THE EFFECT OF VITAMIN C ON PHARMACOKINETIC PARAMETERS OF CHLOROQUINE IN HEALTHY HUMAN VOLUNTEERS

BY

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DECLARATION

I declare that the work in this dissertation entitled THE EFFECT OF VITAMIN C ON PHARMACOKINETIC PARAMETERS OF CHLOROQUINE 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.

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CERTIFICATION

This Desertification entitled “The effect of vitamin C on the pharmacokinetic parameters of chloroquine” by Abdulkarim Umar Abubakar, 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 of knowledge and literary presentation.

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ABSTRACT

This study was undertaken to compare the effect of vitamin C on the pharmacokinetics of chloroquine using human volunteers. Quality control studies for both chloroquine and vitamin C; identification test, assay, weight variation, friability, disintegration time and dissolution rate were carried out according to BP 2009. Jayanathi *et al.*, 1994 method was adopted and validated based on ICH guideline for this study. Calibration curve was constructed within the range of 2 – 10 µg/ml and validated. The adopted method was then employed in determining the effect of vitamin C on the pharmacokinetics parameters (C_{max}, T_{max}, Ka, Ke, $t_{1/2}a$, $t_{1/2}e$, Cl, Vd, lag time and AUC) of chloroquine using human volunteers of 30 years and above and the study was divided into three phases with a washout period of three weeks. Both chloroquine and vitamin C 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 chloroquine and vitamin C 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 5 mins and also both chloroquine and vitamin C 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 – 10 µg/ml as the correlation coefficient was 0.9905. The regression equation was y= 0.001x + 0.120. When chloroquine was administered alone no significant difference ($p<0.05$) at lower values was observed, however, on co-administration of chloroquine and vitamin C no significant difference ($p<0.05$) at lower values was also observed, except for clearance (Cl) which was statistically significant

($p<0.05$) at lower values. On the other hand, when an hour delayed administration of chloroquine and vitamin C no significant difference ($p<0.05$) was observed, except for clearance (Cl) which was also statistically significant ($p<0.05$) at lower values. This finding indicates that vitamin C may influence the pharmacokinetics of chloroquine when co-administered together depending on the time of administration of the two drugs.

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ABBREVIATIONS

Centigrade	^0C
Hydrochloric acid	HCl
Sodium Hydroxide	NaOH
Ultraviolet	UV
High performance liquid chromatography	HPLC
British pharmacopeia	BP
Gram	G
Microgram	µg
Kilogram	Kg
Milligram	mg
Millimeter	ml
Minute	min
Hour	Hr
Hydrogen ion concentration	pH
Molarities	M
Normality (Normal)	N
Percentage	%
Natural Log	Ln
Litre	L
Standard deviation	SD
Standard error of Mean	SEM
Related Standard Deviation	RSD
Coefficient of variation	CV

Chloroquine- CQ

Vitamin C- Vit C

World Health Organization- WHO

CHAPTER ONE

1.0 INTRODUCTION

1.1 Drug interaction

It is evident that in some cases when two or more drugs are administered concurrently or at an interval of time, the expected pharmacological action is not being obtained. Sometimes potentiation of action of one of the drug may be observed. While on the other hand a diminished in action of the other drug occurs in some cases. This could be best explained through the study of possible interaction between the drugs in question. This is termed as drug - drug interaction study. (Pixtrim *et al.*, 1995)

There are many ways in which drug administrated by any route may interact in the patient to produce a harmful effect. Some of these interactions occur only when two drugs are administered within hours, days or weeks of each other; and sometimes the interaction take place only after one of the drugs has been taken for several weeks. There are biochemical explanations for some of this interaction, but for others there is yet no information available about the basic mechanism involved.

1.1.1 General mechanism of action

The mechanism of drug interaction development can be best explained into 2 ways.

1.1.1.1 Pharmacokinetics interactions

A). Absorption

b). Distribution

c). Elimination

1.1.1.2 Pharmacodynamics interactions

a. Enhanced effect produced by 2 drugs acting at the same site

b. The increase effect produced by 2 drugs 'at different receptors site.

c. Enhanced effect of 2 drugs by one which is devoid of action itself.

d Antagonism of the effect of one drug by another.

Pharmacokinetics

Most of drugs interactions are kinetics in origin, one of the most useful pharmacokinetics concepts to have emerged in recent years particular in understanding interaction is that of the area inscribed by the plasma concentration. Time curve of a drug. After a single dose, this area (AUC) is a function of the dose, the fraction of the dose entering the general circulation and the drug clearance (Lappin *et al.*, 2006)

This can be expressed as follows;

AUC = D x F/C---------- (1.1)

Where;

AUC = area under curve

D = dose

C = drug clearance

F = fraction of the dose entering the general circulation

When a drug is given repetitively, accumulation occurs and the plasma concentration increase after each dose until a steady state is reached provided that:

a. Absorption is not altered.

b. Drug binding to plasma proteins remains constant over a wide concentration range.

c. Elimination rate is not dose dependent.

d. The drug does not induced its metabolism, then

$AUC_{(SD)} = AUC_{(MD)}$ ---------- (1.2)

$AUC_{(SD)}$ = Area under curve during' single dose.

$AUC_{(MD)}$ = Area under curve during multiple dose.

1.2 Drugs Absorption Influence

Many drugs influence gastrointestinal function; the usual result of absorption interaction is a reduction in the rate of absorption or in the total amount of drug absorbed, so that drug effect are reduced or abolished. Interactions that cause therapeutic failure are obviously important but are unlikely to be recognized unless significantly looked for, on the other hand the risk of drug toxicity may be increased if absorption is enhanced. From the practical point of view, it is important to differentiate between interaction that alter the rate of drug absorption and those that increase or decrease the total amount of drug absorbed since the consequences may be quite different. A change in the rate of absorption of a long - acting drug such as warfarin would probably have little effect or no effect, whereas a

change in total amount absorbed may be disastrous. In contrast, if the absorption of a drug with a short biological half life such as procainamide is slowed down, therapeutic plasma concentration may never be reached. The absorption of drug from gastrointestinal tract is a complex process that depends on many physiological and physicochemical factors. This leads to many pathways within which the mechanisms of drugs absorption interaction are proposed (Skipper *et al.,* 1996)

1.3 Mechanism of Drug Absorption Interaction

There are many proposed mechanism for drug absorption interaction. The possible ones are as follows:

1.3.1 PH effect on dissolution and ionization

Drug induced change in pH of gastrointestinal fluids may have complex and unpredictable effect on the absorption of other drugs taken at the same time.

According to the pH partition theory, weak organic acids are largely absorbed from the stomach, while weak bases are absorbed best from the more alkaline contents of the upper small intestine it is sometimes stated that the absorption of weak acid is reduced if they are given with alkaline drug, since less drug would be present in the unionized lipid soluble diffusible state (Amidon *et al.,* 1995)

1.3.2 Change in gastric emptying and Gastro intestinal motility.

The stomach is not an important site of drug absorption. Basic drugs and compounds absorbed by active transport are not absorbed from the stomach to any extent, and even weakly acid drugs such as aspirin, warfarin, and barbiturates and low molecular weight

natural compounds such as ethanol are absorbed much more slowly from the stomach than from the small intestine. Drugs are probably absorbed more rapidly from the upper small intestine than from the stomach, because of the much greater surface area of the intestine. The rate of gastric emptying may therefore limit to the rate of drug absorption and particularly important in the context of interactions since it can be influenced by many drugs. Drug effects can be reduced dramatically or even abolished if gastric emptying is retarded by food. In contrast, absorption is more rapid and toxicity can be greatly increased when drugs are given orally in the same dose in dilute form rather than concentrated solutions, and this effect has been attributed in part to rapid gastric emptying. (Amidon *et al.,* 1995)

1.3.3 Formation of complexes, ion - pairs and chelates

Drugs may interact in the gastrointestinal tract to form complexes, ion pairs and chelates which may be absorbed more rapidly or more slowly than the parent drugs. The absorption of tetracycline is inhibited by the formation of insoluble chelates with metals such as Calcium and iron. Dicoumarol absorption is increased by the formation of more soluble chelates with magnesium hydroxide, and the absorption of quaternary ammonium anti arrhythmia agent is enhanced by ion-pair formation with salicylate and trichloroacetate. The absorption of drugs may also be reduced by absorption on to Kaolin or charcoal or binding to ionic exchange resins (Quintanar *et al.,* 1997)

1.3.4 Interference with active transport

Drugs that are analogues of naturally occurring purines, pyramidines, sugar and amino acid may be absorbed by small intestine active transport; and it has been suggested that

absorption may be reduced by competition between substrates such as L-dopa and phenyl amino derived from dietary sources. It is possible for one drug to inhibit enzymes involved in the active transport of another drug and such an interaction has been postulated between chlorpromazine and L-dopa (Prescott, 1969).

1.3.5 Distribution of Lipid Micelles

Interferences with micelles formation may limit the solubility of lipids and inhibition of absorption of cholesterol, bile acids and Vitamin A by neomycin has been attributed to this mechanism (Shirley *et al.*, 2000)

1.3.5 Change in portal blood flow

The splanchnic blood flow may occasionally be a rate limiting factor in drug absorption and this may be of clinical significance because many drugs could have direct effect on the local gastrointestinal blood flow (Prescott, 1969).

1.3.6 Toxic effect on gastrointestinal Mucosa

Toxic effect on gastrointestinal mucosa may cause mal-absorption syndrome with impaired absorption of other drugs. Neomycin, P-Amino salicylic acid and colchicines may cause megaloblastic anaemia through interference with vitamin B_{12} absorption (Amidon *et al.,* 1995)

1.3.7 Change in Volume, composition and viscosity of secretion

Drugs can also influence the volume and composition of gastrointestinal secretions (including bile) and changes in viscosity may modify drug absorption. It is now recognized that many drugs interact through this mechanism. (Amidon *et al.*, 1995)

1.3.8 Effect on mucosal and bacterial drug metabolism

Recent findings have shown that many drugs are extensively metabolized by the gastrointestinal mucosa and the gut bacterial flora, and this process might be influenced by the concurrent administration of other drugs (Prescott, 1969).

1.3.9 Change in membrane permeability

During oral therapy, the intestinal mucosa is exposed intermittently to very high drug concentration that many alter the permeability of the gastrointestinal epitheliums. ln this context it is interesting to note that insulin and many polypeptides greatly enhanced the membrane transport of ranitidine, isoniazid, salicylate and chlorpromazine (drugs normally considered to cross cell membrane by passive diffusion). Thus, intestinal up take of ionized is enhanced by insulin and this effects is antagonized by ouabain. (Amidon *et al.,* 1995)

1.4 Drugs Distribution Interaction

Competition between co-administered drugs at non specific binding sites in the body can result in displacement of one drug by another, with a resulting rise in the free and active fraction of a drug. lt is widely believed that this displacement or redistribution phenomenon causes the enhanced clinical effects and toxicity seen when certain drugs and other substances interact in man.

The distribution of drugs is dependent on the following factors:- Regional blood, flow Partition coefficient of drug between blood and tissue, Binding plasma proteins and tissues macromolecules and Active transport, (Alstead *et al.,* 1971)

This mechanism of drug interactions can be broadly divided into those occurring at the receptor level or beyond and those occurring by pharmacokinetic process prior to the receptor. In the intact body, more than one mechanism may operate simultaneously.

Many drugs reversibly bound to plasma proteins. Drugs may compete for common binding sites on the binding proteins and when administered together they may displace each other. As a result, there is tendency towards an increased in the free (unbound) plasma concentration. The potential importance of this interaction lies in the fact that only the unbound (free) fraction of drug in plasma is available for distribution to tissue (Alstead *et al.,* 1971)

Although in theory this mechanism could produce potent drugs interaction. lt has been overemphasized in the past because unwanted extrapolations have been made from in-vivo studies. If a drug A displaced a second drug B from its plasma protein binding site, the increase in the unbound fraction of B will occur in-vivo will always be less than that observed in vitro. The reason for this is that a proportion of displaced drug molecules will diffuse extravascularly into the drugs distribution volume. Consequently the greater the distribution volume, the less will displacement interactions results in clinically important effect. In addition, displacement interaction will only be significant if the displaced drug is itself highly protein bound (more than 90 %) otherwise increase in free concentration will be too trivial to be relevant.

The following considerations would help to determine whether a given interaction was purely re-distributional one involving plasma protein.

a. The whole interaction should be mimicked by any drugs with comparable displacement ability.
b. Absorptive interaction should be excluded e.g. the interaction should be shown to occur when both drugs are administered parentally or alternatively formed pharmacokinetic analysis of absorption studies.

Metabolic and excretory rates should be measured. Any changes should be entirely predictable from changes in the concentration of free drug. These conditions are not satisfied, some quantitative allowance must be made for the contributions of these and other pharmacokinetic models of interaction. (Alstead *et al.*, 1971)

1.5 Drugs Elimination Interaction

The elimination rate of a drug can best be described by its clearance. Changes in renal function can modify a number of pharmacokinetic processes in the body and there by lead to unanticipated drug effects or drug interaction. Most of drugs are eliminated by renal excretion, by metabolism or by a combination of the two processes. Total clearance is equal to the sum of the individual clearance for renal excretion and metabolism.

1.5.1 Renal Excretion

Drug loss by the kidneys is determined by the net effect of Glomerular filtration, tubular secretion and tubular re-absorption,

Glomerular filtration is not greatly influenced by other drugs although displacement for protein binding sites may lead to increase concentration of drug in Glomerular fluid and enhance renal elimination. Tubular secretion of acid drugs is mediated by relatively non -

specific active transport system and a variety of endogenous and exogenous compounds are potential substrate. Competition for tubular secretion may lead to unpaired renal excretion of drugs whose major route of elimination is via this pathway. Thus probenecid impairs the elimination of penicillin salicylate and indomethacin. Tubular re-absorption is passive and dependent upon the lipid solubility with which a drug can cross the tubular epithelium and on the concentration gradient between tubular fluid and plasma water. Since many drugs are weak electrolytes, the pH of the tubular fluid will be a determinant of drug re-absorption. Consequently, the clearance of acid drug (e.g. salicylate) will be more rapidly excreted in acid urine. These changes in urine pH however will only be of practical importance for the elimination of those drugs for which renal excretion plays a significant role. (Alstead *et al.,* 1971)

1.5.2 Drug Metabolism

Metabolism of drugs generally leads to the formation of polar (lipid - insoluble) derivatives which can undergo renal excretion. Many commonly used drugs are oxidized in the hepatic endoplasmic reticulum. The activity of the enzyme systems involved is subject to wide inter-individual variability. Moreover, the activity of this system may be enhanced (induced) or diminished (inhibited) by other drugs or environmental pollutants.

Induction of hepatic microsomal oxidizing system leads to increased drug clearance and a reduced steady state level during multiple dose therapy. This may result in therapeutic failure. By contrast, inhibition of hepatic microsomal oxidation leads to reduced drug clearance and increased steady state plasma levels. Inhibition of microsomal oxidation is therefore liable to precipitate toxicity. The mechanisms involved in inhibition of drug

metabolism are unclear. It is important, therefore to realize that a drug which inhibits the metabolism of one oxidized drug does not necessarily inhibit the metabolism of other oxidized drug. There are several factors that could influence metabolism, the most important ones are, genetic physiological factors, pharmacodynamics factors and environmental factors (Prescott, 1969).

1.6 Statement of Research Problem

Inappropriate poly-pharmacy has been reported by several studies to be an independent predictor of harmful drug-drug interaction and the consequent adverse drug reaction (Rodriguez and Oliveira, 2016; Nguyen *et al.*, 2006). Ascorbic acid which is an antioxidant and a urinary acidifier, has been reported to affect the pharmacokinetic parameters of Pefloxacin which is also as weakly basic as Chloroquine (Awofisayo *et al.*, 2012).Common co-prescription of vitamin C with Chloroquine (Ezenduka *et al.*, 2014) may have arisen concern for possibility of pharmacokinetic interaction owing to the antioxidant and urinary acidifying properties of the former that may modify the absorption, metabolism and excretion of Chloroquine.

1.7 Justification

Poly-pharmacy in our healthcare settings may result in drug-drug interaction which may cause modification of pharmacokinetic parameters. Based on common co-prescription of vitamin C with chloroquine in our healthcare settings, there may be interaction which may result in the modification of the pharmacokinetic parameters of chloroquine. There is little information on whether the pharmacokinetics of chloroquine may be significantly modified

by co-administration with Ascorbic acid. This study is designed to provide an insight on this identified gap.

1.8 Aim and Objectives of the Study

1.8.1 Aim

To determine the effect of vitamin C on the pharmacokinetic parameters of Chloroquine (CQ).

1.8.2 Objectives

The objectives of this study is to:

- Sample and carry out quality control assessment tests of both chloroquine and vitamin C tablets according to BP, 2009.
- adopt and validate analytical method for the determination of CQ in saliva.
- determine the pharmacokinetics of Chloroquine alone after concurrent administration with Vit C delayed administration with vitamin C in healthy human volunteers.

1.9 Research Hypothesis

Vitamin C has no significant effect on the pharmacokinetics of Chloroquine (CQ) when the two drugs are co-administered.

CHAPTER TWO

2.0 LITERATURE REVIEW

This research work concerns the modification of one drug by the presence of another whether by direct or indirect means with reference to chloroquine and vitamin C. In general drug-drug interaction may affect absorption, distribution, receptor action, metabolism and excretion. This type of interaction may be beneficial or hazardous. They may vary from person to person and form species to species. They may also be of major chemical significance or of no consequence.

2.1 Chloroquine

Chloroquine is an outstanding antimalarial drug which is a synthetic product and was among the first synthetic compounds employed in chemotherapy of malaria (Hong, 1976).

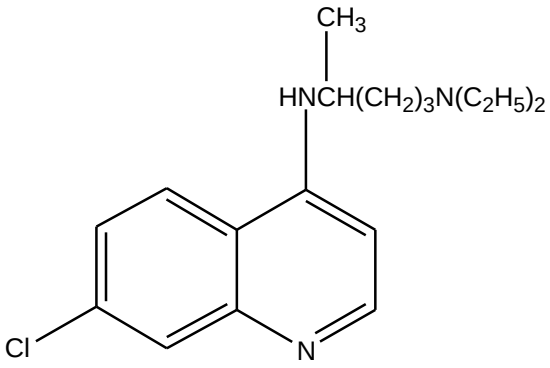
2.1.1 Physical properties of chloroquine

Chloroquine phosphate is a white, odorless, crystalline powder having a bitter taste, it discolors gradually on exposure to light. It is freely soluble in water, practically insoluble in alcohol, chloroform and in ether. It is less soluble at neutral and alkaline pH. It is stable to heat while in solution at pH 4 - 6.5. Chloroquine phosphate exists in two polymorphic forms giving rise to two melting ranges. The Meta stable is between (193^0C - 195°C) and the stable polymorph melts between (210°C - 215^0 C) Mixture of these form melt between (193^0 C-215°C) It is possible to obtain one form selectively by regulating the rate of crystallization (Hong, 1976).

Chloroquine phosphate exhibits essentially no optical activity, existing as a racemic mixture. Riegel and Sherwood (1949) have shown that neither of the optically active enantiomorphs showed any significant differences in antimalarial activity in birds and for toxicity in dogs.

2.1.2 Chemistry of chloroquine

Chloroquine is a member of the 4- aminoquinoline. The chemical name of chloroquine phosphate is 7-chIoro-[4-[4~dithylamino]-1-methylbutylamino] quinoline diphosphate. Chloroquine phosphate has a molecular weight of 515.9 and a molecular formula $C_{18}H_{32}CIN_3O_8P_2$.



Chloroquine

Fig. 1.1: Chloroquine

Chloroquine has a quinoline nucleus and an alkyl side chain. It has chlorine atom attached in position 7 of the quinoline nucleus. When there is addition of methyl group on carbon 3, the antimalarial activity is reduced, while the addition of another methyl group on carbon 8 leads to complete loss of antimalarial activity. Carbon 7 was found to be the best tor

halogen substitution. The 4 –aminoquinoline showing the most marked antimalarial activity in man have a chlorine atom in position 7 on the quinoline nucleus.

Introduction of other groups on the quinoline nucleus reduces antimalarial activity. The 4-diethylamino -1-methylbutylamino side chain in chloroquine is optimum for activity. It was later found that introduction of hydroxyl groups on the side chain particularly on the ethyl group of the terminal nitrogen, tends to reduce toxicity and produce higher blood level concentration as seen in hydroxyl chloroquine

The d, l and dl isomers of chloroquine are indistinguishable in potency tests in duck malaria, but in mammals the d form is somewhat less toxic than the l (White, 1977).

2.1.3 Metabolism of Chloroquine

Chloroquine is metabolised by side chain de-ethylation leading successively to desethyl and then bisdesethyl chloroquine, which is a primary amine. This compound can undergo oxidation to the 4-carboxylic acid derivative.

Chloroquine is eliminated from the body slowly due to extensive tissue binding and after and oral dose of 300 mg, the drug and its metabolite can be detected in plasma for up to 56 days depending on the sensitivity of the method used (Garba, 1992).

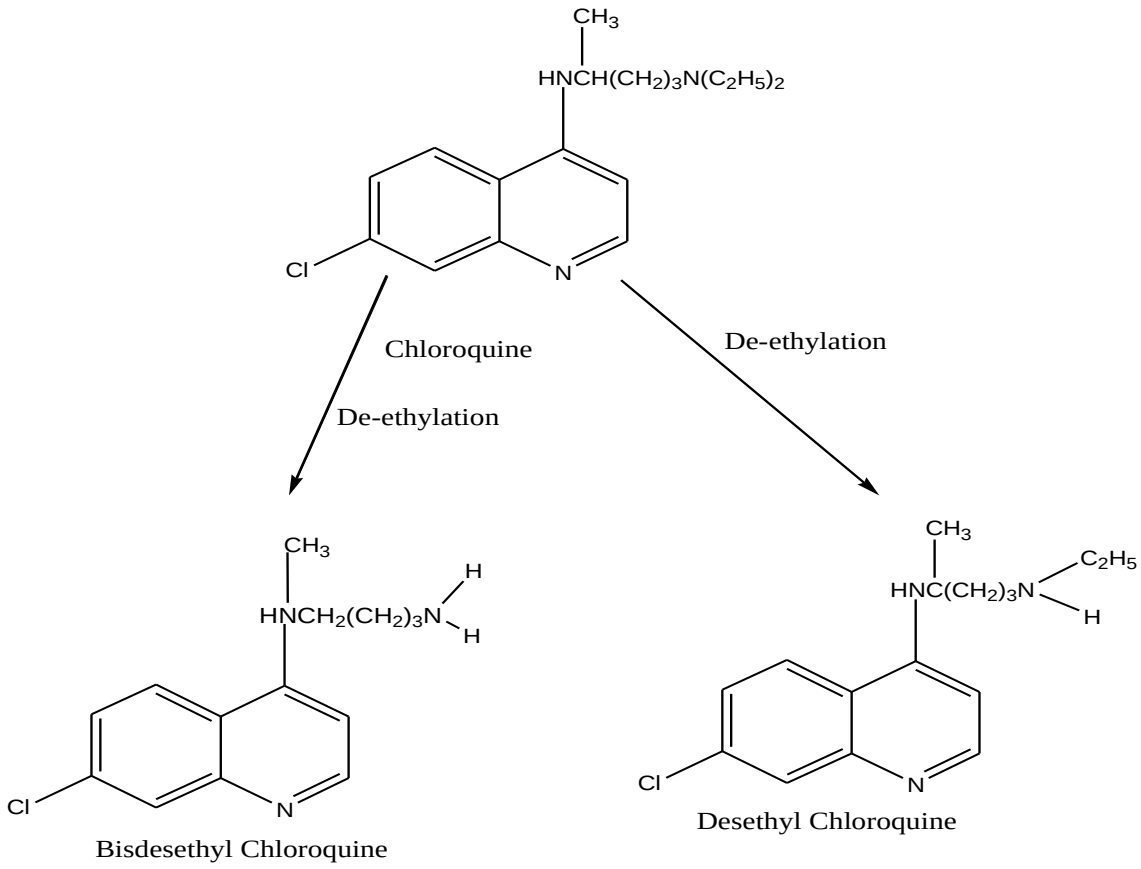


Fig.1.2: Metabolic Pathway of Chloroquine (Garba, 1992)

2.1.4 Mechanism Of Action.

Chloroquine is bactericidal for Bacillus megaterium, inhibiting the synthesis of DNA by blocking DNA replication and degrading the ribosome. The same mechanism is probably responsible for its plasmodicidal action. Resistance to the drug may be the result of impermeability to the drug or impairment of the mechanism for concentrating the drug within the infecting organism (Auram *et al.*, 1974).

Chloroquine is an example of the quick acting schizonticides. While preventing nucleic acid biosynthesis, act directly by forming molecular complexes with DNA thereby preventing the DNA primed DNA polymerase reaction.

The complex formation with DNA renders the nucleic acid incapable of acting as a template for its own replication or for the transcription of RNA, and this occurs regardless of the source of DNA (Rollo, 1968). It has been postulated that chloroquine causes the lysosomes to increase in number and volume, decrease in density and develop lamellar membrane structures called myeloid (myelin) bodies, and thus leading to the formation of large quantities of cellular membranes which are sequestrated within the cell in the increased number of lysosomal vesicles, thereby inhibiting the normal functions of the cell (Mac-Intyre *et al.,* 1986).

Chloroquine and related antimalarial agents causes the clumping of malarial pigments that forms as the parasites digest haemoglobin of the hosts erythrocytes. It was concluded that clumping of pigment disrupts the amino acid metabolism of the parasite and leads to its death (Ciak *et al.,* 1966).

Also according to Homewood and colleagues (1972); chloroquine is a weak base which produced marked swelling of the parasites food vacuole (secondary lysosomes) and they proposed that chloroquine may produce its anti-malarial effects by raising the pH of the vesicle. They proposed that by raising the vesicle pH above the optimal level for the acid proteases of parasite, parasite growth may be prevented, as the proteolysis of haemoglobin is essential for normal parasite growth and replication. Chronic administration of high doses of chloroquine for the treatment of rheumatoid arthritis has been reported to produce a

reduction in body weight of the individual. This effect has been shown to be mediated through the inhibitory action of chloroquine in the central control, hypothalamic feeding centre of food (glucostatic mechanism) and water intake. Also this chronic administration of chloroquine is associated with skeletal muscle myopathy.

Such administration brings about changes in reactivity of the hemi diaphragm muscle to electrical stimulation and drugs with a resultant decrease in muscle strength and tolerance to chloroquine in-vitro. Thus, the direct inhibitory action on the skeletal muscle by chloroquine interferes with Ca^{2+} mobilization within the muscle (Midala, 1972).

2.1.5 Pharmacologic actions

Chloroquine following its introduction was considered as the drug of choice in the treatment of falciparum malaria, abolishing the acute attack and completely curing the infection. As an antimalarial agent, chloroquine is highly effective against the asexual blood forms of P. vivax,

P. malariae and susceptible strains P. falciparum. Chloroquine and other 4-aminoquinoline are effective against protogametoctes but not mature gametocytes of P, falciparum. They are effective against the gametocytes of P. vivax, P. ovale, P. malariae at all stages of their development. Hence chloroquine is highly effective against erythrocytic parasites. It is a suppressive drug that produce complete cure in falciparum malaria but will not eliminate the exoerythrocytic forms of P. vivax. Consequently relapse occurs in vivax treated with chloroquine, although the drug can terminate the clinical attacks very efficiently and it does not prevent the establishment of vivax infection when administered prophylactically. Although by 1960 strain resistant to the drug had appeared and at present as many as 20%

are refractory to the drug and require new drugs for its eradication. Chloroquine has other antiparasitic actions. Chloroquine phosphate is also effective in amoebiasis, because of its lesser toxicity, may be used instead of emetine for the treatment of systemic amoebiasis but in order to affect a cure it must be combined with an arsenical or oxyquinoline to eradicate the infection from the lower bowel (Mac-Kenzie, 1983).

a. To administer a priming or loading dose initially to saturate the tissues and rapidly obtained the desired tissue levels over a 2-3 week period and
b. To exceed dose levels proposed for antimalarial use to the point of developing mildly toxic symptoms. Chloroquine phosphate is also effective in creeping eruption (larva migrans), a cutaneous disorder caused by Ancylostoma braxiliense. Chloroquine has also been used in the treatment of sarcodiosis and as a dislodging agent in obstructive vascular disease but its value in these conditions is questionable.

In infectious mononucleosis, chloroquine shortens the course of the disease but its beneficial effects are neither absolute nor dramatic nor does it affect the degree and duration of the lymphocytosis and heterophile antibody titre.

Chloroquine and its congeners have been widely used in the treatment of chronic discoid lupus erythematosus and rheumatoid arthritis, in discoid lupus; it was found out that about 60% of patients exhibit a complete remission or marked improvement. In rheumatoid arthritis, some symptomatic relief is obtained when the drug is used but the response is delayed with maximum benefit appearing only after 6 - 12 months of treatment.

Chloroquine has also been recommended for use in polymorphic light eruption, in solar urticaria, and in auto sensitivity to deoxyribonucleic acid (Goldstein *et al.,* 1974).

Chloroquine stabilizes lysosomal membranes, leading to what is referred to as lysosomal storage disease (LSD). As these membranes are stabilized, residual matter left in the lysosomes after digestive processes cannot be eradicated.

Chloroquine reduces conduction velocity of the cardiac muscle and it is known to be a cardio depressant. In very high doses particularly when administered parenterally, with plasma concentration of greater than 1000 mg/ml, the drug may cause vasodilatation and hypotension which could precipitate cardio vascular collapse leading to death (Midala, 1992).

2.1.6 Pharmacokinetics of chloroquine

Chloroquine is readily absorbed from the gastro-intestinal tract and about 55% in the circulation is bound to plasma proteins. It accumulates in high concentrations in some tissues, such as the kidneys, liver, lungs and spleen, and is strongly bound in melanin - containing cells such as those in the eyes and the skin. It is also bound to double-stranded DNA, present in red blood cells containing schizoints. Chloroquine is eliminated very slowly from the body and it may persist in tissues for a prolonged period up to 70 % of a dose may be excreted unchanged in the urine and up to 25 % may be excreted also in the urine as the desethyl metabolite. The rate of urinary excretion of chloroquine is increased at low pH values (Salako *et al.*, 1984).

Chloroquine accumulates in smaller concentration in fat, bone, tendons and brain, and the lowest tissue. Concentrations are found in skeletal muscles, skin, and sclera. Because it is concentrated in the tissues, it has a very large apparent volume of distribution of about 13, 000 L. From these sites it is slowly released and metabolised.

The drug readily crosses the placenta. It is excreted in the urine with a half life of 3-5 days. Renal excretion is increased by acidification of the urine.

Because of its very large volume of distribution a loading dose must be given when an effective schizonticidal plasma level of chloroquine is urgently needed in the treatment of acute attacks. To avoid life threatening toxicity when chloroquine is given parenterally, it should be provided by slow intravenous infusion or by small incremental doses intramuscularly. The therapeutically effective plasma concentration appears to be approximately 30 µg/L against P. falciparum and 15 µg/L against P vivax. After oral intake of a single dose of 300 mg, chloroquine base produces peak plasma concentrations of 56-102 µg/L (mean 75 µg/L] within 1 – 6 hr [mean 3 hr] with a 75 % bioavailability.

There is wide variation in plasma half-life which is 21/22 days when sampling is carried out for 7 days as against ten days for a sampling period of 56 days. The shorter half-life pertains to the elimination of the drug from those body sites to which it has a low affinity and therefore describes the plasma concentration rate, on the other hand the longer half-life depicts the elimination rate from tissues to which chloroquine is highly bound. Chloroquine is mainly eliminated from the body by urinary excretion and can be detected in the urine for 120 days after a 300 mg dose. In the study conducted in Uganda by (Ogwal and Okeng, 2003) 39 % failed the active content test for chloroquine tablets. They concluded that variations in the chloroquine amounts in the tablet may contribute to chloroquine toxicity or poor response during treatment. Also another study was conducted by (Patel *et al.*, 2005) in India to ascertain the equivalence of seven brands of chloroquine phosphate tablets sourced from retail pharmacies were determined using invitro methods. This study was aimed at

obtaining baseline data towards establishment of bioequivalence of the tablets. They reported that six brands passed the assay test while one brand failed.

2.1.7 Dosage and Administration

For the treatment of non-severe malaria or susceptible P. falciparum. Malaria oral dose is given in a dose of 25 mg base/kg body weight over 3 days as follows for both adult and children.

Day 1: 10 mg base/kg body weight as single dose

Day 2: 10 mg base /kg body weight as single dose.

Day 3: 5 mg base/kg body weight as a single dose.

However, it has been claimed that better results are obtained by boosting the first dose as follows.

Day 1: First dose 10 mg/kg body weight, 6 - 8 hours later 5 mg/kg body weight.

Day 2 and 3: 5 mg/kg body weight.

The 3 day course is sufficient to eliminate susceptible P. falciparum infections since effective anti malarial plasma concentrations are sustained for several weeks. No further treatment is required in patients removed from risk of infection. For children less than 1 year or under 9.9 kg – 75 mg base in one dose.

1 - 3 years or 10 ~ 14.4 kg – 150 mg base in one dose

4 - 6 years or 14.5 - 18.4 kg – 225 mg base in one dose.

7 - 11 years or 18.5 - 34.9 kg – 300 mg base in one dose.

12 years and above or 35 kg and over – 600 mg base in one dose.

2.2 Chemistry of Vitamin C

Vitamin C is a water soluble Vitamin, it is a synthetic product with both antioxidant and urinary acidifying properties.

2.2.1 Physical properties of vitamin C

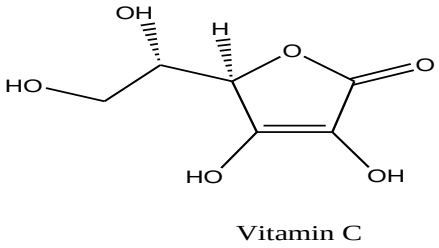
Vitamin C is a white or slightly yellow crystal powder on exposure to light it gradually darkens. In the dry state, it remains stable in air but in solution it rapidly dissolves. It is freely soluble in water, soluble in alcohol, insoluble in chloroform, ether and hexane. It bears pH of 2.1 to 2.6. It is stored in nonmetallic air tight containers. It also protected from light. It has a melting point of above 190 ^{0}C with decomposing (Michael *et al.,* 1991)

2.2.2 Structure activity relationship

Vitamin C (L-ascorbic acid) is a disaccharide and with enediol group built into a five measured heterocyclic lactone ring, the chemical and physical properties of ascorbic acid are related to its structure. The chemical name of ascorbic acid is (2R)-2-[(IS)-1, 2-dihydroxyethy1]-3, 4-dihydroxy-2H-furan-5-one (B.D Michael et al., 1991)

Vitamin C has a molecular weight of 176.1 with a molecular formula of $C_6 H_8 0_6$.

2.2.3 Structure of vitamin C



Vitamin C

Fig 1.3: Vitamin C

The first oxidation product of ascorbic acid has been analyzed by X-ray crystallography to be a dimmer.

Electrochemical studies done on ascorbic acid can be a reduced form of vitamin C, electrochemical studies have indicated that ascorbic acid and dehydroascorbic acid forms a reversible redox couple.

The ascorbic acid molecule consists of two asymmetric carbon atoms, C-4 and C-5(5) therefore in addition to L-ascorbic and itself, there is three other stereo isomers: D ascorbic acid, D- isoascorbic acid and L-isoascorbic acid. These three isomers have very little or no antiascorbic acid activity.

2.2.4 Metabolism of vitamin C

Ascorbic acid is metabolized in the liver, and to some extent in the kidney, in a series of reactions, the principal metabolism of ascorbic acid in where there is loss of the electrons. The intermediate free radicals reversible forms dehydroascorbic acid, leading to the irreversible formation of the physiological inactive 2, 3-dehydroascorbic acid. Dehydroascorbic acid may be either cleaved to ascorbic acid and the other acid, or

decarboxylated to carbon dioxide, lactone and glucose lactones to ascorbic acid, all there metabolites and ascorbic acid itself are excreted in the urine.

2.2.5 Mechanism of Action

Vitamin C, is a water soluble compound, is essential for the synthesis of collagen and intracellular material, vitamin C has a role in cellular metabolism which is accounted for by its reducing properties to protect cellular component from evidence damage (Michael *et al.*, 1991)

The exogenous form of vitamin C is required for collagen formation and tissue repair. Ascorbic acid is reversibly oxidized to dehydroascorbic acid in the body. These two forms of vitamin C are important in oxidation reduction. The vitamin C is involved in tyrosine metabolism, conversion of folic acid to folinic acid, carbohydrate metabolism, synthesis of lipid and proteins, iron metabolism, resistance to infection and cellular respiration.

2.2.6 Pharmacological actions of vitamin C

Vitamin C is concentrated for the synthesis of collagen and intracellular material. Vitamin C deficiency develops when the dietary intake is inadequate. It is rear in adult and may occur in infants or the elderly. Deficiency lead to scurvy, this is characterized by wavy fragile, bleeding from the gums, normocytic or macroytic anaemia, cartilage and bone degenerations and slow healing of wounds. (Michael *et al.*, 1991)

Vitamin C is used in the treatment and protection of deficiency, it completely reverse deficiency if it is normally given by mouth (orally) as ascorbic acid. Ascorbic acid or sodium ascorbate may be given preferably by intramuscular route, but also by intravenous or

subcutaneous route. Doses of 25 to 75 mg or more daily, in the prevention of deficiency and 25mg or more daily may be given with desferrioxamine in the treatment of patient with thelassaaemia to improve the dilating action of desferrioxamine, thereby increasing the excretion of iron. In two deficiency states ascorbic acid may increase gastrointestinal iron absorption. Ascorbic acids or sodium ascorbate have been used in treating methemoglobinemia. Ascorbic acid is also used to acidify the urine.

Eye drops containing potassium ascorbate have been used for the treatment of chemical burns in the eye (Potassium ascorbate 10 %) while in the alternate sodium citrate 10 % is also used. It is also believed that ascorbate works by mopping up free oxygen radicals thus aiding in the prevention of corneal epithelial damage (Sweetman, 2007).

Ascorbic acid, calcium and sodium ascorbate are used as antioxidant in pharmacological manufacturing and in food industries.

2.2.7 Pharmacokinetics of vitamin C

Ascorbic acid is readily absorbed from the gastrointestinal tract and is widely distributed in the tissue. Plasma concentration of ascorbic acid rise as the dose injected is increased until plateau is reached in the doses of about 90-150 mg daily. Body stores of ascorbic acid in health are 1.5 g, although more may be stored at above 200 mg daily. The concentration is higher in leucocytes and platelets than in every part and plasma. In deficiency states the concentration in leucocytes declines later and at a slower rate. And thus has been considered to be better criterion for the evaluation of deficiency than the concentration in plasma.

Ascorbic acid is reversibly oxidized to dehydro ascorbic acid, some is metabolized to ascorbate -2- sulfate, which is active, and oxalic acid which are excreted in the urine. It is

also rapidly eliminated unchanged in the urine, this generally occur with intakes exceeding 100 mg daily. Ascorbic acid crosses the placenta and is distributed into breast milk. It is removed by haemodialysis (Sweetman, 2007)

2.2.8 Dosage and administration of vitamin C

A daily requirement of 30-100 mg of vitamin C is recommended for adult. There is however wide variation in individual requirements. For humans a dietary source of vitamin C is from fruit and vegetable. An amount of 250-500 mg of vitamin C cube administered by adult for any benefit, pregnant and breast feeding are administered between 80-120 mg daily, for pediatric the dosage from birth to 6 months is 40 mg daily and children up to adolescent age the dosage are tailored to 75 mg daily.

2.2.9 Adverse effects of vitamin C

Vitamin C is actually well tolerated. Large dose reported to cause diarrhea and other gastrointestinal disturbances. Large dose also causes hyperoxaluria and the formation of renal calcium oxalate calculi and ascorbic acid should therefore be given with care to patient with prolong use of large doses, resulting in symptoms of deficiency when intake is reduced to normal. Prolonged or unnecessary usage of vitamin C preparation may cause erosion of tooth enamel. Large dose of ascorbic have resulted in haemolysis in patients with Gastro esophageal reflux disease (GERD).

2.2.10 Contraindications and caution

Vitamin C and supplement have a diuretic effect; they help the body to get rid of excess fluids. Vitamin C increases the amount of ion absorbed from foods, as such it is

contraindicated in people with haemochromatosis. Vitamin C is safe at regular dose but at higher dose more than 2000 mg it can cause diarrhea or gastrointestinal upset. Caution is observed in people with compound kidney effects born to mothers taking, 6000 mg or more of vitamin C may develop rebound scurvy, regular intake of vitamin c drops after birth. People with sickle cell anemia and metabolic disorder such as glucose phosphate dehydrogenase can potentially have serious effects with high levels of vitamin c. vitamin C may raise blood sugar levels in diabetics. Vitamin c may potentially interact with some chemotherapy drugs.

2.2.11 Vitamin C drug interactions

Possible drug interaction of vitamin C and aspirin as well as nonsteroidal anti-inflammatory drug is observed, amount of vitamin C is lower in the body when these drugs are administered, the vitamin C is found in the urine.

High dose of vitamin C may lower the amount of acetaminophen found in the urine which could cause the level of the drugs to rise in your blood. Vitamin C can increase the amount of aluminum your body absorbs which could possibly increase the adverse effect of this drug. Barbiturates such as Phenobarbital and pentobarbital may decrease the effect of vitamin C (Sweetman, 2007).

Vitamin C being an antioxidant may interfere with drugs taken for chemotherapy. Vitamin C when taken with oral contraceptives and hormonal replacement therapy may cause a rise in levels of estrogen. Vitamin C slightly lowers the level of indinavir an antiretroviral and may also increase the levels of tetracycline in the body (Sweetman, 2007).

2.2.12 Use of vitamin C in pregnancy

Higher doses of vitamin C are not recommended in pregnancy, limited evidence is available in taking too much of vitamin c in the form of supplements during pregnancy May increase the risk of premature birth. The recommended dosage of vitamin C during pregnancy is 85 mg per day for women age 19 and above and 80 mg per day for women age 14 to 18. The maximum daily amount that is considered safe for pregnant women in their same age is 2000 mg and 1800 mg respectively. Vitamin C is likely safe for pregnant or breast feeding women when taken in the recommended dose of 120 mg per day (Martindale, 2010).

2.2.13 Storage of vitamin C

Ascorbic acid should be protected from air and light and be stored in a tightly closed nonmetallic container.

2.3 Basic Pharmacoklnetlcs

The term pharmacokinetics is concerned with study and characterization of the time course of drug absorption distribution, metabolism, excretion and with relationship of these processes to the intensity and time course of therapeutic and adverse effect of drugs. A drug exert 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 physiological barrier (GIT) for orally administered drug and the process being known as absorption, while its 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 into 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 an erythrocyte and plasma protein 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 the distribution of drugs into the various tissues, several elimination processes occur. These processes include urinary excretion, billiary excretion and conversion into 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. (Katzung, 1980)

To ascertain the pharmacokinetics characteristics of a drug, the different stages of absorption, distribution and elimination must be quantified and denoted 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 or different routes.

The most important pharmacokinetics parameters that are being considered in drug absorption are:-

a. Coefficient of absorption
b. First pass effect
c. Area under curve
d. Half-life,

These parameters determine to a large extent the pharmacological responses of individual patient. Pharmacokinetics helps determine the frequency of administration while excluding any risk of toxicity. Physiological state of the individual related to his age, sex, genetic makeup and morphology are also taken into consideration. In order to estimate the effect on the pharmacokinetic properties ‘of the drug, the pathological state of the patient whether transient or permanent is assessed. This clinical pharmacokinetics is referred to as the application of pharmacokinetics therapy to the management of the patient. (Katzung, 1980).

2.4 Compartmental Models

In pharmacokinetics experimental data on drug concentration in plasma are title into mathematical equations that represent two 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:-

a. Drug enters the system via a central compartment and is eliminated only from the compartment.

b. Reversible transfer occurs between central and peripheral compartments.

c. The exit of drug from all compartments in line system is described by a first order kinetics.

d. The third assumption is true for most drugs excepts for drugs eliminated by biotransformation whereby saturation of metabolite enzymes makes elimination a zero order process

2.4.1 Single compartment model

Here, exchange of drug 'between the plasma and tissue proceeds rapidly compare with the rates elimination with the whole body considered mathematically as a single compartment. We assume an instantaneous distention after an injection of a drug into the models.

This equation then holds true:

$C_0 = D / V_{dc}$ ---------- (1.3)

Where;

C_0 = Concentration immediately after injection

D = Dose

V_{dc}= 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; we get an exponential curve, while on a semi ~ logarithm scale, we get an exponential curve, while on a logarithm scale; we get a straight line. (Goodman *et al.,* 1990)

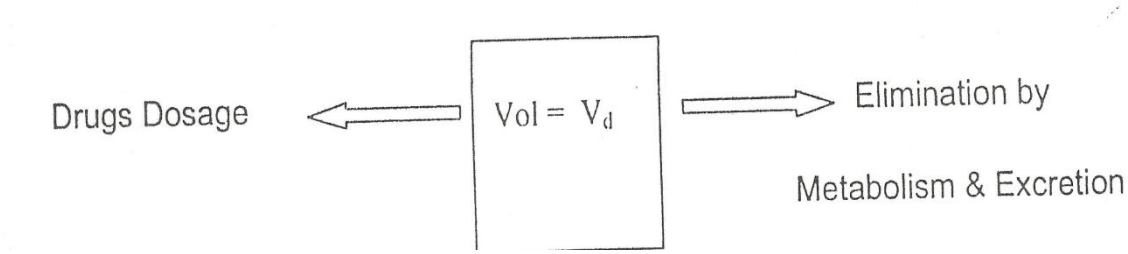


Fig 1.4: Rapid IV injection into a single compartmental model

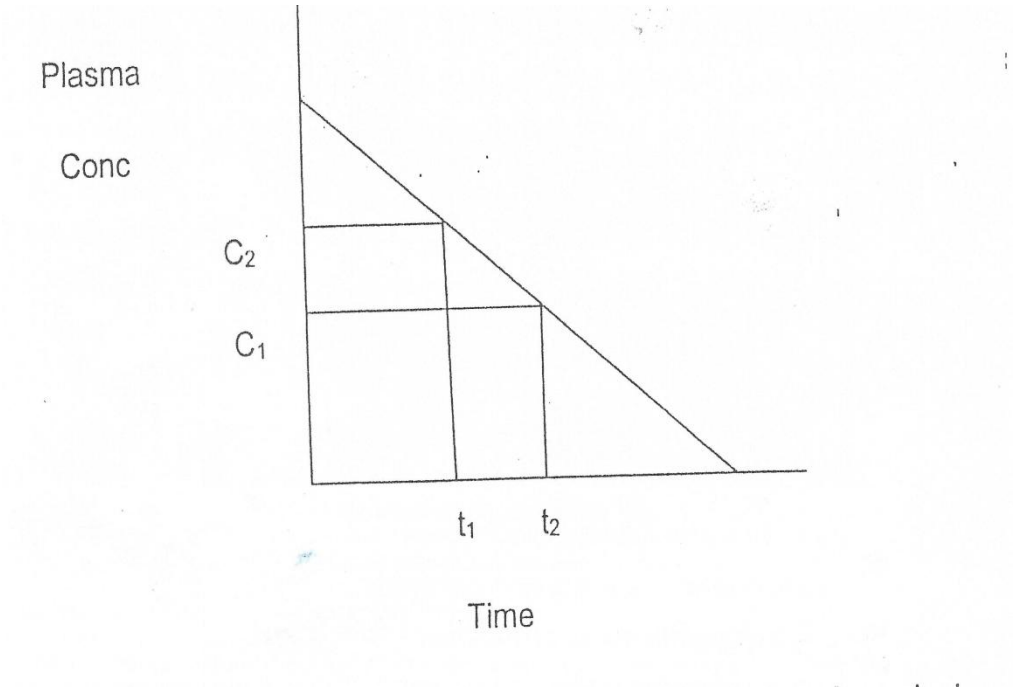


Fig 1.5: Plots of plasma drug concentration against time for a single Compartmental model on log scale

The decline in drug concentration may be expressed as in equation (1.4)

$dc/dt = -B.ct$ ---------- (1.4)

Where B = Elimination rate constant

On integration,

$Ct = Coe^{Bt}$= e = base of natural log

In Ct= In Co - Bt

Converting logarithm to the base 10

$\text{Log } C_{10} = \text{Log } C_0 - B+/2.303$ ---------- (1.5)

2.4.2 Multiple compartmental models

It is Worthy to note that the plasma concentration time curve of a drug is often not a straight line on a semi -- log plot for a slow distribution of drug, a single Compartmental model cannot be fitted into; hence a peripheral compartment in addition to the central must be considered (Paxton, 1981)

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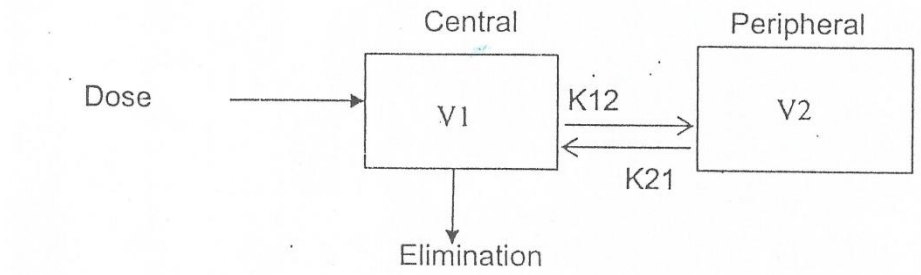


Fig 1.6: Rapid IV injection into a two compartments

V_1 and V_2 = apparent volume of distribution.

K_{12} and K_{21} = distribution rate constants

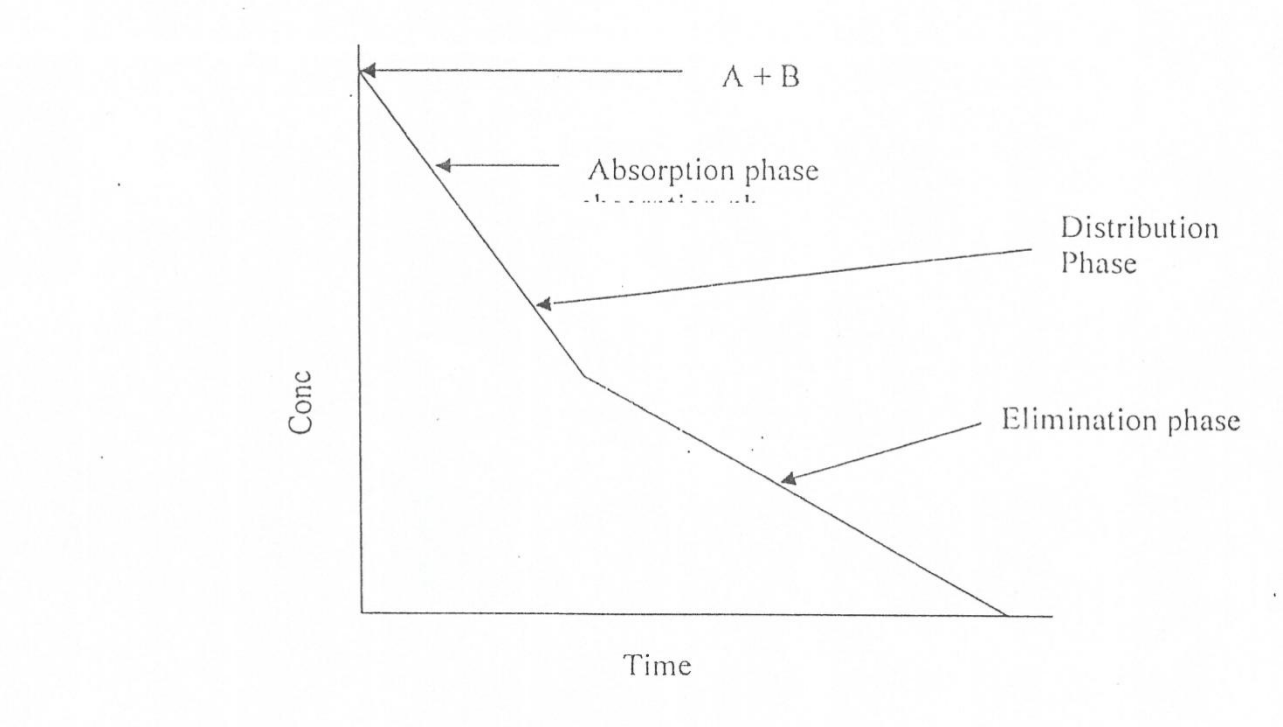


Fig 1.7: Plot of plasma drug concentration against time for a two Compartmental model on a logarithm scale.

This may be represented by the equations (1.6) below.

$C_t = Ae^{xi} + Be^{BT}$ ---------- (1.6)

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

A + B is the actual intercept of the concentration curve at T = 0

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

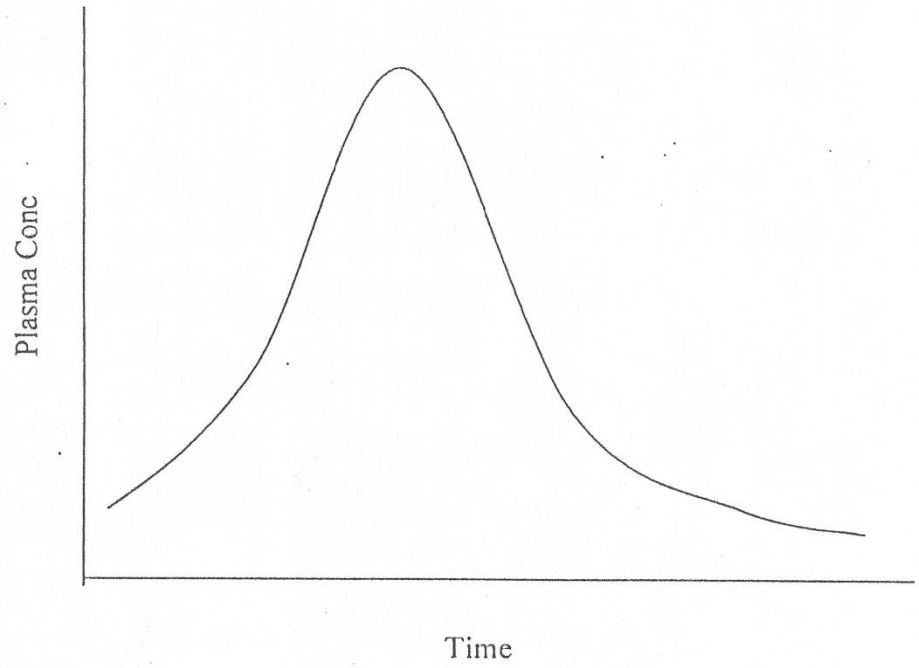


Fig 5: Plot of plasma drug concentration against time for three compartment models on logarithm scale.

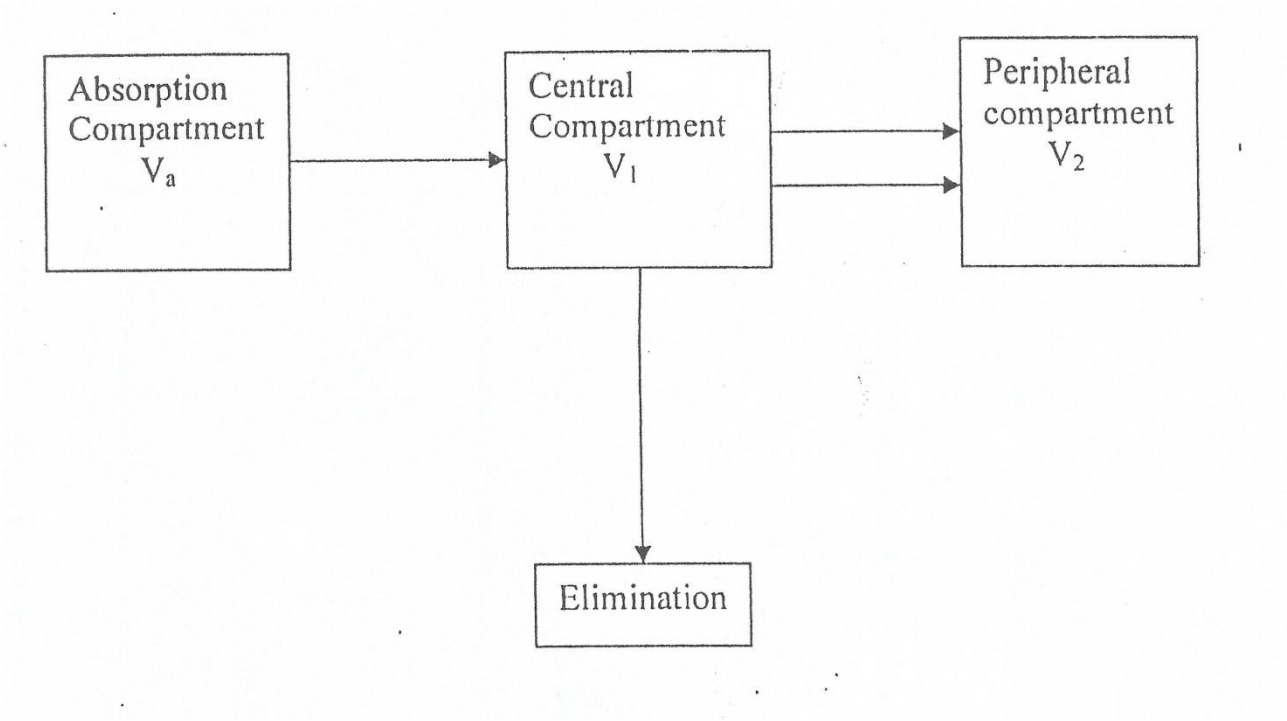


Fig 1.9: Three Compartment model after a single oral dose

2.5 Non Linear Pharmacokinetics

The pharmacokinetics of a drug are said to be nonlinear when one or more of the pharmacokinetics parameters vary with dose, the concentration at a given time. Nonlinear can be related to absorption, the hepatic first pass effect, distribution, urinary excretion billiary elimination or metabolism. (Katzung, 1980)

The most frequent reasons for nonlinear Kinetics are:-

a. Saturation of the hepatic first pass effect
b. Saturation of the plasma protein binding site
c. Saturation of the process of re-absorption of tubular secretion.

c. Saturation of the enzymes involved in biotransformation. `

Examples of nonlinear Kinetics are;

a. Absorption - clomidine, Griseofulvin, chlorthiazide
b. First pass effect - propranolol, hydrallazine.
c. Tissue Distribution - Quinidine, Disopyramide
d. Protein Binding - Quinidine, ceftriazone.
e. Metabolism - Phenytoin, Theophylline, erythromycin, probenicid, 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 nonlinear generally occurs in accordance with the Michele - Menten process (Labanne 1989) as represented in equation (1.7).

$dt/dt = V_m .C/K_m .t$ ---------- (1.7)

dt = Elimination rate

\/m = Maximum velocity of enzyme process

Km = Michael -Menten constant

2.6 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:

2.6.1 Absorption rate constant (K_a)

This is the rate constant of the entire process of drug transfer into the body through all biological membrane. lt has units of reciprocal of time (hr^{-1}) it is calculated using equation (1.8) below.

$$K_a = \frac{0.693}{t\,½} \quad \text{---------- (1.8)}$$

2.6.2 Area under the curve (AUC)

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 to infinity. It measures the quantity of the drug, which has been absorbed and has entered the general circulation. Thus it is a measure of the amount of circulating drug. It has units of mg /hr/ml it can be calculated by the trapezoidal method as represented in equation (1.9) below.

$$A \text{ Area} = \frac{C_1 + C_2\ (t_2 - t_1)}{2} \quad \text{---------- (1.9)}$$

$$AUC^a o = AUC^t + \frac{C}{B}$$

Where B = Elimination rate constant

Mathematical methods

After striping the curve, it is determined by the formula

$$AUC^a_O = A + b$$

2.6.3 Absorption half life ($T_{1/2}$)

This is the time taken for half of the total drug absorbed to be achieved (half of the difference between theoretical and experimental values). lt has units of time in hours.

2.6.4 Elimination rate constant (Ke)

This is the rate constant of the process (es) leading to the elimination of the drug from the body. Lt is the sum of all the individual administration rate constants it has units of reciprocal of time (hr)

2.6.5 Elimination half-life ($t_{1/2}$ e)

This is the time take for a quantity of drug to be reduced by 50%. The half life can be determined using the formula described in equation (1.10).

$$t_{1/2} = \frac{0.6923}{ke} \quad \text{---------- (1.10)}$$

Where K_e = rate constant

With the first order, process, $t_{1/2e}$ is constant and independent of the initial concentration and the administered dose. It may be calculated from elimination phase of concentration time curve on a logarithm scale or from equation (1.11) below.

$$t_{1/2}e = \frac{0.6923}{Cl} \times V_d \text{ or } t_{1/2}e = \frac{0.6923}{Ke} \text{ ---------- (1.11)}$$

Where B = elimination rate constant

Cl = Clearance

V_d = Volume of distribution

It is used to estimate the appropriate dosage interval during maintenance therapy.

2.6.6 Absorption lag time

It begins where extrapolated straight line and that of the residual intersect. It reflects the time taken between the administration of a drug and the time absorption begins. It has unit of time (hr).

2.6.7 Total body clearance

This is the capacity of the body to eliminate a substance after it has reached the general circulation. It is the total sum of all the individual clearance by the various organs. 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 (Labanne 1989). The total body clearance can be calculated using equation (1.12) below.

Total clearance = Cl (renal) + Cl (hepatic) + Cl (other organs of metabolism) --------- (1.12)

Clearance by any organ depends on the blood flow through the organs and the extraction ratios of the drug by the same organ can be determined using equation (1.13) below.

$$Cl = \frac{\text{Dose iv}}{\text{AUC}} = \frac{\text{f x oral dose}}{\text{AUCoral}} \quad \text{---------- (1.13)}$$

Where F = Bioavailability

It has unit of L/hrs

Clearance relates the concentration to the rate of drug elimination.

Rate of elimination = clearance x concentration

2.6.8 Volume of distribution

a. Initial volume of distribution,

This is the ratio of the administered dose to the plasma drug concentration extrapolated to zero time (after an intravenous or intra - arterial injection).

b. Apparent volume of distribution (V_d)

This is the ratio of the amount of drug in the body to its plasma concentration equilibrium. Volume of distribution (V_d) rates the amount of drug in the body to the concentration of drug in blood plasma. Volume of distribution has unit of L or L/Kg and can be determined using equation (1.14) below.

Initial $V_d = V = \frac{D}{Co}$ ---------- (1.14)

Where D = Dose administered by rapid IV. Injection

C_o = Theoretical blood Conc. at time O

Apparent $V_d = V = V_p = V_t$

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

3.1.1 Drugs

Standard powder of vitamin C and chloroquine (Emzor pharmaceuticals, Nigeria), 2 drug products: Tab Chloroquine (150mg) and Vitamin C (100mg)

3.1.2 Glass Wares

- Extraction tubes (Glass fin. W. Germany)
- Pipettes: 0.02 ml, 0.2 ml, 1 ml, 2 ml, 3 ml, 5 ml and 10 ml
- Test tubes: 10 ml and 20 ml (Pyrex England)
- Measuring cylinders: 5 ml, 10 ml, 50 ml and 100 ml
- Volumetric flasks: 25 ml, 50 ml, 250 ml, and 500 ml
- Crucible
- Conical flask: 25 ml, 50 ml and 250 ml (Pyrex, England)
- Beakers: 50 ml, 100 ml and 250 ml (Pyrex, England)
- Round bottom flask (Pyrex, England)
- Syringes and needle: 2 ml, 5 ml and 10 ml
- Glass funnel and filter paper, (Whatman)

- Stirring Rod

Sample Bottle: 10 ml and 20 ml

3.1.3 Equipment and instruments

- Vortex mixer (Kanke and kunke, Germany)
- Centrifuge (Gallenkamp, England)
- Flask shaker (Gallenkamp, England)
- Hot Air Oven (Gallenkamp, England)
- Electric Weighing balance
- UV spectrophotometer (Helios Zeta, Model 164617)
- Disintegration rate apparatus (Erweka, Germany)
- Dissolution rate apparatus (Erweka, Germany)
- Friabiliator (Erweka, England)
- Melting point study apparatus (Erweka, Germany)

3.1.4 Reagents

- Methanol, analar BDH chemicals England
- Sodium hydroxide pellet May and Baker, England
- Potassium hydroxide May and Baker, England
- Picric acid analar BDH chemicals, England
- Ferric chloride, May and Baker, England
- Distilled and filtered water
- Acetic anhydride, May and Baker England
- Crystal violet, Harns reagent, England

- Glacial acetic acid, Harns, England
- Hydrochloric acid, analar, BDH chemicals, England
- Acetone, May and Baker, England

3.2 Methodology

3.2.1 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 B.P 2009 specification.

3.2.1.1 Identification test

Identification test of chloroquine

The identification test was carried in accordance with the BP 2009 specification. 20 tablets of CQ were randomly selected and crushed in porcelain pestle and mortar. Then 2.5 g of powdered CQ was taken and extracted with distilled water (50ml), it was then filtered and 8 mls of picric acid was added to the filtrate.

Identification test of vitamin C

The identification test for vitamin c was carried out according to BP 2009. 20 tablets of vitamin c were randomly selected and crushed in porcelain mortar and pestle. A quantity of the finely powdered tablets was triturated and alcohol was added to make approximately the equivalent of 1 in 50 solution of ascorbic acid and filtered.

To the filtrate (2ml), 4 drops of methylene blue TS was added and warm to 40^{o}c.

3.2.1.2 Assay

Assay of Chloroquine tablet

Twenty (20) tablets of chloroquine were randomly selected weighed and crushed using a porcelain pestle and mortar, dissolve a quantity of the powder containing 0.5 g of Chloroquine phosphate in 20ml of NaOH and the extracted with four 25ml of chloroform. the four chloroform extracts were combined together and evaporated to a volume of about 10 ml, then 40 ml of anhydride acetic acid was added and titrated with 0.1M perchloric acid, using acetic crystal violet solution as an indicator.

Assay of Chloroquine standard powder

Aliquots (0.1 - 3.0 ml) of standard CQ (50 µg/ml) solution were accurately transferred into series of 5 ml calibration flask. To each flask 1 ml of 0.025 % perchloric acid solution was added and the solution made up to mark with chloroform. The content was mixed well and the absorbance was measured at 220 nm against a reagent blank.

Assay of vitamin C tablet

Twenty (20) tablets of vitamin C were randomly selected and crushed using a porcelain pestle and mortar, 0.15 g was taken and dissolved in a mixture of dilute sulphuric acid and some of carbon dioxide free water. Then 1 ml of stock solution was added, the mixture was then titrated with iodine.

3.2.1.3 Weight variation test

The method used was in accordance to BP 2009, 20 tablets of chloroquine were selected at random 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 tablets of vitamin C. the result obtained were recorded accordingly.

3.2.1.4 Disintegration test

The disintegration test was also carried out in accordance with the BP 2009 procedure. Six tablets of CQ were picked at random. The Vander Kamp disintegration tester was used which consist of a basket rack holding six plastic tubes, which are open at both ends; the bottom of the tubes is covered with 10 mesh screen. For testing one tablet was placed in each of the six tubes and covered with plastic disc which forced the soft mass of disintegration tablets through the bottom screen. For operation, the disintegration tester was placed in a water bath maintained at 37±0.5 ^{o}c

The time needed for the tablet to disintegrate completely was noted and recorded. The same procedure was repeated for vitamin C.

3.2.1.5 Friability test

20 tablets of test drug chloroquine were weighed and put into a Friabiliator (Erweka) which was set to rotate at 25 rpm for 4 minutes, at the end of 100 revolutions the tablets were removed and weighed again. The two weights were compared and the percentage friability determined. The same procedure was done for tablet of vitamin C. The percentage friability was determined using equation (3.1) below.

$$\text{Percentage friability} = \frac{(\text{initial weight} - \text{final weight}) \quad x100}{\text{Initial weight}} \quad \text{---------- (3.1)}$$

3.2.1.6 Dissolution test

The Rocking Basket method was used as described in the BP 2009. One tablet of CQ was placed in the Basket and then placed in the flask containing 100 ml of 0.1M hydrochloric acid, it was warmed and maintained between 36.5 and 37 ^{o}C with an electric heater. The stirrer was set at 100 rpm and the apparatus was operated for 30 minutes, the sample of the dissolution medium was withdrawn, filtered and assayed using a UV spectrometer at wavelength 220 nm. By the same procedure, six different tablets were subjected to the same test. The percentage of Chloroquine released from the dosage after 30 min was calculated, the same procedure was done for the tablet of vitamin C. The percentage released of both tablet of chloroquine and vitamin C was determined using equation (3.2) below.

$$\text{Percentage released} = \frac{\text{Amount of sample released}}{\text{Content of standard CQ tablet}} \quad \text{---------- (3.2)}$$

3.2.2 Analytical method

Jayanathi *et al.,* (1999) method was adopted, modified and validated based on ICH guideline, (1995);

A stock solution (1000 µg/mL) was prepared, from the stock solution a working solution of 10 µg/mL was prepared at different pH ranges (4-9). The maximum wavelength was determined when 10 µg/mL was scanned between 200-365 nm.

3.2.2.1 Calibration Curve

A five point calibration curve was constructed from the stock solution by preparing solution of concentration (2-10 µg/mL). in five labeled test tubes, blank saliva (2 ml) was spiked with 0.1, 0.2, 0.3, 0.4 and 0.5 ml of the stock solution transferred into 5 ml volumetric flask

and then make up to mark with distilled water to give concentration of 2, 4, 6, 8 and 10 µg/mL respectively.

Chloroquine was extracted from the solution using a modified and validated version of Jayanathi *et al.* (1999) method of chloroquine extraction which is as follows:

To 2 mL of saliva sample in alkaline medium (5N NaOH) was mixed with 3 mL of the extracting solvent (chloroform), and vortex mixed for 10 min. The mixture was then centrifuged at 2500 rpm for 5 min, the aqueous layer was carefully removed by Pasteur pipette and the absorbance was measured at λmax 220 nm, the absorbance value was converted to concentration from the calibration curve. Percentage extraction recovery was expressed using equation (1.19) below.

$$\% \text{ extraction recovery} = \frac{\text{Added concentration}}{\text{Expected concentration}} \times 100 \quad \text{---------- (1.19)}$$

3.2.3 Validation of analytical method

3.2.3.1 Precision

The precision test was done at intraday and inter-day periods. For the intraday, six different solutions of same concentration of the stock were analyzed three times a day at an hourly interval. This was done using standard powder of chloroquine (0.01g). The solvent used was 0.1M HCl, 0.6 ml of stock solution was taken and made up to 100 ml with distilled water then 1ml of the prepared solution was then measured and 3ml of the prepared buffered pH solution was added to make up to 4ml. absorbance was then taken for the intraday, hourly

for 3 hours, for the inter-day it was done at 24 hour interval for 3 days. The precision of the method was expressed in terms of %RSD.

3.2.3.2 Percentage accuracy

Was done by spiking 2 ml each of 8 µg/ml, 10 µg/ml and 12 µg/ml of chloroquine corresponding to concentration of 80 %, 100 % and 120 %. 3 ml of pH buffer was added, vortex mixed for I0 minutes and extracted with 3 ml chloroform, followed by addition of 5ml of 5N NaOH, the aqueous layer was collected and absorbance for each concentrations were taken at 220 nm. Percentage accuracy was expressed using equation (1.17) below.

$$\text{Percentage Accuracy} = \frac{\text{Added concentration - expected concentration}}{\text{Expected Concentration}} \times 100\% \text{ ------ (1.17)}$$

3.2.3.3 Percentage extraction recovery

The percentage extraction recovery of analytical method gives the efficiency of the extraction procedure to be employed in the analyses. It also gives the assurance of the .reproducibility of the extraction method employed. Percentage extraction recovery was expressed using equation (1.18) below.

$$\% \text{ extraction recovery} = \frac{\text{Added concentration}}{\text{Expected concentration}} \times 100 \text{ ---------- (1.18)}$$

3.2.4 In-Vivo studies

The last segment of the study involves the participation of 6 healthy male volunteers aged between 28 -32 years, weight between 55-64 kg. Healthy volunteers were used for the study; they were all nonsmokers and non-alcohol consumers. The volunteers were fully briefed on

the study and were informed on their role in the practical. A wash out period of 3 weeks was observed between the phases of the study. The cross over study was divided into 3 phases.

Using the modified and validated method, saliva collected was analyzed for their chloroquine content using extraction procedure mentioned.

3.2.4.1 Protocol of the studies

The protocol of the study was developed to have a wash out period of 3 weeks between the phases of the study

Phase 1

This phase involves administration of chloroquine tablet (300 mg) alone to six healthy volunteers with a glass of water after overnight fasting. Saliva samples were then collected at 0mins, 15mins, 30mins, 1, 2, 3, 4, 5, 6,7,8,9,10,11 and 12 hours. Thereafter, the saliva samples were then stored at -4 ^{o}c before analysis.

Phase 2

The second phase involves concurrent administration of chloroquine tablet (300 mg) with vitamin C (200 mg) tablets to each of the 6 volunteers after the wash out period (3 weeks) from phase 1. Saliva samples were collected and stored at same time frame as done in Phase 1.

Phase 3

The third phase involves delayed administration of chloroquine tablet (300 mg) an hour after administration of vitamin C (200 mg). The samples were collected and stored as done in the previous phase.

3.2.5 Analysis of sample

Absorbance obtained were converted to concentration and using the calibration curve. Pharmacokinetic parameters were generated by residual method and AUC was determined by trapezoid rule. All pharmacokinetic data obtained were compared and the statistical significance of their difference was determined using the student t-test of paired data, values of $p < 0.05$ considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Quality Control

4.1.1 Sampling

From the results obtained table 4.1 represent drugs label description for both tab CQ phosphate 150 mg and tab vitamin C 100 mg.

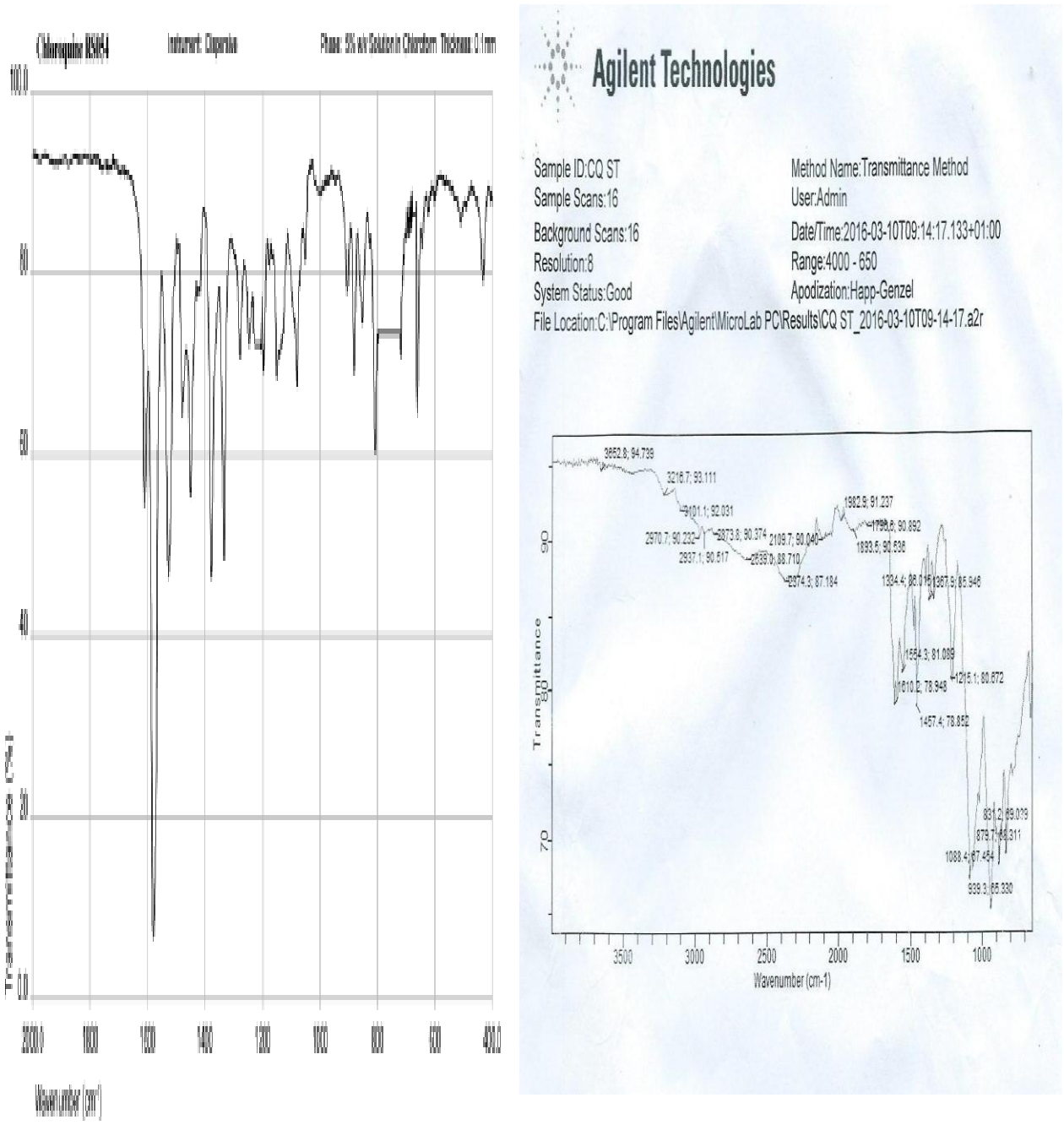
Table 4.1: **Drug Products Description**

S/NO	PRODUCTS	NAFDAC NO	BATCH NO	MFG Date	Exp. Date
1	Tab Chloroquine Phosphate 150mg	29615	005	01/2015	04/2019
2	Tab. Vitamin C 100 mg	39854	036	08/2015	08/2018

4.1.2 Identification of Samples

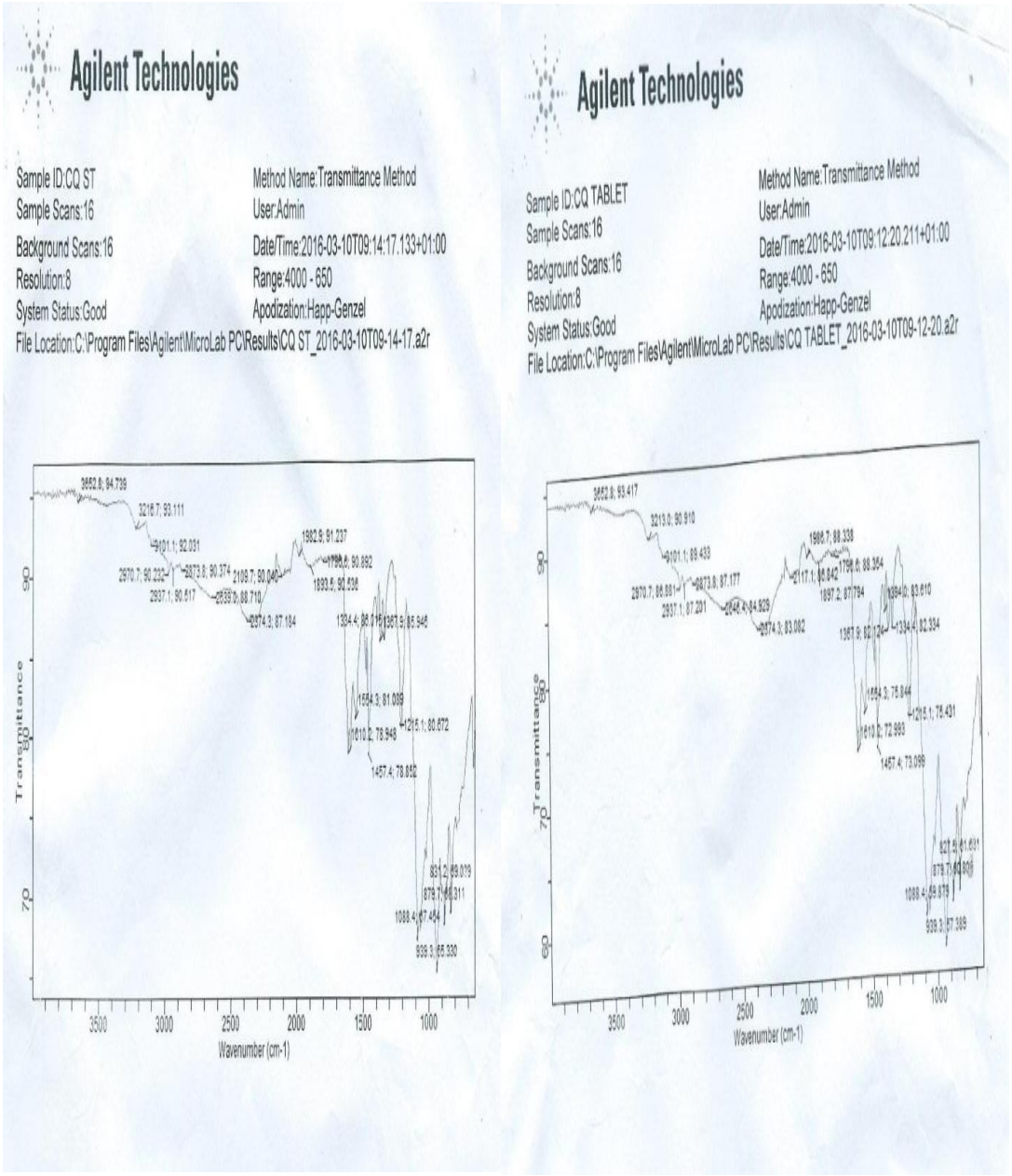
Chloroquine was found to be present in the sample tablet as indicated by the formation of a yellow precipitate with picric acid. Furthermore, an IR spectrum of the standard (Figure 4.1b) and the sample (figure 4.2a) were superimposable with the reference spectrum of chloroquine (figure 4.1a)

Vitamin C was found to be present in the sample tablet as indicated by the formation of a deep blue colour by trituration with alcohol using methylene blue TS as an indicator.



4.1a **4.1b**

Fig 4.1: IR spectrum of test drug CQ (Both standard powder and Reference standard BP 2009)



4.2a **4.2b**

Fig 4.2: IR spectrum of test drug CQ (Both standard powder and Tablet)

4.1.3 Assay

The table 4.3 shows results of assay test (% contents) of tab and standard powder of both Chloroquine and Vitamin C

Table 4.3: **Assay test or % content of CQ and Vit C**

Drug	**% Content**	**Remarks**
Tab CQ	103.10	Passed
Standard CQ	103.10	Passed
Tab Vit C	98.30	Passed
Standard Vit C	100.50	Passed

Acceptable limit (95-105 %)

4.1.4 Weight Variation

From the result below (table 4.4) represent weight variation of CQ using twenty tablets.

Table 4.4: **Weight Variation of Chloroquine and Vitamin C using 20 tablets**

No of tablets used	MEAN ± SD (Observed)	% MD	Remarks
CQ (20)	352 ± 18.867	≤ 5	Passed
Vit C (20)	296 ±10.012	≤ 5	Passed

The percentage mean deviation (MD) is ≤ 5 %

4.1.5 Disintegration Test

Table 4.5 shows disintegration time of chloroquine (CQ) using six tablets

Table 4.5: **Disintegration test of CQ and Vit C with acceptable value ≤ 15 min**

Tablets No		1	2	3	4	5	6
Disintegration (min) (CQ)	time	0.41	0.44	0.46	0.51	0.65	0.70
Disintegration (min) Vit C	time	2.32	2.41	2.55	2.65	2.82	3.02
Remarks CQ and Vit C		Passed	Passed	Passed	Passed	Passed	Passed

Acceptable value: The disintegration time is ≤ 15 min (BP, 2009)

4.1.6 Friability Test

Table 4.6 shows results of friability test of both tab Chloroquine and Vitamin C using 20 tablets subjected to a Friabiliator

Table 4.6: **Friability test**

No of tablets	Initial total weight	Final total weight	Total weight loss	% Friability	BP 2009 Specification	Remarks
20 CQ	3.46 g	3.43 g	0.03 g	0.867	< 1.0 %	Passed
20 Vit C	2.96g	2.94g	0.02g	0.680	< 1.0 %	Passed

The % friability of the test of both tab CQ and Vit C is less than 1.0 % (BP, 2009)

4.1.7 Dissolution Test

The table below (4.7) shows results of dissolution test using six tablets for both Chloroquine and Vitamin C.

Table 4.7: **Dissolution test**

No of replication	Time of Sampling (t)	Mean % released	BP 2009 Specification	Remarks
6 CQ	30 mins	79.34	≥ 70 %	Passed
6 Vit C	30 mins	82.34	≥ 70 %	Passed

The mean % released for both CQ and Vit C at 30 minutes was greater than 70 %, (BP 2009)

4.2 Analytical Method

The wavelength of maximum absorption of chloroquine in water was found to be 220 nm. At pH 6 it was found to be of optimal absorption of chloroquine at this wavelength (Figure 4.3). The calibration curve showing the regression equation is shown in figure 4.4, while the validation parameters are shown in table (4.8)

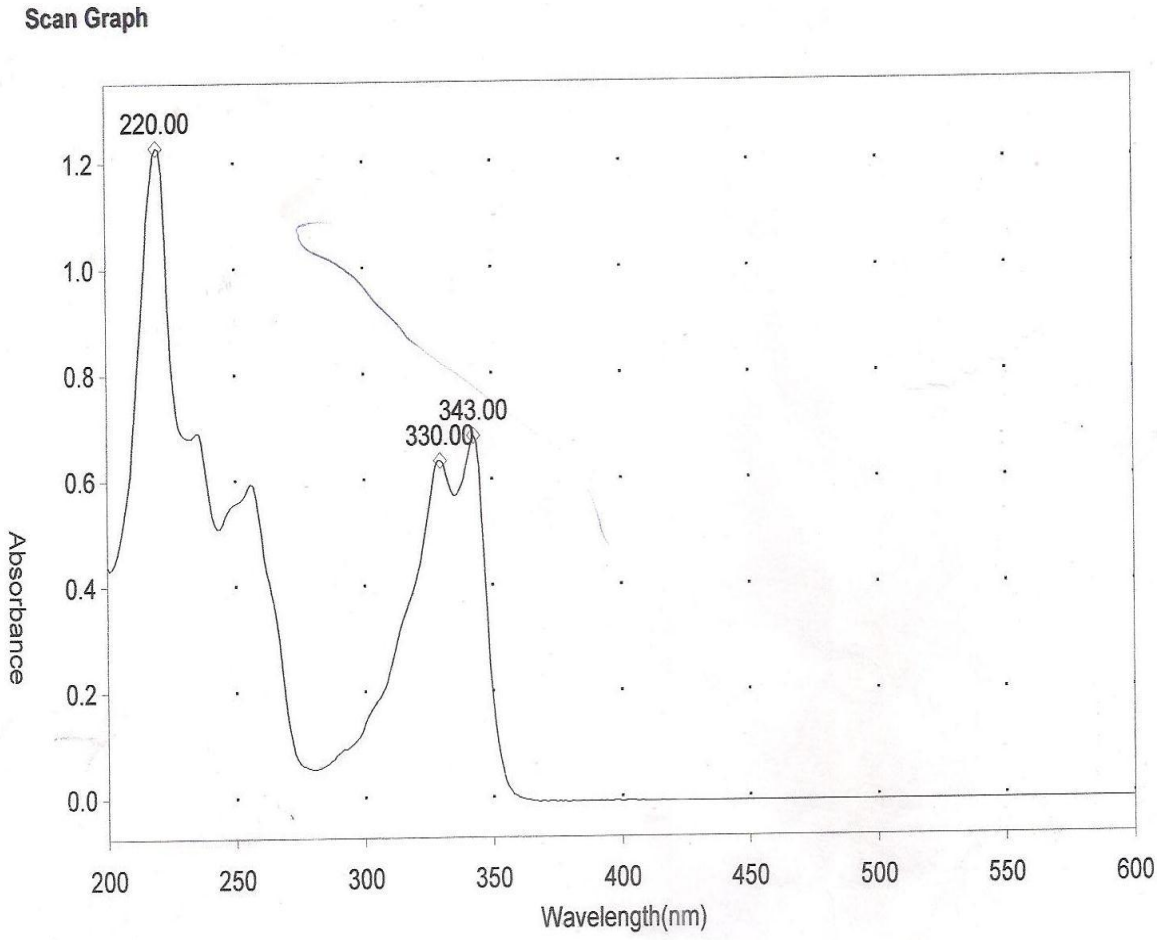


Fig 4.3: UV spectrum of Chloroquine in water at pH 6

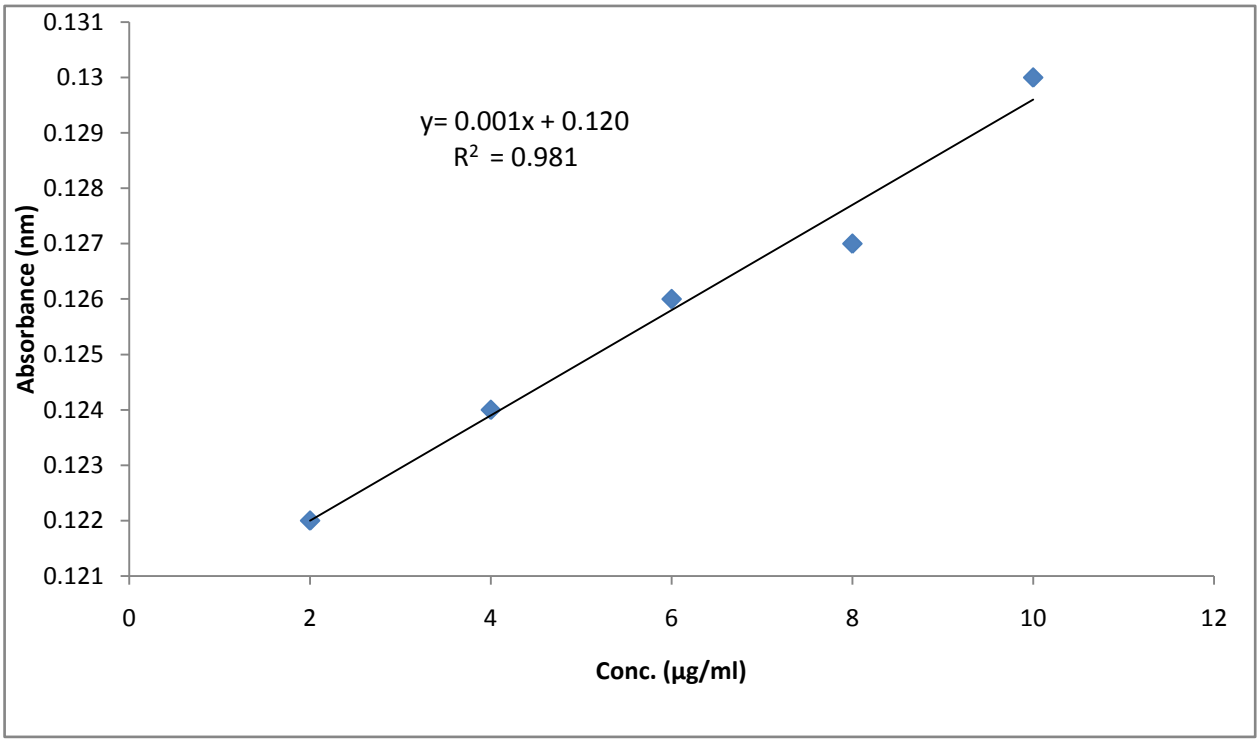


Fig 4.4: Calibration curve of chloroquine in water at pH 6

Table 4.8: **Summary of Validation Parameter of the developed method**

S/NO	Parameters		Result obtained	Official specification
1	Percentage extraction recovery (PER)		96.4	95 – 105
2	Percentage accuracy		3.72	1 – 5
3	Precision			
4		Within-day (% RSD)	0.682	< 2.0
5		Between-day (% RSD)	0.667	< 2.0

4.3 In-Vivo Results

Table 4.8 shows the mean salivary concentration for all the three phases from time 0 – 12 hrs for the six volunteers which was determined using regression equation from calibration curve (figure 4.4). The graph of mean salivary concentration against time for all the three phases is shown in (figure 4.5).

Table 4.8: **Mean salivary concentration of all the three phases, n = 6**

Time (hours)	Phase 1	Phase 2	Phase 3
0.00	7	23.5	23.33
0.25	20.33	37.5	43.33
0.5	60.67	54.66	66.5
1	101.5	92.66	92.33
2	190	143.5	143.5
3	312.83	197.16	212.16
4	203.67	168.33	167.83
5	104	143.83	135.5
6	67.33	125.33	126.67
7	52.5	109.5	120.16
8	31.83	85	108.67
9	26.33	55.67	101.83
10	19.16	34.83	75.67
11	16	28	68.83
12	10.83	18.33	59.16

The figure below (fig 4.5) shows a graph of mean salivary concentration against time for the three phases

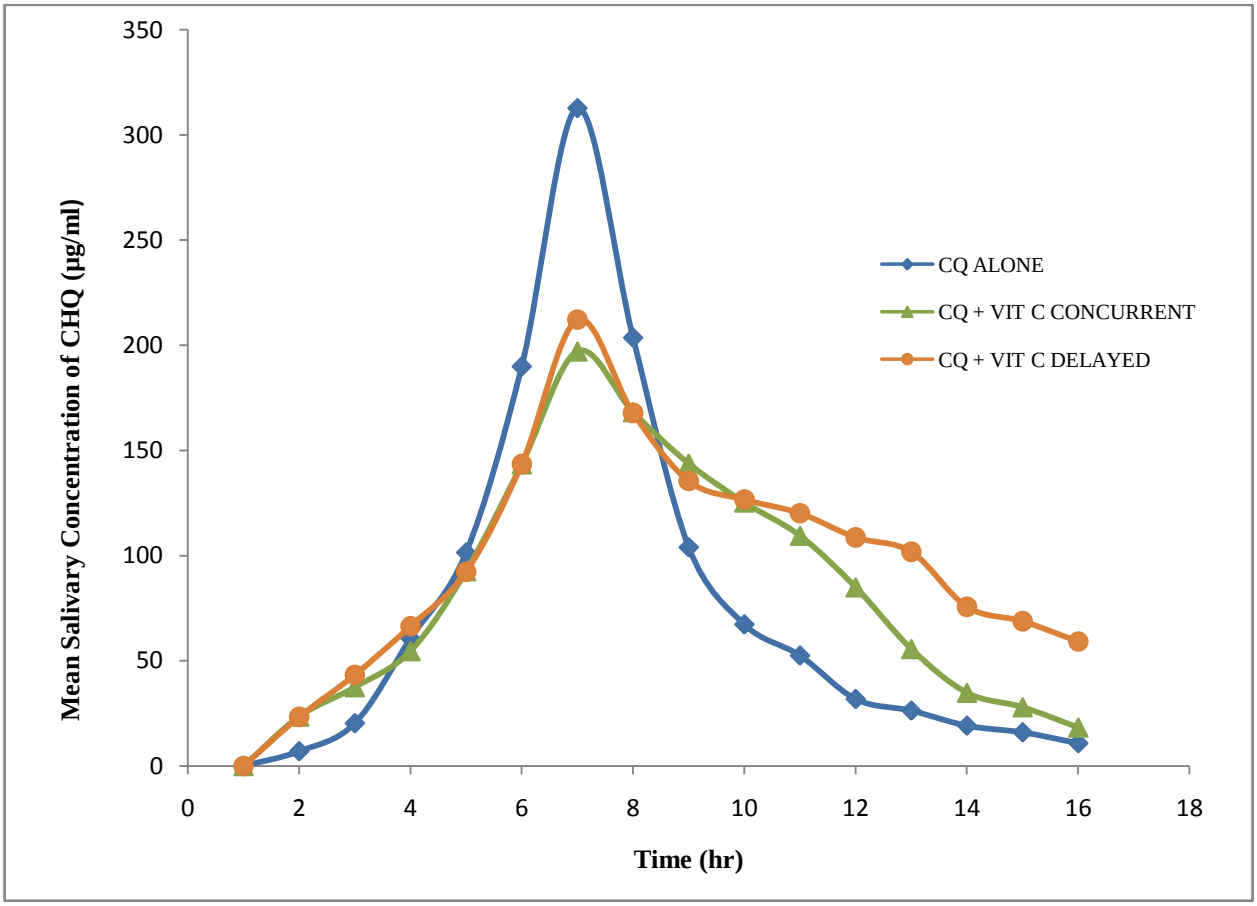


Fig. 4.5: Graph of mean salivary concentration against time for the 3 phases

Table 4.9: **Comparing Mean Pharmacokinetic Parameters of CQ alone with co-administered CQ and Vitamin C**

Pharmacokinetic parameters **n= 6**	**CQ Alone**	**Co-administered CQ and Vitamin C**
C_{max} (µg/ml)	312.27±53.90126	197.11 ± 54.18728 ↓
T_{max} (hr)	1.7133 ± 1.10551	2.0957 ± 0.93917 ↑
K_e (µg/ml)	0.3262 ± 0.24611	0.1080 ± 0.11247 ↓
K_a (µg/ml)	1.6987 ± 1.36107	1.8150 ± 0.86952 ↑
$T_{e1/2}$ (h)	3.1803 ± 1.89871	5.2420 ± 2.74241 ↑
$T_{a1/2}$ (h)	0.4938 ± 0.33882	0.3998 ± 0.16817 ↓
A U $C_{0-\infty}$	1235.1583 ± 349.58638	1189.5717 ± 350.24812 ↓
Vd (ml)	4.9683 ± 2.76079	2.5237 ± 1.03060 ↓
Cl (ml/h)	2,0413 ± 0.77028	0.2862 ± 0.18716* ↓
Lag time (h)	0.2670 ± 0.46263	0.0938 ± 0.15513 ↓

*Significant difference at P □ 0.05

Table 4.10: **Comparing Mean Pharmacokinetic Parameters of CQ alone and delayed co-administered CQ and vitamin C**

Pharmacokinetic parameters	**n=6**	**CQ Alone**	**Delayed CQ and Vitamin C**
C_{max} (µg/ml)		312.27±53.90126	212.08 ± 45.68085 ↓
T_{max} (hr)		1.7143 ± 0.45111	2.0292 ± 0.51842 ↑
K_e (µg/ml)		0.3262 ± 0.24611	0.1573 ± 0.06034 ↓
K_a (µg/ml)		1.6987 ± 1.3607	1.9065 ± 0.37203 ↑
$T_{e1/2}$ (h)		3.1800 ± 1.89868	4.9517 ± 1.82917 ↑
$T_{a1/2}$ (h)		0.4438 ± 0.37075	0.5917 ± 0.55021 ↑
A U $C_{0-\infty}$		1235.1583 ± 349.58638	1156.9400 ± 314.99987 ↓
Vd (ml)		4.9683 ± 2.76079	3.3388 ± 1.76041 ↓
Cl (ml/h)		2.0413 ± 0.31446	0.6336 ± 0.64467* ↓
Lag time (h)		0.2670 ± 0.46263	0.0963 ±0.11652 ↓

* Significant difference at P □ *0.05*

CHAPTER FIVE

5.0 DISCUSSION

5.1 Quality Control

From the result of the quality control carried out for both chloroquine and vitamin C. Both drugs passed the quality test conducted (BP, 2009).

The identification test conducted indicated the presence of chloroquine and vitamin C in their respective tablet. Infrared spectroscopy of both the standard powder and tablet of the test drug chloroquine confirmed the presence of chloroquine, in comparism to the reference standard the infra red spectra were found to be super impossible at finger print region.

The weight Variation test of both drugs chloroquine vitamin C indicated that their average percentage deviation determined 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 chloroquine and vitamin C 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 both drugs would be able to withstand transportation and handling stresses involved. Reference to literature on similar results is non-existent

The disintegration time of both chloroquine and vitamin C shows that they comply with good manufacturing practice; they both disintegrate within 15mins, 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 chloroquine and vitamin C, 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 chloroquine and vitamin C was released within 30 minutes.

The drug Assay test was conducted to measure the percentage content of both standard powder of chloroquine and vitamin C, the percentage content of both drugs determined is between 95-100% of the their active ingredients of BP 2009 specification.

5.2 Analytical Method

The analytical method of Jayanathi *et al.* (1999) was adopted, modified and validated based on ICH guidelines for this study. A working solution of 10 µg/ml was determined and scanned through (200-365 nm) to obtained the working (λ) max.

A five point calibration curve within the range of 2-10 µg/ml was constructed from the stock solution using serial dilution. The drug was extracted using the validated extraction method and absorbance also measured at 220 nm, this was then plotted against concentration.

The analytical method was validated via precision, percentage accuracy a percentage extraction recovery. The degree of precision of the analytical method was done both at intraday hourly interval for three hours and inter day twenty four hourly (24hrs) interval period. Percentage relative standard deviation (%RSD) was determined for both intraday and inter day, it was found to be less than 2 % which indicates good precision based on ICH

guidelines (1995), Percentage extraction recovery was found to be 96.4 % which is within the BP 2009 specification. Percentage accuracy determined was 3.72 % which is within the specification of ICH guidelines (1995).

5.3 In Vivo Studies

The most important pharmacokinetics parameters considered in order to evaluate the rate and extend of absorption of chloroquine in this study are absorption rate coefficient (Ka), time corresponding to maximum concentration (Tmax), maximum concentration absorbed (Cmax), lag time and half-life $T^1/_2$. While those parameters indicative of metabolism and/or excretion were also considered, this includes the following time line for a quantity of drug available to be reduced by half ($T^1/_2$) coefficient of elimination (Ke), clearance (CL).

It was observed that the mean maximum concentration (Cmax) of chloroquine achieved after oral administration of 300 mg of chloroquine alone in phase 1 was found to be 312.27 µg/ml which is less than 335 µg/ml reported by Jayanathi *et al* (1999), this may be due to inter subject variability, this value consequently decreased with both concurrent and one hour delayed administration with vitamin C to 197.11 µg/ml and 212.08 µg/ml respectively This may be due to acidic properties of vitamin C which increased the stomach pH and absorption of chloroquine.

The absorption half-life ($T^1/_2$a) for chloroquine alone was found to be 0.493 hrs with corresponding absorption constant (Ka) 1.6987 µg/ml. Jayanathi *et al* (1999) reported a value of 0.513 hrs for half-life and (Ka) of 1.662 µg/ml which indicates a good correlation. While in both concurrent and one hour delayed administration with vitamin C, the absorption constant (Ka) increased to 1.8150 µg/ml and 1.9063 µg/ml. while the $T^1/_2$ for

both phases also decreased to 0.399 hrs and 0.5917 hrs, this is due to acidic and oxidative properties of vitamin C.

The time taken to reach peak absorption (Tmax) in this study for phase 1 was found to be 1.713 hrs, the value observed by Jayanathi *et al*. (1999) is 2 hrs while Goodman and Gilman (1990) reported a value of 120 minutes, which indicates a good correlation.

The AUC_o was found to be 1235.15 µg/hr/ml regularly for chloroquine alone, while Jayanathi *et al*. (1999) also reported a value of 1289.10µg/hr/ml.

On concurrent and one hour delayed administration with vitamin C, the value of AUC observed decreased to 1189.5717 µg/hr/ml and 1157.9400 µg/hr/ml regularly, this is consistent with the findings of Raina *et al*. (1993) on the effect of Aspirin an acidic drug on the pharmacokinetics of chloroquine. The elimination half-life and corresponding elimination rate constant for phase 1 were found to be 3.1803hrs and 0.3262 µg/ml regularly which is in the range reported in some literatures (Goodman and Gilman 1990, Payan and Katzung, 1995). It can also be seen on concurrent and delayed administration with vitamin C the values for both Ke decreased to 0.1080 µg/ml regularly and 0.1573 µg/ml and the Te½ also increased for both phase II and phase III to 5.2420 hrs and 4.9517 hrs respectively this is due to the urinary acidifying properties of vitamin C which increased the elimination rate of chloroquine.

However the volume of distribution found in this study for phase I was 4.9683ml and in Comparism to both phase II and phase III the values obtained decreased to 2.5237 ml and 3.338 ml.

In this study, it was observed that clearance (cl) in both phase II and phase III significantly decreased at $p < 0.05$. the observed clearance (cl) in phase I was 2.0413 ml/hrs while for both Phase II and phase III the value decreased significantly to 0.2862 ml/hrs and 0.6336 ml/hrs respectively this is because chloroquine is a lipophilic compound on administration of a hydrophilic compound like vitamin C. it becomes more preferentially cleared.

It is observed from the data comparing the mean pharmacokinetic parameters in all the phases only clearance significantly changed at $p < 0.05$ for both phases II and phase III as compared to phase I, this indicates that there is influence in the absorption of chloroquine by vitamin C when they are orally administered concurrently or one hour delayed. Jayanathi *et al*. (1999) and Payan katzung, (1995) reported that the time to reach peak plasma and other biotic fluids of chloroquine is between 2-3hrs this support the study that there is interaction or inhibition of pharmacokinetic parameters of chloroquine when administered concurrently or one hour delayed with vitamin C.

In summary, the decrease in Cmax, Ke, $Ta^{1}/_{2}$, $AUC_{o,}$ and lag time as well as the increase in Tmax, Ke, and $Te^{1}/_{2}$ in both phase II and phase III as compared to phase I in this study lead to a suggestion that absorption of chloroquine a weakly basic drugs was impaired by vitamin C and acidic drug. The theoretic implication of the impairment of absorption of chloroquine by vitamin C are increase rate of absorption which can increase pharmacological effects of chloroquine in that it may be absorbed rapidly leading to maximum effective concentration which increases the onset of the therapeutic activities of Chloroquine.

The parameters obtained when chloroquine was administered alone and when administered concurrently and one hour delayed with vitamin C was compared.

It can be seen that in all the phases there was no significant changes in the pharmacokinetics parameters except clearance (cl) which was significant at $p < 0.05$. This simply means that there is a significant interaction between chloroquine and vitamin C when taken concurrently or delayed, the little changes observed in some pharmacokinetic parameters may be attributed to individual difference and other physiochemical factors earlier mentioned.

CHAPTER SIX

6.0 CONCLUSION

It can been seen from the study that the both chloroquine and vitamin C passed the quality control test, i.e. identification, assay, friability, disintegration, weight variation and dissolution according to BP 2009.

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

In the study it was also observed that there was a significant change in clearance (cl) of chloroquine when vitamin C was concurrently administered. Clearance (cl) decreased in these phase in comparism to phase 1 which is chloroquine alone.

In this study it was also observed that there was a significant effect of one hour delayed administration of vitamin C with chloroquine, because clearance was also significantly lower in the phase with delayed co administration of vitamin C with chloroquine than in the phase with chloroquine alone.

In conclusion, it can be seen that co administration of vitamin C with chloroquine whether concurrent or delayed may change the pharmacokinetics of chloroquine.

6.1 RECOMMENDATIONS

- Routine quality control and pharmacokinetic studies should be carried out to ascertain the claim of the manufacturer of this pharmaceutical products
- Similar pharmacokinetic study should be conducted with long without period periodically with advance technique like HPLC.
- A prospective clinical trial should be conducted to investigate the clinical significance of the reduced Cmax, AUC and Cl of chloroquine when administered with Vitamin C.
- Similar studies should be desighned to assess the effect of observed reduced Cmax, Tmax, Ke, AUC and Cl, following repeated doses and Chronic administration of chloroquine in patient with either systemic lupus erythematosus or (SLE) or rheumatoid arthritis.
- Regulatory bodies like NAFDAC should periodically carry out quality control test to manage and maintain good quality product registration.

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APPENDIXES

Appendix I

Table 14: **Mean salivary concentration of all the three phases**

Time (hours)	Phase 1	Phase 2	Phase 3
0.00	7	23.5	23.33
0.25	20.33	37.5	43.33
0.5	60.67	54.66	66.5
1	101.5	92.66	92.33
2	190	143.5	143.5
3	312.83	197.16	212.16
4	203.67	168.33	167.83
5	104	143.83	135.5
6	67.33	125.33	126.67
7	52.5	109.5	120.16
8	31.83	85	108.67
9	26.33	55.67	101.83
10	19.16	34.83	75.67
11	16	28	68.83
12	10.83	18.33	59.16

Appendix II

Table 15: **Comparing Mean Pharmacokinetic Parameters of CQ alone with concurrent CQ and Vitamin C**

Pharmacokinetic parameters n= 6	**CQ Alone**	**Concurrent CQ and Vitamin C**
C_{max} (µg/ml)	312.27±53.90126	197.11 ± 54.18728 ↓
T_{max} (hr)	1.7133 ± 1.10551	2.0957 ± 0.93917
K_e (µg/ml)	0.3262 ± 0.24611	0.1080 ± 0.11247
K_a (µg/ml)	1.6987 ± 1.36107	1.8150 ± 0.86952
$T_{e1/2}$ (h)	3.1803 ± 1.89871	5.2420 ± 2.74241
$T_{a1/2}$ (h)	0.4938 ± 0.33882	0.3998 ± 0.16817
A U $C_{0-\infty}$	1235.1583 ± 349.58638	1189.5717 ± 350.24812
Vd (ml)	4.9683 ± 2.76079	2.5237 ± 1.03060
Cl (ml/h)	2,0413 ± 0.77028	0.2862 ± 0.18716*
Lag time (h)	0.2670 ± 0.46263	0.0938 ± 0.15513

*Significant difference at P □ 0.05

Appendix III

Table 16: **Comparing Mean Pharmacokinetic Parameters of CQ alone and delayed co-administered CQ and vitamin C**

Pharmacokinetic parameters **n=6**	**CQ Alone**	**Delayed CQ and Vitamin C**
C_{max} (µg/ml)	312.27±53.90126	212.08 ± 45.68085 ↓
T_{max} (hr)	1.7143 ± 0.45111	2.0292 ± 0.51842
K_e (µg/ml)	0.3262 ± 0.24611	0.1573 ± 0.06034
K_a (µg/ml)	1.6987 ± 1.3607	1.9065 ± 0.37203
$T_{e1/2}$ (h)	3.1800 ± 1.89868	4.9517 ± 1.82917
$T_{a1/2}$ (h)	0.4438 ± 0.37075	0.5917 ± 0.55021
A U $C_{0-\infty}$	1235.1583 ± 349.58638	1156.9400 ± 314.99987
Vd (ml)	4.9683 ± 2.76079	3.3388 ± 1.76041
Cl (ml/h)	2.0413 ± 0.31446	0.6336 ± 0.64467*
Lag time (h)	0.2670 ± 0.46263	0.0963 ±0.11652

* Significant difference at P □ 0.05