine may also be involved in the repair of damaged tissue. Acetylcysteine is most effective when given during the first 8 hrs in an initial dose of 150mg per Kg body weight as a 20% solution in 200ml of 5% glucose over the next 4 hours finally 100mg per Kg in one litre over the next 16 hours.

Methionine is given by mouth at 2.5g every four hours for four doses starting less than 10 - 12 hours after ingestion of the paracetamol.

The H₂ – receptor antagonist cimetidine which blocks the hepatic cytochrome P450 mixed function oxidase enzyme system might be of use as an adjunct to acetyl cysteine for patients whose production of the toxic metabolite of paracetamol is increased due to enzyme induction. However the addition of cimetidine 300mg given every 6 hours intravenously to standard therapy with acetyl cysteine in 41 patients was started later than 8 hours after overdose did not provide additional hepato protection when compared with the use of acetyl cysteine alone in 66 similar patients (Burkhart for acetaminophen over dosage. Hum. Exp. Toxicol 1995, 14: 299 – 304.)

2.1.10 *Pharmacodynamics*

Although the mechanism and site of action have not been definitely established (Goodman Gilman, 1986) it appears to involve central prostaglandin inhibition. Paracetamol does not inhibit neutrophil activation as do other NSAIDs. (Abramson and Weissman, 1983). It has a central action for antipyresis in the hypothalamus (Vandrman *et al*, 1985) and is believed to exert its analgesic effect by acting on receptors involving N-methyl-d-aspartate (NMDA) and substance P in the spinal cord (Pilleta *et al*, 1991).

Recent studies have shown the presence of a new previously unknown cyclooxygenase enzyme, COX-3 found in the brain and spinal cord which is selectively inhibited by paracetamol and is distinct from the two already known

enzymes COX-1 and COX-2, it is now believed that their selective inhibition of the enzyme COX-3 in the brain and spinal cord explains the effectiveness of paracetamol in relieving pain and reducing fever without having unwanted GI side effects (Chandra-selcharan *et al*, 2002).

2.1.11 *Dosage*

Orally 0.5 – 1 g every 4 – 6 hours upto a maximum of 4 g daily.

Paediatrics:

2 month: 60 mg for post immunization pyrexia

3 – 12 month: 60 – 120 mg

1 – 5 years: 120 – 250 mg

6 – 12 years: 250 – 500 mg

There doses may be repeated every 4 – 6 hours when necessary upto a maximum of 4 doses in any given 24 hours.

Source: British National formulary (BNF, March 2005).

2.1.12 *Salivary concentrations of paracetamol and assay methods*

In drug analysis, research involving the use of saliva sampling as a non-invasive qualitative and quantitative techniques have become increasingly important. Being readily accessible and collectible, saliva may show many advantages over classical biological fluids such as blood and urine. Drug levels in saliva can be used to estimate absorption pattern of drugs.

A study by Danhof and Breimer (1978) shows that for many drugs there is a high degree of correlation between the saliva and plasma concentration of drug.

The concentration of acetaminophen in saliva is high enough to obviate the need for highly sensitive analytical procedures, furthermore, its half life is short enough so that a fairly good characterization of the time course of the drug in the body may be obtained in three hours.

Several methods have been described for the collection of saliva. Example Dawes and Macpherson (1992) have recommended chewing paraffin wax, rubber bands, pieces of Teflon and this will usually elicit a flow of 1 – 3 ml/min.

When these types of stimuli are used, the subject should allow saliva to accumulate in the mouth until the desire to swallow occurs at which time fluid can be expelled smoothly into a vessel.

Once the samples are collected, they must be stored properly unless analysis is to be performed immediately (Chen *et al*, 1999).

Most drugs appear to enter saliva through one or more of the following mechanisms:

- Passive diffusion process which is characterized by transfer of drug molecules down a concentrated gradient with no expenditure of energy.
- Ultra filtration (or Para cellular transport)
- Active transport mechanism
- Pinocytosis

In passive transcellular diffusion highly lipid soluble material may cross capillary wall with the lipid layer of the epithelial cell wall providing the rate limiting

barrier. The salivary concentration of the lipid soluble un-conjugated steroids like oestriol and testosterone approximate the unbound plasma concentration (Vining and Maccinley, 1999). Recoveries of the method ranged from 97.7 % 107%. Except for salicylates, other anti-inflammatory drugs did not interfere.

Garba *et al* (1999) reported a UV method to determine the concentration of paracetamol in human saliva. According to the method a calibration graph of absorbance versus paracetamol concentrations in saliva was plotted as follows:

5 ml of ethyl acetate was added to each of 12 saliva samples containing different concentrations of paracetamol (i.e. 0.00, 10.00, 20.00, 30.00, 40.00 and 50.00 $\mu\text{g/ml}$) in duplicate. The mixtures in the tube were then vortex mixed for 1 minute at 2500 rpm for 5 minutes. Ethyl acetate layer (upper layer) was removed from each of the tube using a Pasteur pipette and its absorbance measured at 262 nm. Using 1 cm silica cuvette with a double beam UV spectrophotometer. The absorbance of the blank saliva sample were then subtracted from those of the samples containing serial concentrations of paracetamol in order to obtain a set of absorbance readings corresponding to the serial concentration of paracetamol in saliva samples.

The calibration curve was constructed by plotting the total absorbance readings obtained against the corresponding concentrations of paracetamol (10 – 50 $\mu\text{g/ml}$) in saliva samples. The plot was then used to determine the concentration of ingested paracetamol from saliva. Percentage recoveries ranged 96 – 104 %.

2.1.13 Paracetamol drug interactions

Most of the reported drug interaction with paracetamol occur at the sites of metabolism and absorption. Food especially if high in carbohydrate has been shown to delay paracetamol absorption, an effect associated to delayed entry of the drug into the main site of absorption, the small intestine or to delay in tablet disintegration or dissolution (MacGilveray and Mattok, 1972). Similarly activated charcoal in large oral doses (5 – 10 g) (Levy and Houston, 1976) and oral cholestyramine (Hansten and Horn, 1989d) were reported to reduce the GIT absorption of paracetamol.

Anticholinergics

May delay the onset of the response to paracetamol by delaying gastric emptying. Other agents with anticholinergic activity like TCA's, antihistamines and phenothiazines also probably delay paracetamol absorption. However this interaction may not be of clinical significance in patients receiving repetitive doses of paracetamol because the extent of absorption is not affected.

Barbiturates

This group of drugs seems to enhance the metabolism of paracetamol therefore patients who chronically receive barbiturates may experience a somewhat reduced effect from therapeutic doses. Phenobarbital has been shown to increase the hepatotoxicity and nephrotoxicity of paracetamol overdose in rats (Pessayre *et al*, 1980, Mcleen *et al*, 1976) but evidence from human is limited to isolated case reports (Wilson *et al*, 1971).

Diazepam

Paracetamol may reduce diazepam bioavailability though it might not be of any clinical significance as such no special precaution is necessary during co-administration of diazepam and paracetamol (Phillip and John, 1989).

Metochlorpramide

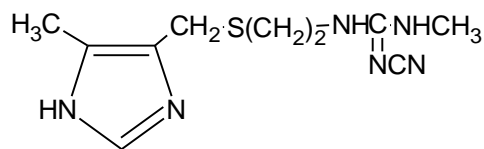
This hastens the onset of action of paracetamol by increasing gastric emptying rate. The rate but not extent of absorption is affected (Nimmo *et al*, 1973).

2.2 Cimetidine

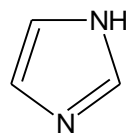
2.2.1 Introduction and chemistry

Cimetidine is a histamine H₂-receptor antagonist used in conditions where inhibition of gastric acid secretion may be beneficial such as duodenal and gastric ulcers. Its principal action is on parietal cell histamine H₂-receptors. Generally, H₂-receptor antagonists are congeners of histamine that contain a bulky side chain in place of the ethylamine moiety. Cimetidine retains the imidazole ring of histamine but more recently developed H₂-antagonist like ranitidine and famotidine have the imidazole ring replaced by furan and thiazole respectively.

Chemically cimetidine is a substituted imidazole compound with a chemical nomenclature: 2-cyano-1-methyl-3-[2-(5-methyl imidazole-4-yl methyl thio) ethyl] guanidine (B.P., 1993). It has a chemical structure as shown below.



Cimetidine



Imidazole ring

It is a weak base with a high degree of water solubility. These properties affect many of the drug's pharmacokinetic characteristics. Its solubility in water is greatly increased with the addition of dilute acid which protonates the imidazole ring.

2.2.2 *Physicochemical properties*

1. Appearance: White to off-white crystalline powder
2. Polymorphism: Exist in 3 polymorphic forms
3. Odour: Odourless or with a faint odour
4. Molecular formula: $C_{10}H_{16}N_6S$
5. Molecular weight: 252.34
6. Melting point: 141 – 143 °C
7. Pka: 6.80
8. pH: a 5.0mg/ml in CO_2 free water is 8 – 9.5
9. Loss on drying: Loses not more than 0.5 % of its weight when dried to constant weight at 100 – 105 °C
10. Solubility: Solubility in acetonitrile is 0.27 % at 27 °C, slightly soluble in water (1.14 % at 37 °C) but hydrochloride is more soluble in water, very soluble in methanol 14.1% at 37 °C, practically insoluble in dichloromethane and in ether, dissolves in dilute mineral acids.

11 Stability: Stable for 48 hrs at room temperature when added to commonly used I.V. solutions e.g. 5% Dextrose in Water

Source: The pharmaceutical Codex 1994.

It is found to be stable both visually and chemically for at least one week at ambient room temperature when combined with commonly used IV fluids (Rosenberg *et al*, 1980, Yuhas *et al*, 1981).

2.2.3 Assay methods in biological fluids

Because of its high degree of water solubility, the extraction of cimetidine into most organic solvents is not complete and averages about 60% though Zeimniac *et al* 1981 reported almost 100% extraction.

Cimetidine has been assayed in various biological fluids by high performance liquid chromatography (HPLC) employing both normal and reverse phase columns (Radolph *et al*, 1971, Larsen *et al*, 1979, Soldin *et al*, 1979, Chiarmonete and Schentag, 1970, Lorenzo and Drayer, 1981).

The only method which measures the metabolite in plasma is that of Ziemniak *et al*, 1981.

A method for the measurement of cimetidine sulphoxide in blood and urine has also been reported by Lee and Osborne (1978).

2.2.4 Pharmacokinetics

Absorption and bioavailability

Cimetidine is rapidly and well absorbed after oral administration, peak plasma concentrations are attained within 1 or 2 hours. First pass hepatic metabolism limits the bioavailability to about 50%

Following oral ingestion of cimetidine, two plasma level maxima appear between 1 and 2 hours and between 3 and 4 hours. Given the relative short elimination half life and the fact that this double peak time frame coincides with intestinal transit time, variable absorption rate down the length of the intestine is anticipated to contribute to drug plasma level observations. Enterohepatic circulation (Veng Pederson and Miller, 1980), intestinal bacterial reconversion of biliary metabolite (Gugler *et al*, 1981), Variable gastric emptying (Oberle and Amindon, 1987) and regional dependent absorption (Hui *et al*, 1994) have all been proposed to account for these observations (Piyapolrunroj *et al*, 2000).

In another study (Logan *et al*, 1978) a good correlation between gastric emptying of a liquid meal and cimetidine absorption at 1 hour in healthy subjects. This suggests that the individual variations in gastric emptying rate was responsible for the variation in cimetidine absorption.

The bioavailability of cimetidine is significantly reduced by antacids by upto one third. Metochlopramide also reduces the bioavailability by an average of 22 % (Gugler *et al*, 1981).

Distribution

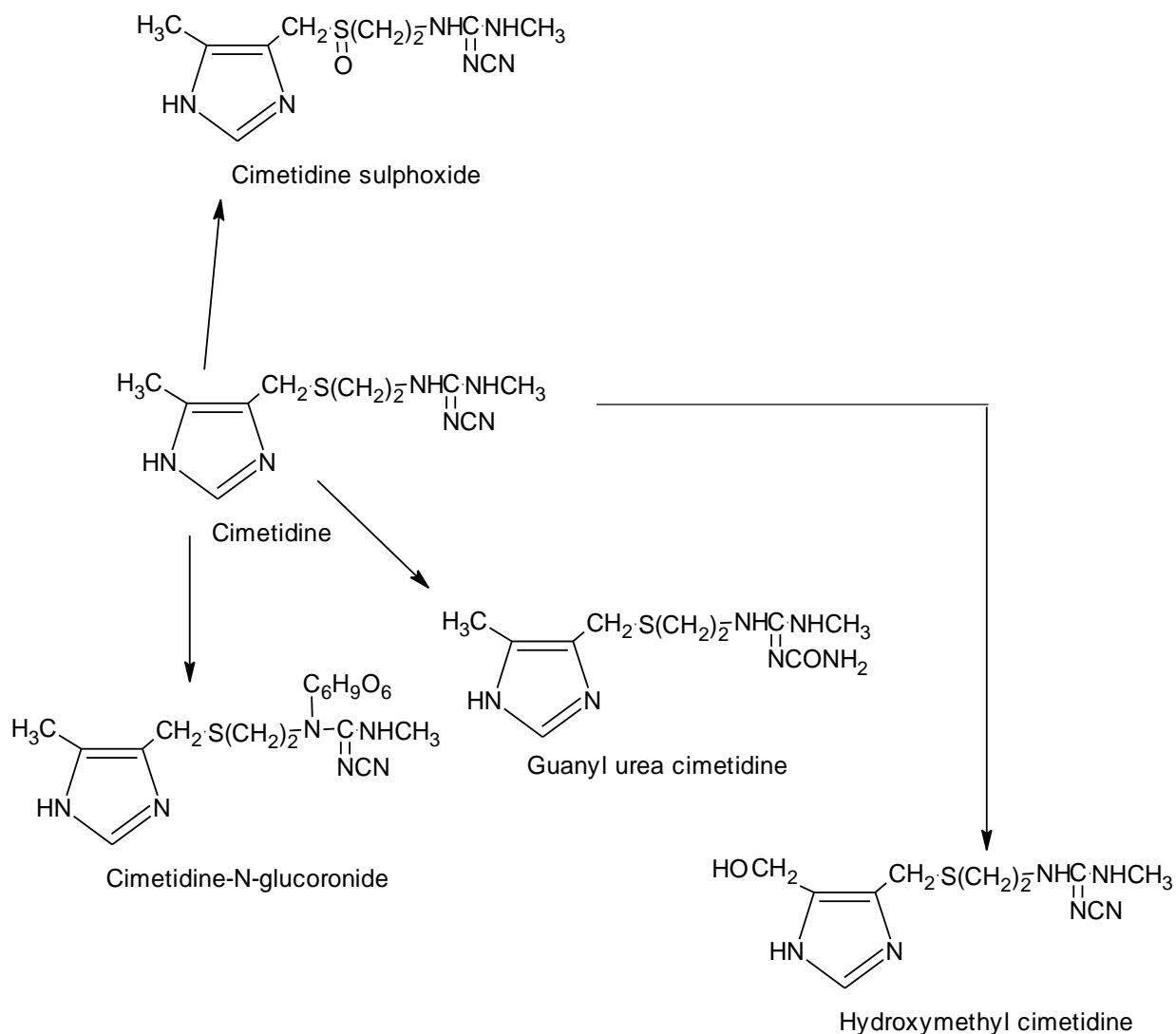
Cimetidine is widely distributed and has a volume of distribution of about 1 litre per kg and is weakly bound (about 20 %) to plasma proteins (Martinade-The extra pharmacopoeia 39, 1996). Uptake into selected organs (kidneys, lungs) and tissues is very extensive (Schentag *et al*, 1981). It distributes into the cerebrospinal fluid at a ratio of 0.1 to 0.2 compared to plasma. Higher ratios have been observed in patients with liver disease (Schentag *et al*, 1981). This explains some of the adverse effects observed in patients with liver disease.

Cimetidine crosses the placenta barrier and is detectable in the foetus in considerable amounts. The ratio of foetus to maternal blood concentration of cimetidine was time dependent (Howe *et al*, 1981). It also appears in breast milk and may reach the infant in amounts in several milligrams daily (Somogyi and Gugler, 1979).

Elimination

The elimination half-life from plasma is about 2 hours and is increased in renal impairment. It is eliminated from the body by renal, metabolic and biliary routes, the renal route being the most important route (Granen *et al*, 1979). The relatively long half life of the drug despite its very high renal clearance is attributable to its large volume of distribution (Weiner and Roth, 1981). Biliary excretion accounts for only 2 % of the dose (Gugler *et al*, 1981). It is therefore negligible and of no clinical significance.

Representation of the metabolic pathways of cimetidine



Phenobarbitone accelerates the elimination of cimetidine by an average of 15 % due to induction of metabolism.

2.2.5 Pharmacological properties and indications

Cimetidine is a histamine H_2 -receptor antagonist. Accordingly, it inhibits gastric acid secretion and reduces pepsin output. It has also been known to inhibit other actions of histamine mediated by H_2 -receptors. It is used in conditions where inhibition of gastric acid secretion may be beneficial. Such conditions include gastric and duodenal ulcer, gastroesophageal reflux disease, selected cases of

persistent dyspepsia pathological hyper secretion states such as Zollinger Ellison syndrome, stress ulceration and in patients at risk of acid aspiration (Mendelson's syndrome) during general an aesthesia.

Cimetidine may also be used to reduce malabsorption and fluid loss in patients with the short bowel syndrome and to reduce the degradation of enzyme supplements give to patients with pancreatic insufficiency.

2.2.6 Administration and dosage

Cimetidine may be given by mouth, by the nasogastric route, or parenterally by intravenous or intramuscular route.

The total daily dose by any route should not normally exceed 2.4 g. When cimetidine is given by mouth, day time doses should generally be taken with meals.

Duodenal and Gastric ulcers - single daily dose of 800 mg for 6 and 8 weeks respectively.

Gastrooesophageal reflux disease (GERD), 400mg by mouth qid with meals and at bedtime for 4-8 weeks

Zollinger –Ellison Syndrome, 300-400 mg by mouth qid, although some times higher dose may be required.

2.2.7 Adverse effects

A variety of adverse reactions have been ascribed to cimetidine reflecting in part the very large number of patients who have been treated with the drug. The

incidence of reactions is low and the reactions generally are minor. The low incidence is attributable in part to the limited function of H₂-receptors in organs other than the stomach and to the poor penetration of these agents across the normal blood brain (BBB).

The incidence of adverse effects with cimetidine is less than 3 %; reactions are usually less intense and less frequent (< 1 %) or absent with normal clinical dose of the other H₂-receptor antagonist (Goodman and Gilman, 1996). In many clinical studies, the incidence of side effects with H₂-receptor antagonist is not greater than that for patients treated with placebo.

Side effects of cimetidine include altered bowel habit, interstitial nephritis, acute pancreatitis, tiredness, and hypersensitivity. Reversible liver damage and haematologic disorders have been reported. Cardiac arrhythmias and arrest following iv bolus may occur. Gynaecomastia is also an occasional problem with cimetidine (but usually only in high dose) and reversible impotence has also been reported.

CNS side effect occur mainly as a result of prescribing high dose to patients with reduced ability to eliminate the drug (Somogyi and Gugler, 1983) These include dizziness, somnolence, headache, hallucinations and delirium, symptoms of brainstem dysfunction and peripheral neuropathy.

2.2.8 Cimetidine drug interactions

All agents that inhibit gastric acid secretion may alter the bioavailability and rate of absorption of certain drugs secondary to changes in gastric pH.

Interactions with cimetidine occur primarily through 3 different mechanisms.

- a. Inhibition of the activity of cytochrome P450 thereby slowing the metabolism of many drug that are substrates for hepatic mixed-function oxidases. The concurrent administration of cimetidine will prolong the half life of many drug including phenytoin, theophyllin, Phenobarbital, cyclosporine, benzodiazepins, Ca^{2+} channel blockers, carbamazepine, propranolol.
- b. Inhibition of tubular secretion via the renal cationic transport system. Competition with other basic compounds renally secreted may occur as in the case of triamteren (Somogyi *et al*, 1989), Chlorpropamide (Maeda *et al*, 1994), Zidovudine (Fletcher *et al*, 1995) and Creatinine (Zeimnialc *et al*, 1956)
- c. The ability of cimetidine to raise gastric pH may have influence on oral drug bioavailability as the disintegration of some tablet formulation and the dissolution and ionization of many drugs is dependent on the pH of the gastric fluid (Mayersohn, 1979).

Effect of cimetidine on disposition of other drugs

Cimetidine have been found to inhibit renal tubular secretion of tramteen in isolated perfused rat kidney and produced a significant change in the tubular secretion of triamteren which will be of clinical significance if extrapolated (Samogyi *et al.*, 1989).

Cimetidine was also found to decrease the bioavailability of albendazole (Nagy J, Schipper H.G., Koopmans RP, Butter JJ, Van Boxtel C.J, Kager P.A, In Am. J. Trop. Med Hyg. 2002 March 66 (3): 260-3). In this study the assumed metabolic breakdown of albendazole by mucosal CYP 3 A4 enzyme was studied by coadministering albendazole sulphoxide (ABZSX), the active metabolite of albendazole were compared with those after albendazole was administered with water, fatty meal or grapefruit Juice plus cimetidine 10 mg/kg. In comparison to water, maximum ABZSX concentration (C_{max}) was enhanced 5-6 fold by a fatty meal and 3-2- fold by grapefruit juice, when grapefruit juice was combined with cimetidine C_{max} was significantly lower than with grapefruit juice alone. The AUC from zero to infinity followed a smearable pattern. $T_{1/2}$ was 8.8 ± 4.2 hrs and 8.2 ± 4 hrs after administration with water or a fatty meal ($P = 1.000$). Grapefruit juice shortened $t_{1/2}$ by 46% ($P = 0.026$)

Cimetidine AUC (0. 240 min) increased by 25% ($P < 0.01$) following itraconazole administration. The GFR and V_d remained unchanged but significant reduction in $Cl(T)$ were observed (Karyekar CS *et al* 2004)

Another study by Boffito *et al* (2002) showed that there was significant increase in saquinivir AUC (0.24) (120%; $P = 0.023$) and C_{max} (179%; $P = 0.019$). When co-administered with cimetidine

Clinical significance of Cimetidine - Drug interaction

Before an effect of cimetidine interaction can be regarded as significant, it is suggested that the change caused by the interaction should be between 20 and 25% (Upton *et al* 1982)

Influence of cimetidine on the pharmacokinetics of drugs (British national formulary, BNF 1994)

Drug class	Influence of cimetidine	Remark
Analgesics	Increase plasma concentration of opioids by inhibiting metabolism	NPH
Antiarrhythmics	Increase plasma concentrations of amiodarone, Lignocaine, procainamide	PH
Antibacterials	Reduces absorption of cefpodoxime, increases plasma concentration of metronidazole by inhibiting metabolism	N.P.H
Anticoagulants	Enhances anticoagulant effect of incoumalone and warfarin by inhibiting their metabolism	NPH
Antidepressants	Increases plasma concentration of amilriptylline, desipramine, doxepin, imipramin, nortriptymine and probably other antidepressants by inhibiting their metabolism	NPH
Antidiabetics	Increases plasma concentration of metformin by inhibiting its renal excretion	NPH
	Increase plasma concentration of phenytoin and carbamazepine by inhibiting their metabolism	P.H
Antifungals	Reduce absorption of itraconazole and ketoconazole. Increase plasma concentration of terbinafine	NPH
Anxiolytics and hypnotics	Increases plasma concentration of benzodiazepine and chlormethiazole by inhibiting their metabolism	NPH
Anti malarials	Increases plasma concentrations of quinine and chloroquine by inhibiting their metabolism	NPH
B- blockers	Increases plasma concentrations of labetolol and propranolol by inhibiting their metabolism	NPH
Calcium channel blockers	Increases plasma concentration of some calcium channel blockers through inhibition of metabolism	NPH
Cyclosporine	Possibly increases plasma cyclosporine concentration	P.H
Cytotoxics	Increase plasma concentration of fluorouracil	NPH
Mebendazole	Enhances plasma concentration by inhibiting its metabolism	NPH

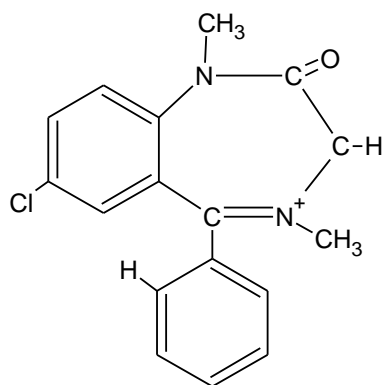
Key: - pH => potentially hazardous interaction
 NPH => Not potentially hazardous interaction

2.3 Diazepam

2.3.1 Introduction and chemistry

Diazepam is a benzodiazepine with anticonvulsant, anxiolytic, sedative, muscle relaxant and amnesic properties.

It has molecular formula as $C_{16}H_{13}ClN_2O$ and molecular weight 284.7.



Diazepam

Chloro-1,3-dihydro-1-methyl-5-phenyl
2H-1,4-benzodiazepine-2one

It is a white, almost white or yellow, odourless or almost odourless, crystalline powder. BP solubilities are: - very slightly soluble in water, soluble in alcohol and freely soluble in chloroform.

USP solubilities are: 1 in 333 of water, water 1 in 16 of alcohol, 1 in 2 of chloroform and 1 in 39 of ether.

The B.P injection has a pH 6.2 to 7.0, the USP injection has a pH of between 6.2 and 6.9. B.P oral solution has a pH has a pH of 4.7 to 5.4

2.3.2 Pharmacokinetics

Diazepam is readily and completely absorbed from the gastro intestinal tract peak plasma concentration occurring within 0.5 to 2 hours of oral administration. Absorption may be erratic following intramuscular administration and lower peak plasma concentrations may be obtained compared with those following oral administration.

Diazepam is highly lipid soluble and crosses the BBB which qualifies it for intravenous use in short term anaesthetic procedure since it acts promptly on administration.

The brain and its initial effects decrease rapidly as it is redistributed into fat depots and tissues.

Diazepam has a biphasic half life with an initial rapid distribution phase followed by a prolonged terminal elimination phase of 1 or 2 days of its principal active metabolite, desmethyldiazepam and nordiazepam.

Diazepam and nordiazepam accumulate on repeated administration and the relative proportion of nordiazepam in the body increases on long term administration. No simple correlation has been found between plasma concentration of diazepam or its metabolites and their therapeutic effect.

Diazepam is extensively metabolized in the liver and in addition to nordiazepam its active metabolite include oxazepam and temazepam, it is excreted in the form

of its metabolites either free or in conjugated form .It is also extensively bound to plasma protein(98-99%).

The plasma half life of diazepam is prolonged in neonates elderly and in patients with liver disease. In addition to crossing the blood brain barrier diazepam and its metabolites also cross the placental barrier and are excreted in breast milk.

In a study of 36 patients who had received diazepam 2-30mg, diazepam concentrations were directly related to dose and inversely related to age (Rutherford D.M. *et al*).Most of the patients were also receiving other drugs.

There was a close association between the plasma concentrations of diazepam and its metabolite desmethyldiazepam and both concentrations were independent of the duration of therapy. Plasma diazepam concentrations ranges were 0.02 to 1.01 µg/ml and plasma-desmethyldiazepam concentration ranges were 0.055 to 1.765 µg/ml. A similar study by Greenblat *et al* (1981) has reached the same general conclusions.

2.3.3 Pharmacodynamics

Diazepam actions are mediated by enhancement of the activity of aminobutyric acid (GABA), a major inhibitory neurotransmitter in the brain. It is used in the treatment of severe anxiety disorders, as a hypnotic in the short term management of insomnia as an anticonvulsant in the management of status epilepticus. Its function within the CNS includes involvement in sleep induction

and in the control of neuronal excitability, epilepsy, anxiety, memory and hypnosis (Greenblatt D.Z *et al*, 1989).

Two subtypes of GABA receptors have been identified A and B. Diazepam interacts with GABA receptors to produce its effects. The GABA receptors within the CNS are composed of different subunits, four of which have been described α , β , γ and δ . These subunits combine to form macromolecular complexes which include a chloride ion channel and regions to which GABA, diazepam and other benzodiazepines bind as well as binding sites for other substances.

There are two types of diazepam receptors suggested;

Type I: found throughout the brain and in large concentrations in the cerebrum and Type II: found mainly in the cerebral cortex, spinal cord and hippocampus.

Diazepam may be given for severe anxiety in oral doses of 2mg three times daily to a maximum of 30mg daily. Doses of 1-5mg at bedtime have been used in children to control night terrors and sleep walking.

2.3.5 Toxicity and adverse effects

Drowsiness, sedation and ataxia are the most frequent adverse effects of diazepam use. They generally decrease on continued administration and are a consequence of CNS depression, mental depression, slurred speech, and changes in libido, urinary retention or incontinence, visual disturbances.

The use of diazepam in the first trimester of pregnancy has been associated with various congenital malformations in the infant but no clear relationship has been established. Administration of diazepam in late pregnancy has been associated with intoxication of the neonate.

2.3.6 Drug Interactions

Enhanced sedation or respiratory and cardiovascular depression may occur if diazepam is given with other drugs that have CNS-depressant properties or with agents that interfere with their metabolism. Benzodiazepines such as diazepam which is primarily metabolized by hepatic microsomal oxidation may be more susceptible to pharmacokinetic changes than those eliminated primarily by glucuronide conjugation.

Paracetamol produced no significant change in plasma concentration of diazepam or its major metabolite and only marginal changes in urine concentration in four healthy subjects (Mulley BA *et al*, 1978).

Diazepam and other benzodiazepines such as lorazepam and midazolam may be used with opioid analgesics in anaesthetic or analgesic regimens. An additive sedative effect is to be expected but there are also reports of severe respiratory depression with midazolam and fentanyl (Yaster M. *et al* 1990) or sudden hypotension with midazolam and fentanyl (Burtin P. *et al* 1991).

Pretreatment with morphine or pethidine has decreased the rate of oral absorption of diazepam. This has been attributed to the effect of opioid analgesics on gastrointestinal mobility (Gamble Jas *et al*).

Isoniazid has been reported to increase the half life of a single dose of diazepam in healthy subjects (Ochs H.R. *et al*, 1981). In patients receiving therapy for tuberculosis with a combination of isoniazid, rifampicin and ethambutol the half life of a single diazepam dose was shortened and its clearance increased (Ochs HR *et al* 1981).

Reduced plasma binding of diazepam and desmethyldiazepam and increases in the free concentrations without changes in the total blood or plasma concentrations occurred immediately following heparin intravenously (Routledge PA *et al*, 1980). Fluoxetine, an antidepressant affects the plasma concentration of diazepam (Peruca E *et al* 1994) therefore it has been suggested that patients taking fluoxetine who require benzodiazepines should preferentially receive a benzodiazepine such as Lorazepam which has a different metabolic pathway (Peruca E *et al*. 1994).

Antacids have variable effects on the absorption of benzodiazepines but any resulting interaction is unlikely to be of major clinical significance (Nair S.G *et al*. 1976). Famotidine and nizatidine do not appear to inhibit the hepatic metabolism of diazepam (Lozniskar A *et al* 1986 and Klotz U. *et al*. 1987).

Oral diazepam was absorbed more rapidly after intravenous administration of metochlorpramide (Gamble JA S *et al* 1976). Enhanced mobility of the GI tract was implicated.

Studies of continuous omeprazole administration on the pharmacokinetic of a single dose of diazepam in healthy subjects indicate inhibition of diazepam metabolism in a similar manner to cimetidine (Gugler R, Jensen JC, 1984) and Anderson T. *et al.*1990).

Omeprazole decreases the clearance and prolongs the elimination half life of diazepam, in addition both the formation and elimination of desmethyldiazepam appear to be decreased. The effects may be greater in rapid than in slow metabolizers of omeprazole (Anderson T. *et al* 1990) but the clinical significance remains to be established.

Lansoprazole has been reported not to affect the pharmacokinetics of diazepam (Lefebure R.A *et al.* 1992).

The Boston collaborative drug surveillance program reported drowsiness as a side effect of diazepam or chlordiazepoxide less frequency in smokers than non-smokers (Boston collaborative Drug surveillance program, 1973). Pharmacokinetic studies have however been divided between those indicating that smoking induces the hepatic metabolism of benzodiazepines and those showing no effect on benzodiazepine pharmacokinetics (Miller L.G.1990).

Hence diminished end-organ responsiveness may in part account for the observed clinical effects. Concomitant consumption of large amounts of xanthine-containing beverages may decrease any enzymes-inducing effects of smoking (Downing R W, Rickels K. 1981).

2.4 Aims and objectives of the study

Many researchers have studied the effects of cimetidine on the pharmacokinetics of paracetamol and it is well established and generally agreed that cimetidine delays and reduces the absorption of paracetamol when 1 g of paracetamol is given as single dose 1 hour after a 400 mg dose of cimetidine (Garba *et al* 1999).

However the mechanism of this action has not been fully elucidated nor understood.

This study is an attempt to see whether diazepam will give a likely insight as to how cimetidine causes its action.

The combination of diazepam and paracetamol in clinical practice is a common phenomenon so the study will further throw more light as to the suitability or otherwise of such a combination in one prescription.

3.1 Materials

The following materials were used in carrying out the project:

3.1.1 Glassware

10ml extraction tubes, Pyrex, England

20ml burette, Pyrex, England

Screw-capped sample bottles: 5ml, 10ml and 20ml sizes

Pipettes: 0.02ml, 0.1ml, 1ml, 5ml and 10ml sizes, Pyrex, England.

Test tubes: 10ml and 20ml sizes, Pyrex, England.

Measuring cylinders: 5ml, 10ml, 50ml and 100ml sizes, Pyrex, England.

Volumetric flasks; 25ml, 50ml, 100ml, 250ml, 500ml and 1000ml sizes,

Technico, England.

Glass weighing pan

Crucible

Conical flasks: 25ml, 50ml, and 250ml sizes, Pyrex, England.

Beakers: 50ml, 100ml and 250 sizes, Pyrex, England.

Reflux condenser, Pyrex, England.

Round bottom flask, Pyrex, England.

Syringes and needles (2ml, 5ml and 20ml sizes syringes and 21 g needles).

Glass funnel and filter papers (Whatman).

Pasteur pipette.

3.1.2 *Equipment*

Auto-vortex mixer, Stuart, England

Centrifuge, CS-15 Beckman

Flask shaker, Gallenkamp, England

Disintegration rate study apparatus, Erweka, Germany

Dissolution rate study apparatus, Erweka, Germany

Gilson pipetteman, France

Hot air oven BS size one, Gallenkamp, England

HPLC priming syringe, Hamilton, England

Injection microsyringe, Hamilton, England

Electronic balance, mettler, A.E 240

LG refrigerator

Solvent filters, 0.45um, Waters, Massachusetts, USA.

Schimadzu UV-visible recording spectrophotometer (Serial number A113 32034752)

Melting point apparatus, Gallenkamp, England

Hotplate, B2 12, Bibby, U.K.

Water bath, compenstat, Gallenkamp, England

Friabilator, Erweka, Germany

3.1.3 *Chemicals and standard samples*

Chemicals

Methanol, Analar, BDH Chemicals, England

Perchloric acid, Analar, BDH Chemicals, England

Glacial acetic acid, Harris reagent, England

Ethylacetate, May and Baker, England

Distilled and filtered water

Sodium hydroxide pellets, May and Baker, England

Acetone, May and Baker, England

Hydrochloric acid, Analar, BDH Chemicals, England

Potassium dichromate, May and Baker, England

Ferric chloride, May and Baker, England

Acetic anhydride, Analar, BDH Chemicals, England

Crystal violet, Harris reagent, England

Lead Nitrate, May and Baker, England

Potassium hydrogen Phthalate, BDH Chemicals, England

Potassium hydroxide, May and Baker, England

Bismuth oxynitrate, Analar, BDH Chemicals, England

Standard Samples

Standard pure samples used in the project were obtained as gift samples.

Standard paracetamol powder was obtained from National Institute for Pharmaceutical Research and Development, Abuja

3.1.4 Tablet samples

The following are the particulars of the tablet samples used in the study:

Paracetamol tablets

Source: Purchased from a registered Pharmacy
Brand name: Panadol[®]
Manufacturer: GSK Nig PL RC 8729, Igbesa Road Agbarra, Ogun State
Manuf. Date: July 2006
Exp. Date: July 2009
Strength: 500 mg per tablet
Batch No.: 05090

Cimetidine tablets

Source: Purchased from a registered Pharmacy
Brand name: Altramet[®]
Manufacturer: Lek Pharmaceutical and Chemical Company, B.D. Verovskova, 57 Ljubltana, Slovenia
Manuf. Date: Dec. 2004
Exp. Date: Dec. 2009
Strength: 200 mg per tablet
Batch No.: 3424712 F

Diazepam tablets

Source: Purchased from a registered Pharmacy
Brand name: Valium[®]

Manufacturer: Swisspharma, 5 Dopemu Road Agege, Lagos
Manuf. Date: May 2006
Exp. Date: May 20011
Strength: 5 mg per tablet
Batch No.: L26061

3.2 Methods

3.2.1 *Preparation of reagents, solutions and disintegration/dissolution medium*

5% Crystal violet

10ml of 5 % crystal violet solution in glacial acetic acid was prepared by weighing accurately and dissolving 0.5 mg crystal violet in 10 ml of glacial acetic acid.

Ferric chloride test solution

10 ml of ferric chloride test solution used for the identification test for paracetamol tablet was prepared by weighing accurately and dissolving 0.45 g ferric chloride in sufficient distilled water to produce a solution of 10 ml.

Lead nitrate paper

Lead nitrate paper used for the identification test for cimetidine was prepared by the following procedure:

10 % lead nitrate solution was first prepared by dissolving 5 g of lead nitrate powder in 50 ml distilled water. Filter paper (Whatman qualitative) was then soaked into the solution, removed and allowed to dry.

0.1M Perchloric acid

250 ml of 0.1 M perchloric acid was prepared according to the method described in I.P. (1979) volume I, general method of analysis.

To 225ml glacial acetic acid, 2.05ml of 70% w/w perchloric acid was added, mixed and to the content was added 8ml of acetic anhydride and thoroughly mixed. The solution was then cooled and sufficient glacial acetic acid was added to produce 250ml. The solution was allowed to stand for 24 hours.

The solution was then standardised with potassium hydrogen phthalate (previously dried at 120°C for 2 hours) dissolved in acetic acid, and was stored, pending use.

Potassium iodobismuthate/acetic acid test solution

Potassium iodobismuthate/acetic acid test solution used for the identification test for cimetidine tablet was prepared by dissolving 4g of potassium hydroxide in 2ml of water. The solution was then added to another solution containing 0.085g of bismuth oxynitrate dissolved in 4ml of water and 1ml of glacial acetic acid.

0.1M Sodium hydroxide

0.1M solution of sodium hydroxide was prepared by dissolving accurately weighed 4.5g of sodium hydroxide pellets in sufficient distilled water to produce 1000ml solution.

Preparation of dissolution/disintegration medium

0.1M hydrochloric acid was used as the disintegration and dissolution medium and was prepared as follows:

8.5ml of concentrated hydrochloric acid was accurately measured and was diluted with distilled water sufficiently enough to produce 1000ml.

3.2.2 Quality Control

Identification Tests

PARACETAMOL TABLETS: B.P (2002) was used for identification of paracetamol.

Powdered tablet (0.59 g) containing 0.5g of paracetamol was extracted with 20 ml of acetone. It was then filtered and the filtrate evaporated to dryness. The residue obtained was dried at 105 °C and then subjected to the following test:

- (i) Melting point determination
- (ii) 0.1 g of the residue was boiled with 1ml of concentrated hydrochloric acid for 3 minutes, 10ml of water was then added and the content cooled. 0.05ml of 0.0167M potassium dichromate was then added and observed for the appearance of a violet colour or any other change.

CIMETIDINE TABLET: The method described in basic tests for Pharmaceutical dosage forms (WHO, 1991) was followed for the identification test for Cimetidine. One tablet was weighed and the amount (0.12 g) equivalent to 60 mg Cimetidine calculated. The tablet was finely powdered and the amount equivalent to 60 mg Cimetidine weighed, divided into 2 equal parts and used

directly as the test substance. The test substance was subjected to the following tests.

1. One part of the test substance was ignited. Lead nitrate paper was then exposed to the vapour evolved and observed for the darkening of the lead nitrate paper.
2. To another part of the test substance was added 10ml of water, and stirred. Few drops of potassium iodobismuthate/acetic acid test solution was added and observed for an orange precipitation.

Test for Uniformity of Weight

B.P. (2002) procedure was followed:

Twenty (20) tablets of paracetamol were selected randomly and their average weight determined. The tablets were then weighed individually and the percentage weight deviation of each tablet from the mean (average) weight calculated.

The same procedure above was repeated using sample of Cimetidine tablets and Diazepam.

Friability Test

Ten (10) tablets were weighed and introduced into a friabilator (Erweka, Germany) which was set to rotate at 25 rpm for 4 minutes. At the end of 100 revolutions (4 minutes), the tablets were removed and re-weighed and compared to the initial weight. The procedure was carried out for all samples of

paracetamol, cimetidine and diazepam tablets. The percentage loss in weight was subsequently calculated and represents the friability;

$$\text{Percentage friability} = \frac{\text{Loss in weight} \times 100}{\text{Initial weight}}$$

Assay for Content of Active Ingredient

PARACETAMOL TABLET: B.P.(2002) method was adopted:

Twenty (20) tablets of paracetamol were randomly selected, weighed and crushed into a fine powder. A quantity of the powder (0.178 g) equivalent to 0.15g of paracetamol was accurately weighed on an analytical balance and was added to 50ml of 0.1M sodium hydroxide in a 200ml volumetric flask. The content was diluted with 100ml of water shaken for 15 minutes and sufficient water added to make 200ml. It was mixed, filtered and 10ml of the filtrate was diluted to 100ml with distilled water 10ml of the resulting solution was added to 10ml of 0.1 M sodium hydroxide, diluted to 100ml with distilled water and the absorbance of the resulting solution was measured at the wavelength maximum at 257nm. Content of paracetamol was calculated taking 715 as the value of A (1%, 1cm) at the Wavelength of 257nm. Thus,

$$A (1\%, 1\text{cm}) \text{ of paracetamol at } 257\text{nm} = 715.$$

$$\text{Expected conc. of sample after dilutions} = 0.00075\% \text{ w/v}$$

$$\text{Expected absorbance} = \frac{0.00075 \times 715}{1}$$

$$\text{So percentage content} = \frac{\text{Absorbance given by sample} \times 100}{\text{Expected absorbance}}$$

The results for the assay are presented in Chapter 4.

CIMETIDINE TABLET: Non-aqueous titrimetric method, using 0.1M perchloric acid as titrant was adopted to assay for content of active ingredient of the cimetidine tablet.

0.1M perchloric acid was prepared by mixing acetic acid, 70%w/w perchloric acid and acetic anhydride, and allowed to stand for 24 hours. It was then standardised by titrating it with potassium hydrogen phthalate (previously dried at 120°C for 2 hours) dissolved in glacial acetic acid.

Twenty (20) tablets of cimetidine were randomly selected accurately weighed and finely powdered. Powder (0.501 g) equivalent to 0.25g cimetidine was weighed and dissolved (by gentle warming and cooling) in 30ml glacial acetic acid. The content was then titrated with the previously prepared and standardized 0.1M perchloric acid using 5% crystal violet dissolved in glacial acetic acid as indicator for end point determination. The titration was performed 5 times and the average value calculated.

Cimetidine content was determined by using the relationship: Each ml of 0.1M perchloric acid consumed in the titration is equivalent to 25.23mg of cimetidine.

The results for the assay are presented in Chapter 4.

Disintegration Rate Test

The test was performed for both paracetamol and cimetidine tablet samples.

DIAZEPAM TABLET ASSAY

The method described in BP (1993) was adopted.

So tablets were weighted and powdered. To a quantity of the powder containing 10 mg of diazepam 5 ml of distilled water was added, mixed and allowed to stand for 15 minutes. 70 ml of 0.5 %^{w/v} solution of sulphuric acid in methanol was added. The mixture was shaken for 15 minutes and then add sufficient solution of the methanolic sulphuric acid to produce 100 ml and then filter. 10 ml of the filtrate was diluted to 50 ml with the same solvent and the absorbance measured at 284 nm.

Disintegration Rate Test

The test was performed for paracetamol, cimetidine and diazepam tablet sample. Six (6) tablets were placed in 6 tubes (one tablet per tube) of the tablet disintegration apparatus (Erweka, Germany). The apparatus was operated in accordance with procedure in B.P. (2002) to determine the disintegration time of the tablets as follows:

The assembly of tubes was suspended in a beaker containing the disintegration medium (0.1M HCl) that was maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ by an electrically heated water bath. The assembly of tubes was made to move up and down in the medium so that the tablets were constantly agitated. The time taken for all the particles of the disintegrated tablets to pass freely through the mesh at the lower end of the tubes is usually considered to be the disintegration rate time.

The procedure was repeated, but with cimetidine tablets and diazepam. The results for the disintegration rate tests for both paracetamol, cimetidine and diazepam tablets are presented in Chapter 4.

Dissolution Rate Test

The rotary basket method described in B.P. (1993), and using 0.1M HCl as the dissolution medium was adopted.

One tablet of paracetamol was placed in the basket and the assembly was placed in the flask containing 1000ml of 0.1 M Hydrochloric acid, warmed and maintained between 36.5 and 37.5°C by an electric heater. The stirrer was set at 100 r.p.m. and the apparatus operated for 45 minutes. Sample of the dissolution medium was then withdrawn, filtered and assayed spectrophotometrically using a U.V. spectrophotometer at wavelength of 257nm. The test was further repeated 4 times and the average determined. The amount of paracetamol released after 45 minutes was calculated by comparing the absorbance of the dissolution medium obtained with the absorbance given by a known concentration of paracetamol in 0.1 M hydrochloric acid at 257 nm. The percentage of paracetamol released from the dosage form after 45 minutes was calculated using the relationship below:

$$\text{Percentage released} = \frac{\text{Amount of sample released}}{\text{Content of standard paracetamol tablet}} \times 100$$

The dissolution rate test was also carried out for cimetidine and diazepam tablets, in 5 replicate using the same operating conditions and procedure as above for paracetamol. Sample of dissolution medium was also analysed

spectrophotometrically at a wavelength of 218nm. The amount of drugs released after 45 minutes was calculated by comparing the absorbance given by the sample of the dissolution medium with the absorbance given by a known concentration of cimetidine and diazepam in 0.1M hydrochloric acid at 218nm.

The percentage of drugs released from the dosage form after 45 minutes was calculated using the relationship below:

$$\text{Percentage released} = \frac{\text{Amount of sample released}}{\text{Content of standard cimetidine tablet}} \times 100$$

3.2.3 Preparation and validation of calibration curve

Preparation of paracetamol Stock solution

A stock solution of pure paracetamol powder in methanol with a concentration of 4mg/ml w/v (4000ug/ml) used for the construction and validation of calibration curve was prepared as follows:

A 100 mg of pure paracetamol powder was accurately weighed using an electronic analytical balance. The powder was carefully and completely transferred into a 25ml volumetric flask and dissolved with about 15ml of methanol. The volume of the solution was then made to 25ml by the addition of a sufficient quantity of methanol. The stock solution was then stored in a refrigerator, pending the time of its use.

Construction of Calibration Curve

Calibration curve of concentration of paracetamol in human saliva based on absorbance was prepared by spiking drug-free (blank) saliva with standard

solution of paracetamol to give a concentration range of 0 to 50 µg/ml. The procedure involved for the construction of the calibration curve is as follows:

Sufficient quantity of blank (drug-free) saliva sample was collected with the aid of chewing a piece of rubber band. The collected saliva sample was pooled and then distributed into twelve 10ml extraction (centrifuge) tubes in two milliliter volumes, using an auto-pipette. Two of the twelve tubes were kept as blanks (Drug-free), and the remaining ten tubes were spiked with different (increasing) concentrations of paracetamol in methanol from the standard stock solution, using a microlitre Hamilton syringe. Each concentration was spiked in duplicate (as indicated in Table 3.1) and each duplicate was repeated four times on different days, resulting in 8 replicate data for each concentration and blanks.

Table 3.1 shows the volumes of stock solution of paracetamol spiked into 2ml saliva samples and the concentrations of paracetamol in saliva sample obtained. The amount (volume) of paracetamol stock solution spiked to 2ml saliva to obtain a desired concentration was determined from the following relationship:

$$C_1V_1 = C_2V_2$$

Where,

C_1 = Concentration of paracetamol stock solution (i.e. 4mg/ml or 4000ug/ml)

V_1 = Volume of stock solution to be spiked (unknown) to saliva sample.

V_2 = Volume of saliva samples (i.e. 2ml)

C2 = Concentration ranges of paracetamol in saliva samples (i.e. 0, 10, 20, 30, 40 and 50ml), for the construction of the calibration curve.

In order to obtain absorbance readings for the various paracetamol concentrations in saliva, the following procedure was followed.

Five milliliters (5 ml) of ethyl acetate was added to each of the twelve saliva samples and the extraction (centrifuge) tubes were well stoppered. The mixtures in the tubes were vortex-mixed for 1 minute and centrifuge at 2500 rpm for 5 minutes. Ethyl acetate layer (upper) was removed from each of the tube using a Pasteur pipette and its absorbance measured at 262nm, using 1cm silica cuvette, using a double beam U.V spectrophotometer. The absorbance for the blank (drug-free) saliva samples were then subtracted from those of the samples containing serial concentrations of paracetamol in order to obtain a set of absorbance readings corresponding to the serial concentrations of paracetamol in saliva samples (i.e. 0.00, 10.00, 20.00, 30.00, 40.00 and 50.00 $\mu\text{g/ml}$ concentrations).

The calibration curve was constructed by plotting the total mean absorbance readings obtained against the corresponding concentrations of paracetamol (10 - 50 $\mu\text{g/ml}$) in saliva samples, and correlation coefficient calculated.

Table 3.1 Volumes of stock solution of paracetamol spiked to saliva samples and the concentrations obtained for the construction of the calibration curve

Sample serial No.	Volume of stock solution spiked to 2ml saliva sample (μl)	Concentration of paracetamol in saliva sample obtained ($\mu\text{g}/\text{ml}$)
1	0.00	0.00
2	0.00	0.00
3	5.00	10.00
4	5.00	10.00
5	10.00	20.00
6	10.00	20.00
7	15.00	30.00
8	15.00	30.00
9	20.00	40.00
10	20.00	40.00
11	25.00	50.00
12	25.00	50.00

Validation of the calibration curve

The calibration curve constructed was validated using the following procedure: Sufficient quantity of blank (drug-free) saliva was obtained with the aid of chewing a piece of rubber band. The saliva sample was then pooled and distributed into eight, 10ml extraction (centrifuge) tubes into 2ml-volumes using an auto-pipette. Two of the tubes were kept as blanks and the remaining six (6) tubes were spiked with different volumes of paracetamol stock solution to give a concentration range of 5 to 55 $\mu\text{g}/\text{ml}$ (as indicated by Table 3.2). The concentrations used for the construction of the calibration curve (i.e. 10, 20, 30, 40 and 50 $\mu\text{g}/\text{ml}$ concentrations) were not selected.

Each sample spiked with the stock solution was then extracted with ethylacetate and the ethylacetate extract analysed using the same analytical procedure as described for the construction of calibration curve.

Table 3.2 Volumes of paracetamol stock solution spiked to saliva samples and the concentrations obtained for the validation of the calibration curve

Sample serial No.	Volume of stock solution spiked to 2ml saliva sample (μ l)	Concentration of paracetamol in saliva sample obtained (μ g/ml)
1	0.00	0.00
2	2.50	5.00
3	7.50	15.00
4	12.50	25.00
5	17.50	35.00
6	22.50	45.00
7	27.50	55.00
8	0.00	0.00

Absorbance readings obtained were then converted directly to the corresponding concentrations of paracetamol in saliva using the calibration curve. The concentrations estimated from the calibration curve were then compared with the actual concentrations spiked to the saliva samples. The results are presented in chapter four.

3.2.4 *In vivo pharmacokinetic studies*

Volunteers and Protocol of Study

Eight healthy male volunteers aged between 25 – 29 (mean \pm SD; 27.32 \pm 1.41) years, weighing between 65 - 70 (mean \pm SD; 67.45 \pm 3.32) kg participated in the study. The volunteers were clinically certified fit for the study and were

asked to abstain from taking any drug for at least two weeks before the commencement of, and during the study. They were all non-smokers and did not take alcohol or kolanut at all before and during the study period. The volunteers were fully briefed on the study and their informed consent was obtained before the commencement of the study.

The protocol of the study was divided into 5 phases and a wash-out period of 2 weeks was allowed between the phases of the study.

The first phase of the study involved the ingestion of 1 g dose of paracetamol tablets with about 100ml of water after overnight fasting. Food but not water was withheld for the next 4 hours. Saliva samples (4ml) were immediately collected prior to the drug ingestion and at 0.25, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00 and 6.00 hour intervals with the aid of chewing a rubber band. The saliva samples were then stored at -10°C, pending analysis.

The second phase involved the concomitant ingestion of 1 g of paracetamol and 400 mg single dose of cimetidine after overnight fasting. Food but not water was withheld for the next 4 hours as before and saliva samples were collected and stored as described before.

The third phase of the study involved ingestion of 400 mg single dose of cimetidine, followed by 1 g oral dose of paracetamol tablets one hour after, still under fasting state. Food but not water was withheld for the next 4 hours and saliva samples (4 ml) were collected and stored as before.

The fourth phase involved ingestion of 5 mg diazepam and 1 g paracetamol concomitantly after overnight fasting. Food but not water was withheld for the next four hours. Salivary samples were collected and stored as described earlier.

The fifth phase involved administration of 5 mg diazepam followed by 1 g paracetamol tablets one hour later after overnight fasting. Food but not water was withheld for the next four hours and saliva samples were collected and stored accordingly.

Extraction and analysis

The method used for the extraction and analysis of paracetamol in saliva samples in this study was adopted from Garba *et al* (1999).

Saliva samples (2 ml) were placed in 10ml extraction (centrifuge) tube using auto-pipette. Ethylacetate (5 ml) were added to the content of the tubes and well stoppered. The mixture was vortex-mixed for one minute and centrifuged at 2500 rpm for 5 minutes. The ethylacetate layer (upper) was removed with Pasteur pipette and its absorbance measured at 262nm, using 1 cm silica cuvettes using a double beam Shimadzu UV-spectrophotometer.

Data Handling

The values for the concentration of paracetamol in saliva samples were plotted against time on a logarithmic scale. The method of residuals was then used to determine the following pharmacokinetic parameters, using salivary concentration-time curves as preliminary data: Lag time, Absorption and

elimination half-lives ($t_{1/2ab}$ and $t_{1/2el}$ respectively), absorption and elimination constants (K_{ab} and K_{el} respectively).

The remaining pharmacokinetic parameters were determined as follows:

(i) AUC (from time 0 to 6 hours) were calculated using the triangular-trapezoidal method,

(ii) AUC (From time 0 to infinity) were calculated as follows:

$$AUC_{(0-\infty)} = AUC_{(0-6)} + AUC_{(6-\infty)}$$

$$AUC_{(6-\infty)} = \frac{\text{Conc. at 6 hours}}{K_{el}}$$

(iii) Volume of distribution (Vd) = $\frac{F \cdot D}{AUC \cdot K_e}$

(iv) Clearance (Cl) = $Vd \cdot k_e = \frac{0.693 \cdot Vd}{t_{1/2 el}}$

Where,

AUC = Area under the salivary concentration - time curve

K_{el} = Elimination rate constant

$t_{1/2 el}$ = Elimination half life

F = Bioavailability (assumed to be 100%)

D = Dose of paracetamol ingested

The mean pharmacokinetic parameters for the study were then calculated from the results obtained for the 8 volunteers for the 5 phases of the study.

All the mean pharmacokinetic parameters obtained in the study were analysed statistically using the student two tailed t-test of paired data. Values of $p < 0.05$

were considered as significant and $p > 0.05$ insignificant for the differences between the controls and treated groups.

3.3 Analytical method

The analytical method of Garba *et al* (1999) was adopted and modified. The method uses ethylacetate as solvent with UV spectrophotometry at λ max of 262 nm.

Precision of the analytical method

The precision of the analytical method was determined by assaying a number of aliquots homogeneous samples (50 $\mu\text{g/ml}$ and 20 $\mu\text{g/ml}$) paracetamol in saliva in order to calculate statistically, valid estimates of mean among individual tests applied. This is repeated five times with estimates of standard deviation (SD) and coefficient of variation (CV) both within a day and between day precision for building confidence in the application of the analytical method.

Chapter 4

RESULTS

4.1 Quality control assessment

Table 4.1 Quality control assessment results for paracetamol, cimetidine and Diazepam

S/N	Quality assessment	Paracetamol	Cimetidine	Diazepam	Remark
1.	Identification	Positive	Positive	Positive	Pass
2.	Weight uniformity (mean)	0.5903	0.5413	0.5917	Pass
3.	Assay for content (mean \pm SEM)	99.47 \pm 0.319	98.12 \pm 0.173	98.71 \pm 0.1	Pass
4.	Disintegration rate (mean)	6.7	5.3	7.2	Pass
5.	Dissolution rate (mean \pm SEM)	93.23 \pm 0.942	-	-	Pass

Key:

SEM = Standard error of the mean

4.2 Construction and validation of calibration curve

4.2.1 Calibration curve

Table 4.2 Calibration data for spiked saliva samples containing paracetamol

Concentration ($\mu\text{g/ml}$)	Mean absorbance	Standard deviation	Mean absorbance(blank)
0	0.012	0	0
10	0.22	0.0461	0.227
20	0.43	0.0312	0.431
30	0.62	0.0786	0.675
40	0.86	0.0532	0.891
50	1.12	0.0951	1.197

4.2.2 Validation of calibration curve

Table 4.3 Results obtained from the validation of the calibration curve

Vol. of stock (ml)	Absorbance (nm)	Conc. of pcm (mg/ml)	Conc. from calibration curve ($\mu\text{g/ml}$)	% Recovery
0	0	0	0	0
0.025	0.09	5	4.8	96.00
0.075	0.31	15	14.8	98.66
0.125	0.55	25	25.2	100.8
0.175	0.75	35	34.7	99.14
0.225	0.98	45	46.0	102.22
0.275	1.18	55	53.4	97.09

Table 4.4 Data obtained for the construction of the calibration curve

Conc. of stock solution of paracetamol in saliva ($\mu\text{g/ml}$)	1A	1B	2A	2B	3A	3B	4A	4B
0.00	0.034	0.0370	0.0470	0.0430	0.050	0.051	0.0410	0.0330
10.00	0.130	0.1500	0.290	0.2600	0.230	0.210	0.1800	0.3100
20.00	0.470	0.4600	0.390	0.4000	0.440	0.450	0.4100	0.4200
30.00	0.690	0.7100	0.570	0.5500	0.630	0.670	0.5300	0.6100
40.00	0.930	0.9100	0.900	0.8200	0.790	0.810	0.8100	0.8700
50.00	1.150	1.1700	1.270	1.1300	0.970	1.090	1.0700	1.1100

4.3 Validation of analytic method

Table 4.5 Results for the validation of analytic method

Sample	Concentration ($\mu\text{g/ml}$)	CV % coefficient of variation	N
Within-day run paracetamol	30	1.7	5
	40	2.1	5
Between day run paracetamol	30	1.6	5
	40	2.4	5

4.4 Percentage extraction recovery

Table 4.6 Results for the percentage extraction

Sample	Concentration ($\mu\text{g/ml}$)	Recovery % \pm SD	N
Paracetamol	30	95 \pm 6.2	5
	40	95 \pm 5.5	5

4.5 In-vivo pharmacokinetic studies

Paracetamol concentrations under two protocols of concomitant and delayed administration of cimetidine are presented below

Table 4.7 Mean salivary concentration of paracetamol \pm SD for paracetamol alone and with cimetidine phase II and III ($\mu\text{g/ml}$)

Time (hr)	PCM alone (Phase I)	PCM + cimetidine Concomitant (Phase II)	PCM + Cimetidine Delayed (phase III)
0.00	0.00	0.00	0.00
0.25	11.34 \pm 0.41	11.03 \pm 0.46	4.6 \pm 0.22
0.5	12.90 \pm 0.93	12.37 \pm 2.22	5.30 \pm 0.33
1.00	14.39 \pm 0.91	14.28 \pm 1.30	6.50 \pm 0.16
2.00	11.81 \pm 0.79	11.92 \pm 0.87	8.20 \pm 0.26
3.00	8.30 \pm 0.25	8.26 \pm 0.32	8.00 \pm 0.33
4.00	6.52 \pm 0.47	6.50 \pm 0.27	7.82 \pm 0.25
5.00	4.37 \pm 0.20	4.29 \pm 0.07	6.80 \pm 0.33
6.00	2.10 \pm 0.18	2.00 \pm 0.17	6.0 \pm 0.39

Table 4.8 Mean salivary concentration of paracetamol \pm SD for paracetamol alone and with diazepam phase IV and V ($\mu\text{g/ml}$)

Time (hr)	PCM alone (Phase I)	PCM + diazepam Concomitant (Phase IV)	PCM + diazepam Delayed (phase V)
0.00	0.00	0.00	0.00
0.25	11.34 \pm 0.41	11.20 \pm 0.40	13.65 \pm 0.64
0.5	12.90 \pm 0.93	12.85 \pm 0.31	17.50 \pm 1.86
1.00	14.39 \pm 0.91	14.50 \pm 0.58	14.50 \pm 2.11
2.00	11.81 \pm 0.79	10.90 \pm 0.63	12.90 \pm 0.49
3.00	8.30 \pm 0.25	8.45 \pm 0.34	8.15 \pm 1.68
4.00	6.52 \pm 0.47	6.30 \pm 0.28	6.40 \pm 1.15
5.00	4.37 \pm 0.20	4.50 \pm 0.33	4.12 \pm 0.70
6.00	2.10 \pm 0.18	1.90 \pm 0.41	2.83 \pm 0.28

4.6 Pharmacokinetic parameters

Mean \pm SEM salivary pharmacokinetic parameters of paracetamol ingested alone concomitant, and delayed administration profiles for diazepam and cimetidine.

Table 4.9 Mean value \pm SEM of pharmacokinetic parameters of paracetamol given alone and paracetamol + cimetidine concomitant (Phase II)

Pharmacokinetic parameters	Pcm alone (phase I)	Pcm + cimetidine Concomitant phase (IV)	P-value
C_{max} ($\mu\text{g/ml}$)	14.39 ± 1.39	14.27 ± 1.31	$P > 0.05$
T_{max} (hr)	0.90 ± 0.06	0.93 ± 0.09	$P > 0.05$
$t_{1/2ab}$ (hr)	0.312 ± 0.02	0.33 ± 0.10	$P > 0.05$
K_{ab} ($\mu\text{g/ml/hr}$)	2.25 ± 0.29	2.13 ± 0.21	$P > 0.05$
$t_{1/2el}$ (el)	1.47 ± 0.04	1.39 ± 0.06	$P > 0.05$
K_{el} ($\mu\text{g/ml/hr}$)	0.477 ± 0.03	0.43 ± 0.07	$P > 0.05$
V_d (l)	34.40 ± 2.19	33.29 ± 2.04	$P > 0.05$
Cl (l/hr)	20.09 ± 1.02	20.17 ± 1.00	$P > 0.05$
Lag time (hr)	0.124 ± 0.03	0.11 ± 0.05	$P > 0.05$
$AUC_{0 \rightarrow 6}$ ($\mu\text{gh/ml}$)	50.52 ± 2.62	50.69 ± 2.30	$P > 0.05$

Table 4.10 Mean value \pm SEM of pharmacokinetic parameters of paracetamol given alone and paracetamol + cimetidine delay (Phase III)

Pharmacokinetic parameters	Pcm alone (phase I)	Pcm + cimetidine delay phase (III)	P-value
C_{max} ($\mu\text{g/ml}$)	14.39 \pm 1.39	10.07 \pm 1.02	P < 0.05
T_{max} (hr)	0.90 \pm 0.06	1.73 \pm 0.08	P < 0.05
$t_{1/2ab}$ (hr)	0.312 \pm 0.02	0.541 \pm 3.4	P < 0.05
K_{ab} ($\mu\text{g/ml/hr}$)	2.25 \pm 0.29	1.236 \pm 3.2	P < 0.05
$t_{1/2el}$ (el)	1.47 \pm 0.04	2.418 \pm 0.92	P < 0.05
K_{el} ($\mu\text{g/ml/hr}$)	0.477 \pm 0.03	0.273 \pm 0.08	P < 0.05
V_d (l)	34.40 \pm 2.19	57.36 \pm 3.2	P < 0.05
Clearance (l/hr)	20.09 \pm 1.02	15.92 \pm 4.61	P < 0.05
Lag time (hr)	0.124 \pm 0.03	0.301 \pm 1.14	P < 0.05
$AUC_{0 \rightarrow 6}$ ($\mu\text{gh/ml}$)	50.52 \pm 2.62	71.24 \pm 2.32	P < 0.05

Table 4.11 Mean value \pm SEM of pharmacokinetic parameters of paracetamol given alone and paracetamol + diazepam concomitant (Phase IV)

Pharmacokinetic parameters	Pcm alone (phase I)	Pcm + Diazepam Concomitant phase (IV)	P-value
C_{max} ($\mu\text{g/ml}$)	14.39 \pm 1.39	14.36 \pm 1.32	P > 0.05
T_{max} (hr)	0.90 \pm 0.06	0.85 \pm 0.04	P > 0.05
$t_{1/2ab}$ (hr)	0.312 \pm 0.02	0.301 \pm 0.06	P > 0.05
K_{ab} ($\mu\text{g/ml/hr}$)	2.25 \pm 0.29	2.10 \pm 0.21	P > 0.05
$t_{1/2el}$ (el)	1.47 \pm 0.04	1.39 \pm 0.14	P > 0.05
K_{el} ($\mu\text{g/ml/hr}$)	0.477 \pm 0.03	0.452 \pm 0.02	P > 0.05
V_d (l)	34.40 \pm 2.19	33.90 \pm 2.09	P > 0.05
Clearance (l/hr)	20.09 \pm 1.02	19.90 \pm 1.03	P > 0.05
Lag time (hr)	0.124 \pm 0.03	0.121 \pm 0.031	P > 0.05
$AUC_{0 \rightarrow 6}$ ($\mu\text{gh/ml}$)	50.52 \pm 2.62	49.93 \pm 2.60	P > 0.05

Table 4.12 Mean value \pm SEM of pharmacokinetic parameters of paracetamol given alone and paracetamol + diazepam delay (Phase V)

Pharmacokinetic parameters	Pcm alone (phase I)	Pcm + Diazepam delay phase (V)	P-value
C_{max} ($\mu\text{g/ml}$)	14.39 ± 1.39	17.5 ± 1.41	$P < 0.05$
T_{max} (hr)	0.90 ± 0.06	0.62 ± 0.12	$P < 0.05$
$t_{1/2ab}$ (hr)	0.312 ± 0.02	0.605 ± 0.17	$P < 0.05$
K_{ab} ($\mu\text{g/ml/hr}$)	2.25 ± 0.29	3.05 ± 1.04	$P < 0.05$
$t_{1/2el}$ (el)	1.47 ± 0.04	1.83 ± 0.14	$P < 0.05$
K_{el} ($\mu\text{g/ml/hr}$)	0.477 ± 0.03	1.055 ± 0.09	$P < 0.05$
V_d (l)	34.40 ± 2.19	33.20 ± 2.16	$P > 0.05$
Clearance (l/hr)	20.09 ± 1.02	20.15 ± 1.17	$P > 0.05$
Lag time (hr)	0.124 ± 0.03	0.06 ± 1.02	$P < 0.05$
$AUC_{0 \rightarrow 6}$ ($\mu\text{gh/ml}$)	50.52 ± 2.62	65.81 ± 2.16	$P < 0.05$

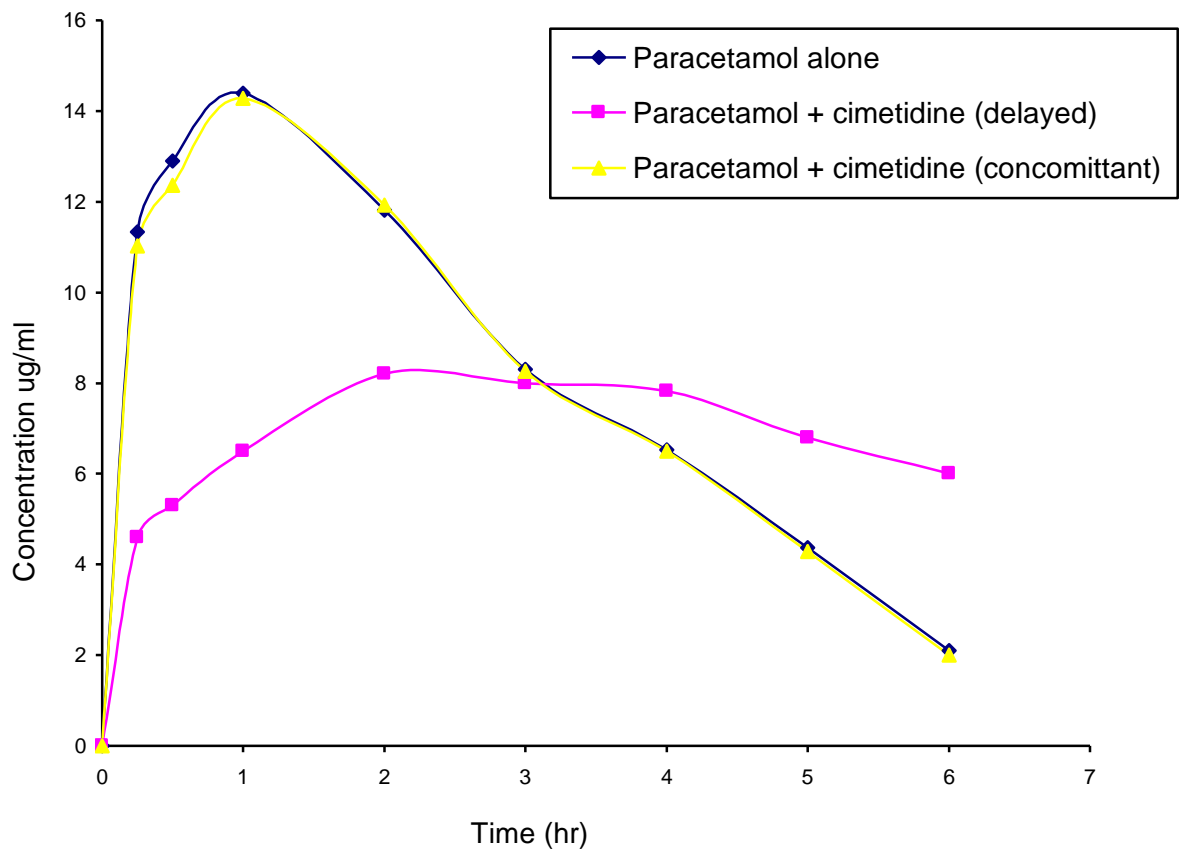


Fig 4.1: Mean Salivary concentration-time profile for paracetamol alone and for the influence of cimetidine under concomitant and delayed administration

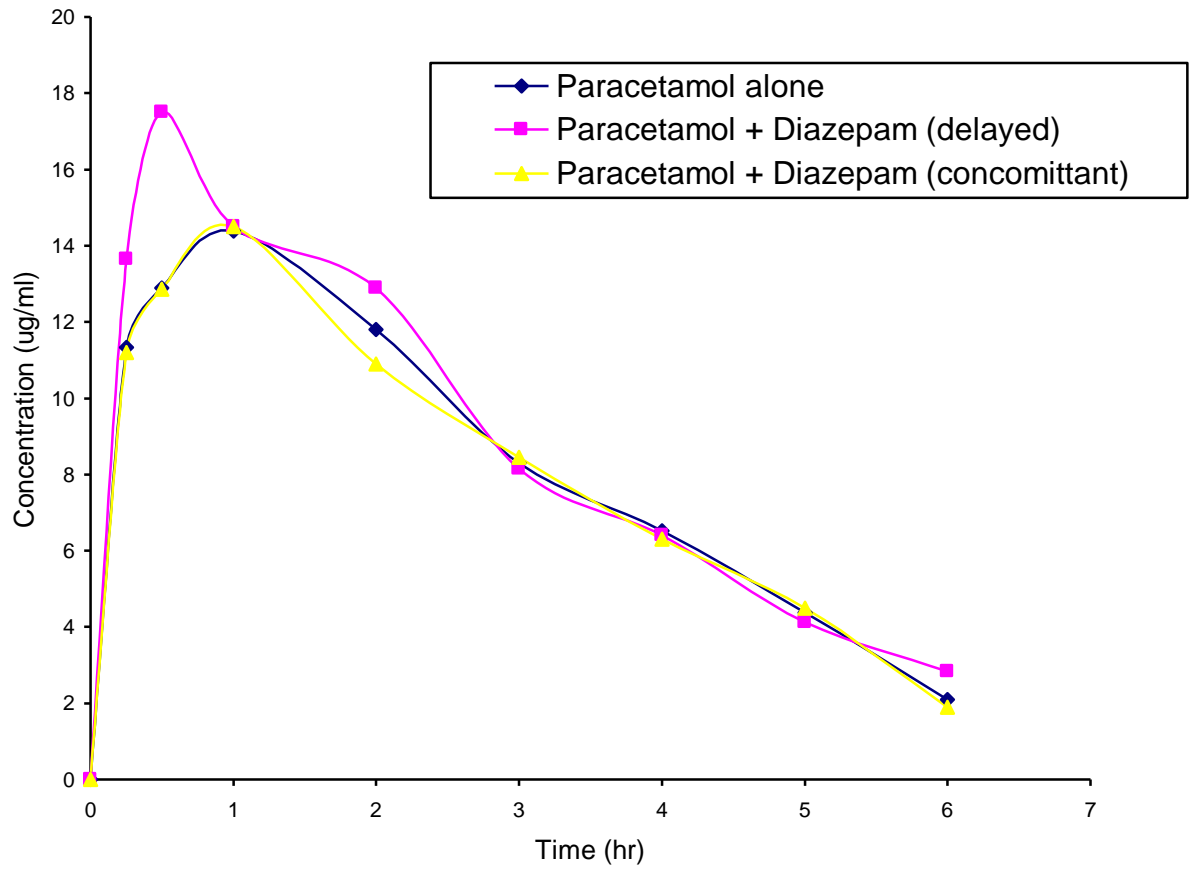
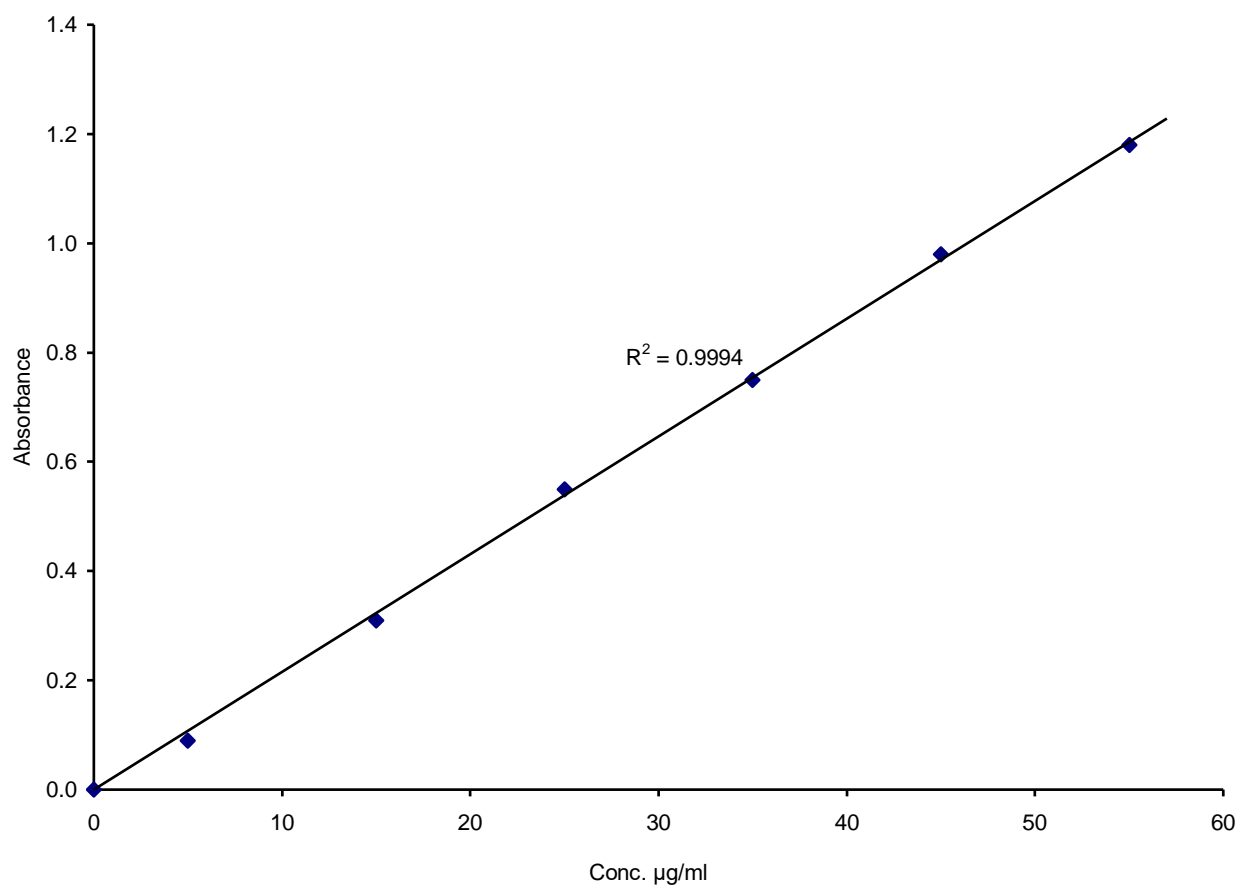


Fig 4.2: Mean Salivary concentration-time profile for paracetamol alone and for the influence of diazepam under concomittant and delayed administration

Concentration ($\mu\text{g/ml}$)	0	5	15	25	35	45	55
Absorbance	0	0.09	0.31	0.55	0.75	0.98	1.18



Validation of calibration curve

5.1 Quality control assessments**IDENTIFICATION TESTS**

The procedure for identification was carried in conformity to recommendation as outlined in BP 2002.

The standard Paracetamol powder gave a violet colour in 0.0167m potassium dichromate solution. The melting was 170⁰C which fell within the range of 168 - 172⁰C. As given by the official book:

The residue obtained from extraction of the tablets with 0.0167m potassium dichromate gave the same violet colour and also a melting point of 168⁰C. All these verified the identity of the Paracetamol tablets.

Cimetidine gave an orange with potassium iodobismuthate and acetic acid solution and a darkened paper was obtained after igniting under lead nitrate paper, these tests are in consonance with requirements by WHO (1991) IN Basic tests for Pharmaceutical dosage forms.

Uniformity of weight

To ensure that accurate doses were administered, test for uniformity of weight was our formed. Average weight for Paracetamol, diazepam and cimetidine were 0.59, 0.169 and 0.6502 respectively, the maxim percentage derivations from the mean weight were 1.59 for diazepam, 0.428 for cimetidine and 4.83 for Paracetamol.

According to the BP. 2002 for tablets with average weight of 250mg or more to pass the test, not more than 2 of the individual weight deviate from the mean weight by more than 5% and none deviate by more than twice that percentage this shows that for the tablets has passed the test.

Assay for content of active ingredient paracetamol gave 99.47% which is in conformity with B. P. 2002 which specifies a range of between 95% - 105%. Cimetidine gave 98.12% while diazepam gave 98, 71% both of which fall within the official range.

DISSOLUTION TEST

The percentage of paracetamol released after 45 minutes in 96.23%. The B.P (2002) recommends that not less 70% be relieved within 45 minutes.

DISINTEGRATION TEST

Cimetidine, paracetamol and diazepam tablets took 5.3 min, 6.7 min, and 7.2 minutes respectively. The BP 2002 recommends that disintegrate time should not exceed 15 minutes.

5.2 Validation of Analytical Methods

The results of validation of the analytical method is presented in Table 4.5. They have ensured that confidence was built in the method adopted and that it is also reproducible.

5.3 Percentage extraction recovery

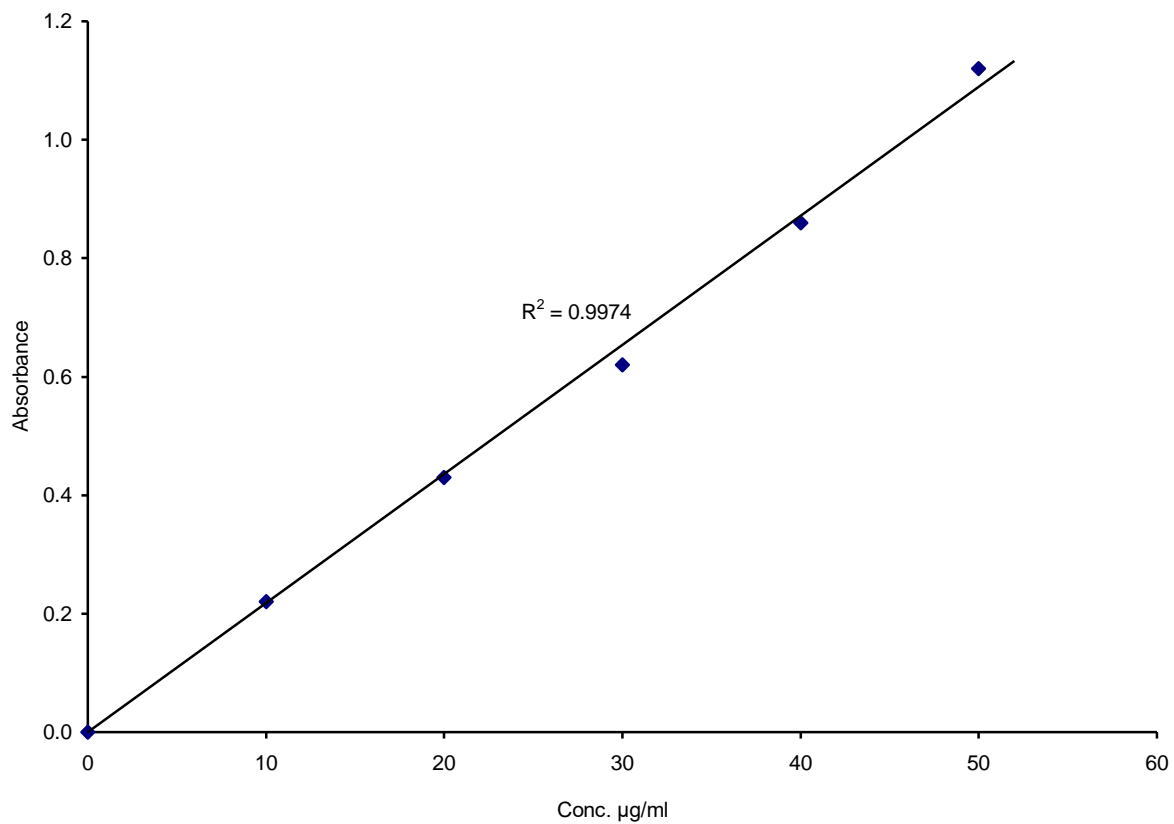
Results of the above are presented in Table 4.6. The active ingredient is extracted in high percentage (95%) to ensure efficient analysis

5.4 Construction and validation of calibration curve

Linear calibration curve with good correlation coefficient ($r = 0.9974$) of paracetamol in ethyl acetate over concentration range of 10-50 $\mu\text{g/ml}$ using UV spectrometer is shown in figure. This indicates that with a good precision in measurement, accurate quantitative estimation of paracetamol in saliva will be obtained using the calibration curve. Due to recovery loss, and variability inherent in measurement in biological fluids, calibration curves are always required.

The plot is usually between an independent variable usually drug concentration on the X-axis and a dependent variable usually some analysis measurement in this case the absorbance on the Y-axis.

Concentration ($\mu\text{g/ml}$)	0	10	20	30	40	50
Absorbance	0	0.22	0.43	0.62	0.86	1.12



Calibration curve

5.4.1 Validation of the calibration curve

Table show the result obtained for the validation of the calibration curve and these have demonstrated a good correlation between the spiked concentrations and those estimated directly from the calibration curve. The relative recoveries of range between 96% and 102.22% are good enough for accurate quantitative determination of paracetamol in saliva sample in the concentration range of the calibration curve. The relative recovery is a measure of the accuracy of the assay method (Smith and Steward, 1981).

5.5 In-vivo Pharmacokinetic studies

Table 4.7 and 4.8 show the mean \pm SD salivary concentration – time data for paracetamol alone (control) and for the influence of diazepam and cimetidine on paracetamol under concomitant and delayed administration.

5.5.1 Single dose salivary pharmacokinetics of paracetamol

Various researches have earlier been conducted to study the pharmacokinetics of paracetamol (Garba *et al* 1999, Thomas S.H.L: 1993, Clement and Prescott 1982). As expected, the result revealed some inter individual variability. Difference in gastric empty rate (Heading *et al* 1973) is attributed to this variability.

The $t_{1/2(ab)}$, of 0.312 obtained showed some similarity with earlier studies (Garba M. *et al*, 1998) which gave a $t_{1/2(ab)}$, of 0.34.

The time to reach peak concentration, t_{\max} was 0.9hrs. This is also closer to the one reported by Garba M. *et al* (1998) at 0.88hrs but slightly differed from another study by Garba M. *et al* (1999) of 0.84.

The value however is within the range of 0.5-1hour reported by most literature (Ameer and Greenblatt 1997, Goodman and Gilman 1996).

The C_{\max} was 14.39 $\mu\text{g/ml}$ lower than Garba M. *et al* (1998) and Garba M. *et al* (1999). This could be attributable to inter individual variability.

The lag time in this study was 0.12hrs which closely agrees with Garba *et al* (1999) of 0.14.

Elimination half life $t_{1/2(\text{ab})}$ obtained was 1.47hrs with corresponding elimination rate constant K_{el} of 0.477 $\mu\text{g/ml/hr}$. Both figures vary only slightly with other reported figures but have however fallen within the range of most established literature $t_{1/2(\text{el})}$ 1-2 hours (Payan and Katzung 1995, Goodman and Gilman 1996).

Volume of distribution (V_d) was 34.40 and clearance of 20.09 L/hr. These figures came close to those reported by Garba M. *et al*, (1999) with a V_d of 29.1931L and clearance of 18.1861 L/hr. The V_d is however lower than the one reported by Bukhari K.A.(1996) of 40.75L and Bello (1990) of 43.012L.

The clearance 20.09 L/hr obtained is however much higher than 12.07L/hr and 12.904L/hr as reported by Bukhari (1999) and Bello (1990) respectively. The

AUC from time zero to infinity was 50.52 $\mu\text{ml/hr}$ is lower than the 128.98 $\mu\text{ml/hr}$ reported by Garba M. *et al*, (1998) and 57.36 $\mu\text{ml/hr}$ as reported by Bukhari 1996 and 77.497 $\mu\text{ml/hr}$ by Bello (1990).

These result as well as others reported earlier showed a lot of inter individual variability probably due to difference in gastric emptying rate (Heading *et al* 1973), age, bodyweight, environmental factors can contribute to the variations in the Pharmacokinetics of Paracetamol.

5.5.2 Influence of diazepam in pharmacokinetics of paracetamol

The results of this protocol are presented in Table 4.8 both under concomitant and delayed administration.

When paracetamol administration is delayed by one hour after diazepam there were significant changes in some of the mean pharmacokinetic parameters. The $t_{1/2(ab)}$ was significantly increased 48.42% ($P<.05$) and T_{max} was reduced from 0.9hrs to 0.62hrs ($P<0.05$) a percentage of 31.11%. The K_{ab} was significant increased to 3.05 ($P<.05$) about 26.22%.

Diazepam was reported to increase the gastric emptying rate and enhance amplitude of contraction (Schurizek B.A *et al* 1988). The result obtained in this study corroborates the earlier findings by Schurizek B.A *et al*.

The elimination parameter $t_{1/2(el)}$ and K_{el} was also significant increased. The lag time of paracetamol was also shortened to from 0.12 hrs to 0.06 hrs probably because the increased gastric motility thereby releasing the paracetamol into the

intestines where most of the absorption takes place. AUC was also increased significantly because of this reason these increase might be of clinical significance

When paracetamol was administered concomitantly with diazepam, there were no significant changes in any of the pharmacokinetic parameters. There indicate that diazepam has no effect on the pharmacokinetics of paracetamol when administered concentrate. Since the two drugs require about the same time to reach plasma concentration (0.5-2 hrs for diazepam Martindale 1996) it follows therefore that for any changes to be seen, diazepam needs to be administered at least 0.5-1 hrs before paracetamol.

5.5.3 Influence of cimetidine on the salivary pharmacokinetics of paracetamol

The mean estimated salivary concentration-time data and the salivary concentration-time profiles for paracetamol alone and for the influence of cimetidine on paracetamol under concomitantly and delayed administration of paracetamol are shown in Table 4.7.

The administration of paracetamol concomitantly with cimetidine gave no significant changes in the pharmacokinetic parameters of paracetamol ($P > .05$). this is as expected because for cimetidine to have any effect (based on knowledge of its own pharmacokinetics) it must be administered at least one hour before paracetamol.

The time to reach peak plasma concentration for paracetamol is 10.60 minutes (Martindale 1996) while cimetidine requires 60-120 minutes. Thus, when paracetamol and cimetidine are administered concurrently, the former will appear in plasma at reasonable concentrations before the latter and so its effect on the pharmacokinetics of paracetamol will not be evident.

But the administration of paracetamol one hour after cimetidine produced a significant change in the pharmacokinetic parameters as presented in Table 4.7.

As shown, C_{max} and K_{ab} were significantly reduced by 30.02% and 45.06% respectively ($P < 0.05$). T_{max} , $t_{1/2(ab)}$ and lag time were significantly increased by 47.97%, 42.32% and 60.13% respectively ($P < 0.05$).

This study showed that cimetidine reduced the absorption of paracetamol if administration of paracetamol is delayed by 1 hour after cimetidine. Delay in gastric emptying (Heading *et al* 1973 and Nimmo *et al* 1973) might be responsible for the impaired absorption of paracetamol. Cimetidine has also been shown to relax guinea pig ileum *in-vitro* (Kwanashie *et al* 1992), a factor that can further explain the delayed absorption of paracetamol.

The outcome of this might mean that the absorption of paracetamol might be so slow that the minimum effective concentration will not be reached on time, thereby stalling the onset of analgesia.

Because cimetidine is a substituted imidazole, it is a strong inhibitor of microsomal P 450 enzymes, the $t_{1/2(ee)}$ was greatly increased by 39.20% ($P = 0.05$).

while the elimination rate constant K_{el} was reduced by about 42.76% ($P < 0.05$). The total body clearance was reduced by 20.75 ($P < 0.05$) while vol. of distribution (V_d) was increased by 40.02 % ($P < 0.05$). AUC was increased by 29.08% ($P < 0.05$).

It is obvious from the above that cimetidine inhibits the metabolism of paracetamol. The concurrent occurrence of both inhibition of metabolism and impairment of absorption lead to increase in salivary concentrations of paracetamol. The strength of the reduced clearance (metabolic inhibition is confirmed by the increase in the area under the curve (AUC). The increased AUC could also be the result of prolonged contact with absorption surfaces of the intestine due to smooth muscle relaxation

5.6 CONCLUSION

Generally, this study agreed with the pharmacokinetic profile of the three drug and earlier works carried out where it was revealed that paracetamol absorption was delayed by cimetidine, an effect attributed to the indirect action of cimetidine on gastric emptying and by its direct action on GIT smooth muscle.

Diazepam and cimetidine have no effect on the pharmacokinetics of paracetamol when administered concurrently. However, paracetamol administered one hour after diazepam shows increase in absorption due to enhanced gastric emptying and amplitude of contraction. While paracetamol given one hour after cimetidine show decrease in absorption due to delay in gastric emptying while delayed elimination is due to metabolic inhibition.

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APPENDICES

Uniformity of weight if diazepam tablets

S/N	Weight (g)	% deviation from mean
1	0.170	0.176
2	0.170	0.176
3	0.170	0.176
4	0.169	0.412
5	0.169	0.412
6	0.168	1.001
7	0.168	1.001
8	0.171	0.760
9	0.171	0.760
10	0.170	0.176
11	0.170	0.176
12	0.170	0.176
13	0.167	1.590
14	0.171	0.760
15	0.172	1.337
16	0.170	0.176
17	0.170	0.176
18	0.168	1.001
19	0.170	0.176
20	0.170	0.176

Total 3.394

Mean 0.1697

Uniformity of weight of cimetidine tablets

S/N	Weight (g)	% deviation from mean
1	0.650	0.030
2	0.650	0.030
3	0.651	0.122
4	0.653	0.428
5	0.651	0.122
6	0.651	0.122
7	0.652	0.276
8	0.649	0.184
9	0.651	0.122
10	0.652	0.276
11	0.649	0.184
12	0.649	0.184
13	0.650	0.030
14	0.650	0.030
15	0.650	0.030
16	0.649	0.184
17	0.649	0.184
18	0.649	0.184
19	0.650	0.030
20	0.650	0.030

Total 13.005

Mean 0.6502

Uniformity of weight of paracetamol 500 mg tablets

S/N	Weight (g)	% variation from mean
1	0.60	1.66
2	0.61	3.27
3	0.59	0.00
4	0.59	0.00
5	0.58	1.69
6	0.60	1.66
7	0.60	1.66
8	0.62	4.83
9	0.59	0.00
10	0.58	1.69
11	0.57	3.38
12	0.62	4.83
13	0.61	3.27
14	0.57	3.38
15	0.59	0.00
16	0.59	0.00
17	0.58	1.69
18	0.60	1.66
19	0.60	1.66
20	0.61	3.27

Total 11.93

Mean 0.59

Assay of contents of diazepam tablets

S/N	Content (mg) per tablet	Percentage content	Remark
1	4.92	98.4	Pass
2	4.95	99.0	Pass
3	4.89	97.8	Pass
4	4.91	98.2	Pass
5	4.93	98.6	Pass
Mean \pm SEM	4.92 \pm 0 .010	98.4 \pm 0.200	

Assay of contents of paracetamol tablets

S/N	Content (mg) per tablet	Percentage content	Remark
1	496.7	99.20	Pass
2	498.8	99.76	Pass
3	497.1	99.42	Pass
4	495.0	99.00	Pass
5	499.1	99.82	Pass
Mean \pm SEM	497.34 \pm 0.747	99.44 \pm 0.158	

Assay of contents of cimetidine tablets

S/N	Content (mg) per tablet	Percentage content	Remark
1	397.8	99.45	Pass
2	395.6	98.90	Pass
3	396.9	99.22	Pass
4	397.1	99.27	Pass
5	395.2	98.80	Pass
Mean \pm SEM	396.52 \pm 0.485	99.13 \pm 0.121	

Salivary concentrations obtained for Paracetamol alone

Time	I	II	III	IV	V	VI	VII	VIII	Σ	\bar{X}
0	0	0	0	0	0	0	0	0	0	0
0.25	10.79	11.34	11.70	10.90	11.63	11.50	10.87	11.91	90.64	11.33
0.5	12.30	11.98	12.10	13.02	12.56	14.70	13.81	12.78	103.25	12.90
1	13.80	15.20	14.62	12.84	15.70	14.32	14.92	13.79	115.19	14.39
2	12.70	11.92	11.87	10.91	12.76	10.53	11.64	12.20	94.53	11.81
3	8.58	7.92	8.24	8.33	8.67	8.02	8.36	8.27	66.40	8.30
4	6.82	6.92	6.12	6.75	6.22	7.20	6.29	5.84	52.16	6.52
5	4.32	4.24	4.02	4.44	4.42	4.30	4.50	4.72	34.96	4.37
6	1.90	1.85	2.30	1.98	2.15	2.35	2.22	2.05	16.80	2.10

Table Paracetamol + Diazepam (delayed)

Time	I	II	III	IV	V	VI	VII	VIII	Σ	\bar{X}
0	0	0	0	0	0	0	0	0	0	0
0.25	12.80	14.30	13.20	13.00	13.55	14.21	13.75	14.50	109.31	13.65
0.5	16.80	12.20	17.75	17.60	17.80	18.20	17.25	12.40	140.00	17.50
1	14.90	10.95	18.35	14.60	15.05	13.65	14.10	14.40	116.00	14.50
2	13.72	12.98	12.50	12.65	12.16	13.38	12.94	12.90	103.23	12.90
3	10.35	8.27	9.70	8.65	5.95	8.03	7.65	6.60	65.20	8.15
4	7.42	5.75	7.05	8.10	5.38	6.90	4.70	5.90	51.20	6.40
5	3.75	4.20	5.35	3.95	4.45	4.20	4.25	2.85	33.00	4.12
6	2.45	3.15	3.02	2.84	3.21	2.64	2.82	2.51	22.64	2.83