# EFFECT OF RANITIDINE ON PHARMACOKINETIC PARAMETERS OF METRONIDAZOLE IN HEALTHY HUMAN VOLUNTEERS

**BY**

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# ZARIA

**FEBRUARY, 2018**

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**DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY, FACULTY OF PHARMACEUTICAL SCIENCE,**

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# DECLARATION

I declare that the work in this dissertation entitled “Effect of Ranitidine on the Pharmacokinetic Parameters of Metronidazole in Healthy Human Volunteers” has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Inusa Babanladi MuhammadSignature Date

# CERTIFICATION

This dissertation entitled “The effect of ranitidine on the pharmacokinetics parameters of metronidazole in healthy human volunteers” by Inusa Babanladi Muhammad meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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# ABSTRACT

Pharmacokinetics is the study of the time course of drug movement in the body during absorption, distribution, metabolism and elimination.Drug interactions are pharmacodynamic, pharmacokinetic, or clinical responses to the administration of a drugcombination that differs from the known effects of the individual drugs administered alone. The clinicalconsequences of drug interactions may be antagonistic, additive, synergistic, or idiosyncratic, resulting in treatmentfailure, increased pharmacologic effect, or toxic reactions, which may be serious or fatal. Due to the fact that mostdrugs are detoxified by the liver, the most important of the pharmacokinetic drug interactions involve drugmetabolism usually entailing the induction or inhibition of the cytochrome P450 (CYP450) enzyme system which isresponsible for oxidative-reductive metabolism.

This study was undertaken to compare the effect of ranitidine on the pharmacokinetic parameters of metronidazole in saliva sample of normal healthy human volunteers by applying UV spectrophotometric analytical method for the estimation of metronidazole.This was done by preparing different concentrations of pure metronidazole in 0.1 N HCl solutions and their respective absorbance determined at 277 nm. Quality control studies (identification test, assay test, weight variation, friability test, disintegration and dissolution rate) for both ranitidine and metronidazole were carried out according to BP2009. The method of Kolawole and Ameh, 2004 was adopted and validated based on ICH guideline for this study. Both metronidazole and ranitidine tablets passed all the quality control studies and were thus found to be fit for the purpose of this study.The percentage recovery of the method was within the accepted range of 98 – 102 %. Calibration curve was linear within the range of 2.5–15 µg/ml adopted and validated. The adopted method was then employed in

determining the effect of ranitidine on the pharmacokinetics parameters (Cmax, Tmax, Kab, Kel, t1/2ab, t1/2el, Cl, Vd, lag time and AUC) of metronidazole using human volunteers of 30 years and above and the study was divided into three phases with a washout period of two weeks. Both metronidazole and ranitidine used for this study were found to have the labeled active ingredient.They all passed the assay test as they were within the acceptable limits 95 – 105

%. Weight variation was conducted for both metronidazole and ranitidine and they all passed as their percentage mean deviation was less than 5 %. Similarly, both drugs passed the friability test which was less than 1%. Disintegration tests for both drugs reveals that they disintegrate in less 15 mins and also both metronidazole and ranitidine passed the dissolution rate as more than 70 % of the active ingredient was released in 30 mins. The percentage recovery of the method was within the accepted range of 98 – 102 %. Calibration curve was linear within the range of 2.5 – 15 µg/ml as the correlation coefficient was 0.9681. The regression equation was y= 0.027x + 0.002. After concurrent and delayed administration, Cmax of metronidazole was found to be statistically significant *(p<0.05)* at a value of 51.57 μg/ml and 36.27 μg/ml. Other pharmacokinetic parameters of metronidazole like AUC were not significantly (*p < 0.05*) affected by ranitidine. This finding indicates that ranitidine may influence the pharmacokinetics of metronidazole when co-administered together depending on the time of administration of the two drugs. Based on the finding the dosage of the metronidazole has to be reduced when concurrently administered with ranitidine so as to overcome the problem of toxicity due to significant increase of the drug in the systemic circulation.

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# ABBREVIATIONS

|  |
| --- |
| UV UltravioletHPLC High Performace Liquid Chromatography BP British Pharmacopeia |
| SDStandard Deviation |
| SEMStandard Error of the Mean |
| RSDRelative StandardDeviation |
| MDZ Metronidazole |
| RNTRanitidine |
| WHOWorld Health Organization |
| APAAmerican Pharmaceutical Association |
| AUCArea Under Concentration Time CurveICHInternational Conference on Harmonization |

IPInternationalPharmacopeia

# CHAPTER ONE

# INTRODUCTION

# Drug Interaction

When two or more drugs are administered simultaneously or in quick succession, the response of one drug may be modified by another (Nidhi, 2012),also the response may be greater or smaller than the sum of the effects of the drugs given separately (Klaus and Jouni, 2001). One drug may potentiate or antagonize the effects of the other and in some cases there may also be qualitative difference in their response (Klaus and Jouni, 2001).

Doctors have always practiced poly pharmacy and a sound combination of drugs help to increase the efficacy and safety of drug treatment. The true prevalence of undesirable drug interactions is substantial but largely unknown.

It has been estimated that the number of death attributed to adverse drug reactions may be as high as 200,000 deaths per year in the United States (Chyka, 2000). A pharmaco- epidemiological study conducted by Kennedy *et al.* (2000) demonstrated that half the population of 1225 adult general surgical patients were taking medicines that were not related to surgery. On average these patients received nine different drugs which may interact. The Boston collaborative drug surveillance program reported a study of 9,900 patients with 83,200 drug exposures and found 3,600 adverse drug reactions, 234 (6.5%) of which were attributable to druginteractions. In a study where the medical charts of 1,800 surgical patients were reviewed researchers found at least one potential drug interaction in 17% of patient (Durrence *et al.,* 1985).

Many adverse drug interactions are the result of concomitant therapy with potent drugs. Patients treated with phenothiazines, corticosteroids, antineoplastics and many other drugs must frequently be subjected to certain adverse effects in order to obtain therapeutic benefit. A possible correlation has been noted between the significant increases in adverse effects and the use of multiple drug therapy (APA, 1997). The incidence of drug reaction increased with the number of drugs prescribed simultaneously, and drug interaction makes a small but significant contribution to the overall morbidity and mortality due to drugs.

Many of the drug interactions reported in the literature are anecdotal and have not been confirmed, nor does there exist any sound pharmacological basis for believing they could occur (Griffin and D’Arcy, 1979).

Nevertheless, individual variability is such that factors like pharmacokinetic differences and effects of disease states may have contributed to a unique reaction. Environmental factors such as smoking and atmospheric pollution or even the hardness of the water supply have also been reported to influence drug metabolism and may also be involved in contributing to a drug interaction. Other causes are dietary factors and particularly herbal remedies, of which there is increase in their usage by the population due to the mistaken belief that they are free of adverse effect, when in fact their usage is surrounded by ignorance of their pharmacology and toxicology (Dukes, 1973). Outside the hospital less information is available on the multiple usage of drugs. Patients frequently use over the counter drugs (OTC), which they prescribed for themselves along with the doctor’s prescription. These drugs frequently interacts thereby complicating drug treatment.

In the light of this information, it seems reasonable to speculate that a large number of patients may be at risk of having potentially harmful drug interaction and that an important problem in modern therapeutics might exist (Graham-Smith, 1977).

# Statement of Research Problems

Inappropriate poly-pharmacy has been reported by several studies to be an independent predictor of harmful dug-drug interaction and consequence adverse drug reaction (Nguyen *et al*., 2006; Rodriguez and Oliveira, 2016). It was reported by JensenandGugler (1983); Hasten (1984) that co-prescription of cimetidine with metronidazole resulted to pharmacokinetic interaction which led to prolonged half-life and decreased clearance of metronidazole. Ranitidine which is H2 antagonist has been reported to affect the pharmacokinetics parameters of metoprolol which led to increased Cmax and AUC (Kelly *et al*., 1983).

# Justification

Polypharmacy in our health care settings may result in drug-drug interaction which may cause modification of pharmacokinetic parameters. Common co-prescription of ranitidine with metronidazole may result to modification of the pharmacokinetic of metronidazole. There is little information on whether the pharmacokinetics of metronidazole may be significantly modified by co-administration with ranitidine.

# Aim and Objectives of the study

# Aim

The aim of study was to determine the effect of ranitidine on the pharmacokinetic parameters of metronidazole in healthy human volunteers.

# Objectives

The objectives of this study were to:-

1. sample and carryout quality control assessment test of both metronidazole and ranitidine tablets according to BP, 2009.
2. adopt and validate UV spectrophotometric method (Kolawole and Ameh, 2004) for the analysis of metronidazole in a dosage form from a biological samples via precision, accuracy and percentage extraction recovery.
3. determinethe pharmacokinetic parameters of metronidazole alone and compare between co-administered anddelayed and ranitidine in healthy human volunteers.

# 1.6 Research Hypothesis

Ranitidine has no significant effect on the pharmacokinetic parameters of metronidazole when the two drugs are co-administered.

# CHAPTER TWO

# LITERATURE REVIEW

# Drug Interaction

# Mechanism of drug interaction

Drugs may interact in a number of ways such as pharmaceutical, pharmacodynamic or pharmacokinetic basis. A number of drugs may also interact at several different sites. Such cases represents drug – drug interactions. Other forms of interactions also do occur. Therefore, the concept of drug interactions also includes the modification of drug effects by food or dietary items (drug – food interactions).

* + - 1. *Pharmaceutical drug interactions*

Pharmaceutical interactions normally occur before the drug in given to the patient. They may be caused by several different mechanisms. When thiopentone with vecuronium were injected through the same given set, a precipitate will form instantaneously. Numerous incompatibilities have been demonstrated, and drugs should never be mixed in this fashion unless the absence of reaction has been clearly established (Trisel, 1994).

Pharmaceutical interactions can also be described as physicochemical incompatibilities, which are unintentional interactions that occur *in vitro* between drug and other component of medicinal products during their preparation, storage or administration. Drug-drug, drug- excipient, excipient-excipient, drug-packaging and excipient-packaging are all interactions that may cause adverse effects on bioavailability, efficacy or toxicity.

Another important area of drug interaction of clinical important occurs when drugs are added to intravenous infusion. Over the last ten years the practice of administering drug by continuous intravenous infusion has become more common, particularly in surgical unit.

The problem is mainly produced by incompatibility between the drug added and component of the infusion fluid (Kramer *et al.,* 1974).

* + - 1. *Pharmacokinetic drug interactions*

Pharmacokinetic drug interactions can be divided into interactions that occur during absorption, distribution, metabolism and elimination. In some cases, drugs may interact simultaneously during several different phases of passage of the drug through the body (Wandalkar *et al.,* 2015).

* + - 1. *Absorption interactions*

Drugs can influence the absorption of other drugs at least by altering the gastrointestinal pH and motility, by intraluminal binding or the chelation of drug, by changing the regional blood flow, by inhibition or stimulation of first pass metabolism, or through toxic effects on the gastrointestinal canal. The subcutaneous and intramuscular absorption of drugs can be delayed or decreased after the administration of drugs affecting regional blood flow (vasoactive agents). However, these mechanisms have no major importance in pharmacokinetics (Klaus and Jouni, 2001).

* + - 1. *Distribution interactions*

The mechanisms of drug interactions may alter drug distribution by competition for plasma protein binding, displacement from tissue binding sites, and alterations in local tissue barriers, for example, P-glycoprotein inhibition in the blood-brain barrier (Wandalkar *et al.,* 2015)

* + - 1. *Metabolism interaction*

The metabolism of drugs may be stimulated or inhibited by concurrent therapy, and the importance of the effect varies from negligible to dramatic (Wandalkar *et al.,* 2015).The

biotransformation of drugs during the first – pass and during elimination from the systemic circulations in the liver, is usually divided into phase I and phase II reactions. Many drugs are lipophilic and cannot be excreted through the kidneys until they have been transformed into more favourable water soluble forms. Phase I reactions include oxidation, reduction and hydrolysis. Phase I reactions add a functional group to the drug, where as phase II reaction are conjugation reactions in which the drug or its metabolite is attached to a water soluble molecule, such as glucuronic acid, glutathione, sulphatic group, acetyl group, methyl group or glucosamine, making the whole complex more hydrophilic. Oxidation is the most important phase I reaction catalysed by cytochrome P450 (CYP450) enzymes. Most metabolic drug interactions involve either the induction or inhibition of cytochrome P450 enzymes (Levy *et al*., 2000). Oxidation reactions include aromatic and aliphatic hydroxylation, oxide formation, desulfurization, deamination, dehalogenation, NO and S dealkylation and sulfoxidation. Reduction reactions include azoreduction, aldehyde reduction, and nitro reduction while hydrolytic reactions include de-esterification and deamination. Oxidative and reducing enzymes are found primarily in the liver microsomes. Hydrolytic enzymes are located in the plasma, liver microsomes and many other tissues. Cytochrome P450 (CYP450) enzymes are characterised by a maximum absorption wavelength of 450nm in the reduced state in the presence of carbon monoxide. According to the homology of their amino acid sequence, the CYP enzymes are divided into families, sub families and specific iso enzymes. CYPI, CYP2 and CYP3 are involved mainly in the metabolism of drugs and other xenobiotics, where as those belonging to the families CYP4, CYP5 and CYP7 have endogenous functions (Levy *et al.*, 2000). So many drugs and environmental chemicals are implicated in cytochrome P450 enzymes induction and

inhibition. Cytochrome P450 enzyme system inducers include phenobarbital and many other drugs and environmental chemicals, including chlorinated hydrocarbon, insecticides carcinogenic hydrocarbons, food additives and cigarette smoke (Conney and Burns, 1972). Inhibitors of cytochrome P450 enzymes such as phenylbutazone and imidazole compounds have since been recognized (Powell and Donn, 1984). The activities of the cytochrome P450 dependent system are extremely sensitive to difference in sex, age strain and species and to differences in the hormonal and nutritional state of animal (Conney and Burns, 1972).

Co-administration of the inhibitor and the substrate of any CYP enzyme will result in an increase of the substrate concentrations. The magnitude of the increase depends on the inhibitor and its dose. An example is ketoconazole which increased the AUC for oral triazolam approximately 30times compared with the administration of triazolam with placebo (Varhe *et al*., 1994). But the AUC of oral midazolam was increased approximately

16 times (Olkkola *et al.,* 1999). Many lipid-soluble compounds such as barbiturates, phenytoin, carbamazepine and also ethanol cause a stimulation of drug metabolism through the induction of hepatic microsomal enzymes. The administration of the inducing drug causes stimulation not only of its own metabolism, but also the metabolism of many unrelated drugs which are substrates for microsomal enzymes. Ritonavir is a protease inhibitor used in the treatment of HIV infection. A 2-day ritonavir treatment greatly increases the concentration of intravenous fentanyl by reducing fentanyl clearance. Because fentanyl clearance was reduced by 67%, it can be calculated that ritonavir treatment results in approximately threefold increase in fentanyl concentrations (Olkkola *et al.,* 1999). However, the AUC of norpethidine was increased suggesting the induction of hepatic pethidine metabolism by ritonavir (Piscitelli *et al.,* 2000). It was shown that paracetamol, a

substrate of the CYP2E1 andCYP3A family does not affect the pharmacokinetics of fentanyl at clinically relevant concentrations (Feierman, 2000). Ropivacaine is a local anaesthetic, which is metabolised mainly by CYP1A2 but also by CYP3A4. It’s clearance is reduced by 77% by concomitant CYP1A2 inhibitor, fluvoxamine. Erythromycine a CYP3A4 inhibitor alone only had a minor effect on the pharmacokinetics of ropivacaine. However, compared with fluvoxamine alone, the combination of fluvoxamine and erythromycine further increased the area under the drug plasma concentration time curve by 50% (Jokinen, 2000). Recent studies have shown that many dietary supplements and natural products can modify the pharmacokinetics of drug. For instance, St. Johns wort (Hyperuricum perforatum) a plant used as an antidepressant in the United States is a potent inhibitor of CYP3A4 and can have potentially hazardous drug interactions when used with the substrates for CYP3A4 (Fugh – Berman, 2000).

* + - 1. *Excretion interactions*

Drugs are eliminated by renal excretionthrough glomerular filtration, tubularreabsorption and active tubular secretion. Aco-administered drug can influence any ofthese processes and the resultant change indrug elimination will increase or decreasesome pharmacokinetic parameters (Wandalkar *et al.,* 2015)

Drug interactionsinvolving this mechanism are gradual (notimmediate) and continue for some time afterwithdrawal of the inducing agent. There is alsoa wide range of drugs that inhibit metabolismof other drugs. Cimetidine (a popular antiulcerdrug) for instance, inhibits thebiotransformation, and hence increases theplasma levels of a variety of drugs includingopioid analgesics, mebendazole, warfarin,imipramine, nortriptylline, metronidazole (Wandalkar *et al.,* 2915). Changes in urine pH increase the ionized fraction of

the drug in the urine which increases the excretion rate of the drug. This has been extensively investigated with Phenobarbital and alkalinization of the urine (Waddel and Buter, 1957) and had been used for treating patients with phenobarbital poisoning (Lassen, 1960). Change in renal function can modify a number of pharmacokinetic processes in the body and thereby lead to unanticipated drug effect or interaction (Reidenberg, 1977). Drug excretion is slowed in patients with impaired renal function.

* + - 1. *Pharmacodynamic interactions*

Pharmacodynamic interactions are those where the effects of one drug are changed by the presence of another drug at its site of action. Sometimes the drugs directly compete for particular receptors (e.g. beta2 agonists, such as salbutamol, and beta blockers, such as propranolol) but often the reaction is more indirect and involves interference with physiological mechanisms (Pharmaceutical Press, 2008). Other drugs can occupy these specific receptors without producing any response and in this way prevent or reverse the effect. Example is reversal of the antihypertensive effect of guanethidine by amphetamine. Guainethidine act by blocking nerve transmission on adrenergic neurounes. Amphetamine displaces it from these neurones and thus abolished its action. Frequently overlooked is the multiplicity of effect of many drugs. Thus, phenothiazines are effective α-adrenergic antagonists; many antihistamines and tricyclic antidepressants, are potent antagonists at muscarinic receptors. These “minor” actions of drugs may be the cause of drug interactions (Goodman andGilman, 1996). When drugs with similar pharmacologic effects are administered concurrently, an additive or synergistic response is usually seen. Conversely, drugs with opposing pharmacologic effects may reduce the response to one or both drugs. Pharmacodynamic drug interactions are relatively common in clinical practice, but adverse

effects can usually be minimized if one understands the pharmacology of the drugs involved (Wandalkar *et al.,* 2015). From the foregoing we see four different kind of pharmacodynamic drug interactions.

1. Enhanced effects produced by two drugs acting at same site. An example is streptomycin (weak depolarising properties) in the presence of a depolarizing muscle relaxant (Toivakka and Hokkanen, 1965).
2. The increased effects produced by two drugs acting at different receptor sites (potentiation). This usually results in an effect, which is greater than the sum of the component effectse.g.Antihypertensive drugs.
3. Enhanced effects of a drug by one which is devoid of action itself. Example of increased anticoagulant effect of warfarin with clofibrate (Solomon and Shrogie, 1967).
4. Antagonsim of the effect of one drug by another. An example is naloxone action at opioid receptors.

# Forces involved in drug macromolecular interaction

Generally, the interaction between a therapeutically useful drug and macromolecule is reversible

Viz:-

Macromolecule + drug Macromolecule-drug complex (Unbound) (Bound drug)

This means that covalent bonds, which are very stable at body temperature, are not involved in most interactions between drug and macromolecules in humans. Because of the high bond strengths, covalent bonds are not likely to be broken down, unless specific enzyme is present to break the bond and this may lead to serious drug interactions. However, there are two

types of drug-macromolecule interaction that involve the formation of covalent bonds. The first type is the alkylation or arylation of cell constituents by certain drugs that are respectively alkylating or arylating agents.

# Ranitidine

Ranitidine is a histamine H2-receptor antagonist that competitively inhibits gastric acid secretion by parietal cells in the gastric mucosa (Mycek *et al*., 1997).Ranitidine was first prepared as AH19065 by John Bradshaw in the summer of 1977 in the Ware research laboratories of Allen and Hanburys Ltd, part of the Glaxo organization (Lenidcer and Daniel, 1993). It was introduced in 1981 and was the world’s top-selling prescription drug by 1988. It is used in the treatment of Duodenal and Gastric Ulcer caused by *Helicobacter pylori* infection, and for the treatment of Gastroesophageal Reflux Disease (Sweetman, 2009). It has been reported to be four times more active on a molar basis than cemitidine in inhibiting pentagastrin stimulated gastric acid secretion in man (Domschke *et al*., 1979). The drug has a short biological half-life of approximately 2.5-3.0 h an absolute bioavailability of only 50% in oral forms (Dave *et al*., 2004). Its popular trade name is Zantac® from GlazoSmithkline.

# Chemistry of ranitidine

Chemically, ranitidine is a substituted furan compound with a chemical nomenclature of Dimethyl {5-[2-(1-methylamino-2-nitrovinylamino)-ethylthiomethyl]-furfuryl} amine hydrochloride (BP, 2009). Its structural formula is represented in Figure 2.1. The physical and chemical properties are described in Table 2.1.

# Table 2.1 Physicochemical Properties of Ranitidine

|  |  |
| --- | --- |
| 1. Molecular Formula | H22N4O3SHCl |
| 2. Molecular weight | 350.87g/mol |
| 3. Melting point | 133-134oC |
| 4. Appearance | White or pale yellow crystalline powder |
| 5. Odour | Suphur-like odour |
| 6. pH | 4.5-6.0 mg/ml in water |
| 7. Solubility | Freely soluble in water, methanol and ethanol (95%),sparingly soluble in chloroform and in Dichloromethane. |
| 8. Storage and Stability | Store between 2-30oC for tablets, 2-25oC for injection andprotect from light. |
| 9. Loss on drying | Losses not more than 0.75% (1g, in vacuum, 60oC, 3h). |

# Synthesis of ranitidine

Ranitidine is formed from the Mannich based reaction of paraldehyde (II) and dimethyl amine (I) to give 5-(N,N-dimethyl amino methyl) furfuryl alcohol (IV). Thioaminoethylation of (IV) produce (V). Conjugate addition of (V) with α, β- unsaturated nitro compound i.e.1-methylthio-1-(N-methyl amino)-2-nitroethylene (VI) yield the target molecule ranitidine.

H3C

H3C

NH + (CH2O)n +

Paraldehyde

II O

Mannich reaction

CH2OH

H2C

CH2OH

O IV

Dimethylamine hydrochloride

I

2-Furfuryl alcohol III

H3C

N

CH3

HSCH2CH2NH2

VI

H3CS NHCH3

C

H

H3C

CHNO2

+ N

C

H3C H2 O

CH2SCH2CH2N H2

H2C

N

O CH2SCH2CH2NHCNHCH3 V

Ranitidine

H3C

CH3

VII

CHNO2

**Figure 2.1 Synthesis of ranitidine** (Alagasarmy, 2010)

# Structure activity relationship of ranitidine

Ranitidine is a second generation H2-antagonist but differs fundamentally from cimetidine in having a furan ring instead of imidazole ring as represented in Figure 2.3 below;

I H3C CH2SCH2CH2NHCNHCH3

H2CN(H3C)2

CH2S(CH2)2NHCNHCH3 HN N

O

NCN

Ranitidine

CHNO2

II

Cimetidine

# Figure 2.2 Structure of ranitidine and cemetidine

* + - 1. *The Ring System*

The imidazole ring was a key structural feature of cimetidine and related H2-antagonists (Durant*et al*., 1981). It was concluded that this ring appeared to have special importance at H2-receptors. The possibility that a basic heterocyclic might not be essential for H2-

antagonist activity was investigated by replacing it with a non basic ring system to which a basic function might readily 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 H2-blocking activity to the imidazole.Replacing the furan ring with more hydrophobic rings such as phenyl or thiophene reduces activity. This could be explicable in terms of increased lipophilicity down the series.

* + - 1. *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 derivative were shown to have similar activities to that exhibited by the 4-methyl group.

* + - 1. *Modification of Basic Function*

H2CN(H3C)2

Basic Function O

CH2SCH2CH2NHCNHCH3

Methylthio

Ring system

ethyl chain

CHNO2

Neutral end group

# Figure2.3Substituents of Ranitidine Structure

Substitution on the basic (dimethylamino) group attached to furan ring may be varied, showed that the basicity and hydrophobicity of this group are not crucial to activity. Secondary amines are equivalent with tertiary amines and variation in lipophilicity appeared not to have 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.

* + - 1. *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 sulphur atom by a further methylene group led to a compound which possessed one-third of activity. Attachment of the sulphur directly on to the furan ring resulted in an isomer with about 1/10th the potency of the parent compound.

*2.1.2.5 Modification of the Neutral End Group*

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

CH2SHCH2CH2NHCNHCH3

X

# Figure2.4 Terminal N-group of Ranitidine

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

*2.1.3.6 Ring substitution pattern*

Ring substitution pattern in the ranitidine molecules is of the 2, 5-disubstitution type, which is the best substitution pattern for the furan ring. The five other possible isomers all gave compounds that were substantially less active than the original compound.

* + 1. *Pharmacokinetics of ranitidine 2.1.3.1Absorption*

Ranitidine is readily absorbed from the gastrointestinal tract with peak concentration in plasma occurring about 2 to 3 h after oral administration (Hardman, 2001; Sweetman, 2007). The oral bioavailability of ranitidine is about 50%. Drug is rapidly absorbed when

administered via the oral route and absorption after oral administration is linear (Katzung, 2004; Kortejarvi *et al*., 2005; Zhou *et al*., 2006). Ranitidine is highly water soluble and has pKa values of 8.2 and 2.7 (Roberts, 1984). The bioavailability of ranitidine is significantly lower when administered as a solution directly to the colon instead of stomach, jejunum, or ileum. Food in general has no effect on the rate and extent of absorption of drug (Kortejarvi *et al*., 2005). Ranitidine is rapidly absorbed on intramuscular injection, resulting in peak plasma concentration occurring in about 15 minutes (Sweetman, 2007).

* + - 1. *Distribution*

The apparent volume of the distribution for terminal phase is about 1.16-1.87 l/kg. It is weakly bound, about 15% to plasma proteins (Katzung, 2004; Kortejarvi *et al*., 2005). Ranitidine crosses the placental barrier and is distributed into breast milk (Sweetman, 2007).

* + - 1. *Metabolism*

A small proportion of ranitidine is metabolized in the liver (Hardman, 2001; Zhou *et al*., 2006). The hepatic metabolism of the drug results in production of metabolites i.e. N-oxide, the S-oxide and desmethylranitidine as in Figure (2.5); the proportions of the various metabolites varies from one species to another and is also affected by the mode of administration. In man, N-oxide is the major metabolite but accounts for only about 4-6% of a dose. After intravenous administration, the recovery of unchanged ranitidine plus its three major metabolites does not amount to 100%. This has been elucidated as revealing biliary excretion of some metabolites of ranitidine. In most studies, reduced bioavailability of ranitidine and reduced recovery of unchanged ranitidine in the urine after oral administration signify high pre-systemic hepatic metabolism (Koch *et al*., 1997; Katzung, 2004; Zhou *et al*., 2006;Sweetman, 2007).

H3C

N

H3C

H2C

CH2SCH2CH2NHCNHCH3

O

O (I)

C

O2N NO2

H3C

N

H

H2C

CH2SCH2CH2NHCNHCH3

O

(II)

C

O2N NO2

H3C

N

H3C

H2C

CH2SCH2CH2NHCNHCH3

O

Ranitidine

C

O2N H

H3C

N

H3C

H2C

CH2SCH2CH2NHCNHCH3

O

(III)

O

O2N

C

NO2

**Figure2.5 Metabolic pathway of ranitidine in animals and man** (Bell *et al*., 1980)

* + - 1. *Excretion*

About 30% of an oral dose and 70% of an intravenous dose is excreted unchanged in the urine in 24 h, primarily by active tubular secretion. Of orally administrated ranitidine, 26%

is excreted through the faeces also. Elimination half life is about 2 to 3 hour and is increased in renal impairment. In most studies, the elimination half-life after oral administration has been longer than that of after intravenous administration (Roberts, 1984). Renal clearance values amount to some 70% to 80% of total clearance, indicating that renal excretion is the major route of elimination of unchanged ranitidine after intravenous administration (Koch *et al*., 1997;Katzung, 2004; Zhou *et al*., 2006; Sweetman, 2007).

# Mode of action of ranitidine

Ranitidine exhibit competitive inhibition at the parietal cell H2 receptor, and suppress basal and meal stimulated acid secretion. H2-receptor antagonists inhibit acid production by reversibly competing with histamine for binding to H2 receptors on the basolateral membrane of parietal cell(Rang *et al.,* 2003; Katzung *et al*., 2004). H2 antagonists reduce acid secretion stimulated by histamine as well as by gastrin and cholinomimetic agents through two mechanisms. Firstly, histamine released from enterochromaffin-like (ECL) cells by gastrin or vagal stimulation is blocked from binding to the parietal cell H2 receptor. Secondly, direct stimulation of the parietal cell by gastrin or acetylcholine results in diminished acid secretion in the presence of H2 receptor blockade (Katzung *et al*., 2004).

The most prominent effects of H2 receptor antagonist are on basal acid secretion and less profound but still significant are suppression of stimulated acid production. These agents are effective in suppressing nocturnal acid secretion, which reflects mainly basal parietal cell activity (Pahwa *et al*., 2010).

# Administration and dosage

* + - 1. *Duodenal ulcer or benign gastric ulcer*

Dose of 300 mg of ranitidine once daily at bedtime or 150 mg twice daily taken in the morning and before retiring, it is not necessary to time the dose in relation to meals. In most cases of duodenal ulcer and benign gastric ulcer, healing will occur in four weeks. In the small number of patients whose ulcers may not have fully healed, these are likely to respond to a further four week course of therapy. In the treatment of duodenal ulcers, 300 mg twice daily for 4 weeks may be of benefit when more rapid healing is desired.

* + - 1. *Maintenance therapy*

Duodenal ulcers, benign gastric ulcers: Patients who have responded to short-term therapy, particularly those with a history of recurrent ulcer, may benefit from chronic maintenance therapy at a reduced oral dosage of 150 mg once daily at bedtime.

In the management of duodenal ulcers, smoking is associated with a higher rate of ulcer relapse (up to 9.2 times higher in one trial), and such patients should be advised to stop smoking. In those patients who fail to comply with such advice, 300 mg nightly provides additional therapeutic benefit over the 150 mg once daily dosage regimen.

* + - 1. *Reflux esophagitis*

300 mg once daily at bedtime or alternatively 150 mg twice daily, taken in the morning and before retiring for up to eight weeks. In patients with moderate to severe esophagitis, the dosage of ranitidine may be increased to 150 mg four times daily for up to 12 weeks.

# Parenteral administration

In some hospitalized patients with pathological hypersecretory conditions or intractable duodenal ulcers, or in patients unable to take oral medication, ZANTAC® may be administered parenterally according to the following recommendations:

* + - 1. *Intramuscular Injection*

Ranitidine 50 mg equivalent to 2 ml every six to eight hours (no dilution is required).

* + - 1. *Intravenous Injection*

Every six to eight hours50 mg dilute ZANTAC® Injection, the 50 mgis compatible IV solution to a total volume of 20 ml and inject over a period of not less than five minutes. Parenteral administration may continue until oral feeding is commenced and, if there is still a risk, oral ZANTAC® may then commence.

* + - 1. *Intermittent Intravenous Infusion*

Every six to eight hours, dilute ZANTAC® Injection 50 mg in 100 ml of compatible IV solution and infuse over 15 to 20 minutes. In some patients, it may be necessary to increase dosage. When this is required, the increases should be made by more frequent administration of a 50 mg dose, but generally should not exceed 400 mg per day.

In the prophylaxis of upper gastrointestinal hemorrhage from stress ulceration in seriously ill patients, a primary dose of 50 mg as a slow (over a period of not less than five minutes) intravenous injection followed by a continuous intravenous infusion of 0.125 - 0.250 mg/kg/hr may be preferred. The higher infusion concentration (0.25 mg/kg/hr) should be reserved for patients who are unresponsive to a lower concentration (0.125 mg/kg/hr).

In the prophylaxis of hemorrhage from stress ulceration in seriously ill patients or prophylaxis of recurrent hemorrhage in patients bleeding from peptic ulceration, parenteral

administration may continue until oral feeding is commenced and if there is still a risk, oral ZANTAC® may then commence.

For patients considered at risk of developing acid aspiration syndrome (Mendelson's Syndrome), 50 mg by intramuscular or slow (over a period of not less than 5 minutes), intravenous injection45-60 minutes before induction of general anaesthesia. In an emergency situation, the use of alkalis, antacids, and meticulous anaesthetic technique is still necessary as ZANTAC® does not affect the pH and volume of the existing gastric content.

# Toxicity and adverse effect of ranitidine

Ranitidine hydrochloride is one of the most extensively studied and widely used drugs of all time. This has provided an excellent opportunity to define its safety profile. However, the use of this drug may associated with some side effects which include headache, skin rashes, tiredness, constipation, nausea, diarrhea, bradycardia, hypersensitivity, contact dermatitis, urticaria etc. A case of severe anaphylaxis to ranitidine is also reported (Dawson *et al*., 1983; Foti *et al*., 2009; Chopra *et al*., 2014). In addition, a case of ranitidine-induced photosensitivity is also reported (Todd *et al*., 1995). Ranitidine, when given in conventional doses, can cause adverse central nervous system reactions as lethargy, confusion, somnolence and disorientation particularly in older patients who have substantial renal function impairment (Slugg *et al*., 1992). Leucocytosis and easinophilia has been reported by Gelwan *et al.* (1986), in a patient receiving ranitidine theraphy. Acute renal failure is one of the risk factors of ranitidine neurotoxicity, and that increased sensitivity of the central nervous system to the drug which may contribute toward its toxicity in renal failure (Nakada *et al*., 1996).

# Ranitidine hydrochloride interaction with other drugs

The pharmacokinetic interaction occurs when the absorption, clearance or rarely, the distribution of one drug is modified by another. Several drug interactions of ranitidine hydrochloride are listed in Table 2.3 below.

# Table 2.2 Drug Interaction of Ranitidine Hydrochloride

|  |  |
| --- | --- |
| **Interactant** | **Interaction** |
| Metoprolol | Increases mean peak plasma concentration |
| Procainamide | Reduction in renal clearance |
| Warfarin | Decreases clearance |
| Nifedipine | Increases the plasma area under curve |
| Midazolam | Increases bioavailability |
| Chloromethiazole | Prolonged the elimination half-life |
| Ketoconazole | Decrease in bioavailability |
| Glipizide | Increases the plasma area under curve |

Adapted from Pahwa *et al*. (2016)

# Metronidazole

Metronidazole is the drug developed against a parasite, which has gained broad use as an antibacterial agent. Briefly, at Rhone-Poulenc in France, extracts of*Streptomyces* species were screen for activity against *Trichomonas vaginalis*, a cause of vaginalitching. A Nitroimidazole, Azomycin, was identified, and a synthetic derivative, metronidazole,was used to treat chronic trichomonad infections, beginning in 1959. The antibacterial activityof metronidazole was discovered by accident in 1962 when metronidazole cured a patient of both*Trichomonad vaginitis* and bacterial gingivitis.

Also, the isolation of the antibiotic, azomycin (2-nitroimidazole) from a Streptomyces byMaeda and collaborators in 1953 and the demonstration of its trichomonocidal properties byHorie in 1956 led to the chemical synthesis and biological testing of many nitroimidazole such as 1-(β-hydroxyethyl)-2-methyl-5-nitroimidazole(FLAGYL®). It was observed that metronidazole has high activity *in vitro* and *in vivo* againstthe anaerobic protozoa like*Trichomonas viginalis* and *Entamoeba histolytica*. Dunel and associates(1960) reported that oral doses of the drug imparted trichomonacidal activity to semen and urineand that high cure rates could be obtained in both male and female patients with trichomoniasis(Phillips and Stanley, 2006). Later studies revealed that metronidazole has extremely usefulclinical activity against varieties of anaerobic pathogens that include both gram positive andgram negative bacteria, in addition to the protozoan *Giardia lamblia*. In 1964, a dentist knownas Shinn noted that patient with gingivitist was cured with flagyl® (Phillips and Stanley, 2006). Itwas also the first drug to have a cure rate approaching 100 % per cent with systemic treatment(Cudmore, *et al.,* 2004). Presently, metronidazole which is inexpensive has good penetration and produces relatively few side effects, is on the formulary at most hospitals for prophylaxis against anaerobic infectionafter bowel surgery, for treatment of wound abscess and for treatment of associatedcolitis caused by *Clostridium difficile*.

# Chemistry of metronidazole

Metronidazole is (2- (2 methyl-5- nitromidazole-1-yl) ethanol) (Kolawole and Ameh, 2004;BP, 2009;Usman *et al.*, 2011). However, it can be named as [1-(2-hydroxyethyl)-2- methyl-5-nitroimidazole] (Houghton *et al*., 1982 and Phillips and Stanley, 2006;Alves *et al.*, 2007). It is a synthetic antimicrobial agent with activity against obligate anaerobic bacteria

and protozoa (Kolawole, 2004; Mustapha *et al.,* 2006). It is a prototype of nitroimidazole class of antimicrobials (Ezzeldin and El-Nahhas, 2012). It is white or yellowish, odourless crystals or crystalline powder. It darkens on exposure to light. Its formula is C6H9N3O3 with a molecular mass of 171.2 g/mol.

# Synthesis of metronidazole

The synthesis involved in two stages. The first stage, the glyoxal (I) and ammonia (II)under go cyclization reaction to give diimine.In the second stage diimine condenses with formaldehyde (III) to form methylimidazole (IV). 2-methylimidazole is nitrated to give 2- methyl-4(5)-nitroimidazole (V), which is in turn alkylated with 2-chloroethanol in the presence of sodium hydroxide to give metronidazole (VI).

N

CHO CHO

Glyoxal I

+ 2NH3 II

Cyclization

+ CH3CHO

III

H3C

IV

N H

H2SO4

HNO3

H3C

N CH2CH2OH

Metronidazole VI

N

NO2

NaOH HOCH2CH2Cl

H3C

N

N H

V

NO2

**Figure 2.6Synthesis** (Alagarsamy, 2010

# Table 2.3 Physicochemical properties of metronidazole

|  |  |
| --- | --- |
| 1. | Molecular formula: C6H9N3O3 |
| 2. | Description: White or yellowish, crystalline powder with slight odor and bitter taste (BP2009) |
| 3. | Melting point: 159 -163oC (BP 2009) |
| 5. | Pka: 2.5 (Castela-Papin, 1999) |
| 6. | Molecular weight: 171.15 g/mol |
| 7. | pH: 5.8 (saturated aqueous solution) |
| 8. | Solubility: It is sparingly soluble in water, alcohol or chloroform and slightly soluble inether (Mofat, 1986; Gennaro,1990; USP 1990; BP2009) |
| 9. | Stability:Stable in air but darkens on exposure to light |
| 10. | Loss on drying: maximum 0.5% determined on 1.000g by drying in oven at 80oC for 3h |

# Structure activity relationship of metronidazole

The antiprotozoal and antibacterial activity of the compounds resides in the nitroimidazole structure.The 1-(2-hydroxyethyl) side chain at position 1 of metronidazole is readily oxidized metabolically, replacement of this side chain resulted in the development of other clinically useful antibacterial and antiprotozoal drugs, like tinidazole (Kapoor *et al.,* 2003) as shown in Figure 2.7.

CH2CH2SO2CH2CH3

OH

O2N

CH O2N CH3

Metronidazole Tinidazole

N

N

N

N

3

# Figure 2.7Structures of metronidazole and tinidazole

The replacement of ethyl alcohol substituents with methyl group and introduction of carbamate group at position 2 lead to the formation of ronidazole which is used in veterinary practice for the treatment and control of swine dysentery (Kapoor *et al.,* 2003).

CH3

O2N N

N

CH2COONH2

Ronidazole

# Pharmacokinetic of metronidazole

* + - 1. *Absorption*

The absorption of metronidazole has been studied in a variety of dosage forms including oral tablets, infusions, vaginal-rectal suppositories, and topical gel. The oral absorption of metronidazole is excellent, with bioavailability often reported as >90% (Bergan and Arnold, 1980; Bistoletti *et al*., 1987; Loft *et al*., 1987). The peak plasma drug concentration (Cmax) after a single dose of 500 mg is approximately 8 to 13 μg/l, with a corresponding time (Tmax) of 0.25 to 4 h (Ralph *et al.,* 1974; Bergan and Arnold, 1980).

* + - 1. *Distribution*

It is widely distributed in body tissues including saliva, breast milk and cerebrospinal fluid in concentrations equivalent to those in plasma or serum (http://www.nlist.com). It also crosses the placenta and rapidly enters the foetal circulation. Protein binding of metronidazole is less than 20% (Ralph *et al*., 1974; Shwartz and Jeunet, 1976).

* + - 1. *Metabolism*

Metronidazole undergoes extensive hepatic biotransformation with subsequent renal elimination (Stambaugh *et al*., 1968; Jensen and Gugler, 1983). The major metabolites found in urine result from the hydroxylation of the 2-methyl group (2- hydroxymetronidazole), oxidation of the 1-ethyl group (1-metronidazole acetic acid), and glucuronide conjugation on the 1-ethyl group as depicted in Figure 2.8. A sulfate conjugate, a further oxidation product of 2-hydroxymetronidazole and a glucuronide conjugate of 2- hydroxymetronidazole have also been identified as minor metabolites (Stambaugh *et al.*, 1968; Lamp *et al*., 1999). Although the parent compound predominates in plasma, along with smaller concentrations of 2-hydroxymetronidazole (Jensen and Gugler, 1983), in urine 2-hydroxymetronidazole is the primary metabolite formed from metronidazole, and accounts for 40–50% of the total metronidazole species present in 24-hour urine samples (Stambaugh *et al*., 1968). Both metronidazole and 2-hydroxymetronidazole have in vitro activity against most anaerobic bacterial strains (O’Keefe *et al.,* 1982). The acetic acid metabolite is reported to occur at detectable levels only in patients with renal dysfunction (Lamp *et al.,* 1999).

O2N

N

N

CH3

O2N

N

N

CH2COOH

CH2CH2OH

CH3

OH

HOOC OH

1- Metronidazole acetic acid

Metronidazole

O

O

CH2CH2

CH2CH2OH

O2N

N

CH3

O2N CH2OH N

N

N

Metronidazole glucuronide

2-Hydroxymetronidazole

# Figure 2.8 Metabolic pathways for the conversion of metronidazole to its major metabolites (Pearce *et al*., 2013)

* + - 1. *Excretion*

The elimination half-life of metronidazole is about 8 hours andthat of hydroxyl metabolite is slightly longer. The half-life of metronidazole is reported to be longer in neonates and in patients with severe hepatic impairment; that of the hydroxyl metabolite is prolonged in patients with substantial renal impairment. The majority of a dose of metronidazole is excreted in the urine, mainly as metabolites; a small amount appears in the faeces (Martindale, 1999).

# Mode of action of metronidazole

Metronidazole and other nitroimidazoles are only toxic once they become reduced, and bioreduction requires low redox potential electron-transfer proteins (e.g nitroreductases such as ferredoxin) to unidentified polar products which lack the nitro group. The reduction

products appear to be responsible for the cytotoxic and antimicrobial effects of the drug which include disruption of DNA and inhibition of nucleic acid synthesis (IP, 2010; Metronidazole Data Sheet, 2013).

.In general, the single-electron reduction potential of nitroimidazoles lies outside the normal range of mammalian redox systems. One-electron reduction of nitroimidazoles leads to an unstable nitro radical anion that can either decompose to a nitrite anion and an imidazole radical or be further reduced by accepting a second electron (Edwards, 1993).

# Toxicity and adverse effect

Metronidazole is a nitroimidazole based drug used as an antiprotozoal and antibacterialagent (especially for anaerobes). It is registered for pet animals and birds, but forbidden for the use in animals used for food production. Metronidazole exhibits much adverse effects, including anorexia, nausea, vomiting and diarrhea, neurological symptoms especially cerebella and vestibular dysfunction, changes in blood count and toxic action on liver (Hsu, 2008). These symptoms may appear in acute overdose, but also during the chronic treatment with therapeutic doses (Plumb, 1999). Reports on neurotoxicity are known for dogs, cats and also humans (Caylor and Cassimatis, 2001; Evans *et al.*, 2003; Olson *et al*., 2005; Kuriyama *et al*., 2011) but the mechanism of toxic action remains unclear. In overdose, the spectrum of clinical signs involves also mydriasis, proprioception deficit, rigidity or seizures. Less common effects described in humans include pancreatitis, pseudomembranous colitis, peripheral neuropathy (Caylor and Cassimatis, 2001). Moreover, metronidazole was revealed as a potential teratogen in the studies on laboratory animals. It has not been proven in dogs and cats, but it is still not recommended to use this substance in pregnant animals

(Plumb, 1999). In a study on cats its genotoxicity at therapeutic doses was reported (Sekis *et al*., 2009).

# Metronidazole interaction with some drugs

* + - 1. *Alcohol*

Metronidazole may provoke a disulfiram like reaction in some individuals when given with alcohol; reactions have occurred after the use of preparations formulated with alcohol, including injections, as well as after drinking alcohol. Acute psychosis or confessional state was reported in 6 of 29 alcoholic patients who were also receiving disulfiram. However an analysis of published reports and a study in healthy subjects both found that there was no convincing evidence of a disulfiram-like reaction between metronidazole and alcohol although caution was still advised (Martindale, 1999).

* + - 1. *Busulfan*

The use of busulfan with metronidazole significantly increased plasma concentrations of busulfan and the degree of associated toxicity, including elevations of liver function tests, venoocclusive disease and mucositis (Martindale, 1999).

* + - 1. *Antacid, kaolin- pectin or colestyramine*

The absorption of metronidazole from the gut is unaffected by kaolin- pectin, but a small reduction occurs if either an aluminium hydroxide antacid or colestyramine is given concurrently (Pharmaceutical Press, 2005).

* + - 1. *Barbiturates*

Phenobarbital markedly increases the loss of metronidazole from the body so that larger doses are needed. Conventional doses of metronidazole in the presence of Phenobarbital

failed to clear up trichomoniasis in a woman, and giardiasis or amoebiasis in children (Pharmaceutical Press, 2005).

* + - 1. *Disulfiram*

Acute psychosis and confusion can result from the concurrent use of metronidazole and disulfiram (Pharmaceutical Press, 2005).

* + - 1. *Carbamazepine*

A patient receiving carbamazepine for bipolar disorder developed dizziness, diplopia and nausea4 days after the addition of metronidazole for diverticulitis (Martindale, 1999).

* + - 1. *Phenytoin*

In addition to conflicting reports on the effect of metronidazole on the metabolism of phenytoin, increased metabolism of metronidazole was reported in patient during treatment with phenytoin(Martindale,1999).

# Basic Pharmacokinetics

Pharmacokinetics is the study and characterization of the time course of drug absorption, distribution, metabolism and excretion, and with the relationship of these processes to the intensity and time course of therapeutic and adverse effects of drugs (Gibaldi and Levy, 1976). A drug exerts its pharmacological effect only when it reaches its site of action, thus it must first reach the general circulation and this requires the crossing of the physiological barrier (GIT) for orally administered drug and the process being known as absorption, the fraction absorbed is known as the absorption coefficient of the drug. The drug enters the portal circulation after absorption, then to the liver where it is metabolized or transformed in to metabolite that are more water soluble, hence more easily eliminated, a phenomenon known as hepatic first pass effect. The net effect of absorption and first pass effect

determines greatly the bioavailability of a drug. On reaching the systemic circulation, a drug will first interact with erythrocytes and plasma proteins where it undergoes some binding. It is then transported to all the tissues to a different extent and this phase is referred to as distribution phase of drug. After distribution of drugs in to various tissues, several elimination processes occur. These processes include urinary excretion, billiary excretion and conversion in to metabolites by organs like the liver, intestine, lungs and kidney. These elimination processes sum up what is known as the total clearance of the drug. To ascertain the pharmacokinetics characteristics of a drug, the different stages of absorption, distribution and elimination must be quantified and donated by specific parameters that can be determined mathematically using methods based on plasma and or urinary kinetic data obtained after the administration on the compound on different routes. The most important pharmacokinetics parameters that are being considered in drug absorption are:

1. Coefficient of absorption
2. First pass effect
3. Area under curve
4. Half life

These parameters determined to the large extent the pharmacological responses of individual patient. Most of the drugs interactions are kinetics in origin, one of the most useful pharmacokinetics concepts to have emerged in recent years particularly in understanding interaction is that of the area inscribed by plasma concentration.

# Pharmacokinetic Models

Pharmacokinetic models are hypothetical structures that are used to describe the fate of a drug in a biological system following its administration. In pharmacokinetics experimental data on drug concentration in plasma are fit in to mathematical equations that represent flow of drugs and their metabolites through the discrete compartments of a model system (Paxton, 1981).

Even with inter-individual variations certain principles can be generally applied in order to manipulate pharmacokinetics data so as to precisely described drug disposition which is of clinical importance. These principles include the assumption that:-

1. Drug enters the system via central compartment and eliminated only from the compartment.
2. Reversible transfer occurs between central and peripheral compartments.
3. The exit of drug from all compartments in the system is described by a first order kinetics.
4. The third assumption is true for most drugs excepts for drugs eliminated by biotransformation where by saturation of metabolite emzymes makes elimination a zero order process.

# Single compartment model

Following drug administration, the body is depicted as a kinetically homogeneous unit. This assumes that the drug achieves instantaneous distribution throughout the body and that the drug equilibrates instantaneously between tissues.



# Figure 2.9 One compartment model

Here, exchange of drug between the plasma and tissue proceeds rapidly compared with the rates elimination with whole body considered mathematically as a single compartment as described in Figure 2.9 above. We assume an instantaneous distribution after an intravenous

(IV) injection of a drug into the models.

This equation then holds through:

C = D

o

Vdc

equation1.1

Where;

Co = Concentration immediately after injection D = Dose

Vdc = Apparent volume of distribution

After instantaneous distribution, the concentration will fall according to first order kinetics with plot of plasma drug concentration against time on an arithmetic scale as represented in Figure 2.10; we get an exponential curve, while on a semi-logarithmic scale as in Figure

2.11 we get a straight line.



# Figure 2.10Plasma concentration-time plot on a linear arithmetic scale following rapid IV injection



**Figure 2.11Plots of plasma drug concentration against time for a single compartmental model on log scale.**

The differential equation for a first order process:

𝑑𝑦 = −𝐾𝑌equation 1.2

𝑑𝑡

Where 𝑑𝑦

𝑑𝑡

is the negative rate of change of substance overtime.

Applying this equation to the elimination of drug in the body gives:

dc = K

 

Ct equation 1.3

dt el

Where Kel = Elimination rate constant The integrated form of equation (1.3) Ct = Co e Ktequation 1.4

Where e = base of natural log or

In Ct = In Co Kelt equation 1.5

Converting to logarithm to base 10

Log Ct = log Co Kelt/2.303 equation 1.6

# Two compartment model

The two-compartment model resolves the body into a central compartment and a peripheral compartment. Although these compartments have no physiological or anatomical meaning, it is assumed that the central compartment comprises tissues that are highly perfused such as heart, lungs, kidneys, liver and brain. The peripheral compartment comprises less well- perfused tissues such as muscle, fat and skin as indicated in Figure 2.12.



# Figure2.12 Rapid Intravenous (IV) injections in to two compartments

K12 and K21 = Distribution rate constant



# Figure 2.13 Plot of plasma drug concentration against time for two compartmental models on a logarithm scale.

A two-compartment model assumes that, following drug administration into the central compartment, the drug distributes between that compartment and the peripheral compartment. However, the drug does not achieve instantaneous distribution, i.e. equilibration, between the two compartments. The drug concentration–time profiles shows a curve as indicated in Figure 2.12.but the log drug concentration–time plot shows a biphasic response as described in Figure 2.13.

This may be represented by the equations below:

Ct = Aekat Beket equation 1.7

The coefficient B is the intercepts on the ordinate obtained with extrapolation of the elimination phase.

A + B is the actual intercepts of the concentration curve at t=0 katand ket is the distribution and elimination rate constant.

A two compartment model may be expanded to contain additional compartments which can be described mathematically as the sum of many individuals’ exponents’ functions as there are relevant compartments.

# Multi-compartment models

In this model the drug distributes into more than one compartment and the concentration– time profile shows more than one exponential. Each exponential on the concentration–time profile describes a compartment. For example, gentamicin can be described by a three- compartment model following a single IV dose as described in Figure 2.14.

# Figure 2.14 Multi-compartment model showing log Cp versus time.

# Three compartment model

In reality a maximum of three compartments is allowed in assay technique (Paxton,1981). The three-compartment model is a modified model in which anadditional compartment in incorporated to represent the volume from whichabsorption occurs at a first order rate as represented in Figure 2.15. It is assumed the entire dose israpidly introduced into the size of absorption, from which it is absorbed into thecentral compartment. The Figure 2.16 described plasma drug concentrations against time for a three compartmental model on semi-logarithmic scale

Absorption compartment (vo)

Central compartment (V1)

Peripheral compartment (V2)

Elimination

# Figure 2.15 Three Compartment model after a single oral dose



**Figure 2.16 Plot of plasma drug concentrations against time for a three compartmental model on semi-logarithmic scale.**

# Non-linear pharmacokinetics

The pharmacokinetics of a drug are said to be non linear when one or more of the parameters vary with the dose and the concentration at a given time.

Non linear can be related to absorption, the hepatic first pass effect, distribution, urinary excretion, billiary elimination or metabolism.

The most frequent reasons for non linear kinetics are:-

1. Saturation of the hepatic first pass effect.
2. Saturation of the plasma protein binding site.
3. Saturation of the process of re-adsorption of tubular secretion.
4. Saturation of the enzymes system involved in biotransformation.

Examples of non linear kinetics are;-

1. Absorption- Clomidine, Griseofulvin, Chlorthiazide
2. First pass effect- Midazolam, Propranol hydrallazine
3. Tissue Distribution- Quinidine Disopyramide
4. Protein Binding- Quinidine, Ceftriazone
5. Metabolism- Phenytoin, Theophyline, Erythromycin, Probenacid, Penicillin G.

Non linearity has important pharmacological and or clinical consequences when it occurs at therapeutic levels. The decline in the plasma concentration of a drug whose plasma kinetics are non linear generally occurs in accordance with the Micheali-Menten’s process as represented in equation (1.8);

dc = Vm. C/Km .tequation 1.8

dt

Where,

dc = Elimination rate constant

dt

Vm = Maximum velocity of enzyme process Km = Michealis-Menten constant

# Pharmacokinetic Parameters

As it has been earlier mentioned, pharmacokinetic parameters determine to a large extent the pharmacological response of individuals. These parameters include the following:

# Absorption rate constant (Kab)

This is the rate constant of the entire process of drug transfer in to the body through all biological membrane. It has unit of reciprocal of time (h-1), it can be calculated using equation (1.9).

Kab 0.693 equation 1.9

t1/2 ab

# Absorption Half-life (t1/2ab)

This is the time taken for half of the total absorption to be achieved (half of the difference between theoretical and experimental values). It has units of time in hour.

# Absorption Lag Time

This begins where the extrapolated straight line and that of the residual intercept. It reflects the time taken between the administration of a drug and the time absorption begins. It has units of time in hour

A

In ( )

Lag time = B equation 1.10

Kab −Kel

# Area under the curve (AUC)

Area under the plasma concentration time curve from time zero to infinity is a measure of the extent of drug absorbed in to the systemic circulation i.e. measure of bioavailability (Hinna and Bressolle, 1998). This is the area defined by the axis and the curve of blood or plasma concentration versus time. It may be limited to a specific time or be extrapolated to infinity.

It is the total blood or plasma drug concentration from time zero to infinity. It measures the quantity of the drug which has been absorbed into general circulation. Thus, it is a measure of the amount of circulating drug. Method usually employed to determine AUC include use of planimeter, the cut and weight method or the triangular method. The most commonly employed is the trapezoidal method which involves dividing the plasma concentration-time curve in to sections that approximate a series of trapezoids with triangle at each end. The individual areas of the trapezoids and triangle summed to obtain the AUC (Notari, 1980). It has unit of mg.hr/ml, it can be calculated by the trapezoidal method determined according to equation (1.11) and (1.12).

Area (C1 +C2)(t2−t1)equation 1.11

2

AUC0-α = AUC0-t + Ct equation 1.12

K el

Β= Elimination rate constant Mathematical method

After stripping the curve, it is determined by equation (1.13)

AUC0-α = A + Bequation 1.13

𝖺 β

# Elimination rate constant (Kel)

This is the rate constant of the process leading to the elimination of the drug from the body. It is the sum of all individual elimination rates constant. It has units of reciprocal of time (h-

1).

# Elimination half life (t1/2el)

This is the time required to change the amount of drug in the body by one half during elimination (Holfard and Benet, 1995) and can be calculated using the equation (1.14).

t1/2el= 0.693equation 1.14

K el

Where, Kel is the slope of the first order plot based on the equation for one compartment or the final slope of the biphasic plot based on the equation for two compartment models. Half life can be determined graphically from a plot of the drug concentration in the blood against time on a log scale.

# Volume of distribution (Vd)

1. Initial volume of distribution: - this is the ratio of the administered dose to the plasma drug concentration extrapolated to zero time after an IV injection.
2. Apparent volume of distribution: - this is hypothetically defined as the volume of body water which would be required to contain the amount of drug in the body, if it were uniformly present in the same concentration in which it is in the blood or plasma.

Volume of distribution (Vd) relates the amount of drug in the body to the concentration of drug in blood or plasma and has unit of l or l/Kg and it can be calculated using equation

(1.15).

Vd= V = D

Co

D = Dose administered by rapid intravenous injection Co = theoretical blood concentration at time zero (0)

Vdcan be determined from the following mathematical expression

Vd = FD equation 1.16

AUC

Where;

F= Bioavailability D= Dose

equation 1.15

AUC= Area under the curve

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

# Total body clearance (Cl)

This is the capacity of the organism to eliminate a substance after it has reached the general circulation. It is the total sum of all the individual clearances by the organs as represented in equation (1.17). It reflects the volume of blood completely cleared of the drug per unit time. Organ clearance refers to the volume of blood or plasma completely cleared of a drug by the organ per unit time.

Total clearance = Clrenal + Clhepatic + Cl other organs of metabolism equation 1.17

Clearance by any organ depends on the blood flow through the organ and the extraction ratio of the drug by the same organ can be calculated using equation (1.18);

Clearance (Cl) =0.693.Vdequation 1.18

t1/2 el

Where, Vd = Apparent volume of distribution t1/2el = Elemination half life

It has unit of Litre per hour (lh-1).

# Lag time

Lag time in pharmacokinetics corresponds to the finite time taken for a drug to appear in systemic circulation following extravascular administration. Lag time is a reflection of the processes associated with the absorption phase such drug dissolution and / or release from the delivery system and drug migration to the absorbing surface. It can be calculated using equation (1.19);

a

In ( )

Lag time= b

Ke −Kab

equation 1.19

Where, a = Y intercept

b = Intercept of the line of residuals

kel, kab = rate constant of elimination and absortion

# Residual Method in Pharmacokinetics

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

For this graphical approach, the drug concentration data are plotted on a log scale versus time. In one compartment open model, the mono-exponential elimination slope is back extrapolated to the ordinate. The difference i.e. the residuals between the measured plasma concentrations and the corresponding values on the back extrapolated line at each sampling time point at the absorption phase are calculated. These residuals are then plotted semi- logarithmically versus time on the same graph. The slope of this plot yields an estimated absorption rate constant (Kab). This method of residuals is probably a reasonable means of graphically approximating the absorption rate constant if the data are well described by the difference between exponential terms and if the distribution of the drug in the body is truly uni-compartmental (Tozer, 1979). The lag time can also be determined from this method and is determined from the point of interception of the line of residual and the back extrapolation.

# CHAPTER THREE

# MATERIALS AND METHODS

# Materials

# Drugs

* + - 1. Standard metronidazole powder was obtained as gift from Sam Pharmaceuticals Ltd., Ilorin with a batch number W15092105
			2. The two drug samples: metronidazole tablet (400mg) and ranitidine tablet (150 mg) purchased from Lamco pharmacy, were manufactured by Cipla Ltd, India.

# Equipment

1. Vortex mixer (Kanke and Kunke, Germany)
2. Centrifuge (Gallenkamp, England)
3. Flask shaker (Gallenkamp, England)
4. Hot Air Oven (Gallenkamp, England)
5. UV spectrophotometer (Helios Zeta, Model 164617)
6. Disintegration rate apparatus (Erweka, Germany)
7. Dissolution rate apparatus (Erweka, Germany)
8. Friabiliator (Erweka, England)
9. Melting point study apparatus (Erweka, Germany)

# Reagents

All reagents and other chemicals used were of high analytical grade.

# Methods

# Preparation of reagents, solutions, disintegration/dissolution medium

* + - 1. *Crystal Violet (5%)*

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

* + - 1. *Perchloric Acid (0.1 N)*

Perchloric acid (250 ml; 0.1 N) was prepared according to the method described by an Indian Pharmacopeia (2010)volume I, general method of analysis.

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

The solution was standardized with potassium hydrogen phthalate (previously dried at 120oC for 2 hours) dissolved in acetic acid, was stored pending use.

* + - 1. *Sodium Hydroxide (1 N)*

Sodium hydroxide solution (0.1 N) was prepared by dissolving accurately weighedsodium hydroxide pellet (40g) in sufficient distilled water to produced 1000 ml.

* + - 1. *Preparation of Dissolution/disintegration Medium*

Hydrochloric acid (0.1 N) was used as the disintegration and dissolution medium and was prepared by diluting concentrated hydrochloric acid (8.6 ml) with 1000 ml of distilled water. *3.2.1.5Preparation of 0.1 N Boric Acid Solution*

Boric acid solution (0.1 N) was prepared by dissolving accurately weighed 6.183g of H3BO3(mwt. 61.831g/mol) in 1000ml of distilled water.

# Quality control studies

The quality control tests carried out were identification test, weight variation test and assay, dissolution, disintegration and friability tests. These tests were conducted according to BP, 2009 specification.

* + - 1. *Identification test*

*Identification test of metronidazole tablets*

A quantity of the powdered metronidazole tablets (10 mg) was weighed and heated on a waterbath with zinc powder (10 mg), distilledwater (1 ml)and hydrochloric acid (0.25 ml)for 5minutes, cooled in ice, sodium nitrite solution (0.5 ml) was addedand remove the excess nitritewith *sulphamic acid* .2-naphthol solution (0.5 ml) was added to sodium hydroxide (2 ml; 5N). iforange-red colour formed its indicated the presence of metronidazole.

*Infrared Identification test of metronidazole tablets*

The powdered tablet of metronidazole (25 mg) was weighed and transferred in to a beaker containing methanol(5 ml). The mixture was shaken for 5 minutes, filtered and the filtrate evaporated to dryness at 60oC for 10 minutes. Petroleum spirit (1 ml) was added to the residue obtained after evaporation. The infrared absorption spectrumof the dried residue was determined using IR machine.

*Identification test of ranitidine*

The powdered tablet of ranitidine (25 mg) was weighed and transferred in to a beaker containing methanol(5 ml) and shaken for 5 minutes, filteredand the filtrate evaporated to dryness at 60oC for 10 minutes. Petroleum spirit (1 ml) was added to the residue obtained after evaporation. The infrared absorption spectrumof the dried residuewas determined using IR machine.

* + - 1. *Assay*

*Assay of metronidazole tablet*

Twenty tablets of metronidazole tablet were randomly selected and crushed with mortar and a pestle.A quantity of the powdered tablet (0.150 g) was dissolve in 50 ml of anhydrous acetic acid and titrated withperchloric acid (0.1 N) and the end-point was determined potentiometrically.The operation was repeated without the powdered tablet.1ml ofperchloric acid (0.1 N) is equivalent to 17.12 mg of C6H9N3O3.

*Assay of metronidazole pure powder*

Metronidazole pure powder (0.150g) was accurately weighed and dissolved in anhydrous acetic acid (50 ml) and titrated with Perchloric (0.1 N) and the endpoint was determined potentiometrically. Perchloric acids( 1 ml; 0.1 N) is equivalent to 17.12 mg of C6H9N3O3.

*Assay of ranitidine*

Twenty tablets of ranitidine were randomly selected. The tablets were crushed in apestle and mortar. A quantity of powdered tablets (0.280 g) of ranitidine was titrated with sodium hydroxide(0.1 N), and the end-point determined potentiometrically. 1ml of 0.1 N sodium hydroxide is equivalent to 35.09mg of C13H23ClN4O3S.

* + - 1. *Weight variation test*

Twenty tablets of metronidazole were selected randomly and their average weight determined. The tablets were then weighed individually and the percentage weight deviation of each tablet from the mean average weight was calculated.The same procedure was repeated for ranitidine tablets.

* + - 1. *Disintegration test*

The disintegration test was carried out according to BP (2009) in whichone tablet of metronidazole was placed in each of the Six (6) tubes of the basket of the disintegration tester (Euweka, Germany) filled with hydrochloric acid (0.1 N) maintained at 37 ± 1oC by an electrically heated water bath. The assembly of the tube was made to move up and down in the medium so that tablets were constantly agitated. The time taken for each of the individual tablet to pass freely through the mesh at lower end of the tubes was recorded. The same procedure was repeated for ranitidine.

* + - 1. *Friability test*

This wascarried out according to BP (2009) in which twenty metronidazole tablets were taken and carefully de-dusted prior to testing.Each tablet was accurately weighed and placed in the drum of the apparatus. The drum was rotated 100 times at 25 rpm for 4 minutes, and the tablets were removed, the loose dust from the tablets was removed with tissue paper, and accurately reweighed. The same procedure was carried out for ranitidine.The loss in weight was noted and the percentage lost was calculated using the equation (3.1);

Friability = Initial tablet weight −Final tablet weight

Inital tablet weight

× 100 equation 3.1

* + - 1. *Dissolution rate test*

The dissolution rates of the active ingredient from the tablet dosage form were determined as described by BP (2009) using Euweka dissolution apparatus. The dissolution medium was Hydrochloric acid solutions (0.1 N). The medium was maintained at 37± 0.5oC. The paddles of the apparatus were maintained at a speed of 100 rpm and a sample (1 ml) was withdrawn with a syringe after30 minutes and diluted to 10ml with the medium. The absorbance of metronidazole from the solution was determined using double beam UV spectrophotometer

at 277 nm using hydrochloric acid (0.1N) as blank. The same procedure was repeated for ranitidine. The percentage drug released was calculated according the formula described in equation (3.2) , (3.3) and (3.4).

Absorptivity(1%, 1 cm) of metronidazole = 365

# Step 1:

Concentration= Absorbance at selected wavelength

Absorptivity (1%,1 cm )

equation 3.2

# Step 2:

Amount of drugreleased= [Conc.× df × vol. ofdissol. medium] ∕ 1000equation 3.3 Where df = dilution factor

# Step 3:

Percentage drugreleased Amount of drug released

labeled content of MDZ tablet

×100 equation3.4

# Analytical method for determination of metronidazole in saliva

The UV spectrophotometric method developed by Kolawole and Ameh (2004) was adopted based on ICH guideline (1996).

* + - 1. *Preparation of the Stock Solution*

A stock solution (1mg/ml) was prepared by dissolving 100 mg of standard metronidazole powder accurately weighed with aid of analytical weighing balance and made up to 100 ml with distilled water in a volumetric flask.

* + 1. *.2 Determination of λ max*

A working solution of 7µg/ml was prepared at different pH ranges (4-9). The maximum wavelength was determined by scanning the solution between 200-400 nm in a UV spectrophotometer.

*3.2.3.3 Construction of calibration curve*

A six point calibration curve was constructed from the stock solution by preparing solution of concentration (2.5-15 μg/ml). Six labeled test tubes, each containing blank saliva (2 ml) were spiked with 0.25, 0.5, 0.75, 1, 1.25 and 1.5 ml of the stock solution, transferred into 10 ml volumetric flask and made up to mark with distilled water to give concentration ranges of 2.5, 5, 7.5, 10, 12.5 and 15 μg/ml respectively.

The extraction procedure was adopted from the work of Kolawole and Ameh (2004) as follows:

The extraction procedure was adopted from the work of Kolawole and Ameh (2004). Volunteer saliva sample(2ml) were centrifuged at 2500 rpm for 10 minutes, and the supernatant were carefully removed by Pasteur pipette. A 1ml portion of the supernatant was mixed with 3ml of borate buffer solution (pH4) and vortexed mix for few seconds. The resulting solution was extracted with 10 ml x 3 chloroform. The combined chloroform extract was transferred in to a separating funnel and 4ml of 0.1N HCl was added. The aqueous phase was carefully taken and the absorbance measured at λmax of 277nm against a blank obtained by treating drug free pooled saliva the same way as above and then converted to concentration from the calibration graph.

# Validation of analytical method

* + - 1. *Precision*

Precision was determined by repeatability (intra-day) and intermediate precision (inter- day).Repeatability was determined by taking the absorbance of 1ml of 15μg/ml solution with buffer solution (3 ml) at pH 4. The absorbance was taken six times at an interval of 1

hin the first day under the same experimental condition. Inter-day precision was evaluated by taking the absorbance of 1 ml three times in three consecutive days.

* + - 1. *Accuracy*

This was done by spiking 1 ml each of 8 μg/ml, 10 μg/ml and 12 μg/ml of metronidazole corresponding to concentration of 80 %, 100 % and 120 % of a nominal concentration of 10μg/ml solution of metronidazole into 1 ml of supernatant portion of saliva. pH buffer (3 ml) was added, vortex mixed for 1 minute and extracted with 2 x 5mlchloroform, followed by addition of 4 ml of 0.1 N HCl. The aqueous layer was collected and the absorbance for each concentration was taken at 277 nm.

The percentage accuracy was determined using the following equation (3.5);

(i) Accuracy is expressed as percentage relative error (% Er)

% Er = ×−μ × 100 equation 3.5

μ

Where x is the mean concentration and µ is the expected concentration value.

*3.2.3.3 Percentage Extraction Recovery*

The percentage extraction recoveries of analytical method give the efficiency of the extraction procedure to be employed in the analyses. It also gives assurance on the reproducibility of the extraction method employed. The percentage recovery was computed

using the formula described in equation (3.5).

% Recovery = Measured concentration after extraction

Added concentration

× 100 equation 3.6

**3.3*In vivo* Studies**

The last segment of the study involves the participation of 6 healthy male volunteers aged between 30-35 ± 5 years, weight between 55-65 ±5Kg; all nonsmokers and non-alcohol

consumers. The volunteers were fully briefed on the study and were informed of their role in the practical. The study was divided into 3 phases.

# Inclusion and exclusioncriteria

Human volunteers aged 30±5 years, weight 55±5 Kg, non-smokers, non-alcoholic and non- Kola nut consumers were enrolled in the study and Consent of the individual volunteers was sought. Subjects that were overweight (BMI˃25) and those with abnormal liver and kidney functions were excluded from the study.

# Protocol of in vivo studies

*Phase 1*

The first phase of the study involved an overnight fasting of not less than 10 hours, collection of blank saliva followed by administration of 400 mg of metronidazole to each volunteer with volume of water (200 ml). No food or drink allowed 3 hours after administration of the drugs. Saliva sample (4ml) wascollected after administration at the interval of 0.25, 0.5, 1, 2, 3, 4, 5, 6,7,8,9,10,11 and 12 h. Chewing of gum by volunteers aided the production of saliva. The saliva samples were stored at -40C before analysis.

*Phase 2*

The second phaseinvolved the concomitant administration of 400 mg metronidazole tablet with 300 mg of ranitidine to each of the six healthy volunteers after overnight fasting with volume of water (200 ml). Thereafter, saliva samples collected at time interval similar to that of phase one.Same were treated and stored for analysis as mentioned above.

*Phase 3*

The third phase involved administration of 300mg of ranitidine with volume of water (200 ml) to each volunteer. After one hour (delay); 400mg of metronidazole was administered

with volume of water (200 ml) and the saliva samples were collected at the administered time (zero hour) and at time interval mentioned above.Thereafter they were treated and kept for analysis.

The protocol of the study was developed to have a wash out period of 2 weeks between the phases of the study.

# Data analysis

The calibration curve and the regression analysis were carried out using Microsoft Excel® 2007. The absorbance wasconverted to concentrations using the validated calibration curve. Some pharmacokinetic parameters were generated Using PharmPKSoftware®.All the mean pharmacokinetic data obtained in the study were analyzed statistically using student t-test of paired data. Values (*P˂0.05)*considered statistically significant and *P˃0.05* considered statistically insignificant.

# CHAPTER FOUR

# Results

# Quality Control

# Sampling

The labeled information on the drug used for this study (metronidazole tablet 400 mg and ranitidine 150 mg) are presented in Table 4.1.

# Table 4.1Labeled information on drug samples

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **PRODUCT** | **BATCH NO.** | **NAFDAC NO** | **MFG. DATE** | **EXP. DATE** |
| Metronidazole Tablet (200mg) | R140413 | 04-5294 | 08/2014 | 07/2019 |
| Ranitidine Tablet (150mg) | PB50895 | O4-0283 | 05/2015 | 04/2018 |

# Identification of sample

Metronidazole was found to be present in the sample tablet as indicated by the formation of an orange-red color. Furthermore, IR spectrum of the standard and the sample were super imposable with reference spectrum of metronidazole (Appendix 1&2). IR spectrum of ranitidine tablet was found to be super imposable with reference spectrum of ranitidine confirmed the presence of ranitidine (appendix 3)

# Assay test

The Table 4.2 shows the results of assay test (% contents) of metronidazole, ranitidine tablet and metronidazole standard powder.

# Table 4.2Assay test of metronidazole and ranitidine

|  |  |  |
| --- | --- | --- |
| **Drug** | **% Assay** | **Remarks** |
| Metronidazole tablet | 95.87 | Passed |
| MDZ Standard Powder | 102.49 | Passed |
| Ranitidine tablet | 99.13 | Passed |

Acceptable value: metronidazole: 95-105% and ranitidine: 98.5-101.5% (BP, 2009)

# Weight variation test

Table 4.3 Results of weight variation for metronidazole and ranitidine using twenty tablets of each drug product.

# Table 4.3Weight variation of metronidazole

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Drug** | **No. of Table used** | **Mean±SEM (mg)** | **% Deviation** | **Remarks** |
| Metronidazole | 20 | 347±2.60 | 2.37 | Passed |
| Ranitidine | 20 | 314±1.63 | 1.65 | Passed |

Acceptable values: not more than 2 tablets deviate by ≥ 5 % (BP 2009)

# Disintegration test

Table 4.4 shows disintegration time for metronidazole and ranitidine

# Table 4.4 Disintegration test of metronidazole and ranitidine

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Tablet Number** | **1** | **2** | **3** | **4** | **5** | **6** |
| MDZ Disintegration time(mins) | 0.25 | 0.33 | 0.42 | 0.50 | 0.67 | 0.75 |
| RNT Disintegration time(mins) | 6.75 | 7.33 | 7.5 | 7.75 | 7.83 | 7.92 |
| Remarks for MDZ & RNT | Passed | Passed | Passed | Passed | Passed | Passed |

Mean disintegration time for metronidazole &ranitidine is ≤ 15 mins (BP2009)

# Friability test

Table 4.5 shows the results of friability test of both Metronidazole and Ranitidine

# Table 4.5 Friability test of metronidazole and ranitidine

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **No. of****tablets** | **Initial weight (g)** | **Final weight****(g)** | **% Friability** | **Remarks** |
| 20 | 3.43 | 3.41 | 0.58 | Passed |
| 20 | 3.13 | 3.12 | 0.32 | Passed |

Acceptable friability value is ≤ 1 (BP2009)

# Dissolution test of metronidazole and ranitidine

Table 4.6 shows the result of dissolution test of both metronidazole and ranitidine using dissolution tester.

# Table 4.6Dissolution test of metronidazole and ranitidine

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Drug** | **No. of replications** | **Time of sampling****(mins)** | **% released** | **Remarks** |
| MDZ | 6 | 30 | 74.85 | Passed |
| RNT | 6 | 30 | 86.4 | Passed |

Mean dissolution rate of metronidazole is ≥ 70% at 30minutes and ranitidine is not ˂ 80% at 30 minutes(BP 2009)

# Analytical Method

The wavelength of maximum absorption of metronidazole in 0.1N HCl at pH of 4 was found to be 277 nm(Figure 4.4). The calibration curve showing the regression equation is shown in Figure 4.5, while the validation parameters are shown in the Table 4.7.



# Figure 4.4 UV Spectrum of metronidazole in 0.1N HCl at pH 4



**Figure4.5 Calibration curve of absorbance of metronidazole in 0.1N HCl at pH 4 against corresponding concentration.**

# Table 4.7Summary of validation parameter of the developed method

|  |  |  |  |
| --- | --- | --- | --- |
| **S/NO** | **Parameters** | **Result obtained** | **Official specification** |
| 1. | Percentage extraction recovery (%) | 98.40 | 98-102 |
| 2. | Relative error (accuracy) (%) | 1.64 | 1-5 |
| 3. | Precision |  |  |
| I. | Within-day (% RSD) | 0.38 | ˂ 2.0 |
| II. | Between–day (% RSD) | 0.26 | ˂ 2.0 |

* 1. ***In vivo* Studies**

Table 4.7 shows the mean salivary concentration for all the three phases from time 0 – 12 hrs for the six volunteers (Figure 4.5). The graph of mean salivary concentration against time for all the three phases is shown in (Figure 4.6).

# Table 4.8Metronidazole mean salivary concentration (µg/ml)

|  |  |  |  |
| --- | --- | --- | --- |
| **Time** | **Phase 1** | **Phase 2** | **Phase 3** |
| 0 | 0.00 | 0.00 | 0.00 |
| 0.25 | 5.33 | 8.10 | 5.34 |
| 0.5 | 6.87 | 9.68 | 6.92 |
| 1 | 6.91 | 11.38 | 8.29 |
| 2 | 6.95 | 15.26 | 10.5 |
| 3 | 7.13 | 51.57 | 11.91 |
| 4 | 6.68 | 14.23 | 36.27 |
| 5 | 5.75 | 11.77 | 11.28 |
| 6 | 5.42 | 10.21 | 10.42 |
| 7 | 5.34 | 8.41 | 8.96 |
| 8 | 4.14 | 6.13 | 7.79 |
| 9 | 3.99 | 4.44 | 6.30 |
| 10 | 2.79 | 2.82 | 4.74 |
| 11 | 2.05 | 1.52 | 2.97 |
| 12 | 1.76 | 0.96 | 1.15 |



**Figure 4.6Graph ofmetronidazole mean salivary concentration against time for the three phases**

# Mean pharmacokinetic parameters for the 3 phases ± SEM

Table 4.9 shows the result of pharmacokinetic parameters of generated from all the three phases

# Table 4.9 Pharmacokinetics parameters of metronidazole in the phases (1-3)

|  |  |  |  |
| --- | --- | --- | --- |
| **Pharmacokinetic Parameters** | **Metronidazole** | **MDZ+RNT** | **MDZ+RNT** |
| **Alone (400mg)** | **Concurrent****Administration** | **Delayed****Administration** |
| Lag Time (h) | 0.36±0.80 | 0.14±0.79 | 0.14±0.79 |
| Cmax (µg/ml) | 7.13±2.01 | 51.57±18.91 | 36.27±8.66 |
| Tmax (h) | 3.24±0.504 | 1.57±0.17 | 1.98±0.18 |
| T1/2 ab (h) | 1.99±0.42 | 0.84±0.13 | 1.21±0.80 |
| Kab (h-1) | 0.44±0.88 | 0.20±0.37 | 0.29±0.32 |
| AUC0-12 (µg. h/ml) | 104.27±19.86 | 106.64±8.65 | 115.65±9.98 |
| T1/2 el (h) | 3.59±0.47 | 1.62±0.17 | 1.58±0.18 |
| K el (h-1) | 1.22±0.36 | 0.46±0.68 | 0.46±0.45 |
| Vd (L) | 40.42±7.87 | 28.20±2.89 | 26.37±1.42 |
| CL (L/h) | 8.43±2.22 | 5.18±2.46 | 2.65±0.35 |

# Comparism of pharmacokinetic parameters

Table 4.10 shows the comparism of mean pharmacokinetic parameters of phase 1 and phase 2

# Table 4.10 Mean pharmacokinetics parameters of phase I and II

|  |  |  |  |
| --- | --- | --- | --- |
| **Pharmacokinetic****parameters** | **n=6** | **Metronidazole Alone** | **Concurrent Metronidazole and****Ranitidine** |
| Lag Time (h) | 6 | 0.36±0.80 | 0.14±0.79 |
| Cmax (µg/ml) | 6 | 7.13±2.01 | 51.57±18.91\* |
| Tmax (h) | 6 | 3.24±0.504 | 1.57±0.17 |
| T1/2 ab ( h) | 6 | 1.99±0.42 | 0.84±0.13 |
| Kab (hr-1) | 6 | 0.44±0.88 | 0.20±0.37 |
| AUC0-12 (µg. h/ml) | 6 | 104.27±19.86 | 106.64±8.65 |
| T1/2 el (hr) | 6 | 3.59±0.47 | 1.62±0.17 |
| K el (h-1) | 6 | 1.22±0.36 | 0.46±0.68 |
| Vd (L) | 6 | 40.42±7.87 | 28.20±2.89 |
| CL (l/h) | 6 | 8.43±2.22 | 5.18±2.46 |

* Significant difference at *P ˂ 0.05*

Table 4.11 shows the comparism of mean pharmacokinetic parameters of phase 1 and phase 3

# Table 4.11 Mean pharmacokinetic parameters of phase I and III

|  |  |  |  |
| --- | --- | --- | --- |
| **Pharmacokinetic****parameters** | **n=6** | **Metronidazole Alone** | **Delayed Metronidazole and****Ranitidine** |
| Lag Time (h) | 6 | 0.36±0.80 | 0.14±0.79 |
| Cmax (µg/ml) | 6 | 7.13±2.01 | 36.27±8.66\* |
| Tmax (h) | 6 | 3.24±0.504 | 1.98±0.18 |
| T1/2 ab ( h) | 6 | 1.99±0.42 | 1.21±0.80 |
| Kab (h-1) | 6 | 0.44±0.88 | 0.29±0.32 |
| AUC0-12 (µg. h/ml) | 6 | 104.27±19.86 | 115.65±9.98 |
| T1/2 el (h) | 6 | 3.59±0.47 | 1.58±0.18 |
| K el (h-1) | 6 | 1.22±0.36 | 0.46±0.45 |
| Vd (l) | 6 | 40.42±7.87 | 26.37±1.42 |
| CL (l/h) | 6 | 8.43±2.22 | 2.65±0.35 |

* Significant difference at *P ˂ 0.05*

# CHAPTER FIVE

# DISCUSSION

# Quality Control

There has been so many research conducted to prove that some drugs after oral administration appears in saliva equally like in plasma, metronidazole has been shown to be among them, for this reason saliva can be used to determine the pharmacokinetics of metronidazole. From the result of the *in vitro* study carried out for both metronidazole and ranitidine they both passed the quality control test conducted, according to BP 2009. The weight variation test of both drugs indicated that the average percentage deviation for both metronidazole and ranitidine was not greater than 5% suggesting that both drugs exhibit a high level of weight consistency, such that the active ingredients are expected to be consistent in a singular manner. Since uniformity of weight is an attempt to minimize drug variation in tablets. The result of the friability test of both metronidazole and ranitidine was found to be within official specification of BP 2009, the percentage friability was found to be less than 1%. This indicates that the samples were not chipped, abraded or deformed which indicates that there species would be able to withstand transportation and handling stresses involved.

The identification test showed the presence of metronidazole and ranitidine moiety. The infrared spectroscopy of both standard powder and tablet of test drug (metronidazole) confirmed the presence of metronidazole in comparism to the reference standard, the infra red spectra were found to be super imposed. The disintegration time of both metronidazole and ranitidine shows that they comply with good manufacturing practice; they both disintegrate within 15 mins, which is within the official specification of BP 2009. The Biological performance of a tablet is commonly assessed by disintegration time and

dissolution rate. These two parameters described how much of the orally administered tablet may be bioavailable (Wagner, 1971).

From the result of the dissolution rate test of both metronidazole and ranitidine, it was found to be greater than 70%, hence they conformed to the official specification of BP 2009 and this indicates that at least 70% of the active component of both metronidazole and ranitidine was released within 30 minutes.

The drug Assay test was conducted to measure the percentage content of both standard powder of metronidazole and ranitidine, the percentage content of both drugs determined is between 95-105 % of the their active ingredients of BP 2009 specification.

The degree of precision in this study of metronidazole is consistent with ICH guidelines (1996) and the work of Kolawole and Ameh (2004) on the spectrophotometric method for estimation of metronidazole in bulk and tablet dosage form. The % RSD observed for metronidazole in this study was found to be less than 2% which is comparable with work of Musa *et al.*(2011), indicating good precision. The percentage extraction recovery obtained from this study of metronidazole was found to be comparable with the result of Usman *et al.* (2010) on the Rapid spectrophotometric determination of metronidazole tablet.

* 1. ***In vivo* Pharmacokinetic Studies**

The most important pharmacokinetics parameters considered in order to evaluate the rate and extent of absorption of metronidazole in this study are absorption rate coefficient (Kab), time corresponding to maximum concentration (Tmax), maximum concentration (Cmax), lag time and half-life (t1/2). While those parameters indicative of metabolism and or excretion were also considered, this include the following time line for a quantity of drug available to be reduced by half (t1/2), coefficient of elimination (Kel) and clearance (Cl).

# Single dose salivary pharmacokinetics of metronidazole

The pharmacokinetic parameters were found from saliva samples following oral single dose administration of 400 mg of metronidazole tablets to overnight fasted human volunteers. UV visible spectrophotometer was used for the analysis at 277 nm. *In-vivo* study showed that the lag time for metronidazole tablet was found to be 0.358 h. The value obtained was in agreement with value reported by Kolawole and Ameh (2004) who reported 0.24 h and Garba (2015) who also reported 0.45 h. The maximum concentration (Cmax) for metronidazole tablet achieved after oral administration was found to be 8.29 μg/ml which is in agreement with work of Usman *et al*. (2007) who reported 7.20 µg/ml and Kolawole and Ameh (2004) reported 8.72 μg/ml. The mean Tmax for single oral dose of metronidazole tablet extrapolated from the concentration-time curve was 0.50 h, the values were not in harmony with the 3.0 h reported by Ezzeldin and El-Nahhas (2012). The value was also not agreement with Rafindadi (1993) who reported 2.5 h. t1/2ab value was found to be 1.989 h which is higher than 0.51 h reported by Kolawole and Ameh (2004) and 0.665 h reported by Rafindadi (1993). Kab value was found to be 0.437 h-1 which is lower than 1.394 h-1 reported by Kolawole and Ameh (2004) and 1.7 h-1 reported by Garba (2015). AUCwas found to be

104.274 μg.hr/ml which is in agreement with 103.17 μg.h/ml reported by Ashiq *et al.* (2011) and slightly lower than 115.1 μg.h/ml reported by Bergamaschi *et al*. (2014). t1/2el was found to be 3.593 h lower than the 5.19 h reported by Kolawole and Ameh (2004). Kel was found to be 0.217 h-1, the value were in close agreement with study of Kolawole and Ameh (2004) that reported 0.135 hr-1. The Vdwas found to be 40.415 l which is slightly lower than 52 l reported by Katzung *et al*. (2012), this may be due to individual variability. The total body

clearance (Cl) was found to be 8.434 l/h which are in close agreement with 5.4 l/h reported by Katzung *et al.* (2012).

# Effect of ranitidine on the pharmacokinetics of metronidazole

The results of the effect of ranitidineare shown in Table4.10both under concurrent and delayed administration.

The pharmacokinetic parameters may roughly be divided in to two groups:- parameters that mainly related to rate and extent of absorption which are Kab, t1/2ab, Lagtime, Cmax and Tmax and parameters that are mainly indicators of metabolism and/ or excretion are t1/2el, Kel, Vd, area under concentration time curve (AUC) and total body clearance (Cl).

When metronidazole was administered concomitantly with ranitidine there was a statistically significant change in Cmax (51.57μg/ml) when compared with metronidazole administered alone which was found to be 7.13 μg/ml(*P˂0.05*). These findings indicated that ranitidine has effect on the Cmax of metronidazole when the two drugs were taken concomitantly. For the delayed administration of metronidazole one hour after ranitidine the value of Cmax was found to have also increased significantly to 36.27 μg/ml at (*P˂0.05*), when compared to phase 1. The time to reach maximum plasma concentration (Tmax) for administration of metronidazole alone was found to be 3.24 h and on concurrent administration of both metronidazole and ranitidine was found to have decreased to 1.57 h which was not statistically significant (*P˂0.05).* On delayed on administration, Tmax was found to be 1.98 h. The t1/2aband Kab values for the administration of metronidazole alone were found to be 1.99 h and 0.44 h-1 respectively, and in phase 2, thevalues decreased to

0.84 h and0.20 h-1respectively. For phase 3 the values were also found to have decreased to

1.52 h and 0.29 h-1 respectively as compared to phase 1. The value for AUC in phase 1 was

found to be 104.27 µg.h/ml but for phase 2 and phase 3 it’s slightlyincreased to 106.64µg.h/ml and 115.65µg.h/ml respectively. The calculated value for elimination half- life (t1/2el) and elimination rate constant (Kel) for the administration of metronidazole alone (phase 1) were found to be 3.59 h and 1.22h-1 respectively. The values for phase 2 and phase 3 are1.62 h and 0.46 h-1were 1.58 h and 0.46 h-1 respectively, these decreases not statistically significant. The calculated value for both volume of distribution (Vd) and clearance for phase 1 were found to be 40.42 l and 8.43 l/h respectively while phase 2 and phase 3 the values were shown to have decreased to 28.20 l, 5.18 l/h and 26.37, 2.65 l/hrespectively, the decreased were statistically not significant.The calculated value for lag time for phase 1 was found to be 0.36 h, phase 2 and phase 3 the values was shown to have decreased to 0.14 h which is not statistically significant. The lag time and Cmax of test drugs are known to be inversely proportional to each other. The lag time for phase 1, was found to be 0.36 h while that of phase 2 and Phase 3 was found to be 0.14 h and the calculated Cmax for phase 1, phase 2 and phase 3 are 7.17μg/ml, 51.57μg/ml and 36.27 μg/ml respectively. This shown that when lag time increases the Cmax decreases.This implies that decrease in lag time leads to the increase in absorption thereby causing the Cmax to be higher.

The only pharmacokinetic parameter that was found to have increased significantly at (*P˂0.05*) was Cmax for both phase 2 and phase 3. This can be deduced that both concurrent and delayed administration of ranitidine with metronidazole may affect the Cmaxof metronidazole.

# CHAPTER SIX

# SUMMARY,CONCLUSION AND RECOMMENDATIONS

# Summary

An investigation was carried out on the effect of ranitidine on the pharmacokinetic parameters of metronidazole in human subjects.

The quality control studies revealed that the drugs used in this study were of high quality as they complied with B.P (2009) requirements stated in their monographs.

A UV spectrophotometric method was adopted for the analysis of saliva metronidazole concentration.

The delayed and co-administered ranitidine with metronidazole increased maximum plasma concentration (Cmax).

# Conclusion

The results of identification test and chemical assay carried out on the tablets and standard powder of metronidazole and ranitidine showed that the batches of the drugs used satisfied the B.P (2009) official requirements.

The analytical method that was adopted and validated via precision, accuracy and percentage extraction recovery in this study were based on ICH guidelines (1996) and both drugs were within the acceptable limit of validation.

In the study it was also observed that there was a significant change in Cmax of metronidazole when ranitidine was concurrently administered. The absorption parameter,Cmaxwas found toincreased in this phase ascompared to phase 1.

In this study it was observed that there are significant effects of both concurrent and delayed administration of ranitidine with metronidazole, because Cmax was also significantly higher inboth phase2 and phase 3 when compared to phase 1.

In conclusion, it can be seen that co-administration of ranitidine with metronidazole whether concurrent or delayed may change the pharmacokinetics of metronidazole.

# Recommendation

1. Routine quality control and pharmacokinetic studies should be carried out to ascertain the claim of the manufacturer of this pharmaceutical products.
2. Similar pharmacokinetic study should be conducted with long washout period periodically with advance technique like HPLC.
3. Similar studies should be designed to assess the effect of observed increased Cmax.
4. Regulatory bodies like NAFDAC should periodically carry out quality control test to manage and maintain good quality product registration.

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# APPENDICES

**Appendix 1: Reference IR Spectra of Metronidazoleand IR Spectra of Metronidazole Standard Powder.**



# Appendix 2: IR Spectrum of Metronidazole Standard Powder and Tablet



**Appendix 3: Reference IR Spectra of Ranitidineand IR Spectra of Ranitidine Tablet.**



# Appendix 4: Table 4.8 for the metronidazole mean salivary concentration (µg/ml) of all the three phases.

|  |
| --- |
| **Mean Salivary Concentration** |
| **Time** | **Phase 1** | **Phase 2** | **Phase 3** |
| 0.00 | 0.00 | 0.00 | 0.00 |
| 0.25 | 5.33 | 8.10 | 5.34 |
| 0.5 | 6.87 | 9.68 | 6.92 |
| 1 | 6.91 | 11.38 | 8.29 |
| 2 | 6.95 | 15.26 | 10.5 |
| 3 | 7.13 | 51.57 | 11.91 |
| 4 | 6.68 | 14.23 | 36.27 |
| 5 | 5.75 | 11.77 | 11.28 |
| 6 | 5.42 | 10.21 | 10.42 |
| 7 | 5.34 | 8.41 | 8.96 |
| 8 | 4.14 | 6.13 | 7.79 |
| 9 | 3.99 | 4.44 | 6.30 |
| 10 | 2.79 | 2.82 | 4.74 |
| 11 | 2.05 | 1.52 | 2.97 |
| 12 | 1.76 | 0.96 | 1.15 |

**Appendix 5 :Comparism of mean pharmacokinetics parameters of phase I and II Values ± SEM**

|  |  |  |  |
| --- | --- | --- | --- |
| **Pharmacokinetic****parameters** | **n=6** | **Metronidazole Alone** | **Concurrent Metronidazole and****Ranitidine** |
| Lag Time (h) | 6 | 0.36±0.80 | 0.14±0.79 |
| Cmax (µg/ml) | 6 | 7.13±2.01 | 51.57±18.91\* |
| Tmax (h) | 6 | 3.24±0.504 | 1.57±0.17 |
| T1/2 ab ( h) | 6 | 1.99±0.42 | 0.84±0.13 |
| Kab (h-1) | 6 | 0.44±0.88 | 0.20±0.37 |
| AUC0-12 (µg. h/ml) | 6 | 104.27±19.86 | 106.64±8.65 |
| T1/2 el (h) | 6 | 3.59±0.47 | 1.62±0.17 |
| K el (h-1) | 6 | 1.22±0.36 | 0.46±0.68 |
| Vd (L) | 6 | 40.42±7.87 | 28.20±2.89 |
| CL (l/h) | 6 | 8.43±2.22 | 5.18±2.46 |

* Significant difference at *P ˂ 0.05*

# Appendix 6: Comparism of mean pharmacokinetic parameters of phase I and III ± SEM

|  |  |  |  |
| --- | --- | --- | --- |
| **Pharmacokinetic****parameters** | **n=6** | **Metronidazole Alone** | **Delayed Metronidazole and****Ranitidine** |
| Lag Time (h) | 6 | 0.36±0.80 | 0.14±0.79 |
| Cmax (µg/ml) | 6 | 7.13±2.01 | 36.27±8.66\* |
| Tmax (h) | 6 | 3.24±0.504 | 1.98±0.18 |
| T1/2 ab ( h) | 6 | 1.99±0.42 | 1.21±0.80 |
| Kab (hr-1) | 6 | 0.44±0.88 | 0.29±0.32 |
| AUC0-12 (µg. h/ml) | 6 | 104.27±19.86 | 115.65±9.98 |
| T1/2 el (h) | 6 | 3.59±0.47 | 1.58±0.18 |
| K el (h-1) | 6 | 1.22±0.36 | 0.46±0.45 |
| Vd (l) | 6 | 40.42±7.87 | 26.37±1.42 |
| CL (l/h) | 6 | 8.43±2.22 | 2.65±0.35 |

* Significant difference at *P ˂ 0.05*