THE EFFECT OF RANITIDINE ON THE ABSORPTION KINETICS OF METRONIDAZOLE

BY

ABDURRAHMAN YUNUSA RAFINDADI

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

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ABDURRAHMAN YUNUSA RAFINDADI

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

APRIL 1993

DECLARATION

I hereby declare that this thesis has been written by me and it is a record of my research work carried out under the joint supervision of Dr. Abdullahi Mustapha and Dr. Ibrahim Abdu-Aguye. It has not been presented in any previous application for higher degree. The work of other investigators are acknowledged and referred to accordingly.

AbdulRahman Yunusa Rafindadi
Department of Pharmaceutical & Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University, Zaria.

CERTIFICATION

This thesis entitled "THE EFFECT OF RANITIDINE ON THE ABSORPTION KINETICS OF METRONIDAZOLE" by ABDURRAHMAN YUNUSA RAFINDADI meets the regulations governing the award of the degree of MASTER OF SCIENCE (PHARMACEUTICAL CHEMISTRY) of AHMADU BELLO UNIVERSITY, and is approved for its contribution to science, knowledge and literary presentation.

---------- Date: 07/12/93

Dr. A. Mustapha, B.Sc.(Pharm), Ph.D., MPSN.
Internal Examiner,
Head, Dept. of Pharmaceutical & Medicinal Chemistry,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University, Zaria.

---------- Date: 07/12/93

Dr. I. Abdu-Aguye, MBBS, M.Sc., FMCP.
Internal Examiner
Head, Dept. of Pharmacology and Clinical Pharmacy,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University, Zaria.

---------- Date: 07/12/93

Professor A.A. Olaniyi, B.Pharm, Ph.D., FPSN.
External Examiner,
Head, Dept. of Pharmaceutical Chemistry,
University of Ibadan, Ibadan.

---------- Date: 22/12/96

Professor Dalhatu Mohammed
B.A., Dip. Ling., Ph.D.
Dean, Postgraduate School,
Ahmadu Bello University,
Zaria.

DEDICATION

This work is dedicated

to

my father,

Alh. AbdulRahman N. Rafindadi.

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ABSTRACT

The effect of a single oral dose of ranitidine I50mg was studied on the absorption and pharmacokinetic parameters of metronidazole following a single oral dose of 4OOmg in four healthy volunteers (age 28-35, weight 50-65kg) administered one hour after ranitidine in a cross-over study.

The administration of ranitidine l50mg an hour before the dose of metronidazole did not affect significantly the mean absorption and pharmacokinetic parameters of metronidazole ($p>0.5$). Ranitidine increased Kabs by 20.5%, $t^{1/2}$ by 10.15%, Tiag by 60.8% and decreased Tmax and Cmax by 7% and *3,kh%* respectively (in all cases $P>0.05$). Similarly, Kel and ClTBC were decreased by 8.11% and 1.1% ($P> 0.1$ and > 0.5 respectively) while $t^{1/2}$, Vd and AUC were increased by 8.26%, 6.8% and 1.92% respectively ($P>0.1$, > 0.5 and >0.5).

Similar to results obtained in previous studies, there were variations in the plasma concentrations and pharmacokinetic parameters of metronidazole in man. The effect of ranitidine on the kinetics of metronidazole was highly variable and in some volunteers it was substantial. It is concluded that despite the large inter-individual differences, ranitidine does not alter

the mean pharmacokinetics of metronidazole in man, but that simply using the mean data for comparison could be misleading.

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ABBREVIATIONS

B.P.	=	British Pharmacopoeia
HPLC	=	High Performance Liquid Chromatography
HCl	=	Hydrochloric acid
pH	=	Hydrogen-ion concentration
U.V.	=	Ultra-violet
hr	=	hour
min	=	minute
ml	=	milli-litre
ug	=	micro-gramme
g	=	gramme
^{o}C	=	degree celcius
rpm	=	revolutions per minute
log	=	logarithm
ln	=	natural logarithm
mwt	=	molecular weight
MDZ	=	Metronidazole
CTD	=	Cimetidine
RTD	=	Ranitidine
SEM	=	Standard Error of the Mean
ul	=	Micro litres
nm	=	nano meter
NaOH	=	Sodium hydroxide
KH_2PO_4	=	Potassium dihydrogen phosphate
l	=	litre

CHAPTER ONE

INTRODUCTION

1.0 DRUG INTERACTIONS

When two or more drugs are given in combination, the response may be greater or smaller than the sum of the effects of the two drugs given separately. Drugs are of course given in combination for enhanced therapeutic effects with reduced toxicity. Familiar examples include combinations of antihypertensive drugs, antibiotics and cytotoxic drugs. Drug interaction is the modification of an effect of one drug by the presence of another whether by direct or indirect means; in some cases, there may even be qualitative differences in response (Prescott, 1987). The problems of drug interactions have attracted attention in recent years. Doctors have always practised poly-pharmacy and until fairly recently, this mattered little because the majority of drugs had little or no pharmacological activity. At the present time, however, scores of potent drugs are prescribed on an enormous scale to a public with an insatiable appetite for medication. The average hospital in-patient is sometimes treated with 5 or more drugs simultaneously; some with as many as 15 drugs concurrently. In one hospital, one

patient in 10 received 10 drugs at once, while in another, one patient was given more than 50 different drugs during a single admission (Koch-Weser, 1975). The elderly are at particular risk of drug interactions. They are more susceptible to drug effects and receive more drugs more often in combination than younger patients (Law and Chalmers, 1976). Interactions may vary from person to person, and may be of major clinical significance or of no significance at all.

1.1 Mechanisms of Interactions

The mechanisms of interaction may be quite varied and complex. In some cases, more than one mechanism may be involved. Thus any classification of drug-interaction mechanisms is likely to be an over-simplification (Hansten, 1979). Broadly speaking, drugs may interact on a pharmaceutical, pharmacodynamic or pharmacokinetic basis.

1.2 Pharmaceutical Interactions

Pharmaceutical interactions may occur when drugs are inactivated or precipitated when mixed inappropriately in syringes and infusion fluids prior to administration. Numerous incompatibilities have been demonstrated including the inactivation of carbenicillin by gentamicin,

reversibly with receptors and thus preventing the access of the physiological transmitter acetylcholine. Since the drug-receptor combination is reversible, it is possible to overcome the antagonism by increasing the amount of the agonist at the receptor site. Thus muscle paralysis induced by non-depolarising relaxants such as d-tubocurarine can be reversed by neostigmine which inhibits cholinesterase and increases the concentration of acetylcholine at the receptors (Davie, 1977). Serious interactions characterised by extrapyramidal syndromes and irreversible dementia have been reported with α-methyldopa combined with haloperidol (Thornton, 1976). Both drugs act on central dopaminergic receptors. Other examples include bradycardia and atrio-ventricular conduction defects with calcium antagonists given in combination with β-adrenoceptor blockers (Lewis, 1983).

1.3.2 Drugs having similar Pharmacological Effects

Combinations of drugs acting at the same site or influencing the same physiological system may cause exaggerated or reduced responses. For example, the effects of hypnotics on the CNS are potentiated by ethanol, opioid analgesics, antihistamines, etc. Similarly, diuretics, propranolol, anaesthetics and

CNS depressants may potentiate the blood pressure-lowering effect of antihypertensives (Crook and Nies, 1978).

1.3.3 Alteration of Electrolyte Levels

Changes in electrolyte balance may alter the effect of drugs, particularly those acting on the myocardium, neuromuscular transmission and on the kidney. One important interaction is the potentiation of the action of cardiac glycosides by diuretic-induced hypokalaemia (Koch-Weser, 1975). The anti-arrythmic actions of phenytoin, procainamide and lignocaine are antagonised by hypokalaemia and the sudden release of potassium from muscle following injection of suxamethonium could cause ventricular arrythmias in patients receiving digitalis (Dreifus et al., 1974).

1.3.4 Interference with Intracellular Transport Mechanisms

One drug may interfere with the uptake and transport of another to intracellular sites of action. Many aromatic amines are taken up into sympathetic nerve endings by an active transport mechanism which can be blocked competitively by sympathomimetic amines and other compounds. For example, therapeutic doses of ephedrine, phenylphrine, chlorpromazine and

tricyclic antidepressants can inhibit the blood pressure lowering action of such drugs as bethanidine (Oates, et al., 1971). Tricyclic antidepressants such as imipramine may potentiate the pressor effects of adrenaline and nor-adrenaline (Crook and Nies, 1978).

1.4 Pharmacokinetic Interactions

These are interactions in which the absorption, distribution, metabolism or excretion of a drug is altered by another.

1.4.1 Drug-Absorption Interactions

One drug may alter the rate of absorption or the extent of absorption of other drugs from the gastro-intestinal tract (GIT). Because several mechanisms are involved some of which are poorly understood, the prediction of drug absorption interactions is very difficult (Prescott et al., 1977). These mechanisms may also vary from one drug and one formulation to another. It is also important to differentiate between interactions that alter the rate of absorption and those that increase or decrease total amount of drug absorbed (i.e alter bioavailability) since the consequences may be quite different. A change in rate of absorption of a drug with a long plasma half-life such as warfarin would have little or no effect if all

the drug were eventually absorbed whereas a change in the total amount absorbed may be disastrous. On the other hand, if the rate of absorption of a drug with a short plasma half-life such as procainamide is reduced, plasma concentrations may never be reached. Delayed absorption is also important when a rapid effect is required such as with analgesics and hypnotics.

1.4.1.1 Alteration of pH of Gastro-Intestinal (GI) Fluids

Since many drugs are weak acids or bases, the pH of the GI contents may influence the extent of absorption. The rate of absorption of many drugs may be limited by the rate at which the drug passes into solution from tablets or capsules. Basic drugs are more soluble in acid GI contents and acidic drugs are more soluble in alkaline fluids. On the other hand, basic drugs will tend to be ionised and less lipid soluble in acid solution and hence less rapidly absorbed but these theoretical considerations do not always hold in man (Prescott, 1987). For example, weak acids such as aspirin are absorbed more rapidly from bufferred alkaline solutions than from unbufferred solutions at pH 2-8. Drugs that alter pH may therefore have complex and unpredictable effects on the absorption of other drugs (Welling, 1984). For example, the

H_2-antagonist, cimetidine, reduces the absorption of ketoconazole probably through effects on pH-dependent dissolution (Somogyi and Gugler, 1982) while ranitidine increases the oral bioavailability and central depressant effects of midazolam and the mechanism is thought to involve pH-dependent changes in solubility caused by ring closure (Elwood et al., 1983). Factors other than pH seem to be more important determinants of GIT absorption (Hussar, 1985).

1.4.1.2 Complexation and Adsorption

Drugs may react directly within the GIT to form insoluble chelates which cannot be absorbed e.g iron, calcium, aluminium and magnesium and tetracycline (Neuvonen, 1976). In some cases, however, more rapidly absorbed soluble complexes may be formed, for example, the absorption of dicoumarol is increased by the formation of a more soluble complex with magnesium hydroxide (Ambre and Fischer, 1973).

Absorption of drugs may also be reduced if they are given with adsorbents such as kaolin or charcoal or anionic exchange resins such as cholestyramine and colestipol. Digoxin absorption is seriously impaired by some antacids and kaolin-pectin

(Brown and Juhl, 1976) while propranolol absorption is reduced by colestipol and cholestyramine (Hibbard et al, 1984).

1.4.1.3 Alteration of Gastric Emptying and GIT Motility

Drugs are absorbed much more rapidly from the small intestine than from the stomach. It follows that agents which increase or decrease the rate of gastric emptying or GIT motility may influence the rate of absorption and in some cases the extent of absorption of other drugs given at the same time (Nimmo, 1976; Prescott, 1979). Drugs such as levodopa are metabolised by the gastric mucosa and if gastric emptying is delayed, less unchanged drug would be available for absorption.

Opioid analgesics such as pethidine and pentazocine can produce a marked delay in gastric emptying and slow the rate of absorption of paracetamol (Nimmo et al., 1975). The inhibitory effect of strong analgesics on gastric emptying contributes to impaired absorption of oral antiarrythmic agents and therapeutic failure in patients with acute myocardial infarction (Pottage et al., 1978). Metoclopramide accelerates gastric emptying and increases the rate of absorption of paracetamol. Metoclopramide has also been shown to increase the rate of absorption

and peak plasma concentrations of a number of drugs including diazepam and propranolol (Nimmo, 1976). On the other hand, plasma concentrations of digoxin from slowly dissolving tablets are decreased, possibly as a result of the decreased time available for dissolution and absorption of digoxin, a poorly soluble drug (Manninen et al., 1973).

Anticholinergic drugs such as propantheline decrease GI motility and slow down the rate of absorption of paracetamol (Nimmo et al., 1973). On the other hand, the bioavailability of poorly absorbed drugs such as dicoumarol is increased by tricyclic antidepressants (Pond et al., 1975). The tricyclic antidepressants have marked anticholinergic effects which probably slow GI motility. This may increase the time available for dissolution and absorption of dicoumarol. Antacids such as aluminium hydroxide gel delay gastric emptying and can decrease the rate of absorption of highly soluble and rapidly absorbed drugs such as pentobarbitone. By contrast it does not decrease the rate or amount absorbed of ampicillin trihydrate, a drug which is slowly and incompletely absorbed; this further illustrates the unpredictable nature of drug-absorption interactions.

1.4.1.4 Competition for Active Absorption Mechanisms

Drugs which are analogues of naturally occurring purines, pyrimidines, sugars and amino-acids (e.g α-methyldopa) may be absorbed by specialised active transport mechanisms which occur primarily in the small intestine and absorption could be inhibited on a competitive basis. Consequently, a high protein diet decreases the therapeutic effect of levodopa while a low protein diet increases the therapeutic effect (Mena and Cotzias, 1975).

1.4.1.5 Toxic Effect on the GIT

Patients receiving long-term therapy with para-amino salicylic acid, neomycin and colchicine may develop a malabsorption syndrome (Ehrenfeld et al., 1982). In such circumstances, the absorption of other drugs might be impaired. Thus, colchicine may cause megaloblastic anaemia through interference with Vitamin B_{12} absorption.

1.4.1.6 Changes in Bacterial Gut Flora

Some drugs are metabolised extensively (e.g sulfasalazine) or in part (levodopa) by the gut bacterial flora. This process might be altered by concurrent administration of antibacterial drugs.

Suppression of gut flora by antibiotics might limit the metabolic conversion of sulfasalazine to its components (Das and Dubin, 1976). The intestinal metabolism and absorption of levodopa is abnormal in patients after treatment with neomycin (Goldman et al., 1974). Antibiotics may also prevent the intestinal bacterial hydrolysis of drug conjugates into bile and thus reduce the reabsorption of active parent drug. In this way, antibiotics may reduce enterohepatic circulation of oestrogens in oral contraceptives, possibly resulting in therapeutic failure (Back et al., 1981).

1.4.2 Drug Distribution Interactions

One drug may change the distribution of another and thereby alter the concentration of unbound active drug at sites of action. Many drugs and drug metabolites are highly bound to plasma proteins. Generally, acidic drugs bind predominantly to albumin, though not necessarily to the same site while basic drugs such as tricyclic antidepressants and propranolol bind to the acute phase reactant protein α_1-acid glycoprotein as well as albumin. Under certain circumstances, the addition of another drug to a primary drug regimen may result in displacement of the primary drug from its plasma-protein binding sites leading to altered

kinetics and in some instances changed drug effects in the patient. As displacement makes more free (unbound) drug available for metabolism or glomerular filtration, the displaced drug can normally distribute out of the plasma compartment. However, increased free drug concentrations in the plasma are usually transient and therefore do not commonly give rise to altered pharmacological effects in patients. Important determinants of plasma-protein binding displacement interactions and their potential significance include (Prescott, 1987):

(a) The affinity of the interacting drugs for the binding protein and the site at which they bind on the protein molecule: For one drug to competitively displace another, it must have a high affinity for the protein relative to the displaced drug and bind on the same site on the protein molecule.

(b) The concentration of the drug in plasma: Competitive displacement occurs when the product of the free concentration of the displacer and its binding constant is high. This implies that important displacement will only occur with drugs used at plasma concentrations high enough to exceed the binding capacity of their own primary binding sites on the protein molecule.

(c) The elimination characteristics of the drug: For drugs with a low extraction ratio (ER), the organ extraction and consequently clearance are limited by protein binding and thus will be altered by changes in unbound fraction. For drugs with high ER, binding to plasma proteins does not protect the drug from elimination, and in such cases clearance is dependent only on the amount of drug presented to the eliminating organ which is in turn proportional to its blood flow. A highly significant and potentially dangerous drug-distribution interaction has been demonstrated by the drugs warfarin and phenylbutazone. Both are extensively bound to plasma proteins. Phenylbutazone however, has a greater affinity for the binding sites resulting in the displacement of warfarin and making increased quantities of the 'free' drug available. The net concentration of warfarin in the systemic circulation is increased, as a result of which there is increased anticoagulant activity with risks of haemorrhage.

The consequencies of plasma protein binding displacement are likely to be exaggerated in patients with hypoalbuminaemia and renal failure (in which the binding of certain drugs is altered) or certain types of severe liver disease (in which the binding and clearance of acidic drugs can be altered) (Blaschke,1977).

Important interactions can also occur if one drug displaces another from binding sites (other than receptors) in the tissues. Such interactions have a much greater potential for adverse effects as in this case plasma concentrations of free drug will be increased especially if the displacement is extensive (McElnay and D'Arcy, 1983). For example, quinidine causes a variable but often very significant increase in the steady state plasma concentrations of digoxin probably by decreasing it's volume of distribution or by displacing it from tissue binding sites (Fichtl and Doering, 1983).

1.4.3 Drug Metabolism Interactions

Many drug interactions have resulted from the ability of one drug to stimulate or inhibit the metabolism of another through activating or inactivating the hepatic microsomal enzymes involved in the metabolism of numerous therapeutic agents.

By an oversimplification, drugs may be divided into water soluble and lipid soluble compounds. Water soluble drugs are mainly excreted unchanged through the kidneys. Lipid soluble drugs are initially filtered in the glomeruli but may be

fully reabsorbed further in the distal portion of the nephron. Such drugs therefore have to be metabolised to more polar compounds before they can be excreted in the urine (Remmer, 1970). Their rate of metabolism will determine the duration of action of single doses and the intensity of action of multiple doses. This is because the steady state plasma concentration largely depends on the elimination rate constant.

The liver is the most important overall site of drug metabolism. Usually, the metabolites tend to be more water soluble and more readily eliminated from the body and have less pharmacological activity. However, this is not always the case. Chemically, there are two general types of reactions. Initially, there may be the addition of a polar functional group, deletion of a non-polar alkyl group by oxidation, reduction or hydrolysis (Phase I). Water solubility can then further be increased by conjugation with endogenous compounds such as glucuronic acid, sulfate, acetate or glycine (Phase II) (Tucker, 1979). These metabolic reactions are facilitated by enzymes. Within the liver cell, the most important enzymes are the hepatic microsomal enzyme oxidation system located in the smooth endoplasmic reticulum. It is responsible for many Phase I reactions. It is composed of two enzymes, cytochrome P-450 and reduced nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome

P-450 reductase and requires NADPH for its action.

The clearance rate for drugs that are primarily eliminated by microsomal enzymes is variable within and between patients and is affected by patient's genetic background, age, nutritional state, disease and exogenous chemicals. One source of this variability is that multiple forms of cytochrome P-450 exist. These iso-enzymes (as they are called) vary in metabolising activity for a given substrate and exhibit varying degree of overlapping substrate activity. At least six isoenzymes have been identified but there may be more than 60 (Hebert et al., 1981; Waltz et al., 1982).

1.4.3.1 Induction of Drug Metabolism

Many lipid-soluble drugs and foreign compounds stimulate drug metabolism through induction of hepatic microsomal enzymes. The increased activity is probably due to enhanced enzyme synthesis resulting in increased amounts of drug-metabolising enzymes. Induction of drug metabolism is a complex dose-related phenomenom. Induction usually develops over a period of several days or weeks, and persists for a similar period following withdrawal of the drug. Long term exposure to inducing drugs such as phenobarbitone, phenytoin,

cigarrette smoke, etc cause stimulation of not only their own metabolism but also the metabolism of many other unrelated drugs and related physiological compounds which are substrate for these enzymes (Park and Breckenbridge, 1981). Induction leads to significant drug interactions which are of direct relevance to therapy. One example is the clinically significant drug interaction between phenobarbital and warfarin. The induction of metabolism of warfarin by phenobarbital leads to reduced anticoagulant effect and the dose of warfarin may need to be doubled or quadrupled to regain the original effect (Serlin and Breckenbridge, 1981). The stimulation of hepatic enzyme activity is not only a factor in the development of drug interactions but may also be responsible for the development of tolerance to certain drugs that are given for a prolonged period.

1.4.3.2 Inhibition of Drug Metabolism

Inhibition of drug metabolism may result in exaggerated and prolonged responses with an increased risk of toxicity. Many interactions of this type involve liver microsomal enzymes and mechanisms include substrate competition, interference with drug transport and functional impairment of enzyme (Kristensen, 1976).

The predominant types of inhibition are competitive and non-competitive. Competitive inhibition occurs when the inhibitor binds to the enzyme at the same site as the substrate and is also metabolised by the enzyme. Non-competitive inhibition occurs when the inhibitor irreversibly binds to and inactivates the enzyme. This may allow the substrate to bind to the enzyme as usual; however, the maximum velocity of the enzyme-substrate reaction is decreased. Inhibitors are usually lipophilic. Cimetidine is an important inhibitor of drug metabolism and prolongs half-life of many drugs including antipyrine, warfarin, phenytoin, diazepam and theophylline (Powell and Donn, 1984).

1.4.3.3 Changes in Hepatic Blood Flow

Hepatic blood flow is an important determinant of the elimination of drugs which are extensively and rapidly removed from the plasma by the liver (e.g. propranol, lignocaine) such that their disposition can be affected by drug-induced changes in blood flow to the liver (Nies et al., 1976). As a consequence of the reduced cardiac output which they cause, propranolol and other β-blockers decrease hepatic blood flow and decrease their clearance and the clearance of other drugs with a high hepatic extraction ratio.

1.4.4 Drug Excretion Interations

1.4.4.1 Alteration of urinary pH

The renal clearance of weak organic bases with pKa values of 7.5 to 10 is increased if the urine is made acid and decreased in alkaline urine. Conversely, the clearance of weak organic acids (pKa 3 - 7.5) is higher in alkaline than acid urine (Milne et al., 1958). Amphetamine (basic) is normally excreted unchanged in the urine to a significant extent and their effects may be significantly prolonged if the urine is made strongly alkaline. Acetazolamide and other carbonic anhydrase inhibitors render the urine alkaline by interferring with bicarbonate reabsorption. The urinary excretion of calcium is thereby increased and this may lead to renal calculi and aggravation of osteomalacia induced by long-term antiepileptic therapy (Mallette, 1977).

1.4.4.2 Interference with urinary excretion

Many acid drugs and drug metabolites share the same proximal tubular active transport system and can compete with each other for secretion (Kristensen, 1976). One drug may therefore interfere with the renal excretion of another and cause accumulation and toxicity. Drugs which may interact by this mechanism include sulfonamides, thiazide diuretics,

probenicid, penicillins, salicylate, phenylbutazone etc. For example, probenecid is used therapeutically together with penicillins to increase serum concentrations of the latter by altering their distribution and delaying their renal excretion (Kabins, 1972). There is a separate active renal tubular transport system for organic bases and similar competitive interactions may occur at this site. Thus, cimetidine and ranitidine inhibit the renal clearance of procainamide by this mechanism (Christian et al., 1984).

1.5 Basic Concepts of Drug Pharmacokinetics

Pharmacokinetics deals with the time course of drugs in the body (Paxton, 1981). It requires assay methods for the determination of drugs in tissues and body fluids e.g. plasma, urine. The blood is a unique body fluid in that it stays in intimate contact with all tissues. Hence, the drug concentration in blood plasma continuously mirrors the fate of the drug in various tissues and organs.

Drugs are distributed in complex biological systems. Once absorbed, a drug disperses to a variety of locations within the body including the target organs, the plasma and tissue binding sites, and the organs of elimination. The rate of distribution into

a tissue depends on its vascular perfusion, the permeability of the tissue membranes to the drug, and the relative partitioning of drug between tissue and blood. These complex procedures may be simplified by considering the body to consist of a number of compartments. These are postulated to account for the experimental observations that drugs distribute into various fluids and tissues at different rates. These compartments do not have anatomical boundaries, nor necessarily consist of one type of tissue. The number of compartments which can be defined ultimately depends on the number of areas with different rates of drug penetration. Experimentally, it is difficult to demonstrate more than three compartments.

The basic approach used in pharmacokinetics is to fit experimental data on drug concentrations in plasma to mathematical equations that represent the flow of drugs and their metabolites through the discrete compartments of a model system. This results in a precise description of drug disposition which can have major clinical applications.

However, before any equations can be derived, several assumptions have to be made. Firstly, drugs enter the system only via the central compartment and are eliminated only from that compartment.

Secondly, reversible transfer occurs between central and peripheral compartments and thirdly the exit of drugs from all compartments in the model is described by first-order kinetics (i.e the rate at which a drug is removed from a compartment is directly proportional to the drug concentration in it). All three assumptions are relatively valid for most drugs. The majority of drugs enter the body via the blood stream and are eliminated from the blood stream by the liver or kidney. Also, most drugs are eliminated by first-order kinetics.

1.5.1 Single-Compartment Model

The simplest model is the single compartment model. The whole body may be considered mathematically as a single compartment when exchange of drug between plasma and tissues proceeds rapidly compared with rate of elimination. Assuming instantaneous distribution after an intravenous (IV) injection of a drug into this model (Fig. 1.5.1), the concentration Co in the plasma immediately after injection is equal to the dose D divided by the volume of the compartment V_d:

$$C_o = D/vd$$

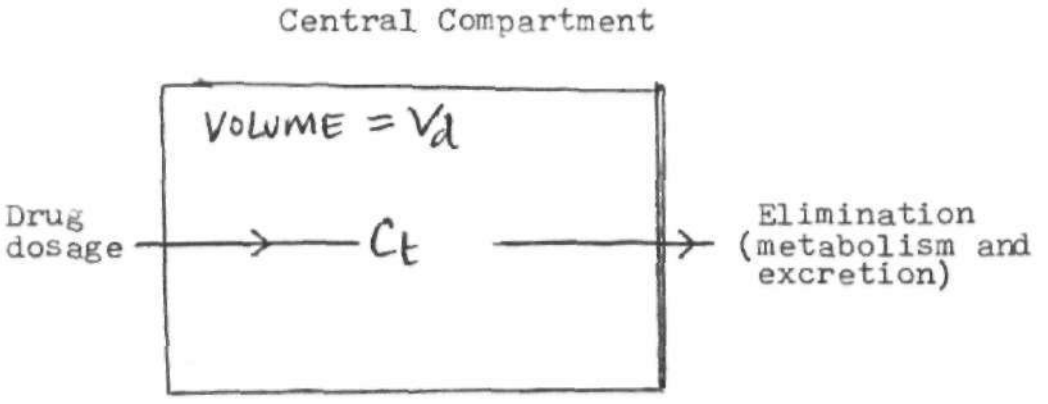


Fig. 1.5.1: Single compartment model.

After instantaneous distribution, the concentration will fall according to first-order kinetics which means a constant fraction of drug is eliminated per unit time. Thus, a plot of plasma drug concentration against time, with each unit representing the time for one half of the drug to be eliminated ($t_{\frac{1}{2}}$), results in an exponential curve:

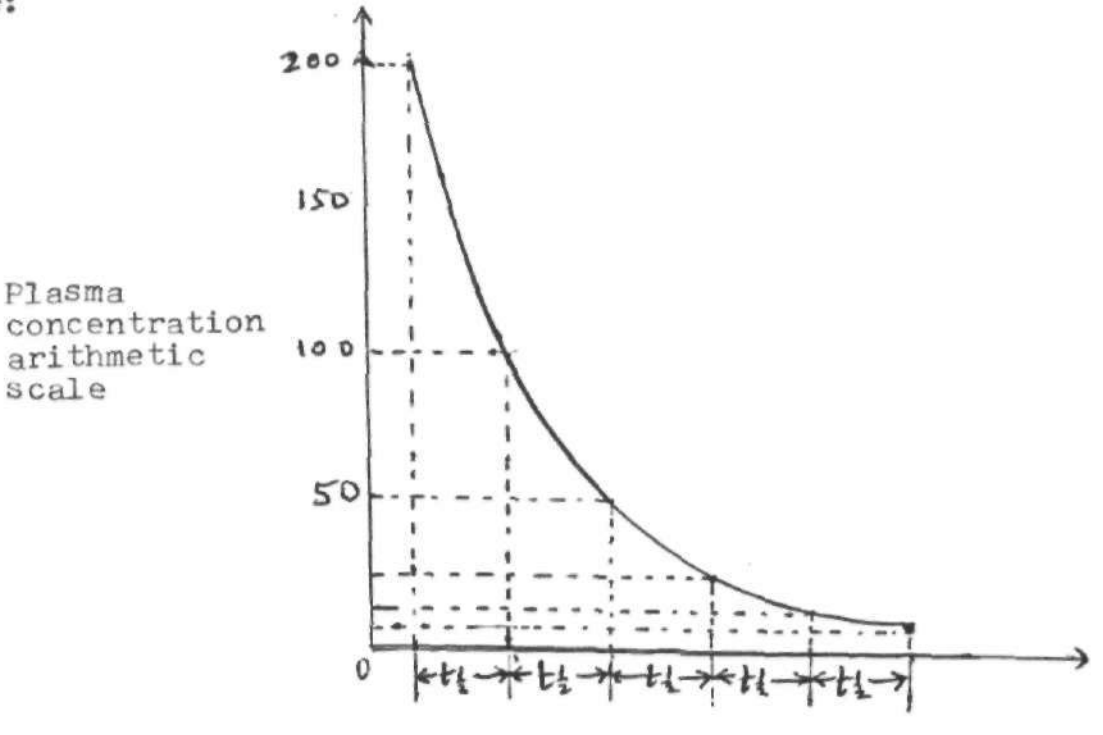


Fig. 1.5.2. Exponential-decay graph for single compartment model

Replotting this curve with concentration on a logarithmic scale results in a straight line:

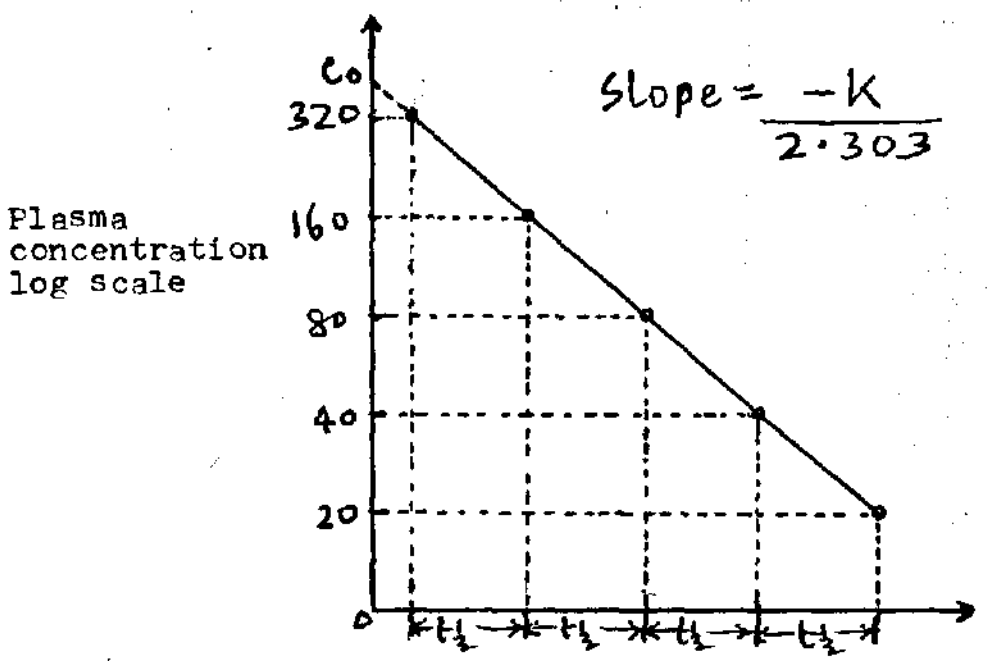


Fig. 1.5.3: Semi-log plot of concentration vs. time for single compartment model.

Extrapolation of this plot to time-zero gives the theoretical initial concentration C_o. Accepting first order kinetics between drug concentration and time, the decline in drug concentration may be expressed mathematically as follows:

$$\frac{dC}{dt} = -\beta C_t$$

Where C_t = Concentration

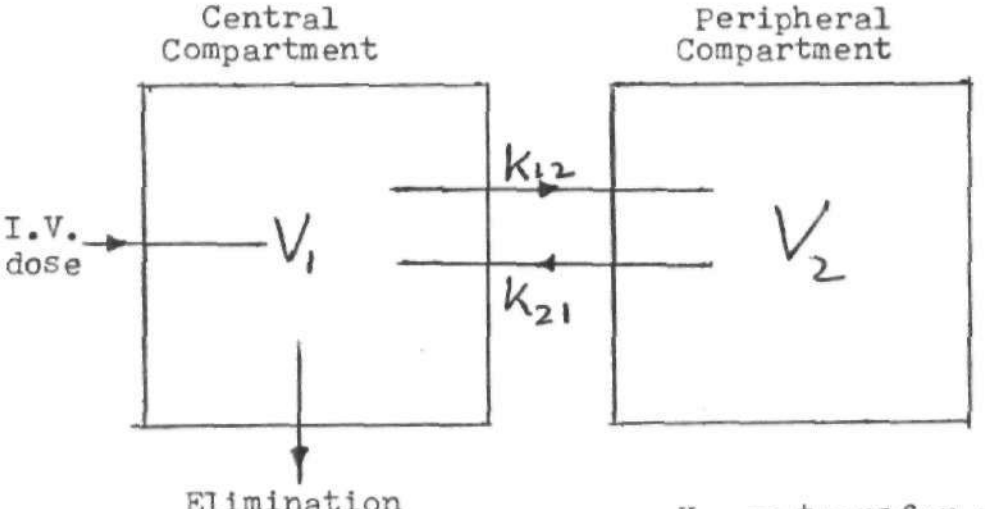
β = Rate constant of elimination

Integration and conversion to logarithms to the base 10 gives:

$$\log C_t = \log C_o - \frac{\beta t}{2.303}$$

1.5.2 Two Compartment Model

The plasma concentration-time curve of a drug is often not a straight line on a semi-logarithmic plot. If distribution of the drug is so slow that it cannot be disregarded, a model must then be considered which contains a central compartment and at least one other peripheral compartment.



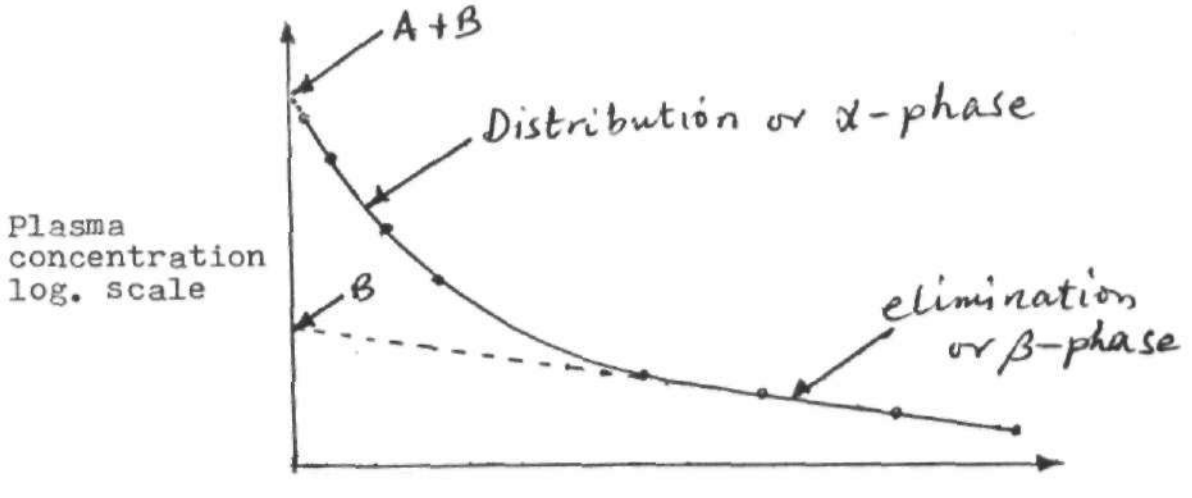


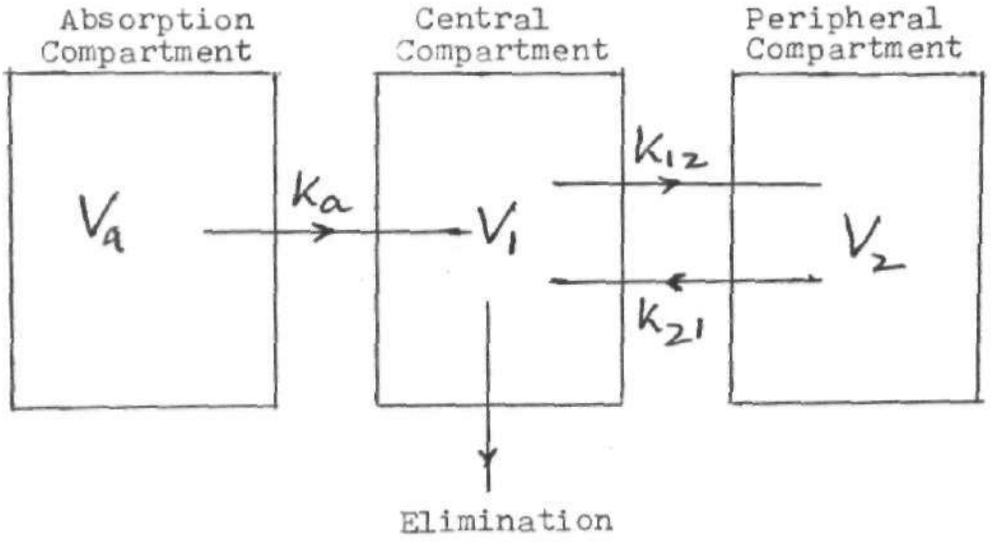
Fig.1.5.4: Plasma log-concentration-time curve for a two compartment model.

Although these compartments lack physical or anatomical reality, for many drugs the central compartment corresponds to the plasma or blood volume, together with the extracellular fluid of highly perfused tissues such as heart, lungs, liver, kidneys and endocrine glands. Drugs distribute within a few minutes through this compartment and equilibrium between plasma and tissue is rapidly established. The peripheral compartment is then formed by less perfused tissues such as skin, muscle or adipose tissue in which drugs enter more slowly. The combined effect of two compartments gives rise to a bi-phasic curve on I.V. injection with two distinct linear portions when drawn on a semi-log scale (see fig. 1.5.4).

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

1.5.3 Modification for Oral Dosing

With an oral dose, an additional absorption compartment is incorporated into the model to represent the volume from which absorption occurs at a first-order rate (see figure 1.5.4). In this model, it's assumed that the entire dose is rapidly introduced into the site of absorption, from which it is absorbed into the central compartment.



Ka = Absorption rate constant

K_{12}, K_{21} = Transfer rate constants between compartments.

Fig. 1.5.4: Plasma log-concentration time curve for a two-compartment model.

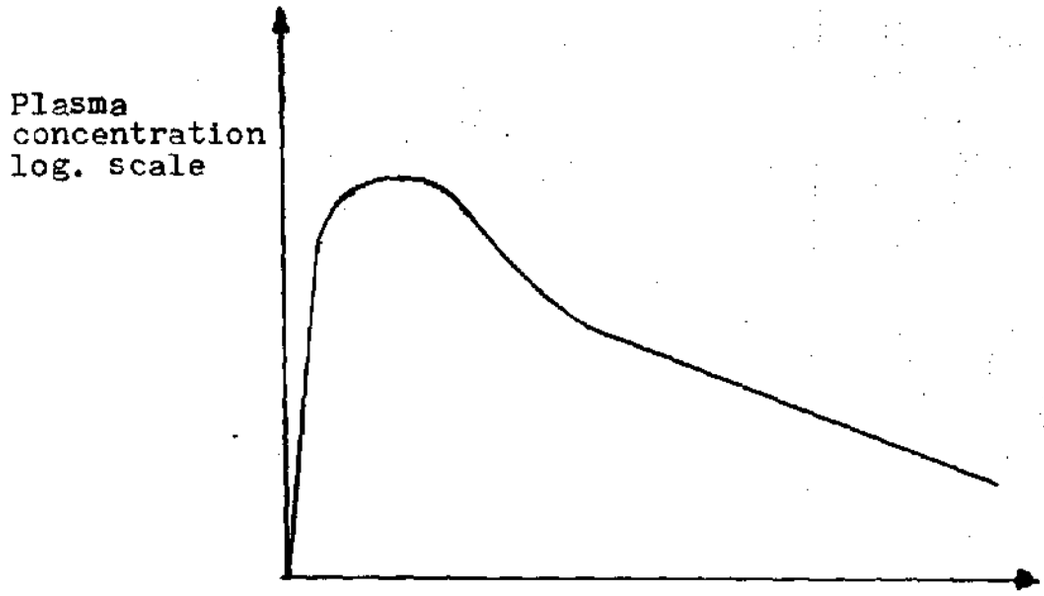


Figure 1.5.5: A three-compartment model which includes a compartment for absorption. A typical log. concentration-time curve/plot after a single oral dose.

1.5.4 Area Under the Concentration-Time Curve (AUC)

The AUC, which is represented by the integral $\int_0^t Cdt$ is a measure of the amount of drug that is available systematically (bioavailable) which is a major determinant of therapeutic efficacy. It is important for calculating such important pharmacokinetic parameters as relative oral bioavailability, plasma clearance and apparent volume of distribution. It can be determined by the trapezoidal rule and is independent of any model proposed.

1.5.5 Plasma clearance (Cls)

Plasma or systemic clearance is defined as the volume of plasma from which a drug is totally and irreversibly removed per unit of time. It is a direct index of drug elimination. Total plasma clearance may be determined after oral administration from the relationship.

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

Where F = Fraction absorbed
D = Dose
AUC = Area under the concentration-time curve.

It may also be calculated from the relationship:

$$Cls = \beta Vd$$

Where β = Elimination rate constant
Vd = Apparent volume of distribution.

Clearance has the dimension of volume per unit time and is made up of the sum of all the clearances due to individual organs. If only one organ is involved, for example the kidney, the plasma clearance is equal to renal clearance.

For any particular organ, the physiological determinants of drug clearance are organ blood-flow,

the inherent ability of the organ to extract the drug from the blood and the extent of binding of the drug to plasma proteins. Considering the liver, for example, hepatic clearance Cl_h is given by the relationship:

$$Cl_h = Q \left[\frac{fCl'int}{Q + fCl'int} \right]$$

Where Q = hepatic blood flow

f = fraction of unbound (free) drug

and $Cl'int$ = intrinsic free drug clearance.

The term in parentheses is referred to as the extraction ratio ER and can vary from Zero to 1. If a drug has a very high intrinsic clearance, ER will tend towards 1 and the hepatic clearance would be equal to Q, the hepatic blood flow, and will demonstrate flow dependent kinetics. Thus, the hepatic clearance of a drug such as propranolol, for which the liver has a high metabolic capacity, is limited by hepatic blood flow and is relatively insensitive to changes in metabolising activity. However, a reduction in the rate at which the drug is transported to the liver such as a decrease in blood flow would result in reduced hepatic clearance.

On the other extreme, when intrinsic hepatic clearance is very low compared to blood flow, then ER is low andthe equation for hepatic clearance reduces to: $Cl_h = fCl'int$

and elimination is dependent on enzyme activity and drug binding. Thus, for a drug such as theophylline for whom there is insufficient enzyme activity present in the liver and which has low ER, its hepatic clearance will be affected by any change of enzyme activity (e.g enzyme inhibition or induction) but will not be affected by changes in blood flow. The hepatic clearance will also be dependent upon the free-fraction of the drug in blood so that increase in binding reduces clearance, and vice versa. In contrast, altered plasma-protein binding will have little effect on organ clearance of drugs with high ER as such drugs can be functionally stripped from their binding sites during passage through the liver.

1.5.6 Apparent Volume of Distribution

Apparent volume of distribution V_d 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. The concept of volume of distribution is used to relate the plasma concentration

of a drug to the dose administered as the body is obviously not homogenous, even if it can be treated as such in a mathematical model. Drug concentrations in the liver, kidney, muscle, fat and other tissues will therefore differ from one another as well as from the plasma concentration. The apparent volume of distribution does not relate to a real physiological or anatomical space, but provides an estimate of the extent of the drug's distribution through the body fluid compartments and its uptake by tissue. A large value for V_d implies wide distribution or extensive tissue uptake or both. After oral administration, V_d can be determined from the relationship:

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

Where F = fraction of dose absorbed

D = dose

β = elimination rate constant

AUC = area under concentration-time curve.

1.5.7 Elimination (Plasma) Half-Life

The elimination half-life ($t_{\frac{1}{2}}$) is the time taken for half the amount of drug present to be eliminated either by excretion or metabolism or both. In practical terms, it is the time taken for the plasma

concentration to be reduced by 50% of its original value, and can be determined for both one and two-compartment models from the terminal data of the log concentration-time plot. It may also be determined from the equation (see page 25).

$$\text{Log } Ct = \log C_o - \frac{\beta t}{2.303}$$

Substituting for $t_{\frac{1}{2}}$ results in

$$\text{Log } X = \log 2x - \frac{\beta t}{2.303}$$

$$\text{i.e } t_{\frac{1}{2}} = \frac{0.693}{\beta}$$

From this equation, it appears that a large value for β corresponds to a short $t_{\frac{1}{2}}$ and is a reflection of the rate at which the drug leaves the body. However, $t_{\frac{1}{2}}$ is a poor index of drug elimination as it depends not only on the clearance but also on the volume through which the drug is distributed (volume of distribution V_d). Obviously, if clearance is increased, one would expect a shorter half-life. Not so obvious is the concept that if clearance is kept constant but the volume into which the drug is distributed is increased, half-life increases; this is supported mathematically by the relationship:

$$t_{\frac{1}{2}} = \frac{0.693\ V_d}{Cl_s}$$

Although $t_{\frac{1}{2}}$ is a poor index of drug elimination processes and dosage requirements in individual patients, it is a useful parameter in the determination of suitable dosage intervals and the time required to attain steady-state during intravenous or chronic oral therapy.

1.5.8 Non-Linear Pharmacokinetics: (Fallibility of Assumptions)

In all the foregoing, it has been assumed that all the systems operating on the drug obey first-order kinetics i.e the drug has linear pharmacokinetic characteristics. This means that elimination half-life, clearance, volume of distribution and bioavailability are independent of dose or concentration of drug. Assumption is also made that the pharmacological effect is elicited by the drug administered (and which is being measured). Even though the general concepts of pharmacokinetics are applicable to many drugs without significant modification, these assumptions do not apply to all drugs. Therefore where these assumptions are not valid, significant clinical consequences accrue and modifications of the models are necessary.

Deviations from linearity are possible with respect to one or more processes in absorption, distribution, binding to biological materials in tissue,

plasma protein binding and elimination of drug (Ritschel, 1980). Deviations from linearity with respect to absorption may be due to low solubility of the drug, low dissolution rate of the drug from the dosage form, changes in gastro-intestinal pH, and, in the case of active absorption processes, due to saturation of carriers.

Deviation from linearity with respect to tissue distribution may occur when tissues or active transport systems in the tissues become saturated. Deviation from linearity with respect to plasma protein binding can occur if increasing dose sizes approach saturation of binding sites or if only limited number of binding sites are available. Non-linearity with respect to excretion can be caused by changes of urinary pH for drugs that are weak acids or weak bases undergoing urinary recycling, by saturation of reabsorptive processes or by diurnal variation in the elimination of drugs.

1.5.8.1 Dose-Dependent Kinetics

One of the commonest causes of non-linearity is the limited capacity of certain drug metabolising enzyme systems. Drug elimination is always linear below the maximal capacity of the drug metabolising enzymes. However, in some situations, drug concentrations

are sufficiently high enough to cause saturation of the enzyme system causing elimination to occur at a fixed rate (i.e zero-order kinetics). In this situation, drug administration will depend on the magnitude of dose, and drug accumulation will not readily be predictable. Once saturation has been reached, the plasma concentration will increase disproportionately with subsequent dosing and the time required to reach the steady state will increase with increasing half-life. A well known example of a drug whose metabolism becomes saturated at clinically achievable concentrations is phenytoin (Richens and Dunlop, 1975).

1.5.8.2 Active Metabolites

Some drugs are biotransformed to a metabolite that has a pharmacological action like that of the parent drug. With these drugs, the combined pharmacodynamic (and sometimes pharmacokinetic) action may rise and fall in a complex way because of the different time courses, distribution, and rates of elimination of the active metabolite. For drugs with such properties e.g. the antiepileptic trimethadione, a study of pharmacokinetics alone would be of little value in predicting a therapeutic regimen (Harvey and Dean, 1985). Two or more active metabolites may greatly increase the complexity.

plasma protein binding and elimination of drug (Ritschel, 1980). Deviations from linearity with respect to absorption may be due to low solubility of the drug, low dissolution rate of the drug from the dosage form, changes in gastro-intestinal pH, and, in the case of active absorption processes, due to saturation of carriers.

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1.5.8.1 Dose-Dependent Kinetics

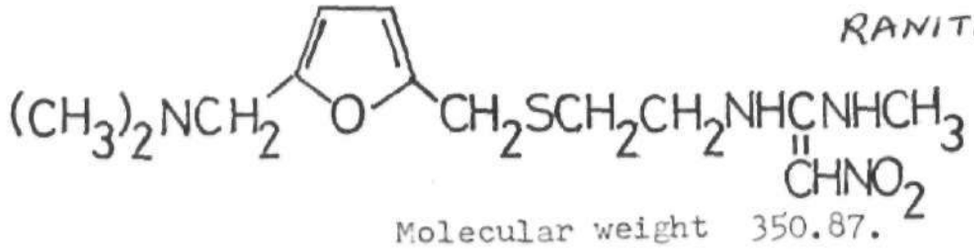
One of the commonest causes of non-linearity is the limited capacity of certain drug metabolising enzyme systems. Drug elimination is always linear below the maximal capacity of the drug metabolising enzymes. However, in some situations, drug concentrations

CHAPTER TWO

LITERATURE REVIEW

2.1 RANITIDINE

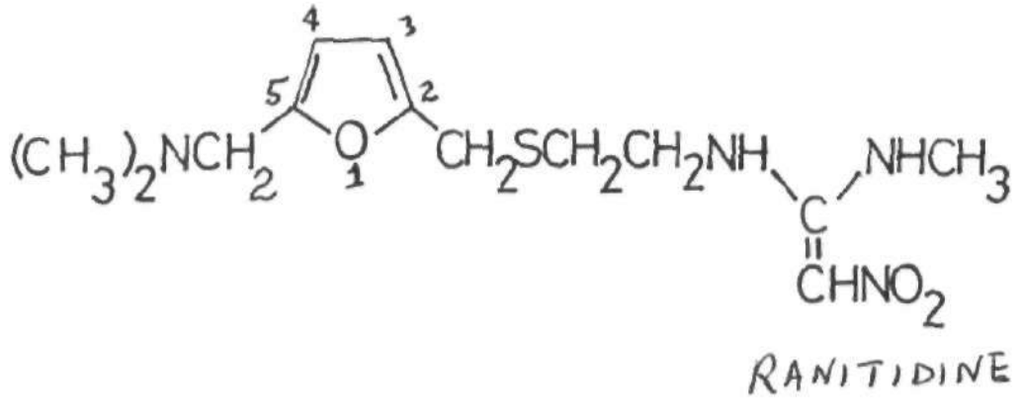
Ranitidine is an aminoalkylfuran derivative of histamine, chemically, it is NN-dimethyl-5-[2-(1-methylamino-2-nitrovinylamine)ethylthiomethyl] furfuryl-amine. It is a white to pale yellow granular substance freely soluble in water with two pKa values 2.7 and 8.2.

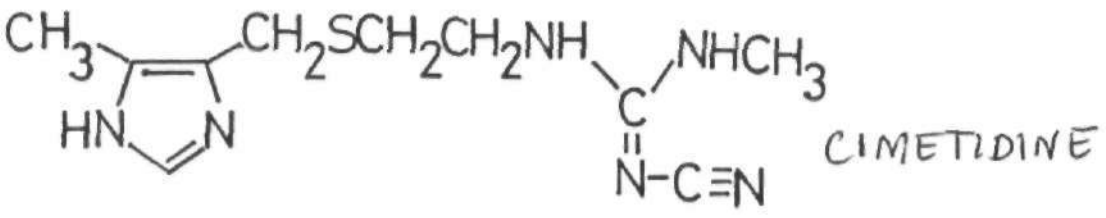


Molecular weight 350.87.

2.1.1 Chemistry (Structure Activity Relationship - SAR):

Ranitidine is a second generation H_2-antagonist but differs fundamentally from cimetidine in having a furan ring instead of an imidazole ring:





CIMETIDINE

The SAR of ranitidine has been reviewed (Bradshaw et al., 1981).

2.1.1.1 The Ring System

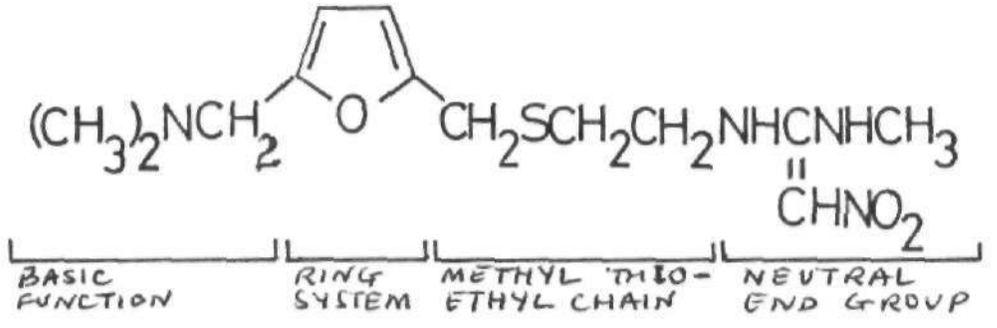
The imidazole ring was a key structural feature of cimetidine and related H_2-antagonists (Durant et al., 1981). It was concluded that this ring appeared to have a special importance at H_2-receptors. The possibility that a basic heterocyclic might not be essential for H_2-antagonist activity was investigated by replacing it with a non-basic ring system to which a basic function might readily be attached (Bradshaw et al., 1981). Replacement of imidazole ring by furan to which a dimethylaminomethyl substituent was attached afforded compounds (such as ranitidine) with comparable H_2-blocking activity to the imidazoles.

Replacement of the furan ring by a thiophene ring gave a compound that was less active; and the benzene derivative was even less active. This could be explicable in terms of increased lipophilicity down the series.

2.1.1.2 Ring Substituents

The 3-methyl analogue was found to be inactive whereas the 4-substituted analogue is highly potent. Both 4-bromo and 4-isopropyl derivatives were shown to have similar activities to that exhibited by the 4-methyl group.

2.1.1.3 Modification of Basic Function



Variation of the basic group attached to the furan ring showed that activity is not particularly sensitive to the nature of the basic function. Secondary amines are equivalent with tertiary amines and variation in lipophilicity appeared not to have a dramatic effect. The trifluoroethyl compound was found to be just as active as the corresponding alkyl amine derivative indicating basicity is not a sensitive parameter. Cyclic amines led to a reduction in potency.

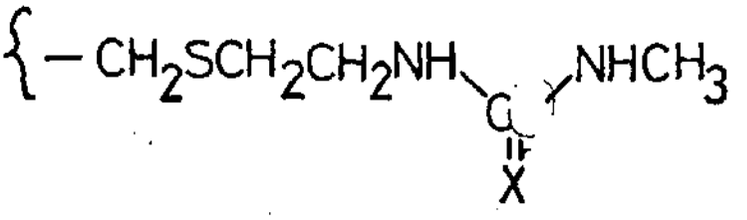
2.1.1.4 Modification of Methyl-thioethyl chain

For optimal activity, the terminal N-group should be separated by the equivalent of a

4-carbon chain. A shorter chain drastically reduces antagonistic activity. Replacement of the sulfur atom by a further methylene group led to a compound which possessed one-third of activity. Attachment of the sulfur atom directly on to the furan ring resulted in an isomer with about 1/10th the potency of the parent compound.

2.1.1.5 Modification of the neutral end group

The terminal N-group should be a polar non-basic substituent for maximal antagonist activity:



For ranitidine, X = nitroethene gave the most potent member of the series as compared to the imidazole derivatives.

2.1.1.6 Ring substitution pattern

Ring substitution pattern in the ranitidine molecule is of the 2,5-disubstituted type. The five other possible isomers all gave compounds that were substantially less active than the original compound.

2.1.2 Pharmacokinetics

Ranitidine is rapidly and well absorbed after oral administration. Peak concentration in plasma occurs 2 hours after per oral administration. The bioavailability of ranitidine following oral dose is about 52% ± 11 due to extensive first-pass metabolism and it increases in patients with liver cirrhosis (Garg et al, 1983; Roberts, 1984). The elimination half-life is 2.1 hrs. ± 0.2 and is significantly prolonged in the aged, in uremia and in patients with liver dysfunction. Ranitidine is weakly bound (15% ± 3) to plasma proteins. IC_{50} for inhibition of gastric secretion is 100 ug/ml. A small proportion of ranitidine is metabolised in the liver to the N-oxide, the S-oxide and des methyl ranitidine but most is excreted unchanged (69% ± 6) and this is decreased in uremia. Volume of distribution is 1.8l/kg and is not affected by cirrhosis. Clearance (10.4ml. min^{-1}/kg^{-1}) is decreased in uremia and in the aged. Ranitidine may undergo enterohepatic recycling. It crosses the placenta and is excreted in breast milk. It does not readily cross the blood-brain barrier and is removable by both peritoneal and haemo-dialysis.

2.1.3 Pharmacological Actions

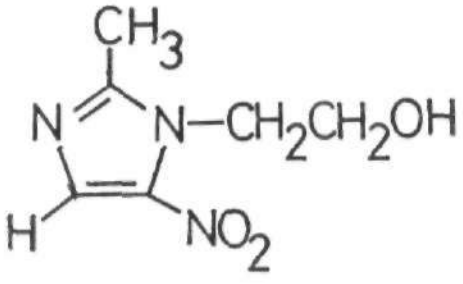
Ranitidine is a histamine H_2-receptor antagonist. It inhibits competitively the interaction of histamine with H_2-receptors and has little or no effect on H_1 or other receptors. Although H_2 receptors are present in numerous tissues, including vascular and bronchial smooth muscle, H_2-antagonists interfere remarkably little with physiological functions other than gastric secretion. Nevertheless, they measurably inhibit effects on the cardiovascular and other systems that are elicited through H_2-receptors by exogenous or endogenous histamine (Brodgen et al., 1982).

H_2-antagonists inhibit gastric secretion elicited by histamine and other H_2-agonists in a dose-dependent manner. The H_2-antagonists also inhibit acid secretion elicited by gastrin and, to a lesser extent, by muscarinic agonists. They also inhibit basal (fasting) and nocturnal acid secretion and that stimulated by food, sham feeding, fundic distention and various pharmacological agents. The H_2-antagonists reduce both the volume of gastric juice secreted and it's H^+ concentration. The output of pepsin, which is secreted by the chief cells of gastric glands (mainly under cholinergic control) generally falls in parallel with the reduction in volume of gastric juice. Secretion of intrinsic

factor is also reduced. The concentration of gastrin is not significantly altered under fasting conditions.

2.2 Metronidazole

Metronidazole is 2-(2-methyl-5-nitroimidazo-1-yl) ethanol. It is a white to pale yellow crystalline powder or crystals, odourless, or with a slight odour. It darkens on exposure to light. It is slightly soluble in water, alcohol, chloroform and ether. The injection has a pH of 4.5 to 7.0.



METRONIDAZOLE

Molecular weight: 171.2

2.2.1 Pharmacokinetics

Metronidazole is readily absorbed following oral administration by mouth and bioavailability approaches 100%. Peak plasma concentrations of 10 ug/ml have been reported an average of 1 hour after an oral dose of 500mg (Ralph, 1983). However, reported metronidazole concentrations can vary considerably depending on the type of assay used. Absorption may be delayed but not overall reduced by co-administration with food.

Metronidazole is widely distributed and its volume of distribution approximates total body water (1.1 ± 0.4 l/kg). It appears in most body tissues and fluids including bile, bone, breast, milk, cerebral abscesses, cerebro-spinal fluid, liver and liver abscesses, saliva, seminal fluid and vaginal secretions, and achieves concentration similar to plasma. It also crosses placenta and enters foetal circulation. About 10% is bound to plasma proteins. The effective plasma concentration is 3-6ug/ml.

Both unchanged metronidazole and several metabolites are excreted in various proportions in the urine after oral-administration. The liver is the main site of metabolism and this accounts for over 50% of the systemic clearance (1.3 ± 0.3 ml. $min^{-1}.kg^{-1}$) of metronidazole. It is metabolised by side-chain oxidation and glucuronide formation. The two principal oxidative metabolites result from oxidation of side chains and both have anti-trichomonal activity (Webster, 1990). Small amounts of reduced metabolites, acetamide and N-(2-hydroxyethyl) oxamic acid have also been detected in urine and are formed by the gut flora (Koch et al., 1981). The urine of some patients may be reddish-brown due to the presence of unidentified pigments derived from the drug.

The plasma elimination half-life of metronidazole is about 8 hours, that of the hydroxymetabolite is slightly longer. The half-life of metronidazole is reported to be longer in neonates and patients with liver disease; that of the metabolite is prolonged in patients with renal failure. The majority of a dose of metronidazole is excreted in the urine mainly as metabolites with less than 10% excreted unchanged. A small amount appears in the faeces. Depending on the assay method employed, up to 80% of a dose has been recovered in the urine within 48 hours.

2.2.2 Pharmacological Actions

Metronidazole is directly active against anaerobic protozoa and anaerobic bacteria. It also has a sensitizing effect on hypoxic tumor cells. It is active against several protozoa including Balantidium coli, Entamoeba histolytica, Giardia lamblia and Trichomonas vaginalis. Metronidazole also displays activity against all anaerobic cocci and both anaerobic gram-negative bacilli, including Bacterioides spp and anaerobic spore-forming gram-positive bacilli. Other effects of nitro-imidazoles include suppression of cellular immunity and mutagenesis.

The mechanism of action of nitroimidazoles is thought to involve interference with DNA by a metabolite in which the nitro group accepts electrons from the electron-transport proteins such as flavo-proteins and diverts them from normal energy-yielding pathways. It is thought that these chemically reactive reduced forms of the drug lead to the formation of cytotoxic products that destroy the cell (Webster, 1990).

2.2.3 Metronidazole-drug interactions

When given in conjunction with alcohol, metronidazole may provoke a disulfiram-like reaction in some individuals. Although metronidazole has no effect on the elimination of antipyrine in healthy patients (Jensen and Gugler, 1987) and, by inference, has little effect on hepatic drug-metabolising enzymes, it enhances the anticoagulant effect of warfarin through selective inhibition of the metabolism of it's S-isomer (Dean and Talbert, 1984) and may impair the clearance of phenytoin (Picard, 1983). There is some evidence that phenytoin might accelerate the metabolism of metronidazole (Wheeler, 1978). Plasma concentrations of metronidazole are decreased by the concommitant administration of phenobarbitone with a consequent reduction in the effectiveness of metronidazole (Mead et al., 1982). Cimetidine has increased plasma

concentration of metronidazole and might increase the risk of neurological side effects (Gugler and Jensen, 1983).

2.3 Ranitidine - Drug Interactions

All agents that inhibit gastric acid secretion have the potential to alter the bioavailability and rate and extent of absorption of certain drugs (weak acids and bases) secondary to changes in gastric pH. However, since the rise in gastric pH is not immediate after administration of ranitidine (and other H_2-antagonists), this type of interaction might be avoided for concommitantly administered rapidly absorbed drugs. For instance, cimetidine has been shown to increase the absorption of lorazepam, diazepam and furosemide and diminish the absorption of indomethacin and chlorpromazine during cimetidine treatment (Powell and Donn, 1984). Cimetidine also greatly reduces the absorption of ketoconazole, probably through effects on pH-dependent dissolution (Somogyi and Gugler, 1982). Ranitidine has been shown to increase the oral bioavailability and central depressant effects of midazolam and the mechanism is thought to involve pH-dependent changes in solubility caused by ring closure (Elwood, et al., 1983). Ranitidine has also been shown to significantly

delay gastric emptying of solids (Scarpignato et al., 1982). Even though such an effect is unlikely to be of any clinical significance, the absorption rate of some drugs may be decreased.

An increase or decrease in gastro-intestinal blood flow should result in a similar change in drug absorption. Although it has been suggested that H_2-antagonists may influence gastro-intestinal blood flow, some studies indicate that such an effect is unlikely (Daneshment et al., 1984).

Because most drugs are absorbed in the intestine where pH is not significantly altered by H_2-antagonists, altered ionisation secondary to pH changes caused by these drugs is unlikely to affect absorption. However, increased gastric pH resulting from H_2-antagonist administration may accelerate dissolution of weak acids or decelerate dissolution of weak bases. By this mechanism, cimetidine has generally been shown to decrease the absorption of tetracycline (weak base) from tablets and capsules by 30 to 40 percent but having no effect on tetracycline absorption from solution (Fischer et al., 1980). It would be expected that the enteric coating of tablets would dissolve more rapidly where gastric pH is elevated. However, the absorption of enteric coated prednisolone (Morrison et al., 1980) and enteric coated aspirin

(Paton et al., 1983) are not influenced by cimetidine. The inability to detect or fully evaluate changes in absorption may be attributed to the study design. Since H_2-antagonists must reach the systemic circulation prior to increasing gastric pH, optimal study conditions to determine the possibility of an absorption interaction would be to administer the H_2-antagonist at least an hour before the test drug. If the affected drug is administered only by the oral route, it may be difficult to differentiate between a change in bioavailability or a decrease in drug elimination clearance. For this reason, absolute proof of mechanism would necessitate administering the affected drug by the oral and intravenous routes in the presence or absence of the H_2-antagonist (Powell and Donn, 1984).

Ranitidine undergoes extensive active renal tubular secretion. It competes with and inhibits the active secretion of procainamide leading to decreased clearance and raised levels of the drug (Christian et al., 1984).

Ranitidine is a second generation H_2-antagonist and was developed after cimetidine. Less than two years after the release of cimetidine in 1976, first reports appeared of interaction between it and warfarin (Flind, 1978). It is now known that

cimetidine inhibits the elimination of more than 25 drugs in man, and the list is certain to increase due to the basic mechanisms of the interaction. Owing to the cimetidine experience, drug interaction studies on subsequent H_2-antagonists have been integral to the development of subsequent H_2-antagonists. The most important of these is ranitidine which has now superseded cimetidine as the most widely used H_2-antagonist all over the world.

Ranitidine is structurally dissimilar from cimetidine in that it has a furan, as opposed to an imidazole ring and a different side chain. Wilkinson et al. (1974) have noted that substituted imidazole compounds powerfully inhibit cytochrome P-450 following either competitive or mixed kinetics, depending on the inhibitor and substrate. Ranitidine has been shown to bind liver microsomes and cytochrome P-450 but with an affinity one-tenth that found for cimetidine (Rendic et al., 1983). Bell and colleagues (1981) have reported an interesting study that compared the inhibitory effect of cimetidine, ranitidine and an investigational molecule with the furan ring of ranitidine and the cyanoguanidine side-chain of cimetidine. When comparing the effects of these drugs on liver microsome binding and prolongation of phenobarbital sleep-time, the hybrid molecule

was intermediate between cimetidine which strongly bound to the microsomes and prolonged sleeping time and ranitidine which was weakly bound and had no effect on sleeping time. They suggested that the ability of cimetidine to inhibit drug metabolism is not solely determined by the imidazole ring and lipophilicity may be an important predicator.

Since ranitidine binds less strongly to cytochrome P-450, ranitidine has fewer significant effects on the metabolism of other concommitantly administered drugs. Several drugs which are known to interact with cimetidine have been found not to interact significantly with ranitidine (see tables). However, a few significant pharmacokinetic interactions have been reported and the possibility that these might be clinically relevant should be borne in mind.

Although ranitidine does not impair drug metabolism at doses that are several fold higher than cimetidine, a very large dose (120 mg/kg) did decrease the elimination rate of aminopyrine in rat (Speeg et al., 1982). Jones et al (1985) have studied the effect of low (50 ug) and high (1mg) doses of cimetidine and ranitidine on the first-pass extraction of propranolol in rat isolated perfused liver. Both

Table 2.3.2: Influence of Ranitidine on Systemic Clearance of High Hepatic Extraction Drugs.

DRUG	PERCENT CHANGE IN CLEARANCE		REFERENCE
Chlormethiazole	-9	(NS)	Mashford et al. (1983)
Indocyamine green	-4	(NS)	Donn et al. (1984)
	-18		Feely and Guy (1982)
	-24		Garg et al. (1982)
	-18		Dunk et al. (1983)
	+2	(NS)	Mashford et al. (1983)
Lidocaine	-1	(NS)	Feely and Guy (1983)
	+5	(NS)	Jackson et al. (1983)
Metoprolol		(NS)	Kelly et al. (1983)
Propranolol		(NS)	Patel and Weerasuriya (1983)

NS = Statistically not significant.

Table 2.3.3: Influence of Ranitidine on Oral Clearance of High Hepatic Extraction Drugs.

DRUG	PERCENT CHANGE IN CLEARANCE		REFERENCE
Chlormethiazole	1	(NS)	Mashford et al. (1983)
	-36		Spahn et al. (1983)
	-27		Kelly et al. (1983)
	-19	(NS)	Kelly et al. (1983)
Nifedipine	-7	(NS)	Kirch et al. (1983)
Propranolol	-7	(NS)	Heagerty et al. (1982)
	-5	(NS)	Donn et al. (1984)

NS = Not statistically significant

low and high cimetidine increased area under the perfusate propranolol concentration-time curve to five fold. Low dose of ranitidine did not alter propranolol AUC. High doses of ranitidine did increase the AUC to the same extent as cimetidine. They concluded that ranitidine has the propensity for microsomal inhibition, but one which is unlikely to be manifest at therapeutic doses. Heagertly et al. (1982) had already reported that ranitidine 150mg b.d. for 2 weeks failed to affect the pharmacokinetics of a single oral dose of propranolol 80mg.

Ranitidine has been shown to significantly reduce the clearance of warfarin single dose (Desmond et al., 1984) presumably due to inhibition of its metabolism. Two other studies found that ranitidine has no effect on warfarin plasma levels (Steady State;Serlin et al., 1981) or single dose (Desmond et al., 1984). The clearance of procainamide and N-acetylprocainamide are significantly reduced by ranitidine (due to inhibition of renal excretion). In addition, the absorption of midazolam appears to be increased by ranitidine, resulting in significantly increased bioavailability, while the absorption of protein-bound cobalamin appears to be decreased (Kirch et al., 1984).

Ranitidine has been reported to induce toxicity of and inhibit clearance of theophylline. Roy et al. (1988) observed the development of clinical and chemical toxicity in three elderly patients (ages 65, 72 and 72 respectively) receiving oral theophylline therapy (250 or 300mg bid) for chromic obstructive pulmonary disease after the administration of 150mg ranitidine for peptic ulcer disease. All three patients had been receiving theophylline for prolonged periods (5, 13 or 20 years) without suffering adverse effects, but when ranitidine was given all the three patients showed symptoms of theophylline toxicity within 36 hrs to one week of starting ranitidine. Serum theophylline levels after ranitidine increased three-fold and theophylline clearance rate decreased by 66%. These symptoms ceased within three days when dosage of theophylline was stopped. However, there is marked inter-individual differences in theophylline metabolism after ranitidine treatment and earlier studies have shown that ranitidine would be unlikely to affect the metabolism of theophylline, but these were carried out with i.v. aminophylline on healthy subjects (Breen et al., 1982; Kelly et al., 1986).

Ranitidine has been found to alter lignocaine kinetics (Robson et al., 1985) in healthy volunteers.

A previous study (Feely et al., 1983) has shown that ranitidine had no effect on kinetics of lignocaine. It has also been found not to have any effect on the pharmacokinetics of cifenline in healthy volunteers (Massarella et al., 1991).

Mean plasma bupivacaine concentration was reported to be higher in 9 women who received ranitidine 150mg by mouth prior to epidural anaesthesia with bupivacaine than in 7 who were not given ranitidine (Wilson et al., 1986). In another study in healthy volunteers, pretreatment with 2 doses of ranitidine 150mg by mouth before an i.v. infusion of bupivacaine 50mg caused a small and insignificant decrease in clearance of bupivacaine while pretreatment with cimetidine 400mg by mouth 13 and 3 hours before the iv infusion of bupivacaine decrease clearance of bupivacaine significantly. Neither drug caused a significant increase in plasma half-life of bupivacaine (Noble et al., 1987).

Administration of cimetidine caused an increase in plasma concentrations of diltiazem in 6 subjects given a single dose of diltiazem 60mg by mouth. Ranitidine produced a similar though less marked effect (Winship et al., 1985). Ranitidine has also been reported not to affect the metabolism of, and

clearance of nifedipine (Schwartz et al., 1988) and tolbutamide (Cate et al., 1986).

The pharmacokinetic and metabolic characteristics of metoprolol are very similar to those of propranolol (Regardh et al., 1980). However, studies on the effects of H_2-antagonists on metoprolol disposition have produced confusing and conflicting results. Kelly et al. (1985) studied the effect of ranitidine on metoprolol disposition. In a single dose study, ranitidine with metoprolol increased peak concentrations of metoprolol significantly (99 $ngml^{-1}$ pre-ranitidine to 158 $ngml^{-1}$ with ranitidine; $P < 0.05$) but half-life and clearance remained unaltered. In the chronic dose study, ranitidine was found to have no effect on the pharmacokinetics of metoprolol. The single dose study suggests that some interaction of unknown nature takes place.

Concurrent administration of ranitidine and pethidine in healthy volunteers resulted in no significant alterations in pethidine elimination rate constant, volume of distribution at steady state, total body clearance or 24-hour urinary excretion. There was also no alteration of oxidation of pethidine to nor-pethidine (Guay et al., 1985).

easily detected. Pharmacokinetic interactions that are significant and detectable require the affected drug to have a narrow therapeutic index and produce more than a 20 to 25 percent change in drug absorption, distribution or elimination. This degree of change is necessary to overcome normal intra-patient variation in drug disposition (Upton et al., 1982). For example, the normal intra-patient variation in theophylline total body clearance and volume of distribution is 15 percent (Powell et al, 1978). Although there may be exceptions to this guideline, it may be useful to remember when evaluating the clinical importance of drug interactions. Drug interactions are generally important only in transition times when the interacting drug (such as cimetidine) is started, stopped or the dosage is changed. Anticipation of these occasions by the clinician can help to prevent therapeutic failure.

One of the more perplexing problems in drug interactions is determination of the clinical significance in a specific patient. The specific conditions under which the two interacting drugs are given are frequently more important than is the supposed significance of the drug interaction per se. These conditions include both the clinical status of

the patient and drug administration factors. The lack of appreciation of the importance of these variables has led to more confusion in the area of drug interactions than any other single factor (Hansten, 1979). The many variables that may influence the activity of a drug and it's ability to interact with other agents may be divided into two-patient and drug administration factors.

2.4.1 Patient Factors

(1) Disease States: Patients with a variety of disorders (such as diabetes, hyperthyroidism etc) may respond differently to drugs than the patient not so affected. Accordingly, these factors may predispose a patient or protect him from the adverse effects of an interaction.

(2) Renal function: A decrease in glomerular filtration rate and/or impairment of renal tubular function may result in increased serum levels of drugs with a resultant increase in the likelihood of adverse drug interactions. Further more, patients with uremia may have altered drug binding to plasma proteins (e.g. phenytoin) and the albuminuria in nephrotic patients may decrease serum albumin

sufficiently to predispose patients to certain interactions.

(3) Hepatic function: A marked decrease in hepatic function could theoritically result in impaired drug metabolism with resultant increased blood levels and likelihood of adverse drug interactions. Also some liver diseases (e.g. cirrhosis) may result in albuminemia of significant magnitude to affect drug action.

(4) Serum protein levels: Hypoalbuminemia probably increases the severity of drug interactions which involve protein binding displacements.

(5) Dietary factors: Food can affect the gastro-intestinal absorption of some drugs thus altering blood levels and interactive potential. In addition, dietary insufficiencies or excesses may predispose to the development of certain adverse drug interactions.

(6) Environmental factors: Drug action, and thus drug interactions, may be affected by a variety of environmental influences such as insecticides, air pollution, and smoking.

(7) Genetic factors: Studies with various drugs during the past several years have provided

ample evidence that an individual's genetic make up is quite important in determining drug disposition. In some cases, the genetic influence is fully defined (e.g. acetylator phenotype on the interaction between isoniazid and phenytoin). In most cases, however, it is not possible to predict, based on genetic considerations, which patient will be predisposed to a given adverse drug interaction.

(8) Age: Older patients are more likely to manifest adverse drug interactions. This is due to a variety of factors some of which have already been discussed (e.g. decreased kidney function, multiple diseases etc).

2.4.2. Drug Administraction Factors

(1) Sequence of administration: The order in which the two interacting drugs are given may considerably affect clinical outcome. For example, the administration of a potent inducer like rifampicin after a patient has been stabilised on warfarin will cause increased rate of elimination of warfarin with resultant failure of anticoagulant therapy. On the other hand,

the administration of an inhibitor like cimetidine after a patient has been stabilised on warfarin will result in enhanced warfarin levels and risk of haemorrhage.

(2) Route of administration: Some interactions occur only when both drugs are given orally (antacids and tetracycline) while other interactions may be more severe when one or both drugs are given by the parenteral route.

(3) Time of administration: Some gastrointestinal absorption interactions are more likely to be important when the time between administration of the interacting drugs is short.

(4) Duration of therapy: Seldom do the manifestation of adverse drug interactions occur immediately. Usually, some period of concomittant administration which may vary from hours to many weeks of therapy is required before manifestation.

(5) Dose of drugs: In general, adverse drug interactions are more likely to be significant with larger doses of one or both drugs (e.g. cimetidine inhibition of cytochrome P-450 is dose-dependent; Powell and Donn, 1984).

Some drugs (phenytoin) have dose-dependent kinetics and some interactions involving these drugs may be important only when large doses are used.

(6) Dosage form: A few differences have been noted for drug interactions when comparing tablets versus liquids; sustained release tablets versus standard tablets and enteric coated versus standard tablets e.g. cimetidine decreases absorption of tetracycline capsules but not tetracycline in solution.

(7) Concurrent use of non-prescription drugs (such as analgesics, antacids, expectorants, etc) by the patient.

With the availability of drugs that are getting more and more potent and the widespread practise of poly-pharmacy, a very large number of patients may be at risk of having potentially harmful drug interactions. However, the limitations of the application of pharmacokinetic parameters, which have frequently been derived from single dose studies in a small number of healthy subjects, to individual patients with different pathological conditions must be recognised. It must also be recognised that

drug-nondrug interactions are often of greater clinical importance than drug-drug interactions.

2.5 Objectives of the Present Study

Due to numerous reported cases of drug-drug interactions with cimetidine, drug interaction studies have become integral in the development of subsequent histamine H_2-receptor antagonists. Ranitidine is the most important of these and it has now overtaken cimetidine as the most widely prescribed H_2-antagonist.

Metronidazole is widely used all over the world and it is the drug of choice in amoebiasis among others which is mainly an affliction of the tropics. The study is aimed at finding out the nature and extent of the possible interaction, if any, of ranitidine with metronidazole in healthy volunteers.

2.6 Methodology

The study will be carried out in two phases. Phase I will involve the administration of 400mg of metronidazole after an overnight fast to healthy volunteers. Plasma samples will then be removed after specified time intervals and these will be analysed using High Performance Liquid Chromatography

techniques (Chapter 3). The pharmacokinetic profile of the drug will then be worked out accordingly.

After a wash-out period of 2 weeks, the second phase will involve administration of ranitidine 150mg after an overnight fast followed after an hour by administration of 400mg of metronidazole.

The pharmacokinetic parameters will be worked out using the computer program GRAPHPAD (Chapter 4). Data generated will then be analysed statistically to assess the significance and importance of such observation to therapy.

CHAPTER THREE

MATERIALS AND METHODS

3.1 DRUGS

(i) Metronidazole: FLAGYL^{R}

Manufacturer: May and Baker Nig. PLC.

Batch Number: IC 337

Manufacturing date: September 1988

Expiry date: September 1993

Strength: 200mg.

(ii) Ranitidine: USP

Manufacturer: APOTEX Inc, Toronto, Canada.

Batch Number: L 54020

Expiry date: September 1993.

3.2 MATERIALS

3.2.1 Glassware

- Extraction tubes, Pyrex, England.
- Pippettes: 0.02ml, 0.1ml, 1ml, 5ml, 10ml, Pyrex, England.
- Measuring cylinders: 5ml, 10ml, 100ml, 200ml, 500ml, Pyrex, England.
- Conical flasks: 200ml, 250ml, 500ml, Pyrex, England.
- Volumetric flasks: 10ml, 25ml, 50ml, 100ml, 200ml, 250ml, Pyrex, England.

- Beakers: 200mls, 250ml, Pyrex, England.
- Bijou bottles: 20ml.
- Syringes and needles: 5ml, 20ml.
- Cannulas: 20G, 18G.
- Heparinised sample bottles: 5ml plastic stirring rod; glass.

3.2.2 Equipment

- Vacuum pump EB3A, Edwards, England.
- Flask shaker, Gallenkamp, England.
- Centrifuge, Junior, Gallenkamp, England.
- Auto-vortex mixer, Stuart, England.
- Ultra-sonicator.
- Pippetteman Gilson, France.
- Hot Air Oven BS Size One, Gallenkamp, England.
- Solvent Filters, 0.45um (Waters).
- Solvent Filtration kit, Millipore, Waters.
- pH meter, Kent Model 7060, England.
- HPLC Priming Syringe, Hamilton.
- HPLC Injection Syringe, Hamilton.
- Electronic Balance, Mettler AE 240.
- Refrigerator, Premier, Thermocool, Nigeria.
- Disintegration-Rate Study Apparatus, Erweka, England.
- Dissolution-Rate Study Apparatus, Erweka, England.

3.3 REAGENTS AND STANDARD SAMPLES

- Methanol AR, M & B, Rhone-Poulenc, France.
- Acetonitrile AR, BDH Chemicals, England.
- Potassium Di-hydrogen Phosphate Buffer Pro-analysii, E-Merck, Germany.
- Phosphoric acid, BDH Chemicals, England.
- Filtered Distilled Water.
- Metronidazole Powder - obtained as Gift Sample from May and Baker, Dagenham, England.
- Tinidazole: Extracted from FASIGYN^{R} brand Pfizer, Lagos, Batch Number: 12493003.

  Manufactured: 8/91

  Expiry: 8/94

  Strength: 500mg.

3.4 SOLVENT SYSTEM

A buffer system consisting of 0.01M KH_2PO_4 : Acetonitrile : Methanol in the ratio 93 : 3 : 4 respectively was used to analyse metronidazole in plasma. pH was adjusted (from 4.76 to 2.5) with 3M phosphoric acid. The solvent system was filtered through Waters filtration kit with 0.45 µm filter and further degassed by ultrasonication for 10 minutes.

3.5 IN-VITRO STUDIES

3.5.1 ASSAY

The B.P. 1988 specifies that at 277nm, the A 1% 1cm of metronidazole in 0.1N HCl is 380. Therefore at a concentration of 0.001% w/v the absorbance of metronidazole in 0.1N HCl will be 0.38. 1.65275mg of the powdered tablets (average weight of 20 tablets is 6.611mg containing 4.00gm of metronidazole) was dissolved in 100 ml of 0.1N HCl and filtered to give a 0.001% w/v solution. Six replicate solutions were prepared from the sample and the absorbance was determined using a UV spectrophotometer at 277nm using 0.1N HCl as blank. Percentage content is given by the equation:

$$\frac{A}{0.380} \times 100$$

Where A = absorbance of sample and 0.380 = absorbance of a 0.001% w/v solution.

3.5.2 Disintegration Tests

These were carried out as specified by BP 1988 using an Erweka disintegration apparatus (see appendix).

Procedure: One tablet each was placed in the tubes of the basket and a disc was added to each tube. The assembly was suspended in the beaker containing distilled water maintained at $37^{o}C \pm 0.5^{o}C$ and the apparatus was operated for 15 minutes. The test was carried out for five tablets.

3.5.3 Dissolution Tests

The Rotary Basket Method as described in the B.P. 1988 was used.

Procedure: One tablet of metronidazole was placed in the basket and placed in the round-bottom flask containing 1,000mls of 0.1N HCl at $37.5^{o}C \pm 0.5^{o}C$. The stirrer was set at 100 r.p.m. 10 ml samples of the dissolution medium were withdrawn at 5, 10, 15, 30, 45 and 60 minutes. These were at the same time replaced with 10mls of 0.1N HCl previously maintained at $37.5^{o}C \pm 0.5^{o}C$. Samples were withdrawn from a point midway between the basket wall and the side of the vessel to ensure that the normal pattern of flow of the dissolution medium was not disturbed. The samples were filtered using Whatman filter paper size 4 , the first 1ml was rejected and the samples assayed by reference to a standard calibration curve which was prepared by dissolving standard metronidazole

powder in 0.1N HCl to give various concentrations and absorbance measured at 277nm. The whole exercise was carried out for six tablets.

Cumulative concentration at a given time was calculated using the formula of Wurster and Taylor (1965):

$$Cn = C_n \text{ (measured)} + \frac{X}{1,000} \sum_{S=1}^{n=1} C_n \text{ measured}$$

Where C_n (measured) = the spectrophotometrically determined concentration.

C_n = Concentration of n^{th} sampling expected in the medium if previous sample had not been removed.

X = Volume of sample removed at each time withdrawal was made.

1,000 = Volume of dissolution medium in millilitres.

3.5.4 Preparation of Standard Samples

3.5.4.1 Preparation of metronidazole solution

A stock solution of 1mg/ml metronidazole was made in acetonitrile. Working concentrations of 50 ug/ml were prepared by serial dilutions with acetonitrile.

3.5.4.2 Preparation of internal standard (Tinidazole)

Two tablets (strength of each tablet = 500mg) were powdered with mortar and pestle, dissolved in acetonitrile and filtered. A stock solution of 1mg/ml was prepared. Working concentrations of 200 ug/ml were prepared by serial dilution with acetonitrile.

3.5.4.3 Preparation of potassium di-hydrogen phosphate buffer

0.01M solution of potassium di-hydrogen phosphate buffer was prepared by dissolving 1.3609gm of KH_2PO_4 (mwt 136.09) in one litre of filtered distilled water.

3.6 IN-VIVO STUDIES

3.6.1 Protocols of Study

Four healthy volunteers (age 28 - 35 years; weight 50-65kg) were involved in the study. Volunteers were healthy (normal laboratory values) free from liver and kidney disease, non-smoking and non-drinking.

The cross-over study was divided into two phases. In the first phase, 400mg MDZ was administered to each volunteer with 150ml of water after

an overnight fast which was continued for 4 hours after commencement of the study. This was followed by a wash-out period of three weeks. In the second phase, 150mg of ranitidine was administered with 100ml of water. After one hour, 400mg of metronidazole was administered with 150ml of water.

Blood sampling was done via a cubitally-placed indwelling cannula. Blood samples (5ml.) were withdrawn prior to each dose and at 0.5, 1, 2, 3, 4, 6, 8, 9 and 24 hours after the dose. The samples were collected in heparinised tubes, centrifuged at 1,000 gm for 10 minutes and the plasma was harvested into Bijou bottles and kept at -10°C until analysis.

3.6.2 Analysis

3.6.2.1 Extraction

The extraction method of Kaye et al (1979) was adopted. 0.5ml of plasma was placed in a pyrex extraction tube. 0.5ml of acetonitrile containing the internal standard (Tinidazole 200 ug/ml) was then added. The mixture was shaken for 10 minutes on a Gallenkamp flask shaker followed by centrifugation for 10 minutes at 4,000gm. 15 µL of the clear supernatant was injected into the HPLC.

3.6.2.2 Calibration curve

Calibration curve based on peak-height ratio was prepared by spiking drug-free plasma with standard metronidazole solution and internal standard (Tinidazole) to give a concentration range of 5 to 40 ug. The ratios of the peak heights of metronidazole versus the internal standard were plotted against the standard concentrations. A linear regression calibration curve was obtained and the co-efficient of linear regression of the slope was determined. The calibration curve was used to determine the concentrations of metronidazole in individual plasma samples using a SHARP® EL-512H Scientific calculator.

3.6.2.3 Instrument

A Waters Model 204 Liquid Chromatograph equipped with Model 441 fixed-wavelength U.V. detector fitted with 280nm filter and U6K universal septumless injector was used. Analysis was carried out on a radial-pak^{R} reverse-phase colum (8x100mm) packed with 10 um reverse-phase C_{18} support (Waters part No. 85721) placed in a compressed Z-module-compartment. The output of the detector was recorded on an SE 120 BBC Goerz metrawatt recorder.

Conditions of analysis

Solvent system	:	KH_2PO_4 (0.01M) : Acetonitrile : Methanol (93:3:4).
pH	:	2.5 (adjusted with 3M ortho-phosphoric acid).
Flow rate	:	2.0ml min^{-1}.
Pressure	:	2,000 psi.
Sensitivity	:	0.05 a.u.f.s.
Internal standard	:	Tinidazole 200 ug/ml in acetonitrile.
Sample size	:	15 uL
Chart speed	:	0.25cm. min^{-1}
Filter	:	280nm.

3.6.2.4 Data handling

The values for concentration of metronidazole in individual plasma samples were used with the computer program GRAPHPAD® to generate the pharmacokinetic parameters $t_{\frac{1}{2}}$ absorption, $t_{\frac{1}{2}}$ elimination, elimination rate constant, absorption rate constant and AUC 0 → 24. The remaining pharmacokinetic parameters were calculated using the following formulae (Ritschel, 1980):

(i) $AUC_{0 \rightarrow \infty}$ = AUC $0 \rightarrow 24$ + AUC $24 \rightarrow \infty$

$$\text{AUC } 24 \rightarrow \infty = \frac{\text{Conc}_{24}}{\text{Kel}}$$

(ii) Clearance

$$\text{Total Body Clearance} = \frac{D}{AUC} \text{ ml. min.}$$

Where D = Dose administered assuming 100% bioavailability.

(iii) Volume of Distribution :

$$\frac{\text{Clearance TBC}}{\text{Kel}}$$

(iv) $$\text{Lag Time} = \frac{\ln \frac{A}{B}}{\text{Kabs} - \text{Kel}}$$

Where A = Y intercept (Co)

and B = Intercept of line of residuals.

All pharmacokinetic data obtained were compared and the statistical significance of the differences determined using the student t-test of paired data. Values of $P < 0.05$ were considered as significant and $P > 0.05$ considered insignificant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 IN-VITRO STUDIES

4.1.1 Assay

Linear calibration curve with good correlation co-efficient ($r = 0.9995$) of MDZ in 0.1N HCl using U.V. spectrophotometer ($\lambda_{max} = 277nm$) is shown in Figure 4.1.1.

The content of MDZ in six tablets was determined (Table 4.1.1). The acceptable range (B.P. 1988) is between 95% - 105%. The results are within acceptable limits.

Table 4.1.1: Assay of MDZ tablets using U.V. spectrophotometric method of analysis.

Tablet No.	Percent content	Comment
1	98.0	Satisfactory
2	98.5	"
3	99.0	"
4	97.5	"
5	101.0	"
6	100.5	"
Mean ± SEM	99.1 ± 0.52	

SEM = Standard Error of the Mean.

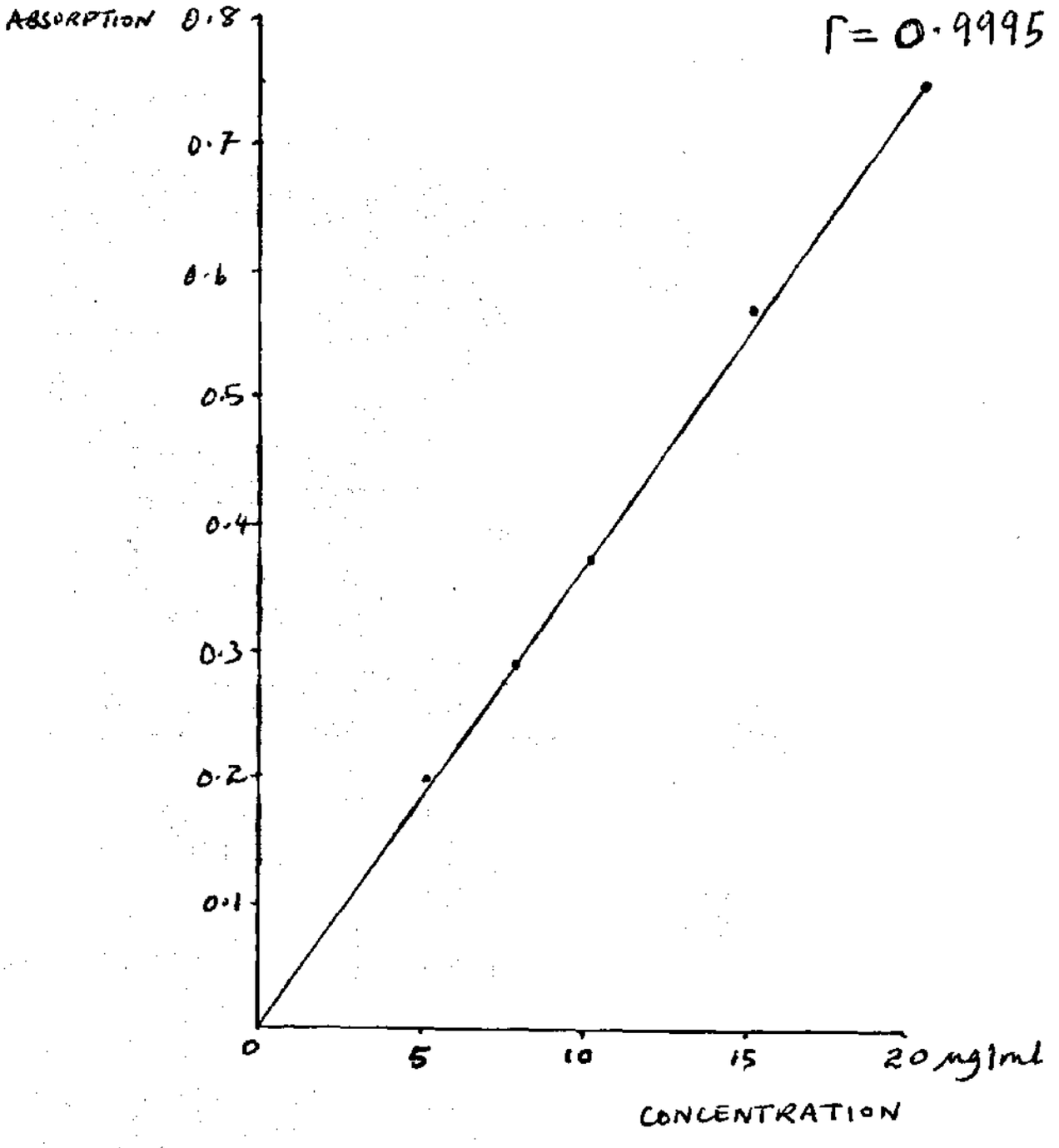


Fig. 4.1.1: Calibration curve for MDZ in 0.1N HCl
λ max = 277nm.

4.1.2 DISINTEGRATION

Results obtained from the disintegration tests are shown in Table 4.1.2. The official B.P. 1988 time-limit for tablet disintegration is 15 minutes. All the tablets tested passed the official requirements for tablet disintegration.

Table 4.1.2: Disintegration times for MDZ tablets.

Tablet No.	Disintegration Time (mins)	Comments
1	7.0	Satisfactory
2	6.5	"
3	6.5	"
4	7.0	"
5	7.0	"
SEM		
Mean ± SEM	6.8 ± 0.11	

SEM = Standard Error of the Mean.

4.1.3 DISSOLUTION

Table 4.1.3 shows the mean dissolution profile of six tablets of MDZ in 0.1N HCl. Total release of active ingredient took place within 30 minutes. The B.P. 1988 requirements for dissolution rate test is that not less than 70% of the stated amount of active ingredient is in solution at the end of 45 minutes

for any dosage form unless otherwise stated in the monograph. The results obtained complied with the official specification.

Table 4.1.3: In-Vitro Dissolution Profile of MDZ tablets in 0.1N HCl using the B.P. 1988 Rotating Basket Method.

Time (Mins)	Cumulative percentage released MEAN ± SEM
5	8.55 ± 0.13
10	19.85 ± 0.45
15	50.30 ± 0.30
30	99.50 ± 0.31
45	99.9 ± 0.22
60	99.5 ± 1.15

SEM = Standard Error of the Mean.
n = 6

4.2 IN VIVO STUDIES

4.2.1 Solvent System

The high-performance liquid chromatograms of blank plasma (with internal standard) and plasma of a volunteer 2 hours after dosing with 400mg MDZ are shown in Figures 4.2.1 and 4.2.2 respectively.

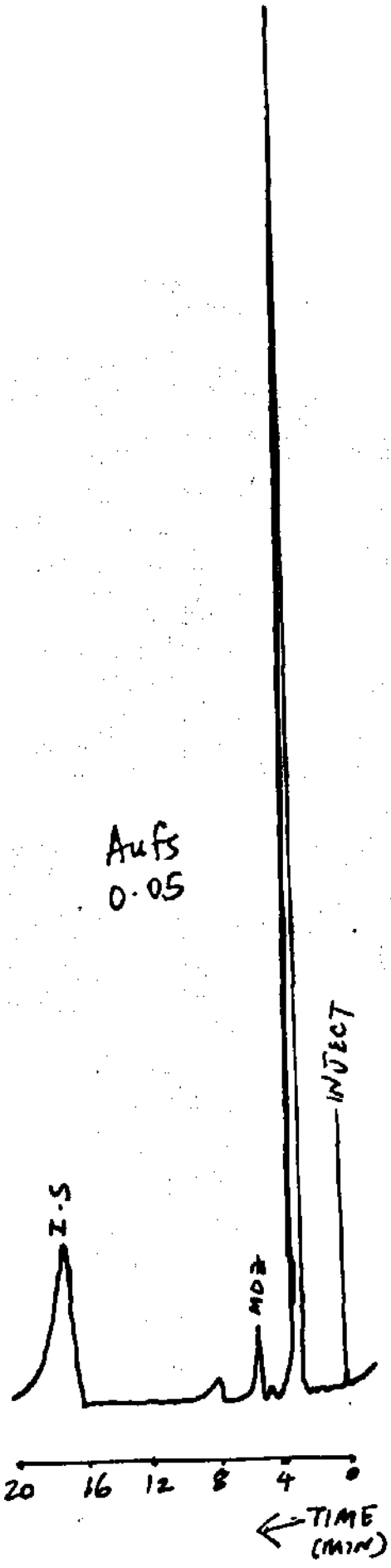


Fig. 4.2.2: High-performance liquid chromatogram of plasma of a volunteer 2 hours post-dosing with MDZ.

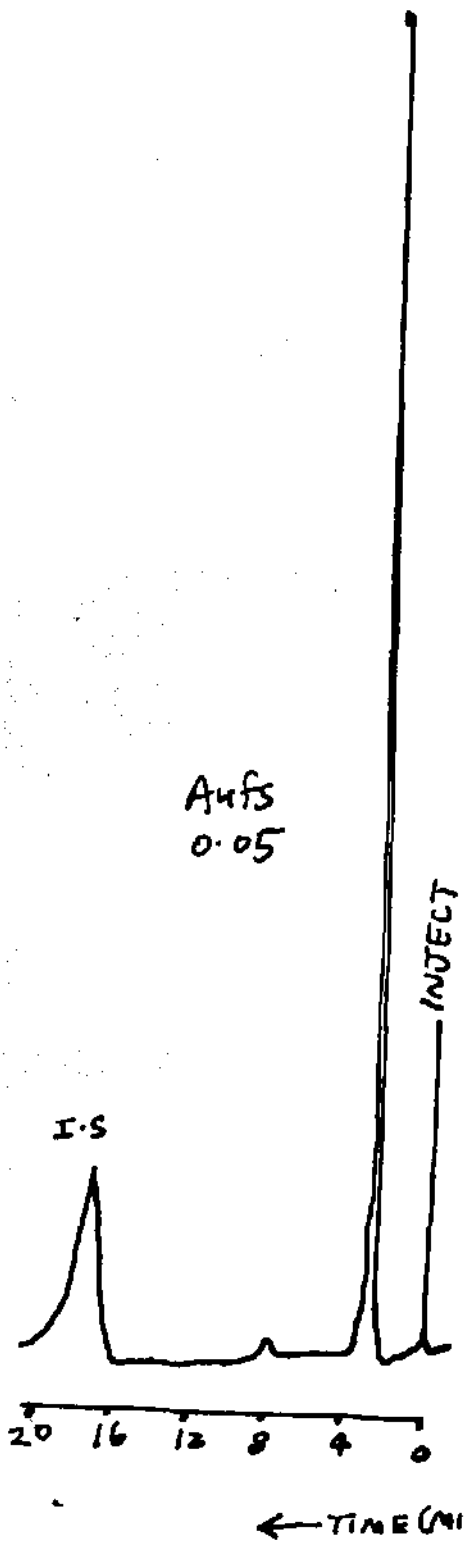


Fig. 4.2.1: High-performance liquid chromatogram of blank plasma.

The solvent system achieved high resolution of MDZ (retention time 5 minutes) from the internal standard tinidazole (retention time 17 minutes). The effects of pH and wavelength of filter on the peak-height of MDZ are presented in Figures 4.2.3 and 4.2.4 respectively. The calibration curve of MDZ in plasma (Fig. 4.2.5) gives a correlation co-efficient r = 0.993 which shows linearity at the relevant concentrations. Results for the precision analysis of the method and the percentage recovery of the extraction method are given in Tables 4.2.1 and 4.2.2 respectively. These results compare well with those obtained by Gulaid et al (1978). Limit of detection was found to be 5 ng.

Table 4.2.1: Precision of Analytical Method

Sample	Conc. ug/ml	C.V%	n
Within run:			
MDZ	5	3.0	5
	10	4.5	5
	25	4.0	5
Between run:			
MDZ	5	3.1	5
	10	3.6	5
	25	3.9	5

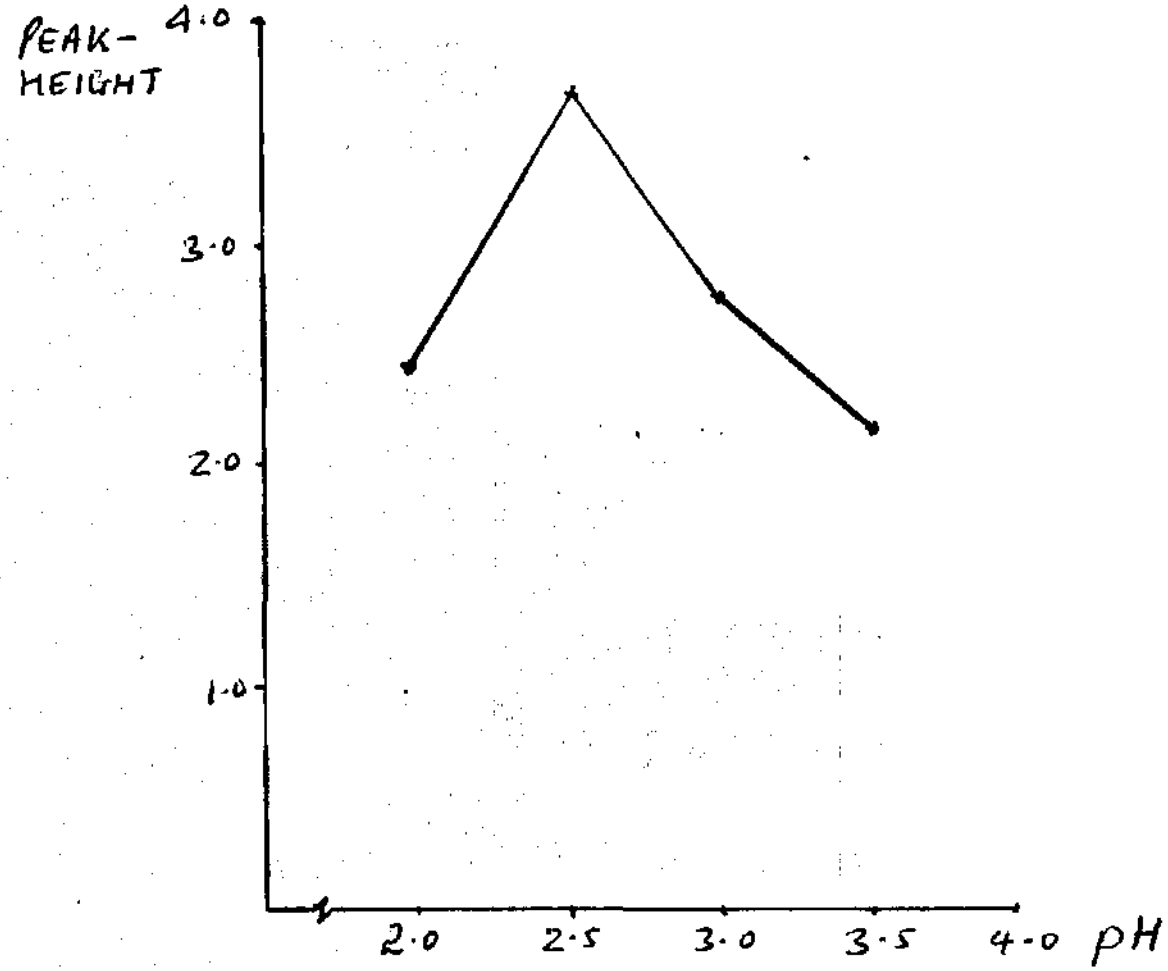


Fig. 4.2.3: Effect of pH on peak-height of MDZ.

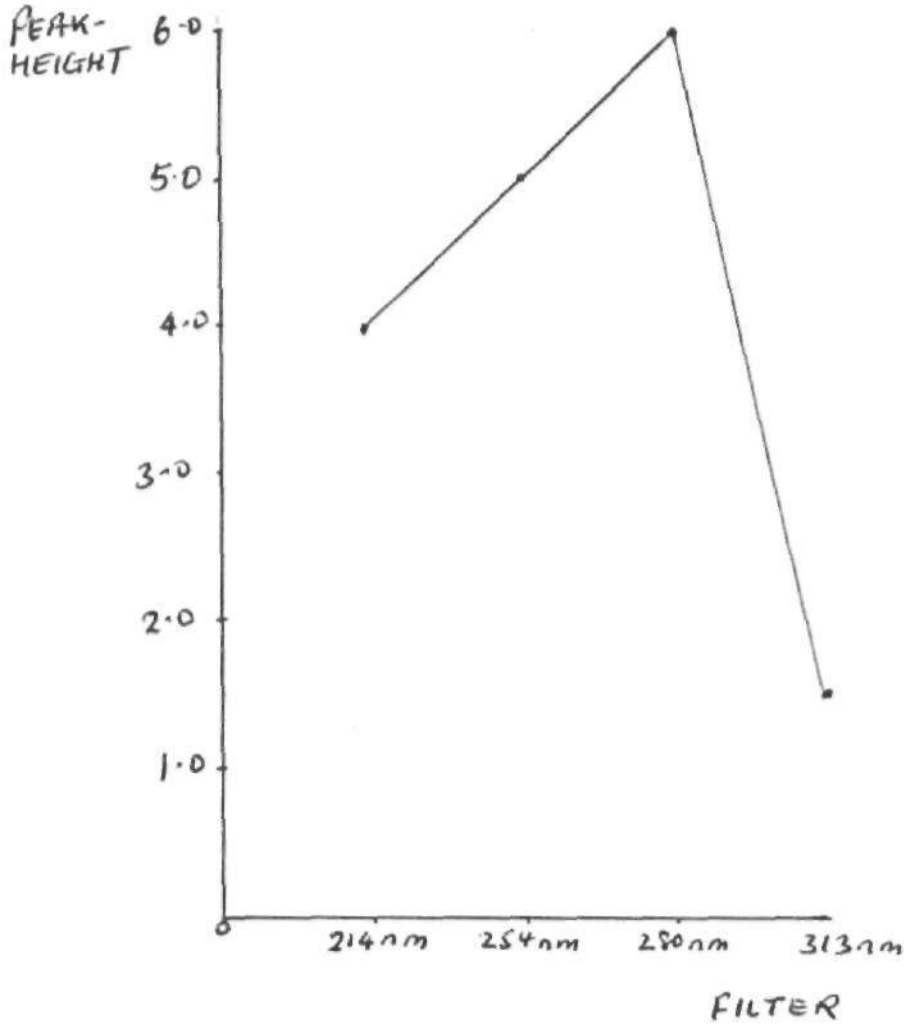


Fig. 4.2.4: Effect of filter on peak-height of MDZ.

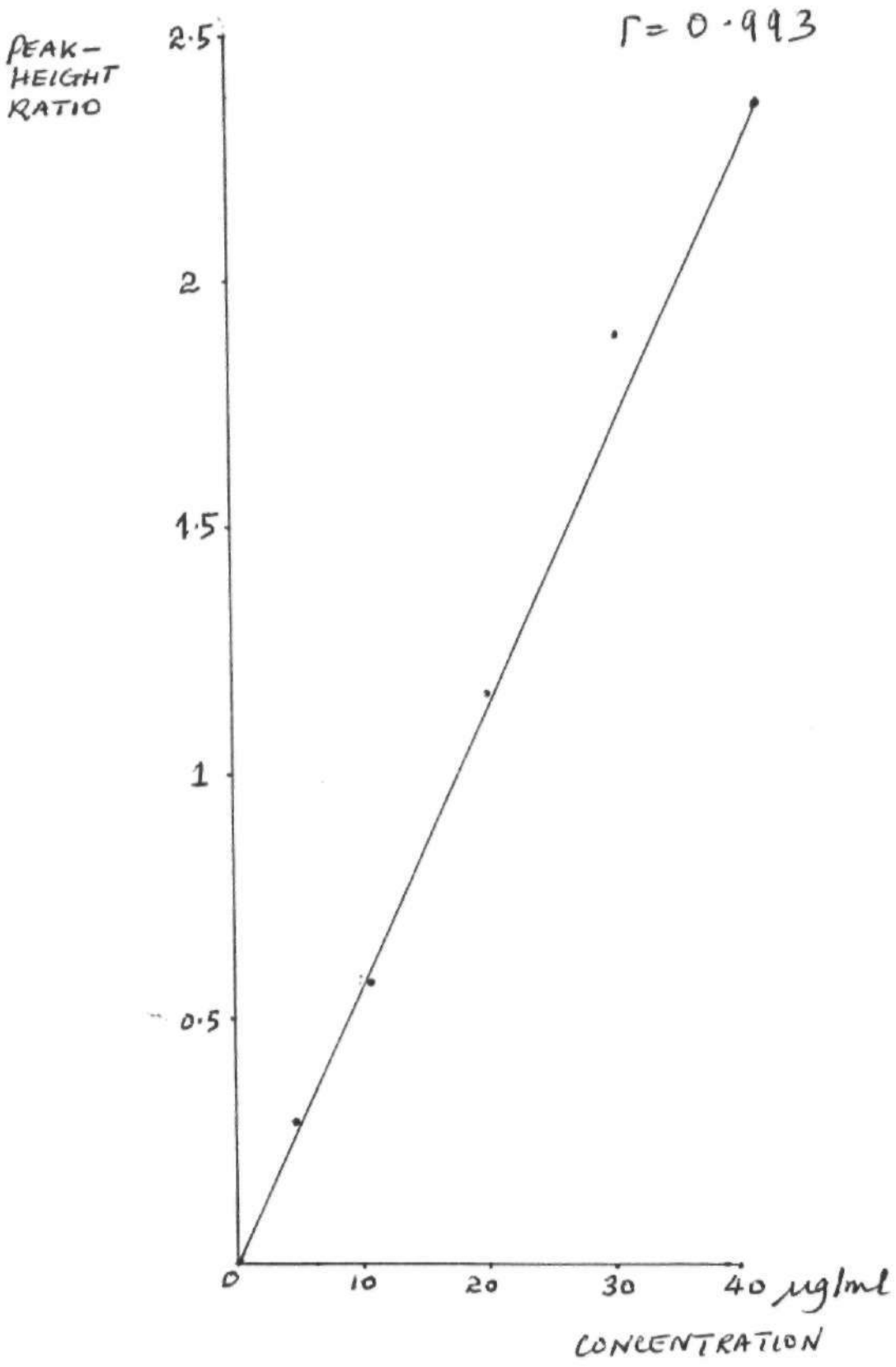


Fig. 4.2.5: Calibration curve for MDZ in plasma

Table 4.2.2: Percent recovery of analytical method

Sample	Conc. ug/ml	Recovery % Mean ± SEM	n
MDZ	5	89.8 ± 3.58	5
	10	87.4 ± 3.35	5
	25	87.0 ± 3.71	5

SEM = Standard Error of the Mean

4.2.2 Pharmacokinetics

Tables 4.2.3 to 4.2.7. show the plasma levels and pharmacokinetic parameters of MDZ following a single oral dose of 400mg and following a single oral dose of 400mg an hour after a 150mg dose of RTD. Fig. 4.2.6. displays the mean plasma-concentration time curves for MDZ alone and MDZ an hour after the dose of RTD.

The pharmacokinetics of MDZ and its major metabolites have been extensively investigated (Templeton, 1977; Brodgen, 1982 ; Houghton, 1979; Molavi, 1982; Jensen and Gugler, 1983; Ralp, 1983; and Loft et al. 1988). Houghton (1979) and Jensen and Gugler (1983) have all shown that there are varying inter-individual differences in the plasma level and pharmacokinetics of MDZ, as has been shown in this study. All the pharmacokinetic parameters derived compare well with already established values.

Table 4.2.3: Drug levels in volunteers after 400mg of MDZ.

Time (hrs)	Metronidazole concentration (ug/ml)				Mean ± SEM*
	Volunteers				
	I	II	III	IV	
0	0	0	0	0	0
0.5	4.5	4.40	1.50	6.00	4.05 ± 0.81
1	5.75	6.80	2.40	7.80	5.69 ± 1.01
2	6.80	7.80	5.95	8.80	7.34 ± 0.53
3	6.10	6.90	8.74	8.10	7.46 ± 0.51
4	5.50	6.12	10.00	7.40	7.255 ± 0.86
6	4.45	4.80	8.00	6.30	5.89 ± 0.70
8	3.60	3.75	6.45	5.30	4.775 ± 0.59
9	3.30	3.34	5.90	4.95	4.37 ± 0.55
24	0.87	1.00	1.74	1.38	1.25 ± 0.17

*SEM = Standard Error of the Mean.

Table 4.2.4: Drug levels in volunteers following 400mg MDZ an hour after a dosa of RTD 150mg.

Time (hrs)	Metronidazole concentration (ug/ml)				Mean ± SEM*
	Volunteers				
	I	II	III	IV	
0	0	0	0	0	0
0.5	3.40	1.55	1.60	8.32	3.72 ± 1.38
1	5.00	2.30	2.50	9.50	4.825 ± 1.25
2	6.27	5.35	6.27	8.63	6.63 ± 0.61
3	6.20	6.11	10.25	8.00	7.64 ± 0.84
4	5.60	5.50	9.31	7.30	6.93 ± 0.78
6	4.60	4.44	7.80	6.20	5.76 ± 0.68
8	3.80	3.60	6.47	5.27	4.8 ± 0.58
9	3.50	3.21	5.95	4.80	4.365 ± 0.55
24	0.81	1.00	1.03	2.00	1.23 ± 0.22

*SEM = Standard Error of the Mean.

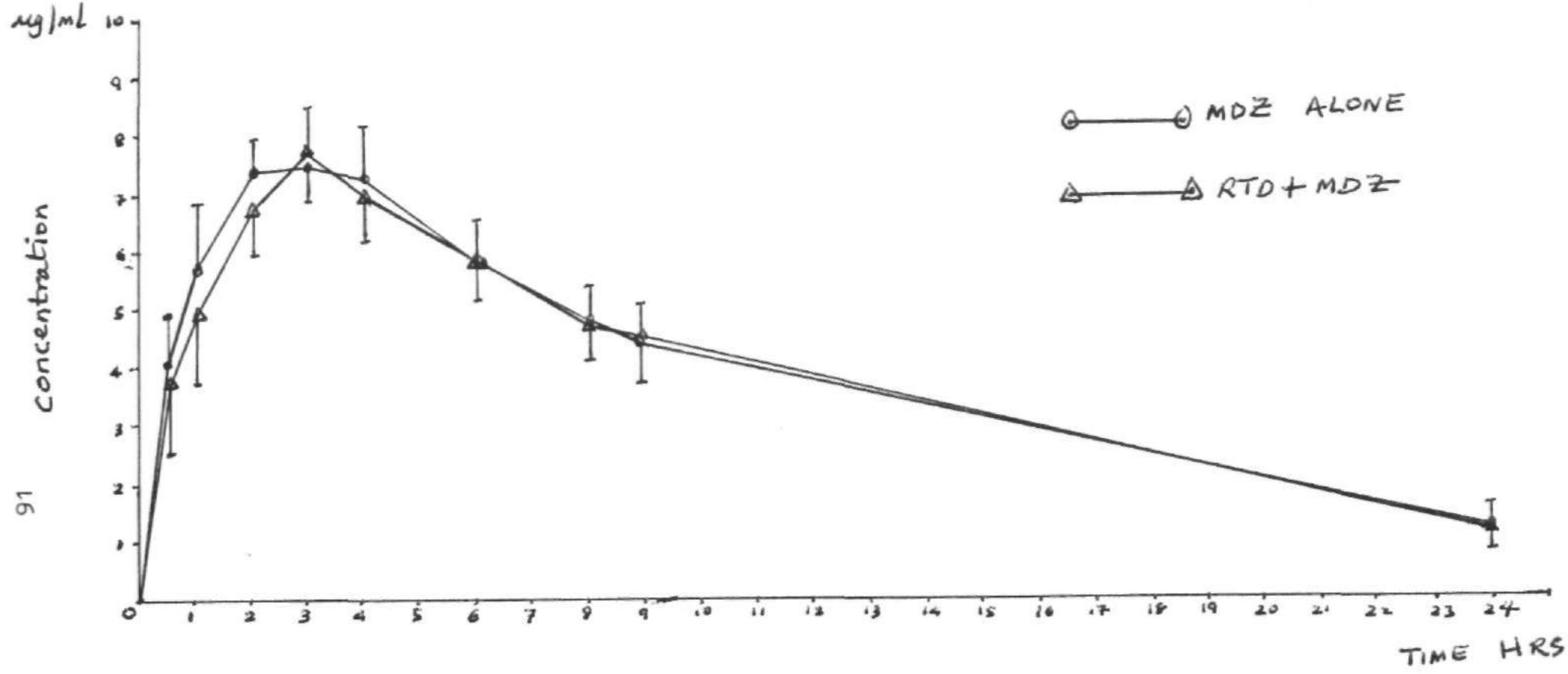


Fig. 4.2.6: Mean plasma concentration-time curves for MDZ 400mg alone and for MDZ 400mg an hour after a dose of RTD 250mg.

Table 4.2.5: Pharmacokinetic parameters after 400mg MDZ in four healthy volunteers.

PARAMETER (UNITS)	VOLUNTEERS				MEAN + SEM
	I	II	III	IV	
WEIGHT Kg	52	70	50	65	59.25 ±4.23
Kel ug/ml/hr	0.0850	0.105	0.088	0.1226	0.100 ±0.0075
$t_{\frac{1}{2}}$ hr	8.15	6.58	7.89	5.65	7.07 ±0.51
Ka ug/ml/hr	1.84	1.59	0.51	1.46	1.35 ±0.25
$t_{\frac{1}{2}}$ abs. hr	0.38	0.44	1.36	0.48	0.665 ±0.25
V_d L/kg	0.767	0.722	0.668	0.656	0.703 ±0.022
Cl_{TBC} ml/min/kg	1.0866	1.264	0.979	1.34	1.1674 ±0.071
$AUC_{0 \rightarrow 24}$ ug/ml/hr	101.89	68.89	115.89	72.09	89.69 ±9.93
$AUC_{0 \rightarrow \infty}$ ug/ml/hr	117.98	75.33	136.19	76.50	101.5 ±13.19
C_{max} ug/ml	8.8	6.8	10.00	7.8	8.35 ±0.59
T_{max} hr.	2.0	2.0	4.0	2.0	2.5 ±0.43
Tlag hr.	0.000918	0.000487	0.2	0.00568	0.0518 ±0.0428

*SEM = Standard Error of the Mean.

Table 4.2.7: Effect of RTD 150mg on the pharmacokinetics of MDZ 400mg administered an hour after the RTD.

PHARMACO-KINETIC PARAMETER (UNITS)	MEAN ± SEM I n = 4 MDZ ALONE	MEAN ± SEM II n = 4 MDZ + RTD	PERCENTAGE CHANGE	P-VALUE (t-test)
Kel	0.10012 ±0.0075	0.92 ±0.0058	-8.11	P > 0.1
$t_{\frac{1}{2}}$ hr	7.07 ±0.51	7.654 ±0.48	±8.26	P > 0.1
Ka hr	1.35 ±0.25	1.76575 ±0.70	+30.8	P > 0.5
$t_{\frac{1}{2}}$ abs hr	0.665 ±0.20	0.7325 ±0.1912	+10.15	P > 0.5
V_d L/Kg	0.703 ±0.022	0.7508 ±0.0182	+6.8	P > 0.1
Cl_{TBC} ml/min/kg	1.1674 ±0.071	1.15455 ±0.088	-1.10	P > 0.5
$AUC_{0 \rightarrow 24}$ ug/ml.hr	89.69 ±9.93	89.42 ±10.826	-0.30	P > 0.5
$AUC_{0 \rightarrow \infty}$ ug/ml.hr	101.5 ±13.19	103.445 ±14.45	+1.92	P > 0.5
C_{max} ug/ml	8.3 ± 0.59	8.01 ± 0.91	-3.49	P > 0.5
T_{max} hr	2.5 ±0.43	2.325 ±0.41	-7.00	P > 0.5
T_{lag} hr.	0.0518 ±0.0428	0.0833 ±0.41	+60.8	P > 0.5

Table 4.2.6: Pharmacokinetic parameters following 400mg MDZ an hour after RTD 150mg.

PARAMETER (UNITS)	VOLUNTEERS				MEAN ± *SEM
	I	II	III	IV	
Kel ug/ml/hr	0.079	0.09995	0.08275	0.1067	0.092 ±0.0058
$t_{\frac{1}{2}}$ hr	8.79	6.94	8.39	6.496	7.654 ±0.48
Kabs ug/ml/hr	4.059	1.73	0.63	0.644	1.76575 ±0.70
$t_{\frac{1}{2}}$ abs hr	0.17	0.59	1.09	1.08	0.7325 ±0.1912
Vd L/Kg	0.774	0.7275	0.705	0.7967	0.7508 ±0.00182
Cl_{TBC} ml/min/kg	1.019	1.212	0.9704	1.4168	1.15455 ±0.088
$AUC_{0 \rightarrow 24}$ ug/ml/hr	106.37	70.50	115.19	65.63	89.42 ±10.826
$AUC_{0 \rightarrow \infty}$ ug/ml/hr	125.72	78.28	137.39	72.39	103.445 ±14.24
C_{max} ug/ml	9.5	6.27	10.25	6.1	8.03 ±0.93
T_{max} hr	1.0	2.3	3.0	3.0	2.325 ±0.41
Tlag hr	0.000135	0.000674	0.25	0.0824	0.0833 ±0.0455

*SEM = Standard Error of the Mean.

Inspection of the plasma concentration-time curves and subsequent calculation of correlation co-efficients of the terminal β-phase indicated that MDZ kinetics are linear. Average half-life was 7.07 hr. Houghton et al. (1979) reported a $t_{\frac{1}{2}}$ of 7.0 hr after a single oral dose of 500mg, Jensen and Gugler (1983) reported a $t_{\frac{1}{2}}$ of 8.03 hr after a dose of 400mg while Nilsson-Ehle et al. (1980) reported a shorter half-life of 5.4 hr. Total body clearance was 1.674 ml/min/kg as against 1.306 reported by Jensen and Gugler (1983). Volume of distribution V_d was 0.703 L/kg (Jensen and Gugler, 1983 reported 0.96L/kg while Houghton et al., 1979 reported 0.49 L/kg after a dose of 500mg MDZ). Kabs was 1.35 hr^{-1} (Jensen and Gugler, 1983 reported 1.453 hr^{-1}). C_{max} was 8.35 ug/ml as compared to 6.9 ug/ml reported by Jensen and Gugler (1983) after a similar dose while Kaye et al. (1983) reported 7.5 ug/ml after the same dose. AUC ($0 \rightarrow \infty$) was 101.5 ug/ml.hr as compared to 79.97 reported by Jensen and Gugler (1983) after a similar dose. Houghton et al (1979) reported an AUC of 159.48 after a dose of 500mg.

Administration of 150mg RTD an hour before the dose of MDZ in a cross-over fashion caused inconsistent and statistically insignificant changes in the MEAN pharmacokinetic parameters. This is a hall-mark of

many drug-interaction studies involving RTD (see Tables 2.3.1 to 2.3.3).

The pharmacokinetic parameters may roughly be divided into two groups - parameters that are mainly related to rate and extent of absorption and these are Kabs, $t_{\frac{1}{2}}$ abs, Tlag, Cmax and Tmax; and parameters that are mainly indicators of metabolism and/or excretion and these are $t_{\frac{1}{2}}$, Kel, V_d, AUC and Total body clearance (Cl_{TBC}).

One possible way administration of RTD an hour before MDZ could affect the absorption kinetics of MDZ is through changes in gut motility and/or gastric emptying (Nimmo et al., 1983). RTD, however, has not been reported to cause diarrhoea and/or constipation or vomiting as side effects. We have also not come across any report on the effect of RTD on isolated smooth muscle. Another possible route of interaction is through the changes in gastric acidity (decrease) caused by administration of RTD. Peak plasma concentrations of RTD (and cimetidine CTD) and suppression of gastric acid secretion are generally not achieved until at least an hour after administration (Garty, et al., 1980). This effect would be expected to be greater for drugs that are either weak acids or weak bases (see page 7). MDZ is, however, neither of the above. Also, because of the small surface area of the

stomach, it is not an important site of absorption even of acidic drugs such as aspirin (Benet, 1985). This is because the stomach is lined by a thick mucus-covered membrane with small surface area and high electrical resistance. The primary function of the stomach is digestion. In contrast the epithelium of the intestine has an extremely large surface area; it is thin, it has low electrical resistance acid it serves the primary function facilitating the absorption of nutrients.

Administration of RTD 150mg an hour before MDZ 400mg increased the mean Kabs by 20.5% and $t_{\frac{1}{2}}$ absorption by 10.15%. Both changes were statistically insignificant ($P > 0.5$). Tlag was increased by 60.8% (from 0.0518 to 0.833 hrs; $P. > 0.5$). Similarly Cmax and T_{max} were decreased insignificantly by 3.4% and 7% respectively ($P > 0.5$).

Changes in pharmacokinetic parameters mainly related to metabolism and/or excretion were generally smaller and similarly insignificant. Kel was decreased by 8.11%, $t_{\frac{1}{2}}$ increased by 8.26% Vd was increased by 6.8%. Total body clearance was decreased by 1.1% and AUC was increased by 0.3%. All changes were statistically insignificant ($P > 0.1$).

These results are consistent with the fact that RTD does not bind to cytochrome P-450 to any significant

extent at therapeutic levels (10-fold lower than the binding affinity of CTD, Speeg et al., 1982). Moreover, MDZ is a low hepatic extraction drug (Powell and Dunn, 1984). It thus does not affect the metabolism of MDZ to any significant extent. On the other hand, Jensen and Gugler (1983) have reported that CTD reduced significantly the clearance of intravenously administered MDZ by 29 percent. Another possible source of interaction between RTD and MDZ is through excretion. RTD, a weak base, has been shown to compete with the active renal tubular secretion of procainamide, another weak base, leading to elevated levels of the latter (Christian et al., 1984). However, MDZ is neither weakly basic nor weakly acidic and so this effect is not expected.

Even though RTD did not affect significantly the mean pharmacokinetic parameters of MDZ ($P > 0.5$), there were variations in plasma levels and pharmacokinetic parameters among the volunteers. The effect of RTD on the kinetics of MDZ was variable and in some volunteers it was substantial. These results are similar to those of Yeung et al. (1992). In a study to determine the pharmacokinetics and metabolism of sustained-release diltiazem (DTZ) in beagle dogs, they reported that pre-treatment of the animals with RTD (150mg bid for 5 doses) did not affect significantly

the mean pharmacokinetic parameters of DTZ ($P > 0.05$). The metabolism profiles of DTZ were also not altered after RTD. However, the effect of RTD on the kinetics of DTZ was highly variable and in some animals it was substantial. They concluded that despite large inter-individual variations, RTD did not alter the metabolism of DTZ in dogs but that simply using the mean data for comparison could be misleading.

CONCLUSION

The brand of MDZ tablets used (FLAGYL^{R} M&B, Batch No. IC 337) passed the official tests for identification, weight variation, disintegration, assay and dissolution according to the B.P. 1988.

The method of extraction and analysis adopted (Kaye et al., 1980) achieved high resolution of MDZ from the internal standard tinidazole and the results for recovery and reproducibility compare well with already published values (Gulaid et al., 1978).

The mean pharmacokinetic parameters of MDZ in healthy Nigerian volunteers is similar to that obtained in Caucasians. As in caucasians also, there are inter-individual variations in plasma levels and pharmacokinetic parameters.

RTD, at a dose of 150mg an hour before a dose of MDZ 400mg has been shown not to affect significantly the mean absorption and pharmacokinetic parameters of MDZ in healthy Nigerian volunteers (P 0.5). However, the effect of RTD on the kinetics of MDZ was variable and in some volunteers it was substantial. Thus simply using the mean data for comparison could be misleading.

This study is especially relevant now that it has been discovered that many cases of antacid-resistant peptic-ulcer (increasingly treated with RTD) are caused by infection with Helicobacter pylori which has been found to be effectively treated with MDZ with or without tetracycline or amoxycillin (Aina, 1992).

REFERENCES

Abernathy, D.R., Greenblatt, D.J., Eshelman, F.N., Shader, R.I. (1984). Ranitidine does not impair oxidative or conjugative metabolism: Non-interaction with antipyrine, diazepam, and lorazepam. Clin. Pharmacol. Therap., 35: 188-1992.

Aina, J.B. (1992). Africa Health, 14(6): 56.

Ambre, J.J. and Fischer, L.J. (1973). Effect of co-administration of aluminium and magnesium hydroxide on adsorption of anti-coagulants in man. Clin. Pharm. Therap., 14: 231.

Back, D.J., Breckenbridge, A.M., Crawford, F.E., McIver, M., Orme, ML'E, Rowe, P.H. (1981). Inter-individual variation and drug interactions with hormonal steroid contraceptives Drugs, 21: 46.

Bell, J.A., Gower, A.J., Martin, L.E., Mills, E.N.S., Smith, W.P. (1981). Interaction of H_2-receptor antagonist with drug metabolising enzymes. Biochem. Soc. Trans., 9: 113-114.

Bell, G.D., Dutka, H.P., Henry, D.A., Langman, M.J.S. (1981). Glucuronidation of 1-menthol in man: the effects of prior treatment with cimetidine and phenobarbitone. Br. J. Clin. Pharmacol., 12: 274P.

Benet, L.Z. (1985). Pharmacokinetics: The dynamics of drug absorption distribution and elimination. In: Goodman and Gilman's Pharmacological Basis of Therapeutics, p. 11, Macmillan, Canada.

Blasche, T.F. (1977). Protein binding and kinetics of drugs in liver disease. Clin. Pharmacokinetics., 2: 32.

Boston (1972). Boston Collaborative Drug Surveillance Programme: Adverse drug reactions. JAMA, 220: 1238.

Bradshaw, J., Butcher, M.E., Cutherow, J.W., Doule, M.D., Hayes, R., Judd, D.B. et al. (1981). Aminoalkyl furans as H_2-receptor antagonists. In Creighton and Turner (Eds). The Biochemical Regulation of Biological Mechanisms, pp. 45-57. The Royal Society of Chemistry, London.

Breen, K.J., Bury, R., Desmond, P.V., Mashford, M.L. Morphett, B., Westwood, B. et al. (1982). Effects of cimetidine and ranitidine on hepatic drug metabolism. Clin. Pharmacol. Therap., 31, 297-300.

Brodgen, R.N., Carmen, A.A., Heel, R.C., Speight, T.S. and Avery, G.S. (1982). Ranitidine: Pharmacology and therapeutic uses in peptic ulcer disease and other allied diseases. Drug, 24, 267-303.

British Pharmacopoiea (1988). Her Majesty's Stationery Office, London.

Brown, D.D. and Juhl, K.P. (1976). Decreased bioavailability of digoxin due to antacids and kaolin-pectin. New Eng. J. Med., 295, 1034.

Christian, C.D., Meredith, C.G., Speeg, K.V. (1984). Cimetidine inhibits renal procainamide clearance. Clin. Pharmacol. and Therap., 36: 221.

Crook, J.E. and Nies, A.S. (1978). Drug interactions with antihypertensive drugs. Drugs 15: 72.

Dalton, M.J., Powell, J.R., Messenheimer, J.A. (1984). The influence of ranitidine on single dose carbamazepine pharmacokinetics. Am. J. Hosp. Pharm., 30:275-9

Daneshmend, T.K., Ene, M.D., Parker, G., Roberts, C.J.C. (1984). Effect of chronic oral cimetidine on apparent liver blood flow and hepatic microsomal enzyme activity in man. Gut., 125-128.

Das, K.M. and Dubin, R. (1976). Clinical pharmacokinetics of sulfasalazine. Clin. Pharmacokinetics, 1: 406.

Davie, I.T. (1977). Specific drug interactions in anaesthesia. Anaesthesia 32: 1000.

Dean, R.P. and Talbert, R.C. (1980). Drug Intell. and Clin. Pharm., 14, 864.

Desmond, P.V., Mashford, M.L., Harman, P.J., Morphett, B.J., Breen, K.J., Wang, Y.M. (1984). Decreased oral warfarin clearance after ranitidine and cimetidine. Clin. Pharmacol. Ther., 32: 338-341.

Donn, K.H., Powell, J.R., Rogers, J.F., Eshelman, F.N. (1984). The influence of H_2-receptor antagonists in steady-state concentrations of propranol and 4-hydroxy propranolol. J. Clin. Pharmacol. 25:480-485.

Donn, K.H., Powell, J.R., Rogers, J.F. Plachetka, J.R. (1984). Lack of effect of histamine H_2-receptor antagonists on indocyanine-green disposition as measured by two methods. J. Clin. Pharmacol. 240: 360-370.

Dreifus, L.S., de-Azevedo, I.M., Watanabe, Y. (1974). Electrolyte and anti-arrhythmic drug interactions. American Heart Journal, 30:88-95.

Dunk, A.A., Jenkins, W.J., Burroughs, A.K. (1983). The effect of ranitidine on the plasma clearance and hepatic extraction of indocyanine-green in patients with chronic liver disease. Br. J. Clin. Pharmacol., 16: 117-120.

Durant, G.J., Brown, T.H., Ernest, J.C., Ganellin, C.R., Prain, H.D., Young, R.C. (1981). Some structure-activity considerations in H_2-receptor antagonists. In Creighton and Turner (Eds). The Chemical Regulation of Biological Mechanisms, pp. 27-44. The Royal Society of Chemistry, London.

Ehrenfeld, M., Levy, M. Shannon, P., Rachmilenitz, D., Eliakin, M. (1982). Gastrointestinal effects of long term colchicine therapy in patients with recurrent polyserositis (familial mediterranean fever). Digestive Diseases and Sciences, 27: 723.

Elwood, R.J., Hilderbrand, P.J., Dundee, J.W. and Collier, P.S. (1983). Ranitidine influences the uptake of oral midozolam. British J. of Clin. Pharmacol., 15: 743.

Feely, J. and Guy, E. (1982). Ranitidine also reduces liver flow. Lancet, 1: 169.

Feely, J. and Guy, E. (1983). Lack of effect of ranitidine on the disposition of lignocaine. Br. J. Pharmacol., 15: 378-379.

Fichtl, B. and Doering, W. (1983). The quinidine-digoxin interaction in perspective. Clin. Pharmacokinetics, 8: 137.

Fisher, P., House, F., Inns, P., Morrison, P.J., Rogers, H.J., Bradbrook, I.D. (1980). Effect of cimetidine on the absorption of orally administered tetracycline. Br. J. Clin. Pharmacol., 9: 153-158.

Flind, A.C. (1978). Cimetidine and oral anticoagulants. Br. Med. J., 2: 1367.

Garg, D.C., Weidler, D.J., Jallad, N.S., Eshelman, F.N. (1982). The effects of ranitidine and cimetidine on hepatic blood flow. Clin. Pharmacol. Ther., 31: 228.

Garty, M. Hurwitz A.(1980) Clin.Pharmacol. Ther, 28:203-207

Goldman, P., Pepperman, M.A., Goldin, B.R. (1974). Drugs metabolised by intestinal flora. In Morselli et al. (Eds). Drug Interactions., p. 91, Raven Press, New York.

Guay, D.R.P., Meatherall, R.C., Chalmers, J.L., Grahame, G.R. and Hudson, R.J. (1985). Br. J. Clin. Pharmacol., 20, 55-59.

Gulaid, A., Houghton, G.W., Lewellen, O.R.W., Smith, J. and Thorne, P.S. (1978). Br. J. Clin. Pharmacol. 6, 430.

Hansten, P.D. (1979). Clinical significance of drug-drug interaction and drug effects on clinical laboratory results. In: Drug Interactions, pp. 2-3. Lea and Febiger, Philadelphia.

Harvey, C.S. and Dean, C.W. (1985). Basic Pharmacokinetics. In Remington's Pharmaceutical Sciences 17th Edition, pp. 74-761, Mack, Pennyslvania, USA.

Heagherty, A.M., Castleden, C.M., Patel, L. (1982). Failure of ranitidine to interact with propranolol. Br. Med. J., 284: 1304.

Hebert, D.W., Eisen, H.J., Negishi, M., Lang, M.A., Hjemaland, L.M., Okey, A.B. (1981). Genetic mechanisms controlling the induction of polysubstrate mono-oxygenase P-450 activities. Ann. Rev. Pharmacol. Toxicol. 21: 431-462.

Hibbard, D.M., Peters, J.R., Hummingshake, D.B. (1984). Effects of cholestyramine and cholestipol on the plasma concentrations of propranolol. Brit. J. Clin. Pharmacol., 18: 337.

Houghton, G.W., Thorne, D.S., Smith, J., Templeton, R. and Colier, J. (1979). Comparison of the pharmacokinetics of metronidazole in healthy adult female volunteers following either single oral or i.v dose. Br. J. Clin. Pharmacol., 8: 337-341.

Hussar, D.A. (1985). Drug interactions. In Remingtons Pharmaceutical Sciences, p. 1801, Mack, Pennysylvania.

Jensen, J.R. and Gugler, R. (1983). Single and multiple dose metronidazole pharmacokinetics. Clin. Pharmacol. Ther., 34(4): 481-486.

Jensen, J.R. and Gugler, R. (1987). Clin. Pharmac. Ther., 34, 407.

Jackson, J.E., Bentley, J.B., Glass, S., Banner, W., Plachetka, J.R. (1983). The effects of H_2-blockers on lidocaine disposition. Clin. Pharmacol. Ther., 33: 255.

Jones, D.B., Ching, M.S., Morgan, D.J. (1985). J. Pharm. Pharmacol., 37/3: 196-198.

Kabins, S.A. (1972). Interactions among antibiotics and other drugs. JAMA, 219: 206.

Kaye, C.M., Sankey, M.G., Thomas, L.A. (1980). A rapid and specific semi-micro method involving HPLC for the assay of metronidazole in plasma, saliva, serum, urine and whole blood. Br. J. Clin. Pharmacol., 9: 528-529.

Kelly, H.W., Powell, J.R., Donahue, J.F. (1980). Ranitidine at very large doses does not inhibit theophylline elimination. Clin. Pharmacol. Ther., 39, 577-588.

Kelly, J.G., Shanks, R.G., McDeritt, D.G. (1983). Influence of ranitidine on plasma metoprolol concentrations. Br. Med. J., 287: 1218-1219.

Kelly, J.G., Salem, S.A.M., Kenney, C.D., Shanks, R.G. and McDeritt, D.G. (1986). Br. J. Pharmac., 19: 219-224.

Kirch, W., Janisch, H.D., Heidemann, R., Ranisch, K., Ohnhauss, E.E. (1983). Dtsch, Med. Wochenschr.,108: 1757-1761.

Klotz, V., Reimann, I. (1980). Effect of cimetidine on the clearance of benzodiazepines. N. Eng. J. Med., 303: 754.

Knapp, A.B. (1983). Ann. Intern. Med.,98, 1974.

Koch, R.L., Beanhean, B.B., Chrystal, E.J.T. and Goldman, P.A. (1981). A metronidazole metabolite in urine and its risk. Science,211, 398-400.

Koch-Weser, J. (1975). Drug Interactions in cardiovascular therapy. American Heart Journal, 90: 93.

Kramer, W. Inglott, A., Cluxton, R.V. (1971). Drug Intelligence and Clinical Pharmacy, 5: 211.

Kristense, M.B. (1976). Drug interactions and clinical pharmacokinetics. Clinical Pharmacokinetics, 1: 351.

Law, R. and Chalmers, C. (1976). Medicines and elderly people: a general practise survey. Brit. Med. J., 1: 565.

Lewis, J.G. (1983). Adverse drug reactions to calcium antagonists. Drugs,25, 196.

Loft, S., Poulsen, H.E., Sonne, J., Dossing, M. (1988). Metronidazole clearance: a one sample method and influencing factors. Clin. Pharmacol. Ther.,43, 420-428.

Malette, L.E. (1977). Acetazolamide-accelerated anticonvulsant osteomalacia. Archives of Internal Medicine, 137: 1013.

Manninen, V., Merlin, J., Apajalahti, A., Karesoja, M. (1973). Altered absorption of digoxin in patients given propantheline and metoclopramide. Lancet, 1: 398.

Masarella, J.W., Defeo, T.M., Passe, S., Aogaichi, K. (1991). The effects of cimetidine and ranitidine on the pharmacokinetics of cifenline. Br. J. Clin. Pharmacol. 31(4), 841-3.

Mashford, M.L., Harmon, J., Morphett, B.J., Breen, K.J., Desmond, P.V. (1983). Ranitidine does not affect chlormethiozole or indocyanime-green disposition. Clin. Pharmacol. Ther. 34, 231-233.

McElnay, J.C. and D'Arcy, P.F. (1983). Protein-binding displacement interactions and their clinical importance. Drugs 25: 495.

Mead, P.B. (1982). New Eng. J. Med. 306, 1490.

Mena, I. and Coatzias, G.C. (1975). Protein intake and treatment of Parkinsons disease with levodopa. New Eng. J. Med. 24: 709.

Molavi, A. Lefrock, J. and Randal, P. (1982). Metronidazole: Med. Clin. N. Am. 66(1), 121-133.

Morrison, P.J , Rogers, H.J., Bradbrook, I.D., Parsons, C. (1980). Concurrent administration of cimetidine and enteric-coated prednisolone. Effect on plasma levels of prednisolone. Br. J. Clin. Pharmacol. 180: 87-89.

Neuvonen, P.J. (1976). Interactions with the absorption of tetracycline. Drugs 11: 45.

Nies, A.S., Shand, D.G., Wilkinson, G.R. (1976). Altered hepatic blood flow and drug disposition. Clinical Pharmacokinetics, 1: 135.

Nilsson-Ehle, I., Ursing B., Nilsson, Ehle, P. (1980). Antimicrob. Agents Chemotherapy 19: 754-760.

Nimmo, W.S., Heading, R.C., Wilson, J., Tothill, P., Presscott, L.F. (1975). Inhibition of gastric emptying and drug absorption by narcotic analgesics. Brit. J. Clin. Pharmacol. 2: 509.

Nimmo, W.S. (1976). Drugs, diseases and gastric emptying. Clin. Pharmacokinetics, 1: 189.

Nimmo, W.S., Heading, R.C., Tothill, P., Prescott, L.F. (1973). Pharmacological modification of gastric emptying: Effects of propantheline and metoclopramide on paracetamol absorption. Brit. Med. J. 1: 587.

Noble, D.W. (1987). Br. J. Anasthesia 59: 735.

Oates, H. (1971). Annals of the New York Academy of Sciences, 179: 302.

O'Reilly, R.A. (1984). Comparative interaction of cimetidine and ranitidine with racemic warfarin in man. Arch. Intern. Med. 144: 989-991.

Park, B.K., Breckenbridge, A.M. (1981). Clinical implications of enzyme induction and enzyme inhibition. Clinical Pharmacokinetics 6:1.

Patel, L., Weerasuriya, K. (1983). Effect of cimetidine and ranitidine on propranolol clearance. Br. J. Clin. Pharmacol. 15: 152P.

Paton, T.W., Walker, S.E., Lening, F.Y.K., Little, A.H. (1983). Effect of cimetidine on bioavailability of enteric-coated aspirin tablets. Clin. Pharm. 2, 165-166.

Paxton, J.W. (1981). Elementary pharmacokinetics in clinical-practice 2: Basic concepts of drug pharmacokinetics. New Zealand Med. J. 695, 342-5.

Picard, E.H. (1983). Mayo Clin. Proceedings, 58, 401.

Pond, S.M., Graham, G.G., Birkett, D.J., Wade, D.N. (1975). Effect of tricyclic antidepressants on drug metabolism. Clin. Pharmacol. Ther. 18: 191.

Pottage, A. Campbell, R.W.F., Achuff, S.C., Murray, A., Julian, D.G., Prescott, L.F. (1978). The absorption of oral mexiletine in coronary care patients. Eur. J. of Clin. Pharmacol. 13: 393.

Powell, J.R. and Donn, K.H. (1984). Histamine H_2-receptor antagonist drug interaction perspective: Mechanistic concepts and clinical implications. Am. J. Med. 77(5B): 57-84.

Powell, J.R., Vozeh, S., Hopwell, P., Costello, J., Skeiner, L.B., Riegelman, S. (1978). Theophylline disposition in acutely ill hospitalised patients. The effects of smoking, heart failure, severe airway obstruction and pneumonia. Am. Rev. Respir. Dis. 18: 185-187.

Powell, J.R., Rogers, J.F., Wargin, W.A., Cross, R.E., Eshelman, F.N. (1984). Inhibition of theophylline clearance by cimetidine but not ranitidine. Arch. Intern. Med. 144: 484-486.

Prescott, L.F. (1987). Clinically important drug interactions. In: Avery's Drug Treatment 3rd Ed. pp. 256-278, ADIS Press, New Zealand.

Prescott, L.F. and Highley, M.S. (1985). Drugs prescribed for self-poisoners. Brit. Med. J. 290: 1633.

Prescott, L.F., Nimmo, W.S. Heading, R.C. (1977). Drug absorption interactions in Grahame-Smith (Ed). Drug Interactions, p. 45, Macmillan, London.

Prescott, L.F. (1974). Gastric emptying and drug absorption. Brit. J. Clin. Pharmacol. 1: 189.

Ralph, E.D., Clark, J.T., Libke, R.D. (1974). Pharmacokinetics of metronidazole as determined by bioassay. Antimicrob. Agents Chemotherap. 6: 691.

Ralph, E.D. (1983). Clinical pharmacokinetics of metronidazole. Clin. Pharmacok. 8, 43-62.

Remmer, H. (1970). The role of the liver in drug metabolism. Am. J. Med. 49: 617.

Rendic, S., Alebic-Kolbah, Kajfez, F., Ruf, H.H. (1982). Interaction of ranitidine with liver microsomes. Xenobiotics, 12: 9-17.

Richens and Dunlop (1975). Lancet 2: 247-248.

Ritschel, W.A. (1980). Non-linear pharmacokinetics. In: Handbook of Basic Pharmacokinetics, p. 354, Second Edition, Drug Intelligence Publications, Hamilton, Illinois, USA.

Roberts, C.J.C. (1984). Clinical pharmacokinetics of ranitidine. Pharmacokinetics, 9, 211-221.

Robson (1985). Br. J. Clin. Pharmac. 20: 170.

Roy, A.K., Luda, M.P., Levine, R.A. (1988). Induction of theophylline toxicity and inhibition of clearance rates by ranitidine. Am. J. Med. 85, 525-527.

Scarpignato, C., Bertaccini, G., Zimbaro, G., Vitulo, F. (1982). Ranitidine delays gastric emptying of solids in man. Br. J. Clin. Pharm. 13: 252-253.

Schwartz, D.E. and Jennett, F. (1976). Comparative pharmacokinetics of metronidazole and ornidazole in man. Chemotherap. 22: 19.

Seitz, M.K., Bosche, J. Ozygan, P., Veith, S., Simon, B., Kommerell, B. (1983). Increased blood ethanol levels following cimetidine but not ranitidine. Lancet 1: 760.

Serlin, M.J., Sibeon, R.G., Breckenbridge, A.M. (1981). Lack of effect of ranitidine on warfarin action. Br. J. Clin. Pharmacol. 12: 791-794.

Serlin, M.J. and Breckenbridge, A.M. (1981). Drug interactions with warfarin. Drugs 25: 610.

Somogyi, A. and Gugler, R. (1982). Drug interactions with cimetidine. Clinical Pharmacokinetics, 7: 23.

Spahn, H., Mutschler, E., Kirch, W., Ohnhauss, E.E., Janisch, H.D. (1983). Influence of ranitidine on plasma metoprolol and etenolol concentrations. Br. Med. J. 286: 1546-1547.

Speeg, K.V., Potwardhan, R.V., Avant, G.R., Mitchell, M.C., Schenker, S. (1982). Inhibition of microsomal drug metabolisms by histamine H_2-receptor antagonists in-vivo and in-vitro in rodents. Gastroenterology. 82: 89-96.

Staiger, C., Simon, B., deVries, H., Walter, E. (1980). Investigations of the effects of ranitidine on antipyrine metabolism. Z. Gastroenterol. 18: 601-604.

Thornton, W.E. (1976). Dementia induced by methyldopa with haloperidol. N. Eng. J. Med. 294: 1222.

Tucker, G.T. (1979). Drug metabolism. Br. J. Anaesth. 51: 603-618.

Upton, R.A., Thercelin, J.F., Moore, J.K., Riegelman, S. (1982). A method for estimating within-individual variability in clearance and in volume of distribution from standard bioavailability studies. J. Pharmacokinetic Biopharm. 310: 1639-1643.

Waltz, F.G., Vlasyk, G.P., Omiecinski, C.J. (1982). Multiple immuno-identical forms of phenobarbital-induced cytochromes P-450 are encoded on different RNA's. J. Biol. Chem. 257: 4023-4026.

Watts, R.W., Hetzel, D.J., Bochner, F., Hallpike, J.F., Hahn, C.S., Shearman, D.J.C. (1983). Lack of interaction. Br. J. Clin. Pharmacol. 15: 499-50.

Webster, L.T. (1990). Drugs used in chemotherapy of protozoal infections. In: Goodman and Gilman's Pharmacological Basis of Therapeutics, pp. 1002-1005, Pergamon Press, New York.

Welling, P.G. (1984). Interactions affecting drug absorption. Clin. Pharmacokin. 9: 404.

Wilkinson, C.F. Hetnarski, K., Wicks, L.J. (1974). Substituted imidazoles as inhibitors of microsomal oxidation and insecticide synergists. Pest Biochem. Physiol. 4: 299-312.

Wilson, C.M. (1986). Br. J. Anaesth. 58, 1330P.

Winship, L.C. (1985). J. Pharmacotherap. 5: 16.

Wheeler, L.A. (1978). Antimicrob. Ag. Chemother. 13, 205.

Wood, B.A. and Monro, A.M. (1977). Pharmacokinetics of tinidazole and metronidazole in women after a single oral dose. Br. Med. J. 1: 607.

Yeung, P.K.F., Mosher, S.J. and Landriatt, H. (1992). Effects of ranitidine on the pharmacokinetics and metabolism of sustained-release diltiazem in dogs. Proceedings of the Fourth North American ISSX Meeting, 2: 193.

APPENDIX I

SOME IDENTIFICATION TESTS FOR METRONIDAZOLE TABLETS (B.P. 1988)

1. Shake a quantity of the powdered tablets containing 0.25gm MDZ with 4ml 0.5m H_2SO_4 and filter. To the filtrate, add 10ml trinitrophenol solution and allow to stand. The melting point of the precipitate after washing with water and drying at 105°C is 150°C.

2. Heat a quantity of the powdered tablets containing 10mg MDZ in a water-bath with zinc powder, 1ml water and 0.25ml of sodium nitrite solution for 5 minutes, cool in ice, add 0.5ml sodium nitrite solution and remove excess nitrite with sulphamic acid. Add 0.5ml of 2-naphthol solution and 2ml of 5M sodium hydroxide solution. An orange-red colour is produced.

APPENDIX II

WEIGHT VARIATION TEST FOR METRONIDAZOLE TABLETS (B.P. 1988)

For uncoated tablets:
20 tablets should be selected at random and the average weight determined. Not more than two of the individual weights should deviate from the average weight by more than 5% for tablets that have an average weight of more than 250mg.

Tablet No.	Weight (gm)
1	0.3131
2	0.3230
3	0.3182
4	0.3426
5	0.3428
6	0.3310
7	0.3325
8	0.3294
9	0.3379
10	0.3381
11	0.3320
12	0.3240
13	0.3418
14	0.3298
15	0.3185
16	0.3311
17	0.3352
18	0.3204
19	0.3378
20	0.3345
Total weight	6.611gm
Average weight	0.33055gm

Average weight ± 5% (acceptable range):
0.3140225 - 0.3470775

COMMENT: Only one tablet fell outside the acceptable range. Therefore the tablets have passed the weight variation test.

APPENDIX 3

APPARATUS FOR THE DISINTEGRATION OF TABLETS

The apparatus consists of

(a) a rigid basket-rack assembly supporting 6 cylindrical glass tubes 77.5mm long, 21.5mm in internal diameter, and with a wall thickness of about 2mm.

(b) a cylindrical disc for each tube, each 20.7mm in diameter and 9.5mm thick, made up of transparent plastic with a relative density of 1.18 to 1.20, and pierced with five holes, each 2mm in diameter, one in the centre and the other four spaced equally on a circle of radius 6mm from the centre of the disc. Four equally-spaced grooves are cut in the lateral surface of the disc in such a way that at the upper surface of the disc they are 9.5mm wide and 2.55mm deep and at the lower surface 1.6mm square.

(c) The tubes are held vertically by 2 super-imposed transparent plastic plates 90mm in diameter and 6mm thick, perforated by 6 holes having the same diameter as the tubes. The holes are equidistant from the centre of the plate and are equally spaced from one another. Attached to the under-side of the lower plate is a piece of wooven guaze made from stainless steel wire 635 μm in

diameter and having nominal mesh apertures of 2.00mm. The upper plate is covered with a stainless steel disc perforated by six holes each about 22mm in diameter, which fits over the tubes and holds them between the plastic plates. The holes coincide with those of the upper plastic plate and the upper ends of the glass tube.

(d) The plates are held rigidly in position and 77.5mm apart by vertical metal rods at the periphery and a metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to a mechanical device capable of raising and lowering it smoothly at a constant frequency of between 28 and 32 cycles per minute, through a distance of 50 to 60mm.

(e) The assembly is suspended in the liquid medium in a suitable vessel, preferrably a 1000ml beaker. The volume of liquid is such that the wire mesh at its highest point is at least 25mm below the surface of the liquid, and at its lowest point is at least 25mm above the bottom of the beaker.

(f) A suitable device maintains the temperature of the liquid at $37 \pm 0.5^{\circ}C$.