EFFECTS OF CIMETIDINE ON THE PHARMACOKINETICS OF MEFLOQUINE, PROGUANIL AND QUININE IN HEALTHY SUBJECTS AND IN PEPTIC ULCER PATIENTS

BY

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DECLARATION

This is to certify that the work reported in this thesis was carried out by me under the supervision of Dr. A. Mustapha and Prof. I. Abdu-Aguye in the Department of Pharmaceutical and Medicinal Chemistry, and Department of Pharmacology and Clinical Pharmacy respectively, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Dr. N. Ochekpe of National Institute for Pharmaceutical Research and Drug Development, Abuja. And also Professor K.B. Taylor, of The School of Pharmacy, The Robert Gordon University, Aberdeen (where most of the bench work was carried out).

The works of other investigators were acknowledged and referred to accordingly. I declare that no part of this thesis has been submitted elsewhere for the purpose of a degree.

FAKOLAWOLE

CERTIFICATION

This thesis entitled " EFFECTS OF CIMETIDINE ON THE PHARMACOKINETICS OF MEFLOQUINE, PROGUANIL AND QUININE IN HEALTHY SUBJECTS AND IN PEPTIC ULCER PATIENTS" By JACOB ADEGBOYEGA KOLAWOLE meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to,
my late dad. David .A. Kolawole,
Josephine (wife), Olanike, Adeola (daughters) and Jerry(son)
for their love for education.

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To God be the glory. He surrounds us with wonders and encourages us in unravelling some of the wonders.

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ABSTRACT

Considering the endemicity of malaria in Nigeria, there is the possibility of patients being treated for malaria to be undergoing treatment for peptic ulcer. It is therefore possible for the drug(s) used in the peptic ulcer treatment to influence the pharmacokinetic parameters of the antimalarial drug(s).

The effects of oral cimetidine were studied on the pharmacokinetics of mefloquine, proguanil and quinine following single oral doses in six healthy male subjects and six ulcer patients. Single dose pharmacokinetics of each of the drugs were studied in all subjects. After a washout period, the interaction was studied with cimetidine 400mg given twice a day for two days and on the third day 400mg given an hour before the drug. Pharmacokinetic parameters were calculated from blood level data before and after cimetidine. Standard pharmacokinetic parameters were obtained from the computer analysis of the calculated pharmacokinetic data.

Oral administration of cimetidine significantly increased ($P<0.05$) C max in plasma of mefloquine in healthy subjects and ulcer patients. AUC 0-∞ was also significantly increased ($P<0.05$) in healthy subject and in ulcer patients. T max, absorption half-life, elimination half-life, volume of distribution and total body clearance were not significantly altered ($P>0.05$) by oral administration of cimetidine.

Cimetidine significantly increased (P<0.05) Cmax, AUCo-∞ and elimination half-life $t_{\frac{1}{2}}$ of proguanil in plasma of healthy subjects. In ulcer patients cimetidine significantly increased (P<0.05), AUCo-μ, elimination half-life and Cmax. Cimetidine significantly reduced (P<0.05) total body clearance in both healthy subjects and peptic ulcer patients. However, the Cmax and AUCo-∞ of the active metabolite cycloguanil was significantly decreased (P<0.05).

Oral administration of cimetidine did not significantly increase (P>0.05) Cmax, and absorption half-life of quinine. Cimetidine significantly increased (P<0.05) Tmax, AUCo-μ and elimination half-life in plasma of healthy subjects. In the ulcer patients, Cmax and Tmax were not significantly altered (P>0.05) by cimetidine administration while AUCo-∞ and elimination half-life were significantly increased (P<0.05). Total body clearance was [illegible]) after cimetidine administration in healthy subjects and ulcer patients.

These findings indicate [illegible]metidine enhances absorption of oral doses of proguanil and metloquine and prolonged the elimination of proguanil and quinine when these drugs are administered after an oral cimetidine. These effects occured in both healthy subjects and peptic ulcer patients.

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CHAPTER I

1.0 INTRODUCTION

1.1 MALARIA

Malaria is an infectious disease transmitted by mosquitoes. It is characterised by recurring attacks of chills and fever. The causative protozoan was identified to be plasmodium species (*P. falciparum, P. vivax, P. ovale, and P. Malariae*). The transmission of the malaria is well understood in terms of the life cycle of the malaria parasite which can be outlined as follows. The sporozoite form of the plasmodium accumulates in the salivary gland of the anopheles mosquito. This is injected into the subcutaneous tissue of the vertebrate host on a bite during its feeding process. The sporozoites (erythrocyte stage) leaves the peripheral circulation to incubate in the liver and develop into the exo-erythrocyte schizonts stage to form merozoites. Merozoites are budded and released into the blood stream as an asexual stage which invades the red blood cells(WHO Geneva 1987, Peters 1987).

The parasite in the red blood cell ingests the host cell cytoplasm and then transforms rapidly into a young trophozoite which develops into two forms. One is the asexually matured erythrocytic merozoites and the other the sexual form --gametocytes. The erythrocytic merozoites burst out into the blood stream to produce more merozoites which then invade the red blood cells, disintergrate them and release erythrocytic proteins and other cell products thereby causing fever and chill (Ritschel 1980). When

the mosquito takes in the gametocyte while feeding on the host, the gametocyte will transform into gametes and produce the fertilised zygote followed by the formation of oocytes (similar to the asexual stage in the liver). Sphoroblasts are formed which then bud to give the sporozoites. The sporozoites move from the vectors gut to accumulate in the salivary glands in readiness to start a new life cycle.

In *Plasmodium vivax* the sporozoite in the hepatocyte will form hypnozoites in addition to merozoites. These hypnozoites stay in the liver and produce relapse of the disease.

This life cycle of the malaria parasite can be broken at any stage using drugs and chemicals. The insecticide DDT was once used to eradicate the vector (WHO, Geneva 1957) and subsequently malaria in some parts of the world. Today, malaria is found mostly in the tropical countries. The World Health Organisation, UNDP, World bank special programme for research and training in tropical diseases established in 1975 (Peters 1987) has malaria as a priority. The programme places particular emphasis on the chemotherapy and immunology of malaria. To this end many compounds are chemically synthesized and screened for antimalarial activity.

1.2 Malaria Chemotherapy and Chemo-Prophylaxis

Chemotherapy (chemical therapy) is the use of chemical agent in the treatment of diseases while chemoprophylaxis (chemical prophylaxis) is the protection from disease or prevention of disease by chemical agents. Varieties of chemical compounds as occured in plants extracts to synthetic compounds have been used as chemotherapeutic or prophylatic agents in malaria. No single antimalarial has been found to consistently act against all strains of *Plasmodium* since resistant strains usually develop in the malaria endemic areas. The alkaloid extracts of Cinchona ledgeriana back had been used in Peru as febrifuges (Haggis 1941), in traditional medicine. Its use in man was recorded in 1630 and it was included in the London Pharmacopoea in 1677 (Peters 1987).

Quinine is the main alkaloid in Cinchona ledgeriana with antimalarial activity. However due to its "cinchonism" toxicity and the developement of resistant strains of *P. falciparum* as far back as 1910 (White 1988) its monopoly as antimalarial was short lived. Based on the quinoline structure (of quinine) pamaquine was produced by the Germans and so was mepacrine based on acridine nucleus. Pamaquine was withdrawn from use in 1946 due to its toxicity and another quinoline, 8-amino quinoline compound - primaquine was developed (Schmidt et al 1963). Primaquine has been used mainly in radical cure of *P. vivax*, malaria (Peters 1987). Other chemotherapeutic

agents that were developed at about the same time based on the quinoline nucleus were chloroquine and amodiaquine.

The search for the ideal antimalarial agent led to the investigation of the antimalarial activity of the sulphonamides. The mechanism of action of the sulphonamides on micro-organism was thought to be the inhibition of the uptake of para-aminobenzoic acid which is an essential compound in the metabolic pathway. This led to the discovery of the dihydrofolate reductase inhibitors and the biguanides. A few sulphonamides have been used singly or in combination with other compounds with synergistic action for treatment of malaria (White 1985).

Curd et al (1945) introduced a new antimalarial biguanide - proguanil, whose structure differ from the previous ones. Proguanil has been used as prophylatic agent since 1948 (White 1985) and was known to be safe (Maegraith et al 1945), Proguanil was withdrawn from the WHO list of antimalarial prophylatics in 1982 due to resistance problem (Bruce 1982, Peters 1987) but was re-introduced in 1984. Chlorproguanil later introduced after proguanil and it was believed to be more potent than proguanil (Carrington et al 1951).

Artemisinin an extract of *Artermisia annua Linnueus* was isolated in 1972 and studied for its antimalarial activity in China. The Chinese herbal literatures showed that the plant has been used since 2000 years earlier (China Cooperative 1982a).

Antibiotics such as tetracycline, clindamycin, lincomycin and erythromycin have been used with antimalarial drugs for the radical cure of chloroquine resistant strains of *P. falciparum* (Crowther and Levi 1953). They have been shown to have schizonticidal activity and inhibit protein synthesis on ribosomes in malaria parasites (Divo et al 1985).

Halofantrine is a phenanthrene methanol derivative and mefloquine is a quinoline methanol derivative (Strude 1975). They are relatively new clinically used antimalarial agents.

1.3 Drug Interactions

Drug interactions occur when two or more compounds are co-administered or one compound directly or indirectly (through the metabolites) affect the pharmacokinetics or pharmacodynamics of the other(s). Therapeutics more often involves polypharmacy therefore these types of interaction are always provoked. These interactions may be of benefit to the patient or expose the patient to different types of risks. This may be complex and many factors may be involved. A general overview may be considered by the analysis of the fate of the drug in the body, from the time it is administered to the time of total elimination. Four broad stages can be distinguished in this overview and can also be followed with pharmacokinetic parameters;

(a) absorption ------- availability, AUC and ka

(b) distribution ------ Vd, k (distribution)

(c) metabolism

and (d) excretion ------- k (elimination) $t_{½}$ MRT, Cl (renal or total).

Therefore a change in any of these processes or parameters by one compound or a disease condition will affect the pharmacokinetics of the other(s) which may lead to change in the pharmacological response to the drug.

Absorption of drugs from the site of administration into the systemic circulation depends on the physicochemical properties of the drug, the enviromental pH, gastric emptying rate (oral) and intestinal blood flow. A critical change of these factors by a chemical compound, or physiological and pathological process will affect the absorption rate and the extent of absorption (bioavailability). In general alkalinising agents such as sodium and potassium bicarbonate decrease the absorption of weak acids (e.g anti inflamatory drugs, penecillins and acids with pka values of 2.5 to 7.5) while acidifying agents such as citric acid affect the absorption of weak bases (pka 5 to 11).The extent of ionization and solubility of a drug is dependent on its dissolution constant and the pH of the aqueous environment. According to the relationship described in the Henderson-Hasselbelch equation, an increase in gastric pH induced by an H_2-receptor antagonist would result in increased ionization of weak acids and a decrease for weak bases. Co-administration of ketoconazole with cimetidine greatly improved absorption of ketoconazole. It was suggested that cimetidine might probably

have increased the gastric pH thereby facilitating more absorption of ketoconazole which in a weak base (Van der Meer et al 1980). Fischer et al (1980) showed that absorption of tetracycline was decreased by cimetidine due to cimetidine increasing the gastric pH. Bioavailability of tetracycline after 5 days of administration with cimetidine did not show any significant change (Bodemar et al 1979). This was however explained with cimetidine increasing gastric emptying rate thus facilitating tetracycline absorption and counteracting the effect seen in the single dose study. Metoclopramide which increases gastric emptying rate increases the rate of absorption of paracetamol while pethidine and diamorphine which decreases gastric emptying rate delay paracetamol absorption but retain same bioavailability.

Complexation at the point of absorption of drug is illustrated here with the formulation of tetracyline. Tetracycline as tablets formulated with calcium carbonate, led to the formation of insoluble calcium salt of the antibiotic which was not absorbed orally. This interaction was eliminated by changing the tablet formulation (Reigelman et al., 1971).

Gastrointestinal absorption may be hindered by mechanism other than direct complexation. The intestinal flora, for example, are able to alter many drugs through a variety of biochemical alterations such as hydroxylation, decarboxylation and ester hydrolysis (Scheline 1968). Enzymes involved in transport of essential nutrients across

the intestional wall may be inhibited by drugs and this in turn may result in rather complex interactions. Folic acid has to be hydrolysed to the monoglutamate before it can be absorbed. Diphenylhydantoin inhibits this hydrolysis thereby diminishing folic acid absorption (Baylis et al 1971).

Changes in distribution essentially involves changes in the plasma and tissue protein binding of drugs. And knowing that it is the free fraction which exert pharmacological action, then the affinity of two drugs for a particular binding site or plasma protein or erythrocyte may lead to drug interaction at this stage. This may be a competitive or non-competitive form in which concentration of the drug in the body or receptor site affinity becomes important. Patient on warfarin have been known to suffer haemorrhagic accidents following phenylbutazone therapy.This was believed to be due to warfarin displacement at its plasma protein binding site by phenylbutazone leading to high concentration of free warfarin in circulation (O'Reilly et al 1970).

Biotransformation takes place largely in the liver but may also occur in the lung, kidney or intestine. Drug metabolism reactions can be divided into Phase I (functionalization) and Phase II (conjugation) biotransformations. In Phase I, the compound is made more water-soluble by addition of a polar functional group or deletion of a non-polar alkyl group by oxidation, reduction or hydrolysis. The metabolite is not always less active than the parent molecule, e.g. cyclophosphamide. Some metabolites have same activity

with the parent compound e.g. primidone and its metabolite phenobarbital. Phase I reactions may increase drug toxicity as in acetominophen or even carcinogenicity as in benzopyrine. Once a 'handle' has been placed on the drug through phase I reaction, water solubility can be further increased by conjugatiing enzymes (Phase II) that link the metabolite to endogenous compounds such as glucuronic acid, sulphate, acetate or glycine (Powell et al 1983). The liver is by far the most important and most studied site of drug metabolism in the body. Disruption and separation of the liver cell components show that the greatest drug metabolism activity is localized in the microsomes of the endoplasmic reticulum and cytosol. Although numerous enzymes are responsible for drug metabolism, quantitatively the most important is the microsomal enzyme oxidation system which is also referred to as mixed function oxidase system. The microsomal enzyme oxidation system is composed of 2 enzymes, cytochrome P-450 and nicotinamide adenine dinucleotide phosphate (NADPH) for its action. Hepatic enzyme activity is an essential parameter in determining the fate of drug in the body. A host of compounds modulate their activity and can be classified as inducers (enhances enzyme activity) or inhibitors (decreases enzyme activity). Among the inducers are the barbiturates, glutethimide, phenytoin, antipyrine and rifampicin and the inhibitors are disulfiram, phenylbutazone, trimethoprim, chloramphenicol, cimetidine and sulphamethoxazole. Multiple forms of cytochrome p-450 exist in rats, there are at least 16 forms known as isozymes that differ chemically and immunologically. These isozymes metabolize both drugs and normal body substrates. They vary in

metabolizing activity with a given substrate and also exhibit varying degrees of overlapping substrate specificity. The activity of isoenzymes can be induced or inhibited by drugs, environmental chemicals etc. Inducers include phenobarbital and 200 other drugs and chemicals. Environmental chemicals, including chlorinated hydrocarbon, insecticides, carcinogenic hydrocarbons, food additives and cigarette smoke cause enzyme induction (Conney et al 1972). Example of this was increased metabolism of phenylbutazone and antipyrine in workers occupationally exposed to lindane and DDT. Also decreased plasma levels of phenacetin were observed in cigarette smokers (Pantuck et al 1972). Inhibitors such as phenylbutazone and imidazole compounds have since been recognised (Powell et al 1983). Microsomal drug metabolism in human is affected by genetics, age, hormones, diseases, nutrition, stress, temporal factors and exogenous chemicals, and so it is not surprising that the rate of elimination for a drug that is primarily metabolized is far less predictable than for a drug that is eliminated unchanged in the urine (Park et al 1981). In the same manner, one would expect the impact of an enzyme inducer or enzyme inhibitor on drug metabolism to vary within and between patients due to these same modulators of metabolic activity. For example, the magnitude of metabolic inhibition from a drug might be far different in a well-nourished, healthy patient than in a malnourished, severely-ill patient.

Urinary excretion is the major route of excretion of drugs and their metabolite(s) from the body. The elimination of drugs here involves three processes, glomerular filtration,

tubular re-absorption and most importantly tubular secretion. Tubular secretion of drugs is usualy mediated by an active transport system. Thus, two different drugs undergoing tubular secretion by the same active transport system may enter into competition. The excretion of penicillin is known to be retarded by probenecid, phenylbutazone, sulphinpyrazone, aspirin, indomethacin and sulphaphenazole. In general, the administration of an organic acid will slow the tubular secretion of other concurrently administered organic acids and administration of a base will slow the tubular secretion of other bases (Reindenberg 1971). Salicylates, for example, were found to slow methotrexate excretion and phenylbutazone potentiates the hypoglyceamic effect of acetohexamide. The phyenylbutazone did this by slowing down the excretion of hydroxyhexamide, an active metabolite of acetohexamide (Leigler et al 1969). Interaction may also result if a drug affects urine pH. The effect will be on elimination of a drug that is filtered at the glomerulus and reabsorbed by non ionic diffusion back into the blood as the glomerular filtrate passes down the nephron (Waddel and Butler 1957).

The clinical significance of all the mechanisms and processes is the shortening or prolongation of the life of the drugs and its metabolite, reduced or enhanced concentration of either the parent drug or metabolite in the body and subsequently their pharmacological activity which may be therapeutic or toxic.

CHAPTER 2

LITERATURE REVIEW

2.1 H_2-RECEPTOR ANTAGONISTS AND GASTRODUODENAL ULCER

Peptic ulcers are circumscribed breaks in the surface of the gastrointestinal mucosa resulting from a gradual disintegration of mucosal substances. Superficial wounds are acute while deep ulcers are usually chronic. Peptic ulcers can be classified by site, as gastric and duodenal. Although the pathogenesis of ulcers is poorly understood, it is generally believed that the disease develops when an imbalance occurs between offensive factors; gastric acid secretion and defensive mechanism; mucus, in the gastroduodenal mucosa.

This led to two major fields of research and development; H_2-receptor antagonism, which reduces the destructive potential by inhibiting gastric acid secretion by histamine (Dammann et al 1983, Howden et al 1985), and cytoprotective synthetic prostaglandins, which stimulates the synthesis and release of mucus (Bright-Ashare et al 1988).

Recently, a micro-organism, *Helicobacter pylori* has been implicated in the pathogenesis of peptic ulcers. It is now well recognised that true eradication of this organism is associated with a greatly prolonged period of remission from duodenal ulcer (Rauws and Tytgat 1990).

Gastroduodenal ulcers afflict approximately 10% of the adult population in the western world, resulting in serious medical and social problems. Until about 10years ago , ulcer disease caused an average of 40-60 work day lost per patient per year in the United States of America and costing 3-4 billion US dollars. The discovery of H_2-receptor (Black et al 1972), and the subsequent introduction of cimetidine (Brimblecombe et al 1979) are regarded as milestones in the history of ulcer disease. The H_2-receptor antagonists are among the most widely prescribed of all drugs. By any standards, they have been an enormous therapeutic and commercial success and have become regarded as standard treatment for peptic ulceration in most countries. At present, there are four members of this class of drugs (cimetidine, ranitidine, famotidine and nizatidine) which are generally available. Others (e.g. etintidine and roxatidine) have more limited availability. Although the molecular structures of these drugs differ, they have essentially similar pharmacology; all currently available H_2-receptor antagonists are competitive and reversible antagonists for histamine at the H_2-receptor and are devoid of activity at the histamine H_1 receptor. Although the H_2 receptor is widely distributed in the body, the only known physiological action of histamine at this receptor is stimulation of acid secretion by parietal cells in the oxyntic mucosa of the stomach. Therefore, the principal pharmacological action of the H_2-receptor antagonist is inhibition of gastric acid secretion. Acids is of central importance in the pathogenesis of peptic ulceration and pharmacological, or less commonly surgical reduction of acid secretion is widely used in the management of peptic ulceration (Howden 1993).

Cimetidine became available for marketing in 1976, ranitidine in 1981, famotidine in 1984 while etintidine, nizatidine and roxatidine followed in that sequence. Oral absorption of all the H_2-receptor antagonists under clinical investigation is fairly rapid. Peak plasma concentrations are usually attained within 1 to 3 hours after the oral dose but a second peak after oral administration has been observed. This effect disappears when the drug (cimetidine) is taken with meal. The mean oral bioavailability for the H_2 antagonists ranged from 40-80% (58-89% for cimetidine). They are eliminated fairly rapidly with a terminal elimination half-life $t_{½}$ of 1 to 3 hours and renal clearance of 24 to 48 L/h.

2.2 Drug Interactions with Cimetidine

Cimetidine, N''-cyano-N-methyl-N'-[2(((5-methyl-1H-imidazol-4-yl)methyl)thio)ethyl]guanide is the first histamine H_2 antagonist with wide clinical application.

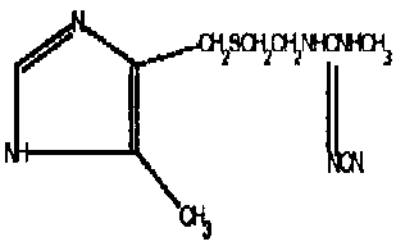


Figure 2. 2 Chemical structure of cimetidine.

It is a weak base and highly water soluble compound. Its principal action is on parietal cell histamine H_2-receptors and as such inhibits the secretion of gastric acid stimulated by histamine, pentagastrin, acetylcholine, insulin, food and other secretagogues (Somogyi and Gugler 1983). The effectiveness of cimetidine as 400mg dose was studied in healing of duodenal ulcer. After four weeks of therapy, complete ulcer healing was achieved in 78% of the patients on 400mg twice-a-day dosage and 79% of the patients on a single dose of 400mg. It was concluded that a more convenient single nocturnal dosage of cimetidine is as effective in the treatment of duodenal ulcer as a twice dosage in a study of an Indian population (Aggrawal et al 1990).

Cimetidine is well absorbed after oral administration with or without food. When taken with food the extent of absorption is unaltered, but a delay occurs with only one peak in the plasma concentration curve. Distribution of cimetidine leads to extensive uptake into the kidney, lung and tissues. Between 50 and 80% of the dose administered intravenously is recovered in urine as unchanged cimetidine and in ulcer patients only 40% is recovered after an oral administration.

Pharmacokinetic drug interactions with cimetidine occur at the sites of gastrointestinal absorption and elimination including metabolism and excretion. In studies on absorption, bezylpenicillin absorption was not altered by cimetidine. The absorption of ketoconazole was reduced by more than half with cimetidine, a consequence of its poor

water solubility which is enhanced in acid solution. Conflicting results are reported with tetracycline, the overall absorption of which does not appear to be significantly altered by cimetidine. Aspirin absorption was halved by cimetidine when gastric pH was raised above 3.5. Cimetidine did not affect the absorption of ampicillin, co-trimoxazole or prednisolone, but absorption of chlorpromazine and indomethacin are reduced (Somogyi and Muirhead 1987).

Cimetidine has presently been found not to inhibit conjugation mechanisms including glucuronidation, sulphation and acetylation or deacetylation (or ethanol dehydrogenation). Caballeria et al 1989b, and DiPadova et al 1992, however, reported an elevation in blood level of ethanol due to inhibition of gastric alcohol dehydrogenase (ADH). Cimetidine binds to the haem portion of cytochrome P-450 and is thus an inhibitor of phase-I drug metabolism. But up till date cimetidine does not inhibit the metabolism of theophylline 8-oxidation, tolbutamide hydroxylation, ibuprofen hydroxylation, misonidazole demethylation and steroid hydroxylation. Back et al 1983a found that antipyrine and tolbutamide are metabolised by different forms of cytochrome P-450 and so was it with diazepam and chlordiazepoxide (Black et al 1972). Cimetidine has also been reported to affect hepatic blood flow and reduce renal blood clearance of drugs with organic cations, by competing for active tubular secretion in the proximal tubule of the kidney, thus reducing the renal clearance of procainamide, ranitidine, triamterene, metformin and flecainide. Cimetidine drug interaction is

complex and a battery of model substrates is needed to investigate the inhibitory effects of a drug in man.

2.3.1 Review of Mefloquine

Mefloquine is a quinolinemethanol antimalarrial found to be effective as single dose therapy for all species of malaria infecting man, including multi-drug-resistant Plasmodium falciparum. It is used in both prophylaxis and treatment. Mefloquine is available in hydrochloride salt form or in combination with sulfadoxine and pyrimethamine tablets. There is no parenteral formulation (Karbwang and White 1990).

Mefloquine, (R^*S^*)-(±)-a-2-piperidinyl-2-8-bis(trifluoromethyl)-4-quinoline-methanol) hydrochloride is a newly developed antimalarial agent. During and immediatietly after the Second World War, the United States Government supported the testing of over 16,000 compounds for antimalarrial activity. From the best class of the methanol-quinoline group SN10275, the best compound WR 142490 was named mefloquine was found to be both efffective and safe. Efficacy studies in experimental animal models of malaria indicated that the drug is a potent schizonticide active against multi-resistant falciparum malaria. The phototoxicity of the earlier antimalarial quinoline-methanols had been eliminated by substituting a trifluoromethyl group on the phenyl group of the quinoline ring (Karbwang and White 1990; Karbwang et al 1987b).

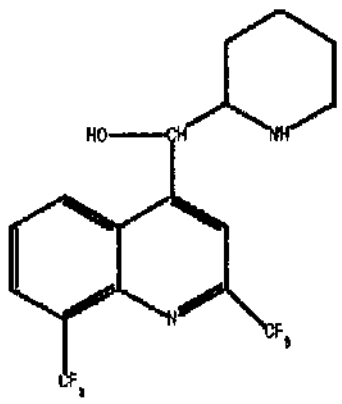
2.3.2 Analytical Methods

Mefloquine has been assayed in biological fluids by different chromatographic methods. Thin layer chromatography assay method, as described by Schwartz (1980) has a lower limit of detection of 50mg/L and can determine both mefloquine and its major carboxymetabolite simultaneously. Gas chromatography methods have been described by Heizman and Genschke (1984), Dadgar et al (1985) and Bergqvist et al (1988). Bergqvist et al (1988) developed an HPLC method using only 100µl sample of plasma with a limit of detection of 25nmol/L and a within-day assay precision of 12%. This method has been improved upon by Bergqvist et al (1993), with mefloquine and its main metabolites assayed in 100µl plasma and capillary blood adsorbed on filter paper. In 1994, Bergqvist et al, reported on the analysis of the enantiomers of mefloquine in 100µl plasma adsorped on filer paper. Arnorld and Stetten (1986) described an HPLC assay method in which mefloquine and the metabolite were analysed in plasma by direct injection of sample into the chromatograph using the pre-column enrichment and column switching techniques. In all the methods described there was no interference with other antimalarial agents.

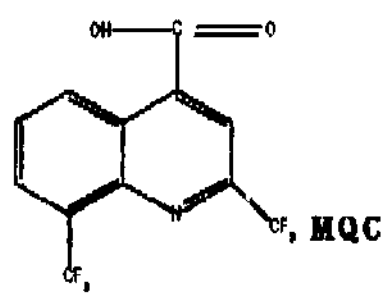
2.3.3 Metabolism

Mefloquine is well distributed in the body. Five metabolites have been isolated from the feaces of rats, of which 2 have been identified as 2,8-bis-trifluoromethylquinoline

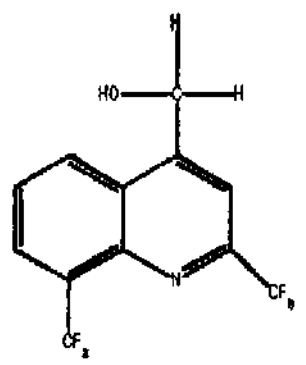
and a carboxylic acid derivative. Schwartz (l980) reported that the major acid metabolite in man has concentrations which exceeded those of the parent drug by a factor of 3.4 in plasma but that only about 26% of the mefloquine was metabolicaly converted to the metabolites in dogs. These workers concluded that the relatively high plasma concentrations of the mefloquine metabolites observed after an oral administration in humans are explained by mefloquine small apparent volume of distribution (Vd/f).



M



MQC



MQA

Figure 2.3.3. Chemical structures of mefloquine (M) acid metabolite (MQC) and hydroxy metabolite (MQA).

2.3.4 Pharmacokinetics of mefloquine

Mefloquine tablets are better absorbed in the gastrointestinal tract. There are no parenteral formulations. Mefloquine is highly insoluble in water. Desjardins et al (1979) studied the pharmacokinetics of mefloquine in healthy male subjects using oral suspension and tablets. They reported an absorption half-life ($t_{½a}$) of 1.1h for oral

suspension and 3.8h for tablets, with AUC which is 35% higher with the oral suspension. The apparent volume of distribution was large 13.3 ± 3.3Lkg^{-1} and the elimination half-life ranged from 6.5 to 22.7 days. Schwartz et al (1982) reported a $t_{½b}$ of 15.6 and 27.3 days in some African adults but 21.1 to 33 days in male Caucasian volunteers with volume of distribution of between 13.5 and 29.1 L/kg respectively, after single oral dose of 1000mg mefloquine tablet. Riviere et al (1985) however reported a $t_{½b}$ of 15.5 ± 10.4 days and a Vd/F of 35.7 ± 30.7 in adult Caucasian subjects, using the same dose and similar HPLC methods. Recently Fanssen et al (1989) studied the blood concentration profile of mefloquine following 500mg and 750mg oral doses followed by 500mg 8h later. They found that the maximum plasma concentration of mefloquine was 1872 ± 362µg/L for 500mg dose and 1900 ± 434µg/L for 750mg dose respectively, after the second dose. The plasma elimination half-life was 20.1 ± 3.7 days and Cl/f of 0.022 ± 0.007L/h/kg. They concluded that their data was similar to the single dose regimen. The time to peak concentration for all the reports range from 7 to 20.7h with corresponding concentration of between 250 and 1872µg/L. Mefloquine like the other quinolines is concentrated in the red cells by high affinity binding to the erythrocyte membranes. San George et al (1984), Schwartz et al (1982) reported a whole blood concentration to be up to double those in the corresponding plasma samples of healthy volunteers. However, Fransen et al (1989) and Karbwang et al (1987b) reported to the contrary, that whole blood and plasma

concentrations are similar and that either plasma or whole blood is suitable for the measurement of mefloquine concentrations.

Desjardins et al (1979) interpreted their result to mean low intrinsic clearance with a very small first pass effect. There is limited information on the blood concentration of the metabolites. All the above reports confirmed that mefloquine is extensively bound to tissues and cleared slowly from the body with great intersubject and ethnic variations.

2.3.5 Drug Interactions

There are few reports of drug interaction of mefloquine in the literature. There was a significant change in the $t_{½b}$ and AUC values obtained for mefloquine alone and when administered as Fansimef - mefloquine, sulfadoxine and pyrimethamine-, (Karbwang and White 1990, Karbwang et al 1987b, Karbwang et al 1988a). Na Bangdang et al (1989b) reported shortened elimination half-life $t_{½b}$ for mefloquine when co-administered with ampicillin showed that no pharmacokinetic changes were observed when concurrently administered with oral contraceptive steroids in women with complicated *P. falciparum* malaria.

2.3.6 Adverse Effects

Mefloquine has been shown to be safe and well tolerated. Mild cases of dizziness, nausea, vomiting and abdominal pain have been reported. These adverse effects are found to be dose related (Franssen et al 1989). Mefloquine worsens the orthostatic hypotension associated with malaria and asymptomatic sinus bradicardia with marked sinus arrhythmia have been noted in some studies with incident ranging from 7 to 49% in adults and 66% in children (Bergqvist et al 1993). Harinaseuta et al (1983) reported cases of serious abrupt psychosis in the second week of drug treatment, sometimes leading to abnormal behaviour, convulsion and loss of consciousness.

2.4.1 Review of proguanil

Proguanil (chlorguanide) [1(p-chlorophenyl)-5-isopropyl biguanide] HCl was synthesized and introduced as antimalarial compound in 1945 by Curd et al (1945), and used in man by 1948 (White et al 1985). Proguanil acts on the pre-erthrocytic stage of the parasite through the inhibition of dihydrofolate reductase (Curd et al 1945). Proguanil is however believed to be a pro-drug in which the in vivo metabolite cycloguanil has the antimalarial activity. Cycloguanil has been shown to be a potent antimalarial compound (Carrington et al 1951). It is however not found to be suitable for direct use as antimalarial agent because of its high water solubility and therefore poor absorption in the gastrointestinal tract and rapid excretion from the body.

The proguanil structure is based on para-chloroaniline and the biguanide group (see Figure 2.4.1). It was found that ortho- substitution in the aniline nucleus destroyed the antimalarial activity but halogen substitution at both the meta and para positions increased the activity (Crowther et al 1951, Curd et al 1945). Crowther and Levi 1953 and Carrington et al 1951, described the isolation and identification of this active species, cycloguanil from urine of treated rabbits. They also pointed out that cycloguanil can easily be converted to the inactive isomer (2-amino-4,4-dimethyl-6-((p-chlorophenylamino)-3,4-dihydro-1,3,5-triazine) and can also be hydrolysed in dilute acid to 4-chlorophenylbiguanide. They suggested that the primary product of proguanil metabolism seemed to be cycloguanil which was subsequently metabolised to form both inactive species. They also claim that cycloguanil is about ten times and chlorcycloguanil is about a hundred times as active as proguanil. However Schmidt et al (1963), reported a contradictory finding. They found that proguanil is about four (4) times more active than cycloguanil while chlorcycloguanil was about the same activity as proguanil. Their experiment was carried out on infected monkeys with proguanil and cycloguanil administered both intramuscularly and as stomach tube dosage forms. Smith et al (1961) suggested that proguanil was more active than cycloguanil and stated that 4-chlorophenylbiguanide has no activity. Smith et al (1961), suggested that the high activity of cycloguanil in proguanil treated animal serum was due to the fact that proguanil is extensively localised in certain tissues such as the liver, where it undergoes continuous transformation to cycloguanil. This metabolite then slowly

returns to systemic circulation to perform its antimalarial activity before being rapidly excreted. It was suggested that proguanil may be transformed to one or more unstable metabolites with higher activity than cycloguanil and possibly the metabolite is a precursor of cycloguanil. Edstein and Veenedaal (1988) suggested the possibility of a third metabolite on the basis of chromatography of urine sample containing chlorproguanil.

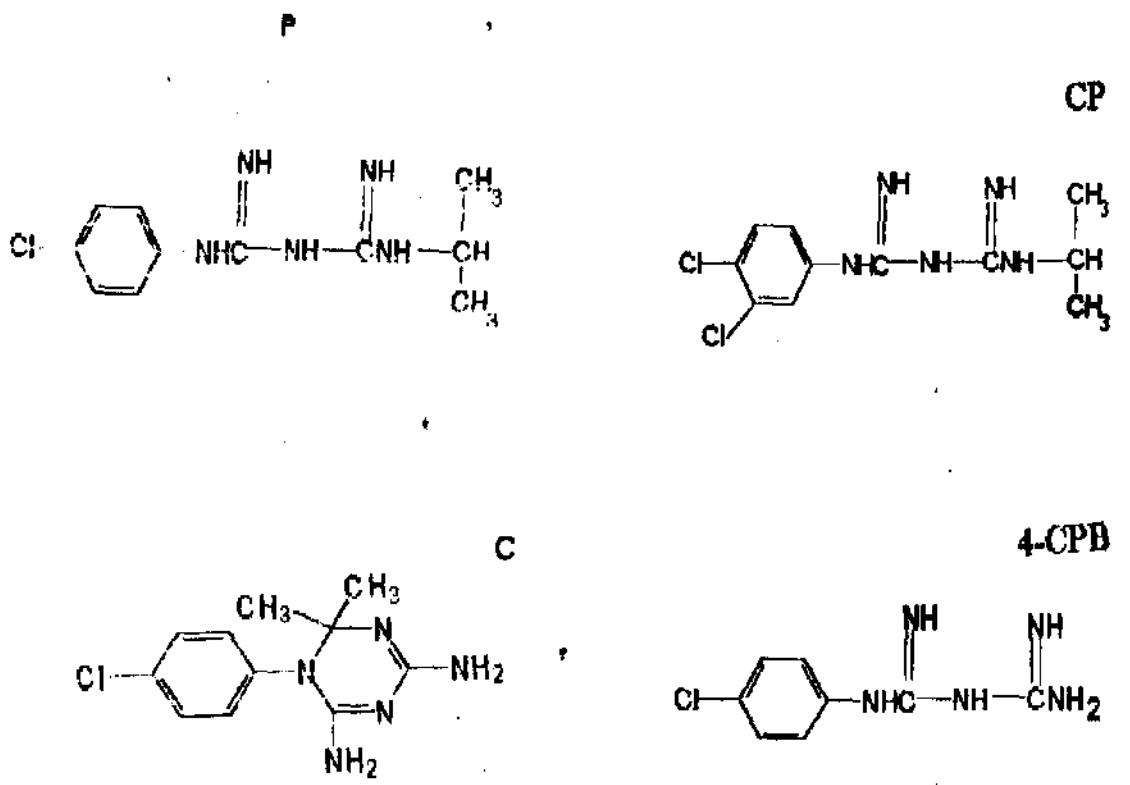


Figure 2.4.1. Chemical structures of proguanil (P), chlorproguanil (CP), cycloguanil (C) and 4- chlorophenylbiguanide (4CPB).

2.4.3 Metabolism studies

The orally administered proguanil was found to have higher concentration of metabolites than the parenteral route (Hawking 1947, Hawking and Perry 1948). It

was then concluded that the drug has a high first pass effect and it is metabolised in the liver. The metabolism was suggested to be cyclization of proguanil by the enzyme cytochrome P450 (Briggs and Briggs 1974) which is present at a high level in liver microsomal fraction. Little is known about the mechanism metabolism of proguanil and less evidence is available on the metabolism of the dichloro analogue of proguanil, - chlorproguanil.

2.4.4 Assay Methods

Smith et al (1961) developed a microbiological analytical method for proguanil while Watkins et al (1990) and Scott et al (1988) developed very specific parasitological bio-analytical methods. Spinks and Tutley (1946) developed a chemical analytical method for biguanide compounds. In their method the biguanides were hydrolysed in acid at high temperature to form para-chloroaniline followed by diazotization and coupling with N--sulphatoethyl-m-toluidine. Detection was by spectrophotometry. This method however did not discriminate between proguanil and its metabolites . Moody et al (1980) developed a HPLC analytical method in 1980. This method was modified and improved upon to give a very sensitive and highly selective analytical method for proguanil and its metabolites in biological fluids. Ion pairing reverse phase HPLC was used in combination with narrow bore columns after solid phase extraction of these compounds from biological fluids. (Taylor et al 1987, 1990). Their reported recoveries for proguanil, cycloguanil and 4-chlorophenylbiguanide in plasma were 99%, 74% and

75% respectively with a detection limit of about 1ng/ml for all compounds and a maximum intra-assay coefficient of variation of about 6.2%. The method was proven to be reliable and appropriate for pharmacokinetic studies (Wangboonskul et al 1993). Bygbjerg and Flachs (1986) also developed a HPLC assay method for proguanil and metabolites. Edstein (1986), Kelly and Fletcher (1986) had also developed a selective and sensitive HPLC analytical method for proguanil and cycloguanil using an ion pairing system and sample pre-treatment step of liquid-liquid extraction.

2.4.4 Pharmacokinetics of proguanil

Maegraith et al (1946) reported the plasma concentration time profile of proguanil in healthy and malaria infected patients. Their report has been the foundation for subsequent pharmacokinetic information on proguanil. They studied single dose and repeated doses of both oral and parenteral dosage forms of the drug. It was found that the drug was well absorbed following oral administration reaching peak plasma concentration in 4 hours with elimination half-life of 15 hours. Smith et al (1961) reported the proportion of proguanil, cycloguanil and 4-chlorophenylbiguanide excreted in urine to be 59, 30 and 8% respectively. They also reported a biphasic pattern of decline in plasma proguanil and cycloguanil concentration after an intravenous injection. They also showed that proguanil is concentrated in human liver and kidneys. Following the advent of sensitive and selective analytical methods, including HPLC, Edstein (1986), Bygbjerg et al (1986), Moody et al(1980) and Wattanagoon et al

(1987) at different times carried out pharmacokinetics studies on healthy patients following oral dosing in the single dose regimen or for steady state pharmacokinetics. Most of the information obtained were consistent with those of Maegraith et al (1946) and Smith et al (1961). The time to peak concentration for proguanil was 2-6 hours while cycloguanil peak concentration occurred about 1-2 hours later and 4-chlorophenyl biguanide after a further 1-2 hours. Edstein (1986), suggested that the maximum antimalarial activity should be of the time to peak concentration of cycloguanil and that concentration of proguanil will reach steady state after the third day as earlier reported by Maegraith et al and Kelly and Fletcher (1986) The peak plasma concentrations of proguanil quoted in all the reports were almost the same, varying from 150-220 ng/ml. The ratio of concentration of proguanil in whole blood to that in plasma was 3 from Edstein (1986) and Maegraith et al (1946) and 5 from Wattanagoon et al (1987), while the ratio of the corresponding erythrocyte to plasma concentrations were 5.8 from Edstein (1986) and 4 from Bygbjerg (1986). The mean elimination half-life in plasma of proguanil from recent publications ranged from 11 to 20 hours. These authors observed large inter-subject variability in circulating plasma cycloguanil concentration following the administration of standard dose of proguanil orally, but with the cause of variation remaining unknown. Wattanagoon et al (1987) reported peak cyclogunil concentration range of 12 to 69ng/ml in 9 healthy male subjects and Esdtein(1986) reported 27 to 70ng/ml in 6 subjects. Interestingly, Ward et al(1989) in their extensive investigation into inter-subject variation found a range of 20

to 545ng/ml in over 100 subjects. The elimination half-life of cycloguanil in plasma was found to be about 11hours. All recent investigations had shown that the level of cycloguanil in plasma is comparable to that of whole blood. These levels indicate that the plasma and erythrocyte cycloguanil levels attainable by the usual proguanil dose (200mg) will always be above the minimum inhibitory concentration of 8ng/ml (Spencer et al 1986).

Proguanil plasma clearance following single dose was reported to be $1.69Lh^{-1}kg^{-1}$ and renal clearance of $0.33Lh^{-1}kg^{-1}$ with renal clearance of $0.35Lh^{-1}kg^{-1}$ for cycloguanil. This shows that both compounds are excreted by active secretion into the renal tubule since the renal clearance value is greater than glumerular filtration rate (Kelly and Fletcher 1986).

2.4.5 Drug-drug Interaction Involving the Biguinides

Very few drug-drug interaction reports on proguanil are found in literature. Kelly and Fletcher (1986) mentioned a likely interaction of oral contraceptive steroids on proguanil metabolism but they did not give details.

2.4.6 Adverse Effect and Toxicity

Biguanide antimalarials are safe and lack serious toxicity or side effects. However it was observed that proguanil was implicated with mouth ulcers and may also cause serious nausea and vomiting (Maegraith et al 1946).

2.5.1 Review of Quinine

The clinical use of cinchona preceded information on the disposition of the alkaloids by over three centuries. The bark of the cinchona tree (Arbor febrifuga) was introduced to European medicine in the first part of the seventeenth century (White 1988), before the discovery of the malaria parasite. The four principal alkaloids of cinchona bark, the laevorotatory diastereoisomers quinine and cinchonidine and the dextrorotatory quinidine and cinchonine are all effective antimalarials. The dextrorotatory diastereoisomers are more active. Quinidine is twice as active as quinine while cinchonine and cinchonidine are less active. The cinchona alkaloids rapidly kill mature intra-erythrocytic malaria parasites with little effect on the gametocytes and no effect on pre-erythrocytic development of the malaria parasites (White 1987). Quinine has been and is still the drug of choice for severe and complicated *Plasmodium falciparum* malaria. But it has an array of side effects including 'cinchonism', cardiovascular toxicity, gastrointestinal irritation and hypersensitivity reactions. Because of the development of *P. falciparum* resistance which was observed in 1910 and its "cinchonism" toxic effect, quinine fell into disuse (White 1985).

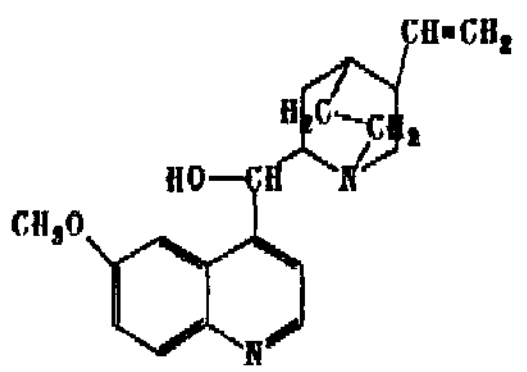


Figure 2.5.1 Chemical structure of quinine and quinidine

{ Quinine.....(-)8S :9R Isomer and Quindine.....(+)8R : 9S Isomer }

Shortly after the war the 4-aminoquinoline antimalarials (chloroquine and amodiaquine) were introduced and the folic acid synthesis inhibitors (proguanil and pyrimethamine) were synthesized. Chloroquine became the drug of choice for the treatment of malaria. Chloroquine resistant *P. falciparum* strains did not appear till the late 60s. This therefore necessitated more research in the antimalarials and the re-introduction of the natural cinchona alkaloids.

2.5.2 Analytical Methods

Early workers used colorimetric or turbidometric techniques after extraction of quinine and usually along with quinidine by organic solvent liquid-liquid extraction method. The early colorimetric assays were imprecise but later more specific methods were

developed. This was based on the natural fluorescence properties of the cinchonna alkaloids. Initially, extraction methods used for the measurement of quinine in body fluids proved insensitive, and so plasma proteins were precipitated out, and the fluorescence of the acidified layer was compared with standard quinine solutions. This protein-precipitation method also quantified other fluorescence polar metabolites present in blood, and therefore gave an overestimation of the concentrations of the parent compound (White 1985). Fluorescence assays were considered less sensitive until Brodie and Udenfriend (1943) introduced the protein precipitation fluorescence assay. This was also relatively non-specific as it gave values up to 3 fold greater than the double extraction fluorescence (EF) method introduced by Spinks and Tottey (1948) and later modified by Cramer and Isaksson (1963).

Methods for determining quinine levels in biological fluids were commonly based on the fluorescence procedures. Until recently, the pharmacokinetics of quinine (and quinidine) in healthy volunteers or malaria infected patients were estimated from plasma and erythrocyte concentration data based on the widely used extraction fluorometric (EF) method of Cramer and Isaksson (1963). These methods are however non-specific and not very selective since the commercial contaminant and the metabolites were presumably determined together with the quinine resulting in higher levels of quinine. Specific RP-HPLC and normal phase HPLC techniques have now been developed. In some of the analytical methods, interference from metabolites,

Edstein et al 1983, dihydroquinine (commercial contaminant) Mberu et al 1991 and/or quinidine, Taylor et al 1992; Salvadori et al 1982; Mihaly et al 1987; Hellgren et al 1990 were not examined. Suitable methods for clinical and pharmacokinetics studies should be able to distinguish the parent drug from the metabolites and the commercial impurity dihydroquinine. This contaminant has been reported to be as high as 9% or more in commercial quinine.

2.5.3 Pharmacokinetics of quinine

The disposition of the four principal alkaloids (quinine, quinidine, cinchonine and cinchonidine) had been compared in healthy volunteers. The laevorotatory diastereomers quinine and cinchonidine reached plasma concentrations of 2-4mg/L after a 10mg base/kg dose, whereas quinidine reached 1-2mg/L and the plasma concentration of cinchonine were less than 1mg/L. Increased absorption from an alkaline milieu, and increased elimination in acid urine in accordance with the pH-partition theory had also been confirmed. However, the New York University investigations were the first detailed comparative studies of disposition and efficacy during infections (White 1987).

Quinine is well absorbed in both healthy subjects and patients with uncomplicated malaria. Estimated oral systemic availability exceeds 80% both in healthy subjects and in uncomplicated malaria (Sabchreon et al 1982). Clearance is predominantly by hepatic metabolism with the metabolites (predominantly 2- or 3-hydroxylated quinine

as obtained in the urine) having less antimalarial activity than quinine. Haag (1943) reported that urinary clearance of quinine comprises of only 20% of the total clearance but is increased as urine pH falls as would be expected from the pka value of 8.4.

Pharmacokinetics of quinine in healthy subjects has been severally reported as being fairly different from those found in malaria infected patients. Dua et al (1993) reported that the average quinine concentration in P. falciparum malaria cases are three to four times greater than those in healthy volunteers at the start of the treatment, and that this factor fell to 1.2 in all blood phases after 32h of treatment as parasitaemia declined. The quinine level in red cell was also reported to decline faster than elimination from plasma. The quinine concentration was found to be highest in the first few hours of treatment in malaria cases. The ratio of quinine levels in plasma to red cells or in serum to red cell varies from 3 to 7 which therefore implies that the accumulation of quinine in erythrocytes is much less than that in plasma or serum during malaria infection. Hall et al (1973) compared plasma concentrations obtained in 12 healthy male volunteers given quinine tablets, capsules and intravenous infusions in a random cross over study. Plasma concentrations obtained after capsule and tablet administration were comparable and between 10 and 20% lower than those obtained after intravenous infusion. Similar values were later reported by Sabchareon et al (1982). Garnham et al (1976) studied the different salts of quinine and reported that the bioavailability of plain tablets were in the order dihydrochloride > bisulphate > sulphate. Sugar coated tablets gave delayed and sometimes incomplete absorption.

as obtained in the urine) having less antimalarial activity than quinine. Haag (1943) reported that urinary clearance of quinine comprises of only 20% of the total clearance but is increased as urine pH falls as would be expected from the pka value of 8.4.

Pharmacokinetics of quinine in healthy subjects has been severally reported as being fairly different from those found in malaria infected patients. Dua et al (1993) reported that the average quinine concentration in P. falciparum malaria cases are three to four times greater than those in healthy volunteers at the start of the treatment, and that this factor fell to 1.2 in all blood phases after 32h of treatment as parasitaemia declined. The quinine level in red cell was also reported to decline faster than elimination from plasma. The quinine concentration was found to be highest in the first few hours of treatment in malaria cases. The ratio of quinine levels in plasma to red cells or in serum to red cell varies from 3 to 7 which therefore implies that the accumulation of quinine in erythrocytes is much less than that in plasma or serum during malaria infection. Hall et al (1973) compared plasma concentrations obtained in 12 healthy male volunteers given quinine tablets, capsules and intravenous infusions in a random cross over study. Plasma concentrations obtained after capsule and tablet administration were comparable and between 10 and 20% lower than those obtained after intravenous infusion. Similar values were later reported by Sabchareon et al (1982). Garnham et al (1976) studied the different salts of quinine and reported that the bioavailability of plain tablets were in the order dihydrochloride > bisulphate > sulphate. Sugar coated tablets gave delayed and sometimes incomplete absorption.

White et al (1983) reported that after slow intravenous injection to healthy volunteers, the disappearance of quinine followed biexponential terms (2-compartment open model) with a rapid distribution phase t½a 1.9 minutes and a slow terminal elimination phase $t_{½b}$ 11.1hr. The apparent volume of distribution Vd/f was 1.80+0.37L/kg. The total clearance was estimated at between 1.3 and 2.66mL/min/kg Garnham et al (1976) reported similar half-lives after an oral administration of quinine.

2.5.4 Drug Interactions

Very little is available on quinine drug interaction. It appears to reduce the renal clearance of digoxin as does quinidine. Some reports have suggested that quinine and quinidine potentiate the oral anticoagulants by inhibition of prothrombin synthesis, this was however disputed by Williams et al (1976).

2.5.5 Adverse Effects

Plasma concentration of quinine above 5mg/ml was reported by Powell and MenNamara (1972) to cause a characteristic complex symptoms called "cinchonism" which consist of dysphoria and tinitus with variable headache, nausea, diarrhoea and occasional visual disturbance. Quinine self-poisoning is associated with deafness and a visual deficit that ranges from a slight reduction in the peripheral visual field to complete blindness (Bateman et al 1986). Quinine is a potent stimulant of pancreatic beta cell causing release of insulin thereby resulting in hyperinsulinaemia and

consequently hypoglycaemia (White et al 1983). Allergic reactions to quinine such as skin reactions, thrombocytopenia or Coombs' positive haemolysis are not usual in antimalarial therapy.

2.6.1 Review of Pharmacokinetic Data of Drugs

The objective of pharmacokinetic analysis is to characterise the absorption, distribution, metabolism, and excretion of a drug in a quantitative manner. This is achieved by using mathematic models, either compartmental or non-compartmental. The compartment model approaches presuppose that drug movement can be represented in terms of system comprised of flow rates and distribution volumes. These models can be simple or extremely complex. The non-compartmental approach presupposes no particular physical model but describes movement in terms of mathematical expressions derived directly from the data. The systemic absorption of a drug from the gastrointestinal (GI) tract or any other extravascular site is dependent on the physicochemical properties of the drug, dosage form of the drug, anatomy and physiology of the absorption site. Such factors as surface area of the gut, stomach-emptying rate, gastrointestinal tract mobility, blood flow to the absorption site all affect the rate and the extent of drug absorption. In spite of these variations, the overall rate of drug absorption may be described mathematically as a first-order or zero-order input process. Most pharmacokinetic models assume first-order absorption unless an

assumption of zero-order absorption improves the model significantly or has been verified experimentally. The rate of change in the amount of drug in the body, dD_B/dt, is dependent on the rates of drug absorption and elimination (Fig. 2.6.1a). The rates of drug accumulation in the body at any time is equal to the rate of drug absorption less the rate of drug elimination.

$$d D_B /dt = dD_{GI}/dt - dDe/dt \qquad \text{---------1}$$

D_{GI} --ABSORPTION--- D_B, V_D --ELIMINATION------- De

Figure 2.6.1a Model of drug absorption and elimination.

D_{GI} ----k_a---- D_B, V_D ----k----

Figure 2.6.1b One compartment pharmcokinetic model for first -order drug absorption and first-order elimination.

This model assumes a first-order input across the gut wall and first-order elimination from the body (Figure 2.6.1b). This model applies mostly to the oral absorption of drugs in solution or rapidly dissolving dosage forms such as tablets, capsules, and suppositories. In addition, drugs given by intramuscular aqueous injections may also be described using a first-order process.

After administration, the drug is absorbed from the absorption site by a first-order process. In the case of a drug given orally, the drug dissolves in the fluids of the GI

tract and is absorbed into the body according to a first-order rate process. The rate of disappearance of drug from the gastrointestinal tract is described by the following:

$$d D_B / dt = -K_a D_{GI} \quad \text{--------- 2}$$

where K_a is the first-order absorption rate constant from the GI tract and D_{GI} is the amount of drug in the GI tract at any time (t). Integration of the differential equation--2 gives

$$D_{GI} = D_o e^{-kat} \quad \text{-------- 3}$$

where D_o is the dose of the drug.

The rate of drug elimination is described by a first-order rate process for most drugs and is equal to $-KD_B$. The rate of drug change in the body, dD_B/dt, is therefore the rate of drug in minus the rate of drug out as given by the differential equation ---4

$$dD_B/dt = \text{Rate in} - \text{Rate out}, \quad dD_B/dt = FK_a D_{GI} - KD_B \quad \text{----4}$$

where F is the fraction of drug systemically absorbed.

Since the drug in the gastrointestinal tract also follows a first-order decline process (i.e. it is absorbed across the gastrointestinal wall), the amount of drug in the gastrointestinal tract is equal to $D_o e$.

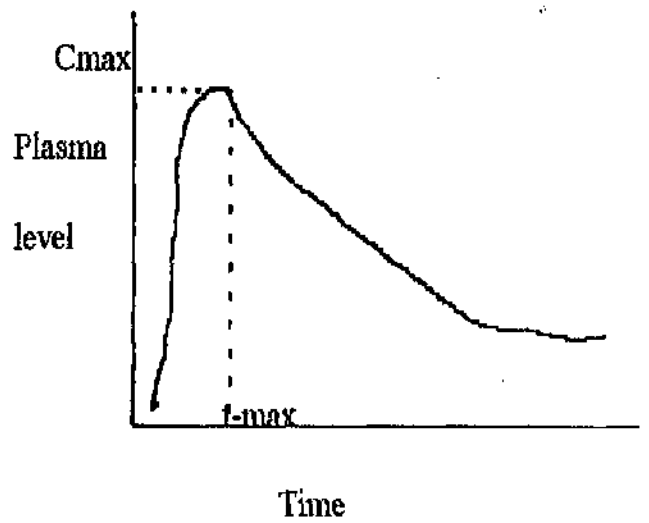


Figure 2.6.1c Typical plasma level-time curve for a drug given in a single oral dose.

F may vary from 1 for a fully absorbed drug to zero for a drug completely unabsorbed. This equation can be integrated to give general oral absorption equation for calculation of the drug concentration (C_p) in the plasma at any time t, as shown below.

$$C_p = \frac{FK_aD_o(e^{-kt} - e^{-kat})}{V_D(K_a-K)} \qquad \text{--------5}$$

A typical plot of the concentration of drug in the body after dose is presented in Figure 2.6.1c.

The maximum concentration is C_{max} and the time needed to reach maximum concentration is t_{max}. The time needed to reach maximum concentration is independent of dose and is dependent on the rate constants for absorption (K_a) and elimination (K). At the maximum concentration, sometimes called peak concentration, the rate of drug absorbed is equal to the rate of drug eliminated. Therefore, the rate of concentration change is equal to zero.

The first-order elimination rate constant may be determined from the elimination phase of the plasma level-time curve (Figure 2.6.1c). At the later time intervals, when drug absorption has been completed, Equation --5 reduces to the following expression:

$$C_p = \frac{FK_aD_o\ e^{-kt}}{V_D(K_a - K)} \qquad \text{--------6}$$

Taking the natural logarithm of this expression.

$$\ln C_p = \frac{FK_aDo - Kt}{V_D(K_a - K)} \qquad \text{-------7}$$

Substitution of common logarithms gives

$$\log C_p = \frac{FK_aDo - Kt}{V_D(K_a - K)\ 2.3}$$

With this equation, a graph constructed by plotting log C_p versus time will yield a straight line with a slope of - K/2.3 (Figure 2.6.1d).

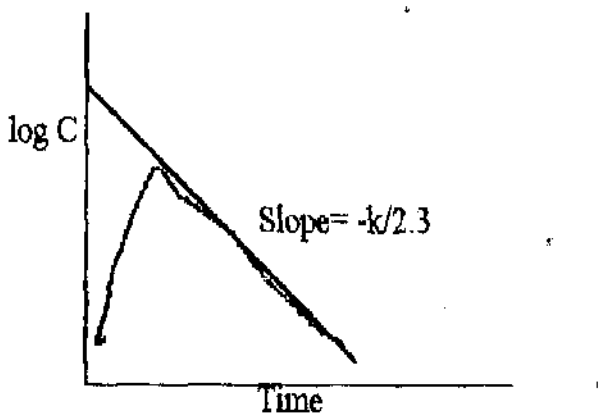


Figure 2.6.1d Aplot of log plasma drug concentration against time after single oral dose

Determination of Absorption Rate constants from Oral Absorption Data

Method of Residuals

Assuming $K_a > K$ in Equation --5, the value for the second exponential will become insignificantly small with time and can therefore be omitted. When this is the case, drug absorption is virtually complete. Equation--5 then reduces to Equation --9.

$$C_p = \frac{FK_aD_o\ e^{-kt}}{V_D(K_a - K)}$$

From this one may also obtain the intercept of the y axis (Figure 2.6.1e).

$$\frac{FK_aD_o}{V_D(K_a - K)} = A$$

where A is a constant. Thus, Equation 9 becomes

$$C_p = Ae^{-kt} \text{ ----------10}$$

Considering an equation which describe the time course of an intravenous bolus of a drug in one-compartment, two-compartment and three-compartment respectively,

$C_p = A\,e^{-kt}$

$C_p = Ae^{-at} + Be^{-bt}$

$C_p = Pe^{-pt} + Ae^{-at} + Be^{-bt}$

Where A, B and P are constants. The same additive principle applies to absorption and elimination for orally administered drug. Thus, for a one compartment model the equation:

$$C_p = \frac{FK_aD_o\,(e^{-kt} - e^{-kat})}{V_D(K_a - K)} \qquad \text{--------5}$$

is seen to have the form of

$Cp = Ye^{-kt} + Ze^{-kat}$

where the constant Y substitutes for [FDo kab / Vd(kab - kel)]

and the constant Z = -Y.

Because elimination and absorption work in opposite directions an exponential absorption process will increase Cp whereas the elimination function decreases it.

Therefore the equation for first-order absorption and elimination from a two compartment model has the form :

$Cp = Me^{-at} + Ne^{-bt} + Re^{-kat}$

where M, N and R are constants. Since Re^{-kat} is the function describing absorption, the R constant will have a negative value.

Non-linear least squares regression analysis programs are used to provide a set of parameter estimations by minimising the differences between observed and predicted

concentration values. Computer programs such as NONLIN,SAS, RSTRIP and BMDP-AR or BMDP-3R (Dixon 1985) can be useful in non-linear regression analysis.

The equation--1(), which represents first-order drug elimination, will yield a linear plot on semilog paper. The slope is equal to -K/2.3. The value for K_a can be obtained by using the method of residuals or a feathering technique. The value of K_a is obtained by means of the following procedure.

1. Plot the drug concentration versus time on semilog paper with the concentration values on the logarithmic axis Figure 2.6.1e).

2. Obtain the slope of the terminal phase (line BC, Figure 2.6.1e) by extrapolation.

3. Take any points on the upper part of line BC (e.g., X_1, X_2, X_3, ...) and drop vertically to obtain corresponding points on the curve (e.g., X_1, X_2, X_3,...).

4. Read off the values X_1 and X_1, X_2 and X_2, X_3, and X_3, and so on. Plot the values of the differences at the corresponding time . A straight line will be obtained with a slope of - K_a/2.3 (Figure 2.6.1f).

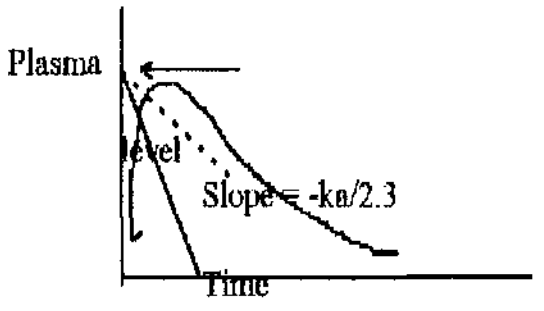


Figure 2.6.1f Plasma level-time curve for a drug demonstrating first order absorption and elimination kinetics.

Body clearance $= K_e V_D + K_1 V_D + K_m V_D$

$Cl_T = V_D(K_e + K_1 + K_m) = V_D K$ ---------- 11

From Equation 11, Cl_T of a drug is the product of two constants, K and V_D, which reflect all the distribution and elimination processes of the drug in the body. The volume of distribution and elimination rate constant are affected by blood flow, which will be demonstrated when clearance is considered using a physiologic model.

Clearance values are often normalized on a per-kilogram body weight basis, such as mL/min per kg. This approach is similar to the method for expressing V_D, because both pharmacokinetic parameters vary with body weight. the clearance for an individual patient is estimated as the product of the clearance per kilogram multiplied by the body weight (kg) of the patient.

Pharmacokinetics is concerned with obtaining the appropriate amount of drug at the receptor site for the appropriate length of time for patient, thus obtaining maximum therapeutic benefit with minimum side effects. Knowledge of the pharmacokinetic profile of any given drug plus the factor capable of modifying it is very useful in the development of rational dosage regimen.

A drug-drug interaction from an isolated in vitro enzyme system does not necessarily predict a significant change in drug plasma concentration in the patient. This prediction might be more accurate by also considering the clearance concept of pharmacokinetics (Rowland et al 1973 and 1980). When the drug dose (D) and

dosing frequency (II) are not changed, an increase in the steady-state drug plasma concentration (Css) will occur when the fraction of the drug dose absorbed (F) increases or the total body drug clearance (Cl_T) decreases:

$$Css = \frac{F.D}{Cl_T}$$

For a drug that is totally eliminated by hepatic metabolism, Cl_T is equivalent to hepatic drug clearance (Cl_H) which is a function of hepatic blood flow (Q, portal vein, hepatic artery), and the ability of the liver to extract the drug.

$$Cl_H = \frac{Q\ Ca - Cv}{Ca} = QE$$

Where Ca is the mixed portal vein and hepatic artery drug concentration leading to the liver, Cv is the hepatic venous drug concentration leaving the liver and E is the hepatic drug extraction ratio that varies between O when no drug and I when all drug is removed on crossing the organ respectively.

$$Cl_h = \frac{Q\ f\ Clintrinsic}{Q + f\ Clintrinsic}$$

Cl_h - is the hepatic clearance

f - is the fraction of free drug (unbound) in blood.

^{Cl}intrinsic - is the intrinsic free drug clearance (practically a function of drug metabolizing enzymes in the liver).

Q - hepatic blood flow.

According to hepatic clearance, drugs can be divided into 2 groups:

1. Drugs with high Extraction Ratio (ER).

2. Drugs with low extraction Ratio.

ER can vary between O and 1 and is defined by:

$$ER = \frac{Cl_{int.}}{Q + Cl_{int.}}$$

If a drug has a very high intrinsic clearance, the ER will tend towards 1 and it means that $Cl_h = Q$; and it will demonstrate the so called flow-dependent kinetics. Thus Cl_h of a drug, such as propranolol, lignocaine for which the liver has a high metabolic capacity, is related to hepatic blood flow and it is relatively insensitive to changes of drug metabolising enzyme system in the liver. At the other extreme, when intrinsic clearance is very low compared to blood flow, then hepatic extraction is low; the Cl_h = f. $Cl_{int.}$, and in this case, elimination is dependent only on enzyme activity and drug binding. For drugs such as theophylline, phenytoin which have low ER, the hepatic clearance will be affected by any change of enzyme activity but will not be affected by changes in hepatic blood flow. In practice, drugs in the hepatic ratio higher than 0.7 (ER > 0.7) are affected only by hepatic blood flow. Drugs with ER 0.2, will be affected by any change in drug metabolising enzyme and drug binding but not by hepatic blood flow.

Another important concept in the studies of drugs bioavailability and pharmacokinetics in body fluids is the apparent volume of distribution (Vd). This is defined as the

volume which the amount of drug in the body would need to be uniformly distributed to produce the observed concentration. The concept of Vd is used to relate the plasma concentration of a drug to the dose administered if no elimination has taken place. It does not relate to real physiological or anatomical spaces. A large Vd implies wide distribution or extensive tissue uptake or both. The simplest method to calculate Vd is:

$$Vd = \frac{D}{Co} \qquad D = \text{dose}, \quad Co = \text{initial conc.}$$

Also

$$Vd = \frac{FD}{ß.AUC}$$

Another important parameter in the study of pharmacokinetics in vivo is the plasma elimination half-life ($t_{½}$). Plasma half-life is the time taken for half of the amount of drug present in plasma to be eliminated either by excretion or metabolism or both. In practical terms, it is the time taken for the plasma concentration to be reduced by 50% of its original value

$$t_{½} = \frac{\ln}{ß} \qquad \ln 2 = 0.693$$

$$= \frac{0.693}{ß}$$

$$ß = \frac{\ln 2}{t_{1/2}}$$

From the above equation, it can be seen that large value for ß corresponds to a shorter half-life and its reflection on the rate at which the drug leaves the body. However, $t_{½}$ is a full index of drug elimination and depends not only on the clearance (elimination processes) but also on the volume through which the drug is distribution. Obviously if the clearance is increased, one would expect a shorter half-life for the drug.

Half-life is related to Vd by the following relationship:

$$t_{1/2} = \frac{0.693\ Vd}{Cls}$$

The above pharmacokinetic parameters play a major role in drug bioavailability in the body which is a function of drug effectiveness. The understanding of these parameters and their application will go a long way in designing an effective dosage regimen.

2.6.2 *Sample Collection, Storage and Pretreatment*

Common biological samples used in drug analysis for routine drug monitoring or pharmacokinetic work are plasma, whole blood, saliva and urine. Ritschel (1980) however reported that plasma sample is more suitable for pharmacokinetic studies. The samples are either analysed immediately or stored for a period of time. It has become generally accepted that sample pretreatment is an important stage in the determination of drugs in biological fluids in addition to storage. Most biological samples are collected and stored in glass or plastic containers at low temperatures or frozen prior to analysis. In some studies, the blood sampling point or source of the biological fluid

might be geographically remote from the laboratory of analysis, thereby requiring a good preservation and transportation system. Recently, determination of antimalarial drugs in capillary blood, plasma, whole blood and urine samples dried on filter paper has been reported. This convenient, cheap and reliable method of sample collection, preservation and transportation has been reported for mefloquine (Bergqvist et al 1993, 1994), chloroquine (Lindstrom et al 1985, Patchen et al 1983), pyrimethamine and sulfadoxine (Winstanley et al 1992, Bergqvist et al 1987) and quinine (Hellgren et al 1990, Mberu et al 1991, Dua et al 1991, Ericsson et al 1993). This method of sampling has been successfully applied in the determination of mefloquine enantiomers (Berggvist et al 1993) and the monitoring of blood levels of chloroquine (Mount et al 1991, Patchen et al 1983) and quinine (Hellgren et al 1990, Dua et al 1991, Ericsson et al 1993). This method of sample collection and storage offers an advantage of small sample size with no loss of drug due to adsorption to glass or plastic. It is also inexpensive and convenient for transportation.

2.6.3 Solid Phase Extraction of Basic Drugs using Bonded Silica

Pretreatment of biological samples prior to analysis is always aimed at having clean analysable sample. Protein-free samples were achieved through protein precipitation with different types of reagents. Conventional liquid-liquid extraction technique has been well developed. This involves the partitioning of the drug and the biological matrix into solvents of different polarity. Factors such as polarity and pH of solvents,

and pa of drug had been exploited in the separation of drugs into water/organic solvents. However solid phase extraction (SPA) is being used increasingly to replace liquid-liquid extraction. The application of the knowledge of bonded silica chemistry transferred from HPLC column technology to sample preparation was a major break-through in changing a biological sample to an analytical one. The deviation from the traditional tedious, time-consuming and costly liquid-liquid extraction to a simpler (fast and less costly) alternative solid phase extraction was introduced in the late 70s and it has since been accepted as a vital pre-sample treatment technique. A number of drugs have been isolated by this type of extraction. Problems such as phase emulsions, large solvent volumes and impure and wet extracts are not associated with this extraction technique. However this simple technique involves a much more complex mechanism of adsorption and elution of analyte (from normal or reverse phase silica and polyamides), where several factors may be responsible for observed results. SPE has a few advantages over liquid-liquid extraction. This includes ease of handling, decrease of extraction time and hence analysis time, preservation of compound integrity, cost reduction and the possibility for on-line arrangement and automation. However the variability of modified silica used as material in SPE is a great disadvantage. Variation in maunfacture-to-maunfacture, batch-to-batch and phase-to-phase has been reported (Marko et al 1986).

2.7.1 Aim of Present Study

Nigeria is malaria endemic and antimalarial drugs are non-prescriptive drugs. Therefore the antimalarials are available to the people at will. Antimalarials are used for curative or as prophylactics in all types of patients. In Nigeria, millions of peptic ulcer and epigastric pain patients receive cimetidine. Since peptic ulcer patients present feverish symptoms, antimalarials are usually prescribed along with cimetidine. Literature search showed that cimetidine interaction with the antimalarials have not been reported in healthy and peptic ulcer patients.

The presence of drug in the biophase leads to interactions between drug molecules and receptors to exert a biological response. The magnitude of the response depends not only on the affinity of the drug for the receptor but also on its concentration in the biophase which is in turn determined by a variety of pharmacokinetic processes such as absorption and disposition.

This study is therefore aimed at understanding the nature and extent of drug interaction (if any) between cimetidine and these selected antimalarials (mefloquine, proguanil and quinine) and also to undertake a pharmacokinetic study of mefloquine, proguanil and quinine when administered individually and when administered with cimetidine in healthy Nigerian males and in peptic ulcer patients. This is with a view to assessing the effect of cimetidine on the absorption and disposition of the antimalarial drugs. The

study also involves the development of an extraction technique for the antimalarials in plasma and whole blood absorbed on filter paper.

CHAPTER 3

EXPERIMENTAL

3.0 Materials and Method

3.1 Materials

The following materials were used in carrying out the project.

3.1.1 Glassware

-- Measuring cylinders, size range: 5 -1000ml.

-- Micropipettes and tits size range: 0.05 -10ml

-- Conical flasks, size range: 25 -500ml.

-- Plastic and glass centrifuge tubes: 1.5ml, 10ml.

-- Pasteur pipettes.

-- Beakers, size range: 5ml-1000ml.

-- Syringes and needles 2ml, 5ml, 10ml, and 20ml, with 21G needle.

-- Cannula, 21G.

-- EDTA blood sample bottle 5ml.

-- C-18 Bond Elut cartridge (Varián, USA).

-- C-8 Bond Elut cartridge (Varian, USA).

-- Vac-Elut, ten cartridge manifold. (Analytichem international, UK).

Whatman No1 filter paper from Whatman International (Maidson, England, UK).

3.1.2 Equipment

-- Solvent filtering kits.

-- Kent pH meter.

-- Metler electrical ballance.

-- Sanssui refrigerator.

-- A Jasco liquid chromatograph consisting of Jasco 980 intelligent pump and Jasco UV 975 intelligent detector(Jasco Tokyo, Japan) fitted with a nominal 18ul flow cell was used. Injection was by Rheodyne 7125 valve(Cotati CA USA) incorporating a 20ul loop.

-- The column was 100mm x 2mm I.D packed in the laboratory (The Robert Gordon University, School of Pharmacy, analytical laboratory), with slurry of 3μm ODS Hypersil (HETP Macclesfied, U.K).

-- SC-3 Sample concentrator (Techne, England, UK).

3.1.3 Chemicals and Standards

Mefloquine hydrochloride, mefloquine metabolites were obtained from Roche (Switzerland). Mefloquine tablets were gifts from Roche (Lagos, Nigeria).

Chlorproguanil, proguanil, cycloguanil, 4-chlorophenylbiguanide and 3,4-dichlorophenylbiguanide were obtained from ICI (Zeneca, Macclesfield, UK). Proguanil tablets (Paludrin) were purchased from Dilimi Pharmacy (Jos, Nigeria).

Quinine sulphate monohydrate, dihydroquinine (hydroquinine) and tetrabutylammonium bromide were obtained from Aldrich (Gillingham, Dorset, UK). Quinidine was from Evans Medical (Greenford, Middlesex, UK). Quinine sulphate tablets were gifts from Our Lady of Apostle Hospital, Jos, Nigeria.

Cimetidine, cimetidine sulphoxide (SKF 92452LRS2) and cimetidine amide (SKF92422LRS2) were gifts from SmithKline-Beecham (West Sussex, England, UK). Cimetidine tablets (Tagamet)were gifts from SmithKline-Beecham (Lagos, Nigeria).

Acetonitrile and methanol were obtained from Rathburn Chemicals(Walkerburn, UK), sodium lauryl sulphate (SLS) by Fison (Loughborough U.K), ammonium hydroxide and ortho-phosphoric acid by BDH chemical (England, UK). Sodium dihydrogen phosphate was obtained from Merck (UK). Water was distilled and further purified by using a Millipore Milli-Q system (Millipore-Waters, Milford, USA). Aquasil was obtained from Pierce (Illinois USA). All other reagents were of AnalaR or equivalent grade.

3.2 Methods

3.2.1 Chromatographic Separation

3.2.1a Mefloquine

The ion-pair system of Taylor et al (1992) for multi-drug assay of antimalarials was modified and optimised. The mobile phase was, Acetonitrile : 20mM phosphate buffer (42:58) containing 12mM TBA and 120mM SLS at pH 2.4 (with ortho-phosphoric acid). The mobile phase was pumped with system pressure of 180kg/cm^2 at a flow rate of 0.4ml/min. The eluent was monitored with ultraviolet detector at wavelength 285nm and sensitivity set at 0.01AUFS and chart speed of 0.2cm/min.

3.2.1b Proguanil and Metabolites

The chromatographic separation system for the determination of proguanil in biological fluids as described by Taylor et al (1987) was adopted. The chromatographic solvent used was acetonitrile: 10mM phosphate buffer pH 2.0 (50:50), containing 200mM SLS. A flow rate of 0.4ml/min was used. A 20ul sample was injected with the aid of Rheodyne 7125 valve injection loop. Peak heights were measured at detector sensitivity set at 0.005AUFS and detector wavelength of 254 nm. The chart speed was set at 0.25cm/min. and the pressure of the operation, was 200kg/cm2.

3.2.1c Quinine

The ion-pairing system of Taylor et al (1992) was modified. The mobile phase was acetonitrile : 20mM phosphate buffer (58:42) containing 6mM TBA and 90mM SLS pH 2.4. The mobile phase was pumped at 0.4ml/ min and the eluent monitored with ultraviolet detector at wavelength 254nm. Sensitivity was set at 0.01AUFS and chart speed at 0.2cm/min.

Fluorescence detection of the effluent was monitored on Varian 2070 Spectrofluorimeter, with range setting at 8-32 at an excitation and emission wavelength of 340 and 425nm respectively, at chart speed of 0.25cm/min.

3.2.2 Method Validation Procedures

3.2.2a Calibration Procedure for Mefloquine

Linearity of chromatographic response following extraction was established by preparing five standards of mefloquine, in concentration range of 0.1 to 4μgml^{-1}) in plasma. Plasma (0.1ml) was pipetted using a micropipette and adsorbed onto filter paper. The filter papers were air dried at room temperature 26°C, then subjected to the sample pre-treatment step and chromatography.

3.2.2b Calibration Procedure for Proguanil

Plasma (0.9ml) was spiked with compounds by adding 0.1ml standard aqueous solution of compounds. Five standard concentrations of compounds were prepared in plasma within these ranges, proguanil (54.4-1000µg/ml), cycloguanil (11.6-232ng/ml), 4-chlorophenylbiguanide (4.2-42ng/ml). Samples (0.1ml) of plasma spiked with compounds were applied to filter papers (about $6cm^2$) with a micropipette and allowed to dry at room temperature(26°C). The compounds were subsequently extracted and injected into the chromatograph as described under sample pre-treatment below.

3.2.2c Calibration Procedure for Quinine

Linearity of chromatographic response following extraction was established by preparing five standards (concentration range of 0.1 to 5μgml^{-1}) in plasma. Plasma (0.1ml) of the resulting biological solution was pipetted with a micropipette and adsorbed onto filter paper. The air dried filter paper at room temperature(26°C) was then subjected to the sample pre-treatment step and chromatography.

3.2.3a Mefloquine Sample Pre-treatment

The blood-spotted parts of the filter paper were cut into pieces (2-3mm diameter a piece) and placed into a plastic centrifuge tube (pre-treated with 0.2% aquasil to minimise drug adsorption) and 2M ammonium hydroxide (3ml) was added. A volume (0.1ml) of the internal standard, 3,4-dichlorophenylbiguanide (1µg/ml), was immediately added. The centrifuge tube was stoppered and vortexed for 1minute and

allowed to stand at ambient temperature for 2hours before subjecting to ultrasonication treatment for 1hour at room temperature (26°C). The Bond Elut C18 cartridge was activated by adding methanol (2ml) to the cartridge and allowing it slowly pass through. The Bond Elut was subsequently conditioned by passing 2M ammonium hydroxide (0.5ml) through it.

The supernatant from the filter paper extract was transferred to the C18 Bond Elut cartridge which had previously been activated with methanol (2ml) and subsequently conditioned with ammonium hydroxide (0.5ml). The residual filter paper was washed with a further 2M ammonium hydroxide (1ml) and the washings also applied to the cartridge. A stream of air was allowed to pass through the cartridge. The cartridge was washed with ammonium hydroxide (1ml), followed by water (1ml) and finally with 65% methanol in water. The compounds, mefloquine and 3,4-dichlorophenylbiguanide were eluted with 0.1% w/v perchloric acid in methanol (1ml). The extracts were collected in small conical plastic centrifuge tubes, evaporated in stream of nitrogen gas at ambient temperature and the residue reconstituted in mobile phase (100µL) just before injection of 20µL into the chromatograph.

3.2.3b Proguanil-Sample pre-treatment

The blood spotted parts of the filter paper were cut into pieces into glass test tubes. Chlorproguanil (0.1ml) at concentration 824ngml^{-1} as internal standard was added onto the cut filter papers followed by 0.9M ammonia hydroxide (5.0ml) and stoppered. This

was allowed to stand for thirty minutes at about 30°C or whirlimixed immediately for 30sec before subjecting it to 30min ultrasonication treatment. The resulting supernatant was extracted for analyte using C8 Bond Elute cartridges in a similar way as previously described for proguanil and metabolites. The Bond Elut C8 cartridge was activated by adding methanol (2ml) and conditioned with 0.9M ammonium hydroxide (0.5ml). Supernatant of the mixture was added onto the cartridge with pasteur pipette. The residue was rinsed with the ammonia solution (2ml) and added onto the cartridge. The cartridge was washed with methanol (1.0ml). The compounds were eluted with 0.1%w/v perchloric acid in methanol (1.0ml). The collected extract was evaporated to dryness under stream of air at 30°C. The residue was reconstituted in 100µL water just before injection of 20µL into the chromatograph.

3.2.3c Quinine-Sample pre-treatment

The blood-spotted parts of the filter paper were cut into pieces (2-3mm diameter a piece) and placed into a glass test tube (pre-treated with 0.2% aquasil to minimise drug adsorption) and 2M ammonium hydroxide (3ml) was added. A volume (0.1ml) of each of the internal standards, chlorproguanil (1µg/ml) and quinidine (0.8µg/ml) were immediately added. The glass tube was stoppered and vortexed for 1min before subjecting to ultrasonication treatment for 30min at room temperature.

The supernatant from the filter paper extract was transferred to the C8 Bond Elut cartridge which was activated by adding methanol (2ml) and conditioned subsequently

by addition of ammonium hydroxide (0.5ml). The residual filter paper was washed with a further 2M ammonium hydroxide (1ml) and the washings also applied to the cartridge. A stream of air was allowed to pass through the cartridge. The cartridge was washed with 0.2ml (for plasma samples) or 0.5ml (for whole blood samples) 75% methanol in water. The compounds, quinine, quinidine and chlorproguanil were eluted with 0.1%w/v perchloric acid in methanol (1ml). The collected extracts were evaporated in stream of nitrogen gas at ambient temperature (30°C) and the residue reconstituted in water (0.1ml) just before injection of 20µl into the chromatograph.

3.2.4 Recovery

The recoveries obtained by the above treatment of spiked samples were determined by spiking plasma with the antimalarials at different concentrations (as stated below). Samples (plasma, 0.1ml) of these were adsorbed on filter paper, dried and subjected to the sample pre-treatment and calibration steps. The peak heights obtained during the subsequent chromatography were compared with those obtained by direct injection of aqueous standards of the compounds containing the theoretical mass corresponding to complete recovery of each of the compounds.

(Mefloquine standard was prepared in the mobile phase)

Mefloquine ..... 0.5, 1.0 and 2.0µg/ml.

Proguanil ..... 217.6, 108.8, and 54.4ng/ml.

Cycloguanil ..... 92.8, 46.4 and 23.2ng/ml

4-CPB ..... 42.0 21.0 and 10.5ng/ml.

Quinine ..... 0.5, 1.0 and 2.0µg/ml

3.2.5 Determination Limit

Determination limit for the antimalarials was determined by preparing serial dilutions of mefloquine in aqueous solution and subsequently in plasma and applying 0.1ml or of these to the filter papers. Extraction and chromatography was carried out as described above and the resulting peak height measured. Detection limit was taken as the concentration that gave signal to noise ratio of 3 to 1. While determination limit was taken as two times the detection limit.

3.2.6 Accuracy and Precision

The within day (intra-day) accuracy and precision of the method was determined by assaying ten samples of filter paper spiked with treated plasma using a single calibration procedure. Calibration equations were obtained by linear regression of peak height ratio (compound/internal standard) on concentration.

The day to day (inter-day) precision was taken as the variation in the slope of the calibration lines obtained on different days over a period of five days.

3.3 Effect of Temperature on the Antimalarials in Plasma Dried on Filter Paper

A 0.1ml plasma spiked with compound at concentrations; 5µg/ml mefloquine, 5ng/ml Quinine, 544ng/ml proguanil, 92ng/ml cycloguanil and 42ng/ml 4-CPB was applied to the filter papers. The blood spotted filter papers were stored at 25°C, 37°C, 55°C, 65°C for two months and at -20°C for 10 months. Samples were analysed following the sample pre-treatment and recovery procedures described above. The three groups of antimalarials were studied separately.

3.4 Pharmacokinetic Procedure

3.4.1 Subject Selection and Treatment

Two groups of subjects were studied, consisting of a healthy male adult group and peptic ulcer patients with each group acting as its own control.

Ethical approval was obtained from the Jos University Teaching Hospital. All subjects were confirmed fit to undertake the study and written consent was obtained. All subjects were non-smokers and none of the subjects took alcohol or any other medication a week before and during the study.

3.4.1.1 First Group (Healthy subjects)

3.4.1a Mefloquine

The subjects were six healthy young adults aged from 30 to 37years (mean ± SD = 32.2 ± 2.6years) and weighing from 58 to 73Kg (mean ± SD = 64.8 ± 6.0Kg). After an overnight fast the subjects were administered with single oral 500mg dose mefloquine tablet (Roche) with approximately 150ml of water. Venous blood (3ml) was then drawn through an indwelling catheter just before dosing and 0.5, 1, 1.5, 2, 2.5, 3, 6, 9, 12, 24, 48hours, 7,15,30 and 45days after dosing. Blood was collected in EDTA-pre-treated plastic blood sample bottles.

3.4.1b Proguanil

The subjects were six healthy young adults aged from 23 to 36years (mean ± SD = 28.67 ± 5.75years) and weighing from 68 to 72Kg (mean ± SD = 70.67 ± 1.51Kg). After an overnight fast the subjects were administered with single oral 200mg dose proguanil tablet(Paludrine ICI) with approximately 150ml of water. Venous blood (3ml) was then drawn through an indwelling catheter just before dosing and 0.5, 1, 1.5, 2, 2.5, 3, 6, 9, 12, 24, 48, and 60hours after dosing. Blood was collected in EDTA- pre-treated blood sample bottles.

3.4.1c Quinine

The subjects were six healthy young adults aged from 25 to 36years (mean ± SD = 30.2 ± 4.8years) and weighing from 58 to 72Kg (mean ± SD = 65 ± 5.4Kg). After an overnight fast the subjects were administered with single oral 400mg dose quinine sulphate with approximately 150ml of water. Venous blood (3ml) was then drawn through an indwelling catheter just before dosing and 0.5, 1, 1.5, 2, 2.5, 3, 6, 9, 12, 24, and 36hours after dosing. Blood was collected in EDTA- pre-treated blood sample bottles.

General treatment of samples collected

The whole blood sample was centrifuged (at 5g for 5min) and 0.1ml plasma was pipetted out with a micropipette and adsorbed on filter paper. The filter paper was then air dried at room temperature (30°C). Subjects were not allowed to eat or drink within the first 3hours of the study.

After a washout period of two weeks, subjects were made to repeat the above process with cimetidine administered before the antimalarials. Cimetidine (400mg) tablet was administered morning and night for two days and after an overnight fast of the third day subjects were administered with single oral 400mg cimetidine tablet (Tagamet) an hour before the single oral doses of the antimalarial tablets. Samples were collected and treated as above.

3.4.2 Second Group (Peptic Ulcer Patients)

Peptic ulcer patients

The subjects were six endoscopically (or by barium meal x-ray examination) certified peptic ulcer patients (male adults) of which a few could not complete the study. All subjects were non-smokers and were out-patients of the Jos University Teaching Hospital. Subjects were requested to abstain from other medications just before and during the course of the study.

3.4.2a Mefloquine

Subjects aged from 31 to 39years (mean ± SD = 34.8 ± 3.9years) weighing from 63 to 70Kg(mean ± SD = 65 ± 2.1Kg).Four patients completed the first part and three the second part of the study. The other patients could not be reached for the last three sampling times or missed more than five sampling times.

3.4.2b Proguanil

Subjects aged from 36-39 years (mean ± SD = 37.75 ± 1.26years) weighing from 65 to 72Kg (mean ± SD = 68.5 ± 2.9Kg). Four of the patients completed the two phases of the study while two could not complete the study because of gastrointestinal irritation and severe dizziness.

3.4.2c Quinine

Subjects aged from 30 to 36years (mean ± SD = 32.3 ± 3.2years) weighing from 54 to 71Kg(mean ± SD = 63 ± 5.6Kg). Three of the ulcer patients completed the two phases of the study while the other three withdrew from the study because of gastrointestinal pain and cramps.

Subjects were treated as in the healthy volunteers above.

All samples were stored in polyethylene bags not transparent to light. Samples were kept away at -20°C until analysis (except during transportation to the UK).

3.4.3 Pharmacokinetics

The standard pharmacokinetic equations for one and two compartment model are shown below as Equation (a) and Equation (b) respectively.

$C = B.exp^{-\beta.t} - Cpo.exp^{-ka.t}$ **(a)**

$C = B.exp^{-\beta.t} + A.exp^{-\alpha.t} - Cpo.exp^{-ka.t}$ (b)

C= drug concentration at time t [$ngml^{-1}$, μgml^{-1}]

$^{\beta}$ = elimination rate constant [h^{-1}]

ka = absorption rate constant [h^{-1}]

$^{\alpha}$= distribution rate constant [h^{-1}]

Cpo = hypothetical drug concentration at time 0, obtained from A+B =Cpo [$ngml^{-1}$]

B = intercept of back-extrapolated monoexponential elimination slope b with ordinate [ngml^{-1}]

A = intercept of monoexponential distribution slope a with ordinate [ngml^{-1}]

Preliminary pharmacokinetic values were obtained from concentration-time profile. Data were then fitted to these relationships above equation (a) and (b) using the (Dixon 1985), non-linear regression curve fitting program, to obtain the best least squares fit to the individual concentration-time curves. Standard pharmacokinetic parameters were then calculated. Elimination and absorption half lives were calculated from the ratio 0.693/β and 0.693/ka respectively. The trapezoidal rule was used in calculating the area under the curve and the mean residence time. This was done with the non-compartment model. AUC is the area under the curve [ng/ml.h, or mg/L.day], obtained from the integral of drug blood level over the time from zero to infinity. It is a measure of the amount of drug absorbed and into the body. AUCo-∝ was calculated by the trapezoidal rule from the beginning of the drug administration to the last data point and with extrapolation to infinity. The area from the last data point Ct to infinity was obtained as Ct/β

Total body clearance Cl_T, (or Plasma clearance Cl_p) was calculated from the ratio of dose to area under the plasma drug concentration - time curve, assuming bioavailability

was equal to one (F=1). Apparent Volume of distribution V_D/f [L or L/kg] was calculated from the ratio of the plasma clearance to rate of elimination.

Student's two tailed t-test was used to assess differences between paired data. Minitab software package for two group analysis was employed. Group data are presented as mean ±SD.

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Quantitative Aspects of Mefloquine Assay

4.1.1 System Performance

Figure 4.1.1a shows the chromatogram of blank blood on filter paper. Figure 4.1.1b shows representative chromatograms of mefloquine and 3,4-dichlorophenylbiguanide in mobile phase and extract of spiked plasma. There was no indication of endogenous materials interference. The system is selective for mefloquine, there was no interference by other antimalarials, cimetidine or its metabolites. Taylor et al. (1992) in their analysis involving other commonly used antimalarials using a similar system, also reported non interference of system with other antimalarials. The metabolites of mefloquine or any other polar metabolites were not retained on this ion-pair mobile phase system on the RP-HPLC.

4.1.2 Extraction from Filter Paper and Solid Extraction

Ammonium hydroxide 2M was found to be efficient and adequate for the extraction of mefloquine from the filter papers at the experimental temperature. Bergqvist et al (1988) had earlier reported the use of ammonium hydroxide in their assay of mefloquine from plasma dried on chromatographic paper. Their recovery was above 70% and they had good coefficient of variation.

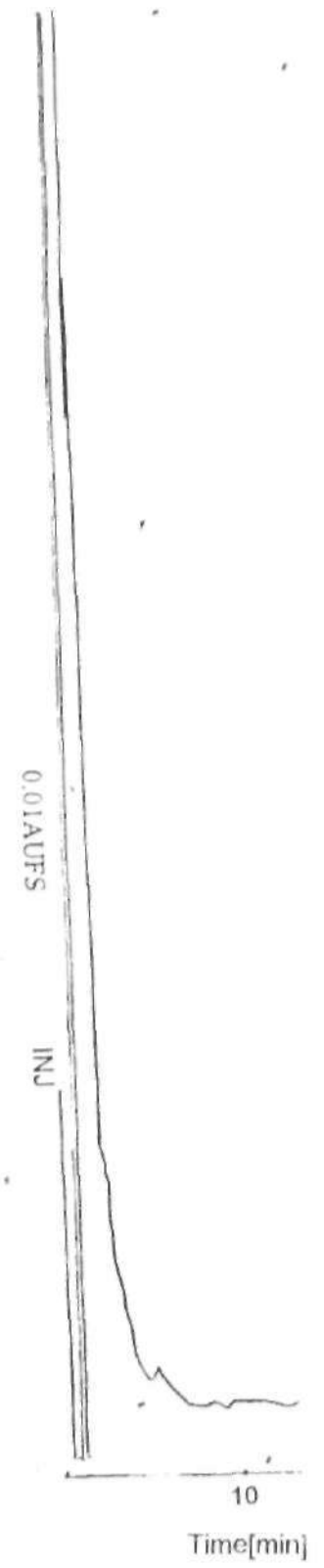


Figure 4.1.1a Representative chromatogram of blank blood on filter paper

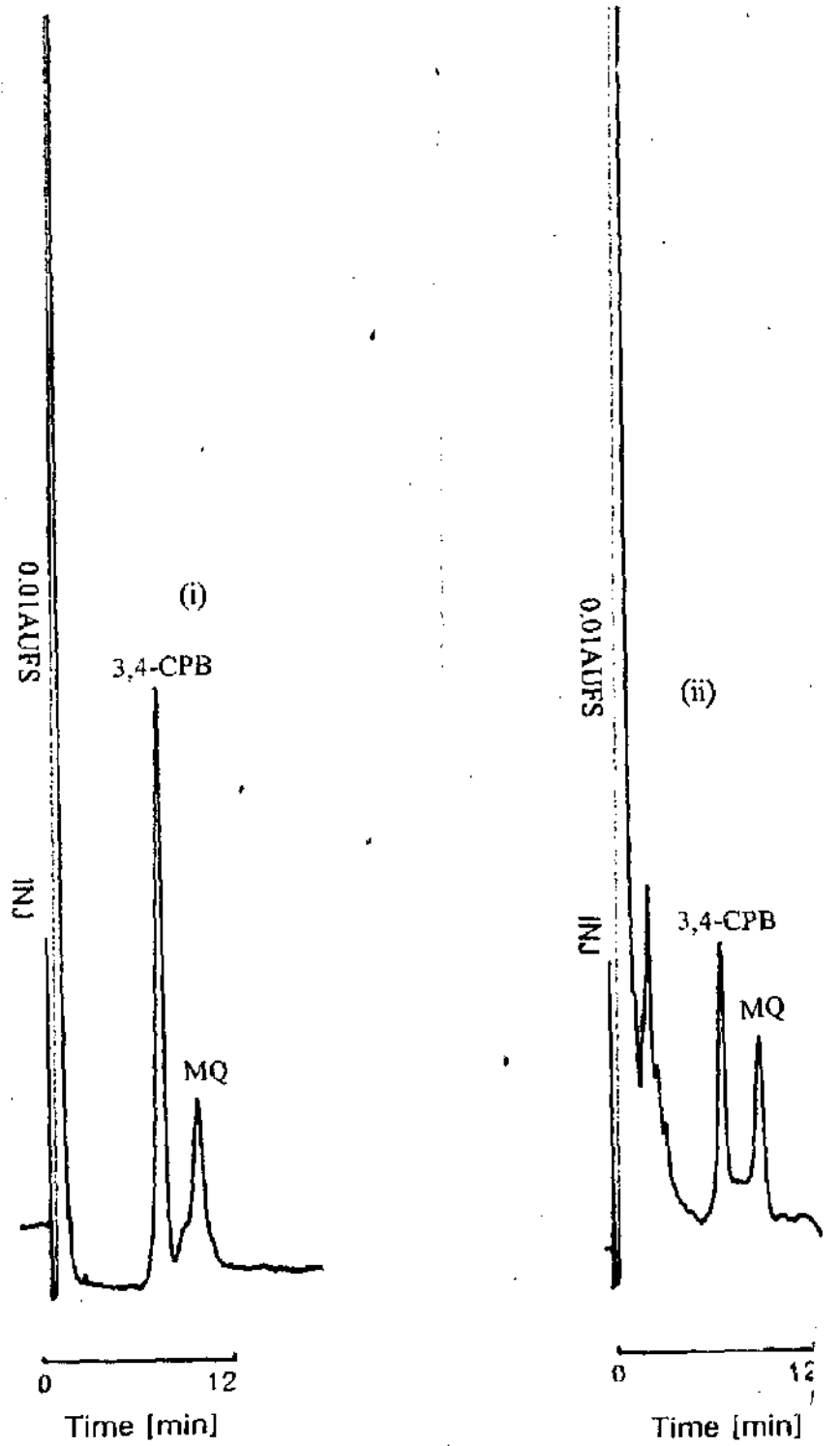


Figure 4.1.1b Representative chromatogram of Mefloquine (1 μgml^{-1}) and 3,4-dichlorophenylbiguanide (1μgml^{-1})
(i) in mobile phase and (ii) extract of spiked plasma sample.

The extraction of mefloquine from the biological-fluid paper mixture in ammonium hydroxide, was carried out by the use of methanol activated C18 Bond Elut cartridge conditioned with ammonium hydroxide. This was to prevent permanent adsorption of mefloquine to the silanol surfaces. The mechanism of extraction and elution of basic drugs have been attributed to a number of factors, of which hydrophobic dispersion forces and electrostatic (ionic) interactions play major roles. Endcapping of the bonded phase reduces the silanol surface available for ionic interaction to about 30% McDowall (1994). Further reduction of the silanol surface activity was achieved by the competing base, ammonium hydroxide solution. The SPE method used avoided the problems of phase emulsion, large solvent volumes, impure and wet extracts associated with liquid-liquid extraction.

4.1.3 Recovery, Precision and Accuracy

Table 4.1.1b show the recovery, precision and accuracy of the assay method. The recovery in plasma ranged from 82.3 to 93%. These recoveries compared well with the reported recoveries of Bergqvist et al (1993) and Franssen et al (1989). The level of recovery is large enough for quantitative work. The intra-day (within-day) assay precision of method averaged 9.83% in plasma. This low coefficient of variation shows the analytical method to be good.

The calibration of the drug in plasma required the use of internal standard from the extraction site. 3,4-dichlorophenylbiguanide is a metabolite of chloproguanil, an antimalarial agent not

commonly used in Nigeria. The retention time of mefloquine was 10min and that of 3,4-CPB was 6.7min in this system. The calibration curve showed good linearity over the concentrations considered (Table 4.1.1a). The mean correlation coefficient was 0.990. The inter-day (day-to-day) assay precision of method was found to be 6.1% (C.V) and intra-day (within day) assay precision averaged 9.8% in plasma.

Table 4.1.1a The analytical characteristics of method (inter-day i.e. day-to-day precision of method in quantification of mefloquine).

Biological fluid	Mean Calibration slope tr=5	C.V. (Day-to-day Precision)	Mean correlation n=5	Determination limit ug/ml
Plasma on filter paper	0.472	6.10	0.990	0.04

Table 4.1.1b. Analytical characteristics of method showing recoveries, intra-day (within-day) precision and accuracy of method.

Biological fluid	Conc. spiked μgml^{-1}	n	Recovery	C.V.	Conc. Obtained μgml^{-1}	C.V.
Plasma	0.5	10	83.3	12.5	0.460	13.0
"	1.0	10	93.3	10.0	1.060	12.2
"	2.0	10	82.3	7.0	2.12	16.5

4.1.4 Effect of Temperature

Plasma spiked with mefloquine at concentration $5\mu gml^{-1}$ stored at -20, 25 37, 55 and 65°C for 2 months was found to be stable to heat.

No decrease in concentration was found during storage at -20°C for 12 months. Storing plasma spiked samples of filter paper for 2 months at 25°C and 37°C did not show any decrease in concentration. Samples subjected to 55°C and 65°C for 2 months lost about 8% of their original concentration.

4.2.0 Quantitative Aspects of Proguanil and Metabolites

4.2.1 System Performance

The chromatographic solvent used was the one developed and used by Moody et al. (1980) and Taylor et al. (1987) for the determination of proguanil, cycloguanil and 4-CPB in

biological fluids. Figure 4.2.1b is the chromatogram of proguanil, cycloguanil, 4-CPB and chloproguanil (internal standard) prepared in water. Figure 4.2.1a is the chromatogram of the extract of blank blood adsorbed on filter paper. There was no indication of interference of endogenous materials or of filter paper additives on the chromatograms. Taylor et al (1987,1990) have earlier shown that the chromatographic separation was selective and sensitive for proguanil and no interference with other antimalarial agents such as chloroquine, quinine and mefloquine or their metabolites were observed.

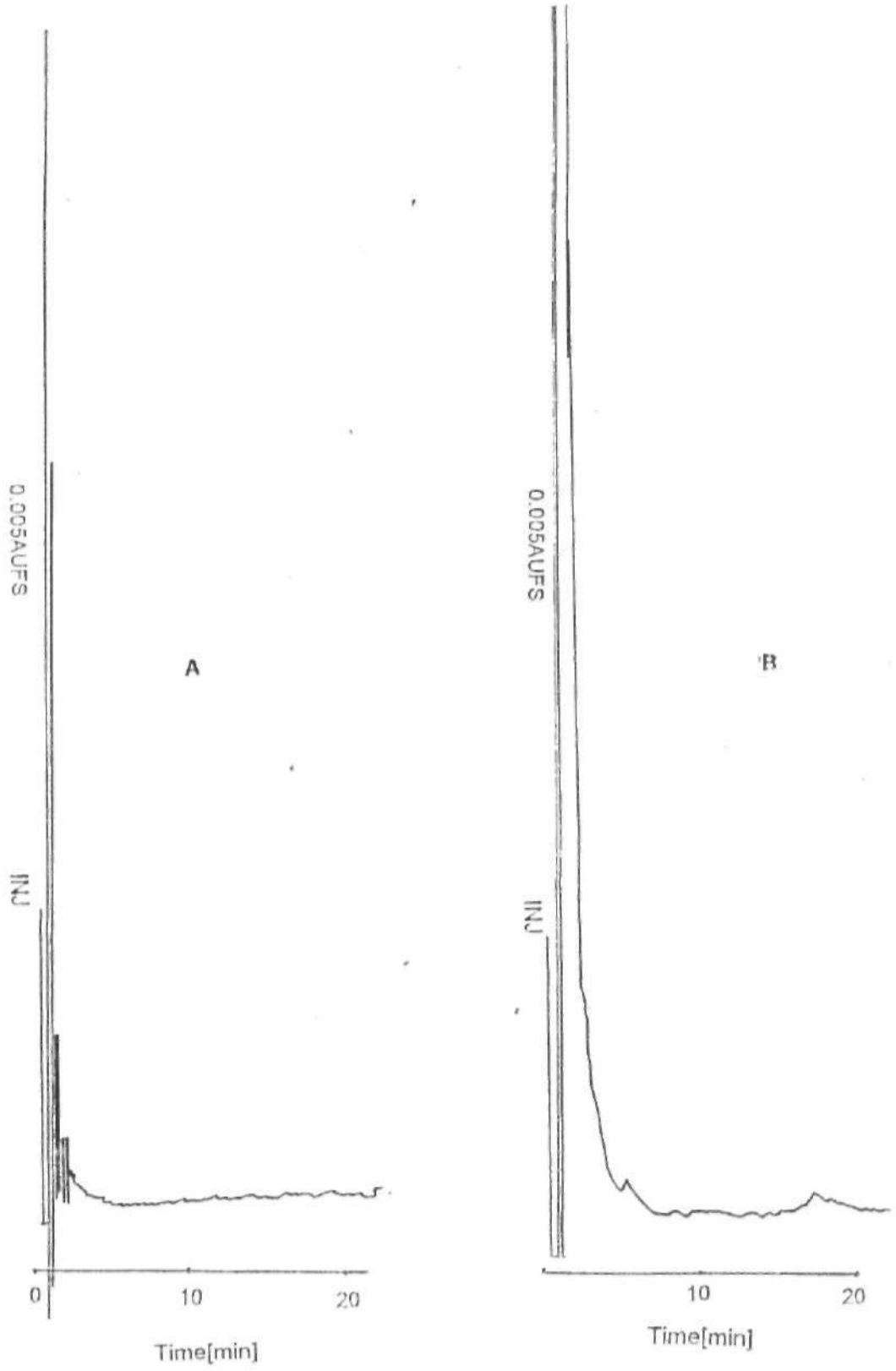


Figure 4.2.1a Representative chromatogram of blank filter paper and blank blood
(A) Blank filter paper (B) Blank blood

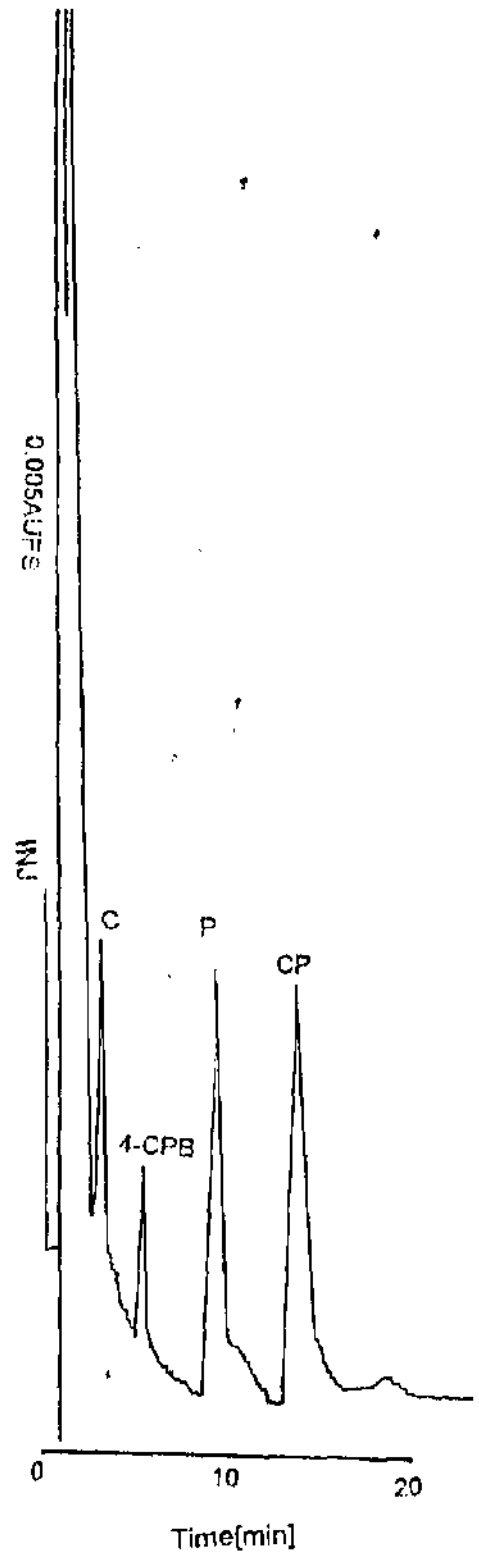


Figure 4.2.1b Representative chromatogram of Proguanil (P) (108.8ng/ml), Chlorproguanil (CP) (824ng/ml), Cycloguanil (C) (46.4ng/ml), 4-Chlorophenylbiguanide (4-CPB) (21ng/ml).

4.2.2 Extraction from Filter Paper/Solid Phase Extraction

Ammonium hydroxide (0.9M) was found to be efficient and adequate for the extraction of proguanil and metabolites from filter paper at the experimental temperature 30°C.

Proguanil and metabolites have pKa values of 10.4 and 2.3. The ammonia hydroxide/matrix extract had a pH of 11.8, this is 1.4 pH units away from the pKa 10.4, therefore the ionisation of these compounds were suppressed. This aided extraction using the non-polar sorbents as found in C8 Bond Elut.

The C8 bond Elut cartridge was activated with methanol and conditioned with ammonia solution .This was to prevent the use of the compounds as conditioning agent on application to the cartridge and to reduce or occupy the exposed surfaces of the silanol (-SiOH) group (McDowall 1994).The mechanism of extraction of basic drugs have been attributed to one or all of the following forces acting at the same time, hydrogen bonding, dipole-dipole interaction, hydrophobic dispersion forces or electrostatic (ionic) interactions. Endcapping of the bonded phase reduces the silanol surface available for ionic interactions to about 30% (McDowall 1994). Further reduction of the silanol activity can be achieved by using a competing base. The ammonium hydroxide therefore reduced secondary (ionic) attraction at the silanol surface of compounds, a likely secondary mechanism of adsorption in solid phase extraction process in C18 and C8 sorbents but increases the primary non-polar interaction and subsequent extraction yield with the less hydrophobic C8 sorbent.

The collection of plasma samples onto filter paper has eliminated the problems of adsorption of proguanil to glass, transportation of samples and scarcity of dry ice which frequently complicate the storage of liquid samples.

4.2.3 Recovery, Precision and Accuracy

Recoveries of proguanil 75% , cycloguanil 82%, 4-chlorophenylbiguinide 87% (1.5, C.V.) and chloproguanil 66% (3.4, C.V) were obtained over the concentration ranged used (Table 4.2.3b). The recovery of proguanil was relatively low when compared with the result for proguanil 99% for plasma reported by Taylor et al (1987), This might be due to the two extraction steps. However it is high enough for quantitative analysis and falls within the range obtained using the filter paper methods for other antimalarial drugs (Bergqvist et al 1993,1987, Hellgreen et al 1990, Mount et al 1991). The calibration of the drug in blood on filter paper required the use of internal standard before extraction. Chloproguanil, a drug similar in structure to proguanil but with a longer retention time (14.5 min) was used.

The intra-day and inter-day precision and accuracy, (see Tables 4.2.3a and 4.2.3b) showed the sample collection, pre-treatment and assay method as efficient and compared well with the result obtained for analyte in blood samples not adsorbed on filter paper (Taylor et al 1987, Wattanagoon et al 1987, Wangboonskul 1993).

Table 4.2.3a Analytical characteristics of method

Compounds	Calibration slope mean±SD($x10^{-3}$) * n=5	Mean r^2	SD($x10^{-3}$)	Typical regression equation y=mx + b
Proguanil	5.110± 0.643	0.998	1.8	$5.5306x10^{-3}x + 9.011x10^{-2}$
Cycloguanil	6.100 ± 0.649	0.995	6.3	$5.675x10^{-3}+0.298 x10^{-3}$
4-CPB	11.03 ± 0.232	0.995	7.3	$10.35x10^{-3}+ 1.7902 x10^{-3}$

*SD of calibration over 5days is taken as the day to day (inter day) precision.

Table 4.2.3b Recoveries(%), precision and accuracy of method.

Compounds	Recovery mean n=10 (%)	C.V.	Concentration spiked ngml-1	Mean conc. found	C.V.	Detection limit ngml-1
Proguanil	75.0	7.5	217.6	217.5	3.0	1.0
			108.8	108.3	7.7	
			54.4	50.0	12.0	
Cycloguanil	82.3	6.0	92.8	95.2	3.8	2.0
			46.4	47.1	7.3	
			23.4	20.7	15.7	
4-CPB	87.0	1.5	42.0	41.7	5.6	0.5
			21.0	20.6	10.2	
			10.5	10.0	11.4	
Chlorproguanil	66.4	3.4	824			

4.3.0 Quinine

4.3.1 System Performance

There are no interfering peaks visible from the extract for the blank filter paper on either UV or fluorescence detection. However some minor UV peaks were observed in the extract for blank blood just adjacent to the position of quinine. These peaks are absent when using fluorescence detection (Figure 4.3.3). Quinidine with a retention time of 7.2min was chosen as internal standard with fluorescence and chlorproguanil as internal standard with UV detection. Chlorproguanil is an antimalarial drug not commonly used and has a long retention time of 12.5min in this system. It is well removed from other possible peaks, therefore making it a suitable (UV detection) internal standard. Typical chromatograms of quinine, quinidine, dihydroquine and chlorproguanil in plasma using ultraviolet and fluorescence detection are illustrated in Figure 4.3.3a and 4.3.3b. The fluorescence detection showed higher sensitivity than the ultraviolet detection, however there was no appreciable difference in the quantitation of quinine (when quinine was estimated with reference to internal standard). The fluorescence detection was used for further validation of system. This was to show the likely position of the metabolites and those of quinidine and dihydroquinine on the chromatograms.

Optimal selectivity and sensitivity was obtained with the mobile phase containing 90mM SLS, 6mM TBA in acetonitrile: 20mM phosphate buffer (58:42). The resolution of the quinidine and dihydroquinine with respect to quinine in each case was greater than 1. The

likely metabolites (peaks 1x, 2x, 3x see Figure 4.3.3b) were well removed from quinine, quinidine and dihydroquinine and also well separated from each other.

Chromatograms obtained after extraction of dried samples of blood spiked with 1 μg/ml quinine and the healthy volunteers blood sample after 1h and 24h of oral quinine sulphate 400mg tablet are shown in Figure 4.3.3b and 4.3.3b. Comparison of these chromatograms obtained with the fluorescence detector showed extra peaks with retention times, 3.6, 4.2 and 4.8min at 24h after ingestion of quinine (see Figure 4.3.3b), which were not obvious in the chromatograms of the spiked blood samples or at 1h after oral quinine ingestion. It is therefore possible that these compounds are metabolites of quinine but this could not be confirmed due to unavailability of authentic samples of the 3-hydroxylated or epoxy metabolites.

4.4.3 Recovery, Precision and Accuracy

The efficient solid phase extraction process with the C8 Bond Elut resulted in high recoveries (90-94.8% of quinine from the filter papers. The HPLC method used for the measurement of quinine with its diastereoisomer quinidine (as internal standard) is simple, selective and sensitive.

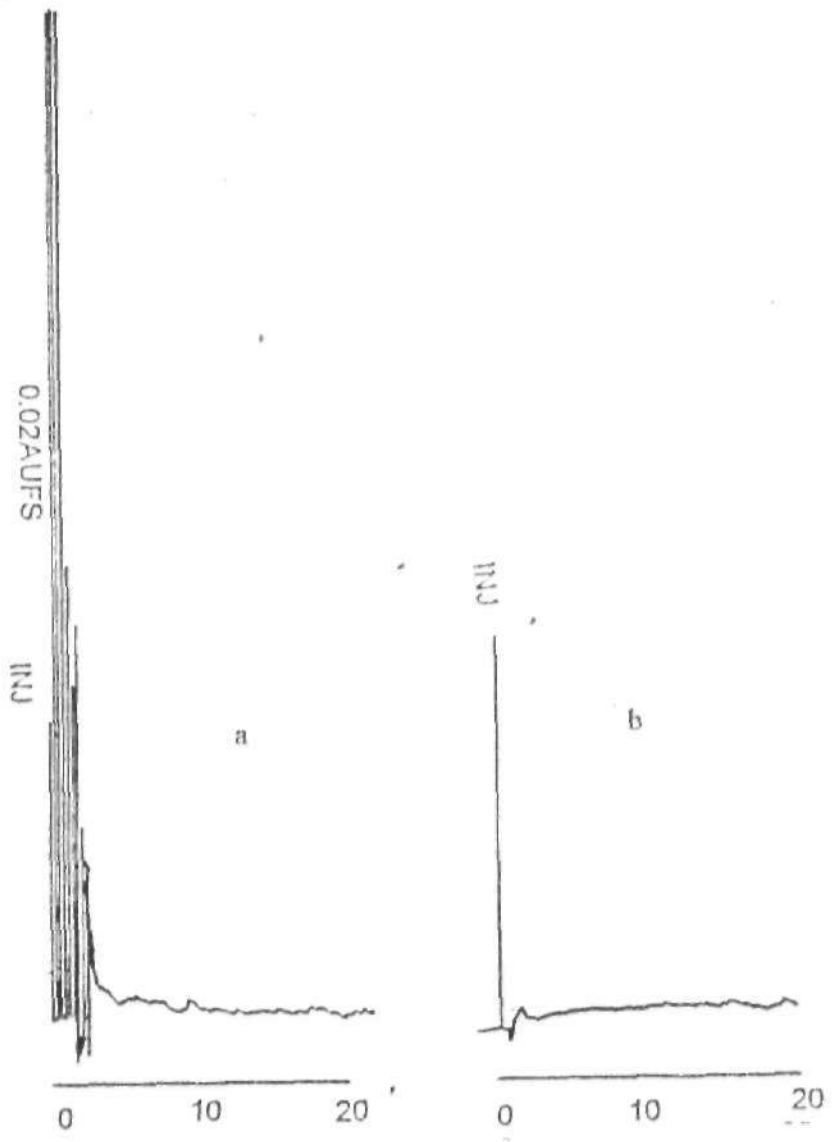


Figure 4.3.3 Chromatogram of extracted filter paper and plasma adsorbed on filter paper.(a) by U.V detection (b) fluorescence detection.

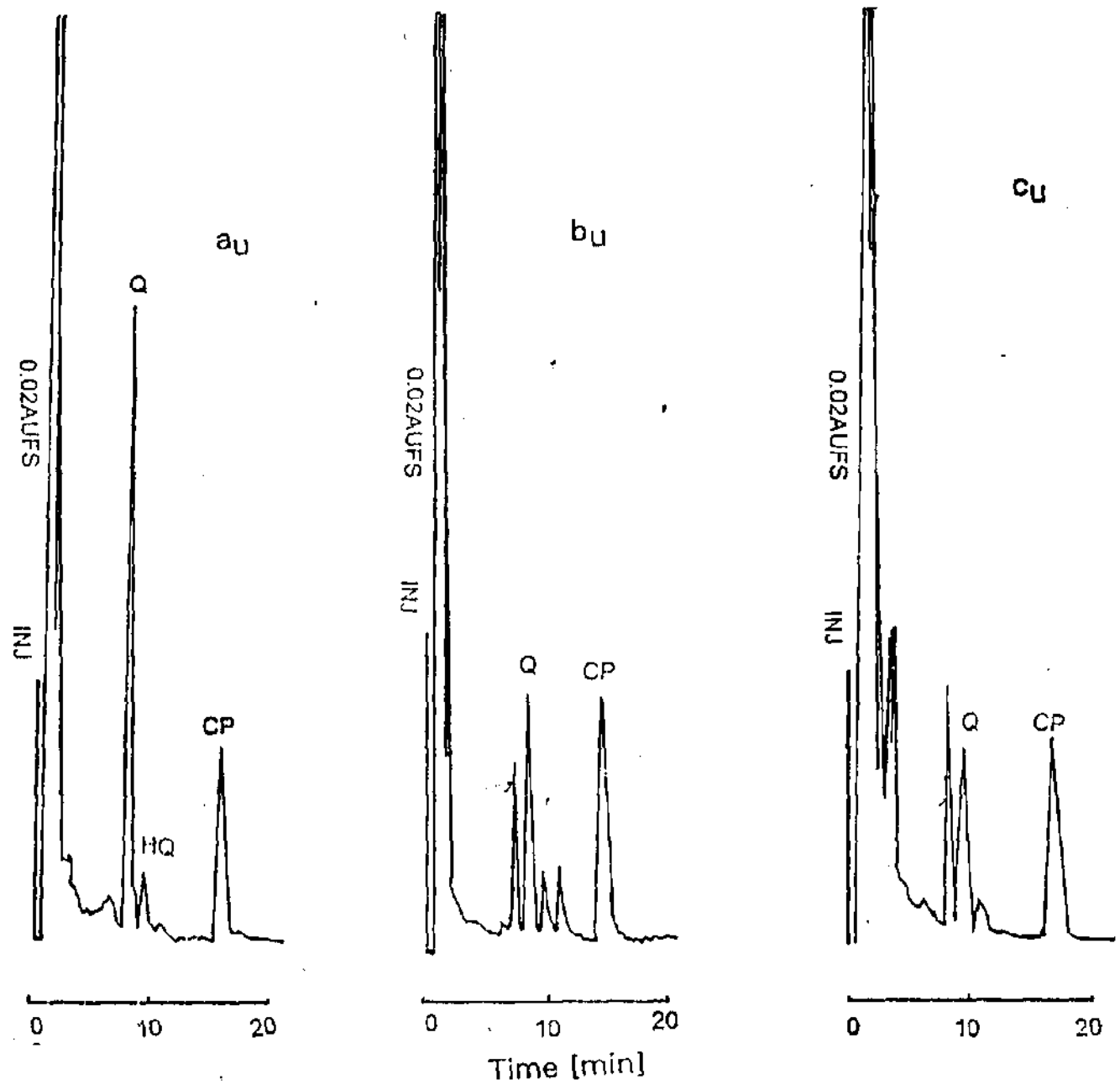


Figure 4.3.3a Chromatograms of extracted plasma samples with U.V detection of quinine.

au.....plasma spiked with 1.0ug/ml quinine [Q] containing internal standard, chlorproguanil [CP] and commercial contaminant hydroquinine [HQ]

bu.....concentration obtained after 1h of oral ingestion of 400mg quinine.

cu.....concentration obtained after 24h of oral ingestion of 400mg quinine

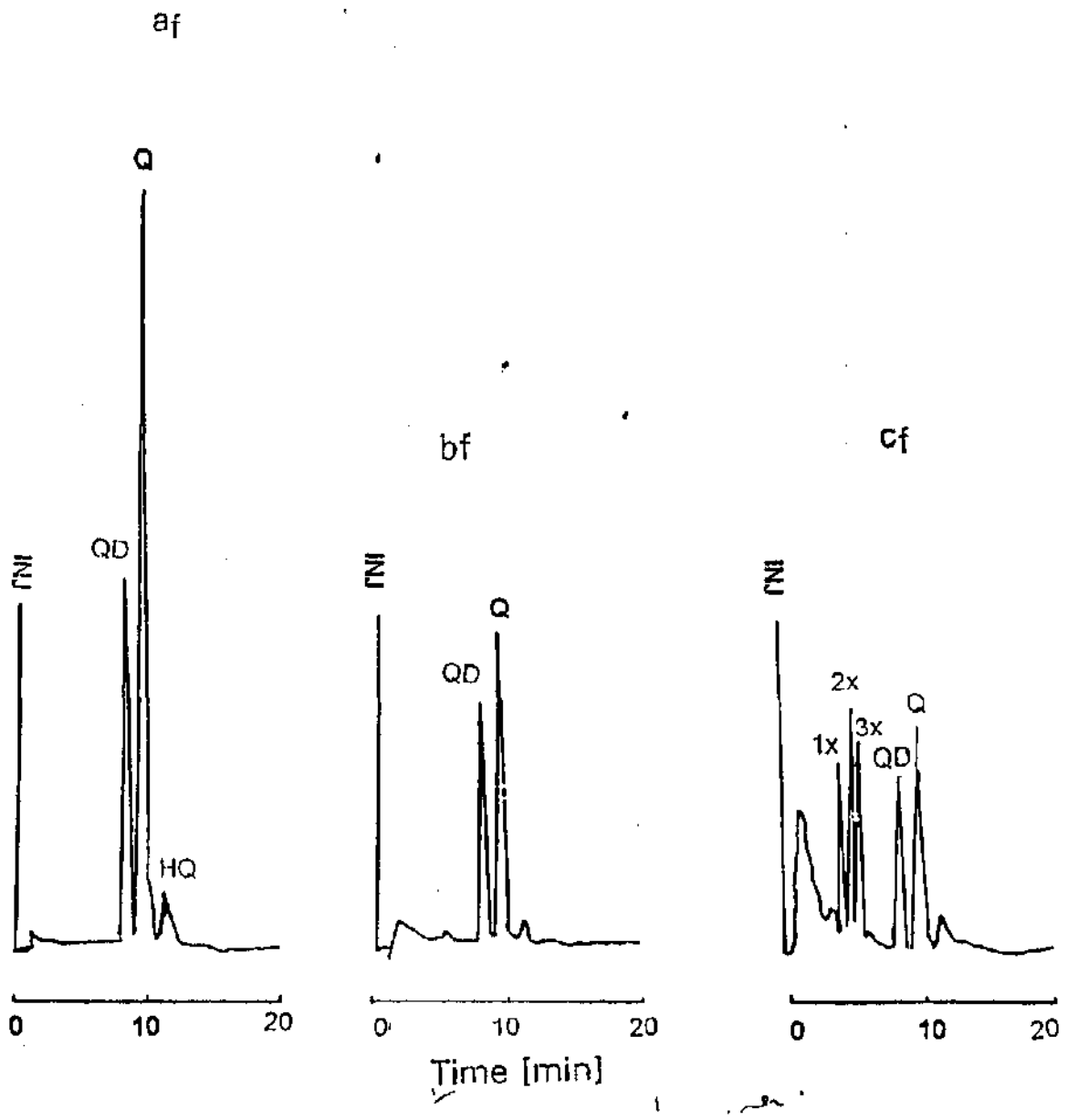


Figure 4.3.3b Chromatograms of extracted plasma samples with fluorescence detection.

af.....plasma spiked with 1.0ug/ml quinine [Q] containing internal standard, quinidine[QD] and commercial contaminant hydroquinine [HQ].

bf.....concentration obtained after 1h of oral ingestion of 400mg quinine.

cf.....concentration obtained after 24h of oral ingestion of 400mg quinine and likely metabolites 1x, 2x and 3x..

Table 4.3.3a & 4.3.3b showed the accuracy, reproducibility and recovery of quinine from spiked plasma (0.1ml) sample. Calibration was linear over the range 0.1 to 5 µg/ml quinine with correlation coefficients of 0.998. The extraction recoveries at 0.5, 1.0 and 2 ug/ml quinine concentration in plasma was 94%, 95% and 95% respectively. This was higher than the reported recoveries of 45 - 83% by Hellgren et al (1990), Mberu et al (1991) or Ericsson et al. (1993). All the authors however used liquid - liquid extraction for the analyte which might be responsible for the low recoveries reported. A determination limit of 0.01 µg/ml was obtained using the ultraviolet and fluorescence detectors respectively. This determination limit combined with the sensitivity and selectivity of the system enabled the determination of quinine in 0.1ml plasma sample even at 36h post oral administration of 400mg quinine sulphate. This is about 6h longer than the 30h sample collection time reported by Hellgren et al (1990). This sensitivity is sufficient for normal monitoring of the drug concentration during antimalarial therapy as therapeutic plasma concentrations are about 5 µg/ml. This technique like that of Mberu et al (1991), Dua et al (1993) and unlike that of Hellgren et al (1990) has the ability to separate quinine from quinidine, thus making it possible to monitor quinine in the presence of quinidine in a patient on both drugs. The method also separates quinine from its commercial contaminant, dihydroquinine and the likely metabolites. These are advantages over the methods of Mberu et al. (1991) and Dua et al. (1993). The sensitivity and selectivity of the method is similar to those of Ericsson et al. (1993).

Measurements of concentrations of quinine in plasma spiked with 5 ug/ml quinine and adsorbed on filter paper for ten (10) months at -20°C (except when in transit to the laboratory of analysis) did not show any changes in concentration. This agrees with earlier reports on

Table 4.3.3a. Analytical characteristics of method (day-to-day precision of method for quantification of quinine) using ultraviolet detection(UV) and fluorescence detection (F).

Biological fluid Plasma	Mean calibration slope	C.V.	Mean correlation coefficient	Determination limit ug/ml
V.U detection	2.470	0.066	0.998	0.01
Fluorescence detection	2.560	0.027	0.998	0.01

stability of quinine in biological matrices, by Hellgren et al. (1990) who stored their filter paper samples at 30°C for 3 months and Edstein at (1983) who stored their samples at -20°C for 4 months.

Table 4.3.3b Recoveries, within-day precision and accuracy of assay method.

Biological fluid	Conc. spiked (ug/mL)	n	Recovery %	C.V	Relative conc. found	C.V
plasma	0.5	10	94	8.4	0.45	8.6
"	1.0	10	95	5.6	1.05	10.4
"	2.0	10	95	6.2	2.15	9.6

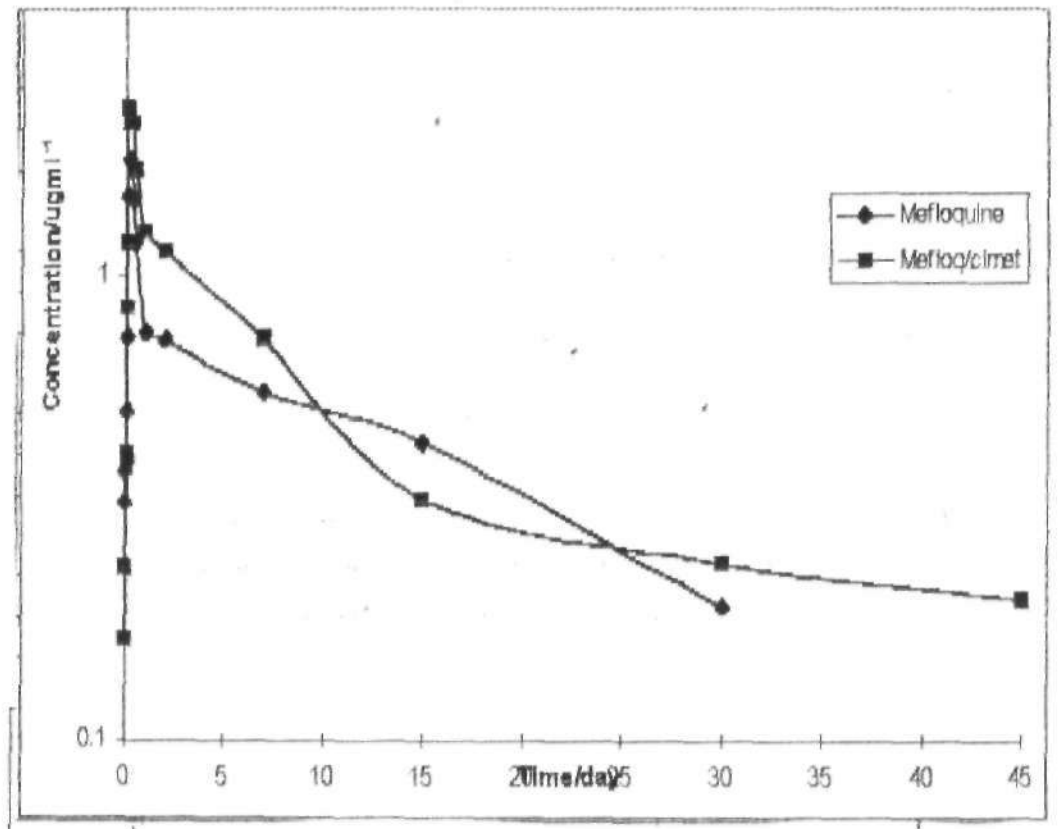
4.4.1 Single Dose Pharmacokinetics of Mefloquine in Healthy Male Subjects

Figures 4.4.2a show the mean (n_{-6}) concentration-time profile of mefloquine in plasma following 500mg mefloquine tablet. The individual and mean concentration-time data for the six healthy subjects are presented in Appendix VA. The mean pharmacokinetic parameters of mefloquine in plasma of healthy subjects were presented in Table 4.4.2a. The rate of absorption k_a was found to be $5.27h^{-1}$ in plasma with the corresponding $t_{½a}$ being 4.2 ± 3.2h. The $t_{½a}$ of 4.2h in plasma is in close agreement with the reported $t_{½a}$, 3.8h of Desjardins et al (1979) but longer than the 2h reported by Schwartz et al (1982) . This absorption phase is fairly rapid. The time to peak concentration Tmax was 10 ± 7h in plasma. This agree with the reported Tmax 10.5h of Desjardins et al (1979) and shorter than the reported cases of about 20h (Karbwang et al 1988) and 14h reported by Schwartz et al (1987). The Cmax was 1.77 ± 0.2µg/ml in plasma. This is higher than the Cmax reported by Mansor et al (1989) of 1.10ug/ml, Karbwang et al (1988) and Desjardins et al (1987) but lower than that of Franssen et al (1989). The AUCo-∞ was found to be 19.05 ± 7.01mg/L.day in plasma. The volume of distribution Vd/f was 9.43 ± 3.77L/kg in plasma, with the corresponding total clearance of $Cl_{T/f}$0.453 ± 0.15L/day/kg in plasma. The terminal

elimination half-life t_{β} of mefloquine in this study was found to be 18.60 ± 9.8days in plasma. The pharmacokinetic properties of mefloquine after a single oral dose of 500mg mefloquine hydrochloride tablet were adequately described by the triexponential function. In this study, the plasma concentration in healthy young Nigerian adults after 500mg single dose oral mefloquine are generally similar to earlier reports of Desjardins et al (1979), Karbwang et al (1988), Riviere et al (1985), Mansor et al (1989) and Franssen et al (1989). The peak plasma concentration (1.77ug/mL) is relatively high when compared with some early reports (1.1ug/mL) using similar dose treatment. The sample collection and treatment in plastic containers, among other things such as sensitivity of assay method might explain the differences in the values. In this study application of aquasil to glass wares did not significantly prevent substantial adsorption of mefloquine to glass. Also intersubject and ethnic variability which has been reported by most of the authors cited above contributed to the differences in the concentration. However these values are still within the general range of Cmax in the literatures. Franssen et al (1989), reported peak plasma concentration of 1.872µg/mL after 500mg dose followed by a further 500mg 8h later.

The volume of distribution Vd/f of 9.43 L/kg in plasma is smaller than the Vd/f of 13.3L/kg reported by Schwartz et al (1980) after 1000mg mefloquine dose. The volume of distribution is generally low as compared to earlier reports, which probably explain the low clearance obtained in this report. The terminal elimination half-life t_{β} in this report was found to be

18.6h in plasma. This agreed with the reported half-life elimination of 17.8days reported by Karbwang et al (1988b) and 16.1days by Mansor et al (1989). These value fall within the range (13.8 to 27.5days) of most of the earlier reports.



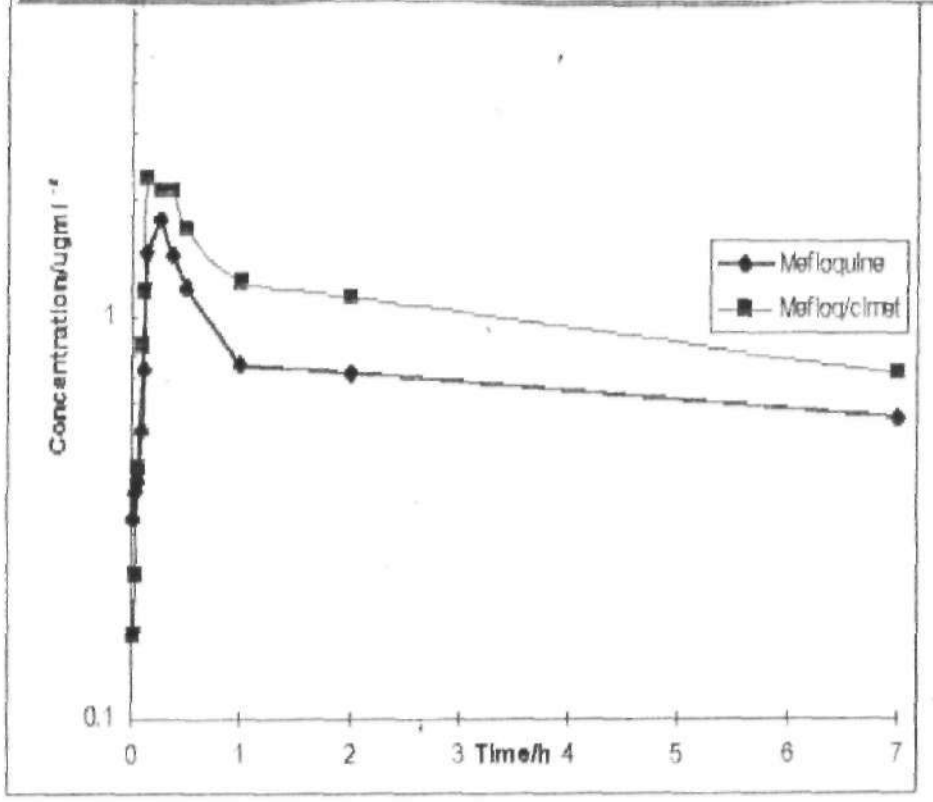


Figure 4.4.2a. (Time scale; Upper 50days, lower scale, first 7days)
The mean concentration-time profile of mefloquine in plasma of six healthy subjects following oral 500mg mefloquine hydrochloride before and after 400mg cimetidine

Table 4.4.2a Pharmacokinetic parameters of mefloquine in six healthy subjects following oral 500mg mefloquine before and after oral cimetidine (plasma data).

Parameter	Mefloquine alone	±SD	Mefloquine Cimetidine	±SD
Ka (h^{-1})	5.27	2.38	7.51	3.18
β (day^{-1})	0.0445	0.018	0.0366	0.011
$t_{½a}$(h)	4.20	3.15	2.70	1.59
$t_{½\beta}$(day)	18.56	9.79	20.38	6.34
Tmax (h)	8.00	3.10	6.50	4.00h
Cmax (ug/mL)	1.77	0.23	2.52	0.27*
AUCo-∞ (mg/L . day)	19.05	7.01	26.20	18.90*
V_D (Lkg^{-1})	9.43	3.77	11.60	6.66
$CL_T/_f$(L/day/kg)	0.453	0.151	0.391	0.18

*P < 0.05.....significant.

4.4.2 Effect of Cimetidine on the Pharmacokinetics of Mefloquine in Healthy Subjects

The plasma concentration-time profile of mefloquine before and after cimetidine for six healthy male subjects are shown in Figure 4.4.2a. Appendix VB (I & II) show the pharmacokinetic data of mefloquine in individual healthy subjects before and after administration of cimetidine. The individual and mean plasma concentration-time data for the four ulcer patients are presented in Appendix VA and VB. The mean mefloquine absorption and elimination kinetics before and after cimetidine treatment are shown in Table4.4.2a... There was an increase in the of absorption of mefloquine resulting in reduced $t_{½a}$ from 4.2h to 2.7h in plasma. The Tmax was reduced by cimetidine from 10 ± 7.3h to 6.5 ± 4.0h in plasma. These changes were not significant (P>0.05). These changes were however accompanied by a significant increase (P<0.05) in Cmax in plasma with a corresponding significant increase (P<0.05) in AUCo-∝ after cimetidine treatment. There was a marginal 21% increase in the Vd/f which is not statistically significant (P>0.05) in plasma. There was no significant change (P>0.05) in plasma clearance after cimetidine administration. Elimination kinetics and half-life were not significantly altered (P>0.05) by cimetidine. Pharmacokinetic drug interactions with cimetidine has been known to occur at the site of

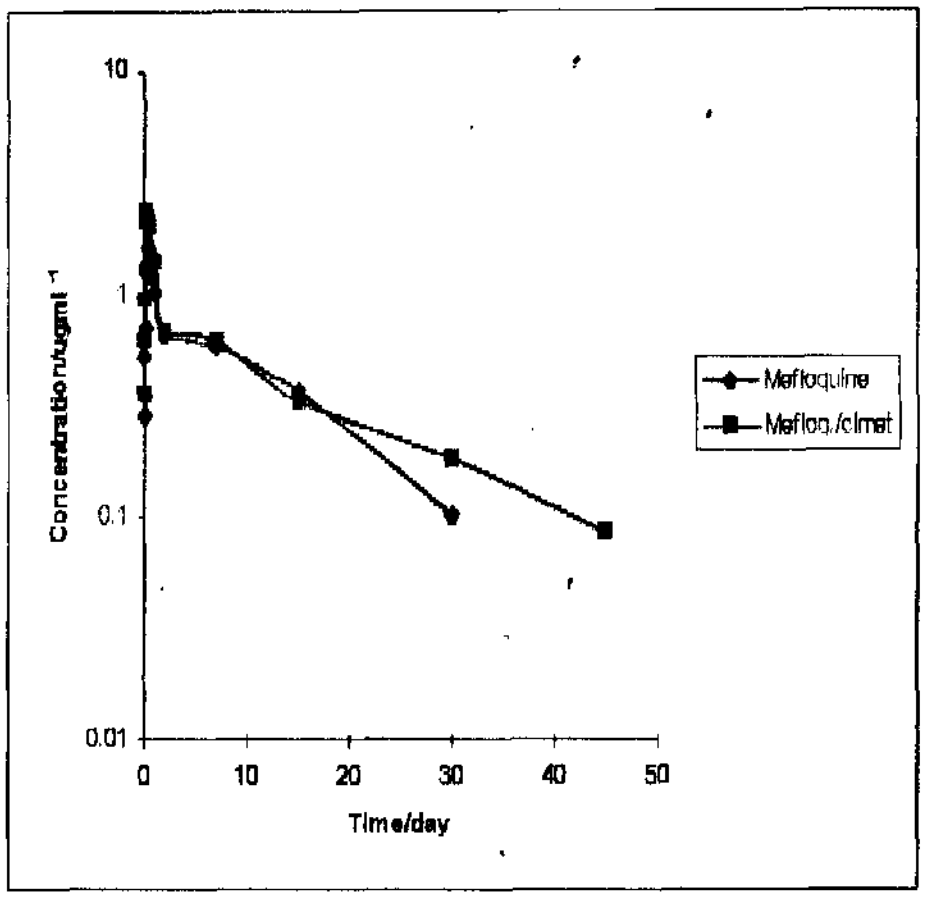
gastrointestinal absorption and elimination (metabolism and excretion). Cimetidine was found in this study to increase the rate of absorption of mefloquine. These effects might probably be due to enhanced dissolution of mefloquine because of the increase in pH caused by cimetidine, rather than its effect on gastric motility. Cimetidine is known to alter the absorption kinetics of some drugs, penicillin, indomethacin and ketoconazole, by increasing the gastric pH (Somogyi and Muirhead 1987). The effect of cimetidine on gastric motility has been reported as not significant (Heading et al.1977). The plasma clearance and elimination half-life were not significantly increased ($P>0.05$) by cimetidine. Cimetidine is a potent inhibitor of cytochrome P450 systems in vivo. It is also known to affect drugs with high first pass effect and low hepatic extraction ratios like quinine and quinidine. Mefloquine has low intrinsic clearance with a very small first pass effect . Therefore the little change in elimination half-life and in clearance show that cimetidine has no effect on the biotransformation of mefloquine.

4.4.3 Effect of Cimetidine on Mefloquine Pharmacokinetics in Peptic Ulcer Patients

Table 4.4.3a - 4.4.3b show the mean pharmacokietic data of mefloquine in ulcer patients following 500mg dose mefloquine before and after oral cimetidine. Appendix VB (III & IV) show the pharmacokinetic data of mefloquine in individual ulcer patient before and after administration of cimetidine. The individual and mean plasma concentration-time data for the four ulcer patients are presented in Appendix VC and VD. The absorption, distribution

and elimination phases of the concentration-time profile in plasma were found to be similar to those of the healthy subjects (Figure 4.4.3a & 4.4.3b). The absorption half-life $t_{½a}$ was 1.3h in plasma. The time to peak concentration Tmax was 7.5 ± 3.0h in plasma while peak concentration was 2.00 ± 0.30μgmL^{-1} in plasma before administration of oral cimetidine. The AUCo-∞ was found to be 19.85mg/L.day in plasma. Elimination half-life was found to be 18.70days in plasma with total body clearance, Cl_T/f of 0.4502L/day/kg before oral administration of cimetidine.

Absorption half-life and Tmax were not significantly altered (P>0.05) after cimetidine treatment. The increase in plasma Cmax and AUCo-∞ after cimetidine treatment were statistically significant (P<0.05). Elimination half-life ($t_{½β}$) was not significantly altered (P>0.05) after cimetidine treatment. Total body clearance was not significantly reduced (P>0.05) and V_d/f was not significantly increased (P<0.05) after administration of cimetidine. Cimetidine is widely used in peptic ulcer patients and in epigastric pains. Although cimetidine did not significantly affect the absorption and elimination kinetics of mefloquine in this study, there were marginal differences in the absorption of mefloquine leading to significant increase in Cmax (P<0.05) and AUCo-∞ (P<0.05). The elimination half-life and total clearance were found to be similar to those in the healthy subjects.



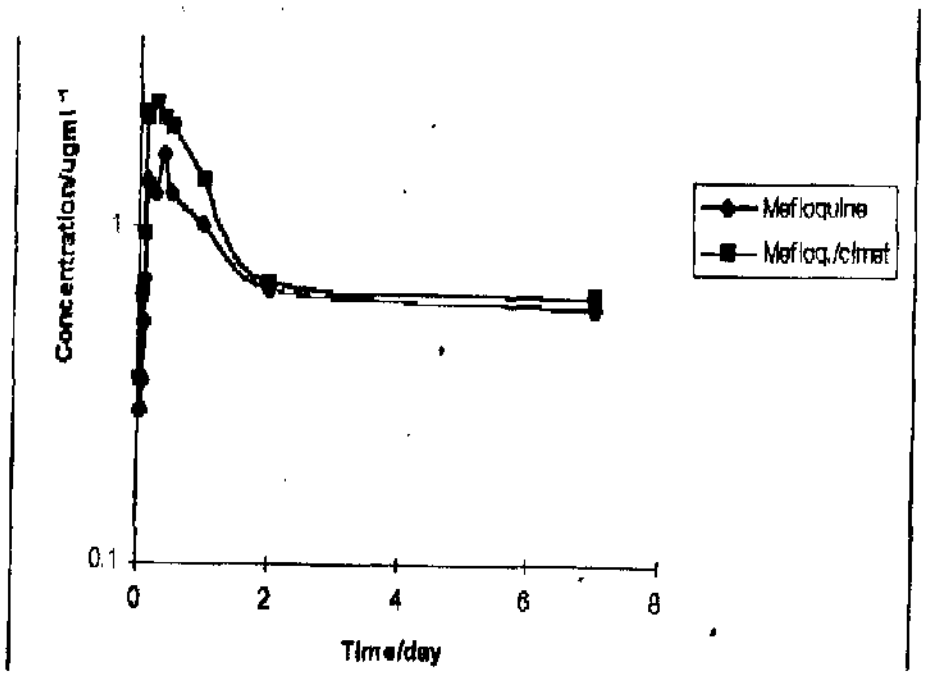


Figure4.4.3a. (Time scale: Upper fig. 50days , lower fig. first 7 days)

The mean concentration-time profile of mefloquine in plasma of peptic ulcer patients following oral 500mg mefloquine hydrochloride before and after cimetidine

Table 4.4.3b Pharmacokinetic parameters of mefloquine in ulcer patients following oral 500mg mefloquine before and after oral cimetidine (Plasma data).

Parameter	Mefloquine alone (n=4)	±SD	Mefloquine /cimetidine	±SD (n=3)
K_a (h^{-1})	11.09	5.82	10.15	2.40
β(day^{-1})	0.0417	0.02	0.0365	0.01
$t_{½}a$ (h)	1.9	1.0	1.7	0.3
$t_{½\,\beta}$ (day)	18.70	7.12	19.40	3.30
Tmax(h)	7.5	3.0	7.0	1.7
Cmax(ug/mL)	2.00	0.30	2.41	0.10
AUCo-∞ (mg/L.day)	19.85	9.48	26.24	9.81
V_d/f (L/day/kg)	11.12	4.04	8.50	2.30
CL_T/f(L/day/kg)	0.4542	0.19	0.315	0.10

*P<0.05, (Significant)



4.4.4 Single Dose Pharmacokinetics of Proguanil and Metabolites in Healthy Nigerian Males.

Table 4.4.4a show the mean pharmacokinetic parameters of proguanil in plasma for the subjects before and after cimetidine. Mean blood concentration - time curves for proguanil and the two metabolites, cycloguanil and 4-CPB are shown in Figure 4.4.4a. Proguanil profile in plasma before and after administration of cimetidine is shown in Figure 4.4.4b.

Proguanil was found to be rapidly absorbed with a mean ± SD absorption half-life of 1.00 ± 0.35)h derived from the plasma drug concentration profile. The time to peak concentration Tmax was 4.18 ± 1.05h at Cmax of 208.3 ± 30.3ng/mL. This agreed with the reports of Edstein et al (1988), Taylor et al (1987) and Wattanagoon et al (1990). The AUCo-∞ was found to be 4670ng/mL.h in plasma. This agreed with the reports of Taylor et al (1987) and Wattanagoon et al (1990). The apparent volume of distribution was estimated to be 14.00 ± 5.04L/kg in plasma with a total clearance $Cl_{T/f}$ estimated to be 10.51 ± 2.17mL/min/kg. The $Cl_{T/f}$ in this study is smaller than the reported 14mL/min/kg reported by Wangboonskul et al 1993 in a study carried out on male Thai subjects. The terminal elimination half-life $t_{½\beta}$ for proguanil was estimated to be 15.27 ± 3.73h in plasma. This compared well with the reported $t_{½\beta}$ of Edstein et al 1988 and Wangboonskul et al 1993.

Tables 4.4.4c - 4.4.4d show the mean pharmacokinetic parameters for cycloguanil and 4-chlorophenylbiguanide. The time to peak concentrations were 5 ± 1.7h and 7.8 ± 1.64h with

the corresponding peak concentrations of 43.67 ± 19ng/mL and 11.25 ± 4.44ng/mL respectively for cycloguanil and 4-CPB in plasma . The corresponding estimations in the whole blood were, 6h and 50.5 ± 14ng/mL for cycloguanil and 7 ± 1.55h and 14.0 ± 1.54ng/mL for 4-CPB. The plasma concentration profile of the metabolites cycloguanil and chlorophenylbiguanide appeared to parallel those of the parent drug, but with a phase shift to the left of approximately 2 hours for cycloguanil and 2.5 hours for 4-CPB.

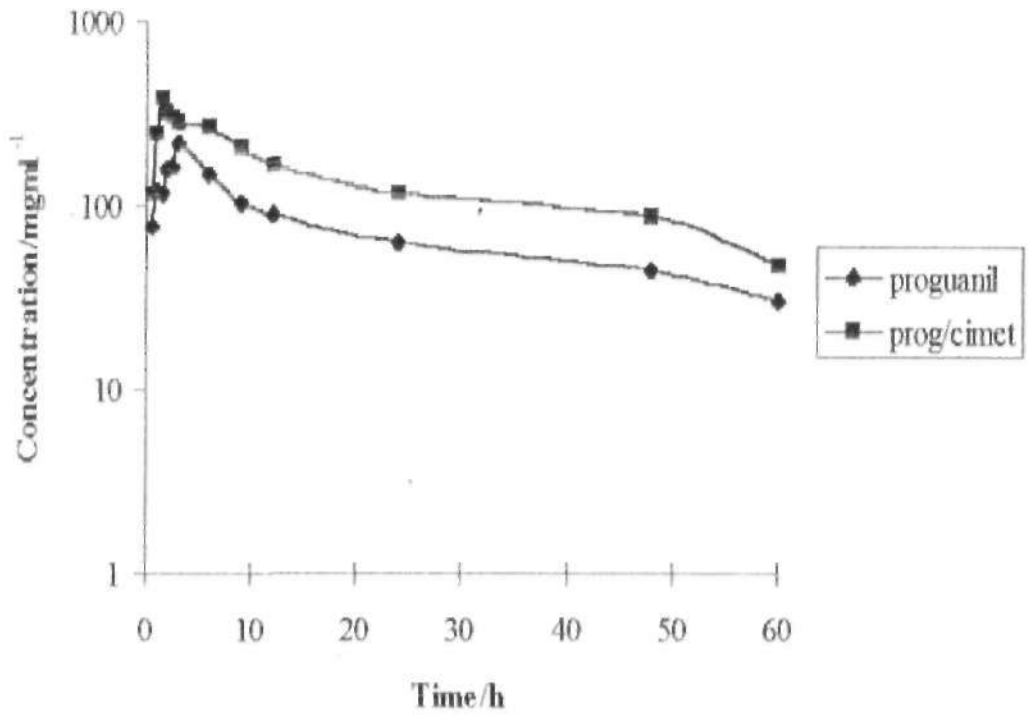


Figure 4.4.4

Mean plasma concentratration-time profile of proguanil in six healthy subjects before and after cimetidine.

Table 4.4.4a Pharmacokinetic parameters of proguanil in six healthy subjects following oral 200mg proguanil before and after oral cimetidine (plasma data).

Parameter	Proguanil alone		Proguanil and Cimetidine	
	mean	± SD	mean	± SD
Ka (h^{-1})	0.793	0.373	1.101	0.813
$t_{½ab}$ (h)	1.00	0.35	0.86	0.42
Tmax (h)	3.3	1.4	3.0	1.6
Cmax($ngml^{-1}$)	08.3	30.3	393.4	104*
AUCo-∞(ng/mL.h)	4670	1049	8991	2101*
V_D (L/kg)	14.00	5.04	10.74	3.37
$\beta(h^{-1})$	0.0473	0.0107	0.0314	0.0058*
$t_{½\beta}$ (h)	15.27	3.73	22.55	4.19*
$Cl_T/_f$(mL/min/kg)	10.51	2.17	5.47	1.14

*P<0.05..... Significant

Table 4.4.4c Pharmacokinetic profile of cycloguanil following 200mg oral proguanil before and after Cimetidine in Healthy subjects.

	Plasma	
	Before	After
Tmax(h)	5.0	8.0
Cmax(ng/mL)	43.7	17.8*
AUCo-∝ (ng/mL.h)	399.7	250.0*

*P<0.05, ...Significant.

Table 4.4.4d

III. Pharmacokinetic profile of 4-chlorophenyibiguanide(4-CPB) in six healthy subjects following 200mg oral proguanil before and after cimetidine.

	Plasma	
	Before	After
Tmax(h)	7.8	9
Cmax(ng/mL)	11.25	5.73*
AUC o-∝ (ng/mL.h)	145.2	114.2

*P<0.05 Significant

4.4.4a Effect of Cimetidine on the Pharmacokinetics of Proguanil in Healthy Subjects

The plasma concentration-time profiles for the six healthy subjects are shown in Figure 4.4.4. The mean proguanil absorption and elimination kinetics before and after cimetidine treatment are listed in Table 4.4.5a. Proguanil absorption was significantly enhanced ($P<0.05$) by the shortening of absorption half-life $t_{½a}$, and a significant increase ($P<0.05$) in Cmax at a shorter Tmax in plasma. There was approximately 92.5% significant increase ($P<0.05$) in plasma AUCo-∞ while V_d/f was marginally reduced and total body clearance $Cl_{T/f}$ was significantly reduced ($P<0.005$). Elimination half-life was significantly increased ($P<0.05$).

Cimetidine was found to affect the absorption of proguanil by probably hastening dissolution and increasing the gastric pH thereby making it favourable for rapid absorption. The $t_{½a}$ and Tmax were significantly ($P<0.05$) reduced with a corresponding increase in Cmax. Cimetidine was found to affect the absorption of other drugs such as ketoconazole, penicillin, indomethacin and aspirin by increasing the pH of the stomach and the proximal small bowel (Somogyi and Muirhead 1987). The effect of cimetidine on gastric motility might have enhanced the observed rapid absorption and the availability, however Heading et al.(1977) showed no significant effect of cimetidine on gastric motility. The rate and extent of absorption of proguanil as measured by $t_{½a}$, Tmax, Cmax and AUCo-∞ were all affected by cimetidine. The significant increase in plasma Cmax and AUCo-∞ and the right shift of $t_{½a}$

and Tmax might be justified by the effect of cimetidine on the gastrointestinal pH rather than its effect on gastrointestinal tract motility. Also since proguanil is a drug with a high first pass metabolism the effect of cimetidine on portal blood flow might have contributed to the increased bioavailability of proguanil after the cimetidine treatment. The total clearance and elimination half life were significantly ($P<0.05$) increased. Proguanil undergoes high first pass mechanism and it is metabolised to cycloguanil through CP450 enzyme systems. It is therefore possible for cimetidine to influence the clearance and elimination (including metabolism) of proguanil. This agreed with the reports on cimetidine interaction through its effect on hepatic blood flow and CP450 inhibition associated with drugs having high first pass metabolism (Reiman et al 1981) and also drugs with low hepatic extraction (Hetzel et al 1981 and Hardy et al 1984). The increase in $t_{1/2}$ elimination of proguanil and the increase in AUCo-∞ suggest that cimetidine inhibits the biotransformation process thereby keeping proguanil concentration high in the blood for a longer period of time. This effect might be clinically significant since the active species is cycloguanil. The plasma concentration of cycloguanil was significantly reduced ($P<0.05$) after the cimetidine treatment. There was also significant decrease in AUCo-∞ ($P<0.05$) of cycloguanil after the cimetidine in plasma (Table 4.4.4c).The changes reflected in significant reduction in the inactive metabolite 4-CPB in plasma (Table 4.4.4d).

4.4.5 Effect of Cimetidine on Proguanil in Peptic Ulcer Subjects

Table 4.4.5a show the pharmacokinetic parameters of proguanil in the ulcer patients before and after administration of cimetidine. Table 4.4.5c and 4.4.5d show the mean pharmacokinetics of cycloguanil and 4-CPB in plasma following 200mg proguanil, before and after oral cimetidine. The concentration-time profiles for four ulcer patients is shown in Figures 4.4.5 Proguanil absorption, distribution and elimination in the ulcer patients are not identical with those of the healthy subjects.

The plasma absorption half-life $t_{½a}$ was 1.53 ± 0.5h and time to peak concentration was 4.5 ± 1.7h at peak concentration 347.1 ± 54.0ng/mL.. Cmax was significantly increased from 347.1ng/mL to 491.45ng/mL after cimetidine treatment. Absorption half-life and Tmax were not significantly altered by cimetidine administration. AUCo-∞ was 8261 ± 1198ng/mL.h in plasma before cimetidine treatment and 12155 ± 2127ng/mL.h after cimetidine administration. Cimetidine significantly ($P<0.05$) increase the AUCo-∞ in plasma.

Cimetidine prolonged the elimination half-life $t_{½\beta}$ of proguanil in the ulcer patient subjects from 14.2 ± 2.7h to 23.1 ± 8.2h in plasma. This change is statistically significant ($P<0.05$). Plasma clearance was significantly reduced ($P<0.05$) after the cimetidine administration. There was no significant change in the volume of distribution.

Cimetidine is widely prescribed for peptic ulcer patients or in epigastric pains. Most ulcer patients have been exposed to some treatment including the use of cimetidine before they are diagnosed with the aid of endoscopy or barium meal X-ray. Cimetidine did not affect proguanil absorption in these patients. The pathologic conditions of this group of subjects might have modulated the effect of cimetidine. The prolongation of the elimination half-life of proguanil might be due to the effect of cimetidine on the metabolic pathways. There was significant decrease ($P<0.05$) in cycloguanil Cmax in plasma and significant decrease in

AUCo-∞ after cimetidine treatment. There was a corresponding decrease in plasma concentration 4-CPB after the cimetidine.

A comparison of the normal pharmacokinetic profiles of the subjects and the ulcer patients before the cimetidine administration shows that the values of Cmax and AUCo-∞ of proguanil were larger in the ulcer patients while the elimination half-life was smaller than those of the normal subjects. These differences might be due to previous exposure of patients to a CP450 inhibitor or any of the H_2-receptor antagonists. The effect of gastric pH might also be a factor since the patients are likely to have more acid in the gut than the normal subjects.

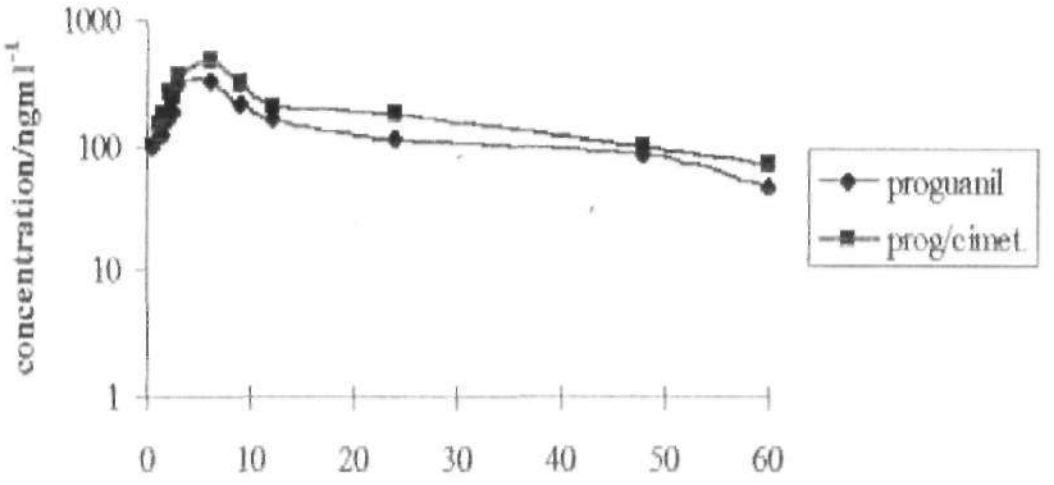


Figure 4.4.5
Mean plasma proguanil concentration-time profile after 200mg oral proguanil in four ulcer patient subjects before and after 300mg cimetidine.

Table 4.4.5a Pharmacokinetic parameters of proguanil in four ulcer patients following oral 200mg proguanil before and after oral cimetidine (Plasma data).

Parameter	Proguanil Mean ± SD		Proguanil / Cimetidine Mean ± SD	
Ka (h^{-1})	0.495	0.184	0.504	0.175
$t_{½}a$ (h)	1.535	0.45	1.46	0.45
Tmax (h)	4.5	1.7	5.3	1.5
Cmax (ng/mL)	347.1	54.0	481.45	69.80
AUC o-∞(ng/mL.h)	8261	1198	12155	2127*
V_D(L/kg)	7.30	1.09	7.94	2.22
$_{\beta}$ (h^{-1})	0.0502	0.0101	0.0344	0.0167
$t_{½\beta}$ (h)	14.22	2.75	23.06	8.17
$Cl_T/_f$(mL/min /kg)	6.00	0.74	4.11	0.68

*P<0.05 Significant.

Table 4.4.5c

Pharmacokinetic profile of cycloguanil in four ulcer patients following 200mg proguanil before and after cimetidine

	Plasma	
	Before	After
T_{max} (h)	6.0	8.0
C_{max}(ng/mL)	38.8	26.1*
AUCo-∝ (ng/mL.h)	495	462.0

P(<0.05) Significant.

Table 4.4.5d

Pharmacokinetic profile of 4-Chlorophenylbiguanide(4CPB) in four ulcer patients following 200mg oral proguanil before and after 400mg cimetidine

	Plasma	
	Before	After
T_{max} (h)	7.5	6.0
C_{max}(ng/mL)	9.8	5.6
AUCo-∝ (ng/mL.h	160.0	125

**(P<0.05) .....significant

4.6.1 Single Dose Pharmacokinetics of Quinine in Healthy Subjects.

Figure 4.6.1 shows the concentration-time profile of quinine in plasma of healthy subjects. The individual and mean concentration-time data of quinine in plasma for the subjects are shown in Appendix VII A&B. Table 4.6.2a shows the mean (± SD) pharmacokinetic data in plasma of healthy subjects following 400mg quinine before and after oral cimetidine.

The plasma concentration-time profile shows a rapid absorption of quinine $t_{½a}$ 0.97 ± 0.4h in plasma with a Cmax of 2.66 ± 0.7mg/mL and Tmax of 2.8 ± 0.7h. The AUCo-∝ was 33.7 ± 10.5mg/L.h The rapid absorption phase was followed by a biexponential decline in concentration. The values obtained for Tmax, Cmax and AUCo-∝ are similar to those of Garnham et al (1976). The Tmax 2.8h is longer than the 1.5h reported by Wanwimolruk et al (1986) but shorter than that reported by Hall et al (1973) in plasma. The apparent volume of distribution was 2.2L/kg in plasma. This is in agreement with the report of Wanwinmolruk et al (1986). The total body clearance, Cl_T/f was 0.21 L/h/kg in plasma, this value is smaller than what Hardy et al (1983) and Kolb et al (1984) reported for its diastereoisomer quinidine but similar to that reported by White et al (1983) for quinine. The elimination half-life of quinine was found to be 8.1h in plasma. This elimination half-life is in agreement with those reported by Trenholme et al (1976) and Wanwinmolruk et al (1986).

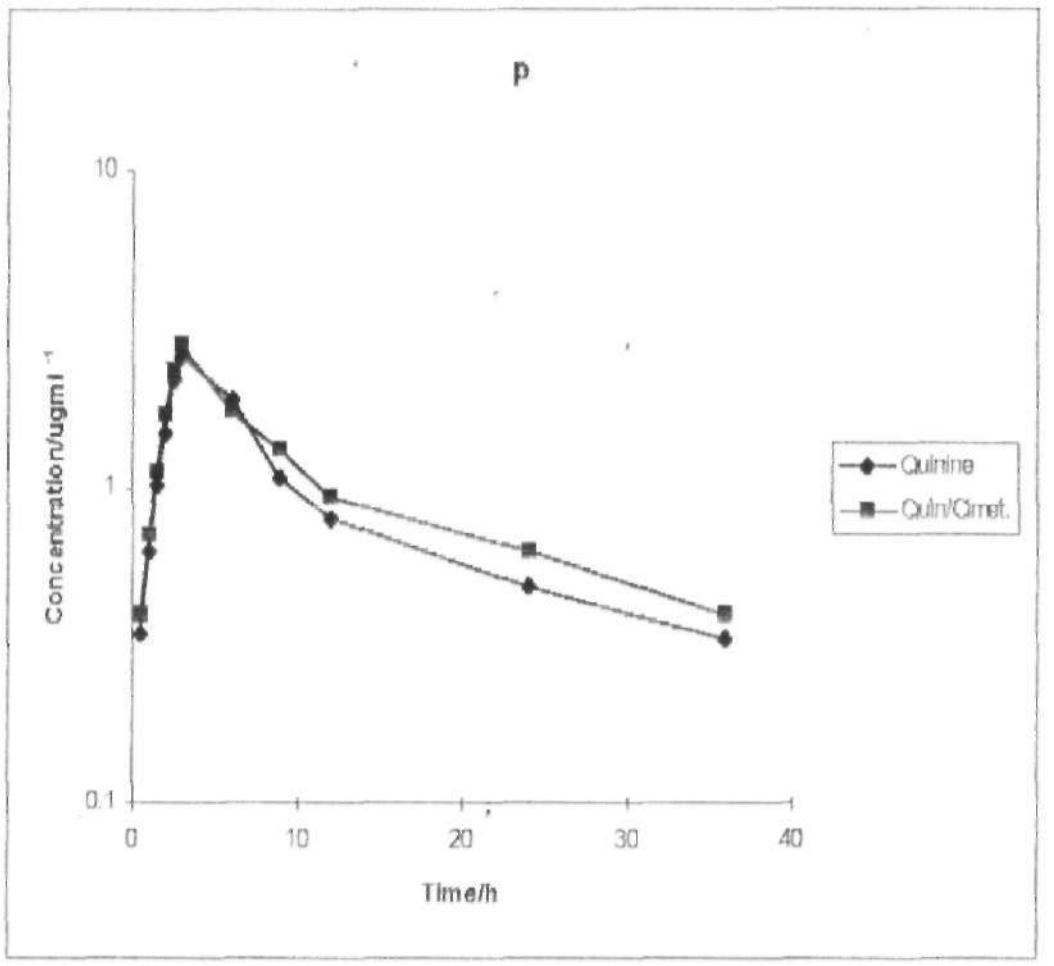


Figure 4.6.1

Mean concentration-time profile of quinine in plasma of six healthy subjects following oral 400mg quinine sulphate before and after cimetidine.

Table 4.6.2a Pharmacokinetic parameters of quinine in six healthy subjects following oral 400mg quinine before and after oral cimetidine (Plasma data ...Mean ± SD).

Parameter	Quinine alone	± SD	Quinine/Cimet.	±SD
Ka (h^{-1})	0.803	0.27	0.855	0.47
β (h^{-1})	0.097	0.043	0.060	0.011
t½a (h)	0.97	0.40	0.95	0.33
t½β (h)	8.10	2.83	11.97*	2.25
Tmax (h)	2.80	0.73	2.83	0.26
Cmax (ug/mL)	2.66	0.70	2.83	0.60
AUCo-∞(mg/L.h)	33.70	10.50	40.50*	13.7
V_D/f (L/kg)	2.19	0.45	3.01	1.61
Cl_T/f(L/h/kg)	0.210	0.11	0.166*	0.06

*P<0.05 Significant

4.6.2 Effect of Cimetidine on Quinine Pharmacokinetics in Healthy Subjects

Figure 4.6.1 shows the mean concentration-time profile for quinine in plasma. And Table.4.6.2a show the mean (± SD) pharmacokinetic data in plasma.

The result shows a rapid absorption of quinine in healthy subjects before and after oral cimetidine. The administration of cimetidine did not significantly alter ($P>0.05$) the absorption half-life or the absorption kinetics. The marginal increase in Tmax from 2.80h to 2.83h and Cmax from 2.66mg/mL to 2.8mg/mL after cimetidine treatment were not statistically significant. This result disagree with the report of Hardy et al (1983) who reported a higher Cmax of quinidine (the diastereoisomer of quinine) after cimetidine administration but agreed with the report of Kolb et al (1984) who found no change in Cmax and Tmax of quinidine. Similarly Wanwimolruk et al (1986) reported no change in Cmax and Tmax of quinine in their study. Cimetidine was found to significantly increase ($P<0.05$) AUCo-∞ in plasma from 33.70mg/L.h to 40.50mg/L.h, this supports the finding of Wanwimolruk et al (1986) who reported an increase in AUCo-∞ of quinine after cimetidine treatment. This might be due to the reduced total body clearance of quinine and prolonged elimination half-life $t_{1/2b}$. However, Mackichan et al (1987) reported decreased bioavailability of quinidine after cimetidine treatment, this was attributed to the effect of cimetidine on the extent of absorption rather than metabolism of quinidine.

The effect of cimetidine on the elimination (Including metabolism) of quinine is in agreement with the report of Wanwimolruk (1986) for quinine and also similar to that reported for quinidine by Faringer et al. (1984), Hardy et al. (1983) and Kolb et al. (1984). Quinine is considered a low clearance drug and low clearance drugs are relatively not sensitive to changes in hepatic blood flow. Therefore the consideration of enzyme activity on quinine metabolism rather than hepatic blood flow might explain the changes observed in the clearance Cl_T/f, $AUC_{0-\infty}$ and elimination half-life in this study. Rowland et al (1973), Wilkinson and Shand (1975) showed that the activity of drug metabolising enzymes is considered to be one of the major determinants for the elimination of low clearance drugs such as quinine. Therefore the significant increase ($P<0.05$) in elimination half-life $t_{1/\beta}$ and the significantly reduced ($P<0.05$) total clearance of quinine after the cimetidine pre-treatment might be due to the inhibitory effect of cimetidine on CP450 mixed function oxidase. The volume of distribution Vd/f was found to increase by a margin of 37.6% in plasma after cimetidine treatment. This change is not statistically significant ($P>0.05$).

4.6.3 Effect of Cimetidine on Quinine Pharmacokinetics in Peptic Ulcer Patients

Figure 4.6.2 shows the mean concentration-time profile of quinine in plasma of ulcer patients following 400mg dose quinine, before and after cimetidine. Table 4.6.3a show

the mean (± SD) pharmcaokinetic data of quinine in ulcer patients before and after cimetidine treatment.

The absorption and distribution kinetics, as well as the absorption half-life were not significantly altered (P>0.05) after cimetidine treatment. The Cmax was increased from 2.71ug/mL to 2.78 ug/mL while the Tmax was decreased from 2.8h to 2.7h after cimetidine treatment. These changes were not significant (P>0.05). The AUCo-∝ was significantly increased (P<0.05) after cimetidine treatment. Also the elimination half-life was significantly increased (P<0.05) after cimetidine treatment. The changes in Cmax and AUCo-∝ might be due to inhibition of hepatic enzymes - CP450 mixed oxidase rather than reduced liver blood flow or enhance absorption due to pH changes.

The elimination half-life was significantly increased (P<0.05) in plasma of the ulcer patients following cimetidine treatment. There was a corresponding significant decrease (p<0.05) in total body clearance after cimetidine treatment in plasma. The significant (P<0.05) changes in elimination half-life and total clearance might be due to the inhibition of the metabolic pathway of quinine in presence of cimetidine (as earlier discussed in the healthy subjects).

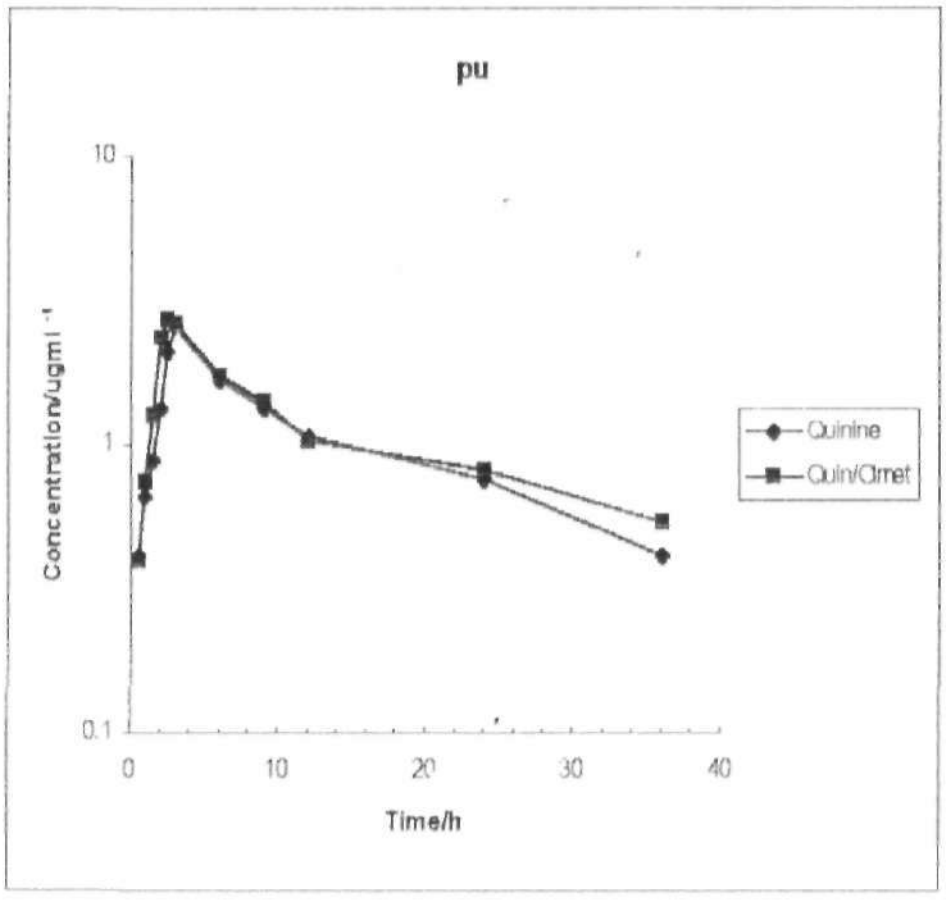


Figure 4.6.2

Mean concentration-time profile of quinine in plasma of three peptic ulcer patients following oral 400mg quinine sulphate before and after cimetidine.

Table 4.6.3a Pharmacokinetic parameters of quinine in ulcer patients following oral 400mg quinine before and after oral cimetidine (plasma data...Mean ± SD)

Parameter	Quinine alone ± SD		Quinine/Cimet. ± SD	
Ka (h^{-1})	0.785	0.386	0.763	0.41
B (h^{-1})	0.0942	0.035	0.0568*	0.013*
$t_{½a}$(h)	1.17	0.76	1.067	0.37
$t_{½b}$(h)	8.10	2.42	12.87*	3.21*
Tmax (h)	2.83	0.29	2.67	0.29
Cmax (ug/mL)	2.71	0.20	2.78	0.2
AUCo-∝(mg/L.h)	35.49	8.99	43.70*	12.2*
V_D (L/kg)	2.13	0.44	2.96	0.05
Cl_T/f (L/h/kg)	0.197	0.086	0.156*	1.38*

*P(<0.05) Significant.

CHAPTER 5

GENERAL DISCUSSION

The tablets used in this study were all branded products, cimetidine (Tagamet^{R} SB), proguanil (paludrine^{R} ICI) , mefloquine (Roche) and quinine (Evans). They were all found to conform with the BP 1988 standards on dissolution at t_{45}, disintegration time, weight uniformity and percent content of drugs. Mefloquine tablets was assayed by spectrophotometric procedure (APPENDIX II).

The method described for sample collection and storage provided a convenient storage and transport system for the plasma samples from point of collection to the laboratory of analysis. The sample pre-treatment step was efficiently carried out using the solid phase extraction technique. High recoveries of the antimalarials were obtained. Standard assay method validation procedures showed the analytical methods to be suitable for pharmacokinetic works.

The doses used in this study were the recommended single oral therapeutic doses and also as have been used for pharmacokinetic works in the literature. The biological fluid sampled for analysis in the study was plasma. Previous works on these antimalarials revealed that whole blood and plasma have been used for pharmacokinetic works. However general pharmacokinetic works were usually preferred in plasma (Ritschel et al. 1980).

Mefloquine, proguanil and quinine were found to be well absorbed after oral administration in this study. This was in conformity with earlier reported investigations. Mefloquine absorption was further enhanced after the oral cimetidine treatment both in the healthy subjects and in the peptic ulcer patients. This gastrointestinal absorption effect of cimetidine on mefloquine resulted in the observed increase in plasma mefloquine concentration. The effect of cimetidine on the metabolism and excretion of mefloquine unlike proguanil and quinine was not found to be statistically significant in this study. The elimination half-life and the clearance of mefloquine is extremely large when compared with that of cimetidine. The single dose design of this project did not adequately take care of the long elimination half-life of mefloquine. The employed dose of cimetidine in the study must have been eliminated appreciably during the distribution and the early elimination phase of mefloquine.

Proguanil was shown in this study to be well absorbed after oral administration and well distributed in the body. The rate and extent of absorption of proguanil were found to be in agreement with reported cases (Wattanagoon et al 1987). The absorption parameters were found to be enhanced by cimetidine administration. Proguanil unlike mefloquine and quinine is a prodrug, with the active metabolite being cycloguanil. The metabolism of proquanil and quinine were possibly inhibited by cimetidine thereby reflecting in the prolonged time of their elimination and reduced total clearance. The effect of cimetidine on proquanil is more than that of quinine. This might be due to the

significant effect of cimetidine on the absorption phase of proguanil but not in quinine. Also the significant effect of cimetidine on proguanil elimination but not mefloquine elimination might have accounted for the significant change in proguanil AUCo-∝ in plasma. The plasma level of cycloguanil- the metabolic product of proguanil, was found to be significantly reduced after cimetidine treatment. This further reflects in the significant reduction in the plasma level of the inactive metabolite 4CPB. These findings further support the observations on pharmacokinetics of proguanil after cimetidine treatment and the reasoning that cimetidine inhibits the metabolic pathway of proguanil.

The pharmacokinetics of all these antimalarials in healthy subjects have been severally reported to be fairly different from the pharmacokinetics data obtained in *P. falciparum* infected patients. The design of this study did not focus on malaria patients or malaria and ulcer patients. Particular attention might be paid to the pharmacokinetics of the drugs in malaria infection especially that of quinine, which was reported to significantly vary in proportion with severity of infection. Also red cell concentration and plasma protein binding are both increased in severe malaria.

Ethnic and genetic factors have been reported to affect the pharmacokinetics of cimetidine (Powell and Donn 1984, Somogyi and Gugler 1983 and Woolhouse 1985).

Also individual variability in the pharmacokinetics of these drugs have been reported.

The present study design has been prepared to take care of most of these factors.

CONCLUSION

The blood sample dried on filter paper and the solid phase extraction SPE pre-treatment procedure were found to be adequate and efficient sample collection, storage and pre-treatment techniques. The antimalarials in their respective solid state in blood sample dried on filter paper afforded the reported good stability of these drugs at the storage and other treatment temperatures. The recoveries of the drugs from the blood sample on filter paper were high and compared well with reported recoveries of the antimalarials from biological fluids. The within day, and day to day precision, accuracy, linearity and limit of detection showed the analytical method to be specific selective and sensitive; therefore adequate and appropriate for plasma drug concentration monitoring. The result of the single dose pharmacokinetic studies on mefloquine, proquanil and quinine in the healthy subjects were found to be in agreement with previously reported cases. Single dose pharmacokinetic parameters often considered in drug therapy and in calculating drug dosage regimen were considered for the purpose of this study. These same parameters were considered for the drug - drug interaction with cimetidine.

Cimetidine was found to enhance the absorption (to different degrees) of these drugs proguanil, mefloquine and quinine through absorption-drug interaction from the gastrointestinal fluid. Cimetidine has been shown to prolong elimination and reduce

total clearance of proquanil and quinine as shown by changes in elimination including metabolism parameters. The effects lead to increased plasma drug concentrations.

The effects have been significant in both the healthy subjects and ulcer patients, and may have some important clinical implications especially in situations where cimetidine and these antimalarials are administered concurrently and in multidose therapy. Pharmacokinetic drug-drug interaction data had provided bases and scientific support to clinically observed interactions in previous times. Therefore in day-to-day or general clinical practice where routine monitoring of therapeutic concentrations of these antimalarials are not feasible, care should be taken in patients taking any of the antimalarials and cimetidine concomitantly.

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APPENDIX I

PROTOCOL OF STUDY ON CIMETIDINE - DRUG INTERACTIONS SUBMITTED TO THE ETHICAL COMMITTEE OF JOS UNIVERSITY TEACHING HOSPITAL FOR APPROVAL FOR STUDIES INVOLVING HUMANS

Title of Project: Cimetidine - Drug Interactions

Introduction

Cimetidine (Tagamet^{R}), a H_2-receptor antagonist is today being prescribed for millions of peptic ulcer patients all over the world due to its efficacy and advantage in regulating gastric pH. Reports indicated that the adminbistration of cimetidine is associated with a direct inhibition of liver microsomal enzymes (1). As to the reduction in liver blood flow, consistent controversies prevailed (2). However, the effects of cimetidine on drugs used in the tropic have been rarely undertaken (3). Where studies have been done, the results are inconclusive (4,5,6). It is with this in mind that we intend to investigate the effects of co-administration of cimetidine with some antimalarial drugs commonly used in Nigeria with a view to assessing the extent of drug-drug interactions, possible mechanisms and implications on drug therapy.

Volunteers

Only adult, healthy and patient volunteers who freely give their informed consent will be involved in the study. Healthy volunteers shall be those certified as such by practising clinicians particularly with respect to liver and renal functions. Patients shall be those diagnosed to be suffering from petic ulcer and body pains (of any aetiology) and malaria. Pregnant subjects will not be involved in the study. If at any point, a volunteer wishes to withdraw from the study, he shall be absolutely free to do so without any hindrance whatever. At least six healthy and six

patients volunteers will be required for each study involving the selected drugs alone and the drugs co-administerred with cimetidine. Mefloquine, proguanil and Quinine tablets.

Outline of the Study

Each volunteer will receive a single oral dose of each of the drugs mentioned except cimetidine. The concentrations of the drugs will be determined at different time intervals over a period of time (depending on the pharmacokinetic profile of each drug). This is the 1st phase of the study. After a wash-out period of at least 2 weeks, the second phase of the study will commence.

The second phase wiill involve giving the volunteers the same drug taken earlier but with cimetidine in this order. Cimetidine 400mg given morning and night (12hourly) for two days and 400mg on the third day after an overnight fast. The drug is then given an hour later and blood samples taken.

Blood samples will be withdrawn (3-5ml of Blood) at timeintervals which will correspond with each drug's assumed pharmacokinetic profile.

Generally the following schedule will be followed:

Mefloquine tab: 0.0, 0.5, 1.0, 1.5, 2, 3, 6, 9, 12, 24, 36, 96, 168 (7th day) 360 (15th day) 720(30th day) 1080hr (45th day) hours.

Quuinine tab: 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 6, 9, 12, 24, 36hours.

Proguanil tab: 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 6, 12, 24, 36, 60, hours.

Ethical Consideration

A 5ml of blood samples will be taken at a time from each volunteer,not exceeding 8 times in24 houurs and not exceeding 12 times altogether during the study following each drug administration.

Benefit of the Study

The study, no doubt, will be beneficial to the patients and the society at large because adverse effects of cimetidine on drugs have now started to be documented and are of direct relevance to therapy. Drug monitoring is an important part of a successful drug therapy in a Hospital setting. This study will introduce a very good dimension in assessing implications of inhibitor drugs such as cimetidine to the clinicians.

Main Investigator

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APPENDIX II

ANALYSIS OF TABLETS USED

All tests are based on the recommendation of the BP 1980 and BP1988. (Except that of mefloquine hydrochloride tablet, where non-aqueous titrimetric method was used).

Content Uniformity

Tablet	Weight uniformity	Strength	Amount found	Percent content	Remark
Mefloquine	0.4998(0.0028)	250mg	257.9mg	103.16	PASS
Proguanil	0.2465(0.0025)	100mg	101mg	101.00	PASS
Quinine	0.4758 (0.014)	200mg	196mg	98.00	PASS

Dissolution rate (Basket method in 0.1M HCL at 37oC)

Tablets	Percent released	Remark
Mefloquine	88%	PASS
Proguanil	95%	PASS
Quinine	78%	PASS

Disintergration test

Tablet	Disintergration time	Remark	
Mefloquine	5.6min (0.6)	PASS	
Proguanil	8.2min (0.5)	PASS	
Quinine	14.1min (2.5)	PASS	

APPENDIX III

VOLUNTEERS (Healthy and Ulcer patients)

Mefloquine healthy

S/N	Code Name	Age	Weight	Remark
1	FI	37	65	Healthy
2	KS	30	71	"
3	KI	35	73	"
4	F2	34	60	"
5	NN	31	58	"
6	M2	32	62	"

Mefloquine ulcer patients

S/N	Code Name	Age	Weight	Remark
1	MK	32	63	Peptic ulcer (acute)
2	OG2	37	65	" (acute)
3	GP	39	68	" (bleeding)
4	OG	31	64	" (acute)
5	MT	28	58	" (chronic)
6	KO	30	70	" (acute)

Proguanil Healthy subjects

S/N	Code Name	Age (years)	Weight kg	Remark
1	KI	35	72	HEALTHY
2	K2	36	71	"
3	KS1	23	72	"
4	KS2	23	70	"
5	K3A	29	68	"
6	SH	26	71	"

Proguanil ulcer patients

S/N	Code Name	Age (years)	Weight kg	Remark
1	KA	39	68	Peptic ulcer (acute)
2	AD	38	72	" (chronic)
3	PA	36	65	" (acute)
4	AU	37	68	" (acute)
5	TF	36	66	" "
6	PK	37	69	" (acute)

Quinine healthy

S/N	Code Name	Age(years)	Weight kg	Remark
1	OG	36	70	Healthy
2	SH	26	60	"
3	AD	27	72	"
4	AF	35	65	"
5	AD2	25	65	"
6	ER	32	65	"

Quinine ulcer patients

S/N	Code Name	Age (years)	Weight kg	Remark
1	GI	31	65	Peptic ulcer (acute)
2	SG	36	54	" (acute)
3	FS	30	61	" (acute)
4	TP	35	62	" (chronic)
5	KP	29	65	" (acute)
6	TE	32	71	" (acute)

APPENDIX IV

STABILITY OF DRUGS IN PLASMA ADSORBED ON FILTER PAPER

Temperature 25°C

Time (Day)	Percentage of initial concentration found		
	Proguanil	Quinine	Mefloquine
0	100	100	100
7	100	100	100
15	98.1	99.0	100
21	98.0	99.0	100
30	98.0	99.0	100
45	98.0	99.0	100
60	98.0	99.0	99.0

Temperature 37°C

Time (Day)	Percentage of initial concentration found		
0	Proguanil	Quinine	Mefloquine
1	100	100	100
7	100	100	100
15	97.5	98.0	100
21	97.0	99.0	99
30	97.0	98.0	99
45	96.4	98.0	99
60	96.4	97.0	98

Temperature 55°C

Time (Day)	Percentage of initial concentration found		
0	Proguanil	Quinine	Mefloquine
1	100	100	100
7	98.6	100	100
15	98.3	98.0	98.6
21	96.6	96.0	98.0
30	96.0	96.0	98.0
45	95.6	95.0	96.0
60	94.0	93.5	94.0

Temperature 65°C

Time (Day)	Percentage of initial concentration found		
0	Proguanil	Quinine	Mefloquine
1	100	100	100
7	98.0	97.0	98.7
15	95.5	98.0	98.0
21	93.7	97.0	96.0
30	93.0	96.0	96.2
45	91.0	95.0	93.1
60	91.0	92.5	91.8

APPENDIX V

A. Plasma concentration-time data of mefloquine for six healthy subjects following oral 500mg dose mefloquine hydrochloride tablet.

Concentration ug /mL. Individual subjects								
Time (h)	S1	S2	S3	S4	S5	S6	Mean	± SD
0.5	0.32	-	-	-	0.33	-	0.33	-
1.0	0.62	-	0.04	0.17	0.52	-	0.36	0.25
1.5	0.64	0.11	0.44	0.45	0.91	0.15	0.45	0.30
2.0	1.20	0.17	0.55	0.28	0.60	0.42	0.53	0.33
2.5	0.90	0.23	0.92	0.60	1.00	0.92	0.76	0.30
3.0	1.13	1.03	1.80	1.00	1.75	1.51	1.37	0.36
6.0	1.55	1.50	1.70	1.43	1.90	1.92	1.67	0.21
9.0	0.88	0.96	1.80	1.55'	1.66	1.80	1.44	0.42
12.0	1.06	0.74	1.90	1.89	0.85	0.76	1.20	0.55
24.0	0.91	0.35	1.22	0.43	0.70	0.90	0.75	0.33
48.0	0.39	0.47	0.97	0.21	0.75	0.85	0.62	0.28
7 days	0.39	0.33	0.60	0.47	0.61	0.76	0.53	0.16
15 days	0.33	0.30	-	0.43	0.56	0.48	0.42	0.11
30 days	0.25	0.07	0.34	0.11	0.25	0.16	0.20	0.10
45 days	-	-	-	-	-	-	-	-

APPENDIX V

B. Plasma concentration-time data of mefloquine for six healthy subjects following oral 500mg dose mefloquine hydrochloride tablet after administration of oral cimetidine.

Concentration ug /mL. Individual subjects								
Time (h)	S1	S2	S3	S4	S5	S6	Mean	± SD
0.5	-	-	-	-	0.09	0.24	0.17	0.11
1.0	0.14	0.22	0.30	0.35	0.12	0.38	0.23	0.12
1.5	0.15	0.39	0.35	0.44	0.72	0.43	0.41	0.18
2.0	1.45	0.95	1.05	0.75	0.80	1.08	0.86	0.24
2.5	0.46	1.21	1.08	1.14	1.50	1.63	1.17	0.41
3.0	2.75	2.26	1.53	2.82	1.90	2.30	2.26	0.51
6.0	2.41	2.10	2.37	2.14	2.06	1.52	2.10	0.32
9.0	2.24	1.95	2.38	0.95	2.22	2.73	2.07	0.62
12.0	1.69	1.72	1.44	0.87	1.69	2.75	1.69	0.61
24.0	0.99	1.20	1.10	0.61	1.44	2.07	1.23	0.50
48.0	-	1.00	1.11	0.20	1.41	1.88	1.12	0.62
7 days	0.38	0.95	0.24	0.60	0.51	1.79	0.73	0.55
15 days	0.28	0.45	0.19	0.30	0.34	0.54	0.34	0.12
30 days	0.12	0.48	0.20	0.14	0.11	0.45	0.25	0.17
45 days	-	0.28	0.15	-	0.05	0.53	0.25	0.20

C. Plasma concentration-time data of mefloquine for four ulcer patients following oral 500mg dose mefloquine hydrochloride tablet.

Concentration ug /mL. Individual subjects							
Time (h)	S1	S2	S3	S4	S6	Mean	± SD
0.5	-	-	-	-		-	-
1.0	-	0.28	0.36	0.21		0.28	0.08
1.5	-	0.35	0.38	0.33		0.35	0.03
2.0	0.22	0.83	0.46	0.62		0.54	0.26
2.5	0.22	1.18	0.62	0.83		0.70	0.40
3.0	0.42	2.30	1.22	1.46		1.35	0.77
6.0	1.69	0.83	1.41	1.15		1.27	0.37
9.0	2.15	0.86	1.61	1.91		1.64	0.56
12.0	1.29	1.21	1.40	1.10		1.25	0.13
24.0	1.34	1.20	1.00	0.70		1.08	0.31
48.0	-	-	-	-		-	-
7 days	0.62	0.47	0.61	0.35		0.58	0.16
15 days	0.44	0.63	0.35	0.25		0.41	0.16
30 days	-	0.35	0.11	0.09		0.18	0.14
45 days	-	-	-	-		-	-

APPENDIX V

D. Plasma concentration-time data of mefloquine for four ulcer patients following oral 500mg dose mefloquine hydrochloride tablet after administration of oral cimetidine.

Concentration ug/mL Individual subjects					
Time (h)	S1	S2	S3	Mean	±SD
0.5	0.08	-	0.09	0.08	-
1.0	0.22	0.60	0.25	0.35	0.22
1.5	0.47	0.96	0.42	0.62	0.29
2.0	0.88	1.02	1.05	0.98	0.10
2.5	2.18	2.20	2.10	2.16	0.10
3.0	2.18	1.90	2.31	2.13	0.20
6.0	2.30	2.57	2.03	2.30	0.27
9.0	2.07	2.10	2.43	2.20	0.20
12.0	2.00	2.55	1.80	2.12	0.34
24.0	1.07	2.12	0.95	1.39	0.65
48.0	0.88	1.25	0.70	0.94	0.28
7 days	0.40	0.90	0.50	0.60	0.27
15 days	0.35	0.70	0.31	0.45	0.21
30 days	0.26	0.32	0.26	0.28	0.04
45 days	-	0.27	-	0.27	-

APPENDIX VB

Pharmacokinetics data of subjects

I. The pharmacokinetic parameter of mefloquine in plasma of individual healthy subjects following oral 500mg mefloquine.

Parameter	Subject No1	Subject No2	Subject No3	Subject No4	Subject No5	Subject No6
ka (h^{-1})	7.4280	1.8700	5.8918	5.8900	3.95	6.60
β(day^{-1})	0.03720	0.0214	0.0617	0.0576	0.063	0.027
$t_{½a}$(h)	2.240	8.894	2.820	2.800	4.20	2.04
$t_{½β}$ (day)	18.63	32.38	11.20	12.04	11.00	25.70
Tmax(h)	6.00	6.00	12.00	12.00	6.00	6.00
Cmax(µg/mL)	1.55	1.50	1.90	1.89	1.70	1.72
AUC$_{0-\infty}$(mg/L.day)	17.10	11.20	31.15	13.71	19.93	21.20
Vd(L/kg)	12.10	9.37	3.57	10.56	6.86	14.09
Cl(L/day/kg	0.450	0.628	0.220	0.608	0.433	0.380

II. The phamacokinetic parameter of mefloquine in plasma of individual healthy subjects following oral 500mg mefloquine after oral 400mg cimetidine.

Parameter	Subject No1	Subject No2	Subject No3	Subject No4	Subject No5	Subject No6
ka (h^{-1})	11.029	10.7	7.595	4.502	8.0912	3.3035
β (day^{-1})	0.04058	0.0476	0.0239	0.026	0.0490	0.0331
$t_{½ß}$(h)	1.50	1.55	2.19	3.70	2.056	5.040
$t_{½ß}$ (day)	17.06	14.55	29.01	26.65	14.50	20.94
Tmax(h)	3.0	3.0	9.0	3.0	9.0	12.0
Cmax(µg/mL)	2.75	2.82	2.38	2.82	2.22	2.75
AUCo-∞(mg/L.day)	14.30	35.10	19.60	17.90	16.36	54.40
Vd(L/kg)	13.25	4.44	14.64	21.96	10.77	4.47
Cl(L/day/kg	0.5378	0.211	0.350	0.571	0.5270	0.1480

III. The pharmacokinetics of mefloquine in plasma of individual ulcer patients following oral 500mg mefloquine.

Parameter	Subject No1	Subject No2	Subject No3	Subject No4
$k_a(h^{-1})$	16.001	16.22	5.4251	6.721
$\beta(day^{-1})$	0.0350	0.025	0.0643	0.04235
$t_{½\alpha}$	0.989	1.03	3.06	2.474
$t_{½\beta}$(day)	19.80	27.80	10.78	16.40
Tmax(h)	9.0	3.0	9.0	9.0
Cmax(μg/ml)	2.15	2.30	1.61	1.95
AUC$_{0-\infty}$(mg/L.day)	17.60	34.00	16.60	11.20
Vd(L/kg)	12.58	8.912	7.08	16.20
Cl(L/day/kg)	0.437	0.223	0.455	0.686

IV The pharmacokinetic parameter of mefloquine in plasma of individual ulcer patients following oral 500mg mefloquine after oral cimetidine.

Parameter	Subject No1	Subject No2	Subject No6
ka(h^{-1})	10.238	12.526	7.6844
ß(day^{-1})	0.0444	0.03215	0.0331
$t_{½ß}$	1.620	1.317	2.16
$t_{½ß}$(day)	15.61	21.60	20.97
Tmax(h)	6.0	6.0	9.0
Cmax(µg/ml)	2.30	2.51	2.43
AUCo-∞(mg/L.day)	19.60	37.50	21.61
Vd(L/kg)	9.12	6.37	10.02
Cl(L/day/kg)	0.405	0.205	0.331

APPENDIX VI

A. Plasma concentration-time data of proguanil for six healthy subjects following oral 200mg dose proguanil hydrochloride tablet.

Concentration ng /mL. Individual subjects								
Time (h)	S1	S2	S3	S4	S5	S6	Mean	± SD
0.5	97.73	-	53.1	62.1	67.0	59.1	67.7	35.0
1.0	115.3	147.2	91.27	196.2	78.7	91.9	120.0	45.0
1.5	112.4	155.2	-	-	89.7	108.5	116.0	23.0
2.0	162.6	109.6	108.0	240.8	106.8	217.0	157.5	60.0
2.5	184.8	169.3	-	123.2	110.3	205.1	158.5	40.5
3.0	191.6	176.4	210.0	230.5	196.8	252.8	206.2	29.0
6.0	85.4	173.3	193.2	118.7	172.3	134.2	146.1	40.1
9.0	78.6	137.3	128.0	77.0	131.2	68.6	103.3	31.3
12.0	75.7	105.4	112.7	66.5	112.0	56.0	88.3	25.5
24.0	45.2	-	77.0	51.6	94.8	41.2	62.0	23.0
48.0	-	48.9	-	-	39.4	-	44.1	6.7
60.0	17.0	46.3	32.0	27.0	10.0	21.5	25.6	12.7

APPENDIX VI

B. Plasma concentration-time data of proguanil for six healthy subjects following oral 200mg dose proguanil hydrochloride tablet after oral cimetidine.

Concentration ng /mL. Individual subjects								
Time (h)	S1	S2	S3	S4	S5	S6	Mean	± SD
0.5	76.7	96.1	156.5	127.3	110.6	120.0	114.5	27.4
1.0	186.6	214.7	368.0	294.6	150.5	245.6	243.3	78.5
1.5	538.6	176.6	419.0	-	-	-	378.1	184.
2.0	321.3	335.6	425.0	308.0	220.0	280.5	315.0	67.6
2.5	-	-	-	-	-	-	-	-
3.0	267.0	288.9	202.0	340.0	285.6	355.2	289.8	55.0
6.0	198.8	416.2	348.0	171.7	210.4	258.2	267.2	96.0
9.0	125.8	221.5	316.0	231.1	160.8	160.0	202.0	68.0
12.0	111.3	114.1	239.2	281.4	120.0	140.0	167.6	13.7
24.0	90.0	78.0	110.5	209.0	89.6	110.0	114.5	48.0
48.0	-	-	-	96.1	75.6	85.0	85.5	10.2
60.0	33.4	35.0	48.0	70.1	48.5	51.2	47.7	13.0

APPENDIX VI

C. Plasma concentration-time data of proguanil for four ulcer patients following oral 200mg dose proguanil hydrochloride tablet.

Concentration ng /mL. Individual subjects						
Time (h)	S1	S2	S3	S4	Mean	±SD
0.5	109.0	95.0	-	98.0	100.7	7.4
1.0	131.5	111.0	-	109.0	117.2	12.1
1.5	144.1	122.2	100.0	131.6	124.5	18.0
2.0	225.9	149.0	111.0	164.5	162.6	49.0
2.5	-	173.0	226.4	170.5	190.0	32.2
3.0	260.1	287.0	393.4	303.7	311.1	58.0
6.0	392.2	232.0	375.5	315.9	328.9	72.0
9.0	307.1	157.5	209.6	208.9	220.8	62.0
12.0	182.0	105.3	180.0	178.5	161.5	37.0
24.0	77.2	83.0	123.9	163.6	111.2	41.0
48.0	-	65.0	81.0	73.0	78.0	11.3
60.0	48.0	55.0	57.2	30.0	47.6	12.3

APPENDIX VI

D. Plasma concentration-time data of proguanil for four ulcer patients following oral 200mg dose proguanil hydrochloride tablet after oral cimetidine.

Concentration ng /mL. Individual subjects						
Time (h)	S1	S2	S3	S4	Mean	±SD
0.5	-	-	103.6	-	103.6	-
1.0	-	129.0	150.0	174.1	151.2	23.0
1.5	143.0	172.0	203.0	191.0	177.3	26.1
2.0	250.0	201.2	230.0	395.6	269.2	87.5
2.5	138.0	211.2	402.0	-	250.4	136.0
3.0	191.5	279.6	465.0	536.0	367.9	160
6.0	439.5	405.4	545.3	528.2	479.6	67.0
9.0	211.2	295.7	335.6	409.7	313.0	83.0
12.0	265.0	196.3	134.6	228.7	206.2	55.0
24.0	233.5	106.6	75.5	170.0	146.4	70.1
48.0	85.0	113.7	-	-	99.4	14.3
60.0	58.0	80.0	81.1	70.0	72.3	11.0

APPENDIX VI

I. Table showing pharmacokinetic paranieters of proguanil in plasma of individual healthy subjects after an oral 200mg dose of proguanil.

Parameter	Subject No1	Subject No2	Subject No3	Subject No4	Subject No5	Subject No6
ka (h^{-1})	0.7699	0.5760	0.5280	1.4990	0.5173	0.8650
B (h^{-1})	0.0333	0.0369	0.06133	0.0550	0.0509	0.0465
$t_{1/2a}$ (h)	0.9000	1.200	1.313	0.450	1.340	0.801
$t_{1/2B}$ (h)	20.80	18.70	11.30	12.33	13.62	14.90
Tmax (h)	3.0	3.0	6.0	2.0	3.0	3.0
Cmax (ng/mL)	191.6	176.0	193.2	240.0	196.8	252.0
AUCo-∞ (ng/mL.h)	3634.2	6320.6	5380.3	4114.4	4832.3	3740.2
Vd/f (L/kg)	22.95	12.03	8.42	12.36	11.97	16.20
$Cl_{T/f}$ (mL/min/kg)	12.74	7.43	8.61	11.57	10.14	12.56

APPENDIX VI

II. Table showing pharmacokinetic parameters of proguanil in plasma of individual healthy subjects after an oral cimetidine followed by 200mg dose of proguanil.

Parameter	Subject No1	Subject No2	Subject No3	Subject No4	Subject No5	Subject No6
ka (h^{-1})	1.3200	0.8863	2.6460	0.5175	0.5410	0.6930
ß (h^{-1})	0.02234	0.03939	0.03150	0.03425	0.0330	0.0279
$t_{1/2\alpha}$ (h)	0.5250	0.782	0.262	1.340	1.280	1.000
$t_{1/2\beta}$ (h)	29.64	17.60	22.03	20.23	21.00	24.84
Tmax (h)	1.5	6.0	2.0	3.0	3.0	3.0
Cmax (ng/mL)	538.9	416.0	425.0	340.0	285.0	355.2
AUCo-∞ (ng/mL.h)	7174.9	7232.5	10213.4	12547.0	7751.3	9025.0
Vd/f (L/kg)	16.58	9.90	8.66	6.63	11.50	11.12
$Cl_{T/f}$ (mL/min/kg	6.45	6.49	4.54	3.79	6.33	5.20

APPENDIX VII

III. Table showing pharmacokinetic parameters of proguanil in plasma of individual ulcer patient subjects after an oral dose of cimetidine followed by 200mg dose of proguanil.

Parameter	Subject 1	Subject 2	Subject 3	Subject 4
ka (h^{-1})	0.5110	0.7493	0.3666	0.3910
ß (h^{-1})	0.02510	0.05897	0.03060	0.02296
$t_{1/2s}$ (h)	1.256	0.924	1.890	1.770
$t_{1/2\beta}$ (h)	27.65	11.75	22.65	30.20
Tmax (h)	6.0	6.0	6.0	3.0
Cmax (ng/mL)	439.5	405.0	545.0	536.0
AUCo-∞ (ng/mL.h)	13006.0	10148.4	10712.8	14752.0
Vd/f (L/kg)	9.03	4.64	9.39	8.69
$Cl_{T/f}$ (mL/min/kg	3.77	4.56	4.79	3.32

APPENDIX VI

IV. Table showing pharmacokinetic parameters of proguanil in plasma of individual ulcer patient subjects following an oral 200mg dose of proguanil.

Parameter	Subject1	Subject2	Subject3	Subject4
ka (h^{-1})	0.4679	0.7619	0.3556	0.3943
B (h^{-1})	0.0401	0.0631	0.0529	0.0447
$t_{1/2a}$ (h)	1.48	0.91	1.95	1.76
$t_{1/2B}$ (h)	17.28	11.00	13.08	15.50
Tmax (h)	6.0	3.0	3.0	6.0
Cmax (ng/mL)	392.2	287.0	393.4	315.9
AUCo-∞ (ng/mL.h)	8279.3	6562.0	9136.0	9067.0
Vd/f (L/kg)	8.83	6.73	6.36	7.26
$Cl_{T/f}$ (mL/min/kg	5.90	7.06	5.62	5.41

V

Table showing the mean plasma concentration time data of cycloguanil and 4-CPB in six healthy subjects following 200mg oral proguanil before and after cimetidine.

	Before cimetidine		After cimetidine	
Time(h)	C Mean ± SD	4-CPB Mean ± SD	C Mean ± SD	4-CPB Mean ± SD
0.5	-	-	-	-
1	-	-	-	-
1.5	-	-	-	
2	8.15 ± 0.5	1.14 ± 0.7	-	
2.5	11.03 ± 10	2.18 ± 0.8	-	
3	26.90 ± 25	2.73 ± 1.1	2.5 ± 1	5.01 ± 3.3
6	37.0 ± 23	5.18 ± 1.3	9.6 ± 5	9.77 ± 2.3
9	21.70 ± 12	5.03 ± 0.5	15 ± 13	5.34 ± 2.4
12	15.6 ± 10	4.16 ± 0.4	6.2 ± 3.6	5.0 ± 1.6
24	10.1 ± 7	2.28 ± 0.6	5.6 ± 4	2.17 ± 0.6
60	9.8 ± 3	1.14 ± 0.6	5.3 ± 2	1.17 ± 0.8

VI

Table showing the mean plasma concentration-time data of cycloguanil (C)and 4-CPB in four ulcer patient subjects following 200mg oral proguanil before and after cimetidine

	Before cimetidine		After cimetidine	
Time(h)	C Mean ± SD	4-CPB Mean± SD	C Mean ± SD	4-CPB Mean ± SD
0.5		-	-	-
1		-	-	-
1.5		-	-	-
2		-	17.5 ± 4.5	2.3 ± 1.8
2.5	16 ± 6	5.2 ± 1.6	20 ± 8	3.15 ± 1
3	28 ± 16.9	6.8 ± 2.5	29.3 ± 23.0	3.83 ± 2.1
6	34 ± 5	9.0 ± 4.0	22.7 ± 17	5.6 ± 0.5
9	18.8 ±10	7.5 ± 1.1	12.1 ± 2.5	3.73 ± 1.4
12	11 ± 5.9	3.76 ± 2.7	9.2 ± 1.5	1.6 ± 0.6
24	8.1 ± 3	1.8 ± 0.4	5.35 ± 1.0	1.0 ± 0.1
60	7.5 ± 2	1.0 ± 0.4	-	-

APPENDIX VII

A. Plasma concentration-time data of quinine for six healthy subjects following oral 400mg dose quinine sulphate tablet.

Concentration ug /mL. Individual subjects								
Time (h)	S1	S2	S3	S4	S5	S6	Mean	± SD
0.5	0.06	0.18	0.55	-	0.28	0.70	0.35	0.25
1.0	0.20	0.33	0.71	-	0.78	1.10	0.63	0.36
1.5	0.80	0.93	0.80	0.83	0.93	1.80	1.02	0.39
2.0	1.20	1.11	1.40	1.43	1.43	2.40	1.50	0.46
2.5	2.00	1.70	2.40	2.00	2.15	2.90	2.18	0.40
3.0	3.71	1.45	3.00	2.53	2.10	2.70	2.58	0.80
6.0	2.85	1.00	2.06	1.54	1.82	2.10	1.94	0.54
9.0	1.10	0.70	1.14	1.32	1.00	1.20	1.08	0.21
12.0	0.98	0.17	1.00	0.94	0.70	1.00	0.80	0.33
24.0	0.40	0.08	0.61	0.68	0.33	0.80	0.48	0.26
36.0	0.16	0.45	0.36	0.35	0.22	0.40	0.32	0.11

APPENDIX VII

B. Plasma concentration-time data of quinine for six healthy subjects following oral 400mg dose quinine sulphate tablet after oral cimetidine.

Concentration ug /mL. Individual subjects								
Time (h)	S1	S2	S3	S4	S5	S6	Mean	± SD
0.5	0.11	-	0.43	0.80	0.20	-	0.39	-
1.0	0.33	0.90	1.01	0.85	0.33	0.80	0.70	0.29
1.5	0.48	1.31	1.36	1.60	0.38	1.60	1.12	0.55
2.0	1.05	1.85	1.65	1.85	1.32	2.50	1.70	0.50
2.5	1.67	2.64	2.31	3.10	1.60	2.80	2.35	0.61
3.0	2.05	3.30	2.85	3.00	2.15	3.50	2.80	0.59
6.0	1.38	2.06	1.20	2.30	1.50	2.10	1.75	0.44
9.0	0.61	1.70	1.10	1.82	1.10	1.50	1.30	0.45
12.0	0.48	1.16	1.00	1.45	0.50	1.00	0.93	0.38
24.0	0.29	0.80	0.60	0.90	0.40	0.80	0.63	0.24
36.0	0.10	0.64	0.25	0.56	0.30	0.50	0.39	0.21

APPENDIX VII

C. Plasma concentration-time data of quinine for three ulcer patients following oral 400mg dose quinine sulphate tablet.

Concentration ug /mL. Individual subjects					
Time (h)	S1	S2	S3	Mean	±SD
0.5	0.51	0.30	0.41	0.41	0.10
1.0	0.85	0.50	0.62	0.66	0.20
1.5	0.90	0.80	0.91	0.87	0.10
2.0	1.20	1.65	1.10	1.32	0.30
2.5	2.30	2.82	1.20	2.12	0.80
3.0	2.80	2.45	2.50	2.58	0.20
6.0	1.30	1.70	2.10	1.67	0.40
9.0	1.20	1.21	1.60	1.34	0.20
12.0	0.90	0.85	1.40	1.05	0.30
24.0	0.50	0.65	1.10	0.75	0.30
36.0	0.40	0.42	0.45	0.42	0.10

APPENDIX VII

D. Plasma concentration-time data of quinine for three ulcer patients following oral 400mg dose quinine sulphate tablet after oral cimetidine.

Concentration ug /mL. Individual subjects					
Time (h)	S1	S2	S3	Mean	±SD
0.5	0.50	0.25	0.42	0.39	0.10
1.0	0.80	0.65	0.76	0.74	0.10
1.5	1.50	1.20	1.05	1.25	0.20
2.0	2.80	2.50	1.80	2.40	0.50
2.5	2.90	2.85	2.50	2.75	0.20
3.0	2.70	2.68	2.60	2.66	0.10
6.0	1.60	1.55	2.10	1.75	0.30
9.0	1.2	1.21	1.80	1.41	0.30
12.0	1.00	0.85	1.20	1.02	0.20
24.0	0.80	0.72	0.90	0.81	0.10
60.0	*0.45*	*0.55*	*0.60*	*0.53*	*0.10*

APPENDIX VIIB

I. Table showing the individual pharmacokinetic data of quinine in plasma following oral 400mg dose of quinine sulphate in healthy subjects

Parameter	Subject No1	Subject No2	Subject No3	Subject No4	Subject No5	Subject No6
ka (h^{-1})	1.083	1.055	0.850	0.886	0.438	0.502
ß (h^{-1})	0.0588	0.1770	0.1034	0.0650	0.0944	0.0835
$t_{1/2a}$ (h)	0.6400	0.6569	0.8100	0.7820	1.5770	1.3800
$t_{1/2ß}$ (h)	11.78	3.90	6.70	10.60	7.34	8.30
Tmax (h)	3.0	2.5	3.0	3.0	2.5	2.5
Cmax (ug/mL)	3.71	1.70	3.00	2.53	2.15	2.85
AUCo-∞ (mg/L.h)	39.4	15.0	38.3	38.2	27.8	43.4
$Cl_{T/f}$ (L/h/kg)	0.1450	0.4100	0.1700	0.1610	0.2478	0.1280
Vd/f (L/kg)	2.4650	2.3164	1.7000	2.4800	2.6250	1.5400

APPENDIX VIIB

I. Table showing the individual pharmacokinetic data of quinine in plasma following oral 400mg dose of quinine sulphate in healthy subjects

Parameter	Subject No1	Subject No2	Subject No3	Subject No4	Subject No5	Subject No6
ka (h^{-1})	1.083	1.055	0.850	0.886	0.438	0.502
ß (h^{-1})	0.0588	0.1770	0.1034	0.0650	0.0944	0.0835
$t_{1/2\alpha}$ (h)	0.6400	0.6569	0.8100	0.7820	1.5770	1.3800
$t_{1/2\beta}$ (h)	11.78	3.90	6.70	10.60	7.34	8.30
Tmax (h)	3.0	2.5	3.0	3.0	2.5	2.5
Cmax (ug/mL)	3.71	1.70	3.00	2.53	2.15	2.85
AUCo-∞ (mg/L.h)	39.4	15.0	38.3	38.2	27.8	43.4
$Cl_{T/f}$ (L/h/kg)	0.1450	0.4100	0.1700	0.1610	0.2478	0.1280
Vd/f (L/kg)	2.4650	2.3164	1.7000	2.4800	2.6250	1.5400

APPENDIX VIIB

II. Table showing the individual pharmacokinetic data of quinine in plasma following oral 400mg dose of quinine sulphate before and after 400mg cimetidine in healthy subjects.

Parameter	Subject No1	Subject No2	Subject No3	Subject No4	Subject No5	Subject No6
ka (h^{-1})	0.6066	0.8060	0.5682	1.7799	0.5570	0.8130
ß (h^{-1})	0.0499	0.0640	0.0463	0.0701	0.0560	0.0722
$t_{1/2a}$ (h)	1.14	0.86	1.22	0.39	1.24	0.85
$t_{1/2ß}$ (h)	14.15	10.80	14.98	9.89	12.40	9.60
Tmax (h)	3.0	3.0	2.5	2.5	3.0	3.0
Cmax (ug/mL)	2.05	3.30	2.85	3.10	2.15	3.50
AUCo-∞ (mg/L.h)	21.1	52.6	36.9	54.8	29.2	48.6
$Cl_{T/f}$ (L/h/kg)	0.27	0.12	0.18	0.11	0.20	0.11
Vd/f (L/kg)	5.54	1.82	3.91	1.60	3.60	1.58

APPENDIX VIIB

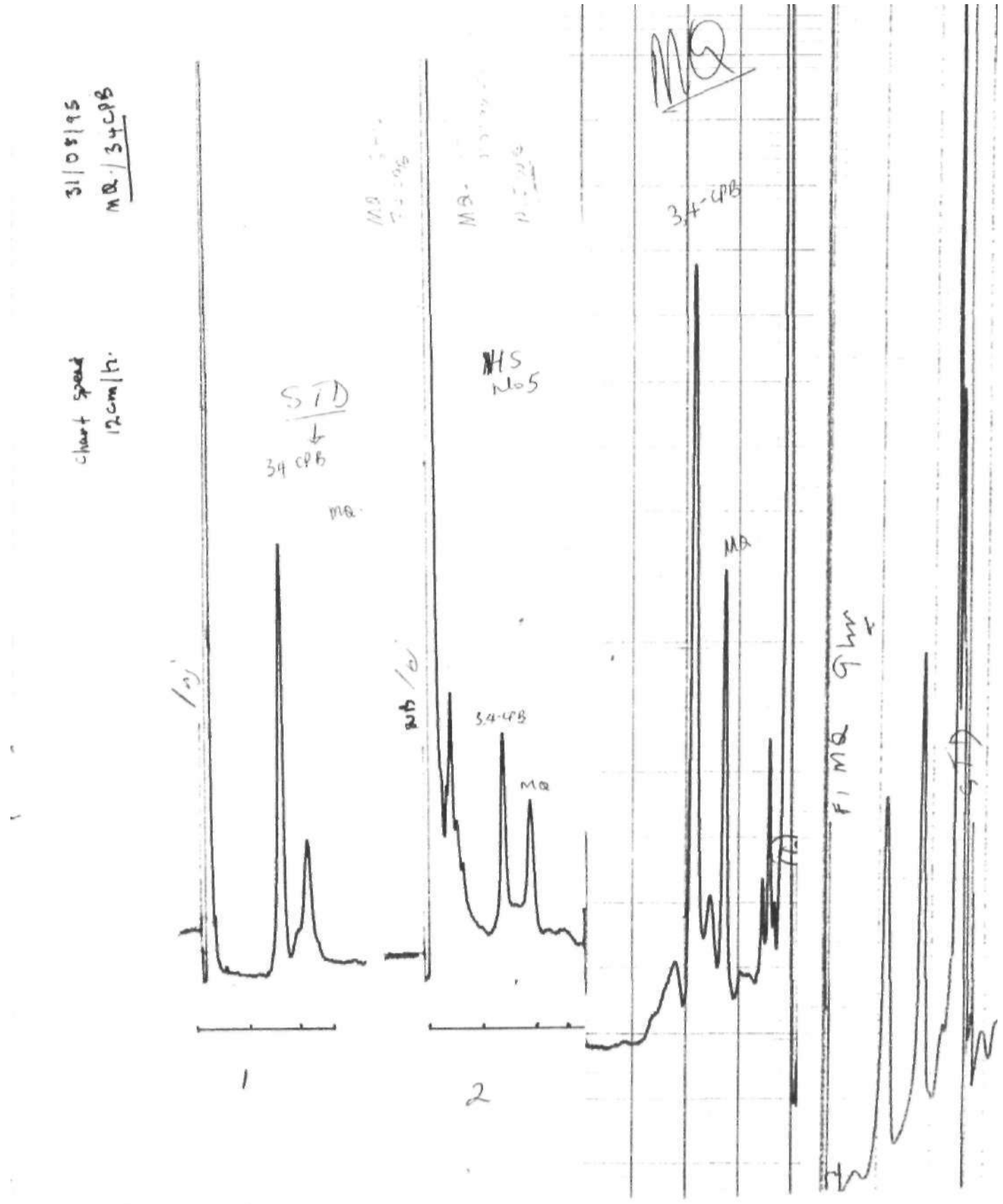
III. Table showing the individual pharmacokinetic data of quinine in plasma of ulcer patients following oral 400mg dose of quinine sulphate.

Parameter	Subject No1	Subject No2	Subject No3
ka (h^{-1})	0.5550	1.4629	0.2352
B (h^{-1})	0.1037	0.680	0.0938
$t_{1/2a}$ (h)	**1.25**	0.49	2.95
$t_{1/2\beta}$ (h)	**6.70**	10.20	7.40
Tmax (h)	3.0	**2.5**	**3.0**
Cmax (ug/mL)	2.80	2.82	2.50
AUCo-∞ (mg/L.h)	34.3	42.0	**41.0**
$Cl_{T/f}$ (L/h/kg)	0.177	0.177	0.160
Vd/f (L/kg)	1.73	2.60	1.74

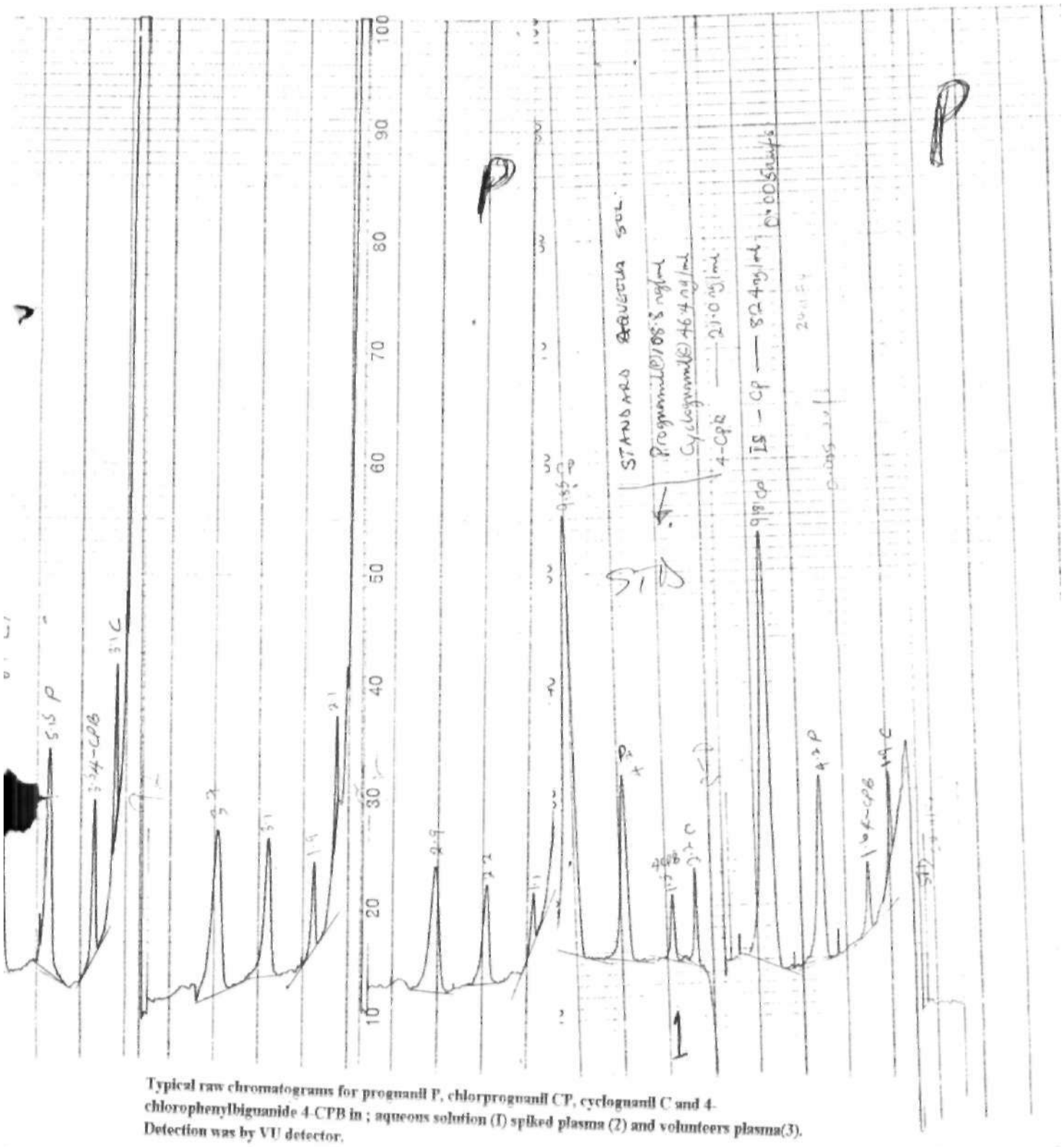
APPENDIX VIIB

IV. Table showing the individual pharmacokinetic data of quinine in plasma of ulcer patients following oral 400mg dose of quinine sulphate before and after 400mg cimetidine

Parameter	Subject No1	Subject No2	Subject No3
ka (h^{-1})	0.8182	0.4792	0.4368
ß (h^{-1})	0.0717	0.0370	0.0443
$t_{1/2a}$ (h)	0.85	1.45	1.60
$t_{1/2\beta}$ (h)	9.67	18.75	15.60
Tmax (h)	2.5	2.5	3.0
Cmax (ug/mL)	2.90	2.85	2.60
AUCo-∞ (mg/L.h)	43.7	50.0	56.5
$Cl_{T/f}$ (L/h/kg)	0.142	0.148	0.116
Vd/f (L/kg)	1.98	4.00	2.62



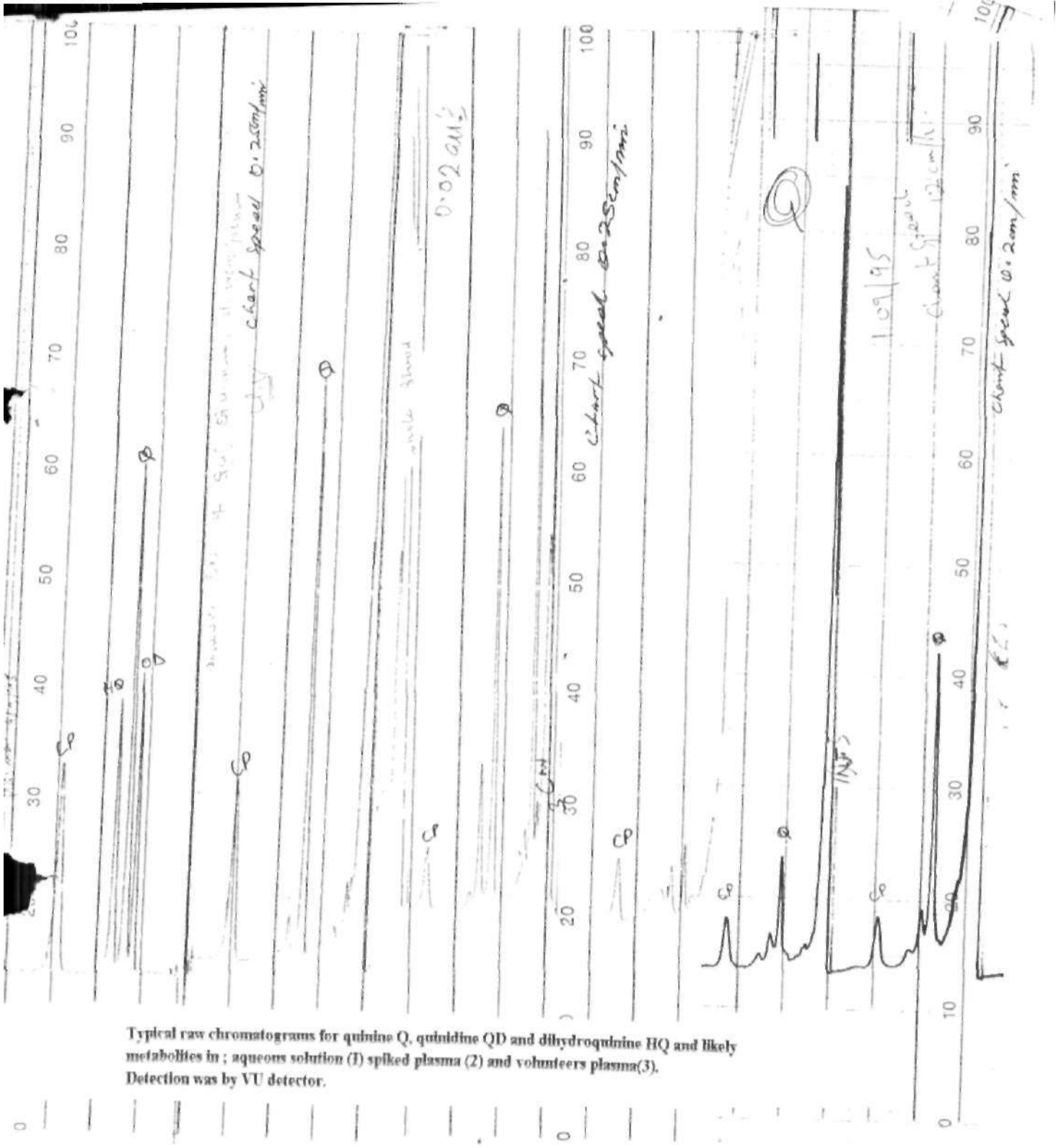
Typical raw chromatograms for mefloquine MQ, and 3,4-dichlorophenylbiguanide in ; aqueous solution (I) spiked plasma (2) and volunteers plasma(3).
Detection was by VU detector.



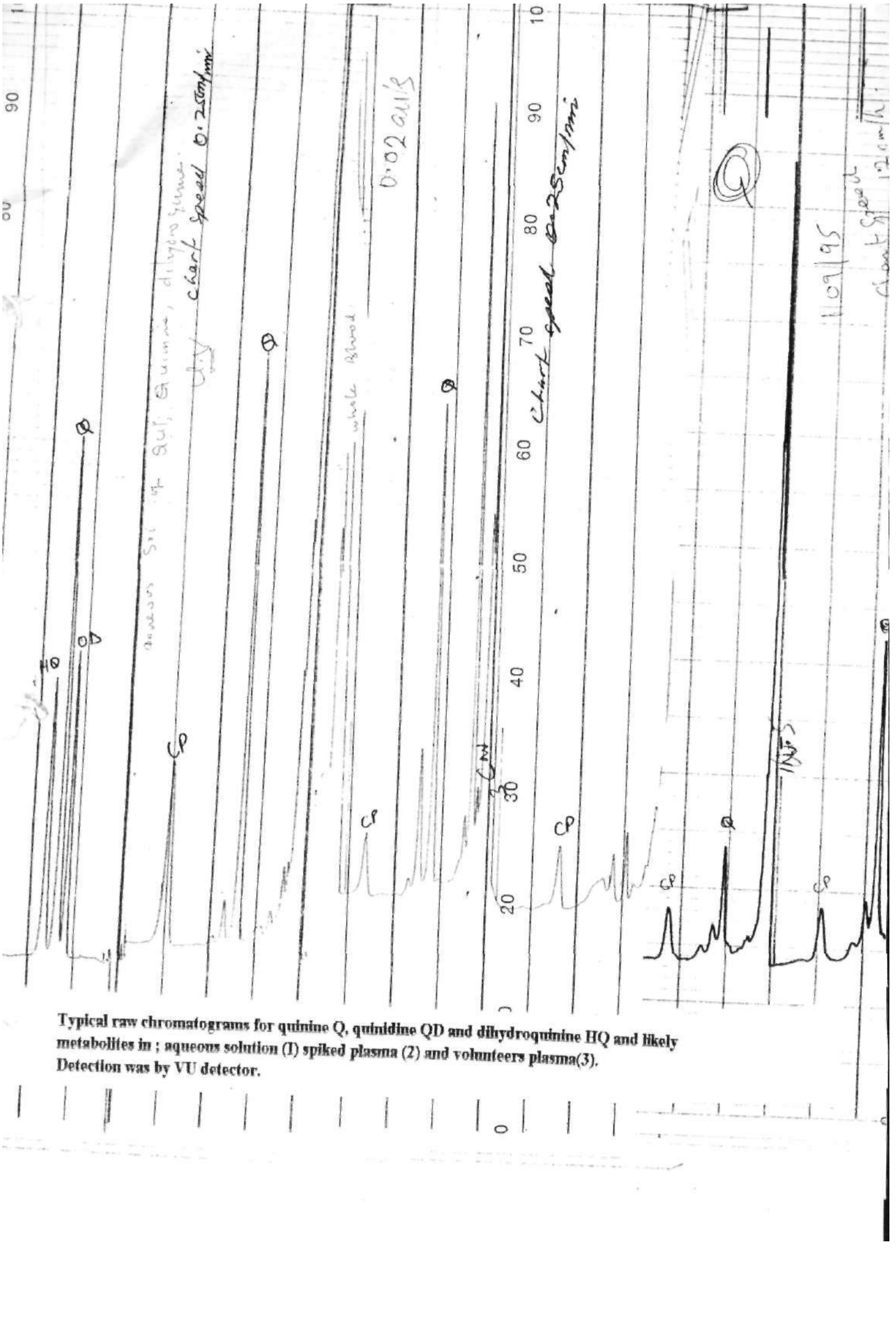
Typical raw chromatograms for proguanil P, chlorproguanil CP, cycloguanil C and 4-chlorophenylbiguanide 4-CPB in ; aqueous solution (I) spiked plasma (2) and volunteers plasma(3). Detection was by VU detector.



Typical raw chromatograms for quinine Q, quinidine QD and dihydroquinine HQ and likely metabolites in ; aqueous solution (I) spiked plasma (2) and volunteers plasma(3). Detection was by fluorescence detector.



Typical raw chromatograms for quinine Q, quinidine QD and dihydroquinine HQ and likely metabolites in ; aqueous solution (1) spiked plasma (2) and volunteers plasma(3). Detection was by VU detector.



Typical raw chromatograms for quinine Q, quinidine QD and dihydroquinine HQ and likely metabolites in ; aqueous solution (I) spiked plasma (2) and volunteers plasma(3). Detection was by VU detector.

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Short communication

Determination of proguanil and metabolites in small sample volumes of whole blood stored on filter paper by high-performance liquid chromatography

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Abstract

A method is reported for the determination of proguanil and its two metabolites cycloguanil and 4-chlorophenylbiguanide in whole blood and plasma samples obtained by thumbprick and stored dry on filter paper. The sample preparation involves liquid extraction from the filter paper and subsequent solid-phase extraction using C_8 Bond-Elut cartridges. Separation and quantification is by a previously reported ion-pairing high-performance liquid chromatographic system with ODS Hypersil as stationary phase and an 50:50 acetonitrile–pH 2 phosphate buffer mobile phase containing 200 m*M* sodium dodecylsulphate as ion-pairing agent. The analytical characteristics of the method are reported. Representative concentrations are shown as a function of time from a human subject after ingestion of a single 200-mg dose of proguanil hydrochloride. Typical ranges of concentration detected by the proposed method in human subjects were proguanil 12–900 ng/ml, cycloguanil 16–44 ng/ml and 4-chlorophenylbiguanide 1.5–10 ng/ml in whole blood.

1. Introduction

It has become generally accepted in recent years that sample pretreatment is an important stage in the determination of drugs in biological fluids at prophylactic and therapeutic levels. In some investigations of drug action it may be that the site of biological sample collection is geographically remote from the laboratory in which the analysis of the sample for specified drugs is to be carried out. In such instances an additional problem arises in the transport of samples from the collection area to the analytical laboratory. The accepted procedure of transporting frozen blood and plasma samples is expensive and subject to possible drug degradation, loss of drug and matrix changes due to thawing of samples during transport.

Pharmacokinetic and clinical studies involving malaria are often carried out in such circumstances. Recently several reports have appeared in the literature dealing with the transport of quinine [1], mefloquine [2,3], chloroquine [4,5] and pyramethamine–sulphadoxime combinations [6,7] by applying capillary blood samples to filter paper and drying. These show good correlation between samples stored in this way and those stored in conventional frozen form. Such a pro-

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2.4. Calibration

Linearity of response following the extraction was established by preparing five standards in whole blood, applying 0.2 ml of the resulting solutions to filter paper and, after drying, subjecting these to the above sample pretreatment and chromatography. The concentration ranges examined were: proguanil 21.8–870 ng ml^{-1}, cycloguanil 9.3–92.8 ng ml^{-1}, and 4-chlorophenylbiguanide 4.2–42.0 ng ml^{-1}. These concentration ranges were chosen as being appro-

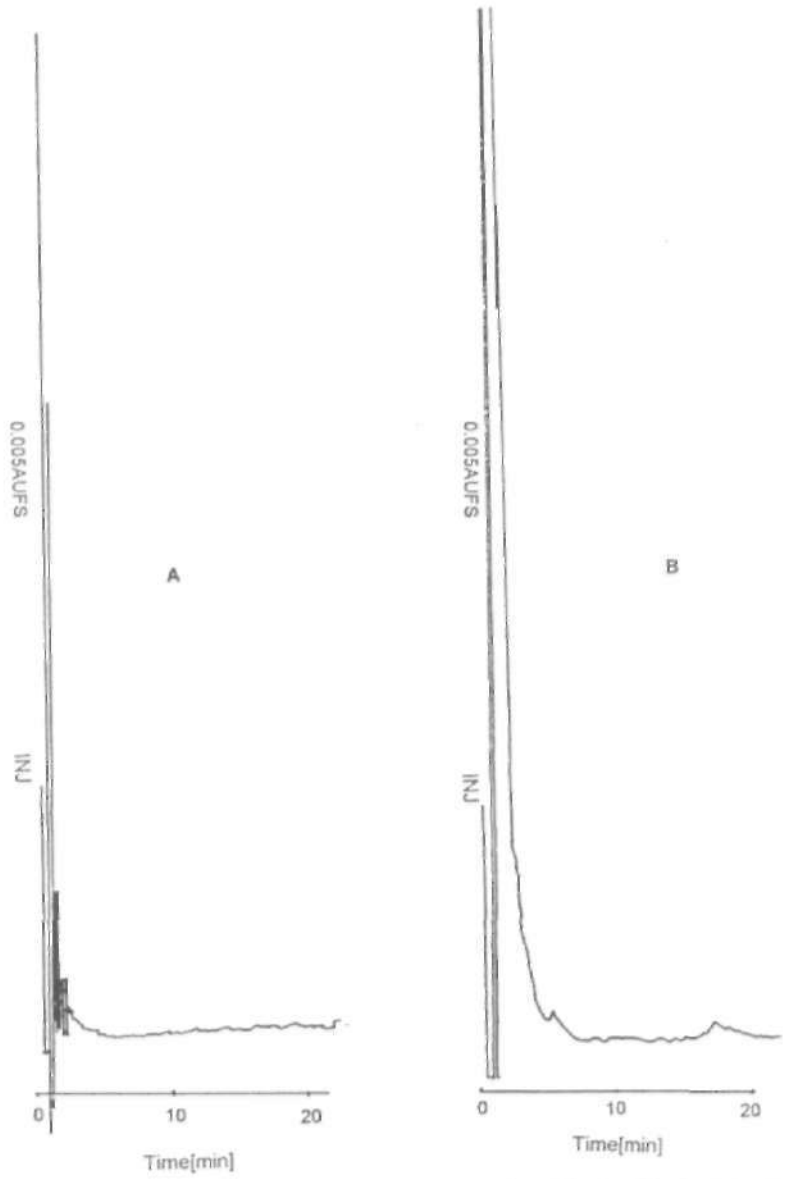


Fig. 1. Chromatograms of (A) blank filter paper and (B) filter paper containing 200 μl of blank whole blood following extraction and chromatography.

chromatogram obtained following analysis of 0.2 ml of whole blood spiked with 160 ng ml^{-1} proguanil, 70 ng ml^{-1} cycloguanil and 30 ng ml^{-1} 4-chlorophenyl biguanide confirms that this minor peak due to a plasma component has a longer retention time than any of the analytes or internal standard.

Table 1 summarises the main analytical characteristics of the overall method. Calibration is linear over the required ranges and the slopes of the calibration lines are of the same relative magnitude and precision to those obtained previously [8]. The recovery, as expected, is lower indicating that the transfer from the filter paper incurs additional losses. The limits of quantification found for proguanil and 4-chlorophenyl biguanide are, however, the same. This may be a consequence of a lower noise level in the detector used in the present determination. The limit of quantification for cycloguanil at the first peak eluted is appreciably higher than that reported previously which may be a result of increased matrix interference following extraction from filter paper. Comparison of the overall accuracy of the method as shown by the comparison of concentrations found compared with spiked values is high being on average 98.6 at fairly representative levels of concentration.

The procedure was applied to blood samples obtained from five subjects after ingestion of the prophylactic dose of proguanil. A representative plot of proguanil, cycloguanil and 4-chlorophenylbiguanide concentrations as a logarithmic function of time following dosing with proguanil (200 mg) is shown as Fig. 3. Detailed pharmacokinetics of these results will be the subject of a separate publication. It was found that mean C_{max} for proguanil was 848 ± 70 ng ml^{-1} which is comparable with the mean value found in previous pharmacokinetic studies [9,10]. Also the mean elimination half life for proguanil using the present technique was determined as 18.8 ± 7.0 h which is within the range previously determined using liquid whole blood samples. The correspondence of the C_{max} values indicates that little if any decomposition of proguanil has occurred

Table 1
Analytical characteristics of the method

	Proguanil	Cycloguanil	4-Chlorophenyl biguanide	Chlorproguanil
Calibration slope (mean ± S.D.)(× 10^3) (n = 5)	5.110 ± 0.643	6.100 ± 0.649	11.030 ± 0.995	–
Mean correlation coefficient (n = 5)	0.998	0.995	0.995	–
Percentage recovery (mean + S.D., n = 10)	75.0 ± 5.6	82.3 ± 4.9	87.0 + 21.0	66.4 ± 2.2
Concentrations spiked (ng/ml)	217.6 108.8 54.4	92.8 46.4 23.2	42.0 21.0 10.5	824.0 824.0 824.0
Corresponding concentrations found (ng/ml) (mean + %R.S.D.) (n = 10)	217.5 ± 3.0 108.3 ± 7.7 50.0 ± 12.0	95.2 ± 3.8 47.5 ± 7.3 20.7 ± 15.7	41.7 ± 5.6 20.6 ± 10.2 10.0 + 11.4	– – –
Limit of quantification (ng/ml)	3.0	15.0	1.5	–

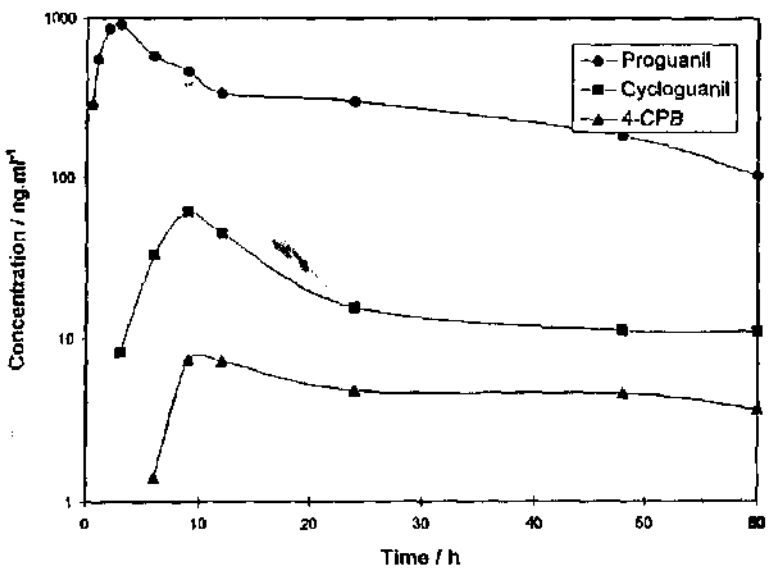


Fig. 3. Representative plots of the variation of the concentrations of proguanil, cycloguanil and 4-chlorophenylbiguanide as a logarithmic function of time for a human subject after ingestion of 200 mg proguanil.

during storage on paper. This is to be expected since, in a previous study [10], proguanil even in solution was shown to be a relatively stable compound.

4. Conclusions

From the above results it appears that whole blood samples of proguanil and its metabolites can be stored on filter paper and assayed reliably following extraction from the paper and that the previously published procedure has the required sensitivity of detection to allow the use of 200-μl blood samples.

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priate for the determination of plasma concentrations in human subjects following ingestion of a single 200-mg oral dose of proguanil [9].

2.5. Recovery

The combined recovery from filter paper and solid-phase extraction was determined by spiking whole blood with proguanil, cycloguanil and 4-chlorophenylbiguanide at concentrations of 108.8, 46.4 and 21 ng ml $^{-1}$, respectively. Samples of this (0.2 ml) were applied to filter paper, dried and subjected to the pretreatment described above. The peak heights recorded during the subsequent chromatography were compared with the corresponding values obtained by injecting directly aqueous solutions of the three biguanides containing the theoretical mass corresponding to complete recovery of each of the analytes from the samples.

2.6. Limit of quantification

The detection limits for the three analytes were determined by preparing serial dilutions of these compounds in blood, applying 0.2 ml of these to filter paper extracting as outlined above and determining the resulting peak heights of the individual analytes. The detection limit was taken as the concentration which gave a signal-to-noise ratio of 3 and the limit of quantification calculated as three times this concentration.

2.7. Precision

The within-day precision was determined by extracting and chromatographing ten 0.2-ml aliquots of a single sample of blood spiked at comparable concentrations to those used for determination of recovery. The day-to-day precision was measured as the variation in slope of the calibration line over a period of five days.

3. Results and discussion

Figs. 1A and 1B show representative chromatograms of a filter paper blank and filter paper spotted with 0.2 ml blank whole blood, respectively, after being subjected to the pretreatment and chromatography described above. No interfering peaks are visible for the blank paper and only a single peak from whole blood. Comparison with Fig. 2 which shows a representative

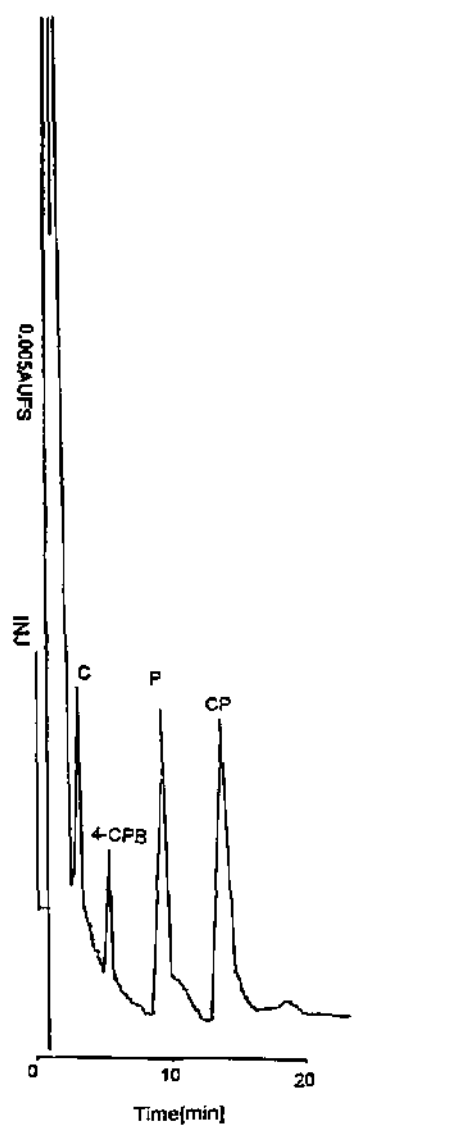


Fig. 2. Typical chromatogram of a 200-μl sample of whole blood spiked with proguanil (P) 160 ng ml $^{-1}$, cycloguanil (C) 70 ng ml $^{-1}$ and 4-chlorophenylbiguanide (4-CPB) 30 ng ml $^{-1}$ using chlorproguanil (CP) as internal standard.

cedure while offering advantages in the transport of drug samples places additional constraints on the analytical procedure. The drug must be removed from the filter paper and the total mass available for analysis will necessarily be smaller since the volume of blood which can be stored in this way is in the region of 75–200 μl so that the sensitivity of detection must be adequate.

The applicability of this procedure for the storing and transport of the biguanide antimalarial proguanil has not so far been reported. It is the purpose of the present paper to describe a procedure for the analysis of this drug and its two metabolites cycloguanil and 4-chlorophenyl biguanide when 200-μl samples of capillary whole blood from human subjects following ingestion of the prophylactic dose of 200 mg are stored in this way. The analysis is based on liquid extraction from the filter paper, subsequent solid-phase extraction and the chromatographic system previously described [8].

2. Experimental

2.1. Materials and equipment

Proguanil, cycloguanil and 4-chlorophenylbiguanide were kindly donated by ICI (Zeneca) (Macclesfield, UK). Acetonitrile was obtained from Rathburn Chemicals (Walkerburn, UK), water was distilled and further purified by a Millipore Milli-Q system (Millipore-Waters, Milford, MA, USA) and all other chemicals were of AnalaR or equivalent grade. The liquid chromatograph used was a modular system consisting of a Jasco 980 pump and Jasco 975 variable wavelength ultraviolet detector (Jasco, Tokyo, Japan) and incorporated a Rheodyne 7125 injection valve (Cotati, CA, USA) fitted with a 20-μl loop. The wavelength of detection used was 254 nm. The chromatographic column was 100 × 2 mm I.D., slurry packed in the laboratory with 3 μm particle size ODS Hypersil (HETP, Macclesfield, UK) at a pressure of 55 MPa in a solvent of propan-2-ol-hexane-methanol.

Samples to be analysed were blood samples (0.2 ml) which had been applied to filter paper and allowed to dry. For assay development and validation the blood samples were prepared by spiking whole blood with appropriate concentrations of proguanil and its two established metabolites. Samples were also assayed of capillary whole blood obtained from human subjects at various times after ingestion of the normal prophylactic dose of 200 mg proguanil.

2.2. Chromatographic separation

The ion-pairing system [8] mobile phase acetonitrile–10 m*M* phosphate buffer pH 2 (50:50) containing 200 m*M* sodium dodecylsulphate as pairing ion was used at a flow-rate of 0.4 ml min^{-1}.

2.3. Sample pretreatment

Spiked samples for calibration and subject samples were subjected to the following pretreatment. The blood spotted parts of the filter paper were cut into 5-mm pieces and placed in a glass test tube. The internal standard chlorproguanil was added as an aqueous solution (0.1 ml) typically of concentration 824 ng ml^{-1}. This was allowed to dry and 0.9 *M* ammonia solution (5 ml) was added. The tube was stoppered and heated at 30°C for 30 min. The mixture was vortex-mixed. After cooling an additional aliquot of 0.9 *M* ammonia solution (2 ml) was added and the tube placed in an ultrasonic bath for 30 min.

A C_8 Bond-Elut cartridge (Analytichem, Harbour City, CA, USA) was activated by washing with methanol (2 ml) and conditioned with ammonia solution (0.5 ml). The supernatant from the filter paper extract was transferred to the cartridge. The filter paper was washed with a further volume of ammonia (2 ml) and the washings also applied to the cartridge. The cartridge was washed with methanol (1 ml) and the biguanides eluted with a 10 m*M* solution of perchloric acid in methanol (1 ml). After evaporating the methanol under a stream of nitrogen the residue was reconstituted in water (0.1 ml) before injection of 20 μl into the chromatograph.