PHARMACEUTICAL EQUIVALENCE AND COMPARATIVE BIOAVAILABILITY OF MULTISOURCED ARTESUNATE AND AMODIAQUINE TABLETS IN SOUTH WESTERN NIGERIA

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2011

PHARMACEUTICAL EQUIVALENCE AND COMPARATIVE BIOAVAILABILITY OF MULTISOURCED ARTESUNATE AND AMODIAQUINE TABLETS IN SOUTH WESTERN NIGERIA

BY

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B.Pharm., M.Sc. (Ibadan)

A thesis in the department of

PHARMACEUTICS AND INDUSTRIAL PHARMACY

Submitted to the Faculty of Pharmacy

in partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

Department of Pharmaceutics and Industrial Pharmacy

University of Ibadan,

Ibadan.

APRIL 2011

CERTIFICATION

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ACKNOWLEDGEMENTS

The *in vitro* study was performed at the Pharmaceutics laboratory, University of Ibadan and the Lagos University Teaching Hospital Research Laboratory. While samples for the cross over study was collected at the Pathology Department, Federal Medical Centre, Abeokuta, they were analysed at the Central Research Laboratory, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria.

I would like to express my sincere gratitude to my supervisor, Dr. Ignatius Onaga. Your vast knowledge of biopharmaceutics and pharmacokinetics is truly inspiring.

Dr. (Mrs) O.A Odeku, my head of department, Prof. O.A Itiola, immediate past head of department, and all colleagues at the Department of Pharmaceutics and Industrial Pharmacy, thank you for creating an inspiring work atmosphere.

Special mention must be made for the invaluable contributions, love and support of Dr. F.T Ajayi, Head of Department of Pharmaceutical Services, Federal Medical Centre, Abeokuta.

I am grateful to my colleagues and friends, Dr. Adegoke of the Department of Pharmaceutical Chemistry, University of Ibadan, Dr. Soyinka of the Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Dr. Oyebola of the Department of Pain and Palliative Medicine, Federal Medical Centre, Abeokuta, Mr Awe and Mr Folarin of the Pathology Department Federal Medical Centre, Abeokuta, Dr. Kunle Odunfa of the Department of General Practice, Queen Mary's Hospital Sidcup, Kent, England and Segun Oduwaye of the University of Agriculture, Abeokuta for their contributions to this work.

I would also like to express my gratitude to my parents Cecelia and Ayinde, my sisters Kemi and Lolade, Kunle my brother for his technical assistance, and thank you Tope Soretire, Funmi Soretire, Tolu and Lanre Akinyemi, Gbolahan Fabiyi, Toke, Laolu, Sheriff, Doyin and Seun Odesesan for your eternal optimism and support.

Financial support was obtained from the Federal Medical Centre, Abeokuta, Ogun State, Nigeria.

ABSTRACT

Malaria is one of the most devastating parasitic diseases in the world and remains a major public health problem in Sub-Saharan Africa. First-line treatment of uncomplicated malaria includes the use of artesunate and amodiaquine. However, the existence of counterfeit and substandard artesunate and amodiaquine in the market may lead to therapeutic failures or development of resistance when consumed. The aim of the study was to examine the pharmaceutical equivalence of different brands of artesunate and amodiaquine tablets and, their bioavailability and tolerability when given as monotherapy and combination therapy.

Fifteen brands of artesunate and five brands of amodiaquine tablets selected randomly were obtained from retail drug outlets in Ogun, Oyo and Lagos states in South western Nigeria and were subjected to various physicochemical tests including drug content, disintegration and dissolution times. The bioavailability after oral administration of single doses of artesunate (200mg), amodiaquine (600mg), their fixed combination (200mg/612.6mg), and non-fixed combination (200mg+600mg) as measured by high performance liquid chromatography of plasma samples and tolerability were compared. Sixteen healthy male volunteers aged between 18 and 45 years distributed into four groups received treatments at four different occasions in an open label, Latin square, 4-phase, cross-over study. Absorption was determined from area under the plasma drug concentration-time curves (AUC), peak plasma concentration (C_{max}) reached and time taken to reach peak plasma concentration (T_{max}). Impairment of absorption was indicated by at least one statistically significant parameter. Biochemical test was measured using serum albumin while body fat was derived from the body mass index. Data collected from physicochemical tests were evaluated using correlation and Chi square analyses while those of serum albumin and body fat, parent drugs and their main metabolites(dihydroartemisinnin and desethylamodiaquine) were assessed using logistic regression, Students' t-test and ANOVA.

Physicochemical tests revealed that 33.0% of the artesunate tablets and 80.0% of the amodiaquine tablets analyzed met compendial standards. Minimum absorption occurred when tablets were given as monotherapy and in combination. The

bioavailability of artesunate when given in fixed combination with amodiaquine was lowered (C_{max} ratio-76.4%, $p<0.001$) compared with monotherapy. Taking amodiaquine in fixed combination with artesunate markedly increased its bioavailability (AUC ratio-159.9%, $p<0.001$) when compared with monotherapy. However, concurrent administration of artesunate in non-fixed combination with amodiaquine reduced its bioavailability (T_{max} ratio-209.7%, $p<0.001$) when compared with monotherapy. The bioavailability of amodiaquine was about twice as high when given in non-fixed combination (AUC ratio-195.4%, $p<0.05$) compared with monotherapy. Adverse events of concern were anaemia (81.25%), asthenia (62.5%) and neutropenia (25%). Asthenia was largely correlated with serum albumin in volunteers that took fixed dose combination (OR=9.3, $p<0.05$). Adverse effects were increased in volunteers that had higher body fat percentage (OR= 0.571, $p>0.05$).

Pharmaceutically inequivalent and subpotent artesunate and amodiaquine tablets are in circulation in Southwestern Nigeria and this suggests the need for regulatory authorities to rigorously monitor their quality. There was severe impairment of rate and extent of absorption as a result of co-administration of the drugs. It is therefore recommended that artesunate should not be coadministered with amodiaquine in clinical setting.

Keywords: Antimalarial tablets, Artesunate, Amodiaquine, Drug bioavailability, Pharmaceutical equivalence.

Word count:497

DEDICATION

This research work is dedicated to the Lord Jesus, the teacher and the only wise

AND

my happy family

Yemisi, Precious, Peace and Price.

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CHAPTER 1

INTRODUCTION

Malaria is perhaps the most devastating parasitic disease the world has ever known (WHO, 2005a). The disease remains a major public health problem and a serious challenge to socioeconomic development. In spite of huge amount of money being spent on the procurement of mosquito nets, antimalarial drugs, training and public enlightenment programmes to control malaria, there is a growing concern globally on the quality of marketed medicines. Studies in Nigeria have shown that there is a proliferation of counterfeit and substandard drugs in circulation (Taylor *et al*., 2001; Mbachu, 2002; Campos and Pradhan, 2007). Some products are substandard due to:

1. deliberate production of such drugs (Chandra, 2011)
2. deterioration during transportation, warehousing and distribution.
3. production processes that do not adhere to GMP standards.

Poor quality, substandard and fake artemisinin combination therapy (ACT) antimalarials may lead to poor bioavailability, therapeutic failure, development of resistance and a rise in morbidity and mortality. Artemisinnin-based combination therapies recommended by the WHO, though highly efficacious are largely unaffordable by the majority of the people due to high cost.

Nigeria, in 2004, adopted the use of ACTs as first line treatment in her national antimalarial treatment policy, for uncomplicated malaria in accordance with WHO guidelines (National Antimalaria Treatment Policy, 2005). Various artesunate formulations may have different bioavailabilities and development of resistance will be accelerated if suboptimal doses are consumed (White, 1997; Na-Bangchang *et al*., 1998). Poor compliance, resulting from adverse drug effects is a drawback in the effort to achieve positive clinical outcomes. The bioavailabilities of drugs have been found to be affected by fluid/food intake, gastrointestinal transit time and changes in release characteristics of the drug from the dosage form.

From drug use perspective, knowledge of factors such as the drug quality, parasite susceptibility, drug dosage and pharmacokinetics, and host factor variability are important in the interpretation of treatment outcomes.

The present study examines some factors that may be responsible for therapeutic failure by assessing the quality and pharmaceutical equivalence of different brands of artesunate and amodiaquine tablets, their bioavailability and tolerability when given singly and in combination. To the best of knowledge, few studies have addressed effect of coadministration on bioavailability and the effect of biochemical and anthropometric factors on development of adverse effects.

1.1 The malaria burden

The disease is caused by infection with protozoa of the genus *Plasmodium.* In humans, four Plasmodial species cause malaria: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae*, with *P. falciparum* causing the most severe infection. *Plasmodium falciparum* worldwide infects 300-500 million people every year causing the highest burden and mortality of Sub-Saharan Africa (SSA), where nearly one million children die yearly from the disease (Snow *et al*., 1999). Worldwide, an estimated 3.3 billion people are at risk of malaria with 90% of deaths occurring in Africa. The overall burden of malaria can reach 2 million casualties per year, mostly among young children (aged under 5 years) and pregnant women in Sub-Saharan Africa (Sachs and Malany, 2002). Malaria constitutes 10% of the overall disease burden. It accounts for 30-50% of in-patient admissions, and up to 50% of outpatient visits in areas with high malaria transmission (Roll Back Malaria-World Health Organization, 2000). The heavy toll among children in SSA is a result of a combination of factors such as:

1. the ideal climatic and ecological condition for the most efficient vector, the *Anopheles gambiae* mosquito, to thrive; 2. the most deadly species of plasmodium parasites, *P. falciparum* is also the most common parasites in the sub-region; 3. and it is in SSA where underdevelopment and lack of good-quality health care have hindered the control and treatment efforts that had significant impact in other regions of the world (Whitehead et al., 2001). The burden of malaria in Africa is influenced by poverty, rapid and transformative economic development, chronically weak health

systems, and highly efficient malaria parasites and vectors. Furthermore, in highly endemic areas, the severity of infection and mortality from *P.falciparum* is highest amongst pre-school age children due to insufficient degree of partial immunity in this age group (Hviid, 2005; Meremikwu *et al*., 2009; Pullan *et al*., 2011).

Nigeria with a population of about 148 million (Focus Nigeria, 2011), contributes a quarter of the malaria burden in Africa – 50% of the population will have at least one malaria attack each year. Malaria is responsible for 30% of childhood deaths yearly and 11% of maternal deaths in Nigeria (Yishua and Chiejna, 2011). Malaria has been referred to as a disease of poverty (Worrall *et al*., 2005). Besides causing ill health and death, malaria also affects the social and economic status of the individual, the family, the community and the nation as a whole. For countries whose economies depend on agricultural activities, malaria causes poverty. Since malaria affects people most during the rainy seasons, this interferes with farm activities, thereby causing a vicious cycle of poverty and disease (Worrall *et al*., 2005). In some countries with a heavy malaria burden, the disease may account for as much as 40% of public health expenditure (Roll Back Malaria-World Health Organization, 2000).

The life cycle of *Plasmodium* is complex, comprising a sexual phase (sporogony) in the mosquito (vector) and an asexual phase (schizogony) in man (Figure 1.1). Infection in man is usually caused by *sporozoites* from the bite of an infected female anopheles mosquito. It may rarely be acquired in other ways, congenitally via the placenta, or through blood transfusion, needle stick injuries, or organ transplantation. After an infected mosquito bite, sporozoites rapidly enter liver parenchymal cells, where they undergo *exoerythrocytic* or *pre-erythrocytic schizogony* forming *tissue schizonts* which mature in 5-15 days and release thousands of *merozoites* into the blood on rupture of the cell. Merozoites enter erythrocytes where they transform into *trophozoites*. These produce *blood schizonts* which, as they mature, rupture and are released. Each of these will provide 8–24 new merozoites, which are released at the burst of the erythrocytes into the circulation, which can infect other erythrocytes. This is termed the *erythrocytic cycle* and it is this periodic release of merozoites that is responsible for the characteristic periodicity of the fever in malaria. After several erythrocytic cycles, depending on the type of malaria, some

erythrocytic forms develop into sexual *gametocytes*. Ingestion of infected blood containing gametocytes by a biting female mosquito allows the life cycle to be completed with the sexual phase in the mosquito.

In *P. vivax* and *P. ovale* infections, some of the sporozoites entering the liver cells enter a latent tissue stage in the form of *hypnozoites* which are responsible for recurrence of malaria caused by these organisms. Recurrences resulting from the persistence of latent tissue forms are often referred to as *relapses* while renewed attacks caused by persistent residual erythrocytic forms are termed *recrudescences*.

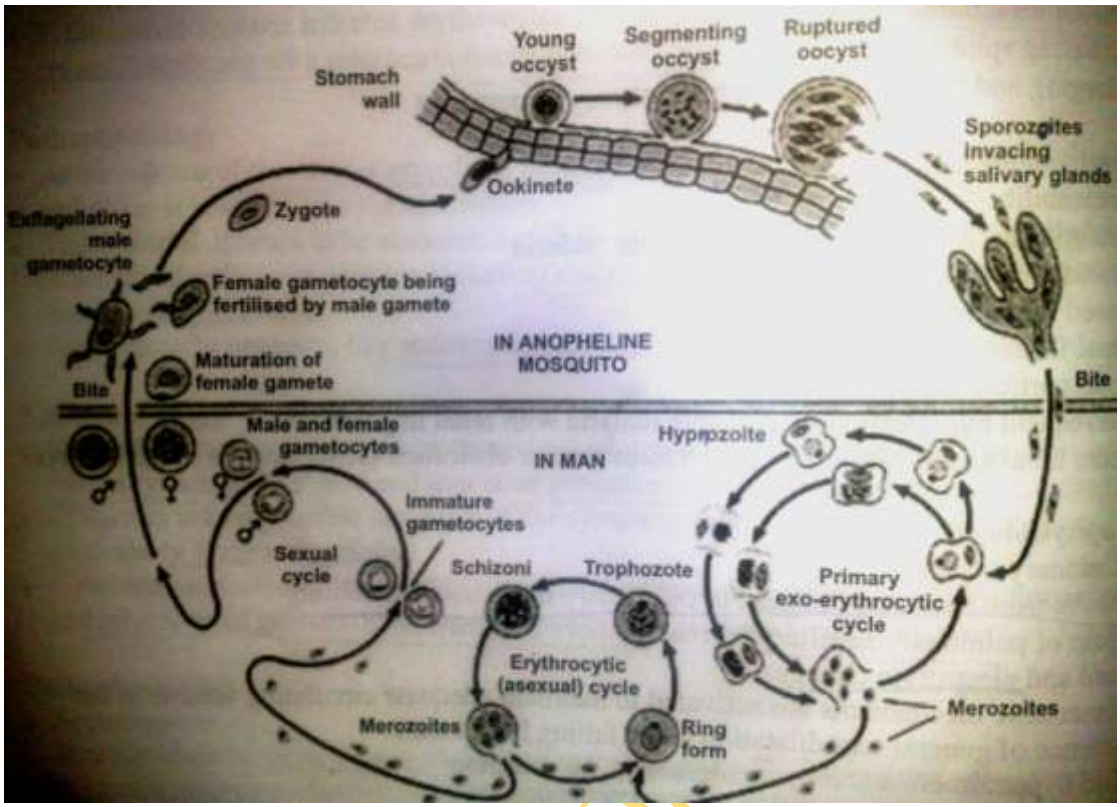


Figure 1.1: A representation of the life cycle of Plasmodium falciparum.

(Life cycle of the malaria parasite, 2011)

1.1.1 Clinical Manifestations of Malaria

The clinical symptoms of malaria are varied and non-specific; they usually include fever, fatigue, malaise, headache, myalgia, and sweating. Vomiting occurs in about 20% of patients, and mild diarrhoea in less than 5%. As the infection progresses, the spleen enlarge and the patient may develop anaemia and thrombocytopenia. The prognosis worsens with increasing parasite counts, more mature parasites, and more neutrophils containing malaria pigment (White, 1996a). Severe falciparum malaria may be complicated by acute renal failure, pulmonary oedema, and cerebral dysfunction. Since none of the clinical features of malaria are diagnostic, a definitive diagnosis depends upon the demonstration of parasites in stained blood films. However, antimalarial drug treatment should not be withheld in the absence of positive blood films if there is clinical suspicion of malaria. Any patient who has been in a malaria area in the previous two months should be considered to have malaria until proved otherwise.

1.1.2 Antimalarial Drugs

Antimalarial drugs can be classified by the stage of the parasitic life cycle they affect. Thus:

Blood schizontocides act on the erythrocytic stages of the parasite that are directly responsible for the clinical symptoms of the disease. They can produce a clinical cure or suppression of infection by susceptible strains of all 4 species of malaria parasite but, since they have no effect on exoerythrocytic forms, do not produce a radical cure of ovale or vivax malaria.

Tissue schizontocides act on the exoerythrocytic stages of the parasite and are used for causal prophylaxis to prevent invasion of the blood cells, or as anti-relapse drugs to produce radical cures of vivax and ovale malaria.

Gametocytocides destroy the sexual forms of the parasite to interrupt transmission of the infection to the mosquito vector.

Sporontocides have no direct effect on the gametocytes in the human host but prevent sporogony in the mosquito.

Antimalarial drugs can also be classified by the chemical group to which they belong, which in turn determines the stage of the life cycle they affect. The principal antimalarials, classified according to drug group and activity are:

1. The **4-methanolquinoline** derivatives, such as the cinchona alkaloids and mefloquine. The main cinchona alkaloid quinine and its optical isomer quinidine are rapid-acting blood schizontocides with some gametocytocidal activity. Mefloquine also acts as a blood schizontocide.
2. The **4-aminoquinolines**, such as chloroquine, hydroxychloroquine, and amodiaquine, are rapid-acting blood schizontocides with some gametocytocidal activity.
3. The **8-aminoquinolines**, such as primaquine and tafenoquine, are tissue schizontocides; primaquine also has gametocytocidal activity and some activity at other stages of the parasite's life cycle.
4. The **biguanides**, such as proguanil and chlorproguanil, have dihydrofolate reductase inhibitory activity and thus inhibit folate synthesis in the parasite. They are tissue schizontocides and slow-acting blood schizontocides with some sporontocidal activity.
5. The **diaminopyrimidines**, such as pyrimethamine. Pyrimethamine is a dihydrofolate reductase inhibitor and has actions similar to those of the biguanides. It is usually administered with other antimalarials that inhibit different stages of folate synthesis, such as a sulfonamide or sulfone, to form synergistic combinations.
6. The **dichlorobenzylidine** lumefantrine, a blood schizontocide given in combination with the artemisinin derivative artemether.
7. The **hydroxynaphthoquinones**, such as atovaquone. Atovaquone has blood schizontocidal activity and is usually given in combination with proguanil.
8. The **9-phenanthrenemethanols**, such as the blood schizontocide halofantrine.
9. The **sesquiterpene lactones**, such as artemisinin and its derivatives, act mainly as blood schizontocides.
10. The **sulfonamides**, such as sulfadoxine and sulfametopyrazine, which are dihydropteroate synthase inhibitors and thus inhibit folate synthesis. They act

mainly as blood schizontocides and are usually administered with pyrimethamine.

11. The **tetracyclines**, such as doxycycline and tetracycline, are blood schizontocides and also have some activity against tissue forms.
12. The **lincosamide**, clindamycin, which is also sometimes used, has a similar action to the tetracyclines.
13. The **sulfone**, dapsone, which has similar antimalarial actions and uses to the sulfonamides.

The differing mechanisms of action of antimalarial drugs sometimes allow the use of combinations of antimalarials to improve efficacy. Such combinations are usually designed to potentiate each other, for instance by acting at sequential steps in the parasite's folic acid pathway (e.g. pyrimethamine with sulfadoxine or dapsone). Alternatively, a combination may be complementary, when the drugs involved act against different stages in the life cycle of the parasite (e.g. the use of chloroquine with primaquine to produce radical cure of *P. vivax* or *P. ovale* infections). The rationale behind the use of such combinations may be to enhance efficacy, particularly when drug resistance is a problem, or it may be an attempt to delay the development of resistance to one or more of the drugs concerned (The Merck Manual, 2011).

1.1.3 Malaria Treatment

There are two main levels of treating malaria infection. At the individual level, treatment is aimed at cure, prevention of severity and complication and minimizing mortality. While at the public level, an additional goal is to prevent emergence and the spread of resistance to antimalarials (WHO, 2005b). In order to achieve these goals, the most efficacious antimalarials are advocated (Attaran *et al*, 2004). In this regards, the ACTs have been recommended by the WHO (WHO, 2005b). However, of all the available antimalarials, ACTs are the most costly (UNICEF, 2007) and this has constrained their availability in the poor countries of SSA (Olliaro and Taylor, 2004). The production of artemisinin has been increased in recent years. There are several initiatives to reduce the cost of ACTs. Development of entirely synthetic antimalarial peroxides is one strategy and a large number of substances have been synthesized

during the last years. However, it has been difficult to find candidates with satisfactory bioavailability which are easily synthesized, stable and inexpensive. Hybrid molecules combining two mechanisms of actions, trioxaquines, have shown to be more stable peroxides and are hence promising developments (White, 2008). The tetraoxanes is another potential group of new peroxides which have been reported to be highly active, inexpensive and demonstrate low toxicity (Opsenica *et al*, 2008). The WHO has set up a multilateral Global Funds to Fight AIDS, malaria and Tuberculosis (GFAMT) to assist resource poor nations especially in SSA to assess these drugs (GFAMT.ORG). While considerable funding is currently invested to scale up anti-malaria tools and interventions, delivery modalities remain a major challenge especially in poor, remote and unstable areas.

Malaria is treated with a *blood schizontocide*, selected with due regard to the prevalence of specific patterns of drug resistance in the area of infection.

Antimalarials are generally given by mouth, although in order to obtain a rapid response in patients with severe or complicated falciparum malaria, it may be necessary to start with parenteral therapy, the patient being transferred to oral therapy when feasible. Where there are only minimal health care facilities and parenteral therapy is not possible or may be delayed, artemisinin or artesunate suppositories may be given.

1.1.4 Treatment of falciparum malaria.

In most parts of the world *P. falciparum* is now resistant to chloroquine and therefore, apart from the rare circumstance of exposure in one of the few remaining areas of chloroquine sensitivity, chloroquine is not suitable for treatment. Resistance to pyrimethamine-sulfadoxine has also occurred in most countries where it was used in place of chloroquine (Greenwood *et al*., 2005). In uncomplicated falciparum malaria (symptomatic malaria without signs of severity or evidence of vital organ dysfunction) combination drug therapy is recommended by WHO for treatment in all *malaria endemic areas* experiencing resistance to monotherapy with chloroquine, amodiaquine, or pyrimethamine-sulfadoxine. Drugs with differing mechanisms of action but with similar half-lives should be used to avoid exposure of the parasite to a single drug for a prolonged period and to prevent the emergence and spread of drug-

resistant parasites. Pyrimethamine-sulfadoxine and atovaquone-proguanil (*Malarone*®) rely on synergy between the 2 components and are operationally considered single products; they should not be considered combination therapy when used alone. The most widely recommended combinations are those that include an artemisinin-based drug as one of the components (ACT). Recommended ACTs are:

- artemether-lumefantrine (*Coartem*®)
- artesunate and amodiaquine (in areas where resistance to amodiaquine is low)
- artesunate and pyrimethamine-sulfadoxine (in areas where pyrimethamine-sulfadoxine efficacy is still high)
- artesunate and mefloquine (should only be used in areas of low transmission)

Amodiaquine and pyrimethamine-sulfadoxine is still an effective option for some areas in West Africa where susceptibility remains high and for those countries unable to supply ACTs. In *non-endemic areas* the British Infection Society and the Treatment Group of the Advisory Committee on Malaria Prophylaxis recommend treating uncomplicated falciparum malaria or mixed infections with one of the following:

- oral quinine sulphate plus follow-on treatment with either doxycycline or clindamycin
- atovaquone-proguanil (*Malarone*®)
- artemether-lumefantrine (*Coartem*®)
- mefloquine; however, mefloquine is now rarely used for treatment because of concerns about resistance.

In the USA, the Centers for Disease Control (Centers for Disease Control and Prevention, 2007) recommend that uncomplicated falciparum malaria acquired in a chloroquine-sensitive area may be treated with oral chloroquine. Malaria acquired in chloroquine-resistant areas is treated with one of the following:

- quinine sulphate plus doxycycline, tetracycline, or clindamycin
- atovaquone-proguanil (*Malarone*®)

- mefloquine (although it should only be considered as a treatment option in patients who cannot use quinine sulphate or atovaquone-proguanil (*Malarone*®) due to a higher rate of severe neuropsychiatric events associated with treatment doses)

In severe or complicated falciparum malaria including cerebral malaria, parenteral treatment is required to produce adequate blood concentrations as quickly as possible(WHO, 2006a). Patients need to be closely monitored while undergoing parenteral therapy and treatment is changed to an oral antimalarial as soon as the patient's condition permits. In *malaria endemic areas* that are chloroquine-resistant, or where sensitivity to chloroquine is not known, quinine is usually given intravenously, starting with a loading dose. Intramuscular artemether is an alternative to intravenous quinine. In areas of low transmission intravenous artesunate is the recommended first choice. Intramuscular artemotil (arteether) should only be used if other above alternatives are not available and intravenous quinidine should only be used if parenteral quinine or artemisinin derivatives are not available. Where there are only minimal health care facilities or when parenteral therapy is not possible or may be delayed, artemisinin, artesunate, artemether, or dihydroartemisinin suppositories may be given. Follow-on treatment is with either doxycycline or clindamycin (in pregnant women and in children) for 7 days.

In *non-endemic areas* intravenous quinine plus either doxycycline or clindamycin are recommended by authorities in the UK (British Infection Society, 2005). The recommendations are similar in the USA (Centers for Disease Control and Prevention, 2007) but intravenous quinidine gluconate may be used in place of quinine. Supportive therapy in patients with severe or complicated malaria needs to be directed at reducing hyperpyrexia, controlling convulsions, maintaining fluid balance, and correcting hypoglycaemia. Severe malaria is associated with rapid development of anaemia, and blood transfusions are recommended for children with a haemoglobin concentration of less than 5 g per 100 mL (haematocrit less than 15%) living in areas of high transmission or for those with a haemoglobin level of 7 g per 100 mL (haematocrit threshold of 20%) living in an area of low transmission. Although the benefit of exchange transfusion has not been proven in comparative trials, CDC

recommends that it be considered for patients with a parasite density of more than 10% or if the patient has cerebral malaria, renal complications, or non-volume overload pulmonary oedema (Centers for Disease Control and Prevention, 2007). Prophylactic use of anticonvulsants is not recommended and the use of intravenous or intramuscular phenobarbital resulted in increased mortality due to respiratory arrest (WHO, 2006b). Since iron might be involved in the pathogenesis of cerebral malaria, the iron chelator desferrioxamine has been tried in addition to standard antimalarial therapy, but any benefit is yet to be established and WHO advises against its use (WHO, 2006b). Anecdotal reports of the value of corticosteroids in cerebral malaria have not been substantiated by controlled studies and they have no place in the management of this condition (Prasad and Garner, 1999; WHO, 2006b). Other approaches such as the use of hyperimmune serum or monoclonal antibody to tumour necrosis factor have also been unsuccessful.

1.1.5 New generation of antimalarial drugs: trioxaquines

As recommended by the WHO, the combination of at least two drugs having different modes of action must be used to prevent the development of drug resistance. Dechy-Cabaret *et al.*, (2000) therefore adopted a covalent bitherapy strategy by preparing new molecules named trioxaquines by covalently attaching a trioxane, the moiety responsible for the activity of artemisinin, to a 4-aminoquinoline entity, a key constituent of chloroquine (Dechy- Cabaret *et al*., 2000; Rodriguez *et al*., 2003). DU-1102, the first trioxaquine proved very active *in vitro* on chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*, both on laboratory strains (Dechy-Cabaret *et al*., 2000) and on human isolates. DU-1102 is a modular molecule that can be prepared through a convergent synthesis from the cis-bicyclo [3.3.0] octane-3, 7-dione in place of the 1,4-cyclohexanedione used to prepare DU-1102 (Dechy-Cabaret *et al*., 2002).

1.1.6 Vaccine and genetic approaches

Over the years, researchers confronting the extraordinarily complex parasite have suffered a string of disappointments interspersed with some high-profile setbacks, as promising candidate vaccines have failed to perform up to expectations.

The scientific obstacles are enormous: Compared to a virus, with its dozen or so genes and relatively monomaniacal approach to evading the human immune system, the malaria parasite has 14 chromosomes, perhaps 7000 genes, and a four-stage life cycle as it passes from humans to mosquitoes and back again. The existing different species of parasites and their multistage life are obstacles for the efficiency of vaccine. Moreover, dozens of new vaccines are in the works, employing a host of technologies that promise to attack the parasite at every vulnerable point of its multistage life. Researchers have predicted that within 5 or 10 years there will be a successful vaccine that will actually prevent malaria infection (Taubes, 2000). With the actual human, related plasmodium species and mosquito genome sequences, researchers now have in hand the genetic blueprints for the parasite, its vector, and its victim. This will provide the ability to take a holistic approach in understanding how the parasite interacts with the human host. With that approach, new antimalarial strategies should be possible (Pennisi, 2000). Transgenic mosquitoes could be one product of these studies.

The goal would be to replace the natural mosquito populations ravaging developing countries by "designer mosquitoes," genetically modified so that they are unable to transmit malaria parasite. The development of this technique is crucial for scientists studying the biology of the mosquito and its interactions with the malaria-causing parasite. Genetic modification of mosquitoes offers exciting possibilities for controlling malaria, but success will depend on how transformation affects the fitness of modified insects (Enserink, 2002; Flaminia *et al*., 2003)

1.2 Administration of artemisinin derivatives.

To overcome the poor solubility of *artemisinin* in water a number of dosage forms and routes have been tried. Also, several more potent derivatives with more suitable pharmaceutical properties have been developed, notably the methyl ether derivative, *artemether*, and the ethyl ether derivative, *artemotil*, which are more lipid soluble; the sodium salt of the hemisuccinate ester, *sodium* artesunate**,** which is soluble in water but appears to have poor stability in aqueous solutions; and *sodium artelinate*, which is both soluble and stable in water. Other derivatives that have been studied include *arteflene*.

Several preparations of artemisinin derivatives are available and these include oral formulations of artemether, artesunate, artemisinin itself, and *dihydroartemisinin*; intramuscular formulations of artemotil, artemether, and artesunate; intravenous formulations of *artelinic acid* and artesunate; and suppositories of artemisinin, artesunate, and dihydroartemisinin. The following doses are recommended by WHO for the treatment of uncomplicated falciparum malaria. Artesunate, when used with other antimalarials (amodiaquine, mefloquine, or pyrimethamine-sulfadoxine), is given orally to adults and children in a dose of 4 mg/kg daily, as a single dose, for 3 days. The problem with the artemisinin drugs is that when they are used alone over short periods i.e. less than 5 days , clearance of parasitaemia from the blood is only temporary in up to 50% of patients. This has been attributed to the short time that it takes for artemisinin drugs to be eliminated from the body. Higher efficacy can be obtained by 5 and especially 7 day regimens but this is associated with reduced compliance in out-patients.

1.2.1 Mechanism of action of artemisinin and its derivatives

Artemisinin was developed from an ancient Chinese herbal remedy. *Artemisia annua* (sweet wormwood or`qinghao') was used by Chinese herbal medicine practitioners for at least 2000 years. In 1596, Li Shi zhen, a famous herbalist, recommended it to patients with fever. In 1967, Chinese scientists screened a series of traditional remedies for drug activities, and found that extracts of qinghao had potent antimalarial activity. In 1972, the active ingredient was purified and first named qinghaosu (essence of qinghao), and then later renamed artemisinin. Western interest in Artemisinin derivatives (artesunate, artemether, dihydroartemisinin, arteether) began to grow as multidrug resistant *Plasmodium falciparum* strains began to spread. Hundreds of synthetic second generation artemisinin derivatives and other natural peroxide compounds with good antimalarial activity have been reported like yingzhaosu and arteflene (Lian *et al*., 1988; Hofneiz *et al*., 1994). Due to their potent antimalarial activity, fast action, and low toxicity, artemisinin and its derivatives have distinguished themselves as a new generation of antimalarial drugs. Actually, it has been established that the dihydroartemisinin combined to the holotransferrin would be a promising drug against cancer (Singh and Lai, 2001). The unusual structure of

artemisinin molecules might be indicative of a different mode of action from those of other antimalarial drugs and hence the high potency against the resistant strains. Although the mechanism of its antimalarial activity is not clear and still under debate, there is general agreement that the endoperoxide bridge is essential for the antimalarial activity of artemisinin since deoxyartemisinin compounds which lack the endoperoxide moiety are inactive. Meshnick *et al*., (1993) proposed a two-step mechanism for the antimalarial action of endoperoxide:
In the first step, artemisinin is activated by intraparasitic heme or free Fe (II) ion to produce free toxic carbon-centred radicals, confirmed by electron paramagnetic resonance (EPR) studies (Meshnick *et al*., 1993; Taranto *et al*., 2002].
In the second step, once formed, the artemisinin-derived free radicals appear to damage specific intracellular targets, possibly via alkylation (Berman and Adams, 1997). But Pandey *et al*. proposed three possible ways for the effect of endoperoxide drugs on malaria (Pandey *et al*., 1999; Kannan *et al*., 2002):

- Inhibition of hemoglobin degradation
- Inhibition of hemozoin biosynthesis
- Interaction of artemisinin with hemozoin leading to the breakdown of the hemozoin pigment which could then form a complex with the heme unity.

These mechanisms are supported by the characterization of a covalent adduct between artemisinin and heme (Robert and Meunier, 1997) and by protein alkylation. Artemisinin also forms covalent adducts with protein but not with DNA (Yang *et al*., 1994). Thus, heme is both an activator and target of the artemisinin derivatives (Posner *et al*., 1995). There is much stronger evidence that carbon-centred free radicals are involved. In fact, monoelectronic transfer from iron (II) to peroxide resulted in the cleavage of endoperoxide bond with primary formation of an unstable oxygen-centred radical, rearrangement and creation of toxic C4-centred free radicals. It has been proposed that heme attacks the endoperoxide linkage of artemisinin either at the O1 or O2 position (Tonmumphean, 2001). In pathway A, heme iron attacks the compound at the O2 position. Later, it rearranges to form C4 free radical. Thus the presence of heme is necessary for the activation of artemisinin into an alkylating agent, which preferentially attacks proteins. The fact that artemisinin becomes

cytotoxic in the presence of ferrous ions, have triggered some researchers to study its effect on the therapy of cancer. Since iron influx is high in cancer cells, artemisinin and its analogues, after incubation with holotransferrin which increase the concentrations of ferrous iron in cancer cells, selectively kill cancer cells (Singh and Lai, 2001). In addition to the more largely accepted mechanisms summarized above, other mechanisms of action have also been proposed. For example, Jefford proposed that peroxides could interrupt the detoxification process of heme by transferring an O atom to heme, creating iron-oxene or oxyheme intermediates, which subsequently disable parasite (Jefford *et al*., 1995).

1.2.2 Artesunate: Physicochemical Properties

1.2.2.1 Drug Nomenclature

Synonyms: Artesunato; Artesunatum

Chemical name: (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano-[4,3-*j*]-1,2-benzodioxepin-10-ol hydrogen succinate

Molecular formula: $C_{19}H_{28}O_8$ =384.4 -Figure 1.2.

Artesunate (ART) act as a weak acid with a pH of an aqueous suspension containing 10 mg/g to be 3.5–4.5, and a p*K*a value of 4.6 for the hydroxyl group. Artesunate in particular is incompatible with basic quinolines by virtue of proton transfer, and has intrinsic chemical instability.

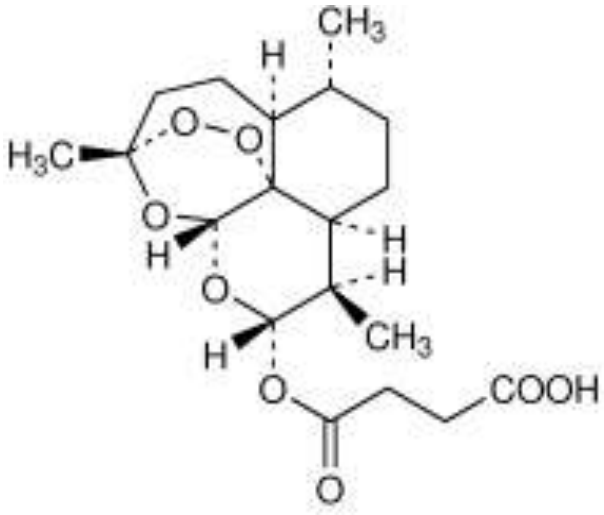


Figure 1.2: Chemical Structure of Artesunate

1.2.2.2 Adverse Effects and Precautions

Artemisinin and its derivatives appear to be generally well tolerated, although there have been reports of mild gastrointestinal disturbance (including nausea, vomiting, diarrhoea, and abdominal pain), dizziness, headache, tinnitus, neutropenia, elevated liver enzyme values, and ECG abnormalities including prolongation of the QT interval. Evidence of severe neurotoxicity has been seen in *animals* given high doses. General references to adverse effects associated with artemisinin derivatives.

1.2.2.3 Effects on the blood.

Severe haemolytic anaemia was reported to occur in patient after taking artemether-lumefantrine.

1.2.2.4 Effects on the heart.

Bradycardia was reported in 10 of 34 patients who received artemether orally for 4 days (Karbwang *et al, 1992*).

1.2.2.5 Effects on the nervous system.

Neurotoxicity has been reported in *animals* given artemotil or artemether (Brewer *et al*., 1994). An *in-vitro* study (Wesche *et al*., 1994) has shown that dihydroartemisinin, the metabolite common to all artemisinin derivatives currently used, is neurotoxic. There has been a report (Miller and Panosian, 1997) of acute cerebellar dysfunction manifesting as ataxia and slurred speech in a patient who took a 5-day course of artesunate by mouth.

1.2.3 Pharmacokinetics

Artemisinin and its derivatives are all rapidly hydrolysed to the active metabolite dihydroartemisinin. Reported elimination half-lives have been about 45 minutes after intravenous doses of artesunate, about 4 hours after rectal artemisinin, and about 4 to 11 hours after intramuscular or oral artemether. At pH 1.2, conversion to dihyroartemisinin (DHA) is rapid, with t1/2 26 min, and at pH 7.4, $t_{1/2}$ is about 10 hours. Because of rapid hydrolysis to dihyroartemisinin (artenimol), artesunate is

considered by many as a prodrug of the latter. With a pKa of 4.6, over 99% of artesunate will be ionized at pH 7.4, and thus uptake by passive diffusion from the intestinal tract will be minimal.

1.3 Amodiaquine: Physicochemical properties

1.3.1 Drug Nomenclature

Synonyms: Amodiaquina, hidrocloruro de; Amodiaquini Hydrochloridum

Chemical name: 4-(7-Chloro-4-quinolylamino)-2-(diethylaminomethyl)phenol dihydrochloride dihydrate

Molecular formula: $C_{20}H_{22}ClN_3O,2HCl,2H_2O$ =464.8- Figure 1.3.

It is slightly acidic and melts at 206^{0}C and has absorption maxima at 343nm in 0.1M hydrochloric acid ; while a 0.001% w/v solution in 0.1M hydrochloric acid has E_{1cm} of 0.37 at 343nm. A yellow, odourless, crystalline powder. Soluble 1 in 25 of water and 1 in 78 of alcohol; very slightly soluble in chloroform, in ether, and in benzene. Store in airtight containers.

1.3.2 Adverse Effects and Precautions

1.3.2.1 Incidence of adverse effects.

Early isolated reports of severe neutropenia with *amodiaquine* usually concerned use in anti-inflammatory doses for rheumatoid arthritis, but there was a cluster of cases in 1986 associated with its use in malaria prophylaxis. In all, 23 cases of agranulocytosis, 7 of which were fatal, were reported in the UK, USA, and Switzerland during a 12-month period ending March 1986. Nearly all of these patients had used the drug at a dosage of 400 mg weekly and the periods of exposure ranged from 3 to 24 weeks. Some of these patients also had evidence of liver damage and there have been other reports of hepatotoxicity associated with the prophylactic use of amodiaquine (Larrey *et al.*, 1986). Examination of data submitted to the UK CSM (Phillips-Howard and West, 1990) suggested that the frequency of adverse reactions

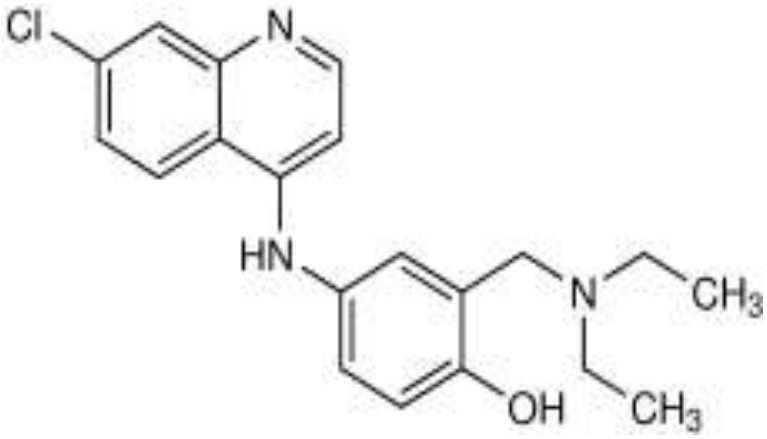


Figure 1.3: Chemical Structure of Amodiaquine

to amodiaquine was about 1 in 1700 for serious reactions, 1 in 2200 for blood disorders, 1 in 15 650 for serious hepatic disorders, and 1 in 15 650 for fatal reactions.

In contrast the frequency of agranulocytosis in users in France has been estimated to be 1 in 25 000. Worldwide (WHO, 1988) the risk of severe reactions appears to be between 1 in 1000 and 1 in 5000. The manufacturers reportedly had 42 cases of serious adverse effects during amodiaquine prophylaxis, between 1985 and 1991; there were 28 cases of agranulocytosis (9 deaths) and 14 of hepatitis (3 deaths) (Olliaro *et al.*, 1996). Whether there was significantly less risk when amodiaquine was given for treatment of malaria rather than prophylaxis was not certain(White, 1996b). It has been suggested that an immunological reaction to amodiaquine quinone imine, which can be produced by autoxidation among other processes, may partially account for amodiaquine**'s** greater tendency to induce agranulocytosis compared with chloroquine (Park and Kitteringham, 1990).

The acute toxicity of amodiaquine appears to differ from that of chloroquine in that there have been no reports of cardiovascular symptoms after overdosage with amodiaquine but intoxication with amodiaquine is also far less frequent than chloroquine poisoning. However, large doses of amodiaquine have been reported to produce syncope, spasticity, convulsions, and involuntary movements (Jaeger *et al*., 1987).

1.3.3 Pharmacokinetics

Amodiaquine hydrochloride is readily absorbed from the gastrointestinal tract. Amodiaquine is rapidly converted in the liver to the active metabolite desethylamodiaquine, only a negligible amount of amodiaquine being excreted unchanged in the urine. The plasma elimination half-life of desethylamodiaquine has varied from 1 to 10 days or more. Amodiaquine and desethylamodiaquine have been detected in the urine several months after use.

1.3.4 Uses and Administration

Amodiaquine is a 4-aminoquinoline antimalarial with an action similar to that of chloroquine. It is as effective as chloroquine against chloroquine-sensitive strains of *Plasmodium falciparum* and is also effective against some chloroquine-resistant strains, although resistance to amodiaquine has developed and there may be partial cross-resistance between amodiaquine and chloroquine. To prevent the emergence and spread of drug resistant parasites WHO recommends that amodiaquine be given with an artemisinin derivative, such as artesunate, for the treatment of falciparum malaria. Amodiaquine is not recommended for the prophylaxis of malaria because of resistance and the risk of major toxicity. Amodiaquine is given by mouth as the hydrochloride, but doses are expressed in terms of amodiaquine base; amodiaquine hydrochloride 260 mg is equivalent to about 200 mg of amodiaquine base. For the treatment of falciparum malaria and uncomplicated chloroquine-resistant vivax malaria a total dose of 30 mg/kg is given over 3 days (10 mg/kg daily for 3 days).

1.4 Treatment failure and parasite resistance

"Treatment failure" in the general context, refers to an unsuccessful treatment outcome from any possible cause, related to the drug, the host and the parasite. "Parasite resistance" on the other hand refers to the ability of the parasite to continue to survive and multiply despite an adequate exposure to the antimalarial drug (White, 2004). Of the various potential causes of malaria treatment failure with either Chloroquine or Sulphadoxine/Pyrimethamine, the most important is resistance-conferring mutations in the parasites (Hyde, 1990; Sibley *et al*., 2001; Sendagire *et al*., 2005). The spread, of multi-drug resistant strains of *P.falciparum,* pose an increasing threat to the effective treatment and prophylaxis of malaria. But the advent of molecular studies has made it possible to identify genetic markers of resistance, differentiating "parasite resistance" from other factors that cause lack of clinical response to antimalarial therapy. Early studies showed that the frequent "treatment failures" that were reported with chloroquine in falciparum malaria in Africa, could have been related to poor drug quality and inadequate dosages (Ogwal-Okeng *et al*., 1998). This may lead to the development of severe, complicated malaria, and death, besides causing selection for resistant strains in the otherwise under-dosed patient. It

has been shown that an important host factor in predicting treatment failure with antimalarials is 'young age' (Staedke *et al*., 2004). Thus children living in malarial areas, especially under high transmission pressure, are at higher risk of treatment failure. Resistance to antimalarials has been documented for *P. falciparum*, *P. vivax*, and *P. malariae* and is seen with all classes of antimalarials except artemisinin and its derivatives (WHO, 2006a). Resistance of *Plasmodium* to antimalarial drugs is of great concern. Chloroquine resistance in *P. falciparum* now occurs virtually everywhere that *P. falciparum* malaria is transmitted, with the exception of certain parts of Central America and the Caribbean (Bloland, 2001). Resistance in *P. falciparum* to proguanil and pyrimethamine is apparent in many endemic areas. Cross-resistance between proguanil and pyrimethamine may also occur. Resistance in *P. falciparum* to the combination pyrimethamine-sulfadoxine has spread rapidly in South-East Asia, but also occurs in other parts of the world including parts of South America, southern China, and Africa (Bloland, 2001). Mefloquine resistance is frequent in some areas of South-East Asia and migrant workers are thought to have spread mefloquine resistance to India and Bangladesh. Resistance has also occurred in the Amazon region of South America and, sporadically, in Africa, but generally, clinical mefloquine resistance outside South-East Asia is rare (WHO, 2006a). Resistance to quinine and halofantrine has also been noted. Cross-resistance between halofantrine and mefloquine may occur, as evidenced by reduced responses to halofantrine in some patients who have experienced treatment failure with mefloquine (Bloland, 2001). The emergence of multidrug resistance in *P. falciparum* makes the selection of effective prophylaxis and treatment difficult. Multidrug resistance is defined as resistance to three or more antimalarial compounds from different chemical classes, of which two of the classes are the 4-aminoquinolines (e.g. chloroquine) and diaminopyrimidines (e.g. pyrimethamine-sulfadoxine). Multidrug resistance occurs in South-East Asia (particularly along the borders of Thailand with Burma and Cambodia) and in the Amazon basin (WHO, 2006a). *P. vivax* has developed resistance rapidly to pyrimethamine-sulfadoxine in many areas and resistance to chloroquine and primaquine has also been reported in several parts of the world (WHO, 2006a). However, it remains sensitive to chloroquine in South-East Asia, the Indian subcontinent, the Korean peninsula, the Middle East, north-east Africa, and most of South and Central America. Resistance of *P. malariae* to chloroquine was

observed recently in Indonesia (WHO, 2006a). A knowledge of the geographical distribution and degree of resistance is important for the selection of appropriate control measures and for the development of policies for the rational use of antimalarial drugs. Resistance can be prevented, or slowed considerably, by selecting effective drugs and drug combinations according to local patterns of resistance, combining antimalarials with different mechanisms of action, preventing indiscriminate and uncontrolled use, and ensuring very high cure rates through full compliance with the correct antimalarial dose regimens. Malaria control strategies also need to involve other measures such as vector control and health education (WHO, 2005c).

1.5 Biopharmaceutics and pharmacokinetics: principles and parameters

Biopharmaceutics and pharmacokinetics studies of drugs and drug products are useful in understanding the relationship between the physicochemical properties of the drug products and the clinical or pharmacologic effect. Figure 1.4 is a general scheme describing this dynamic relationship. The study of biopharmaceutics entails investigation of the factors which influence the release of drug from a drug product, the rate of dissolution of the drug, and the eventual bioavailability of the drug. Pharmacokinetics deals with the changes of drug concentration in the drug product and changes of concentration of a drug and/or its metabolite(s) in the human or animal body following administration of the drug product i.e. the changes of drug concentration in the different body fluids and tissues in the dynamic system of liberation, absorption, distribution, body storage, binding, metabolism and excretion. When administered, a drug must be present in appropriate concentrations at its sites of action to produce its characteristics effects. The concentration of drug attained depends on the amount of drug administered, the extent and the rate of its absorption, distribution, binding, and localization in tissues, biotransformation, and excretion. These factors are depicted in Figure 1.5.

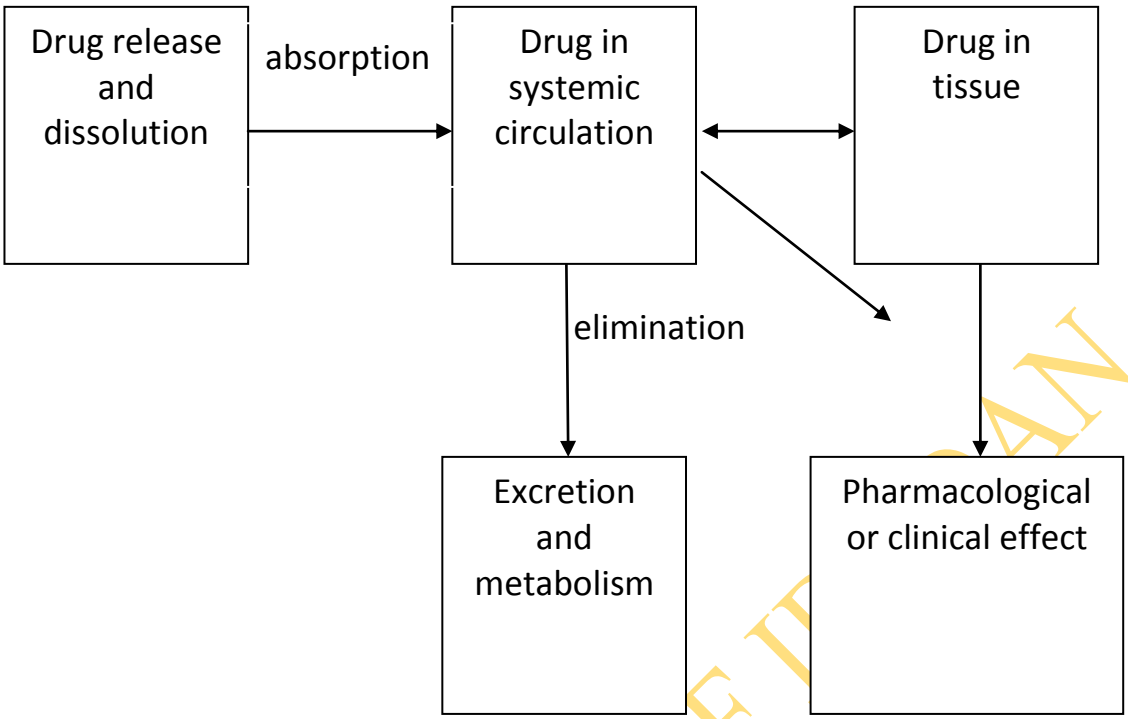


Figure 1.4: Scheme demonstrating the dynamic relationship between the drug, the drug product and the pharmacologic effect

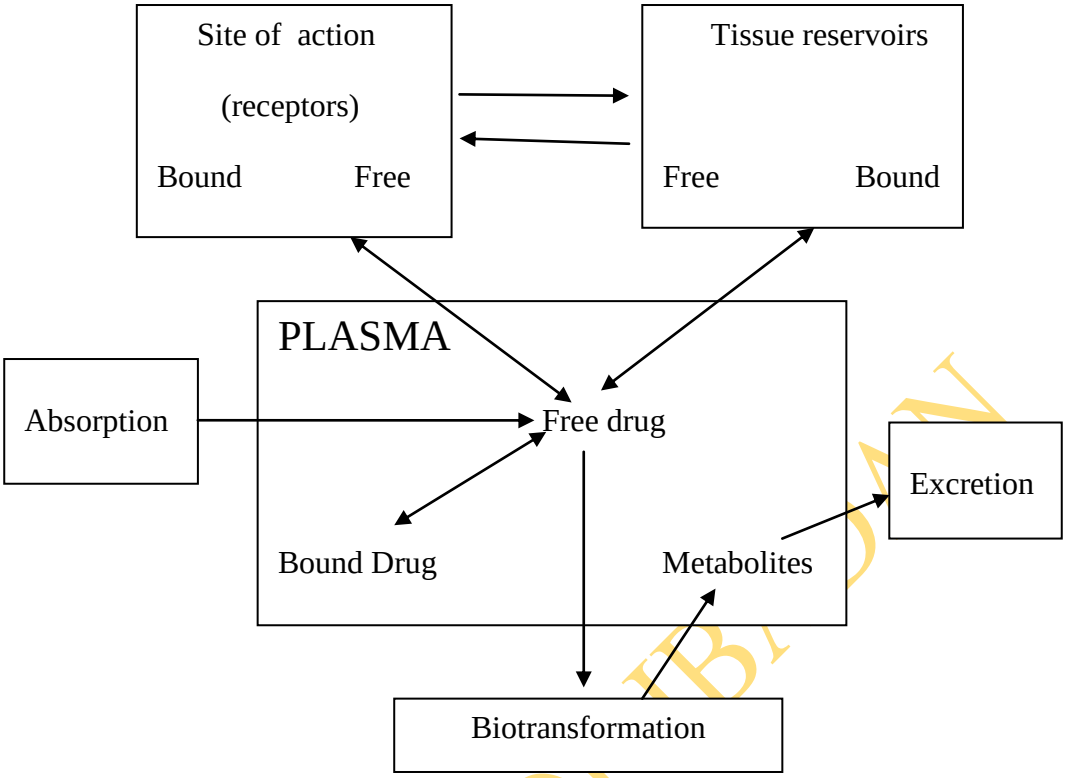


Figure 1.5: Schematic representation of the interrelationship of the absorption, distribution, binding, biotransformation and excretion of a drug and its concentration at its site of action.

Most drugs administered need to be transported to their desired sites of action by the blood. A drug administered extravascularly (orally, topically, rectally, intraperitoneally or intramuscularly) needs to be absorbed before it can enter the systemic circulation. Drugs administered intravenously or intra-arterially do not need to be absorbed. Drugs are not usually specific for a particular tissue and therefore will reach a number of tissues and organs (distribution) unless targeted to a specific site. Elimination is the removal of the drug from the body and may be by renal, and biliary excretion of the intact drug molecule or by metabolism.

1.6 Plasma level-time curve

The plasma level-time curve is generated by measuring the drug concentrations in the plasma samples taken at various time intervals after a drug product is administered (Figure 1.6). As the drug reaches the general (systemic) circulation , plasma drug concentration will rise up to a maximum. Usually absorption of a drug is more rapid than elimination. As the drug is being absorbed into the systemic circulation, the drug is distributed to all the tissues in the body and is also simultaneously being eliminated. Elimination can proceed by excretion or biotransformation or a combination of both. The relationship of the drug level-time curve and various pharmacologic parameters for the drug is shown in Figure 1.6. Assuming the drug concentration in the plasma is in equilibrium with the tissues, the MEC reflects the minimum concentration of a drug needed at the receptors to produce the desired pharmacological effect. Similarly, the MTC represents the drug concentration needed to just barely produce a toxic effect. The *onset time* corresponds to the time required for the drug to reach the MEC. The *intensity* of the pharmacologic effect is proportional to the number of drug receptors occupied, which is reflected in the observation that higher plasma drug concentration produce a greater pharmacologic response, up to a maximum. The *duration* of drug action is the difference between the onset time and the time for the drug to decline back to MEC.

In contrast, the plasma level-time curve can also describe pharmacokinetic terms such as peak plasma level, time for the peak plasma level, and the area under the curve, or AUC (Figure 1.6). The *time of peak plasma level* is roughly proportional to the average rate of drug absorption. The *peak plasma level* or the maximum drug

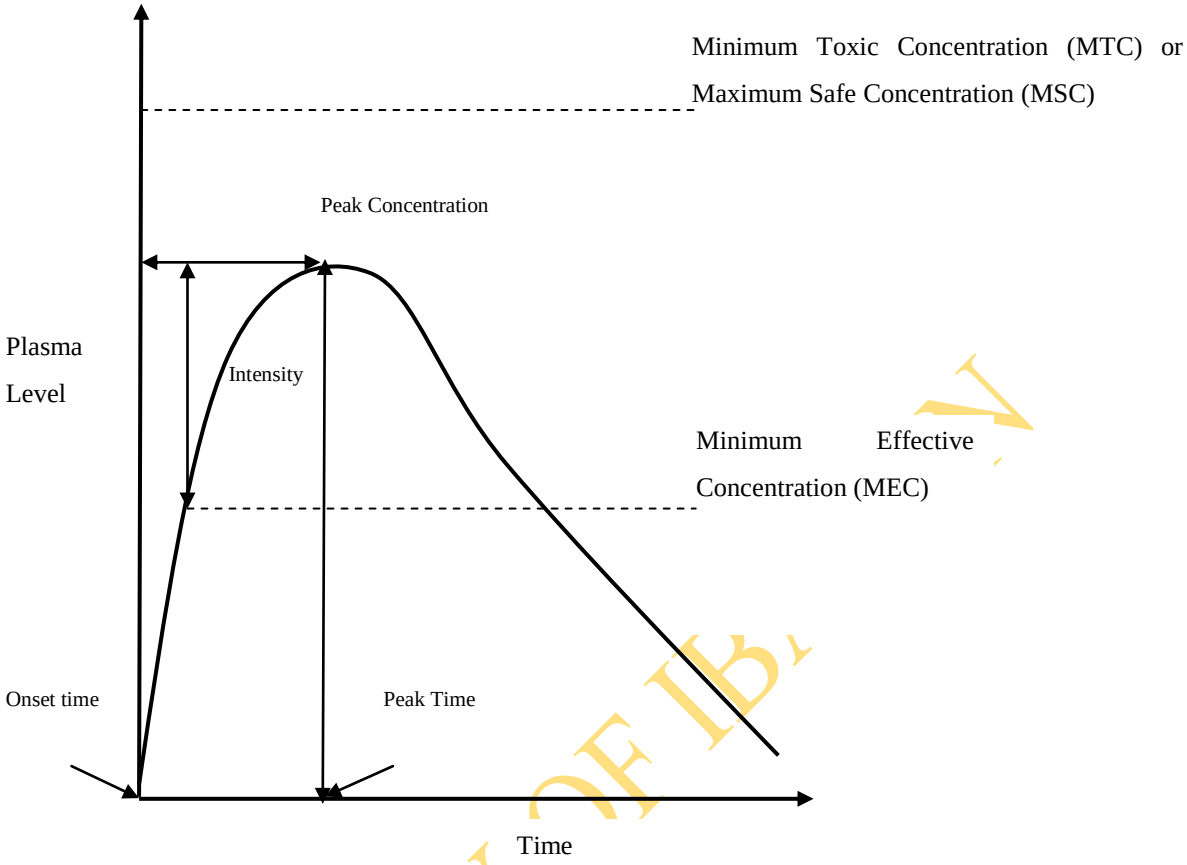


Figure1.6: Plasma level-time curve showing peak time and concentration after oral administration of a drug

concentration is usually related to the dose and the rate constants for absorption and elimination of the drug. The AUC is related to the total amount of drug absorbed systemically.

1.7 Mean Residence Time (MRT)

Mean residence time (MRT) is the average duration of stay of intact drug molecules in the body. For a drug which exhibits linear kinetics, MRT is expressed as the ratio between AUMC (area under the first moment curve is derived mathematically) and AUC (area under the plasma concentration-time curve).

1.8 Compartment model

The body can be represented as a series or system of compartments. A compartment does not need to be a real physiologic or anatomic site but is considered as a tissue or group of different tissues which are similar in blood flow and affinity for a drug. Conceptually, drugs move dynamically in and out of compartments. A drug can move into a compartment at a rate which is usually proportional to the blood flow of that compartment. However, the rate at which the drug leaves the compartment depends upon blood flow and the affinity of the drug for the tissue(s) in that compartment (The Merck Manual, 2011). Several types of compartment models are described in Figure 1.7. The pharmacokinetic rate constants are represented by the letter K. The "1" compartment represents the plasma or central compartment, and the "2" compartment represent the tissue compartment. The drawing of models has three functions: (1) enabling the construction of differential equations to describe drug concentration changes in each compartment; (2) giving a visual representation of the rate processes; and (3) showing how many pharmacokinetic constants are necessary to describe the process adequately. Two parameters are needed to describe model 1 in Figure 1.7: the volume of the compartment and the elimination rate constant, K. In the case of model 4, Figure 1.7, the pharmacokinetic parameters consist of the volumes of compartment I and 2 and the rate constants –Ka, K, K_{12}, and K_{21} for a total of six parameters. In studying these models, it is important to know whether drug concentration data may be obtained directly from each compartment. For models 3 and 4 in Figure 1.7, data concerning compartment 2 cannot be obtained easily because

tissues are not easily sampled and may not contain homogenous concentrations of drugs. If the amount of drug absorbed and eliminated per unit of time is obtained by sampling compartment 1, then the amount of drug contained in the tissue can be estimated mathematically.

1.9 Elimination rate constant

The rate of elimination for most drugs is a first-order process. The elimination rate constant, K, is a first-order elimination rate constant with units of time $^{-1}$(e.g h^{-1}). In general, only the parent or active drug is measured in the vascular compartment. Total removal or elimination of drug from this compartment is effected by metabolism (biotransformation) and excretion. The elimination rate constant represents the sum of each of these processes:

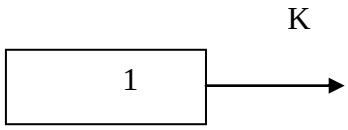
$$K = Km + Ke \qquad \text{----------1.9.1}$$

where Km = first-order rate process of metabolism and Ke = first-order rate process of excretion. There may be several routes of elimination of drug by metabolism or excretion. In such a case, each of the processes has its own first-order rate constant.

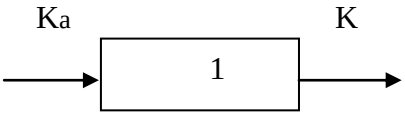
A rate of elimination of Figure 1.8 is:

$$dD_B/dt = -KD_B \qquad \text{----------1.9.2}$$

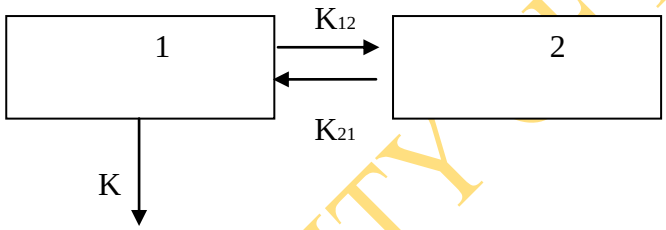
This expression shows that the rate of elimination of drug in the body is a first-order process, depending upon the elimination rate constant, K, and the amount of drug, D_B, remaining.



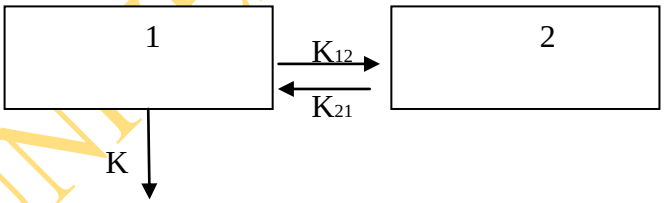
Model 1: One compartment open model, IV injection



Model 2: One-compartment open model with first-order absorption.



Model 3: Two-compartment open model, IV injection



Model 4: Two-compartment open model with first-order absorption

Figure 1.7: Various compartment models (Howard et al., 1995).

Where K=pharmacokinetic rate constant relative to compartment 1, K_a=absorption rate constant, K_{12}=rate constants of compartment 1 realtive to compartment 2, K_{21}=rate constant of compartment 2 realtive to compartment 1, 1=central compartment, 2=tissue compartment.

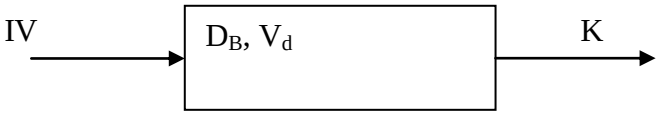


Figure 1.8: Pharmacokinetic model for a drug administered by rapid intravenous injection.

Where D_B, = amount of drug in the body; V_d= apparent volume of distribution; K = elimination rate constant.

Integration of the equation 1.9.2 gives the following equation 1.9.3

$$\text{Log } D_B = -\frac{Kt}{2.3} + log D_B^0 \qquad \text{---------- 1.9.3}$$

where D_B = amount of drug in the body at time t, and D_B^0 = amount of drug in the body at time 0.

1.10 Drug clearance

Drug clearance or total clearance or plasma clearance (Clp) is the volume of plasma in the central compartment that is cleared of drug per unit time. It is a proportionality factor relating plasma drug concentration to the rate of drug elimination.

Rate of elimination = Clp x Cp ----------1.10.1

It can be determined from a model-independent formular:

$$Cl_p = FD_0/(AUC)_0^\infty \qquad \text{----------1.10.2}$$

Clp can also be obtained from the product of volume of distribution (Vd) and elimination rate constant (β) for any compartment.

$$Cl_p = \beta V_d \qquad \text{----------1.10.3}$$

It can also be related to the $t_{1/2}$ of the drug:

$$Cl_p = 0.693 V_d / t_{1/2} \qquad \text{----------1.10.4}$$

Clearance is used as an index of drug elimination from the central compartment and gives an indication of drug removal without reference to the mechanism of the process. Clearance is constant for all drugs that are eliminated by first-order kinetics. In any time interval, a constant fraction of the drug is excreted. A drug excreted by non-first-order kinetics will have changing clearance values. Since the liver is the most important organ for drug biotransformation as well as biliary drug excretion, nonrenal clearance is usually attributed to hepatic clearance. The clearance concept in fact, may be used to describe clearance by any eliminating organ or group of tissues perfused by blood. Clp is the sum of all the clearances in the body such as renal clearance, and non-renal clearance (metabolic and biliary clearances). Clearance is also mathematically defined as follows

$$Clearance = \frac{Excretion\ rate}{plasma\ concentration} = ml/min \qquad \text{----------1.10.5}$$

$$Cl = \left(\frac{dD_u}{dt}\right)/C_p \qquad \text{----------1.10.6}$$

The rate of drug excretion $\left(\frac{dD_u}{dt}\right)$ is equal to the product of clearance (in ml/min) and the concentration of drug in the blood compartment (Cp). Since most drugs are cleared through the kidney by glomerular filtration, the excretion is directly proportional to the plasma drug concentration.

1.11 Apparent volume of distribution

The volume of distribution represents a factor that must be taken into account in estimating the amount of drug in the body from the concentration of the drug found in the sampling compartment. The volume of distribution can also be considered as the apparent volume (V_d) in which the drug is dissolved.

$D_B = V_dC_p$ ----------1.11.1

It is usually assumed that the drug equilibrates rapidly in the body, which is true for most drugs. However, each individual tissue may contain a different concentration of drug, due to differences in drug affinity for that tissue. Since that value of the volume of distribution does not have a true physiological meaning in terms of an anatomic space, the term *apparent volume of distribution* is used. The amount of drug in the body is not determined directly. Instead, a blood sample is removed at periodic intervals and analysed for its concentration of drug. The V_d is useful for relating the concentration of drug in plasma (C_p) and amount of drug in the body (D_B), as in the following equation:

$$\mathbf{D_B = VdCp} \qquad \text{----------1.11.2}$$

By substitution, a similar expression based upon drug concentration in plasma is obtained for the first-order decline of drug plasma levels.

$$\log Cp = -\frac{KT}{2.3} + Log C_p^o \qquad \text{----------1.11.3}$$

1.12 Calculation of volume of distribution

In a one compartment model (IV administration), the Vd is calculated with the following equation:

$$Vd = \frac{Dose}{C_p^o} = \frac{D_B^O}{C_p^o} \text{ or } \frac{FD_B^O}{C_p^o} \qquad \text{----------1.12.1}$$

with rapid IV injection, the dose is identical to D_B^O. C_p^o is the initial plasma concentration of drug at time = 0; its value can be obtained by extrapolation of the regression line to the y axis in the following Figure 1.9; F = bioavailability factor or fraction of the dose absorbed into systemic circulation if drug is given by extravascular route

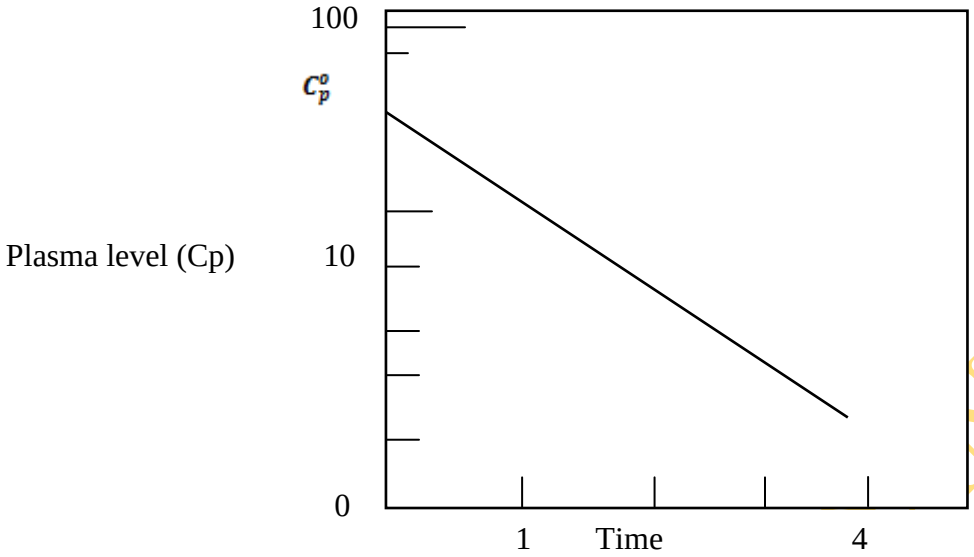


Figure 1.9: Semilog graph giving the values of C_p^o by extrapolation

The apparent volume V*d* may also be calculated from knowledge of the dose, elimination rate constant, and the AUC from t = 0 to
t = ∞.

$$Vd = \frac{Do}{K[AUC]_0^{\infty}} \qquad \text{----------1.12.2}$$

The calculation of V*d* by this equation is a model independent method, no pharmacokinetic model is considered. The AUC may be determined directly by using the trapezoidal rule.

1.13 Multicompartment models

Multi compartment models are needed to explain the observation that after a rapid IV injection the plasma level-time curve does not decline linearly as a single first-order rate process. In a multi compartment model, the drug distributes rapidly into those tissues which have the highest blood flow. These highly perfused tissues and blood make up the central compartment. While this initial drug distribution is taking place, the drug is delivered to one or more peripheral compartments composed of group of tissues with lower but similar blood flow and affinity for the drug. After equilibration of drug within these peripheral tissues, the plasma level-time curve reflects first-order elimination of the drug from the body. A drug will concentrate in a tissue in accordance with the affinity of the drug for that particular tissue. For example, lipid soluble drug tend to accumulate in fat tissues. Drugs which bind proteins may be more concentrated in the plasma, since protein-bound drugs do not diffuse into the tissues. Drugs may also bind with tissue proteins and other macromolecules such as DNA and melanin.

In order to apply kinetic analysis of a multi compartment model, one must assume that all rate processes for the passage of drugs into or out of individual compartments are first-order processes. On the basis of this assumption, the plasma level-time curve for a drug which follows a multi compartment model is best described by the summation of several first-order rate processes. Because of the aforementioned distribution factors, drugs will generally concentrate unevenly in the

tissues, and different groups of tissues will accumulate the drug at different rates. A summary of the approximate blood flow to major human tissues is presented in Table 1.1. After an IV injection, drug concentration in plasma and the highly perfused tissues constituting the central compartment decline rapidly due to distribution of the drug into other, more slowly perfused tissues. This initial rapid decline of drug concentration in the central compartment is known as distribution phase of the curve (may take minutes or hours and may be missed entirely if the blood is sampled too late after administration of drug) (Figure 1.10, line a). In time, the drug attains a state of equilibrium between the central compartment and the more poorly perfused tissue compartment. After this equilibrium is established, the loss of the drug from the central compartment appears to be a single first-order process due to the overall process of elimination of the drug from the body. This second, slower rate process is known as the elimination phase (Figure 1.10, line b). The two-compartment model assumes that at t = 0 there is no drug in the tissue compartment. After an IV dose, drug is rapidly transferred into the tissue compartment while the blood level of drug declines rapidly due to both elimination of the drug and transfer of the drug out of the central compartment into various tissues.

Table 1.1: Blood flow in human tissues

Tissue	% body weight	% cardiac output	Blood flow(ml/100g tissue/min)
Adrenals	0.02	1	550
Kidneys	0.4	24	450
Thyroid	0.04	2	400
Liver			
Hepatic	2.0	5	20
Portal	-	20	75
Portal drained viscera	2.0	20	75
Heart (basal)	0.4	4	70
Brain	2.0	15	55
Skin	7.0	5	5
Muscle (basal)	40.0	15	3
Connective tissue	7.0	1	1
Fat	15.0	2	1

Butler: In La du *et al*., (eds), 1972.

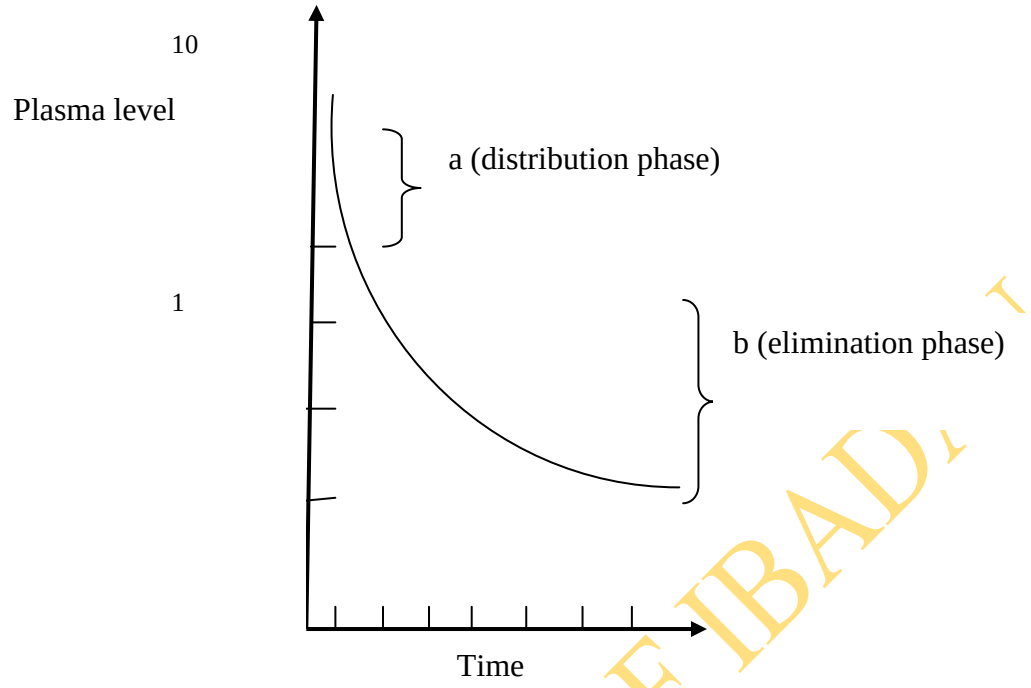


Figure 1.10: Plasma level –Time curve for 2 compartment open model (single IV dose)

The tissue drug level will eventual peak and then start to decline as the concentration gradient between the two compartments narrows. The drug level in the theoretical tissue compartment can be calculated once the parameters for the model are determined. The theoretical tissue concentration, together with the blood concentration, gives an accurate method of calculating the total amount of drug remaining in the body at any time. For the two-compartment open model, elimination occurs simultaneously with distribution; therefore elimination from the central compartment is determined by a complex function, which is determined from the slope of the terminal segment of the semi-logarithm plot of a plasma concentration-time curve of the linear equation.

Equation for two-compartment model with IV bolus dose:

$$C_p = Ae^{\alpha t} + Be^{\beta t} \qquad \text{----------1.13.1}$$

$$t_{1/2} = \frac{0.693}{\beta} \qquad \text{----------1.13.2}$$

where α and β are rate constants for the distribution phase and elimination phase respectively, hybrid constants *A* and *B* are intercepts on Y- axis obtained by the method of residuals (feathering or peeling) or by computer, $t_{1/2}$= elimination half life, Cp=plasma concentration.

$$V_c = \frac{FD_0}{(A+B)} \qquad \text{----------1.13.3}$$

where Vc=the volume of distribution (volume of the central compartment), D_0= dose, F=bioavailability factor, or fraction of the dose absorbed into systemic circulation.
Vc can also be determined from a model independent formula:

$$Vc = FD_0/K_{el}(AUC)_0^{\infty}$$ ----------1.13.4

Where K_{el}= elimination rate constant, AUC=area under the plasma concentration-t

1.14 Three-compartment open model

The three compartment model is an extension of the two-compartment model, with an additional deep tissue compartment. A drug which demonstrates the necessity of a three-compartment open model is distributed most rapidly to a highly perfused central compartment, less rapidly to the second or tissue compartment and very slowly to the third or deep tissue compartment, containing such poorly perfused tissue as bone and fat. The deep tissue may also represent tightly bound drug in the tissues.

1.15 Biopharmaceutic aspects of drug products

Biopharmaceutics is the study of the relationship of the physicochemical properties of a drug formulation to the bioavailability of the drug. Bioavailability refers to the rate and the amount of active drug that reaches the systemic circulation. Because the bioavailability of a drug influences its therapeutic, clinical, and toxic activity, the study of biopharmaceutics is increasingly important. The aim of biopharmaceutics is to adjust the delivery of drug to the systemic circulation in such a way as to provide optional therapeutic activity for a given clinical situation.

The systemic absorption of a drug from an extravascular site is influenced by the anatomic and physiologic properties of the site and the physicochemical property of the drug and the drug products. Biopharmaceutics attempts to control these variables by designing a drug product with a specific therapeutic objective.

1.16 Factors in drug bioavailability

Systemic absorption of most drug products consist of a succession of rate processes (Figure 1.11). These processes include: (1) disintegration of the drug product and subsequent release of the drug; (2) dissolution of the drug in an aqueous environment; and (3) absorption across cell membranes into the systemic circulation. In the process of drug disintegration, dissolution, and absorption, the rate at which drug reaches the circulatory system is determined by the slowest step in the sequence.

The slowest step in a series of kinetic processes is called the *rate-limiting step.* Except for sustained-release or prolonged-action products, disintegration of a solid drug product is usually more rapid than drug dissolution and drug absorption. For drugs which have very poor aqueous solubility, the rate at which the drug dissolves (dissolution) is often the slowest step and therefore exerts a rate-limiting effect on drug bioavailability. In contrast, for a drug which has a high aqueous solubility, the dissolution rate is rapid and the rate at which the drug crosses or permeates cell membranes is the slowest or rate-limiting step.

1.17 Pharmaceutic factors affecting drug bioavailability

The bioavailability of the active drug in a solid dosage form is dependent upon several factors, including (1) disintegration of the drug product and release of the active ingredient; (2) dissolution of the drug; and (3) absorption or permeation of the drug across the cell membranes (Figure 1.11).

1.18 Disintegration

It was generally recognized some years ago that a solid drug product had to disintegrate into small particles and release the drug before absorption could take place. For the purpose of monitoring uniform tablet disintegration, United States Pharmacopeia (USP) established an official disintegration test. Solid drug products which are exempted from disintegration tests include troches, tablets which are intended to be chewed, and drug products intended for sustained release or prolonged or repeat action. The process of disintegration does not imply complete dissolution of the tablet and/or the drug. Complete disintegration is defined by the USPXX as "that state in which any residue of the tablet, except fragment of insoluble coating, remaining on the screen of the test apparatus in the soft mass have no palpably firm core" The official apparatus for the disintegration test and procedure is described in the USPXX. Separate specifications are given for uncoated tablets, plain coated tablets, enteric tablets, buccal tablets, and sublingual tablets. Although disintegration

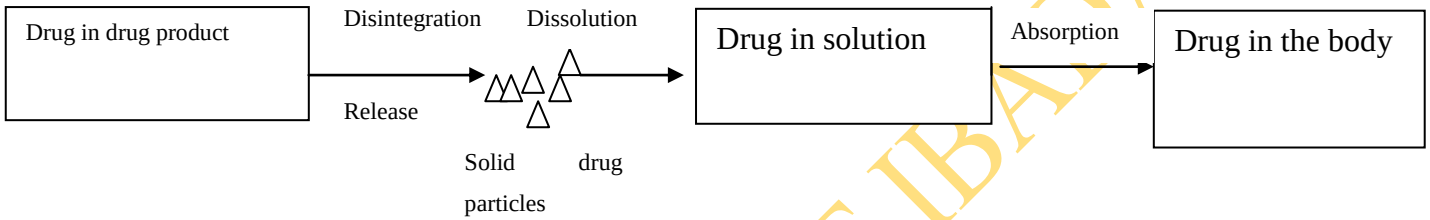


Figure 1.11: Rate processes of drug bioavailability

tests allow for precise measurement of the formation of fragments, granules, or aggregates from solid dosage forms, no information is obtained from this test on the rate of dissolution of the active drug. However, the disintegration tests do serve as a component in the overall quality control of tablet manufacture. The USP tolerance limits for disintegration time test for uncoated tablets is 30minutes.

1.19 Dissolution

In biologic systems, drug dissolution in an aqueous medium is an important prior condition of systemic absorption. The rate at which drugs with poor aqueous solubility dissolve from an intact or disintegrated solid dosage form in the gastrointestinal tract often controls the rate of systemic absorption of the drug. According to the observations of Noyes and Whitney and other investigators, the steps in dissolution include the process of drug dissolution at the surface of the solid particle, thus forming a saturated solution around the particle. The dissolved drug in the saturated solution known as the "stagnant layer" diffuses to the bulk of the solvent from the regions of high drug concentrations to regions of low drug concentrations (Figure 1.12)

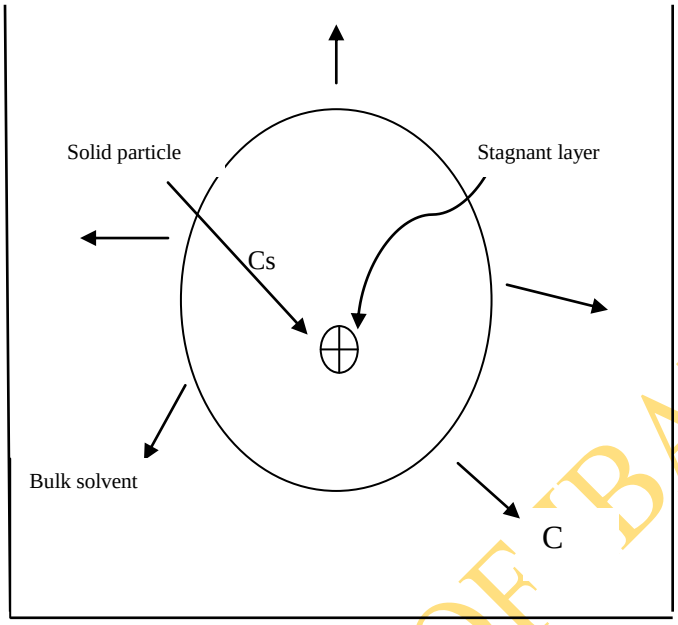


Figure 1.12: Dissolution of a solid drug particle in a solvent.

Cs = concentration of drug in the stagnant layer, and C = concentration of drug in the bulk solvent.

The overall rate of drug dissolution may be described by the Noyes-Whitney equation, which resembles Fick's law of diffusion.

$$\frac{dc}{dt} = \frac{DAK(Cs - C)}{h} \qquad \text{----------1.19.1}$$

where dc/dt = rate of drug dissolution ; D = diffusion rate constant; A = surface area of the particle; Cs = concentration of the drug in the stagnant layer; C = concentration of the drug in the bulk solvent; K = oil/water partition coefficient ; and h = thickness of the stagnant layer. The rate of dissolution,(dc/dt).(1/A), is the amount of drug dissolved per unit area per time (e.g., gm/cm^{2}min).

It can be seen from the Noyes-Whitney equation that dissolution kinetics may be influenced by the physicochemical characteristics of the drug, the formulation and the solvent. Drug in the body, particularly the gastrointestinal tract, is considered to be dissolving in an aqueous environment. In addition to these factors, the temperature of the medium and the agitation rate also affect the rate of drug dissolution. *In vitro* study of dissolution kinetics requires maintenance of constant temperature (37^{0}C) and agitation (specified constant). An increase in temperature will increase the kinetic energy of the molecules and increase the diffusion constant, D. On the other hand, an increase in the agitation of the solvent will reduce the thickness, h, of the stagnant layer, allowing for more rapid dissolution.

1.19.1 Physicochemical nature of the drug

The nature of the physical and chemical properties of the solid drug particles has a great effect on dissolution kinetics. The effective surface area of the drug may be enlarged by a reduction in the particle size. Since dissolution is thought to take place at the surface of the solute, the greater the surface area, the more rapid the rate of drug dissolution. The degree of aqueous solubility of the drug also affects the rate of dissolution. Generally, the ionisable salt of the drug is more water-soluble than the free acid or free base. Moreover, if the drug is in the anhydrous state, the rate of dissolution is usually faster than with the hydrous salt. The drug may also exist in more than one of the crystalline forms known as polymorphs. These polymorphs,

which have identical chemical structures, demonstrate different dissolution kinetics. In general, crystalline structures are more rigid and thermodynamically more stable than the amorphous forms of the drug. Thus the amorphous forms of the drug demonstrate faster dissolution rates than the crystalline forms of the drug. Other factors are complexation, partition coefficient and stability in gastrointestinal fluid of the drug.

1.19.2 Formulation factors affecting drug dissolution

The various excipients in the drug product may also affect dissolution kinetics of the drug by either altering the medium in which the drug is dissolving or by reacting with the drug itself. For example, excipients such as suspending agents increase the viscosity of the drug vehicle and thereby diminish the rate of drug dissolution of suspension. Tablet lubricants such as magnesium stearate may repel water and reduce the dissolution when used in large quantities. Surfactants may, however, affect drug dissolution in an unpredictable fashion. Low concentrations of surfactants lower the surface tension and increase the rate of drug dissolution, whereas higher concentrations of surfactants tend to form micelles with the drug and thus decrease the dissolution rate. Some excipients, such as sodium bicarbonate, may change the pH of the medium. With a solid acid drug, such as aspirin, an alkaline medium adjacent to the acid drug will cause the drug to form a water-soluble salt in which the drug rapidly dissolves. This type of process is called dissolution in a reactive medium. The solid drug may dissolve rapidly in the reactive solvent surrounding the solid particle. However, as the dissolved drug molecules diffuse outward into the bulk solvent, the drug may precipitate out of solution with a very fine particle size. These small particles have enormous collective surface area and disperse easily, coming into contact with the intestinal membrane and dissolving readily for more rapid absorption.

1.20 Bioavailability and bioequivalence

During medication, one of the pharmacokinetic parameters that concern the clinician most is *bioavailability*. This is the fractional extent to which an administered

drug reaches the systemic circulation, where it gains access to its site of action. For a given dose, the bioavailability is therefore determined by the amount, the rate and extent to which it is absorbed and eliminated (first-pass-effect). *Relative bioavailability* on the other hand is a ratio of the bioavailability of different dosage forms or different routes (usually by comparison of the oral or other routes against the intravenous route) of administration of the same drug. It may also involve comparison of the bioavailability of a drug administered during different disease conditions. For resource limited countries especially in SSA, where treatment policies depend to a large extent, if not wholly, on the use of generic drugs, this ratio becomes very important when different formulations of the innovator drugs are to be evaluated for quality. That is, once the patent for a specific drug expires, anybody may manufacture formulations bearing the common scientific name of the patented drug, provided that it is *bioequivalent* to the innovator drug. The product, thus manufactured, is called a generic product. Its manufacture should be guided by the Good Manufacture Practices (GMP) code (European Medicines Agency, 2001).

The major concern with generic products is the ability of a patient to exchange one product for another (*bioequivalence*). Two products are considered bioequivalent if the concentration-time profiles are so similar that they are unlikely to produce clinically relevant differences in either therapeutic or adverse effects (Rowland and Tozer, 1995). For a new product to be considered bioequivalent, the relative bioavailability (AUCtest/AUCreference), and maximum concentration ($C_{max\ test}$/ $C_{max\ reference}$) were within 0.80 and 1.25. should give a ratio between 0.80 – 1.25 within the 90% confidence interval (European Medicines Agency, 2001).

1.20.1 Relative and absolute availability

The area under the drug concentration-time curve is useful as a measure of the total amount of unaltered drug that reaches systemic circulation. The AUC is dependent upon the total quantity of available drug, $FD_{0,}$ divided by the elimination rate constant, K, and the apparent volume of distribution, V_d. F is the fraction of the dose absorbed; after IV administration, F is equal to unity, since the entire dose is placed into the systemic circulation instantaneously. After oral administration of the drug, F may vary from a value of F = 0 (no drug absorption) to F = 1 (complete drug absorption). Relative (apparent) availability is the availability of a drug product as

compared to a recognized standard dosage formulation usually evaluated in a cross over study. The relative availability of two drug products given at the same dosage level and by the same route of administration can be obtained with the following equation.

$$\textbf{Relative availability} = [AUC]_A/[AUC]_B \qquad \text{----------1.20.1}$$

where drug product B is the recognized reference standard. This fraction may be multiplied by 100 to give *percent* relative availability. When different doses are administered, a correction for the size of the dose is made, as in the following equation.

$$\textbf{Relative availability} = \frac{[AUC]_A \div Dose\ A}{[AUC]_B \div Dose\ B} \qquad \text{----------1.20.2}$$

The absolute availability of drug in a drug product may be measured by comparing the respective AUCs after oral and IV administration. This measurement may be performed as long as V_d and K are independent of the route of administration. Absolute availability using plasma data can be determined as follows.

$$\textbf{Absolute availability} = \frac{[AUC]_{PO} \div Dose\ po}{[AUC]_{iv} \div Dose\ iv} \qquad \text{----------1.20.3}$$

Patient and Physiological factors affecting bioavailability are

1. Food intake
2. Fluid intake
3. Gastric emptying and intestinal motility
4. Splanchnic blood flow
5. First pass metabolism
6. Posture and physical activity
7. Drug interaction
8. Disease states (diarrhoea, congestive heart failure, liver and kidney diseases etc.)

1.21 Definitions

1.21.1 Supra-bioavailability

This is a term used when a test product displays an appreciably larger bioavailability than the reference product.

1.21.2 Pharmaceutical equivalents

These are drug products that contain the same therapeutically active drug ingredient(s); contain the same salt or ester, or chemical form; have the same dosage form; and are identical in strength, concentration, and route of administration. Pharmaceutical equivalents may differ in characteristics such as shape, scoring configuration, release mechanisms, packaging, and excipients (including colours, flavouring, and preservatives).

1.21.3 Pharmaceutical alternatives

Pharmaceutical alternatives are drug products that contain the identical therapeutic moiety, or its precursor, but not necessarily in the same amount or dosage form or as the same salt or ester.

1.21.4 Therapeutic equivalents

Therapeutic equivalents are drug products that contain the same active moiety and clinically show the same efficacy and safety when administered to patients under the same conditions specified in the labelling. They contain the same active ingredients in the same dosage form, given by the same route of administration meet compendia standards of strength, quality, purity, and identity; and meet an acceptable in-vitro standard. They are either bioequivalent or have comparable bioavailability.

1.21.5 Pharmacokinetic terms

1.21.5.1 C_{max}

C_{max} is the peak or maximum drug concentration achieved in the systemic circulation following oral drug administration. For many drugs, a relationship is found between the pharmacologic effect of a drug and the plasma drug concentration. C_{max} provides an indication that the drug is sufficiently systemically absorbed to provide a therapeutic response. In addition, C_{max} provides a warning of possibly toxic levels of drug; a guide to the effectiveness, tolerability and the maximum safe concentration of a drug when compared to its minimum toxic concentration. The units are "concentration" units (e.g. µg/ml, ng/ml).

1.21.5.2 C_{min}

C_{min} is the minimum drug concentration achieved in the systemic circulation following a multiple dosing at steady state.

1.21.5.3 C_{pd}

C_{pd} is the pre-dose concentrations determined immediately before a dose is given at steady state.

1.21.5.4 T_{max}

T_{max} is the time required to achieve maximum drug concentration in systemic circulation.

1.21.5.5 AUC_{0-t}

AUC_{0-t} is the area under the plasma concentration - time curve from 0h to the last quantifiable concentration to be calculated using the trapezoidal rule. It is a measurement of the extent of bioavailability of a drug (total amount of active drug that reaches systemic circulation). For many drugs, it is directly proportional to the dose. It may not be proportional to the administered dose in cases where the pathway for drug elimination has become saturated.

1.21.5.6 $AUC_{0-\infty}$

$AUC_{0-\infty}$ is the maximum drug concentration achieved in the systemic circulation following drug administration, from zero time to infinity to be calculated

as the sum of AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant. It is also equal to the amount of unchanged drug reaching the general circulation divided by the clearance:

$$AUC = (\text{antilog } Z/K_{el}) - (\text{antilog } Z/K_{ab}) \quad \text{--------1.21.5.1}$$

where Z is the intercept on the concentration axis, K_{el} is the elimination rate constant and K_{ab} is the absorption rate constant derived from the method of residual (feathering).

$$[AUC]_0^\infty = \int_0^\infty Cp\ dt \quad \text{--------1.21.5.2}$$

$$[AUC]_0^\infty = \frac{FDo}{Clearance} \equiv \frac{FDo}{KVd} \quad \text{--------1.21.5.3}$$

where F = fraction of dose absorbed; Do = dose; K = elimination rate constant; and V_d = volume of distribution. The units of AUC are concentration.time (e.g. µg/ml.hours).

1.21.5.6 $AUC_{0-\tau}$

$AUC_{0-\tau}$ is the area under the plasma concentration - time curve over one dosing interval following single dose for multiple dose study at steady state.

K_{el} Apparent first-order terminal elimination rate constant calculated from a semi-log of the plasma concentration versus time curve.

1.21.5.6 $t_{1/2}$

$t_{1/2}$ is the elimination half life of a drug is the time necessary to reduce the drug concentration in the blood, plasma, or serum to one-half after equilibrium is reached. The biological half-life is the time in which the total amount of drug in the body after equilibration of the plasma with other compartments (fats, muscles etc.) is halved. Half-life is influenced by the route of administration, formulation, diffusion into tissues, plasma protein and tissue binding, metabolism and renal excretion, which are all subject to individual variation. Like plasma concentration, half-life is usually considered in terms of :

(1) Whether the drug is metabolised.

(2) Whether the drug is itself active or is converted to an active metabolite.

(3) Whether the drug has irreversible action.

(4) Presence of disease of the organs of metabolism or excretion.

$t_{1/2} = 0.693/Ke$

where Ke is the elimination rate constant

1.22 *In vitro* demonstration of bioavaialbility

For certain drug products, bioavailability may be demonstrated by evidence obtained *in vitro* in lieu of *in vivo*. For these drugs, bioavailability is largely dependent upon having the drug in the dissolved state. The rate of dissolution of the drug from the product can be measured *in vitro*. This *in vitro* data must be correlated with the *in vivo* bioavailability data for the drug.

1.23 Latin square cross over design

The Latin square design ensures that each volunteer receives each drug product only once in the clinical trial, with adequate time between medications (Table 1.2) for the elimination of the drug from the body. In this case, each volunteer is his own control and subject-to-subject variation is reduced. Moreover, variation due to time is reduced so that all volunteers do not receive the same drug product on the same day.

1.24 Cytochrome p450 enzymes in human tissues

The human genome has 57 CYP genes, and the function for most of the corresponding enzymes is known at least to some degree. Fifteen individual CYP enzymes in families 1, 2 and 3 metabolize xenobiotics, including the majority of small molecule drugs currently in use. A typical feature of these CYPs is broad and overlapping substrate specificity (Guengerich *et al*., 2005). Other CYPs with much narrower substrate specificity are devoted mainly to the metabolism of endogenous substrates, such as sterols, fatty acids, eicosanoids, and vitamins. It has become

Table 1.2: Latin square cross over design for comparative bioavailability study of four treatments in 16 human volunteers

GroupA1 (4volunteers)	**GroupA2 (4volunteers)**	**GroupB1 (4volunteers)**	**GroupB2 (4volunteers)**	**PERIODS**
Artesunate only	Amodiaquine only	Artesunate/Amodiaquine (fixed)	Artesunate+Amodiaquine (non fixed)	1
Amodiaquine only	Artesunate only	Artesunate+Amodiaquine (non fixed)	Artesunate/Amodiaquine (fixed)	2
Artesunate/Amodiaquine (fixed)	Artesunate+Amodiaquine (non fixed)	Artesunate only	Amodiaquine only	3
Artesunate+Amodiaquine (non fixed)	Artesunate/Amodiaquine (fixed)	Amodiaquine only	Artesunate only	4

evident that expression patterns of many individual CYPs in different tissues and cell types of an organ have important physiological roles (Seliskar and Rozman, 2007). Cytochrome P450 enzymes are found in practically all tissues, with highest abundance and largest number of individual CYP forms present in the liver. CYPs reside also in the intestine, lung, kidney, brain, adrenal gland, gonads, heart, nasal and tracheal mucosa, and skin. In human liver CYP enzymes comprise approximately 2% of total microsomal protein (0.3–0.6 nmol of total CYP per mg of microsomal protein). The content of drug-metabolizing CYPs is much lower in other tissues (Table 1.3). While extra hepatic metabolism may have clinically significant local effects, systemic metabolic clearance of drugs occurs in the liver with a significant contribution by the gut wall in special cases.

Metabolism is the main route of clearance for approximately 70% of currently used drugs. Ten individual CYP forms in the adult human liver carry out virtually the whole CYP-mediated metabolism. CYP3A4 is the highest abundance form and it metabolizes the greatest number of drugs and a very large number of other xenobiotics. A minority of Caucasian people have relatively high amount of CYP3A5 in the liver, and CYP3A7 is a foetal enzyme. Also CYP2D6, although of much lower abundance, mediates the metabolism of numerous drugs. Together CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 are responsible for more than 90% of known oxidative drug metabolism reactions (Guengerich, 2008). The CYP enzymes are well known for their capacity to metabolize a vast number of structurally diverse xenobiotics. The genes encoding CYP enzymes are highly polymorphic. Numerous studies have established that several variant alleles of individual CYP genes encode

Table 1.3: Total CYP content in selected human tissues (Hrycay and Bandiera, 2008)

Tissue	CYP content (nmol/mg microsomal protein)
Liver	0.30–0.60
Adrenal	0.23–0.54
Small intestine	0.03–0.21
Brain	0.10
Kidney	0.03
Lung	0.01
Testis	0.01

functionally deficient enzymes, the prime example being CYP2D6. When challenged with a CYP2D6 substrate drug, e.g. dextromethorphan, individuals with a deficient enzyme phenotype [poor metabolizers (PMs)] may experience adverse effects due to excessive serum concentrations of the drug. On the other hand, individuals with multiple copies of the CYP2D6 gene (ultrarapid metabolizers) will have insufficient clinical response since the drug is eliminated during first-pass metabolism (Kirchheiner *et al*., 2005). Most drugs cleared by the CYP system are metabolized through several CYP forms. As a general rule, drugs that are metabolized by a single CYP form are more susceptible to drug interactions than drugs metabolized by multiple forms.

1.25 Drug Metabolism

Metabolism is the principal elimination pathway for a majority of drugs. Lipophilic parent drugs are transformed by enzymes to commonly more hydrophilic metabolites facilitating their excretion into bile or urine. The liver is the central organ for drug metabolism, but other tissues such as the gastrointestinal tract, kidneys, skin and lungs are also involved. Drug metabolism is usually divided into two different types of reactions, phase I and phase II. Phase I, or functionalization reactions, expose or introduce a functional group on a molecule. These reactions include hydrolysis, reduction and oxidation. Phase II metabolism involves conjugation of a functional group of the molecule with hydrophilic endogenous substrates. While phase I reactions generally result in a small increase in hydrophilicity, will the consequence of most phase II reactions be a large increase in hydrophilicity. Glucuronidation, sulfation, acetylation and gluthatione conjugation are examples of phase II metabolism. Glucuronidation is quantitatively the most important conjugation reaction for drugs. Drug metabolizing enzymes are primarily located in the endoplasmatic reticulum and the cytosol. Oxidative phase I enzymes are almost entirely localized in the endoplasmic reticulum together with the phase II enzyme Uridinediphospho-glucuronosyltransferase (UGT), while other phase II enzymes, such as sulfotransferase and glutathione-S-transferase, are found in the cytosol. Phase III is mediated by drug plasma membrane transporters (influx and efflux), such as P-

glycoprotein, multidrug resistance protein (MRP) and organic anion transport protein 2 (OATP2), that are localized in the liver on the endothelium and epithelium membrane, as well as in the gastrointestinal tract, kidney and other organs. They play an important role in drug pharmacokinetics and pharmacodynamics. In the liver, OATP1 and OATP2 are localized in sinusoidal phase membrane of hepatocytes and are responsible for the uptake of many drugs to be metabolized. MRP2 and P-glycoprotein are localized in the canalicular phase membrane of the hepatocytes and are responsible for pumping out xenobiotics (i.e. drugs and their metabolites) to the biliary canals (Lau *et al*., 2004). Since they are present in several types of cells, and mediate cell exposure to drugs, they can influence not only absorption, distribution, metabolism and elimination, but also drug concentrations inside the target cell and affect the therapeutic efficacy. Acute malaria itself has been shown to influence the metabolism and distribution of certain drugs, it seems not by influencing drug absorption but by increasing binding to α-1-glycoprotein in plasma (Krishna and White, 1996). Inhibition of hepatic metabolism, mainly CYP3A4 enzymes in acute malaria, leads to a reduced clearance of quinine (Pukrittayakamee *et al*., 1997).

1.26 Human cytochrome P450 enzymes

The main enzymes involved in phase I drug metabolism are the Cytochrome P450s. They are heme-containing proteins catalysing oxidation reactions by inserting molecular oxygen into substrates. This enables transformation reactions resulting in *N*-, *O*-, and *S*-dealkylation, aliphatic and aromatic hydroxylation. The CYP enzymes also have a key role in the biosynthesis and degradation of many endogenous compounds such as lipids, steroids and vitamins. Broad and often overlapping substrate specificities of the CYP enzymes make it difficult to name them after the type of reaction they catalyse. Individual CYP enzymes are therefore classified on the basis of similarities in their amino acid sequence and are named by a family number, a subfamily letter and a number for each isoform within a subfamily. Individual CYPs responsible for approximately 80% of the metabolism of clinically used drugs in humans are belonging to the CYP1, CYP2 and CYP3 families. CYP3A is the most abundant subfamily in both liver and small intestine. Principal isoforms involved in drug metabolism include CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4.

The CYP isoform 2B6 was initially thought to play a rather insignificant role in overall drug metabolism, most likely due to its presumed low level of expression in human liver. An increasing number of clinically important drugs, including artesunate, cyclophosphamide, nevirapine and efavirenz, have been recognized as CYP2B6 substrates.

1.27 *In vitro* metabolism of drugs

There are several useful experimental systems (primary cultures of hepatocytes, liver tissue slices, subcellular fractions and heterologously expressed enzymes) available for studying the *in vitro* metabolism of drugs. Human liver microsomes, vesicles from fragmented endoplasmic reticulum, are widely used to investigate CYP metabolism, UGT activity and in high-throughput screening for metabolic stability of compounds. They have good long-term stability and associated assays are usually simple, rapid and sensitive (Pearce *et al*., 1996). However, the production of metabolites can differ from *in vivo* conditions due to the closed experimental system (Rodrigues, 1994). Except for glucuronidation, no other phase II reactions are possible. By measuring disappearance rates of known substrates for particular CYP isoforms in liver microsomes, information about the activities of the enzymes of interest can be obtained (Beaune *et al*., 1986). A linear correlation between metabolic rate constants of two different substrates in the same microsomes indicates that the metabolic reactions are principally mediated by the same CYP isoform.

1.28 Induction of CYP enzymes

The drug-metabolizing capacity in man is susceptible to changes due to influence of a variety of chemicals, administered for medical reasons or associated with our lifestyle. Many clinically used drugs from different therapeutic groups and with various chemical structures are known to induce or inhibit the metabolism of other drugs, leading to important drug-drug interactions. In recent years, much effort has been spent in trying to understand the mechanisms leading to induction of drug metabolism. The expression of genes can be increased by an induced gene

transcription, processing or stabilization of mRNA. Induction can also result from enzyme stabilization (Porter and Coon, 1991). The mechanism of induction for most *CYP* genes appears to be an increased gene transcription as a consequence of binding of inducers to intracellular receptors such as, aryl hydrocarbon receptor (AhR), pregnane X-receptor (PXR), constitutive androgen receptor (CAR) and glucocorticoid receptor (GR). PXR is directly activated in the nucleus upon binding of xenobiotics, while CAR undergoes translocation from the cytoplasm to the nucleus (Handschin and Meyer, 2003). GR is suggested to enhance CYP induction by PXR and CAR (Wang *et al*., 2003). Several CYPs are known to be inducible. CYP1A, CYP2A, CYP2B, CYP2C, CYP2E and CYP3A can be induced by dietary factors, drinking, smoking and therapeutic drugs (Hewitt *et al*., 2007). CYP2D6 is considered to be a non-inducible enzyme, but increased CYP2D6 activity has been observed during pregnancy (Wadelius *et al*., 1997).

Enzyme induction is generally a slow process, involving the *de novo* synthesis of proteins. As a consequence the process is expected to be time- and dose-dependent (Lin, 2006). A new enzyme steady-state level will result from a balance between its biosynthesis and degradation, regardless of which underlying induction mechanism is involved (Pelkonen *et al*., 1998). It will also take time for normalization of enzyme activity to base-line levels after discontinuing the inducing agent (Lin, 2006). The time it takes to reach a new steady state level of the enzyme is determined by a change in its half-life, as long as this is longer than the half-life of the inducing agent in the system (Venkatakrishnan *et al*., 2007). Induction of drug metabolism usually results in lower plasma levels of the compound, and becomes important especially for drugs with narrow therapeutic windows. For these drugs previously effective dosages can turn out to be ineffective upon induction. Enzyme induction can be associated with toxicity, due to an increased production of reactive metabolites, but the process is generally considered less important in causing serious adverse effects compared to enzyme inhibition. There are several different examples of pharmacokinetic models describing enzyme induction. Despite this, little is known about the time-course of enzyme activity, including the onset and duration of induction (Magnusson *et al*., 2008). A few models have been presented illustrating the auto-induction phenomena of drugs, including artemisinin (Gordi *et al*., 2005). While most of these models describe changes in clearance of the drug, the latter model predicts the induction in

terms of an increase in intrinsic clearance of the compound, which makes it possible to estimate the time-course of drugs with various degrees of extraction. Also, this model describes the commonly observed lag-time for the initiation of the induction process.

1.29 Inhibition of CYP enzymes

Inhibition of CYP enzymes is the most common cause of harmful drug–drug interactions and has led to the removal of several drugs from the market during the past years (Lasser *et al*. 2002). In contrast to induction, inhibition of drug metabolism is more or less an immediate process. As a result of inhibition, drug plasma concentrations could rapidly increase (increased bioavailability) and cause toxic effects. There are several types of enzyme inhibitors. Reversible inhibitors can be competitive, noncompetitive or uncompetitive, while mechanism-based inactivators or suicide inhibitors are classified as irreversible inhibitors. Competitive inhibition seems to be a relatively common mechanism of action for CYP enzymes. The inhibitor shares structural similarities with the substrates and can thereby compete for the active site of the enzyme. Both noncompetitive and uncompetitive inhibitors bind to the enzyme at a site distinct from that which binds the substrate, but the uncompetitive inhibitor will only bind to the enzyme-substrate complex. However, clear examples of the latter two types of reversible inhibitors are rather uncommon for enzymes involved in drug metabolism (Guengerich, 1999). Inhibition of CYP enzymes is not always mediated by a substrate as in the case of mechanism-based inactivators, where the inhibitory effect is due to a metabolite. These inhibitors are defined as any compound that is catalytically transformed by the enzyme to give a reactive metabolite which inactivates the enzyme without leaving it. It involves the formation of a covalent binding or complex between the reactive metabolite and the enzyme itself, leading to a loss of a variable part of catalytic activity from the enzyme (Pelkonen *et al*., 1998).

1.30 Induction and inhibition of phase II enzymes

In addition to the CYPs, many other enzymes involved in the metabolism of drugs are induced to various extents. However, limited information is available about induction of phase II enzymes compared to the substantial knowledge about induction of CYP enzymes. Nuclear receptors PXR, CAR and AhR seems to be involved the expression of UGTs (Zhou *et al*., 2005) as well as in the induction of glutathione-*S*-transferase (Lindros *et al*., 1998). Several phase II enzymes exist in multiple forms or as homo/heterodimers of two sub-units, which can be differentially induced and thereby dependent on the type of inducer. A number of drugs have been characterized to act as competitive inhibitors of phase II enzymes. Glutathione-*S*-transferase enzymes are very abundant and thought to be competitively inhibited by some hydrophobic compounds (Parkinson, 2001).

1.31 CYP2 family

The human CYP2 family is very diverse and comprises a number of important drug-metabolizing CYPs. Members of this family do not share any common regulation patterns and their substrate specificities and tissue expression vary substantially. CYP2B6, CYP2D6, and CYP2E1 are the only functional enzymes in their subfamilies, whereas CYP2A contains two, and CYP2C four functional members. The clinically most important CYP polymorphisms are found within the CYP2 family (i.e. CYP2C9, CYP2C19, and CYP2D6).

1.31.1 CYP2B6

CYP2B6 was initially considered not to be of importance in drug metabolism, but new investigations indicate high relevance of this enzyme in the metabolism of, e.g., anticancer drugs, like cyclophosphamide and ifosfamide, and anti-HIV drugs, like efavirenz and nevirapine (Owen *et al*., 2006; Turpeinen *et al*., 2006 for recent reviews). The human CYP2B6 gene is highly polymorphic. There appears to be a couple of variant alleles that are associated with lower expression/activity, and these are CYP2B6*6, CYP2B6*16 and CYP2B6*18 in particular (Rotger *et al*., 2007). Of these, CYP2B6*6 is rather common in several different populations (20–30%

frequency), whereas both CYP2B6*16 and CYP2B6*18 are common in Black subjects where the allele frequency is relatively high (Rotger *et al*., 2007).

1.31.2 CYP2C8

Like in the case of CYP2B6, the importance of CYP2C8 for drug metabolism has been elucidated quite recently (Totah and Rettie, 2005). Also a number of functional CYP2C8 polymorphisms have been published during recent years (Dai *et al*., 2001). Some single nucleotide polymorphism (SNPs) or their combinations in the CYP2C8 gene have been associated with certain disease states or adverse drug reactions, but more studies about the importance of CYP2C8 polymorphisms and also the general role of this enzyme in drug metabolism are still needed.

1.31.2.1 Substrates and inhibitors of CYP2C8

Drugs metabolized by CYP2C8 do not share any common structure or chemical pattern. There seems to be some overlapping especially with CYP2C9 and CYP3A4 substrates. Drugs with major importance of CYP2C8 include amodiaquine, paclitaxel, cerivastatin, and several oral antidiabetics such as repaglinide, pioglitazone, and rosiglitazone. Recently, the N-deethylation of the antimalarial amodiaquine was demonstrated as a good model substrate for CYP2C8 with high affinity and turnover rate. So far the applicability of glitazones as model substrates has been restricted by difficulties in obtaining metabolite standards. Known CYP2C8 inhibitors include amodiaquine, quercetin, which has been used for several years for *in vitro* purposes, and leukotriene receptor antagonists montelukast and zafirlukast. Although montelukast and zafirlukast are potent inhibitors of CYP2C8 *in vitro*, they both are highly bound to plasma proteins (99%) resulting in very low free fraction in humans. Thus these two drugs are not suitable for *in vivo* inhibition purposes (Jaakkola *et al*., 2006; Kim *et al*., 2007).

1.31.3 CYP2C19

Drugs metabolized via CYP2C19 are usually amides or weak bases with two HBAs (Lewis, 2004). Compared to CYP2D6, polymorphisms of the CYP2C19 gene represent a smaller proportion and perhaps have less clinical significance in

Caucasians, but in Orientals the frequency of CYP2C19 polymorphisms has been characterized to be up to 20% of the population (Bertilsson, 1995; Ingelman-Sundberg *et al*. 2007).

1.32 CYP3 family

The human CYP3 family represents about 30% of the total hepatic P450 content and is considered to be the most important CYP subfamily in the biotransformation of drugs. This family contains one subfamily including three functional proteins: CYP3A4, CYP3A5, and CYP3A7, and one pseudoprotein, CYP3A34 (Ingelman-Sundberg, 2005). These enzymes have overlapping catalytic specificities and their tissue expression patterns differ. CYP3A5 is a minor polymorphic CYP form in human liver (Westlind-Johnsson *et al*., 2003), but in extra hepatic tissues it is consistently expressed in kidney, lung, colon, and esophagus. Despite a few exceptions, the substrate and inhibitor specificity of CYP3A5 seems to be highly similar to CYP3A4, albeit the catalytic capability might be somewhat lower (Williams *et al*., 2002). CYP3A7 is mainly expressed in embryonic, foetal, and newborn livers, where it is the predominant CYP form (Kitada and Kamataki, 1994; Hakkola *et al*., 2001), whereas in the adult liver, CYP3A7 seems to be a minor form. CYP3A7 has an important role during the foetal period in the hydroxylation of several endogenous substances like retinoic acid and steroid hormones, and therefore it has relevance to normal embryonic development (Hines and McCarver, 2002).

1.33 Albumin

Albumin makes up more than half of the total protein present in serum. Approximately 30 to 40% of the body's total albumin pool is found in the intravascular compartment. The remainder is extravascular and is located in the interstitial spaces, mainly of the muscles and skin. Albumin is also found in small amounts in a variety of body tissue fluids such as sweat, tears, gastric juice, and bile. The normal serum protein level is 6 to 8 g/dl. Albumin makes up 3.5 to 5.0 g/dl, and the remainder is the total globulins. These values may vary according to the individual laboratory. Albumin does not diffuse freely through intact vascular endothelium.

Hence, it is the major protein providing the critical colloid osmotic or oncotic pressure that regulates passage of water and diffusible solutes through the capillaries. Albumin accounts for 70% of the colloid osmotic pressure. It exerts a greater osmotic force than can be accounted for solely on the basis of the number of molecules dissolved in the plasma, and for this reason it cannot be completely replaced by inert substances such as dextran. The reason is that albumin has a negative charge at normal blood pH and attracts and retains cations, especially Na^+ in the vascular compartment. This is called the *Gibbs–Donnan effect*. Albumin also binds a small number of Cl^- ions that increase its negative charge and ability to retain Na^+ ions inside the capillaries. This enhanced osmotic force causes the colloid osmotic pressure to be 50% greater than it would be by protein concentration alone. Albumin serves in the transport of bilirubin, hormones, metals, vitamins, and drugs. It has an important role in fat metabolism by binding fatty acids and keeping them in a soluble form in the plasma. This is one reason why hyperlipemia occurs in clinical situations of hypoalbuminemia. The binding of hormones by albumin regulates the amount of free hormone available at any time. Because of its negative charge, albumin is also able to furnish some of the anions needed to balance the cations of the plasma.

Albumin is synthesized in the liver. The rate of synthesis is constant in normal individuals at 150 to 250 mg/kg/day, resulting in the production of 10 to 18 g of albumin daily in a 70-kg man. The liver produces albumin at less than half of its capacity. The primary factors affecting albumin synthesis include protein and amino acid nutrition, colloidal osmotic pressure, the action of certain hormones, and disease states. The half-life of albumin in the circulation is about 20 days. Fasting or a protein-deficient diet causes a decrease in albumin synthesis as long as the deficiency state is maintained. In the normal individual, the liver increases albumin synthesis in response to the increased availability of amino acids provided by the portal blood following each protein-containing meal. A decrease in extravascular colloidal pressure serves as a stimulus for albumin synthesis and is thought to act within the liver. Thyroid hormone, corticosteroids, growth hormone, and insulin all can increase albumin synthesis. The main site of albumin degradation is not known. Albumin appears to be catabolized in locations that are capable of rapid equilibration with the bloodstream (Busher, 2011).

1.34 Binding of various drugs to plasma proteins

In an ideal therapeutic regimen, a sufficient amount of the drug should reach the locus of action (receptor site) in order to bring about the desired effect, but not so much as to produce toxicity. Furthermore, the drug should not disappear too rapidly from the locus of action, or the therapeutic effects will be transient and hence of limited value. The binding of drugs to plasma proteins and various subcellular components tends to accomplish these objectives. Human plasma contains over 60 different proteins, and the most abundant one is albumin. Other significant proteins include prealbumin, lipoproteins, and various globulins. A number of plasma proteins, especially albumin, have shown a high affinity for binding drugs, so that at a given total plasma concentration only a portion of the total amount of drug is free in the plasma water. The remainder is bound to plasma proteins and in this form does not exert any pharmacological effects. The interaction between proteins and a drug is governed by the law of mass action, in that the proportion of bound drug remains constant provided the binding sites are not saturated. With the possible exception of valprioc acid and disopyramide, the saturability of binding sites does not occur within therapeutic ranges.

The interaction between drug and protein is not a chemical one, but a reversible attachment that is achieved by various forces, including electrostatic, London-van der Waals, and hydrogen binding, or some combination of these. This drug-protein complex is readily reversible (the half-life being on the order of milliseconds). There is a continuous shift of bound to unbound drugs, and, unlike receptor binding, no pharmacologic response occurs as a result of the association. The binding site of endogenously occurring acidic substances (e.g., bilirubin, vitamin C, and bile acids) and acidic drugs (e.g., phenylbutazone, penicillins, sulfonamides, warfarin, and salicylic acid) is the N-terminal amino acid. The basic drugs (e.g., diphenhydramine, streptomycin, chloramphenicol, and coumarin anticoagulants) bind nonspecifically.

1.34.1 Alpha$_1$-acid glucoprotein

Alpha$_1$-acid glycoprotein exists in concentrations that are 50 to 100 times lower than those of albumin. Basic drugs (quinidine, imipramine, propranol, and lidocaine) bind to the single site present on alpha$_1$-acid glycoprotein.

1.34.2 Other glycoproteins

Corticosteroid-binding globulin and thyroxine-binding globulin are both alpha globulins that possess high affinities but low capacities for their respective substrates. Methadone (a narcotic analgesic) binds to the gamma-, beta-, and alpha-globulins, as well as to albumin.

1.35 Pharmacokinetics and drug metabolism of artesunate

Multiple dose studies in both healthy subjects and patients have shown remarkable time-dependent pharmacokinetics of artemisinin, with an up to five-fold increase in oral clearance of the drug (Svensson *et al*., 1998). A pronounced, unusual capacity for auto-induction of drug metabolism appears to be the explanation of this time-dependency (Ashton *et al*., 1996). Absence of a corresponding change in elimination half-life indicates the compound to be highly extracted by the liver, with the increase in hepatic clearance primarily affecting its bioavailability. Artemisinin has demonstrated a capacity to increase the metabolism of other drugs mediated by different Cytochrome P450 (CYP) enzymes, including CYP2C19 and CYP2B6 (Svensson *et al*., 1998; Simonsson *et al*., 2003). The mechanism of induction is suggested to entail activation of nuclear receptors, pregnane X-receptor (PXR) and/or constitutive androgen receptor (CAR) (Burk *et al*., 2005; Simonsson *et al*., 2006) Artemisinin is also an inhibitor of drug metabolism. Artemisinin and DHA were found to be potent inhibitors of CYP1A2 and CYP2C19 in an *in vitro* screening study (Bapiro *et al*., 2001). In addition, artemisinin has been suggested to be an inhibitor of glucuronidation in healthy volunteers (Zhang *et al*., 2001). The *in vitro* metabolism of artemisinin is primarily mediated by CYP2B6, with a secondary contribution of CYP3A4 in individuals with low expression of CYP2B6, and a minor involvement of CYP2A6 (Svensson and Ashton, 1999). The elimination half-life of artemisinin is reported to be 2-3 hours after oral administration in healthy subjects and patients with

falciparum malaria (de Vries and Dien, 1996).

Artemether, arteether and artesunate are all rapidly converted back to DHA after oral and parenteral administration (Figure 1.13). DHA is metabolized by glucuronidation, most likely mediated by UGT1A9 and UGT2B7. The *in vitro* metabolism of artemether is suggested to involve CYP1A2, CYP2B6, CYP2C19 and CYP3A4 (Navaratnam *et al*, 2000). In healthy subjects, intestinal CYP3A4 appears to be involved in its first-pass metabolism (van Agtmael., *et al* 1999). Declining concentrations of DHA and artesunate have, although less convincingly, been reported after multiple administration of artesunate to malaria patients (Khanh *et al*.,1999). CYP3A4 is the primary enzyme involved in the *in vitro* metabolism of arteether, with a minor contribution of CYP3A5 and CYP2B6 (Grace *et al*., 1998). The water-soluble artesunate, is considered as a pro-drug because of its very rapid conversion by hydrolysis to DHA *in vivo* (Lee and Hufford, 1990). After intravenous administration, hydrolysis of the drug appears to be mediated by esterase in the blood (Navaratnam, *et al*., 2000). The *in vitro* metabolism of artesunate has been reported to involve CYP2A6 (Li *et al*, 2003).

In general, absorption of the artemisinin drugs following oral administration appears to be rapid but incomplete. Data on intravenous administration is only available for artesunate and high relative bioavailability (82%) has been reported of DHA after intravenous administration of artesunate to malaria patients (Batty et al., 1998). Compared to oral treatment with artemisinin, relative bioavailability following rectal administration was approximately 30% in malaria patients. The relative bioavailability of intramuscular and intrarectal artemether has been reported to be 25% and 35%, respectively, compared to oral artemether in healthy volunteers. Arteether is available for intramuscular injection only, and has an elimination half-life of > 20 h due to a slow absorption from the injection site (Looareesuwan et al., 2002). The other derivatives appear to be rapidly eliminated after administration. Artemether

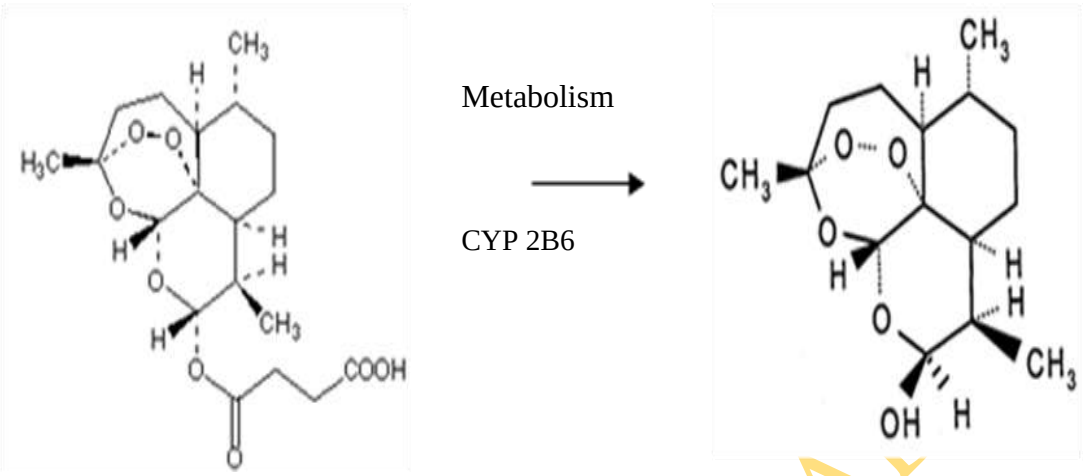


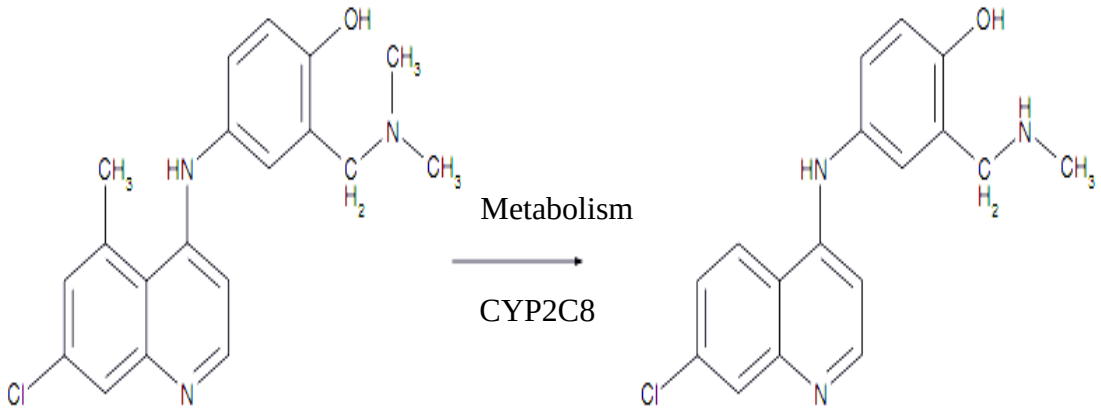
Figure 1.13: Conversion of parent drug (artesunate) to main metabolite (dihydroartemisinin)

has an elimination half-life of approximately 1 hour after oral administration. Half-lives of approximately 3 min and 40 min have been reported for artesunate and DHA, respectively, following oral and intravenous administration of artesunate (Batty *et al*., 1998). The protein binding of [^{14}C] labelled ART was studied in human and rat plasma using equilibrium dialysis method over a concentration range of 0.2 to 78125 ng/mL (Li *et al*., 2006). The binding profile was shown to be concentration-dependent. In both species, the 12 percent of drug bound to plasma proteins ranged from 73% to 81%. At drug concentrations more than 125 ng/mL, the percent bound declined to 62-66%. The protein binding of [^{14}C] labelled DHA was also studied in human and rat plasma using similar method over a concentration range of 0.15 to 57800 ng/mL (Xie *et al*, 2009). The binding of [^{14}C] DHA was concentration-dependent in human plasma but not in rat plasma. At higher concentrations, the binding percentage in human plasma decreased from 82% to 66%.

1.36 Pharmacokinetics of amodiaquine (AMQ)

AMQ pharmacokinetics has mainly been studied in adults. This should be kept in mind when extrapolating the pharmacokinetic data to the clinical situation as the children may have higher or lower clearance/kg body weight, depending on their age and stage of maturation. The plasma concentration time course after IV administration is biphasic with a fast distribution phase and a slower elimination phase. After an intravenous 4-h infusion of 10mg base/kg to ten adult patients with *P. falciparum* malaria, the half-life of the first disposition phase was 22min (geometric mean; range 5–126 min) and the half-life of the terminal phase 10h (geometric mean; range 3–33h). Clearance was 6 L/h/kg (range 2–17) (White, 1997). After an intravenous injection of 3 mg base/kg to six healthy volunteers, similar characteristics were observed but clearance was higher (geometric mean 13L/h/kg; range 5–56) and the terminal half-life shorter (geometric mean 2 h, range 0.5–6 h) than in patients. These differences in observed clearance may be due to a reduced hepatic blood flow or reduced enzyme activity in acute malaria. It is possible that there is another disposition phase of AMQ with a longer half-life. This is indicated by the detection of AMQ in urine for longer time than expected by Winstanley and colleagues (Winstanley *et al*., 1987). AMQ pharmacokinetics is independent of dose in the dose-

range 200–600mg administered as single doses to healthy volunteers. The drug appears to be eliminated mainly through metabolism with some contribution of renal excretion. The main metabolite observed is the pharmacologically active desethylamodiaquine (DEAQ) (Figure 1.14), which is found in large amounts in the circulation. Two other metabolites, 2-hydroxy-DEAQ and *N*-bisdesethyl-AMQ, have also been found in the circulation in similar or higher concentrations than AMQ (Churchill *et al*., 1985), (Figure 1.15). After oral administration, the exposure of DEAQ is many-fold higher than the exposure of AMQ. After a 600 mg oral dose of AMQ to healthy volunteers, the DEAQ:AMQ area under the plasma–time curve $(AUC)_{0-\infty}$ ratio was approximately 52 (the estimate is rough as both $AUC_{0-\infty}$ values included a large area extrapolated from the last data point). DEAQ accumulates in the red blood cells, reaching a red blood cells/plasma ratio of 3 (Winstanley, 1987). Both AMQ and DEAQ are highly (>90%) protein-bound (Pussard and Verdia, 1994). DEAQ is assumed to be the main entity responsible for the therapeutic effect of AMQ treatment. The disposition of DEAQ in healthy volunteers after a 10 mg/kg oral dose of AMQ base has been observed to have three phases; two initial disposition phases with half-lives of 3–11 h and 23–50h and a terminal phase with a half-life of 9–18days in plasma and 12–32days in whole blood (Pussard *et al*., 1987).



Amodiaquine

Desethylamodiaquine

Figure 1.14: Conversion of parent drug (amodiaquine) to main metabolite (desethylamodiaquine)

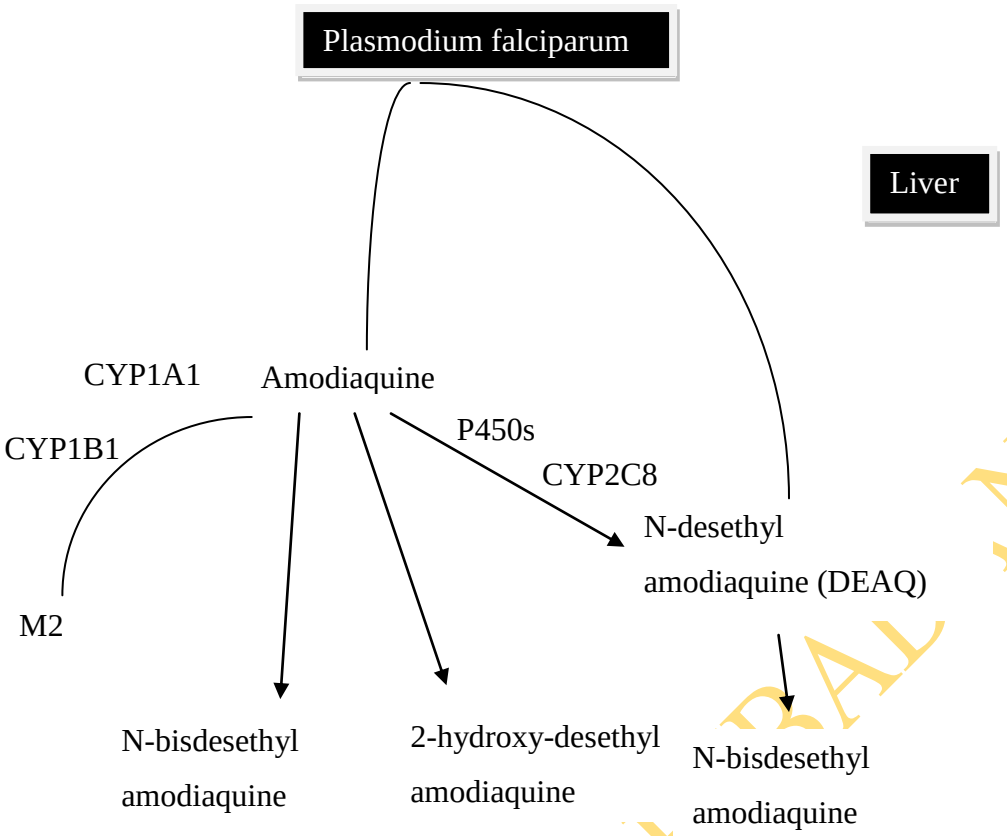


Figure 1.15: Enzymatic Conversion of amodiaquine to desethylamodiaquine in the liver

In vitro metabolism studies with enzyme expression systems, human liver microsomes, enzyme activity correlation analysis and enzyme docking analysis, strongly indicate that CYP2C8 is the only hepatic CYP isoform involved in the formation of DEAQ, and the main hepatic enzyme responsible for AMQ metabolism. DEAQ appears to be eliminated through renal excretion as well as further metabolized by other, probably extrahepatic, CYP enzymes, namely CYP1A1.

1.37 Metabolism of amodiaquine

After oral administration, amodiaqine is rapidly absorbed from the gastrointestinal tract. In the liver it undergoes rapid and extensive metabolism to N-desethyl-amodiaquine (DEAQ) which concentrates in blood cells. Amodiaquine is three-times more potent than DEAQ but the concentration of amodiaquine in blood is quite low. Therefore, DEAQ is responsible for most of the observed antimalarial activity. *In vitro*, amodiaquine showed synergistic activity and enhanced the efficacy of DEAQ against P. falciparum. Amodiaquine and DEAQ are over 90% bound to plasma proteins, which is a potential site for drug interaction. An *in vitro* study using human liver microsomes and two sets of recombinant human cytochrome P450 isoforms (from lymphoblastoids and yeast) established that the oxidation step from amodiaquine to DEAQ in the liver is mainly performed by CYP2C8. These experiments also showed that there is an unidentified metabolite (M2), which is a product of amodiaquine metabolism by the extra hepatic CYP1A1 and CYP1B1 enzymes. Several studies isolated N-bis-desethyl-amodiaquine and 2-hydroxydesethylamodiaquine as minor metabolites compared to DEAQ in human urine and blood, but the pharmacological importance of these metabolites remains unclear. Further metabolism of DEAQ to N-bis-desethyl-amodiaquine has been suggested although the plasma and urine concentrations of this metabolite were low in healthy volunteers.

1.38 Mechanism of action

In vitro studies suggest that amodiaquine inhibits the digestion of haemoglobin as the antimalarial mode of action. The drug also inhibits the glutathione-dependent destruction of ferriprotoporphyrin IX in the malaria parasite, resulting in the accumulation of this peptide, which is toxic for the parasite.

1.39 Pharmacogenetics of amodiaquine

Pharmacokinetic studies on amodiaquine and DEAQ showed that there is great interindividual variability in kinetic parameters. This variation could have implications in the therapeutic and toxicological response to the drug. CYP2C8*2 was only found in populations of African descent. CYP2C8*3 was most prevalent among Caucasians and much less common in African populations. *In vitro* experiments showed that CYP2C8*2, CYP2C8*3 and CYP2C8*4 had a threefold higher Km and six fold lower intrinsic clearance for amodiaquine compared with the wild type enzyme(CYP2C8 activity) and the CYP2C8*3 variant had an even more profound decreased activity. As part of the same study, the association of the CYP2C8 genotype with treatment outcome and adverse events was investigated in 275 African malaria patients treated with amodiaquine monotherapy. Interestingly, no difference in antimalarial efficacy was noted in individuals who were homozygous or heterozygous for CYP2C8*2 alleles. CYP2C8*2 carriers had a higher frequency of treatment-associated mild side effects (abdominal pain) but no other associations were seen between CYP2C8*2 genotype and specific adverse events, including nausea, vomiting, fatigue, and jaundice. These variants were found in several countries in Africa (1-4% of African population). In Burkina Faso the frequency of CYP2C8*2 allele was 0.155 and 0.003 for CYP2C8*3 (Parikh *et al*. 2007). These variants with a reduced activity to metabolize amodiaquine could be responsible probably showing a higher risk of adverse drug reactions (ADRs) occurrence. Clinical studies suggest that *CYP2C8*2* is not a major determinant of amodiaquine efficacy or toxicity (not a significant predictor of the pharmacokinetics or clinical response) in adults or children.

1.40 Drug-Drug interaction

A study in twelve healthy Swedish volunteers showed that a single dose of amodiaquine decreased CYP2D6 and CYP2C9 activities significantly compared to baseline values using debrisoquine and losartan as probe drugs. The antiretroviral drugs efavirenz, saquinavir, lopinavir, and tipranavir were potent CYP2C8 inhibitors at clinically relevant concentrations. A study in two healthy volunteers compared the metabolism of artesunate-amodiaquine and the combination of artesunate-amodiaquine with efavirenz. The addition of efavirenz to the artesunate-amodiaquine treatment increased amodiaquine exposure and decreased DEAQ exposure in both subjects. Both volunteers developed asymptomatic hepatotoxicity several weeks after study completion. This finding suggests that liver function monitoring may be especially necessary in HIV-positive patients receiving efavirenz together with amodiaquine.

1.41 *P. falciparum* amodiaquine/desethylamodiaquine resistance

Malaria therapy with AMQ is in most cases effective against CQ resistance *P. falciparum*. Nevertheless, events of the parasite being simultaneously refractory *in vivo* to both drugs are not rare, pointing for a certain degree of cross-resistance (Bjorkman and Phillips-Howard, 1990). *In vivo P. falciparum* resistance to AMQ regimens seems to be associated with mutations in the parasite *P. falciparum* CQ resistance transporter (*Pfcrt*) gene, coherent with the occasional observation of CQ/AMQ clinical cross-resistance. *Pfcrt* codes for a trans membrane protein located in the parasite food vacuole, the central organelle in the metabolism of haemoglobin, an essential requirement for the progression of the parasite cycle. During the metabolism of haemoglobin the toxic heme is liberated from its molecular scaffold. CQ (and possibly AMQ or DEAQ) binds heme, disturbing its inactivation by the parasite (Ginsburg *et al*., 1999). *Pfcrt* SNPs, in particular a K76T variation, render the parasite resistant to the drug, probably through the efflux of CQ from the organelle. CQ-resistance is reversible *in vitro* by the action of the calcium blocker verapamil (VP), known to interfere specifically with K76T harbouring PfCRT proteins. Interestingly, although DEAQ sensitivity is reversible with VP, AMQ response is not

significantly affected (Bray *et al*., 1996). This data points for *pfcrt* K76T to be associated mainly with DEAQ response, partially explaining previously observed *in vivo* CQ/AMQ cross-resistance *in vivo*, which should maybe be better interpreted as CQ/DEAQ. Furthermore, due to the aforementioned 'tail effect', the slowly eliminated DEAQ is predicted to be the weak link promoting resistance in the AMQ–ART combination.

1.42 Body fat percentage

There are a number of alternative formulae that relate body fat to body mass index (BMI). Although these calculations are based on equations published in peer reviewed journals they are only an estimate and there will be variations around the results, as slightly over for obesity. See Table 1.4.

Note: The body mass index is calculated from an individual's weight (Kg) divided by the square of the height (m). The relationship between densitometrically-determined body fat percentage (BF%) and BMI, taking age and sex into account internal and external cross-validation of the prediction formulas showed that they gave valid estimates of body fat in males and females at all ages. In obese subjects however, the prediction formulas slightly overestimated the BF%. The prediction error is comparable to the prediction error obtained with other methods of estimating BF%, such as skinfold thickness measurements or bioelectrical impedance.

Table 1.4: General Body Fat Percentage Categories

Classification	Women (% fat)	Men (% fat)
Essential Fat	10-12%	2-4%
Athletes	14-20%	6-13%
Fitness	21-24%	14-17%
Acceptable	25-31%	18-25%
Obese	32% plus	25% plus

The formula for children was different and the relationship differed from that in adults, due to the height-related increase in BMI in children.

Child Body Fat % = (1.51 x BMI) - (0.70 x Age) - (3.6 x gender) + 1.4 ---------1.42.1

Adult Body Fat % = (1.20 x BMI) + (0.23 x Age) - (10.8 x gender) - 5.4 ---------1.42.2

where male gender= 1, female=0. (Body fat percentage, 2011)

1.43 Rationale for the study

The availability of numerous brands of artesunate and amodiaquine in the Nigerian drug market today places clinicians and pharmacists in a difficult situation of the choice of a suitable brand and/or alternatives to be used to treat malaria patients. Reports of distribution of counterfeit artesunate tablets in some parts of the world are of great public health concern (Alter *et al*., 2006). This suggests the possibility of the existence of commercial brands that will neither pass compendial tests nor demonstrate similar physicochemical qualities with predominant brand of proven quality. As effective as artesunate is in the treatment of uncomplicated malaria, it is becoming the target of an extremely sophisticated and prolific counterfeit drug trade that includes the counterfeiting of both artesunate tablets and packaging, with fake products looking very similar to authentic ones (Dondorp *et al*., 2004; Aldhous, 2005).

The chaotic drug distribution system as well as slack domestic drug law enforcement, are factors in the infiltration of artesunate and amodiaquine tablets of doubtful quality. The need, therefore, to monitor the physicochemical and biopharmaceutical qualities of the generic antimalarial agents cannot be over-emphasized especially when these drugs are coformulated. Altered pharmacokinetics may result due to coadministration of these antimalarials and this may change the course of tolerability. It leaves us no choice but to agree with the fact that there should be evidence of pharmaceutical and comparative bioavailability before a drug or its coformulation is interchanged/ substituted with another in treating patients.

The aim of the present study is to examine the pharmaceutical equivalence of different brands of artesunate and amodiaquine tablets and, their bioavailability and tolerability when given as monotherapy and in combination.

1.44 Objectives of study

1.44.1 General objectives:

To determine the causes of antimalarial therapy failure with commercial artesunate and amodiaquine tablets commonly available in Southwest Nigeria.

1.44.2 Specific objectives:

1. To determine relative quality and pharmaceutical equivalence of some commercial brands of artesunate and amodiaquine tablets.
2. To investigate the bioavailability, pharmacokinetics and tolerability of artesunate, amodiaquine, their co-formulations and their primary metabolites i.e. dihydroartemisinin and N-desethylamodiaquine .
3. To determine the relative bioavailability and equivalence of the two pharmaceutical alternatives (fixed and non fixed co-formulations of artesunate and amodiaquine tablets).
4. To explore possible pharmacokinetic drug interactions and the effect of concurrent administration of artesunate and amodiaquine tablets on time dependent plasma concentrations profile.
5. To ascertain their possible interchangeability in clinical use.
6. To determine the effect of serum albumin and percentage body fat on tolerability of amodiaquine tablets.

1.45 Research Hypotheses:

1. Concomitant usage of artesunate and amodiaquine in antimalarial therapy affects their respective *in vivo* bioavailabilites.
2. Higher plasma albumin predisposes users to adverse effects of amodiaquine and its combinations.

3. Higher adiposity predisposes users to adverse effects of amodiaquine and its combinations.

1.46 Null Hypotheses:

1. Concomitant usage of artesunate and amodiaquine has no effect on their respective *in vivo* bioavailabilites.
2. Higher plasma albumin does not predispose users to adverse effects of amodiaquine and its combinations.
3. Higher adiposity does not predispose users to adverse effects of amodiaquine and its combinations.

CHAPTER 2

MATERIALS AND METHODS

2.1 EXPERIMENTAL

2.1.1 Materials/Equipment

Artesunate reference powder was a donation from Greenlife Pharmaceuticals Nigeria Plc, (Lagos) and amodiaquine reference powder was donated by Pfizer Specialties Ltd, (Lagos, Nigeria). Sodium hydroxide, hydroxylamine hydrochloride, ethanol, acetone, ethyl acetate, toluene, methanol, ferric chloride, vanillin (all of analytical reagent grades from BDH, Poole, England). Samples of different brands of artesunate 50 mg and amodiaquine HCl (153.1 mg and 200 mg) tablets were randomly purchased from pharmacy retail outlets in South western Nigeria. All samples were within their shelf-life (Table 2.1 and Table 2.2). The purchase costs were noted. Fifteen samples that cut across 9 different artesunate brands (designated A-I) and five different amodiaquine brands (AA, BB, CC, DD, EE) were randomly selected for this study. Randomization was done in the proportion of 50:30:20 (Lagos:Ogun:Oyo states). Hardness Tester (BTB 301 Pharmatest, Switzerland); Erweka tablet disintegration apparatus (Erweka, Apparabetau, Germany); Erweka DT-D dissolution test apparatus (Erweka, U.K.); TLC plates (Merck, Germany); UV-VIS spectrophotometer (Hitachi, Japan) were used in this study.

For the high performance liquid chromatography (HPLC) study, artesunate, dihydroartemisinnin, amodiaquine and artemisinnin reference samples were donated by NAFDAC (National Agency for Food and Drugs Administration and Control, Lagos, Nigeria) while desethyl amodiaquine was purchased from Isosciences LLC USA. HPLC grade acetonitrile and methanol (analytical grade) were purchased from Sigma (Sigma-Aldrich Chemical Company, Germany). Camosunate® (Non-fixed co-formulation of artesunate and amodiaquine) and Diasunate® (fixed co-formulation of artesunate and amodiaquine) were sourced from reputable pharmacies.

Table 2.1: Artesunate drug products used

Code	Brand names	Country of manufacture	NAFDAC Registration No.	Batch No.	Cost of full course(₦)
A	Aretmed	India	04-4213	MP-004	260
B	Artesunat	China	04-3397	06054FX	270
B1	Artesunat	China	04-3397	07009FX	270
B2	Artesunat	China	04-3397	0170805	270
B3	Artesunat	China	04-3397	06027FX	270
C	Arthlon	India	04-5210	NA61103	300
D	Malasunate	China	04-7569	061115	280
E	Arinate	Belgium	04-7884	05G077	240
F	Vami's	Nigeria	04-8174	TVA506	280
F1	Vami's	Nigeria	04-8174	TVA505	280
G	Lever	China	04-5865	091005	260
G1	Lever	China	04-5865	051206	250
H	Malmeter	India	04-6964	K60444	280
H1	Malmeter	India	04-6964	K60893	280
I	Larimal	India	04-6154	BDV7002F	300

Table 2.2: Amodiaquine drug products used

Code	Brand names	Country of manufacture	NAFDAC Registration No.	Batch No.	Cost of full course(₦)
AA	Camoquine	USA	04-0679	040679	450
BB	Larimal	India	04-6154	BDV7002F	350
CC	Timec	India	04-9796	1005	150
DD	Loquine	England	04-7304	LC5001	160
EE	Dart	Nigeria	04-7956	047956	360

2.2 Subjective physical assessment of products

The labels, external packages, unique identification marks, leaflet inserts, foils and tablets were carefully examined for unique markings, odour and colour and recorded. For colour assessment, the tablets were placed on a white sheet of paper and both sides of each tablet examined. Identification of the white colour (for artesunate) and yellow colour (for amodiaquine) was carried out by matching with the closest colour in Federal Standard Number 595 colours, (2008). Deviations from the chosen colours were rated from a scale of 1 - 10 with 10 being the chosen white and yellow colours and 1 being the highest level of deviation. The tablets were also assessed for their odour.

2.3 Weight uniformity test and Uniformity of diameter

Evaluation of tablet weight variation was carried out using the weight uniformity test outlined in the British Pharmacopoeia (B.P, 2001). Twenty tablets were randomly selected for each brand evaluated and their weights determined. The mean weight and deviation (%) were calculated. A micrometre screw gauge (SMC 20326, Sterling Manufacturing Company, India) was used to measure the diameter of 12 randomly selected tablets. The mean diameter and deviation (%) from the mean were calculated.

2.4 Identification test for artesunate and amodiaquine

Artesunate in the tablets were identified using methods B, C and D of International Pharmacopoeia (I.P, 2009) based on a validated TLC-Thin Layer Chromatography.

Method B:

Silica gel was used as the coating substance and a mixture of 5 volumes of ethyl acetate and 95 volumes of toluene was used as the mobile phase. Solution A was prepared as follows: a quantity of the powdered tablets, equivalent to about 0.1mg of artesunate, was mixed well with dehydrated ethanol and filtered through a Whatman

no.1 filter paper, discarding the first few ml of the filtrate. The filtrate was evaporated and the residue dissolved in 1.0ml of toluene. Solution B consisted of about 0.1mg of artesunate RS per ml of toluene. 2µl each of Solutions A and B were spotted on the TLC and developed in the chamber. After removal of plate from the chamber, it was air-dried, sprayed with anisaldehyade methanol TS, and heated to 120 ^{o}C for 5min. The chromatograms were examined in ultraviolet light at a wavelength of 254nm and the R_f values (distance travelled by spot / distance travelled by solvent front) of the various samples were calculated.

Method C:

To a quantity of the powdered tablets equivalent to 0.1g of artesunate, 40ml of dehydrated ethanol was added, shaken and then filtered through a Whatman no.1 filter paper, discarding the first few ml of the filtrate. To half of the filtrate, about 0.5ml of hydroxylamine hydrochloride TS2 and 0.25ml of NaOH (~80g/L) TS were added. The mixture was heated in a water bath to boiling, cooled and two drops of HCl (~70 g/L) TS and two drops of ferric chloride (50g/L) TS was added.

Method D:

The remaining filtrate in Method C was evaporated on a water bath to a volume of about 5ml. Few drops of the mixture was placed on a white porcelain dish and one drop of vanillin/sulphuric acid TS2 was added and allowed to stand for 30min. A sample that contains artesunate yields a principal spot on TLC with the same Rf, appearance and intensity as the reference artesunate (method B), a light red-violet colour (method C) and a red colour (method D). Failure to yield these colours constitutes a negative result. Amodiaquine was identified using United States Pharmacopoeia (USP) method B (UV/VIS absorption) (U.S.P, 2008) whereby an amount equivalent to 300mg of amodiaquine was transferred to a 250ml beaker. Dilute hydrochloric acid 100ml of 1 in 100 was added and heated on a steam bath for about 15min with occasional stirring. The mixture was cooled to room temperature and transferred to a 200ml volumetric flask. Dilute hydrochloric acid (1 in100) was added to make it up to 200ml and mixed; 10ml of the clear supernatant was mixed with 10ml of dilute hydrochloric acid (1 in 100) in a 125ml separator. It was washed with 20ml of chloroform, the washing discarded, and 4.5ml of 1N sodium hydroxide was added followed by extraction with four 25ml portions of chloroform. The

combined chloroform extract portions were further extracted with three 50ml portions of dilute hydrochloric acid (1 in 100). The acid extracts were combined in a 200ml volumetric flask, dilute hydrochloric acid (1 in 100) was added to volume and mixed. A portion (20ml) of this solution was mixed with dilute hydrochloric acid (1 in 100) and made up to volume with the latter in a 100ml volumetric flask. The absorbance of this solution was concomitantly determined with a solution of the undried amodiaquine reference sample in dilute hydrochloric acid (1 in 100) having a known concentration of 15µg per ml in 1cm cells at 342nm with ultraviolet spectrophotometer (Hitachi, Japan) using dilute hydrochloric acid (1 in 100) as blank.

2.5 Assay of artesunate and amodiaquine tablets

2.5.1 Artesunate assay

Artesunate tablets were analysed for their content of artesunate using titrimetric procedure B of IP (I.P, 2009) whereby 50ml of neutralized ethanol (ethanol ~ 750g/L containing 0.5ml of phenolphthalein and carbonate-free 0.1mol/l sodium hydroxide and shows a faint pink colour) was mixed well with a quantity of crushed artesunate tablets equivalent to 0.5g of artesunate and the filtrate titrated against sodium hydroxide. Standardization of NaOH was with potassium hydrogen phthalate

2.5.2 Amodiaquine assay

For the amodiaquine assay, the USP spectrophotometric procedure (method B for identity test described above) was used. Six replicate determinations were carried out. The quantity in mg, (W), of amodiaquine hydrochloride present was calculated in the tablets taken by the formula:

$$W = 21.68\ C(Au/As) \qquad \text{----------2.5.2.1}$$

where C = concentration in µg per ml (calculated on anhydrous basis) of the reference sample; and Au and As are the absorbances of the solution obtained from the tablets and the standard solution, respectively.

2.6 Hardness and disintegration tests

2.6.1 Hardness test

BTB 301 hardness tester was employed. 10 tablets per brand were used for the test and mean hardness values (KgF) were recorded.

2.6.2 Disintegration test

For the disintegration test, the disintegration time of six tablets per brand were determined in distilled water maintained at 37±0.5 ^{0}C using Erweka tablet disintegration apparatus, and the mean values calculated.

2.7 Dissolution test

Dissolution tests were carried out on the tablets using the USP general method in 0.1M HCl and water (900 ml) with the aid of Erweka DT-D dissolution test apparatus (Erweka, U.K.) fitted with a paddle rotated at 100 rpm for artesunate tablets and 50 rpm for amodiaquine tablets as prescribed by IP (I.P, 2009) and USP (U.S.P, 2008), respectively. Samples were withdrawn from a zone midway between the surface of the dissolution medium and the top of the rotating basket, and replaced with fresh aliquot of dissolution medium, in order to maintain sink conditions. The samples were filtered and diluted appropriately with 0.1N HCl and water for artesunate and amodiaquine, respectively. The absorbance of the solutions were measured at 216nm and 342nm, respectively. Drug released (% of the labelled claim) against time was plotted for each formulation and the t_{50} and t_{75} (time required for 50% and 75% of drug dissolution, respectively) were derived from the plots by extrapolation. The amount dissolved in 45 and 30min was obtained for each brand of artesunate and amodiaquine, respectively. All determinations were in quadruplicate.

2.8 Data analysis

Correlation analysis was adopted to study the interrelationship between various physical parameters while the dissolution data were analysed using ANOVA. Results are expressed as mean + standard deviation(SD) of four determinations.

2.9 Bioavailability studies in humans

2.9.1 Ethical approval

Data from two studies are included in this thesis. This clinical study was conducted in accordance with the principles laid down by the World Health Assembly of 1975 on Ethics in Human Experimentation, the Helsinki Declaration and Good Clinical Practice. The protocol was approved by the Health Research Committee of the Federal Medical Centre, Abeokuta (see Appendix 4, page 295). Each volunteer was informed of the objectives, nature and possible risks of the trial. Written informed consent was obtained from every volunteer participating in the study. The volunteers were informed that they were free to withdraw consent at any time.

2.9.2 Entry criteria for study volunteers and study design

Volunteers were recruited by means of advertisement placed at the Federal Medical Centre, Abeokuta, Ogun State, Nigeria. Healthy normal male volunteers who provided written informed consent and met the following criteria were eligible to participate in the study: aged 18–45 years; no abnormalities on medical history, clinical examination, laboratory safety assessment (full blood count, differential white cell count, liver function tests not more than three times the upper limit of normal) as judged by a physician. Volunteers were excluded if they were smokers (>5 cigarettes/day), had taken antimalarials or been in a malarial area in the preceding 8 weeks, had malaria parasites on a thick smear, used recreational drugs, or had ingested any alcohol or any medicines (including over-the-counter preparations) two weeks preceding study commencement.

2.9.3 Design of study, drug administration and sample collection

This was an open label, Latin square four-phase/period crossover comparative clinical trial with a 40 days (5 × $t_{½}$ DEAQ 12–90 days) wash out period in between phases in which the PK properties, tolerability and bioavailability of ART + AMQ when administered as single ingredient, and in combination (fixed-dose or non-fixed dose preparation were studied. Volunteers were allocated into four groups using a table of random numbers to select 16 consecutive, unduplicated numbers between 01

and 16. All volunteers took standard preparations of artesunate (the drug with the shorter elimination half-life), amodiaquine(the drug with the longer elimination half-life), fixed dose combination of artesunate and amodiaquine and non fixed(loose) combination of artesunate and amodiaquine in a restricted order. In the first period/phase, group 1 were given artesunate tablets (200mg which was coblistered with amodiaquine in Camosunate, Adams, batch number-090408). Group 2 received amodiaquine (600mg which was coblistered with artesunate in Camosunate, Adams, batch number-090408). Group 3 received fixed dosage of artesunate/amodiaquine (200mg/612.6mg as Diasunate, Emzor, batch number-653P) and group 4 were given the loose combination of artesunate + amodiaquine (200mg+600mg as Camosunate, Adams, batch number-090408). These doses represent 1 day of a 3-day therapeutic course and were selected to be as close as possible to the target treatment doses of 4 and 10 mg kg^{-1} day^{-1} for ART and AMQ, respectively. The doses of AMQ in the fixed and non-fixed formulations were different because of the different strengths of the formulations used. In the second period, regimens were alternated within the first 2 groups and within the last 2 groups. In the third phase, group 1 received fixed dosage of artesunate/amodiaquine (200mg/612.6mg) and group 2 were given the loose combination of artesunate + amodiaquine (200mg+600mg). Group 3 were given artesunate tablets (200mg) and group 4 received amodiaquine (600mg). This was however alternated similarly within the groups as before in the fourth period. All trial drugs were given under direct supervision with 200ml tap water on an empty stomach after an overnight fast.

No caffeinated drinks were allowed before and during the study period. Standardised meals were offered, with breakfast at 3h post-dose consisting of light breakfast of a cup of xanthine- free beverage with a cube of sugar (no milk) and bread. Thereafter, limited quantity of water (not more than 200ml) was given if required by the volunteer. Volunteers on artesunate had blood samples collected via venipuncture from the forearm prior to drug administration and at 5mins, 10mins, 15mins, 30mins, 1.5h, 3h, 5h, 12h and 24h. The volunteers on amodiaquine products had blood samples collected via venipuncture from the forearm prior to drug administration and at 5mins, 10mins, 15mins, 30mins, 1.5h, 3h, 5h, 12h, 24h, 48h, 72h, day 5, day 8, day 21 and day 28. At each time 10-ml venous blood samples were collected in tubes containing lithium heparin anticoagulant. After centrifugation (at

2000 x g at 25^0C for 10mins), the plasma was immediately transferred on each occasion to separate plastic cryotubes and frozen at −20°C until analysed for artesunate, dihydroartemisinin, amodiaquine and desethylamodiaquine. Volunteers remained in the study ward for the first 12 h of each phase; thereafter specimens were collected at outpatient visits.

Serum albumin was sampled 48h post dose in each period. Serum albumin, total protein, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were quantified at the Chemistry Unit, Department of Pathology, Federal Medical Centre, Abeokuta, Ogun State.

2.9.4 Determination of body fat

The percentage body fat was determined from the formular: Adult Body Fat % = (1.20 x body mass index) + (0.23 x Age) - (10.8 x 1) - 5.4 (Body fat percentage, 2011)

2.9.5 Determination of haemoglobin concentration

Blood sample was obtained for the determination of haemoglobin concentration (Hb) during assessment for inclusion and at the end of each period. The Hb concentrations were determined using a portable Elaehaem 1 Haemoglobinometer (Lovibond®, John Morrison Scientific, Australia).

2.9.6 Preparation of amodiaquine (1mg/ml)

It is available as amodiaquine dihydrochloride dihydrate, MWT 464.8g. MWT of amodiaquine base is 355.86g.

65.306mg of salt will contain 50mg of amodiaquine base.

About 65.306mg of salt was dissolved in a 50ml volumetric flask and made up to volume with distilled water.

2.9.7 Preparation of desethyl amodiaquine (1mg/ml)

It is available as dihydrochloride, MWT 405.8g

MWT of DEAQ base 332.8g

12.194mg of salt ≡10mg of base .

About 12.194mg of salt was dissolved in a 10ml volumetric flask and made up to volume with methanol.

2.9.8 Preparation of quinidine ISTD (1mg/ml)

It is available as quinidine hydrochloride monohydrate, MWT 378.9g.

MWT of Quinidine base =325.4g

58.22mg of salt ≡ 50mg of base.

About 58.22mg of salt was dissolved in a 50ml volumetric flask and made up to volume with methanol.

2.9.9 Preparation of artesunate (1mg/ml)

About 50.128mg of artesunate was dissolved in 50ml volumetric flask and made up to volume with the mobile phase. MWT 384.4g.

2.9.10 Preparation of dihydroartemisinin (1mg /ml) stock

Some 50.214mg of DHA was dissolved in 50ml volumetric flask and made up to volume with the mobile phase. MWT 284.4g

2.9.11 Preparation of artemisinin ISTD (1mg/ml)

50.171mg of artemisinin was dissolved in 50ml volumetric flask and made up to volume with the mobile phase. MWT 282.3g

2.10 Analytical methods

2.10.1 Analysis of artesunate, amodiaquine and metabolites:

ART, AMQ and its major metabolites were detected and quantified using HPLC with UV detection. The HPLC system consisted of an Agilent 1100 Series System with an HP G1311A quaternary pump, an HP G1322A vacuum degasser, an HP G1314A and UV/Vis detector. A reverse-phase C18 column (250×4.6mm ID) was used for analyte separation.

Artesunate was quantitatively analysed using an HPLC-UV method comprising a mobile phase of acetonitrile and 0.05M acetic acid 40:60%v/v (adjusted to pH 4.0 with 1.0 M NaOH) using artemisinin as an internal standard at a flow rate of 1.0ml/min. The chromatogram was run at ambient temperature with a UV detection at 290nm. Amodiaquine was quantitatively analysed using an HPLC-UV method comprising a mobile phase of acetonitrile and 0.05MKH_2PO_4 10:90%V/V (adjusted to pH 4.0 with orthophosphoric acid) using quinidine as an internal standard at a flow rate of 1.0ml/min. The chromatogram was run at ambient temperature with UV detection at 340nm. Both methods are modification of Navaratnam *et al*., 2009.

2.11 Precision studies for artesunate

The lower limit of quantification for both artesunate and dihydroartemisinin was 10ng/ml and inter-day CV for two quality control levels were below 10% (n=6/level) with accuracies varying between 92% and 96%. (Table 2.3-Table 2.5)

Table 2.3: Precision data for artesunate. (n=6)

Sample	Conc (ng/ml)	Coefficient of variation(%)
Intraday		
Artesunate	10	4.48
	100	4.04
DihydroArtemisinin	50	3.89
	400	3.76
Interday		
Artesunate	10	4.86
	100	4.37
Dihydroartemisinin	50	4.46
	400	4.30

Table 2.4: Recovery data for Artesunate (Mean±S.D, n=6)

Sample	Conc (ng/ml)	Recovery(%)
Artesunate	10	90±6.2
	100	92±8.7
Dihydroartemisinin	50	88±5.4
	400	89±5.8

Table 2.5: Accuracy data for Artesunate. (Mean±S.D, n=6)

Sample	Conc (ng/ml)	Accuracy(%)
Artesunate	10	96±5.0
	100	94±8.0
Dihydroartemisinin	50	92±4.6
	400	95±3.8

2.12 Precision studies for Amodiaquine

The lower limit of quantification was 2ng/ml and inter-day CV for two quality control levels were below 10% (n=6/level) with accuracies varying between 90% and 94%. (Table 2.6-Table 2.8)

Table 2.6: Precision data for Amodiaquine. (n=6)

Sample	Conc (ng/ml)	Coefficient of variation %
Intraday		
Amodiaquine	2	4.6
	20	3.9
Desethylamodiaquine	20	3.6
	200	3.2
Interday		
Amodiaquine	2	4.8
	20	4.2
Desethylamodiaquine	20	5.0
	200	4.7

Table 2.7: Recovery data for amodiaquine. (Mean±S.D, n=6)

Sample	Conc (ng/ml)	Recovery(%)
Amodiaquine	2	89
	20	86
Desethylamodiaquine	20	84
	200	88

Table 2.8: Accuracy data for Amodiaquine. (Mean±S.D, n=6)

Sample	Conc (ng /ml)	Accuracy(%)
Amodiaquine	2	92
	20	94
Desethylamodiaquine	20	90
	200	92

2.13 Analytical procedure : amodiaquine

All glassware were pre-treated with dichlorodimethylsilane 5% in toluene, in order to minimize adsorption of amodiaquine and desethylamodiaquine. To avoid photodecomposition, tubes were wrapped in aluminium foil.

To 1ml of plasma placed in a 15ml screw capped extraction tube was added a 200µL volume of 5000ng/ml quinidine (internal standard) solution and the sample was subjected to protein precipitation with acetonitrile 2ml. This mixture was then vortexed for 15min. After centrifugation (3000g, for 5min) the liquid phase was transferred to a clean tube to which was added 2ml ammonia. The mixture was then extracted by mechanical tumbling (15min) with diethylether (5.0ml×2). After centrifugation(3000g,10min) and separation, the combined organic phases were evaporated to dryness in a water bath at 40°C. The residue was reconstituted in 200µL methanol and a 50µL aliquot was injected onto the HPLC column.

2.14 Analysis of Samples:

The plasma samples were analysed for amodiaquine and desethylamodiaquine The HPLC method used for the analysis was a modification of that described by Navaratnam *et al* (2009) in that a C18 column was used and parent drugs/metabolites were separately determined. The Hypersil ODS (C-18) column used was a 5µm particle size with dimension of 250×4.6mm ID. A mobile phase consisting of acetonitrile and 0.05MKH_2PO_4 10:90%V/V (adjusted to pH 4.0 with orthophosphoric acid), was pumped through the column at a flow rate of 1.0ml/min. The chromatogram was run at ambient temperature with UV detection at 340nm.

2.15 Analytical procedure : artesunate

To 1ml of plasma placed in a 15ml screw capped extraction tube were added 50µL of 3M sodium nitrite containing 1% Acetic acid (pH 4.3) to prevent apparent degradation of artesunate and dihydroartemisinin. A 200uL volume of 5000ng/ml artemisinin (internal standard) solution was added and the resulting sample was subjected to protein precipitation with acetonitrile 800µL. Sample was vortex-mixed

and centrifuged for 10min at 3000g. The supernatant was transferred into a polypropylene tube and evaporated to dryness in a water bath at 40°C. The solid residue was reconstituted in 200µL of methanol, vortex-mixed and centrifuged again under the above condition. A 50µL aliquot of the supernatant was injected onto the HPLC column.

2.16 Analysis of Samples:

The plasma samples were analysed for artesunate and dihydroartemisinin. The HPLC method used for the analysis was a modification of that described by Navaratnam *et al*., (2009). The column used was a Hypersil ODS (C-18) 5µm particle size with dimension of 250×4.6mm ID. A mobile phase consisting of acetonitrile and 0.05M acetic acid 40:60%v/v (adjusted to pH 4.0 with 1.0M NaOH) was pumped through the column at a flow rate of 1.0ml/min . The chromatogram was run at ambient temperature with a UV detection at 290nm.

2.17 Pharmacokinetic calculations, statistical and pharmacokinetic analyses

In the bioavailability study, data capture and preliminary analysis was in Microsoft Excel spread sheet, further analysis was done using SPSS version 17.0 (SPSS Inc. Chicago, IL). Toxicity profile was dichotomised into presence or absence of adverse drug effects. Logistic regressions were performed to explore associations between individual PK parameters (Clearance, Volume of distribution) against the dichotomised covariates of presence or absence of adverse effects in order to assess the influence of various biochemical and anthropometric factors (serum albumin and percentage body fat). Categorical variables were compared using the Chi square test, and continuous variables by the independent samples *t* test, ANOVA and statistical significance assumed when $p \leq 0.05$. Half-life ($t_{1/2}$) was calculated from the terminal slope (λ) of the concentration-time profile.

$t_{1/2} = 0.693/k_{el}$ where K_{el},the elimination rate constant = $-2.303(\lambda)$.

Because there was no intravenous comparator arm in this study, equivalent bioavailability was assumed for comparisons of apparent volume of distribution

[Vd/F =Dose/(K_{el} x AUC)] and clearance (CL/F = Dose/AUC), where f is the oral bioavailability or fraction of the drug absorbed (unknown). All four compounds were first analysed separately. Because both artesunate and dihydroartemisinin are highly parasiticidal in malaria patients, the pharmacokinetic parameters associated with therapeutic response [area under the plasma concentration time curve (AUC) and peak concentration (C_{max})] are also reported together as dihydroartemisinin equivalents, stoichiometrically calculated as the sum of the measured dihydroartemisinin concentrations plus the artesunate concentration converted to dihydroartemisinin equivalents (using molecular weights of 384 and 284 for artesunate and dihydroartemisinin respectively). As amodiaquine undergoes a rapid and extensive conversion to its active metabolite, desethylamodiaquine was considered the primary analyte. The AUC was calculated using the method of residuals (feathering or peeling). The pharmacokinetic parameters data following administration of artesunate and amodiaquine alone and in combination were log-transformed and then compared using the analysis of variance (ANOVA) for a cross-over design to take into account the repeated measures by study subject, treatment period and treatment groups. The treatment effects generated from the ANOVA were exponentiated in order to express comparisons between monotherapy and combination therapy as a ratio. Any apparent discrepancies between the difference in the group means and these ratios are due to the fact that the ratios are based on the within-patient differences in log-transformed values and not the group arithmetic means. Area under the curve (AUC) calculated using the method of residuals (feathering). Clearance (CL/F) and volume of distribution (Vd/F) were however not normalized by body weight.

2.18 Safety

For the safety analysis, haematological parameters (haemoglobin, white cell count, absolute lymphocyte and neutrophil count) were noted at baseline and at the end of each treatment phase. Changes in these values from baseline were determined. Reporting of symptoms was solicited during the course of the study. Clinical Adverse Effects(AEs) were described by the attending physician in terms of signs and symptoms and categorized by body system: gastrointestinal (GI), central nervous system (CNS), general, nutritional etc. They were assessed for severity [mild (Grade

1), moderate (Grade 2), severe (Grade 3), very severe (Grade 4)] using the Common Toxicity Criteria (CTC V 3.0)

2.19 Bioavailability

Bioavailability was measured by Area Under the plasma drug concentration-time curves (AUC), peak plasma concentration (C_{max}) reached and time taken to reach peak plasma concentration (T_{max}); impairment on absorption was indicated by at least one statistically significant parameter. Statistical analysis for pharmacokinetic measures, such as AUC, C_{max}, and T_{max} were based on the two 2-sided tests procedure that examined whether the average values for the pharmacokinetic measures determined after administration of the test (co-formulation) and reference (monotherapy) products were comparable. This approach is termed average bioequivalence or comparative bioavailability and involved the calculation of a 90% confidence interval (CI) for the ratio of the averages (geometric means) of the measures for the test and reference products. To establish comparative bioavailability, the calculated confidence interval should fall within a limit, usually between 80 and 125%, for the ratio of the product averages. This method is equivalent to the corresponding two 2-sided tests procedure with the null hypothesis of bioinequivalence at the 5% significance level.

CHAPTER 3

RESULTS

3.1 Physical and organoleptic properties of artesunate and amodiaquine products

The results of physical and organoleptic assessment of the label, packaging, etc., of artesunate products, Table 3.1, indicate that they were attractively packaged with unique identification markings, except samples B2 and B3 which were poorly packaged and had a dull green colour. All the artesunate samples were marked with the logo and/or brand name of their claimed manufacturer. The tablets were odourless with colours ranging from white to off-white or a tint of yellow. As Table 3.2 shows, all the amodiaquine products were attractively packaged; the tablets were yellow in colour, scored and also marked with the manufacturer's logo, with the exception of sample DD which had no unique logo and was not scored. These physical observations indicate that all the artesunate tablets, except B2, B3 and amodiaquine DD, were satisfactory in appearance with actual identification marks. Based on Table 3.1, thirteen out of the total of fifteen samples of the artesunate brands studied contained artesunate in conformity with International Pharmacopeia (IP) requirements, except B2 and B3 which contained < 50 % of labelled content. All the artesunate brands passed the weight uniformity test as no two tablets deviated by more than 10 % and none deviated by more than 20 %. Furthermore, all the artesunate tablets had mean diameter within the IP limit, and also showed good hardness properties. The unit cost per packet was similar for the artesunate products with the substandard products costing about as much as the genuine ones. There was no statistically significant difference ($p > 0.05$) in cost between imported and locally manufactured tablets. The cost per unit packet of amodiaquine varied much more than that of artesunate tablets, with the latter being more expensive. All amodiaquine tablets had satisfactory physical and organoleptic properties.

Table 3.1: Physicochemical and organoleptic properties of artesunate tablets (Mean±S.D)

Code	Product appearance	Identity test	Mean tablet weight(g)	Assay (mg)	Mean (%) drug content	Odour	Colour	Mean disintegration time (mins)	t_{50}(mins)	t_{75}(mins)	(%)dissolved in 45mins	Mean uniformity of diameter (mm)[a]	Mean hardness test(kgf)[b]
A	Satisfactory	Passed	0.2±0.0	54.0±1.3	108.0	Odourless	White	3.8±0.8	2.9±0.1	3.95±0.0	79.0±1.0	9.5±0.0	5.2±0.6
B	Satisfactory	Passed	0.3±0.0	59.1±1.5	118.2	Odourless	White	3.5±0.5	2.9±0.1	4.0±0.1	89.0±0.9	10.1±0.0	5.3±0.6
B1	Satisfactory	Passed	0.3±0.0	56.7±1.3	113.4	Odourless	White	3.3±1.0	2.98±0.1	4.02±0.0	93.0±1.0	10.1±0.0	5.5±0.9
B2	Unsatisfactory	Failed	0.3±0.0	14.1±1.5	28.2	Odourless	White	34.8±3.3	--	--	--	10.2±0.0	6.6±1.9
B3	Unsatisfactory	Failed	0.2±0.0	24.7±3.4	49.5	Odourless	White	3.8±0.8	--	--	--	10.2±0.0	6.9±1.9
C	Satisfactory	Passed	0.2±0.0	53.0±2.2	106.0	Odourless	off white	4.1±0.8	2.9±0.1	3.9±0.0	81.0±1.4	9.5±0.0	7.6±0.4
D	Satisfactory	Passed	0.3±0.0	54.3±2.2	108.7	Odourless	off white	3.8±0.8	2.6±0.1	3.3±0.1	92.0±1.1	10.5±0.0	3.8±0.4
E	Satisfactory	Passed	0.1±0.0	53.7±1.5	107.4	Odourless	off white	3.0±0.6	3.0±0.1	4.0±0.0	79.0±1.1	8.6±0.0	8.0±1.5
F	Satisfactory	Passed	0.3±0.0	56.0±2.6	112.0	Odourless	off white	7.6±1.2	2.8±0.2	3.8±0.2	93.0±1.5	9.5±0.0	5.5±2.7
F1	Satisfactory	Passed	0.3±0.0	56.4±2.7	112.8	Odourless	off white	11.6±2.0	2.9±0.2	3.97±0.1	95.0±5.4	9.4±0.0	5.2±0.9

Table 3.1 continued:

Code	Product appearance	Identity test	Mean tablet weight(g)	Assay (mg)	Mean (%) drug content	Odour	Colour	Mean disintegration time (mins)	t_{50}(mins)	t_{75}(mins)	(%)dissolved in 45mins	Mean uniformity of diameter (mm)[a]	Mean hardness test(kgf)[b]
G	Satisfactory	Passed	0.2±0.0	56.0±2.6	112.0	Odourless	off white	3.5±0.8	2.7±0.2	3.62±0.2	89.0±0.9	9.2±0.0	5.5±1.5
G 1	Satisfactory	Passed	0.2±0.0	57.4±1.3	114.8	Odourless	off white	3.5±0.8	2.7±0.2	3.57±0.2	88.0±0.7	9.2±0.0	5.4±1.3
H	Satisfactory	Passed	0.2±0.0	55.3±2.6	110.7	Odourless	White	4.8±0.8	2.8±0.3	3.78±0.2	84.0±0.5	9.2±0.0	4.1±1.1
H 1	Satisfactory	Passed	0.2±0.0	54.0±2.6	108.0	Odourless	White	7.6±0.8	2.9±0.1	4.04±0.1	85.0±1.4	9.1±0.0	4.3±0.9
I	Satisfactory	Passed	0.2±0.0	57.0±2.2	114.1	Odourless	White	4.1±0.8	2.9±0.1	3.90±0.1	84.0±0.7	9.5±0.0	5.2±0.5

[a] *n =12;* [b] *n= 10, t_{50} =time taken to attain 50 % dissolution; t_{75} = time taken to attain 75 % dissolution*

3.2 Physicochemical properties of artesunate and amodiaquine

Table 3.2 shows the physicochemical properties of amodiaquine tablet brands. All 5 brands of amodiaquine tablets showed good hardness properties and met USP drug content requirements of 93 – 107 % of labelled content (U.S.P, 2008). Five (33 %) of the artesunate tablet samples contained artesunate within IP range (90 - 110 %) of label claim, 8 (53.3 %) exceeded the upper limit (> 110 %) and 2 brands, B2 and B3, (13.3 %) fell well below the lower limit.

Artesunate tablets disintegrated within 12 min with the exception of brand B2, one of the two substandard samples, which disintegrated in 34.8 min; this is higher than the official USP limit of 30 min. Dissolution profile of artesunate as revealed by Figure 3.1a, Figure 3.1aa and Figure 3.1b shows that in 45 min all the artesunate products except B2 and B3 which failed the drug content test had released over 75 % of their drug content (see Table 3.1 also) and thus satisfied the requirements of BP (B.P, 2001) which states that the amount of active ingredient released in solution for uncoated tablets at 45 min should not be less than 75 % of the labelled strength.

However, all the brands of amodiaquine tablets disintegrated within 21 min which is within the USP limit of 30 min. The t_{50} and t_{75} (USP upper limits of t_{50} and t_{75} are 20 and 30 min, respectively, beyond which dissolution is poor) dissolution values indicate that all the tablets also possess good dissolution properties except amodiaquine brand CC which did not meet the essential minimum within 30 min (Figure 3.1c). In addition, the amodiaquine brands passed drug identification test, as shown in Table 3.2.

Table 3.2: Physicochemical and organoleptic properties of amodiaquine tablets (Mean±S.D)

Code	Product appearance	Identity	Mean tablet weight(g)	Assay(mg)	Mean (%)drug content	Status of assay	Mean disintegration time (mins)	t_{50}(mins)	t_{75}(mins)	(%)dissolved in 30min	Status of dissolution test	Mean uniformity of diameter(mm)[a]	Mean hardness test(kgf)[b]
AA	Satisfactory	Passed	0.3±0.0	245.1±0.3	94.3	Passed	9.1±0.7	3.6±0.1	4.8±0.4	81.1±1.8	passed	11.2±0.0	4.7±0.4
BB	Satisfactory	Passed	0.3±0.0	188.0±0.5	94.0	Passed	5.8±0.4	3.2±0.0	5.1±0.1	82.7±1.2	passed	9.1±0.0	6.3±0.7
CC	Satisfactory	Passed	0.3±0.0	245.9±0.4	94.6	Passed	9.0±0.6	5.0±1.0	31.7±1.0	73.3±1.9	failed	11.2±0.0	4.8±0.4
DD	Unsatisfactory	Passed	0.3±0.0	245.4±0.0	94.4	Passed	20.6±0.8	3.4±0.1	5.9±1.0	82.7±3.7	passed	10.9±0.0	4.3±0.7
EE	Satisfactory	Passed	0.3±0.0	245.9±1.0	94.6	Passed	15.8±1.8	3.1±0.1	6.2±0.8	81.3±3.8	passed	11.5±0.0	4.8±1.0

[a] *n =12;* [b] *n= 10,* t_{50} *= time taken to attain 50 % dissolution;* t_{75} *= time taken to attain 75 % dissolution*

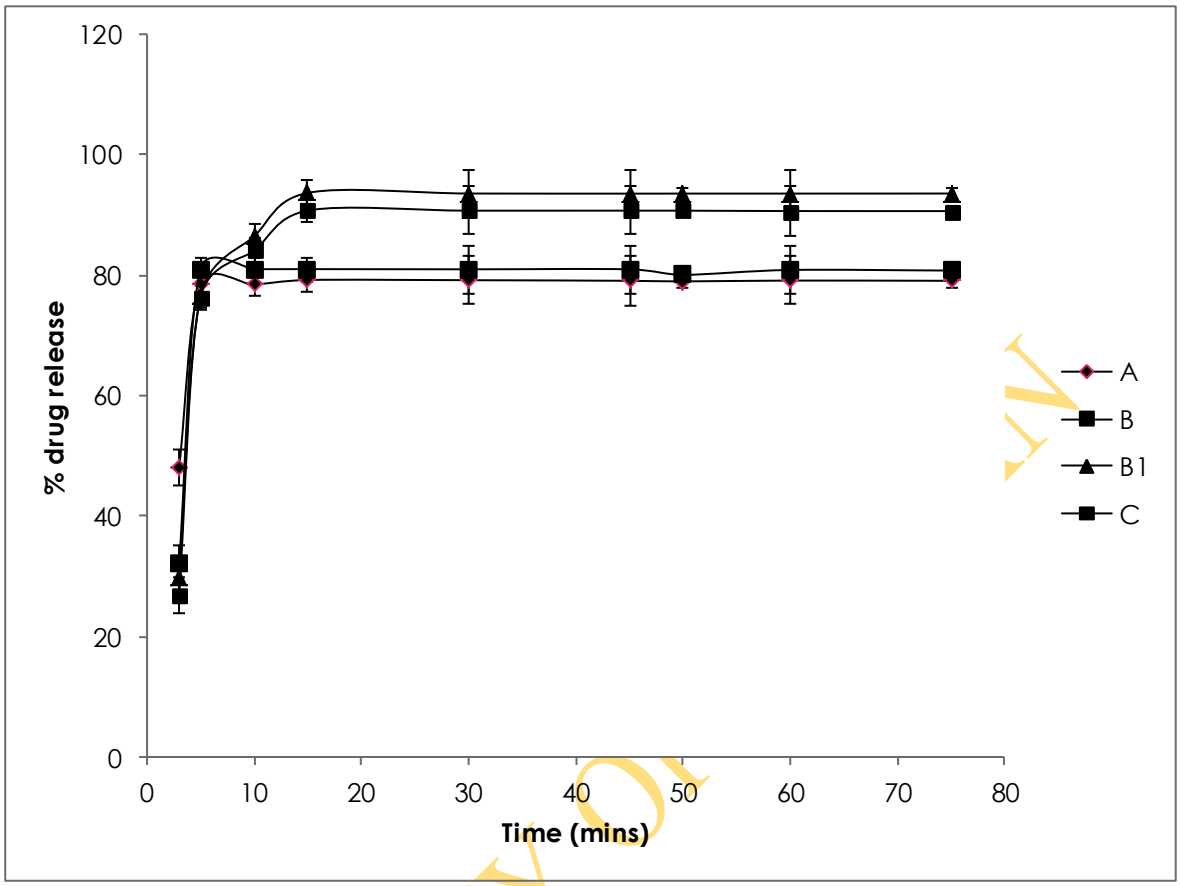


Figure 3.1a: *In vitro* dissolution profile of artesunate tablet brands (Mean±S.E.M, n=6)

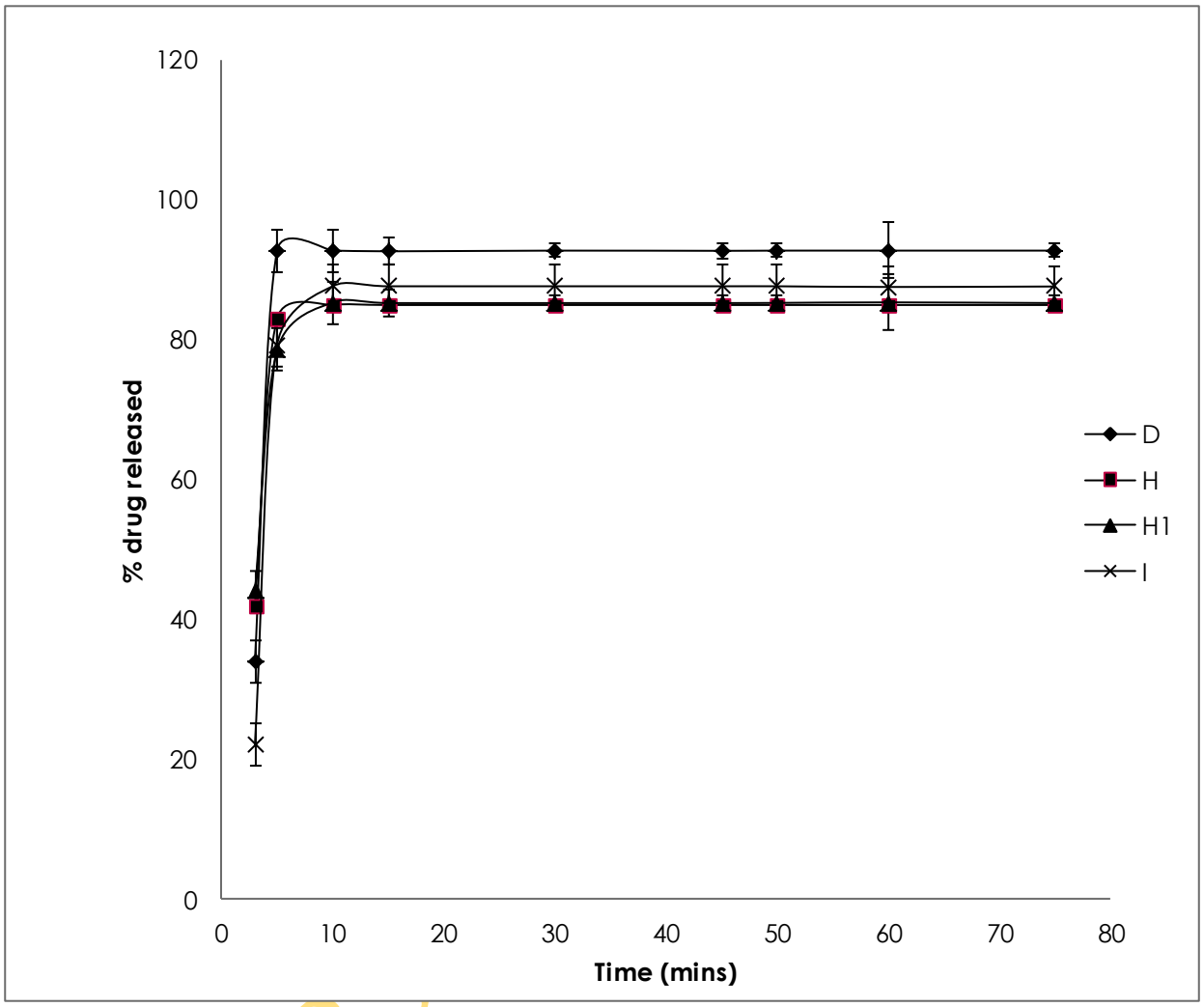


Figure 3.1aa: *In vitro* dissolution profile of artesunate tablet brands (Mean±S.E.M, n=6)

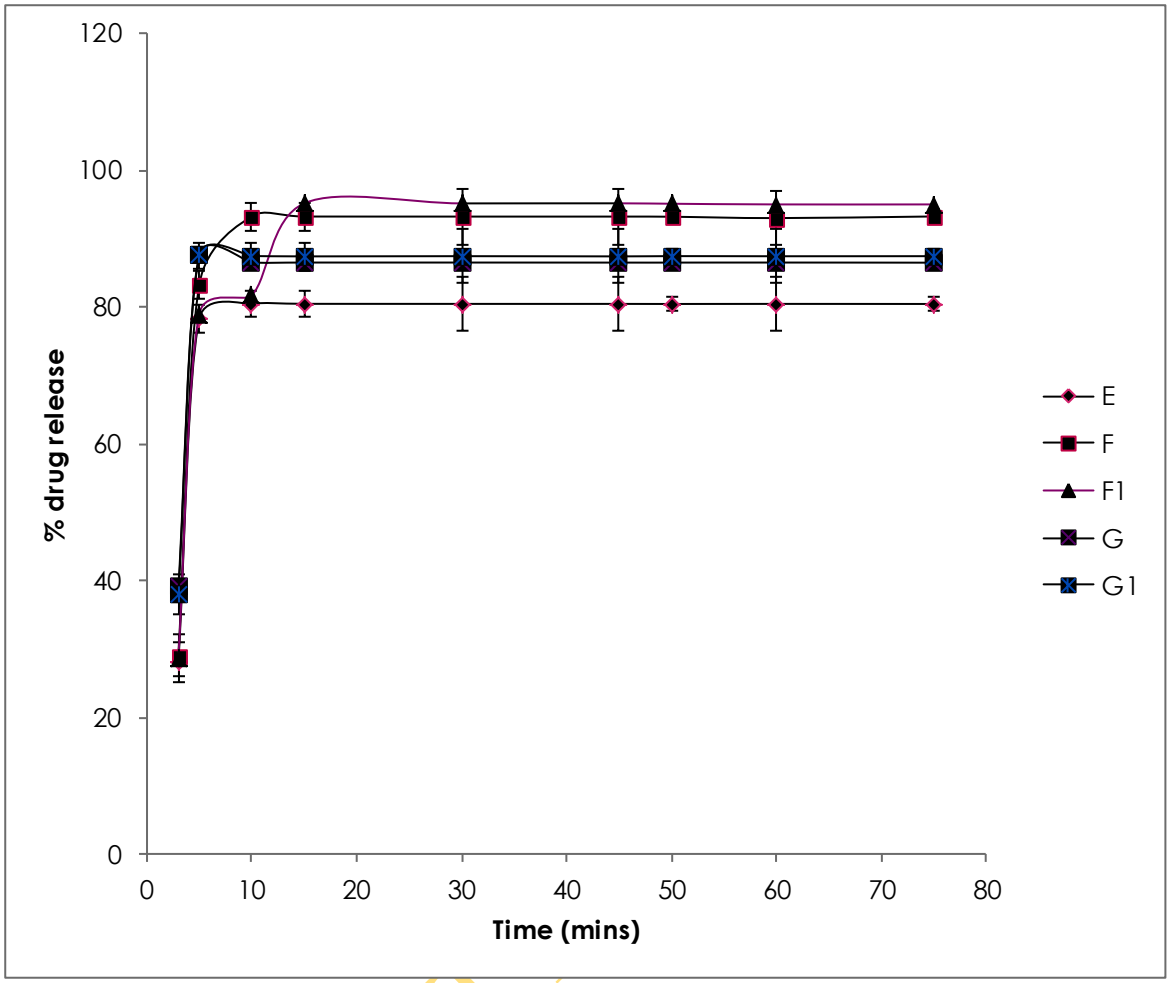


Figure 3.1b: *In vitro* dissolution profile of artesunate tablet brands (Mean±S.E.M, n=6)

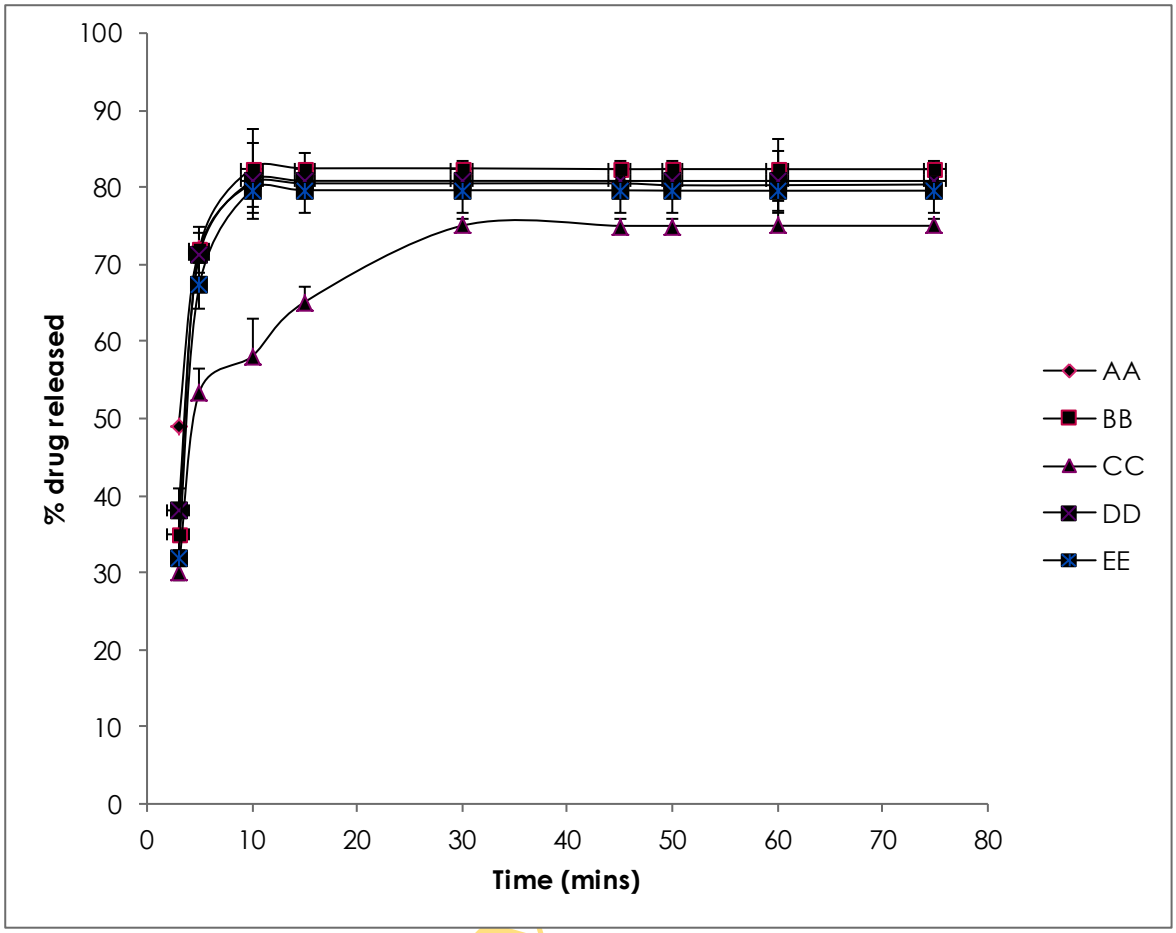


Figure 3.1c: *In vitro* dissolution profile for amodiaquine tablet brands (Mean±S.E.M, n=6)

3.3 Participant Characteristics

Volunteers selected were anatomically and physiologically similar as there were no significant differences in the age, body weight, liver function and haemoglobin among them in the four different groups before medications (Table 3.3, Table 3.4, Table 3.5, Table 3.6, Table 3.7) $p > 0.05$. Sixteen healthy normal male volunteers entered the study with a mean(S.D) of age, weight, height, percentage body fat, pulse, systolic and diastolic blood pressure of 28.94 years(4.61), 64.50 kilograms(5.61), 1.81 meters(0.03), 14.16%(2.87), 77.06 beats per minute(8.07), 112.00 mmHg(11.89) and 73.13 mmHg(6.02) respectively.

3.4 Safety

Standard definitions for adverse events (AEs) and serious adverse events (SAEs) were used (Appendix 1). All subjects were assessed clinically at baseline. Reporting of symptoms was solicited during the course of the study. Clinical AEs were described by the attending physician in terms of signs and symptoms and classed by body system: gastrointestinal (GI), Nutritional (NT), central nervous system (CNS), Respiratory (RS), General (GN) etc. They were assessed for severity [mild (Grade 1), moderate (Grade 2), severe (Grade 3), very severe (Grade 4)] using the Common Toxicity Criteria (CTC V3.0; National Institute of Health, Bethesda, MD). Laboratory examinations (haematology and liver functions) were conducted at Day 0, and at the end of each study period. All AEs that occurred during the study were recorded with the date and time of occurrence and the date the abnormal finding disappeared. The Chi-square test was used to compare the frequencies of: (1) AEs between groups and (2) normal/abnormal laboratory values. The Spearman Correlation test statistic was used to assess the general association of the treatment groups and the occurrence of at least one AE in a subject during the four study periods. Logistic regressions were performed to explore associations between adverse effects and serum albumin, percentage body fat and type of drug.

Table 3.3: Physiological and anthropometric characteristics of volunteers

Voluunteer	Age (yrs)	Weight (kg)	Height (m)	% Body fat	Pulse (beats /min)	Systolic B.P (mmHg)	Diastolic B.P (mmHg)
A	31	67	1.7	16.5	65	132	70
B	31	64	1.8	13.3	90	110	80
C	25	71	1.7	16.4	82	120	80
K	30	63	1.8	13.5	80	110	70
D	28	61	1.7	13.8	80	120	90
F	32	67	1.8	15.9	80	110	70
G	24	67	1.8	14.1	80	130	70
N	30	63	1.8	13.5	80	110	70
H	21	59	1.8	10.4	61	90	70

Table 3.3 continued:

Voluunteer	Age (yrs)	Weight (kg)	Height (m)	% Body fat	Pulse (beats /min)	Systolic B.P (mmHg)	Diastolic B.P (mmHg)
L	41	79	1.8	21.8	70	100	70
M	27	65	1.8	13.3	75	130	70
O	25	56	1.8	10.2	70	100	70
I	30	63	1.8	13.5	80	110	70
J	25	56	1.8	10.2	70	100	70
P	31	64	1.8	13.3	90	110	80
Q	32	67	1.8	15.9	80	110	70
Mean	**28.9**	**64.5**	**1.8**	**14.1**	**77.0**	**112**	**73.1**
Standard deviation	**4.6**	**5.6**	**0.0**	**2.8**	**8.0**	**11.8**	**6.0**
Coefficient of variation	**15.9**	**8.6**	**1.3**	**20.2**	**10.4**	**10.6**	**8.2**

Table 3.4: Occurence of adverse drug effects(ADE) and biochemical parameters of volunteers taking artesunate alone, n=16

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
A	None	None	None	None	None	None	None	None	14	Within normal limits	3.6	3.5	2.0	Within normal limits	1.0	Within normal limit	No
B	None	None	None	None	None	None	None	None	15	Within normal limits	5.0	5.0	12.0	Within normal limits	8.0	Within normal limit	No
C	None	None	None	None	None	None	None	None	16	Within normal limits	4.7	4.7	12.0	Within normal limits	12.0	Within normal limit	No
K	None	None	None	None	None	None	None	None	18	Within normal limits	3.0	3.0	13.0	Within normal limits	10.0	Within normal limit	No
D	None	Mild	None	None	None	None	None	None	15	Within normal limits	3.7	3.7	16.0	Within normal limits	2.0	Within normal limit	Yes
F	None	None	None	None	None	None	None	None	16	Within normal limits	4.2	4.1	20.0	Within normal limits	6.0	Within normal limit	No
G	None	Mild	None	None	None	None	None	None	14	Within normal limits	3.7	3.7	12.0	Within normal limits	2.0	Within normal limit	Yes
N	None	None	None	None	None	None	None	None	15	Within normal limits	3.1	3.1	12.0	Within normal limits	4.0	Within normal limit	No

Table 3.4 continued:

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
I	None	None	None	None	None	None	None	None	14	Within normal limits	3.1	3.1	20.0	Within normal limits	5.0	Within normal limit	No
J	None	None	None	None	None	None	None	None	15	Within normal limits	3.6	3.6	9.0	Within normal limits	4.0	Within normal limit	No
P	None	None	None	None	None	None	None	None	14	Within normal limits	5.0	4.9	16.0	Within normal limits	8.0	Within normal limit	No
Q	None	None	None	None	None	None	None	None	17	Within normal limits	4.2	4.1	9.0	Within normal limits	9.0	Within normal limit	No
H	None	Mild	None	None	None	None	None	None	15	Within normal limits	3.9	3.8	7.0	Within normal limits	10.0	Within normal limit	Yes
L	None	None	None	None	None	None	None	None	14	Within normal limits	3.5	3.4	7.0	Within normal limits	7.0	Within normal limit	No
M	None	None	None	None	None	None	None	None	18	Within normal limits	3.4	3.4	2.0	Within normal limits	1.0	Within normal limit	No
0	None	Mild	None	None	None	None	None	None	14	Within normal limits	3.5	3.5	10.0	Within normal limits	12.0	Within normal limit	Yes

Table 3.5: Occurrence of adverse drug effects(ADE) and biochemical parameters of volunteers taking amodiaquine alone, n=16

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
A	None	None	None	None	None	None	None	None	14	Mild	3.6	3.5	6.1	Within normal limits	1.0	Within normal limit	Yes
B	None	None	None	None	None	None	None	Grade 1	16	Within normal limits	5.0	3.8	6.0	Within normal limits	8.0	Within normal limit	Yes
C	None	None	None	None	None	None	Grade 1	None	15	Mild	4.7	3.0	6.3	Within normal limits	8.0	Grade 1	Yes
K	None	None	None	None	None	None	None	None	14	Mild	3.0	3.0	5.8	Within normal limits	10.0	Within normal limit	Yes
D	None	None	None	None	None	None	None	None	14	Mild	3.7	3.5	6.8	Within normal limits	2.0	Within normal limit	Yes
F	None	Mild	None	Mild	Moderate	None	Grade 1	None	15	Mild	4.2	3.8	6.7	Within normal limits	6.0	Within normal limit	Yes
G	None	None	None	None	None	None	None	None	18	Mild	3.7	3.0	6.4	Within normal limits	5.0	Within normal limit	Yes

Table 3.5 continued:

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
N	None	None	None	None	None	None	None	None	16	Mild	3.1	2.8	5.8	Within normal limits	9.0	Within normal limit	Yes
I	None	None	None	None	None	None	None	None	14	Moderate	3.1	3.0	5.8	Within normal limits	5.0	Within normal limit	Yes
J	None	None	None	None	None	None	None	None	14	Mild	3.6	2.8	6.6	Within normal limits	4.0	Within normal limit	Yes
P	None	Mild	None	None	Moderate	None	Grade 1	None	14	Mild	5.0	3.8	6.1	Within normal limits	8.0	Within normal limit	Yes
Q	None	Mild	None	None	Mild	None	Grade 1	None	14	Mild	4.2	3.8	6.6	Within normal limits	9.0	Within normal limit	Yes
H	None	None	None	None	None	None	None	None	15	Within normal limits	3.9	3.3	5.8	Within normal limits	10.0	Within normal limit	No
L	None	None	None	None	None	None	None	None	16	Within normal limits	3.5	3.0	6.6	Within normal limits	10.0	Within normal limit	No
M	None	Moderate	None	None	Moderate	None	None	Grade 1	14	Mild	3.4	2.7	6.9	Within normal limits	1.0	Grade 1	Yes

Table 3.5 continued:

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
0	None	Moderate	None	None	Mild	None	None	None	15	Moderate	3.5	2.7	6.4	Within normal limits	12.0	Within normal limit	Yes

Table 3.6: Occurence of adverse drug effects(ADE) and biochemical parameters of volunteers taking aretesunate and amodiaquine (fixed dose), n=16

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
A	None	Mild	None	None	None	None	None	None	14	Mild	3.6	3.5	2.0	Within normal limits	1.0	Within normal limit	Yes
B	None	Moder ate	None	None	Moder ate	None	None	Grade 1	15	Within normal limits	5.0	2.8	12.0	Within normal limits	8.0	Within normal limit	Yes
C	None	Mild	None	None	Moder ate	None	Grade 1	None	15	Mild	4.7	2.9	12.0	Within normal limits	9.0	Grade 1	Yes
K	None	None	None	None	None	None	None	None	16	Mild	3.0	3.0	14.0	Within normal limits	11.0	Within normal limit	Yes
D	None	Moder ate	None	None	Moder ate	None	None	None	16	Mild	3.7	1.8	20.0	Within normal limits	2.0	Within normal limit	Yes
F	None	Moder ate	None	None	Moder ate	None	Grade 1	None	14	Mild	4.2	2.9	19.0	Within normal limits	9.0	Within normal limit	Yes
G	None	None	None	None	None	None	None	None	14	Mild	3.7	3.0	11.0	Within normal limits	5.0	Within normal	Yes

Table 3.6 continued:

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
																limit	
N	None	None	None	None	None	None	None	None	15	Mild	3.1	2.8	13.0	Within normal limits	6.0	Within normal limit	Yes
I	None	Mild	None	None	Moder ate	None	None	None	15	Moderate	3.1	2.1	20.0	Within normal limits	5.0	Within normal limit	Yes
J	None	Mild	None	None	None	None	None	None	14	Mild	3.6	2.6	21.0	Within normal limits	4.0	Within normal limit	Yes
P	None	Mild	None	None	Moder ate	None	Grade 1	None	14	Mild	5.0	3.8	17.0	Within normal limits	10.0	Within normal limit	Yes
Q	None	Mild	None	None	Mild	None	Grade 1	None	14	Mild	4.2	3.8	11.0	Within normal limits	10.0	Within normal limit	Yes
H	None	Moder ate	None	None	Mild	None	None	None	15	Within normal limits	3.9	3.3	7.0	Within normal limits	10.0	Within normal limit	Yes
L	None	Mild	None	None	Moder ate	None	None	None	15	Within normal limits	3.5	3.0	17.0	Within normal limits	7.0	Within normal limit	Yes
M	None	Moder	None	None	None	None	None	Grade 1	16	Mild	3.4	2.7	2.0	Within	1.0	Grade 1	Yes

Table 3.6 continued:

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
		ate												normal limits			
O	None	Moderate	None	None	Mild	None	None	None	15	Moderate	3.5	2.7	14.0	Within normal limits	4.0	Within normal limit	Yes

Table 3.7: Occurence of adverse drug effects(ADE) and biochemical parameters of volunteers taking aretesunate and amodiaquine (non-fixed dose), n=16

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
A	None	Mild	None	None	Mild	None	None	None	14	Mild	3.6	3.5	2.0	Within normal limits	1.0	Within normal limit	Yes
B	None	None	None	None	None	None	None	Grade 1	14	Within normal limits	5.0	3.8	12.0	Within normal limits	8.0	Within normal limit	Yes
C	None	Moder ate	None	Mild	Moder ate	None	Grade 1	None	16	Mild	4.7	3.0	14.0	Within normal limits	6.0	Grade 1	Yes
K	None	None	None	None	None	None	None	None	15	Mild	3.0	3.0	14.0	Within normal limits	11.0	Within normal limit	Yes
D	None	None	None	None	None	None	None	None	16	Mild	3.7	3.5	12.0	Within normal limits	2.0	Within normal limit	Yes
F	None	None	None	None	None	None	Grade 1	None	14	Mild	4.2	3.8	20.0	Within normal limits	6.0	Within normal limit	Yes

Table 3.7 continued:

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
G	None	None	None	None	None	None	None	None	15	Mild	3.7	3.0	18.0	Within normal limits	5.0	Within normal limit	Yes
N	None	None	None	None	None	None	None	None	16	Mild	3.1	2.8	13.0	Within normal limits	6.0	Within normal limit	Yes
I	None	None	None	None	None	None	None	None	16	Moderate	3.1	3.0	20.0	Within normal limits	5.0	Within normal limit	Yes
J	None	Mild	None	None	None	None	None	None	16	Mild	3.6	2.8	21.0	Within normal limits	4.0	Within normal limit	Yes
P	None	Mild	None	None	Moderate	None	Grade 1	None	15	Mild	5.0	3.8	17.0	Within normal limits	10.0	Within normal limit	Yes
Q	None	Mild	None	None	Mild	None	Grade 1	None	15	Mild	4.2	3.8	11.0	Within normal limits	10.0	Within normal limit	Yes
H	None	Moderate	None	None	Moderate	None	None	None	15	Within normal limits	3.9	3.3	7.0	Within normal limits	10.0	Within normal limit	Yes
L	None	Mild	None	Mild	Moderate	None	None	None	14	Within normal limits	3.5	3.0	15.0	Within normal limits	12.0	Within normal	Yes

Table 3.7 continued:

Volunteer	Nutrit. ADE	Nerv. ADE	Resp. ADE	GI. ADE	Gen. ADE	Leukopenia	Neutropenia	Lymphopenia	Haemoglobin baseline (g/dl)	Haemoglobin end	Alb. Baseline (g/dl)	Alb. Day 2 (g/dl)	ALT baseline (IU)	ALT end (IU)	AST baseline (IU)	AST end (IU)	Occurrence of ADE
																limit	
M	None	Mild	None	None	None	None	None	Grade 1	15	Mild	3.4	2.7	2.0	Within normal limits	1.0	Grade 1	Yes
O	None	Moder ate	None	None	Mild	None	None	None	17	Moderate	3.5	2.7	14.0	Within normal limits	11.0	Within normal limit	Yes

3.4.1 Safety results

Seven clinical adverse events were reported over the course of the study by 13 (81.25%) of the 16 volunteers (Table 3.8). Adverse events including variations in laboratory results were reported in all 16 volunteers (100%). The frequency of adverse effects was significantly dissimilar across all four treatment arms (p=0.000): 4/16 (25%) following artesunate alone, 14/16 (87.5%) following amodiaquine alone, 16/16 (100%) following each of the 2 forms of combinations.

For the artesunate treatment arm, the body systems most commonly affected were the nervous systems 4/16 (25%). The most common was anaemia 13/16 (81.25%) in the amodiaquine treatment arm. Nervous system adverse effects (somnolence and headache) and anaemia commonly occurred in the fixed dosage artesunate/ amodiaquine arm 13/16 (81.25%) while anaemia commonly occurred 13/16 (81.25%) in all products containing amodiaquine. All adverse effects were consistent with the product information available and resolved spontaneously.

Significant haematological changes were confined to the white cell counts (neutrophils and lymphocytes) and haemoglobin. During the amodiaquine (including its combination) treatment period, four volunteers developed asymptomatic, NCI grade 1 neutropenia ($\geq$1.5 to <2.5 $\times 10^9$/L) and two developed asymptomatic, NCI grade 1 lymphopenia (<LLN to 1.5 x 10^9/L). Eleven developed mild anaemia (9.5-13g/dl) while two developed moderate anaemia (8-9.5g/dl) during the amodiaquine (and combination) treatment. None of the other observed changes in haematological parameters were outside the normal range.

Table 3.8: Percentage of volunteers experiencing adverse drug effects, (n=16)

Class Organ	Adverse Effects	Artesunate	Amodiaquine	Artesunate / amodiaquine fixed dose	Artesunate amodiaquine loose combinations
Nutrition	Anorexia, increased appetite	-	-	-	-
Nervous system	Headache, dizziness, somnolence	25%	31.25%	81.25%	56.25%
Gastro intestinal	Abdominal pain, nausea, diarrhoea	-	6.25%	-	12.5%
Skin	Pruritus	-	-	-	-
General disorders	Asthenia	-	31.25%	62.5%	43.75%
Blood and lymphatic disorders	Anaemia	-	81.25%	81.25%	81.25%
Blood and lymphatic disorders	Neutropenia	-	25%	25%	25%
Blood and lymphatic disorders	Lymphopenia	-	12.5%	12.5%	12.5%
Hepatobiliary disorders	Transaminitis	-	12.5%	12.5%	12.5%

3.4.2 Albumin

Considering volunteers that were given artesunate alone, no correlation existed between serum albumin and the occurrence of adverse drug effects ($p>0.05$). Presence of adverse effects was more common in the volunteers who had plasma albumin values above the first 33rd percentile range of normal i.e. (≥3.4g/dL), but this was however not significant OR= 2.052 {df(1,1) C.I (0.184, 22.89) $p>0.05$}.

However, the amodiaquine group experienced a large effect size (coefficient of determination $r^2 = 0.416$) between serum albumin concentration and occurrence of neutropenia and this correlation was significant ($p<0.05$), however a non significant but weak correlation existed between serum albumin and occurrence of adverse drug effects ($r^2= 0.00044$, $p=0.471$). Furthermore, a medium correlation effect occurred between serum albumin and change in haemoglobin ($r^2= 0.141$), but this was however not significant ($p>0.05$). General adverse effects (e.g. asthenia) and neutropenia was largely correlated with serum albumin in the volunteers that took fixed dose artesunate and amodiaquine ($r^2= 0.27, 0.42$, $p<0.05$). However in this treatment, nervous system adverse effects and change in haemoglobin had non significant medium correlation effect with serum albumin ($r^2= 0.12, 0.14$, $p>0.05$). The volunteers that took the non fixed combination experienced a strong correlation between serum albumin and development of neutropenia $r^2= 0.42$ and this was significant ($p<0.05$). However, a non-significant medium effect size correlation existed between general adverse effects (e.g. asthenia), change in haemoglobin and serum albumin ($r^2= 0.14, 0.14$, $p>0.05$). Generally, headache was common in volunteers that had lowered plasma amodiaquine clearance OR=2.45 {df (1,1)C.I (1.1-5.6) $p<0.05$} and high normal serum albumin (>=3.4g/dL) OR=16{df (1,1)C.I (1.8-141.9) $p<0.05$}. Asthenia occurred mostly in the volunteers with higher serum albumin and that took amodiaquine preparations OR=9.3 {df (1,1)C.I (1.1-81.9) $p<0.05$}. A lowered clearance was observed in volunteers that had a high normal serum albumin (≥3.4g/dL), however, this was not significant ($p>0.05$). No correlation existed between the percentage of albumin bound to drug and plasma clearance with volume of distribution of amodiaquine in the three formulations ($r^2 = 0.24, 0.16$, $p>0.05$).

3.4 3 Percentage body fat

Considering the volunteers that received the fixed dose artesunate and amodiaquine tablets, a medium effect correlation existed between development of nervous system adverse drug effect, neutropenia and percentage body fat (r^2=0.1, 0.11, p>0.05). However a negative, non significant medium effect correlation occurred between percentage body fat and development of lymphopenia (r^2=0.1, p>0.05). The users of the non fixed combination experienced a significant large effect size relationship between percentage body fat and gastrointestinal adverse drug effect (r^2=0.29, p= 0.016) while a non significant medium effect size relationship occurred between development of neutropenia and percentage body fat (r^2=0.11, p>0.05). However a negative, non significant medium effect size association existed between percentage body fat and the development of lymphopenia (r^2=0.1, p>0.05). Presence of adverse drug effects were more on volunteers that their body fat percentage fell within the high normal range (14-25% body fat) OR=0.365, {df(1,1), C.I(0.33, 4.059), but this was however not significant (p>0.05).

Development of asthenia were more on volunteers that used amodiaquine and that their body fat percentage fell within the high normal range (14-25% body fat) OR= 0.571, {df(1,1), C.I(0.028, 11.849), but this was however not significant (p>0.05). AUC was weakly correlated with body fat for volunteers that used the fixed and the non fixed amodiaquine (r^2=0.08, 0.002, p>0.05).

3.4.4 Type of drug used

On logistic regression, volunteers that were on amodiaquine appeared to have more of the adverse effects than those that took artesunate alone OR= 0.043,{df (1,1), C.I (0.006,0.297) p<0.05}

3.5 Bioavailability and Pharmacokinetic parameters

3.5.1 Effect of amodiaquine on artesunate pharmacokinetic parameters.

Data were analysed using a non-compartmental model.

Figure 3.2a and 3.2b shows the mean plasma concentration time curves of total dihydroartemisinin equivalents following administration of artesunate alone, artesunate plus amodiaquine (fixed combination) and artesunate plus amodiaquine (non fixed combination). It followed a 2-compartment model kinetics. Figure 3.3a and 3.3b shows the mean plasma concentration time curves of total desethylamodiaquine equivalents following administration of artesunate alone, artesunate plus amodiaquine (fixed combination) and artesunate plus amodiaquine (non fixed combination). It followed a 3-compartment model kinetics. As shown in Table 3.9 the C_{max} of artesunate were dissimilar following monotherapy and fixed combination therapy while the T_{max} of artesunate were likewise found to be dissimilar following monotherapy, fixed and non fixed combination therapy. However the AUC of amodiaquine were dissimilar following monotherapy, fixed and non fixed combination therapy while the T_{max} were dissimilar following monotherapy and non fixed combination therapy.

Following the fixed combination therapy, total dihydroartemisinin equivalents AUC was comparable [ratio 94.4% (95% CI 66.1-122.7%); $p = 0.61$], C_{max} was lower [ratio 76.4% (95% CI 68.9-83.8%); $p = 0.00$], T_{max} was higher [ratio 179.2% (95% CI 133.2-225.2%); $p = 0.00$], CL/F was higher [ratio 117.2% (95% CI 91.2–143.2%); $p = 0.00$], and Vd/F was higher [ratio 118.8% (95% CI 92.8-144.8%); $p = 0.00$], when compared with monotherapy (Table 3.10, Table 3.11).

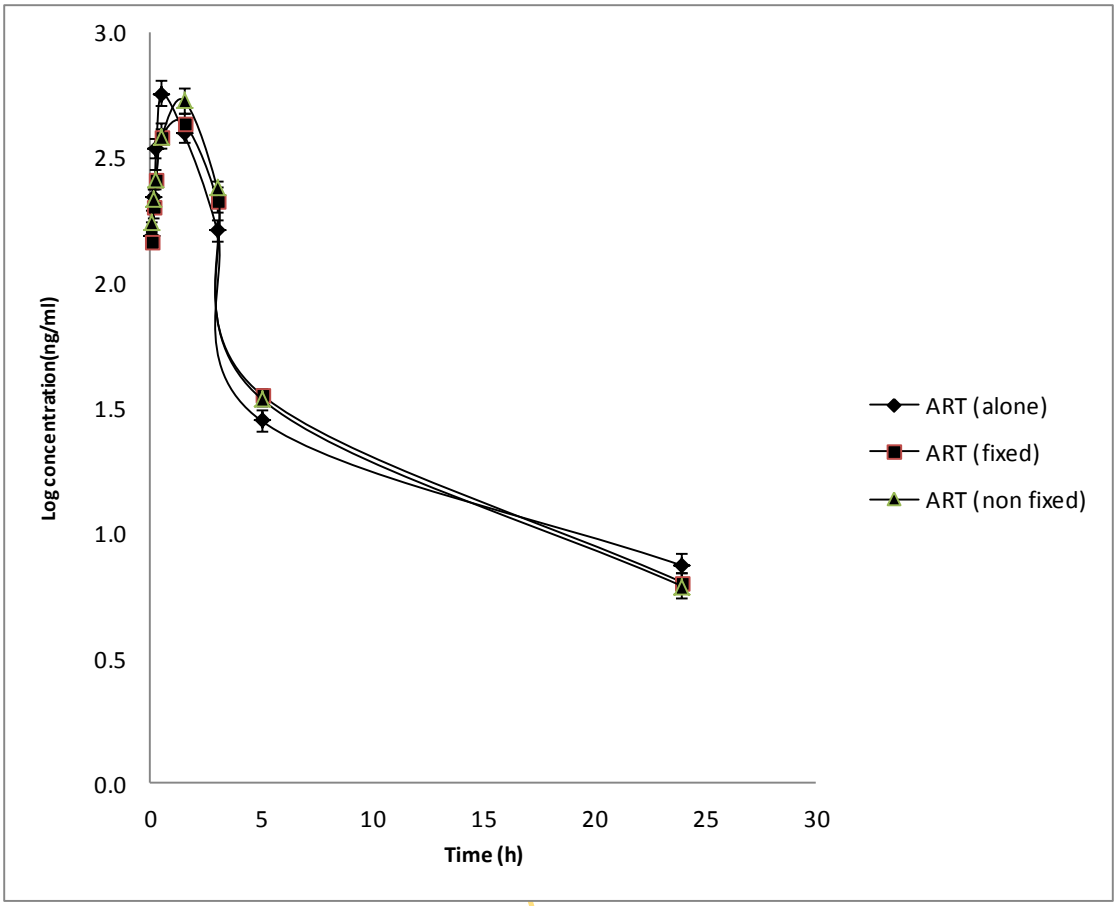


Figure 3.2a: Plasma concentration–time profile for total dihydroartemisinin (DHA) equivalents following single oral administration of artesunate monotherapy (200mg), fixed combination of artesunate /amodiaquine (200mg/612.6mg) and non-fixed combination of artesunate+amodiaquine(200mg+600mg) (Mean±S.E.M, n=16)

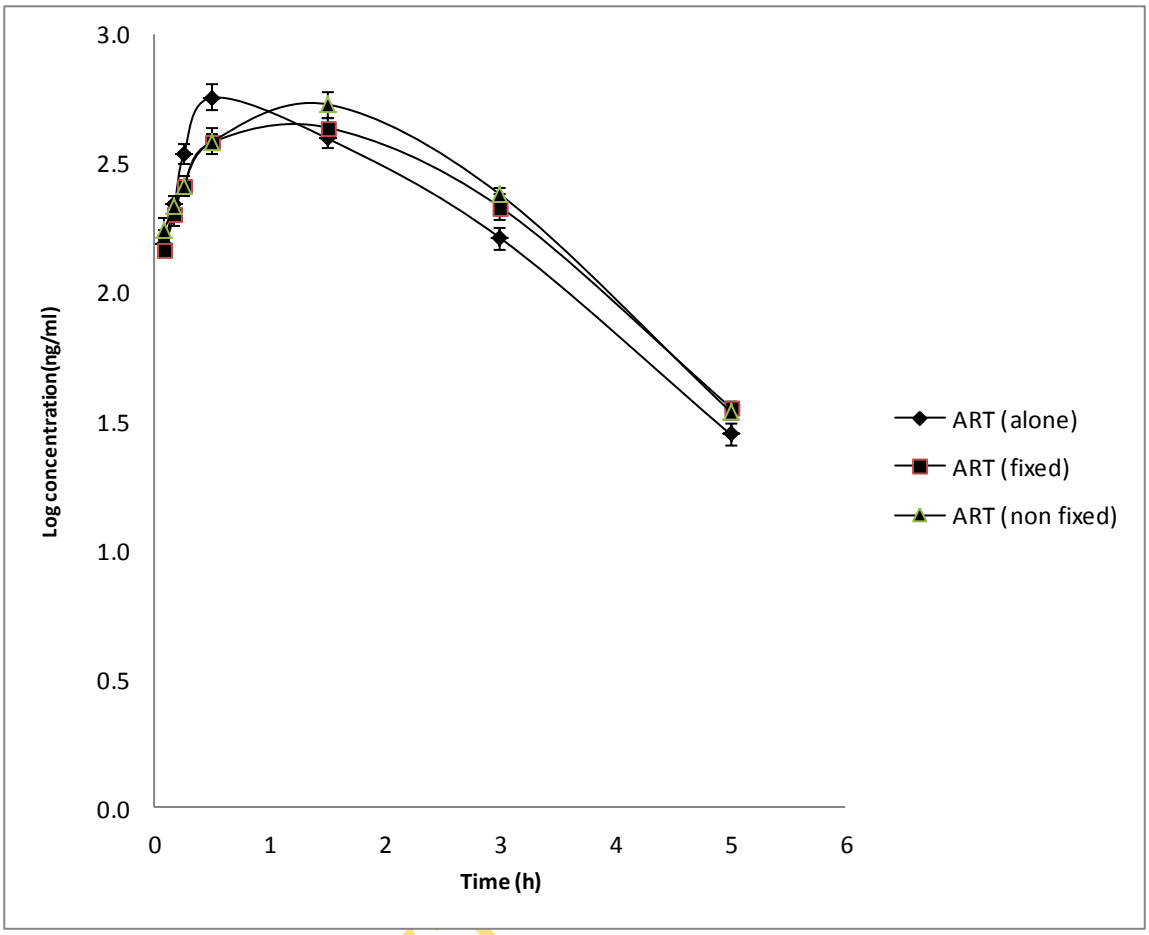


Figure 3.2b: Plasma concentration–time profile for total dihydroartemisinin (DHA) equivalents following single oral administration of artesunate monotherapy (200mg), fixed combination of artesunate /amodiaquine (200mg/612.6mg) and non-fixed combination of artesunate+amodiaquine(200mg+600mg) (Mean±S.E.M, n=16)

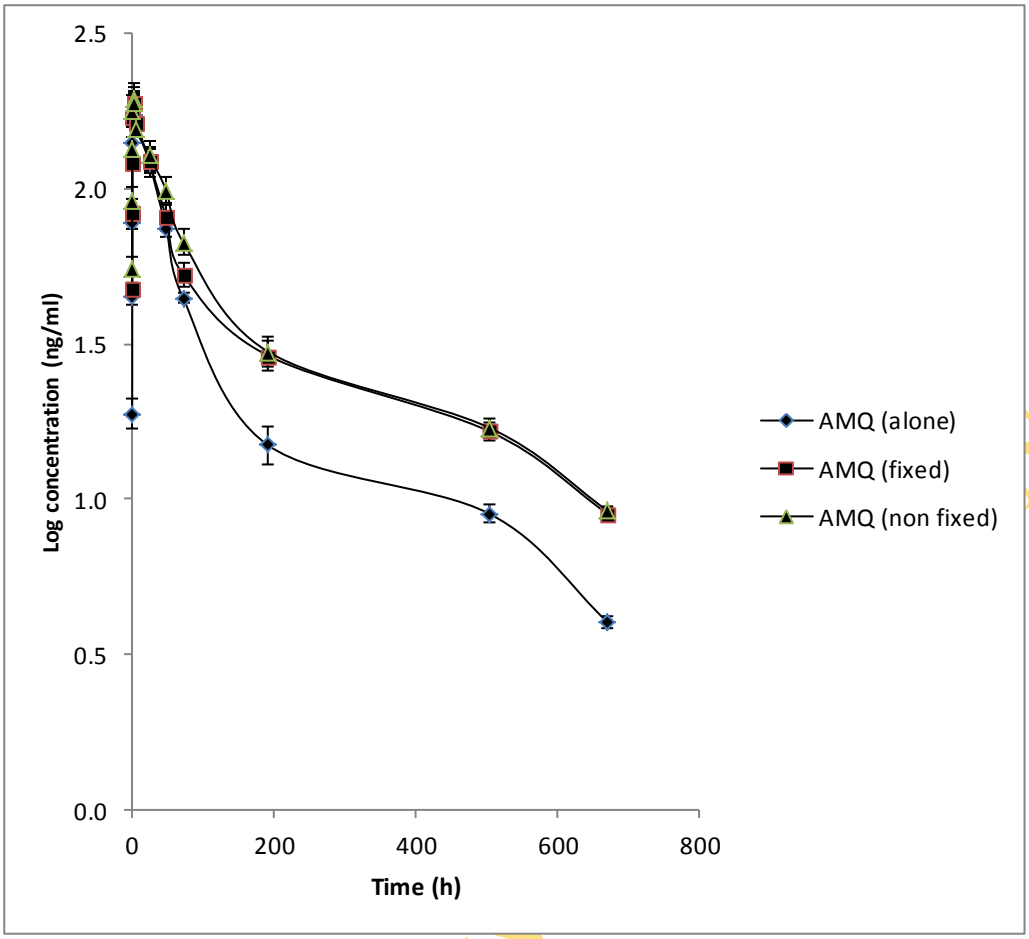


Figure 3.3a: Plasma concentration–time profile for total desethylamodiaquine (DEAQ) equivalents following single oral administration of amodiaquine (AMQ) monotherapy (600mg), fixed combination of artesunate /amodiaquine (200mg/612.6mg) and non-fixed combination of artesunate+amodiaquine(200mg+600mg) (Mean±S.E.M, n=16)

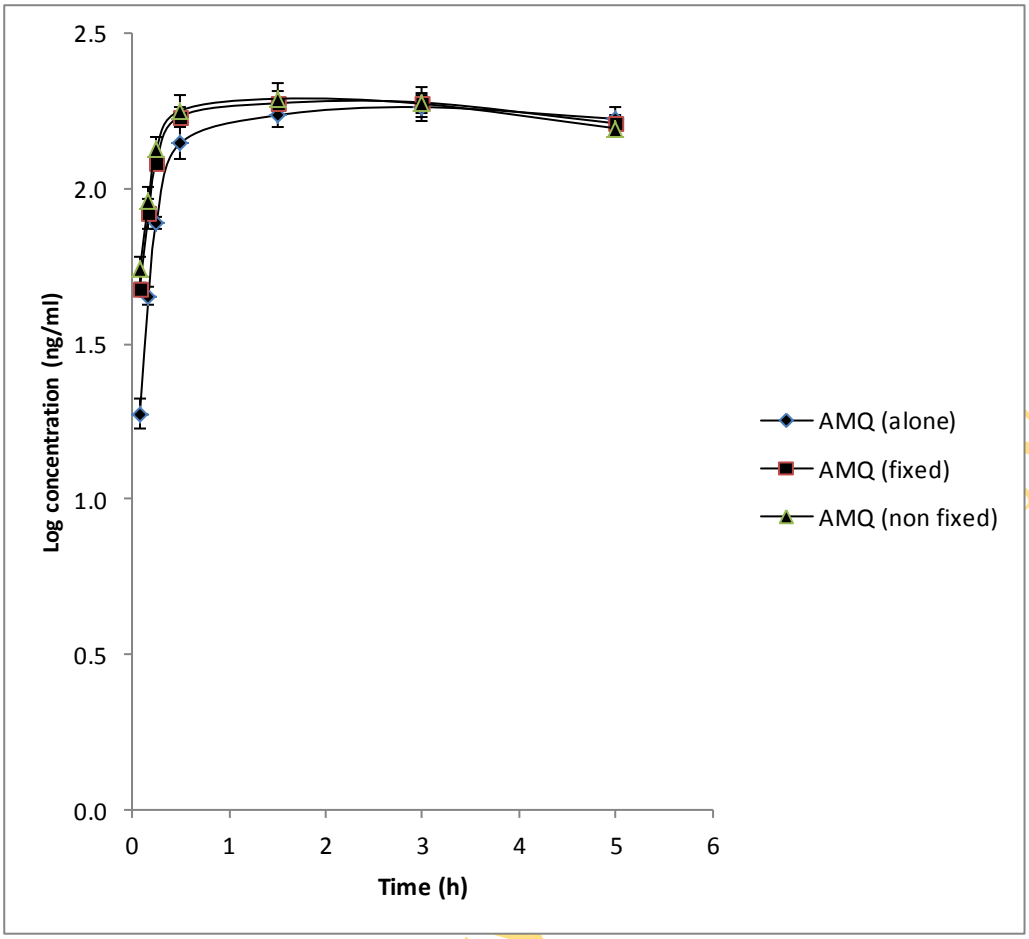


Figure 3.3b: Plasma concentration–time profile for total DEAQ equivalents following single oral administration of AMQ monotherapy (600mg), fixed combination of artesunate /amodiaquine (200mg/612.6mg) non-fixed combination of artesunate+amodiaquine(200mg+600mg) (Mean±S.E.M, n=16)

Table 3.9: Main pharmacokinetic measures associated with monotherapy and co-administration of artesunate and amdiaquine as fixed and non-fixed combination respectively (Mean±S.D, n=16)

Compound	Product	Cmax (ng/ml)	Tmax(h)	AUC (ng.h/ml)	t1/2 (h)	CL/F (L/min)	Vd/F(L)
Artesunate	Monotherapy	163.0± 8.5	0.3±0.0	200.3±91.4	0.9±0.1	24.2±23.9	30.4±31.3
	Fixed	175.5±10.1	0.3±0.0	456.4±129.4	1.1±0.2	7.9±2.7	12.4±4.2
	Non-fixed	186.2±10.5	0.2±0.1	384.0±107.3	0.9±0.0	9.3±2.5	12.9±3.1
Dihydroartemisinin	Monotherapy	544.7±79.1	0.7±0.4	1232.4±371.8	0.9±0.1	2.9±0.9	4.2±1.3
	Fixed	356.3±71.8	1.2±0.3	909.0±247.9	0.9±0.1	4.1±1.8	5.7±2.4
	Non-fixed	434.0±49.8	1.4±0.0	1031.4±123.2	0.8±0.1	3.2±0.3	3.9±0.3
Total dihydro artemisinin Equivalents	Monotherapy	624.7±79.2	0.7±0.3	967.2±298.6	0.9±0.1	3.8±1.5	5.4±2.0
	Fixed	480.3±79.9	1.2±0.2	888.7±198.5	1.0±0.2	3.9±1.1	5.7±1.7
	Non-fixed	537.8±29.9	1.3±0.0	1006.4±108.8	0.8±0.0	3.3±0.3	4.2±0.2
Amodiaquine	Monotherapy	11.9 ±1.6	1.0±0.2	1208.3±385.7	59.5±11.5	9.1±3.0	741.6±118.5
	Fixed	18.0 ±1.9	0.6±0.4	1835.3±263.1	59.8±5.3	5.6±0.8	486.6±62.0
	Non-fixed	20.3±5.1	0.4±0.3	2184.2±768.7	63.0±9.3	5.1±1.7	453±132.0
Desethyl Amodiaquine	Monotherapy	177.2±13.4	2.7±0.6	16527.7±2392.1	57.8±8.8	0.6±0.0	50.0±4.4
	Fixed	191.0±12.1	2.4±1.0	28861.2±5632.3	83.0±12.0	0.3±0.0	43.2±6.7
	Non-fixed	190.2±25.1	2.0±1.2	36579.9±15391.9	85.8±15.6	0.3±0.1	39.5±15.1
Total desethyl amodiaquine equivalents	Monotherapy	181.4±14.5	2.7±0.6	18310.0±2997.2	57.8±8.8	0.5±0.0	46.2±7.5
	Fixed	198.7± 11.6	2.5±1.0	29402.6±5271.4	83.0±12.0	0.3±0.0	42.2±5.6
	Non-fixed	206.0 ±30.6	2.1±1.3	38832.7±17339.1	85.8±15.6	0.3±0.1	37.4±14.5

Table 3.10: Comparative bioavailability parameters of fixed and non-fixed artesunate and amodiaquine products. (Mean±S.D, n=16)

Compound	Formulation	AUC ratio(%)	C_{max} ratio(%)	T_{max} ratio (%)
Artesunate	Fixed	251.5±179.8	107.5±8.0	95.0± 38.7
	Non fixed	212.5±178.3	114.1±6.4	89.9±36.4
Dihydroartemisinin	Fixed	73.6±31.8	64.3±12.9	179.0±95.4
	Non fixed	86.9±31.5	80.0±17.3	206.5±84.1
Total dihydroartemisinin equivalents	Fixed	94.3±57.7	76.4±15.1	179.1±93.9
	Non fixed	109.1±57.3	86.5±12.8	209.6±84.9
Amodiaquine	Fixed	157..9±62.1	151.5±23.3	52.3±45.0
	Non fixed	178.9±127.8	165.9±62.2	36.9±40.5
Desethylamodiaquine	Fixed	173.1±44.1	107.8±11.4	84.1±43.0
	Non fixed	204.7±111.0	106.6±16.8	62.0±50.3
Total desethylamodiaquine equivalents	Fixed	159.9±32.4	109.6±12.7	89.3±42.7
	Non fixed	195.3±97.6	112.6±21.2	65.6±55.4

Table 3.11: Comparative bioavailability parameters of total dihydroartemisinin and desethylamodiaquine in fixed and non-fixed combination products. (Mean±S.D, n=16)

Compound	Formulation	AUC ratio (%)	Level of significance	C_{max} ratio (%)	Level of significance	T_{max} ratio (%)	Level of significance
Total dihydroartemisinin equivalents	Fixed	94.3±57.7	p=0.6	76.4±15.1	p=0.0	179.1±93.9	p=0.0
	Non fixed	109.1±57.3	p=0.1	86.5±12.8	p=0.0	209.6±84.9	p= 0.0
Total desethyl amodiaquine equivalents	Fixed	159.9±32.4	p=0.0	109.6±12.7	p=0.0	89.3 ±42.7	p=0.6
	Non fixed	195.3±97.6	p=0.0	112.6±21.2	p=0.0	65.6±55.4	p=0.1

Following the non fixed combination therapy, total dihydroartemisinin equivalents AUC was similar [ratio 109.2% (95% CI 81.1-137.3%); $p = 0.1$], C_{max} was comparable [ratio 86.6% (95% CI 80.3-92.9%); $p = 0.0$], T_{max} was about two fold [ratio 209.7% (95% CI 168.1 -251.3%); $p = 0.0$], CL/F was lower [ratio 98.28% (95% CI 81.4-115.2%); $p = 0.0$], and Vd/F was lower [ratio 86.4% (95% CI 74.4-98.6%); $p = 0.0$],when compared with monotherapy.

Following the fixed combination therapy, total desethylamodiaquine equivalents AUC was about one and half fold [ratio 159.9% (95% CI 144.0-175.8%); $p = 0.0$], C_{max} was similar [ratio 109.6% (95% CI 103.3-115.9%); $p = 0.0$], T_{max} was comparable [ratio 89.3% (95% CI 68.4-110.2%); $p = 0.6$], CL/F was lower [ratio 63.8% (95% CI 56.9-70.6%); $p = 0.0$], and Vd/F was lower [ratio 91.9% (95% CI 82.1-101.7%); $p = 0.0$],when compared with monotherapy.

Following the non fixed combination therapy, total desethylamodiaquine equivalents AUC was about two fold [ratio 195.4% (95% CI 147.5-243.3%); $p = 0.0$], C_{max} was comparable [ratio 112.7% (95% CI 102.3-123.1%); $p = 0.0$], T_{max} was lower [ratio 65.6% (95% CI 38.4-92.6%); $p = 0.1$], CL/F was lower [ratio 56.5% (95% CI 44.0-68.9%); $p = 0.0$], and Vd/F was lower [ratio 81.1% (95% CI 66.9-95.3%); $p = 0.0$],when compared with monotherapy. The metabolic ratios (as depicted in Table 3.12) of the fixed (2.0±0.6) and non fixed (2.5±1.1) combination therapies of artesunate were lowered compared to monotherapy (9.1±10.5) while the metabolic ratios of the fixed (16.0±3.6) and non fixed (16.9±5.3) combination therapies of amodiaquine were increased compared to monotherapy (14.79±4.4). Table 3.9 shows the main pharmacokinetic data for artesunate and amodiaquine when given as monotherapy and as fixed and non fixed co formulations. The mean T_{max} of total dihydroartemisinin equivalents when given as monotherapy to each of the sixteen volunteers was 0.71±0.36h while the peak plasma concentration (C_{max}) was averaged 624.7±79.2ng/ml (range 524.8-794.3 ng/ml). The drug had an elimination (biological) half life of 0.9±0.1h, a total plasma clearance (CL/F) of 3.8±1.5L/min and an apparent

Table 3.12: Comparison of the ratio of AUC of metabolite to that of unchanged drug, n=16

Volunteers	Metabolic ratios					
	Artesunate monotherapy	Artesunate when fixed	Artesunate when non fixed	Amodiaquine monotherapy	Amodiaquine when fixed	Amodiaquine nonfixed
A	3.1	3.2	2.9	18.4	18.5	20.4
B	11.6	2.9	2.1	12.6	13.8	11.3
C	3.3	2.5	2.4	25.4	18.6	18.6
K	4.5	2.1	2.4	9.0	13.1	14.0
D	5.5	1.1	1.3	13.6	9.3	14.9
F	4.8	2.7	2.1	18.8	19.6	27.3
G	17.1	1.9	2.0	15.6	12.5	10.1
N	7.9	1.4	1.9	9.1	14.1	13.7
I	5.2	1.7	2.0	11.1	17.1	13.9
J	10.9	1.6	2.4	12.5	19.7	12.9
P	7.5	1.7	1.7	12.6	15.1	11.6
Q	5.2	2.7	2.1	20.2	19.8	24.9
H	4.4	1.0	1.4	13.2	15.2	19.1
L	46.3	1.5	6.1	11.1	10.5	18.7
M	4.3	2.2	3.1	18.7	21.9	25.2
O	4.5	2.3	3.3	13.8	16.4	14.5
Mean	**9.1**	**2.0**	**2.5**	**14.7**	**16.0**	**16.9**
Standard deviation	**10.5**	**0.6**	**1.1**	**4.4**	**3.6**	**5.3**

volume of distribution (Vd/F) of 5.4±2.0L. The mean AUC was 967.2±298.6ng.h/ml. On the administration of the co-formulations, the mean T_{max} of total dihydroartemisinin equivalents were both increased, 1.2±0.28h (for fixed combination) and 1.3±0.0h (for non fixed combination) indicating a delayed absorption while the peak plasma concentration (C_{max}) was both decreased and averaged 480.3±79.2ng/ml (range 363.0-602.5ng/ml for fixed combination) and 537.8±29.9ng/ml (range 501.1-602.5ng/ml) for the non fixed combination when compared to monotherapy. The drug had an elimination (biological) half life of total dihydroartemisinin equivalents increased 1.0±0.2h (for the fixed combination) , and decreased (0.88±0.0h) for the non fixed combination compared to monotherapy. The total plasma clearance (CL/F) of dihydroartemisinin increased for the fixed combination 3.9±1.1L/min and decreased for the non fixed combination (3.3±0.3L/min) when compared to monotherapy (see Table 3.13). The apparent volume of distribution (Vd/F) for total dihroartemisinin equivalents increased for the fixed combination 5.7±1.7L and decreased (4.2±0.7L) for the non fixed coformulation when compared to monotherapy. The mean AUC for the fixed combination was decreased 888.7±198.5ng.h/ml but increased (1006.4±108.8ng.h/ml) for the non fixed coformulation compared to monotherapy. Bioavailability of total dihydroartemisinin equivalents significantly decreased in the fixed formulation with respect to the C_{max} ($p<0.001$) compared to monotherapy. Bioavailability of total dihydroartemisinin equivalents significantly decreased in the non fixed formulation with respect to the T_{max} ($p<0.001$) compared to monotherapy.

The mean T_{max} of total desethylamodiaquine equivalents when given as monotherapy to each of the sixteen volunteers was 2.7±0.6h while the peak plasma concentration (C_{max}) was averaged 181.4±14.5ng/ml (range 158.4-199.5ng/ml). The drug had an elimination (biological) half life of 57.8±8.8h, a total plasma clearance (CL/F) of 0.5±0.0L/min and an apparent volume of distribution (Vd/F) of 46.2±7.5L. The mean AUC was 18,310.0±2997.2ng.h/ml

Table 3.13: Clearance and Volume of distribution ratios (for total DHA and DEAQ) compared to monotherapy. (Mean±S.D, n=16)

	Total DHA (fixed)	Total DHA (non fixed)	Total DEAQ (fixed)	Total DEAQ (non fixed)
CL/F ratio	117.2±53.1	98.2± 34.4	63.8±13.8	56.5±25.5
Vd/F ratio	118.8±53.1	86.4±24.6	91.9±20.1	81.1±28.9

On the administration of the co-formulations, the mean T_{max} of total desethylamodiaquine equivalents were both decreased 2.5±1.0h (for fixed combination) and 2.1±1.3h (for non fixed combination) indicating a delayed absorption while the peak plasma concentration (Cmax) was both increased and averaged 198.7±11.6ng/ml (range 165.9-208.9ng/ml for fixed combination) and 206.0±30.5ng/ml (range 158.4-257.0ng/ml) for the non fixed combination when compared to monotherapy. The drug had an elimination (biological) half life of total desethylamodiaquine equivalents both increased 83.0±12.0h (for the fixed combination) , and 85.8±15.6h) for the non fixed combination when compared to monotherapy. The total plasma clearance (CL/F) of desethylamodiaquine decreased for both the fixed combination 0.3±0.0L/min and for the non fixed combination (0.3±0.1L/min) when compared to monotherapy. The apparent volume of distribution (Vd/F) for total desethylamodiaquine equivalents decreased for both the fixed combination (41.3±5.5L) and for the non fixed coformulation (37.4±14.5L) when compared to monotherapy. The mean AUC for the fixed combination was markedly increased 29,402.6±5271.4ng.h/ml. A similar trend occurred with the non fixed co formulation (38,832.7±17339.1ng.h/ml) when compared to monotherapy. Bioavailability of total desethylamodiaquine equivalents significantly increased in the fixed formulation with respect to the AUC ($p<0.001$) compared to monotherapy. Bioavailability of total desethylamodiaquine equivalents significantly increased in the non fixed formulation with respect to the AUC ($p<0.05$) compared to monotherapy. The mean concentration – time profile of the artesunate major metabolite (dihydroartemisinin) following the administration of 200mg oral dose of artesunate as monotherapy and combination therapies is as shown in Figure 3.4. Its relationship with artesunate, the parent drug in monotherapy and as fixed and non fixed combination therapy is also depicted in Figure 3.5, Figure 3.6 and Figure 3.7.

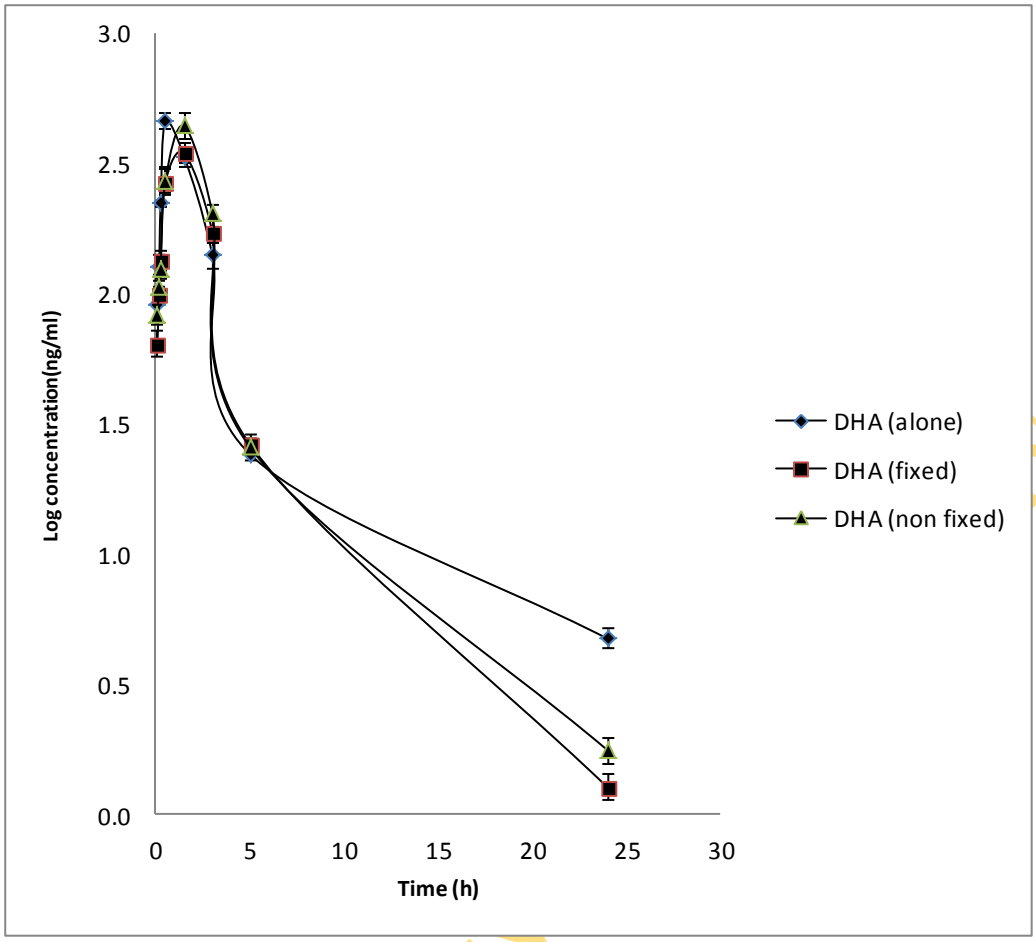


Figure 3.4: Plasma concentration–time profile for dihydroartemisinin (DHA) following single oral administration of artesunate monotherapy (200mg), fixed combination of artesunate /amodiaquine (200mg/612.6mg) non-fixed combination of artesunate+amodiaquine(200mg+600mg) (Mean±S.E.M, n=16)

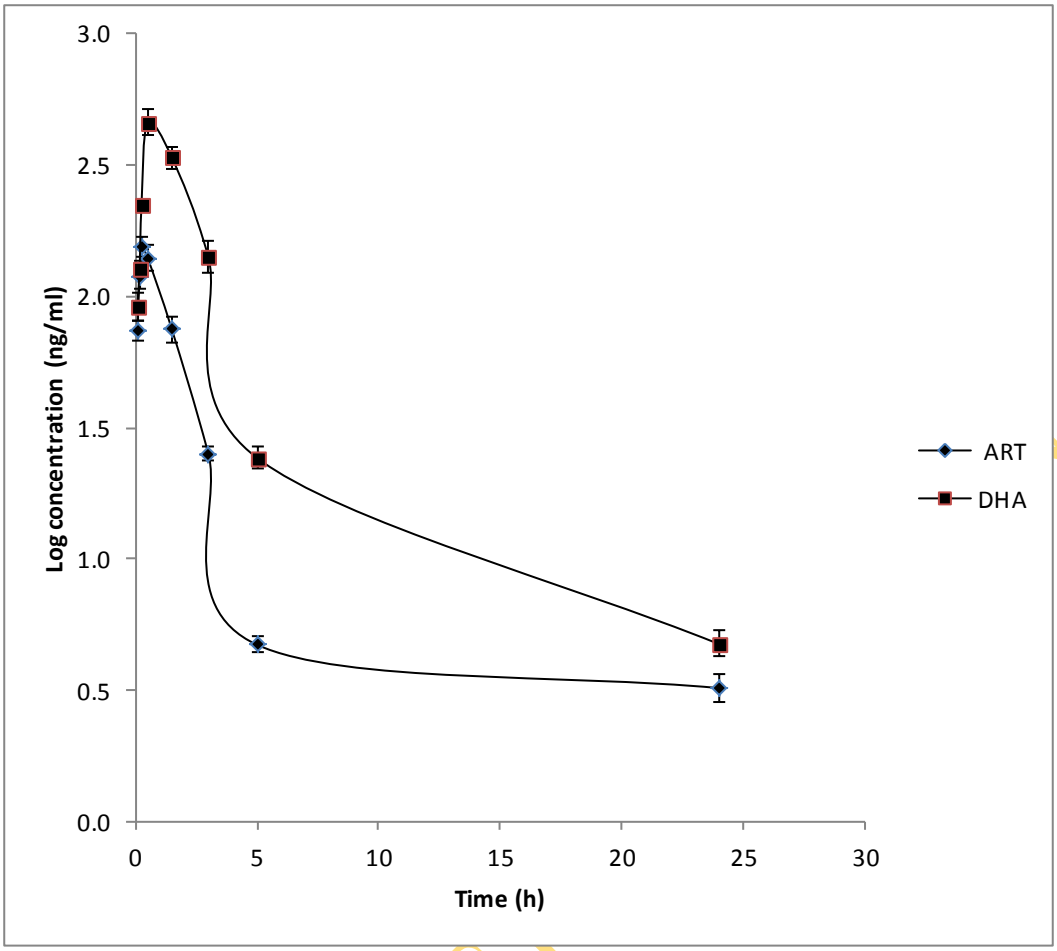


Figure 3.5: Concentration time profile of artesunate (ART) versus dihydroartemisinin (DHA) in monotherapy (Mean±S.E.M, n=16)

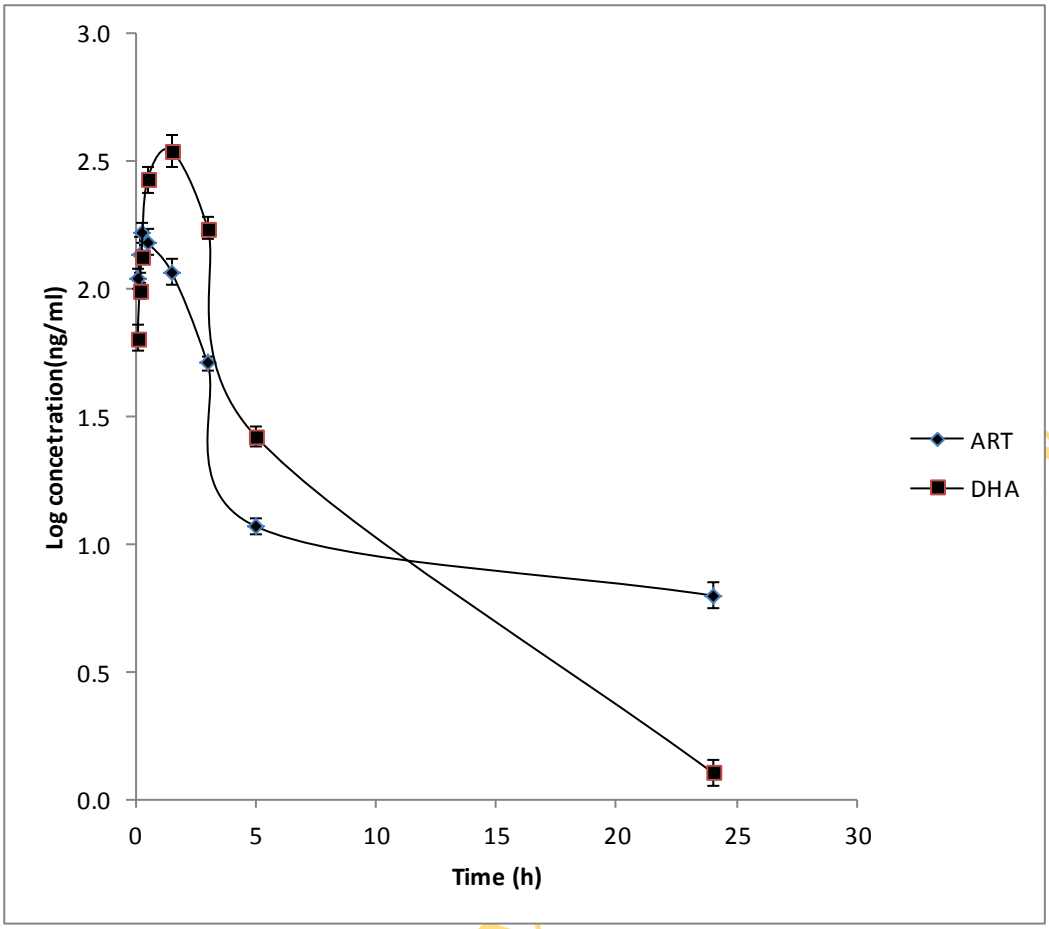


Figure 3.6: Concentration time profile of artesunate (ART) versus dihydroartemisinin (DHA) in fixed combination (Mean±S.E.M, n=16)

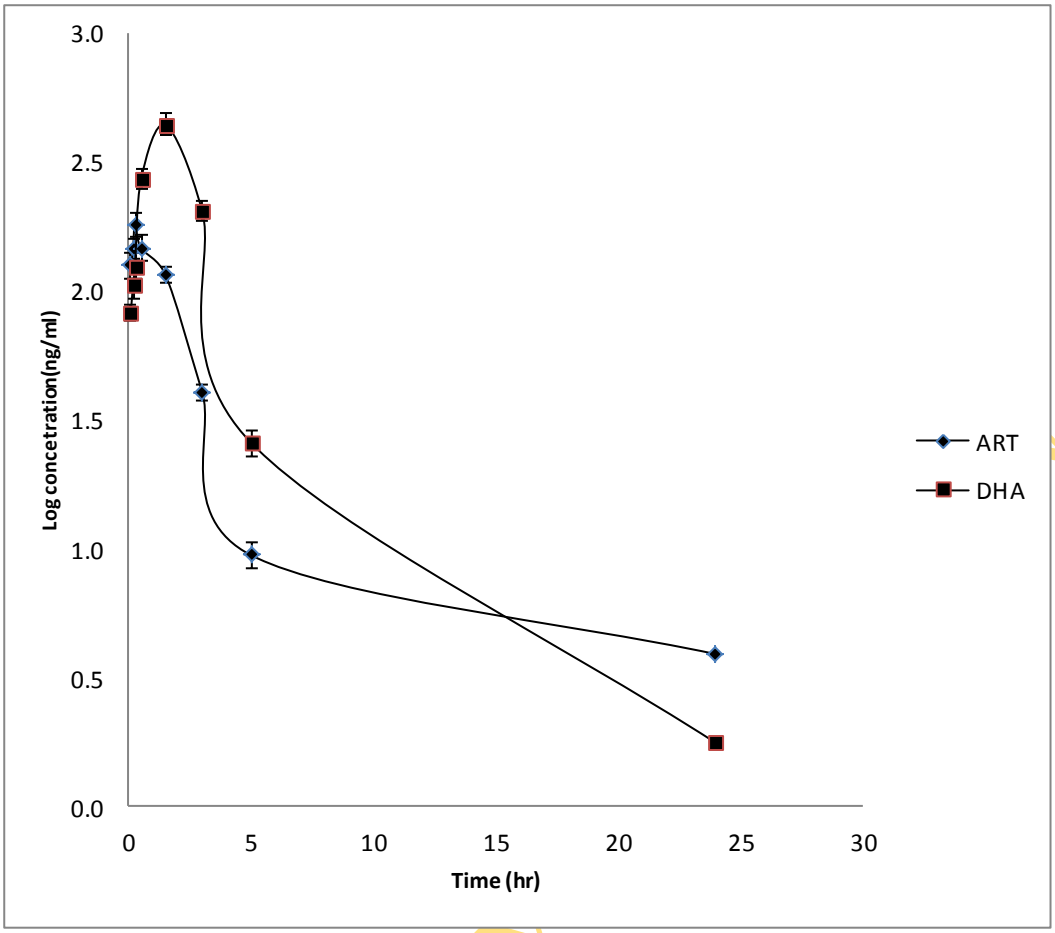


Figure 3.7: Concentration time profile of artesunate (ART) versus dihydroartemisinin (DHA) in non fixed combination (Mean±S.E.M, n=16)

In the absence of amodiaquine, the mean metabolite (dihydroartemisinin) C_{max} was 544.7±79.1ng/ml. This was however lowered both in the fixed combination (356.3±71.8ng/ml) and the non fixed combination (434.0±49.8ng/ml). The AUC of dihydroartemisinin when given as monotherapy was 1232.4±371.8ng.h/ml, the T_{max} was 0.7±0.4h and the elimination half life was 0.9±0.1h (Table 3.9). The mean concentration–time profile of the amodiaquine major metabolite desethylamodiaquine following the administration of 600mg oral dose of amodiaquine as monotherapy and non fixed co combination therapy with 612.6mg of fixed combination therapy is as shown in Figure 3.8. Its relationship with amodiaquine, the parent drug in monotherapy and as fixed and non fixed combination therapy is also depicted in Figure 3.9, Figure 3.10 and Figure 3.11. In the absence of artesunate, the mean metabolite (desethylamodiaquine) C_{max} was 177.2±13.4ng/ml. This was however increased both in the fixed combination (191.0±12.1ng/ml) and the non fixed combination (190.2±25.1ng/ml). The AUC of desethylamodiaquine when given as monotherapy was 16,527.7±2392.1ng.h/ml, the T_{max} was 2.7±0.6h and the elimination half life was 57.8±8.8h (Table 3.9). The concentration–time profile of artesunate when given as monotherapy (as depicted by Figure 3.12) revealed a 2-compartment model kinetics while that of amodiaquine (as depicted by Figure 3.13) showed a 3-compartment model kinetics. Pharmacokinetic parameters comparing the individual volunteers are as shown in Table 3.14-Table 3.19.

The level of significance of the comparative bioavailability of the coformulations compared to monotherapy is as shown in Table 3.11.

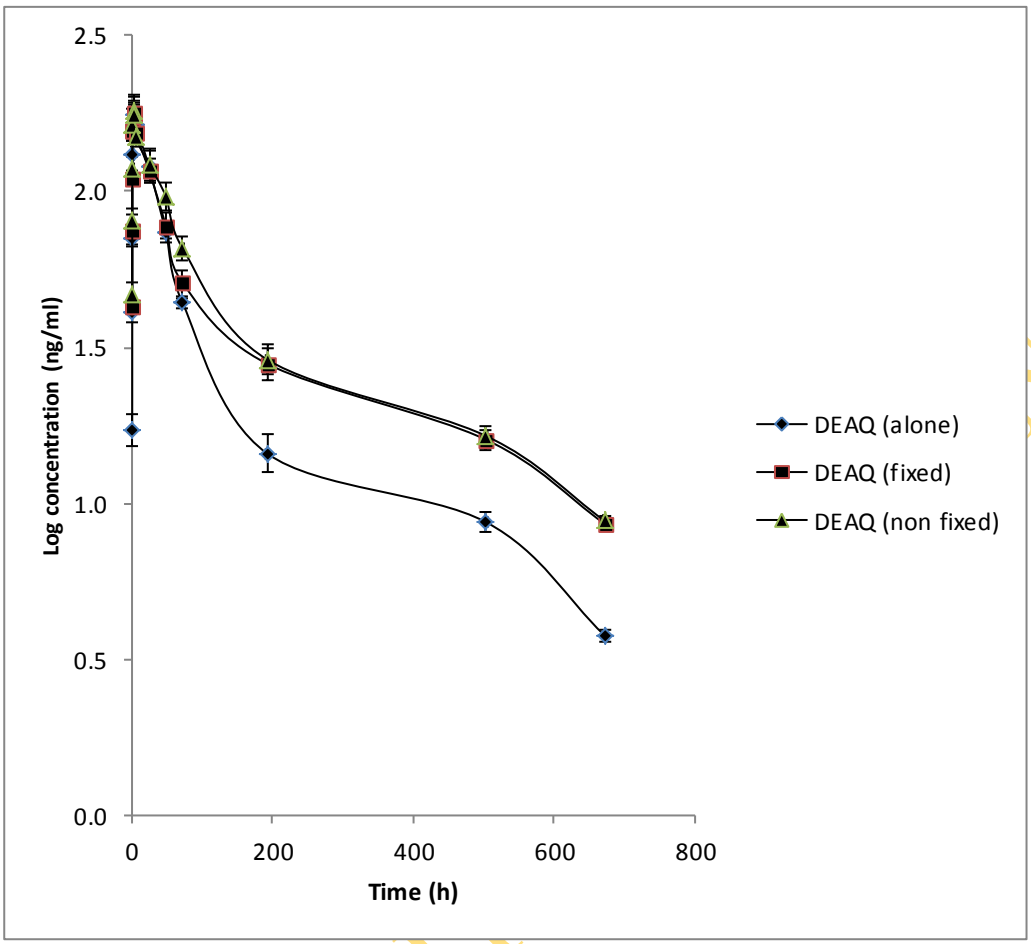


Figure 3.8: Plasma concentration–time profile for desethylamodiaquine (DEAQ) following single oral administration of amodaiquine (AMQ) monotherapy (600mg), fixed combination of artesunate /amodiaquine (200mg/612.6mg) non-fixed combination of artesunate+amodiaquine(200mg+600mg) (Mean±S.E.M, n=16)

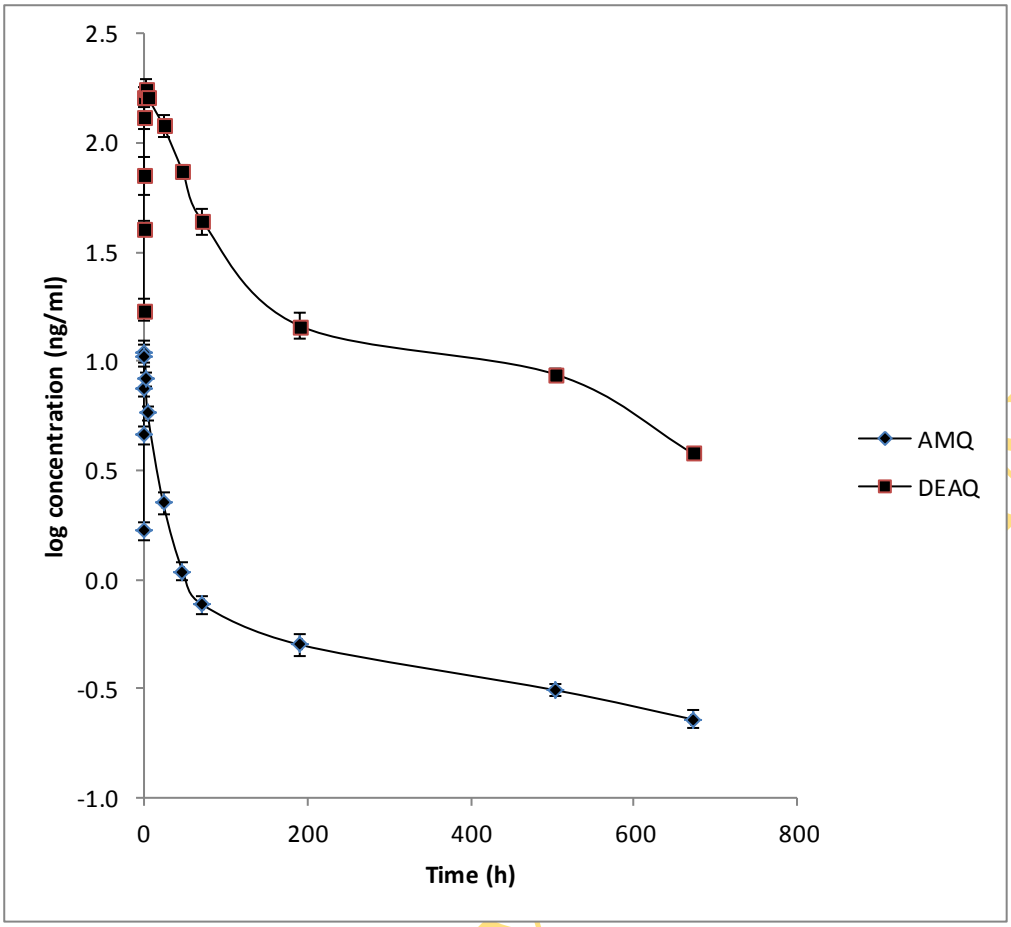


Figure 3.9: Concentration time profile of amodiaquine (AMQ) versus desethylamodiaquine (DEAQ) in monotherapy (Mean±S.E.M, n=16)

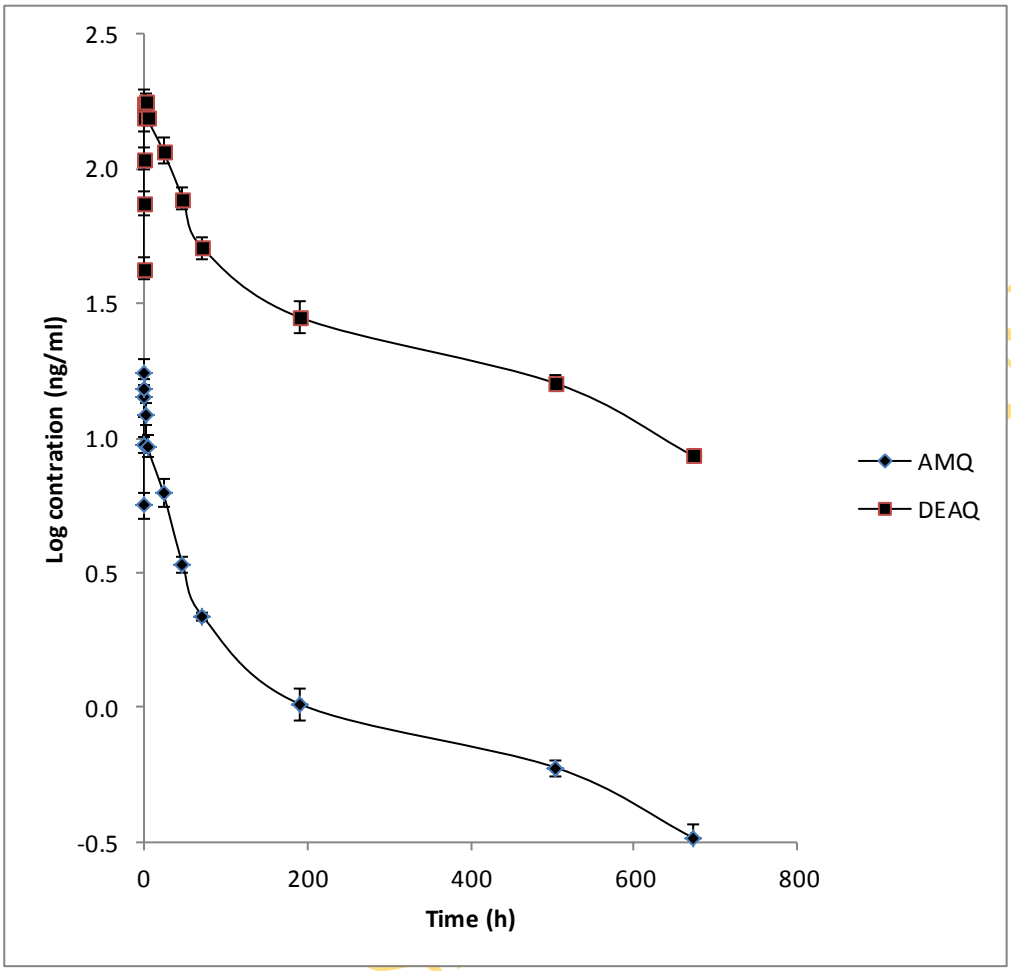


Figure 3.10: Concentration time profile of amodiaquine (AMQ) versus desethylamodiaquine (DEAQ) in fixed combination (Mean±S.E.M, n=16)

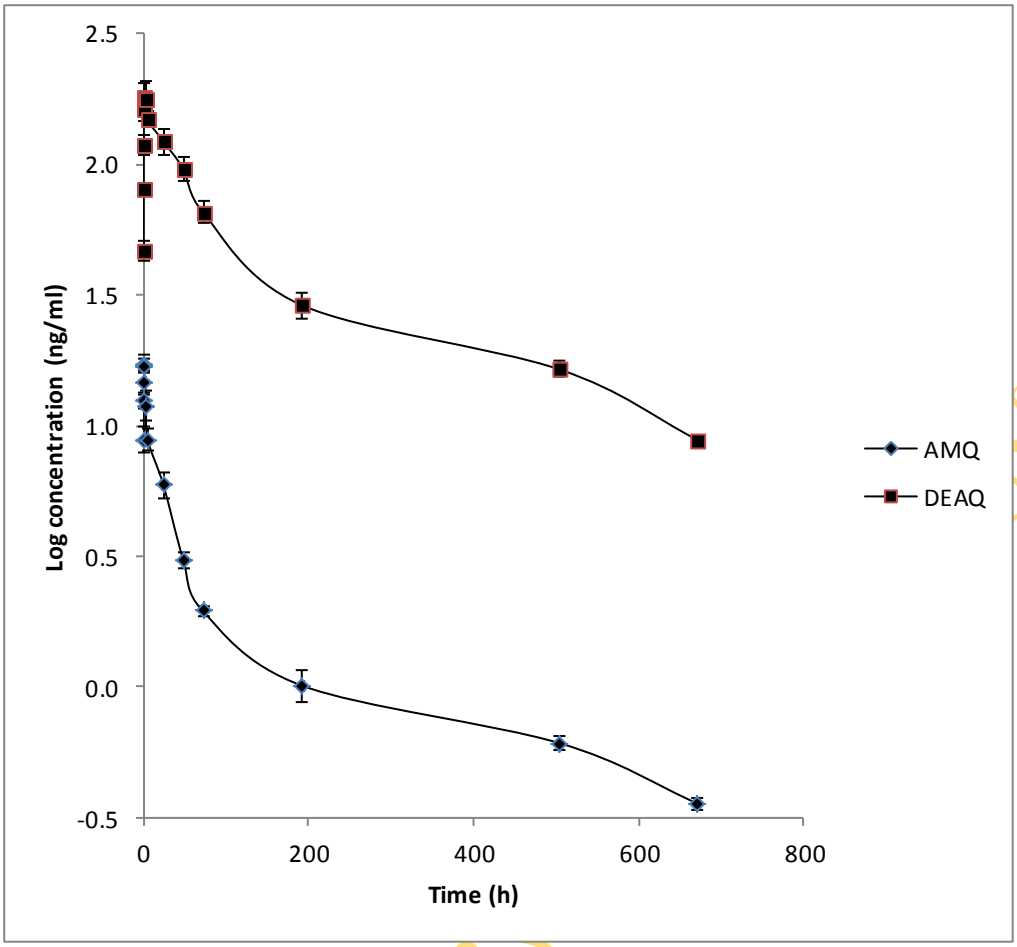


Figure 3.11: Concentration time profile of amodiaquine (AMQ) versus desethylamodiaquine (DEAQ) in non fixed combination (Mean±S.E.M, n=16)

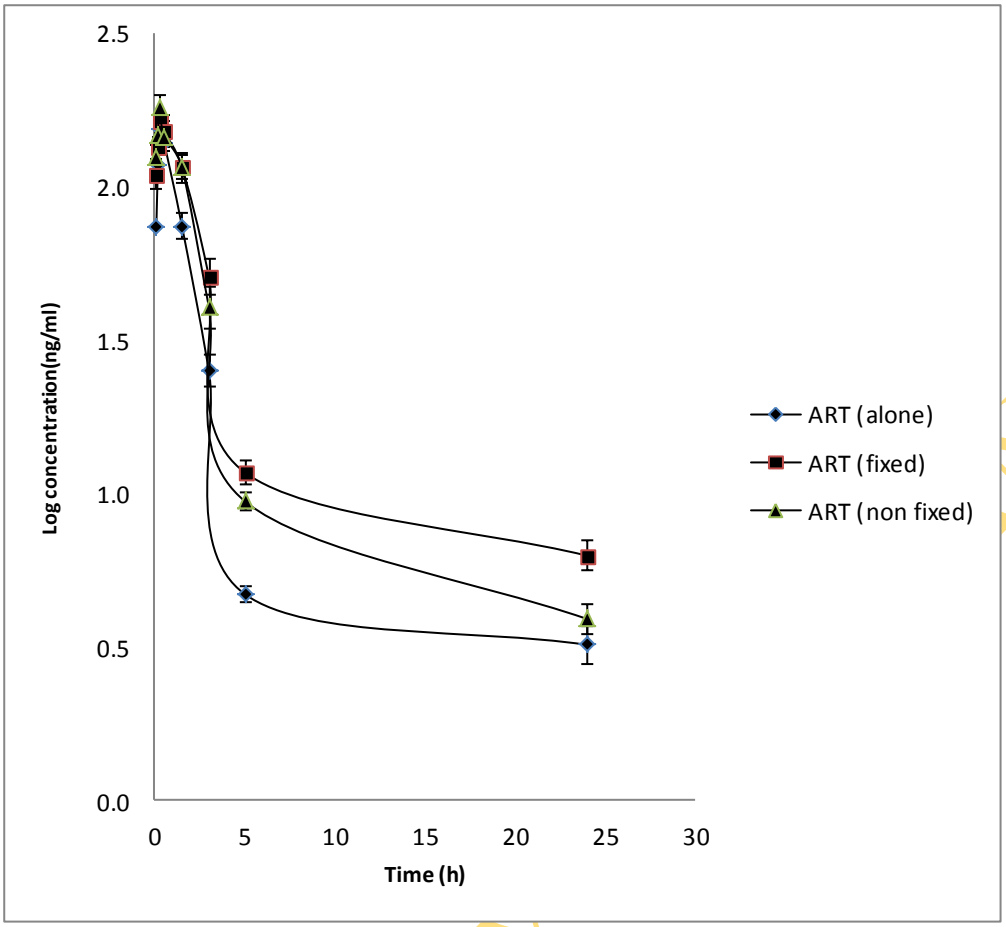


Figure 3.12: Plasma concentration–time profile for artesunate (ART) following single oral administration of artesunate monotherapy (200mg), fixed combination of artesunate /amodiaquine (200mg/612.6mg) non-fixed combination of artesunate+amodiaquine(200mg+600mg) (Mean±S.E.M, n=16)

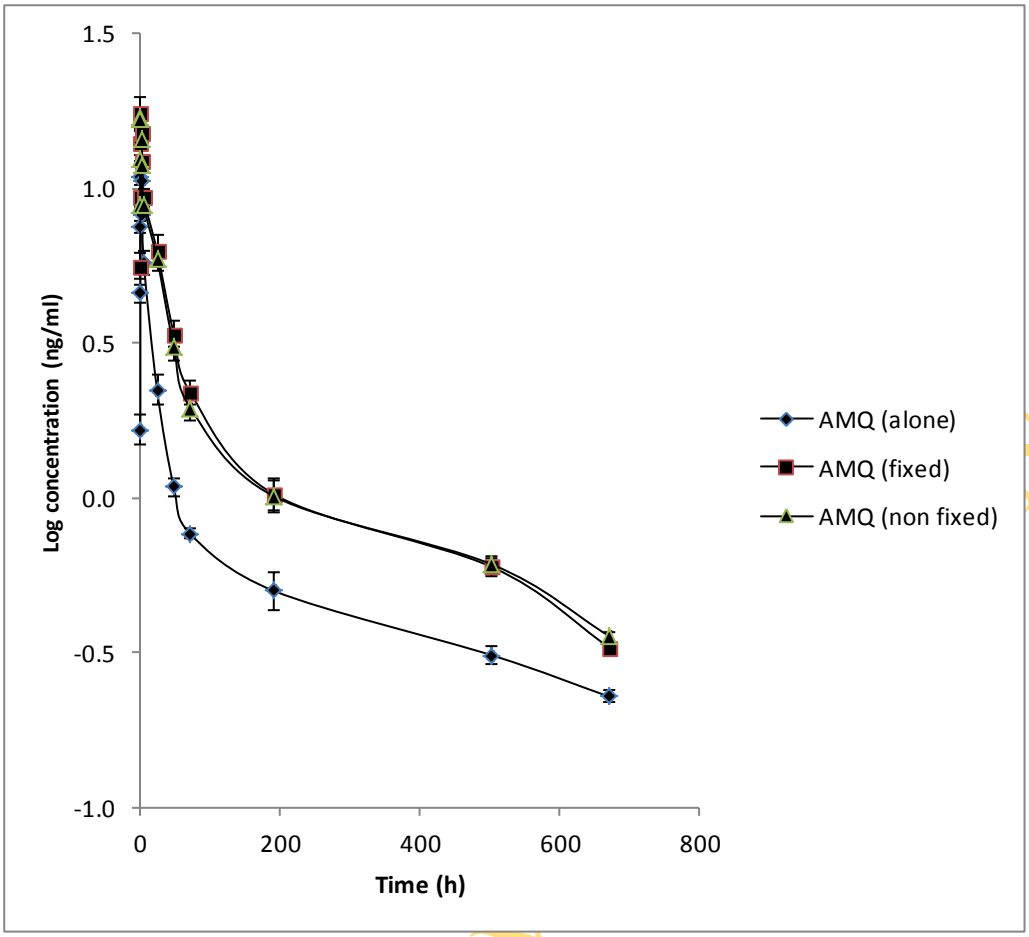


Figure 3.13: Plasma concentration–time profile for amodiaquine (AMQ) following single oral administration of AMQ monotherapy (600mg), fixed combination of artesunate /amodiaquine (200mg/612.6mg) non-fixed combination of artesunate+amodiaquine(200mg+600mg) (Mean±S.E.M, n=16)

Table 3.14: Derived PK parameters of artesunate following single oral administration of 200mg dose of artesunate to each of the volunteers, (n=16)

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
A	67	1.0	707.9	777.5	0.9	4.2	5.6
B	64	0.4	691.8	1522.1	1.4	2.1	4.6
C	71	0.4	630.9	415.9	0.8	8.0	10.0
K	63	0.4	549.5	558.2	1.0	5.9	8.5
D	61	1.3	562.3	1018.1	0.7	3.2	3.4
F	67	1.3	562.3	1146.8	0.9	2.9	3.7
G	67	0.5	794.3	1052.6	1.0	3.1	5.0
N	63	0.4	549.5	610.3	1.0	5.4	7.8
I	63	0.4	524.8	592.7	1.0	5.6	8.0

Table 3.14 continued:

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
J	56	0.4	660.6	1125.9	1.0	2.9	4.2
P	64	0.4	660.6	1096.4	1.0	3.0	4.4
Q	67	1.3	562.3	1146.8	0.9	2.9	3.7
H	59	0.5	549.5	898.6	0.9	3.7	4.9
L	79	1.2	588.8	1217.8	0.9	2.7	3.7
M	65	0.5	707.9	1204.6	0.9	2.7	3.7
O	56	0.4	691.8	1090.6	1.0	3.0	4.4
Mean	**64.5**	**0.7**	**624.7**	**967.2**	**0.9**	**3.8**	**5.4**
Standard deviation	**5.6**	**0.3**	**79.2**	**298.6**	**0.1**	**1.5**	**2.0**

Table 3.15: Derived PK parameters of artesunate following single oral administration of fixed combination of artesunate and amodiaquine to each of the volunteers, (n=16)

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
A	67	1.3	512.8	957.2	0.9	3.4	4.8
B	64	1.2	549.5	1195.8	0.8	2.7	3.3
C	71	1.3	512.8	1125.3	0.8	2.9	3.4
K	63	1.4	398.1	956.6	1.3	3.4	6.5
D	61	1.4	398.1	862.9	1.2	3.8	6.8
F	67	1.2	512.8	1091.4	0.8	3.0	3.8
G	67	1.2	524.8	846.8	0.8	3.9	4.7
N	63	1.4	398.1	956.6	1.3	3.4	6.5
I	63	1.4	398.1	902.0	1.3	3.7	6.9

Table 3.15 continued:

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
J	56	1.4	363.0	853.4	1.1	3.9	6.6
P	64	1.2	549.5	928.6	0.8	3.5	4.2
Q	67	1.2	549.5	1091.4	0.8	3.0	3.8
H	59	0.4	602.5	574.8	0.9	5.8	7.7
L	79	0.5	562.3	483.5	0.8	6.8	8.7
M	65	1.3	489.7	657.7	0.8	5.0	5.9
O	56	1.3	363.0	735.8	1.1	4.5	7.6
Mean	**64.5**	**1.2**	**480.3**	**888.7**	**1.0**	**3.9**	**5.7**
Standard deviation	**5.6**	**0.2**	**79.9**	**198.5**	**0.2**	**1.1**	**1.7**

Table 3.16: Derived PK parameters of artesunate follwing single oral administration of non fixed combination of artesunate and amodiaquine to each of the volunteers, (n=16)

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
A	67	1.3	562.3	890.8	0.8	3.7	4.4
B	64	1.3	524.8	885.1	0.7	3.7	4.2
C	71	1.3	602.5	1091.6	0.9	3.0	4.0
K	63	1.4	562.3	1113.2	1.0	2.9	4.4
D	61	1.3	512.8	962.2	0.8	3.4	4.0
F	67	1.3	549.5	948.9	0.8	3.5	4.2
G	67	1.2	501.1	962.0	0.7	3.4	3.8
N	63	1.4	562.3	1208.7	1.0	2.7	4.0

Table 3.16 continued:

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
I	63	1.4	575.4	1052.9	1.0	3.1	4.6
J	56	1.4	524.8	1015.5	0.8	3.2	4.1
P	64	1.3	512.8	965.5	0.7	3.4	3.9
Q	67	1.3	549.5	948.9	0.8	3.5	4.2
H	59	1.3	501.1	813.8	0.8	4.1	4.7
L	79	1.3	501.1	1198.8	1.0	2.7	4.0
M	65	1.4	524.8	1000.9	0.8	3.3	4.1
O	56	1.4	537.0	1043.6	0.8	3.1	4.0
Mean	**64.5**	**1.3**	**537.8**	**1006.4**	**0.8**	**3.3**	**4.2**
Standard deviation	**5.6**	**0.1**	**29.9**	**108.8**	**0.1**	**0.3**	**0.2**

Table 3.17: Derived PK parameters of amodiaquine following single oral administration of 600mg dose of amodiaquine to each of the volunteers, (n=16)

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
A	67	2.2	158.4	19305.2	42.9	0.5	32.1
B	64	2.5	173.7	13999.8	50.1	0.7	51.6
C	71	2.5	186.2	17774.4	50.1	0.5	40.7
K	63	2.4	199.5	16708.6	60.1	0.6	51.9
D	61	2.5	165.9	15803.0	50.1	0.6	45.7
F	67	2.5	181.9	18958.1	60.1	0.5	45.8
G	67	5.0	173.7	26310.6	75.2	0.3	41.2
N	63	2.8	158.4	16725.8	60.1	0.6	51.9
I	63	2.5	194.9	20534.5	75.2	0.4	52.8

Table 3.17 continued:

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
J	56	2.5	190.5	16006.8	60.1	0.6	54.2
P	64	3.0	173.7	22533.1	50.1	0.4	32.1
Q	67	2.5	186.2	18056.6	60.1	0.5	48.0
H	59	2.5	165.9	20053.6	50.1	0.5	36.0
L	79	2.9	199.5	16611.4	60.1	0.6	52.2
M	65	2.5	194.9	16336.8	60.1	0.6	53.1
O	56	2.6	199.5	17241.3	60.1	0.5	50.3
Mean	**64.5**	**2.7**	**181.4**	**18310.0**	**57.8**	**0.5**	**46.2**
Standard deviation	**5.6**	**0.6**	**14.5**	**2997.2**	**8.8**	**0.0**	**7.5**

Table 3.18: Derived PK parameters of amodiaquine following single oral administration of fixed combination of artesunate and amodiaquine to each of the volunteers, (n=16)

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
A	67	2.6	199.5	34916.5	100.3	0.2	41.4
B	64	3.0	208.9	26699.4	75.2	0.3	40.6
C	71	1.5	204.1	35662.0	100.3	0.2	40.5
K	63	1.5	199.5	25363.9	75.2	0.3	42.8
D	61	1.5	204.1	23586.6	75.2	0.4	46.0
F	67	3.0	199.5	37926.5	100.3	0.2	38.1
G	67	1.4	204.1	28403.3	75.2	0.3	38.2
N	63	1.5	204.1	26637.0	75.2	0.3	40.7
I	63	1.5	204.1	33817.3	75.2	0.3	32.1

Table 3.18 continued:

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
J	56	2.9	199.5	32293.5	75.2	0.3	33.6
P	64	2.9	204.1	26699.4	75.2	0.3	40.6
Q	67	3.0	204.1	37442.6	100.3	0.2	38.6
H	59	2.9	199.5	26108.5	75.2	0.3	41.5
L	79	4.1	173.7	19960.6	75.2	0.5	54.3
M	65	5.0	165.9	28228.6	100.3	0.3	51.2
O	56	2.8	204.1	26696.5	75.2	0.3	40.6
Mean	**64.5**	**2.5**	**198.7**	**29402.6**	**83.0**	**0.3**	**41.3**
Standard deviation	**5.6**	**1.0**	**11.6**	**5271.5**	**12.0**	**0.0**	**5.5**

Table 3.19: Derived PK parameters of amodiaquine following single oral administration of non fixed combination of artesunate and amodiaquine to each of the volunteers, (n=16)

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
A	67	2.6	223.8	72009.1	100.3	0.1	20.1
B	64	1.2	213.8	36166.0	100.3	0.2	40.0
C	71	2.5	257.0	71960.8	100.3	0.1	20.1
K	63	1.0	177.8	21515.2	75.2	0.4	50.4
D	61	1.3	208.9	36520.9	100.3	0.2	39.6
F	67	1.5	199.5	44595.0	100.3	0.2	32.4
G	67	0.5	199.5	29472.2	100.3	0.3	49.1
N	63	1.0	177.8	21515.2	75.2	0.4	50.4
I	63	1.0	181.9	21515.2	75.2	0.4	50.4

Table 3.19 continued:

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (ng/ml)	AUC (ng.h/ml)	$t_{1/2}$ (h)	CL/F (L/min)	Vd/F (L)
J	56	5.0	158.4	20251.3	75.2	0.4	53.6
P	64	1.1	223.8	36166.0	100.3	0.2	40.0
Q	67	1.5	199.5	40655.7	100.3	0.2	35.6
H	59	2.8	257.0	53865.9	75.2	0.1	20.1
L	79	2.9	218.7	43027.8	60.1	0.2	20.1
M	65	2.9	239.8	54260.3	60.1	0.1	16.0
O	56	5.0	158.4	17826.4	75.2	0.5	60.9
Mean	**64.5**	**2.1**	**206.0**	**38832.7**	**85.8**	**0.3**	**37.4**
Standard deviation	**5.6**	**1.3**	**30.5**	**17339.1**	**15.6**	**0.1**	**14.5**

CHAPTER 4

GENERAL DISCUSSION

4.1 Pharmaceutical equivalence of artesunate and amodiaquine

4.1.1 Artesunate products

The results presented in Table 3.1 indicate that a majority (66.7%) of the artesunate tablets studied failed the drug content assay test. The identification test (using TLC) revealed that artesunate was undetectable in brands B2 and B3, probably due to the low content of drug. The packaging of the fake products was similar to some of the genuine brands, except for their colour and unique markings. This suggests that they might have been deliberately counterfeited. Counterfeiting has been reported in a recent survey in Southeast Asia which showed that among 104 tablet brands presented as artesunate, 38% did not contain artesunate. Magnus *et al*., (2007) stated in a previous study that counterfeit or substandard artemisinin-based derivatives were being sold in parts of Africa, presenting a potential route for resistance development in the future. Also in Cambodia in 1990, substandard antimalarials were reported as responsible for the deaths of at least 30 people (Newton, 2000). The results from this work are the first to show, to the best of our knowledge, the circulation of substandard artesunate tablet in Nigeria (Odunfa *et al*., 2009). This poses a serious health danger as Nigeria, in 2004, adopted the use of ACTs, in her national antimalaria treatment policy, for uncomplicated malaria in accordance with WHO guidelines (National Antimalaria Treatment Policy, 2005). In view of the health implications of counterfeit products, including development of resistant strains of plasmodium parasites, this calls for proper monitoring of all commercial brands of artesunate tablets, both locally manufactured and imported, including at ports of entry for imported products. Out of the 10 generic products (66.7%) that failed drug content test, 8 were imported products and 2 (20%) were locally manufactured (F and F1) as shown in Table 3.1. Eight out of these 10 products had excess drug (> 110% of labelled content) and although they may be therapeutically effective, there could be increased risk of toxicity and increased side-effects on ingestion. The two products

with drug content below pharmacopoeial specifications may be regarded as substandard and/or counterfeit products. Chi-square analysis of the unit cost of the products and physicochemical properties revealed that there is no significant correlation between cost and the quality of artesunate products ($p = 0.449$) or between the quality of the drug and country of manufacture ($p = 0.364$). Products F and F1 which are indigenous (local) artesunate tablets had comparable hardness, longer disintegration times compared to product B which served as the reference (Table 3.1). This indicates good formulation protocol for these locally manufactured artesunate products.

4.1.2 Amodiaquine products

All the five amodiaquine brand products studied disintegrated within 21min (Table 3.2) and thus complied with the USP limit of 30min. All the amodiaquine products had good hardness properties. There was no correlation between tablet hardness and disintegration time for these products ($p > 0.05$). The drug release profiles of three of the products (BB, DD and EE) were not statistically different ($p > 0.05$) from that of the reference product AA. However, all but one product, CC, satisfied the USP requirement of a minimum of 75% release within 30min. Most of the amodiaquine tablets sampled must have gone through painstaking GMP.

4.1.3 Pharmaceutical equivalence

Pharmaceutical equivalents are those drug products in identical dosage forms that contain identical amounts of the same active drug and that meet identical compendial or other applicable standards, including potency, content uniformity, disintegration time and dissolution test. If a particular drug product meets the requirements of USP for dissolution and other *in vitro* parameters, it gives assurance that the drug will be released satisfactorily from the dosage formulation *in vivo* and should lead to good bioavailability. From the study, artesunate products A, C, D, E and H1 were pharmaceutical equivalents as were amodiaquine samples AA, BB, DD and EE. Although most of the tested products met compendial standards, including content uniformity, disintegration and dissolution rates, it was shown that not all the products could be referred to as pharmaceutical equivalents. When products are pharmaceutically equivalent, they may have similar profiles in terms of absorption,

clinical effect, tolerability and toxicity. This finding suggests that prescribers cannot freely interchange these artesunate brands to generate consistent or comparable therapeutic and toxicity profiles. It then becomes a challenge for clinicians and pharmacists in using their professional experience to make therapeutic decisions when interchanging generic brands of artesunate and amodiaquine in clinical setting. Hence the need for update seminars and workshops on antimalarials commonly used locally.

4.2 Bioavailability and tolerability study

The preponderance of substandard antimalarial tablets in circulation poses great concerns to the bioavailability of coformulations of artesunate and amodiaquine. With respect to pharmacodynamic and pharmaceutic interaction data on artesunate and amodiaquine which are well documented, limited data is available on their pharmacokinetic drug-drug interaction. The study was designed to assess the bioavailability and tolerability of artesunate and amodiaquine when administered as monotherapy and in combination. It was also aimed at determining the strength of influence of biochemical factors such as serum albumin and anthropometric factors such as percentage body fat on tolerability/development of adverse drug effects. The results of this study on adult Nigerian healthy, male volunteers using a cross-over design shows that ART and AMQ are readily absorbed but not well tolerated when co-administered either as fixed-dose or non-fixed combination. However, the two combinations were not found to have comparable bioavailabilities (Odunfa *et al*., 2011).

Parent compounds and their metabolites were measured separately, but consideration was given to the primary analytes (total DHA for ART, and DEAQ for AMQ), as the biologically and clinically relevant indicators of drug absorption. For ART and DHA, the reason for combining the two measurements (after stoichiometrical conversion of the measured ART values) is that the two compounds have comparable antimalarial potencies and conversion of ART to DHA is rapid (chemical hydrolysis starts in the stomach). The combined measurement may be the better parameter to use for pharmacokinetic–pharmacodynamic analyses. The pharmacokinetic analyses in this Latin square, cross-over study showed statistically significant pharmacokinetic interactions resulting in reductions in the bioavailability

of dihydroartemisinin and increased bioavailability of desethylamodiaquine when artesunate and amodiaquine were given in combination to healthy volunteers. Although AMQ is thrice more potent *in vitro* than its metabolite, the systemic exposure to AMQ is considerably lower than that to DEAQ, which is solely responsible for sustained antimalarial activity after ART and DHA have been eliminated. In this work the ratio between the AUCs of ART to DHA and AMQ to DEAQ was approximately 16.26% and 7.31%, respectively. In this work, modification of the method chosen may have led to the ratios being slightly higher than that reported by Churchill *et al*., 1985 and Orrel *et al*., 2008. This ratio is a measure of abundance of the metabolite drug in the systemic circulation.

4.2.1 Interaction and tolerability of the drugs

The fixed and non fixed co-administration resulted in an approximately 23.60% and 13.41% reduction of the C_{max} for artesunate (with respect to the total dihydroartemisinin equivalents) respectively. C_{max} is a measure of exposure, uptake or absorption rate of the drug. The C_{max} is also a good guide towards the effectiveness or tolerability of a drug when compared with its Minimum Effective Concentration (MEC). One possible explanation of the reduction in C_{max} is that the lower protein binding capacity of artesunate relative to amodiaquine may have led to its displacement from binding site, and consequential exposure to faster elimination (Pussard and Verdia, 1994; Li *et al*., 2006). It is important to note that auto induction of metabolism (Simonsson *et al*., 2003; Gordi *et al*., 2005) and lowering of plasma concentration (Table 3.10) on co administration with amodiaquine are also probable causes of this phenomenon. Enzyme induction, a process considered to be slower relative to enzyme inhibition is reported to be time and dosage dependent (Shorvron, 2004). The lowered C_{max} may pose threats to effective paracitidal activity of artesunate while it remains in the body and attempts to cover the three life cycles of the plasmodium protozoan i.e. the pre-erythrocytic, erythrocytic and gametophyte stages. Hyper metabolism in certain individuals may necessitate the need to increase the dosage of drugs given to them for effectiveness. The consumption of fatty foods was implicated to lower the rate and extent of absorption of artesunate (Fitoussi *et al*.,2009). Immunocompromised patients especially those suffering from HIV already are at risk of therapeutic failure from antimalarial drugs (Cook *et al*., 2009); drugs like

efavirenze and ritonavir when used concurrently with artesunate have been found to induce its metabolism leading to low levels of the drug in the plasma via induction of the CYP 2B6 enzyme, the cytochrome P450 metabolic enzyme of artesunate. While it is a well-accepted fact that co-formulated drugs are desirable for enhancing compliance, pharmacokinetics of individual drugs were altered on co-administration from this present study. The fear of therapeutic failure or recrudescence expressed by many researchers (Orrell *et al*., 2008) may then be justified, because the peak concentrations of DHA being about 43 to 66 times higher (Table 3.9) than those required for maximum and full parasiticidal effect (IC_{50} for *Plasmodium falciparum* isolates taken from patients with primary or recrudescent infections on the north western border of Thailand -up to 8.25ng/ml for DHA) (Brockman *et al*., 2000) may be reduced to sub therapeutic levels leading to failure of therapy or development of resistance on multidosing. The C_{max} above the inhibitory concentration is a good predictor of antimalarial efficacy (Navaratnam *et al*., 2009). *In vitro* IC_{50}s customarily performed was suggested not to be ideal for the artemisinins (Navaratnam *et al*., 2009) and hence caution should be exercised when extrapolating *in vitro* data to i*n vivo* situations.

The peak concentrations of DEAQ being about 1.3 to 1.4 times higher (Table 3.9) than those required for maximum and full parasiticidal effect (IC_{50} for *Plasmodium falciparum* is 135ng/ml) (Adjei *et al*., 2008**).** With this marginal differential (compared to dihydroartemisinin) in the concentration required for maximum and full parasiticidal effect, the worrisome toxicity profile of amodiaquine may justify the reason why its dose must not be increased for fear of resistant mutants selected in some parts of the African continent. Although clinically significant resistance to the artemisinins has not yet been documented and that recrudescence is associated with the drug, amodiaquine resistance is well documented and this is probably the principal explanation for low artesunate plus amodiaquine cure rates observed in a number of African countries (Adjuik *et al*., 2002). C_{max} values from this study were comparable to those reported by Orrel *et al*., 2008. All artemisinin drugs prevent the development of ring stage parasites to the more mature pathogenic stages that rosette and cytoadhere in the capillaries (Watkins *et al*., 1993; Udomsangpetch *et al*., 1996) and the problem with them is that when they are used

alone over short periods i.e. less than 5 days , clearance of parasitaemia from the blood is only temporary in up to 50% of patients. This has been attributed to the short time that it takes for artemisinin drugs to be eliminated from the body. Higher efficacy can be obtained by 5 and especially 7day regimens but this is associated with reduced compliance in out-patients. The $t_{1/2}$ of artesunate from this study averaged about 1h and was similar to that reported by Orrel *et al*., 2008 and Batty *et al*., 1998. Despite the limitations of many of the reported clinical studies, a consistent observation is that artemisinin derivatives produce faster relief of clinical symptoms and clearance of parasites from the blood than other antimalarial drugs (Hien and White, 1993). In around 90% of the patients given these drugs, the fevers resolved and the parasitaemias cleared within 48 hours of treatment. Despite their short elimination half lives, artemisinin drugs are effective when given daily. The development of resistance depends in part on the pharmacokinetic and pharmacodynamic characteristics of drugs. Antimalarial drugs with long terminal half lives such as amodiaquine (averaging about 4h from the study) are particularly vulnerable to the development of resistance because (i) there is an increased chance that a new and unrelated infection may be acquired whilst drug concentrations following treatment have fallen below those sufficient to prevent parasite multiplication and radically cure the new infection and (ii) if the original infection is not radically cured, surviving parasites will be subject to drug pressure as asexual cycles are exposed to decreasing blood concentrations (Watkins and Mosobo, 1993). There is good evidence that short half-life antimalarial drugs like artesunate are less vulnerable to the development of resistance.

Artemisinin and its derivatives have short half-lives and are the most potent and rapidly acting antimalarial drugs known. They reduce the parasite biomass by around 10 000 fold for each asexual cycle and by a factor of 10^6-10^8 over a 3 day course of treatment (White, 1997). Only the residual parasites are exposed to amodiaquine alone so that the selective pressure for the emergence of mutants with reduced sensitivity to amodiaquine is reduced considerably.

Combinations of artemisinin derivatives could have a potential role in slowing the development of resistance to other antimalarial drugs such as amodiaquine, chloroquine and sulfadoxine/pyrimethamine in Africa. Combining drugs may lead to altered pharmacokinetics, decreased efficacy and increased adverse reactions. There is evidence of pharmacokinetic drug-drug interaction from this study and the use of combinations obviously increases the direct cost of treatment. These costs, however, should be offset against the potential indirect savings from both reduced morbidity and the costs of treating recrudescences (Bloland *et al*., 1993). Ideally, the components of combinations should be formulated into a single tablet or capsule but this would be considered as a new drug and require costly pharmacokinetic, toxicological studies required for registration. A less satisfactory but simpler alternative would be to combine the separate components in blister packs (non fixed/loose combinations) as in multiple drug therapy of tuberculosis and leprosy. Licensing would be easier but the drugs in the combination would need regulatory approval. Dual prescription would be the cheapest approach but compliance would be a major problem. Acute malaria reduces the hepatic biotransformation of many drugs (Newton *et al*., 2000). Evidence from some other studies showed that drug disposition may be changed in clinical situations as plasma proteins is altered in malaria patients (Silamut *et al*., 1985; Routledge, 1986; Mansor *et al*., 1991). The DHA concentrations in plasma measured in patients with severe malaria were shown to be considerably lower than those measured in healthy volunteers after i.m. administration (Suputtamongkol *et al*., 2001), suggesting that hepatic metabolism of ART, mainly by CYP2 A6, 2B4, but also by 2B6, 3A4 and 3A5 (Murphy *et al*., 1997; Rajeev *et al*., 2006; Yusof and Siew, 2009), is reduced by malaria infection. Desethylamodiaquine pharmacokinetic parameters in malaria-infected adults in Africa (Winstanley *et al*., 1990) and children with acute malaria in Papua New Guinea (Hombhanje *et al*., 2005) were similar to those of normal volunteers. AUC can be used as a measure of abundance or drug exposure. It is derived from drug concentration and time so it gives a measure how much and how long a drug stays in a body from the toxicological point of view. Dihydroartemisinin was about ten times more abundant (high metabolic ratio) than artesunate when administered on monotherapy basis from this study. Desethylamodiaquine was about fifteen times (14.79) more abundant than amoquiaquine in the systemic circulation when administered on monotherapy basis.

The abundance of dihydroartemisinin, the major metabolite of artesunate was about one quarter (2.08) and about one third (2.5) for the fixed and non fixed combinations respectively compared to artesunate monotherapy (9.19) confirming a notable metabolic interaction on coadministration. Artesunate is rapidly transformed into dihydroartemisinin so that the metabolite is generally present at higher levels than the parent compound (Teja-Isavadharm *et al*., 1996).

Although dihydroartemisinin is the most active derivative on a molar basis, each of the parent drugs is active in the low nM range and known to achieve levels much higher than the minimum inhibitory concentrations in the plasma. In spite of the rapid clearance and extent of transformation, the parent drugs may contribute a significant proportion of the antimalarial effect in the blood (Hien and White, 1993). While the AUC of artesunate was increased more than twice (251.54% and 212.56% for fixed and nonfixed coformulation respectively) in this study, that of dihydroatemisinin was decreased by 26.39% and 13.1% for the fixed and non fixed coformulations respectively. A pharmacokinetic drug interaction can be deduced from this fact as the reduced bioavailability may be via displacement of artesunate from protein binding site by amodiaquine which is about 92% protein bound compared to artesunate which is about 80% bound. Displacement from binding site has been found to expose drugs to a faster elimination from the systemic circulation.

The fixed and non fixed co-administration resulted in an approximately 59.98% and 95.36% increment in the AUC for amodiaquine (with respect to the total desethylamodiaquine equivalents) respectively. One possible explanation of the increased extent of absorption and consequent slowed metabolism of amodiaquine may have resulted from formulation variabilities (Mukeshgohel, 2009; Lee and Thomas, 2003); polymorphisms of the CYP2C8 enzyme may have also contributed to the lowered rates of metabolism. A South African paper reported a 35% decrease in AUC of total DEAQ when ART was coadministered with amodiaquine. These differences could be ascribed to subject populations and analytical assays. One other possible explanation of this observation is that polymorphism of the CYP 2C8 enzyme could contribute to different rates of drug metabolism (Gil and Berglund, 2007). The statistical data available from this study is consistent with the nature and pattern of commonly reported adverse drug effects of amodiaquine in malaria patients in Sub Saharan Africa (Akindele and Odejide, 1976; Rhodes *et al*., 1986; Labro and

Babin-Chevaye, 1988; Clarke *et al*., 1991). Pharmacokinetic elements like the drug concentration at the receptor site (assumed to be that existing in the systemic circulation) and AUC for both a drug with a long residence time such as AMQ/DEAQ, and short-lived drugs, such as ART/DHA is related to the response produced (Navaratnam *et al*., 2009). It is expected that a faster absorption of artesunate will lead to quicker exposure to metabolic enzymes and by extension faster conversion to dihydroartemisinin. The rate of absorption was found to be in the order artesunate monotherapy< fixed combination therapy< non fixed combination therapy (T_{max}= 0.32h, 0.30h and 0.29h respectively). Artesunate according to its total dihydroartemisinin equivalents when administered as non fixed combination had the fastest elimination ($t_{1/2}$ =0.88h) compared to fixed (1.00h) and monotherapy (0.98h). The rate of absorption of artesunate was delayed on coadministration. This implies that in acute conditions when a fast antimalarial action is desired, artesunate should not be administered concurrently with amodiaquine. For amodiaquine, the rate of absorption was also in the order nonfixed combination (T_{max}= 0.46h) >fixed combination(0.62h) > monotherapy (1.09h). The rate of absorption of amodiaquine was increased on coadministration. This may have implication on its toxicity as it may be heightened. Due to the high percentage protein binding of amodiaquine (non fixed combination therapy) being about 92%, it was observed that the Vd/F of amodiaquine was lowest (453L)when compared to fixed combination (486.7L) and monotherapy (741.6L). This indicates a greater protein binding capacity of the nonfixed formulation, the fact which was probably responsible for the longest $t_{1/2}$(85.88h) and poor CL/F (0.31L/min) in the total desethylamodiaquine equivalents when compared to fixed ($t_{1/2}$ = 83.06h, CL/F =0.36L/min) and monotherapy ($t_{1/2}$= 57.85h, CL/F =0.55L/min). The higher the capacity of drugs to bind to plasma proteins, the slower may be their clearance from the body. Adverse reactions common to such drugs may not abate on time because of poor clearance and low volume of distribution.

Amodiaquine is chemically similar to chloroquine and even though it is often effective against chloroquine resistant strains of Plasmodium falciparum, the study carried out supports the greater frequency of adverse reaction commonly observed in those that consumed the coformulations. The 2% increase in the dosage of amodiaquine in the fixed dosage over the non fixed dosage i.e. 612.6mg versus

600mg may have contributed to the increased frequency of its adverse effects on volunteers. Though the fixed coformulation was assessed to be that with lower bioavailability of amodiaquine (Table 3.9), it should be noted however from the study that pharmacokinetics (extent of exposure of the drug) alone may predict clinical response but may not predict adverse reaction/toxicity profile of drugs. It is possible that pharmaceutic and pharmacodynamic indices may be covariates or predictors of toxicity profile. The nature of excipients of tablets formulations may also determine toxicity profile (Mukeshjohel, 2009; Lee and Thomas, 2003). It has been suggested that repeated exposure to AMQ (as in multidosing/malaria therapy) may favour toxicity through the formation of a quinoneimine that may lead to immunoglobulin G immune-mediated liver and neutrophil toxicity; Orrel, *et al*., 2008 reported an asymptomatic, self-resolving hepatitis in a healthy female subject a South African study. Due to circumstances beyond control, limitation to this study arose from the use of only male volunteers.

CHAPTER 5

CONCLUSION

Pharmaceutically inequivalent and subpotent artesunate and amodiaquine tablets existed in circulation and this suggests the need to regularly monitor their quality to avoid therapy failure. Since pharmaceutical equivalence is a basic requirement (among other factors) for substitution when prescribing and/or dispensing drugs, caution should be applied in interchanging or substituting generic brands of artesunate and amodiaquine as shown by the study. The bioavailability study conducted has added to the armamentarium of data on the pharmacokinetics of artesunate and amodiaquine that has earlier been sparse in the early 2000. The rate and extent of absorption of ART was severely lowered on co-administration (T_{max}, C_{max}) $p < 0.05$. The rate and extent of absorption of AMQ was increased on co-administration (T_{max} and AUC) $p<0.05$. The co-formulations cannot be used as therapeutic equivalents of monotherapy as the failure risk may be increased. The fixed and non-fixed co-formulations, though not pharmaceutically equivalent however were not found to have comparable bioavailabilities (more than 20% deviation in their relative bioavailability) and thus cannot be used as interchangeable options. This presupposes that they may not have similar clinical and toxicity profile in clinical setting. Higher serum albumin predisposed volunteers to greater risks of adverse drug effects. Higher adiposity did not predispose volunteers to greater risks of adverse drug effects from this study.

Further pharmacokinetic research on artesunate and amodiaquine tablets when administered to malaria patients on multidosing basis is required to establish the extent and clinical significance of these pharmacokinetic interactions. Population kinetics is necessary to obtain evidence based dosing in certain diseased states. *In vitro*/*in vivo* correlational studies would however be important to put an end to the worrisome concern over expensive bioavailability studies being required for registration of generic substitutes and coformulations of existing drugs moieties.

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APPENDIX 1

Calculations

$C_{19}H_{28}O_8 + NaOH = C_{19}H_{27}O_8Na + H_2O$

384.4g of artesunate($C_{19}H_{28}O_8$) ≡ 1000ml 1M sodium hydroxide(NaOH)

0.0192g of $C_{19}H_{28}O_8$ ≡ 1ml of 0.05MNaOH

$NaOH + C_6H_4(COOK)COOH = C_6H_4(COOK)COONa + H_2O$

204.2g of potassium hydrogen phthalate ($C_6H_4(COOK)COOH$) ≡ 1000ml 1M NaOH

0.01021g of $C_6H_4(COOK)COOH$ ≡ 1ml 0.05M NaOH

Factor = Weight of sample/(Milli equivalent x titre value)

National Cancer Institute Toxicity Criteria:

Haemoglobin –Normal limit 14-18g/dL

Mild anaemia: 9.5-13.0 g/dL

Moderate anaemia: 8-9.5 g/dL

Severe anaemia: 6.5-7.5g/dL

Very Severe anaemia:< 6.5g/dL

White blood cell- Normal limit : 4.0 -11.0 x 10^9 /L

Leucopenia Grade 1: <LLN -3.0 x10^9/L

Grade 2: ≥2.0 - <3.0 x10^9/L

Grade 3: ≥1.0 - <2.0 x10^9/L

Grade 4: <1.0 x10^9/L

Neutrophils- Normal limit: 2.5 -7.5 x 10^9 /L

Neutropenia Grade 1: ≥1.5 -<2.0 x10^9/L

Grade 2: ≥1.0 - <1.5 x10^9/L

Grade 3: ≥0.5 - < 1.0 x10^9/L

Grade 4: <0.5 x10^9/L

Lymphocytes- Normal limit: 1.5 -3.3 x 10^9 /L

Lymphopenia Grade 1: <LLN - 1.0 x10^9/L

Grade 2: ≥0.5 - <1.0 x10^9/L

Grade 3: <0.5 x10^9/L

Basophils- Normal limit: 0 - 0.1 x 10^9 /L

Eosinophils- Normal limit: 0.04- 0.44 x 10^9 /L

Monocytes - Normal limit: 0 - 0.55 x 10^9 /L

Total Protein - Normal limit: 5.8-8.0g/dL

Albumin- Normal limit: 3.0 - 4.5g/dL . Normal (3.0 - <3.4g/dL). High Normal (≥3.4g/dL)

Alanine Aminotransferase (ALT) - Normal limit: 0-22 U/Litre

Transaminitis Grade 1: >ULN – 2.5 x ULN

Grade 2: >2.5 – 5.0 x ULN

Grade 3: >5.0 – 20.0 x ULN

Grade 4: >20 x ULN

Aspartate Aminotransferase (AST) - Normal limit: 0-12U/Litre

Transaminitis Grade 1: >ULN – 2.5 x ULN

Grade 2: >2.5 – 5.0 x ULN

Grade 3: >5.0 – 20.0 x ULN

Grade 4: >20 x ULN

Adverse side effects grouped into Mild, Moderate, Severe, Very severe.

Adverse drug events (clinical) in general practice patients

Mild - a reaction of limited duration not requiring further treatment; minimum impact on daily activities.

Moderate - a reaction of longer duration or which requires further treatment; limits daily activities.

Severe - a reaction of any duration which results in hospitalisation and/or long term limitation of daily activities

Body fat percentage (Adiposity)

Normal for athletes (6%-13%),

High Normal for athletes >13%

APPENDIX 2

Table 6.1: Identity test results of various brands of artesunate tablets, n=15

No.	Code	R_f Values	Status
1	A	0.87	passed
2	B	0.87	passed
3	B1	0.87	passed
4	B2	0.00	failed
5	B3	0.00	failed
6	C	0.87	passed
7	D	0.87	passed
8	E	0.87	passed
9	F	0.87	passed
10	F1	0.87	passed
11	G	0.87	passed
12	G1	0.87	passed
13	H	0.87	passed
14	H1	0.87	passed
15	I	0.87	passed
16	Ref.samp	0.87	passed

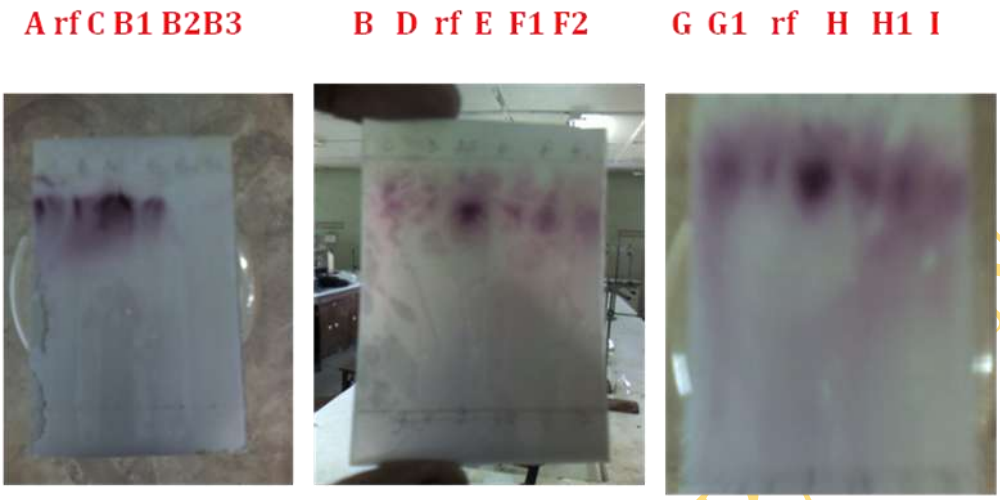


Figure 6.1: Micrograghs of TLC plates of artesunate tablets

Table 6.2: Identity test results of various brands of amodiaquine tablets

No	Code	Absorption Maxima (nm)	Status
1	AA	342.0	Passed
2	BB	342.0	Passed
3	CC	342.0	Passed
4	DD	342.0	Passed
5	EE	342.0	Passed
6	Ref	342.0	Passed

Table 6.3: Hardness test results for artesunate tablets (Kgf), n=10

No	Code	1	2	3	4	5	6	7	8	9	10	Mean	Standard Deviation	(%) Coefficient of Variation
1	A	5.2	4.6	4.2	4.6	5.4	5.9	5.0	5.6	6.0	5.0	5.2	0.6	11.2
2	B	5.4	5.7	5.1	4.9	5.0	6.1	4.0	5.9	5.5	5.6	5.3	0.6	11.2
3	B1	5.8	6.4	7.2	4.9	5.0	4.1	5.0	6.0	5.4	5.2	5.5	0.9	16.1
4	B2	7.2	4.2	8.2	5.2	4.2	10.2	5.2	6.2	7.8	7.2	6.6	1.9	28.9
5	B3	9.6	4.2	8.7	5.3	4.2	8.2	8.4	6.4	8.2	6.2	6.9	1.9	27.7
6	C	7.0	7.4	7.8	7.8	7.4	7.6	8.1	7.4	8.2	7.4	7.6	0.4	5.0
7	D	4.2	3.2	4.5	3.9	3.7	3.9	3.5	3.7	3.8	3.5	3.8	0.4	9.3
8	E	4.4	8.3	8.5	9.6	8.4	8.8	9.0	6.8	8.2	8.1	8.0	1.5	18.0
9	F	12.6	6.1	5.4	6.4	4.2	2.4	4.8	3.9	4.7	4.3	5.5	2.7	49.9

Table 6.3 continued:

No	Code	1	2	3	4	5	6	7	8	9	10	Mean	Standard Deviation	(%) Coefficient of Variation
10	F1	4.2	5.6	6.5	4.2	5.2	4.3	4.5	5.2	6.2	6.4	5.2	0.9	17.4
11	G	5.7	4.0	5.3	7.0	3.8	4.9	3.7	7.6	5.1	7.6	5.5	1.5	26.9
12	G1	5.7	5.3	7.0	3.9	4.5	5.1	7.1	7.2	4.0	4.4	5.4	1.3	23.9
13	H	5.1	2.8	4.4	4.7	3.4	1.9	4.4	4.4	5.3	4.9	4.1	1.1	25.9
14	H1	5.2	3.1	4.5	4.7	3.4	2.9	5.5	4.2	5.1	4.5	4.3	0.9	20.6
15	I	5.2	4.9	5.1	5.7	4.0	5.0	5.8	5.6	5.6	4.9	5.2	0.5	10.0

Table 6.4: Hardness test results for amodiaquine tablets (KgF), n=10

No	Code	1	2	3	4	5	6	7	8	9	10	Mean	Standard Deviation	(%) Coefficient of Variation
1	AA	4.6	4.2	4.4	4.8	4.7	4.5	5.1	5.2	5.3	4.4	4.7	0.4	7.5
2	BB	5.2	5.4	7.8	6.4	6.5	6.4	5.9	6.8	6.4	6.3	6.3	0.7	11.4
3	CC	4.2	4.6	4.8	5.4	4.6	4.7	4.8	4.9	5.3	5.2	4.8	0.4	7.2
4	DD	3.2	4.3	4.3	5.2	3.8	4.2	4.8	4.9	3.4	4.8	4.3	0.7	15.2
5	EE	5.6	4.3	6.2	3.4	4.2	4.7	5.2	6.2	4.3	3.9	4.8	1.0	19.9

Table 6.5: Uniformity of weights of various brands of artesunate tablets (g), n=20

No	Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation	(%) Coefficient of Variation
1	A	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	2.3
2	B	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.0	2.9
3	B1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.0	0.9
4	B2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.0	2.7
5	B3	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	3.4
6	C	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	1.6
7	D	0.3	0.3	0.2	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.0	1.3
8	E	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	1.0

Table 6.5 continued:

No	Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation	(%) Coefficient of Variation
9	F	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.0	4.6
10	F1	0.3	0.3	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.0	4.3
11	G	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.0	3.7
12	G1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.3
13	H	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	3.2
14	H1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0
15	I	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	1.1

Table 6.6: Uniformity of weights of various brands of amodiaquine tablets (g), n=20

No	Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation	(%) Coefficient of Variation
1	AA	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.0	2.1
2	BB	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.0	1.3
3	CC	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.0	0.5
4	DD	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.0	1.2
5	EE	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.0	1.7

Table 6.7: Uniformity of diameter of various brands of artesunate tablets, n=12

No	Code	1	2	3	4	5	6	7	8	9	10	11	12	Mean	Standard deviation	(%) coefficient of variation
1	A	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	0.0	0.0
2	B	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	0.0	0.2
3	B1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	0.0	0.1
4	B2	10.1	10.1	10.2	10.2	10.2	10.2	10.1	10.2	10.1	10.1	10.1	10.2	10.1	0.0	0.1
5	B3	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.1	10.2	0.0	0.1
6	C	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	0.0	0.0
7	D	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	0.0	0.0
8	E	8.8	8.8	8.7	8.7	8.6	8.6	8.7	8.6	8.7	8.6	8.7	8.6	8.6	0.0	0.8
9	F	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	0.0	0.0

Table 6.7 continued:

No	Code	1	2	3	4	5	6	7	8	9	10	11	12	Mean	Standard deviation	(%) coefficient of variation
10	F1	9.5	9.4	9.4	9.5	9.4	9.4	9.4	9.4	9.4	9.4	9.4	9.4	9.4	0.0	0.3
11	G	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	0.0	0.1
12	G1	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	0.0	0.1
13	H	9.1	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	0.0	0.4
14	H1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	0.0	0.1
15	I	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	0.0	0.0

Table 6.8: Uniformity of diameter of various brands of amodiaquine tablets, n=12

No	Code	1	2	3	4	5	6	7	8	9	10	11	12	Mean	Standard deviation	(%) Coefficient of variation
1	AA	11.2	11.3	11.1	11.2	11.1	11.1	11.3	11.2	11.1	11.2	11.2	11.2	11.2	0.0	0.6
2	BB	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	0.0	0.2
3	CC	11.2	11.2	11.3	11.1	11.2	11.2	11.3	11.3	11.2	11.2	11.1	11.1	11.2	0.0	0.6
4	DD	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.8	10.8	10.9	0.0	0.3
5	EE	11.5	11.5	11.5	11.5	11.5	11.4	11.4	11.4	11.5	11.5	11.5	11.5	11.5	0.0	0.3

Table 6.9: Disintegration test results of different brands of artesunate tablets (mins), n=6

No	Code	1	2	3	4	5	6	Mean	Standard deviation
1	A	4	3	4	5	4	3	3.8	0.8
2	B	3	4	3	3	4	4	3.5	0.5
3	B1	2	3	4	3	5	3	3.3	1.0
4	B2	34	30	38	35	39	33	34.8	3.3
5	B3	3	5	4	4	4	3	3.8	0.8
6	C	4	5	4	3	5	4	4.1	0.8

Table 6.9 continued:

No	Code	1	2	3	4	5	6	Mean	Standard deviation
7	D	4	3	4	3	5	4	3.8	0.8
8	E	3	2	3	3	4	3	3.0	0.6
9	F	7	9	6	8	9	7	7.6	1.2
10	F1	11	13	10	9	13	14	11.6	2.0
11	G	3	4	3	3	5	3	3.5	0.8
12	G1	3	3	3	4	5	3	3.5	0.8
13	H	4	4	5	6	5	5	4.8	0.8
14	H1	7	8	7	9	7	8	7.6	0.8
15	I	4	5	4	5	3	4	4.1	0.8

Table 6.10: Disintegration test results of different brands of amodiaquine tablets (mins), n=6

No	Code	1	2	3	4	5	6	Mean	Standard deviation
1	AA	9	9	10	10	9	8	9.1	0.7
2	BB	6	6	5	6	6	6	5.8	0.4
3	CC	9	9	8	9	9	10	9.0	0.6
4	DD	20	21	22	20	21	20	20.6	0.8
5	EE	15	16	18	14	18	14	15.8	1.8

Table 6.11: Assay results of various brands of artesunate tablets

No	Brand name	Titre value(ml)	Amount expected (mg)	Amount Present (mg)	Amount(%)[a]	Standard deviation	Status
1	Aretmed	19.8	50	54.0	108.0	1.3	Passed
2	Artesunat1	21.7	50	59.1	118.2	1.5	failed-above reference range
3	Artesunat2	20.8	50	56.7	113.4	1.3	failed-above reference range
4	Aresunate3	5.2	50	14.1	28.2	1.5	failed- below reference range
5	Artesunat4	9.1	50	24.7	49.5	3.4	failed- below reference range
6	Arthlon	19.5	50	53.0	106.0	2.2	Passed
7	Malasunate	19.9	50	54.3	108.7	2.2	Passed
8	Arinate	19.7	50	53.7	107.4	1.5	Passed
9	Vami's Artesunate1	20.6	50	56.0	112.0	2.6	failed-above reference range

Table 6.11 continued:

No	Brand name	Titre value(ml)	Amount expected (mg)	Amount Present (mg)	Amount(%)[a]	Standard deviation	Status
10	Vami's Artesunate2	20.7	50	56.4	112.8	2.7	failed-above reference range
11	Lever1	20.6	50	56.0	112.0	2.6	failed-above reference range
12	Lever2	21.1	50	57.4	114.8	1.3	failed-above reference range
13	Malmeter1	20.3	50	55.3	110.7	2.6	failed-above reference range
14	Malmeter2	19.8	50	54.0	108.0	2.6	Passed
15	Larimal	20.9	50	57.0	114.1	2.2	failed-above reference range

[a]*IP requirement: 90 -110% of amount stated on label (n=20)*

Table 6.12: Assay results of various brands of amodiaquine tablets

No	Brand name	Amount expected (mg)	Amount present (mg)	Amount (%)[a]	Standard deviation	Status
1	Camoquine	260	245.1	94.3	0.3	passed
2	Larimal	200	188.0	94.0	0.5	passed
3	Timec	260	245.9	94.6	0.4	passed
4	Loquine	260	245.4	94.4	0.9	passed
5	Dart	260	245.9	94.6	1.0	passed

[a]*USP requirement 93%-107% of amount stated on label (n=20)*

Table 6.13: Comparative dissolution profiles of various brands of artesunate, n=6

Time (mins)	Percentage drug release												
	A	B	B1	C	D	E	F	F1	G	G1	H	H1	I
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.0	78.4	76.3	76.5	81.0	92.7	78.2	83.3	79.0	87.6	87.4	82.9	78.4	79.2
10.0	78.4	84.0	86.4	73.3	70.6	80.4	93.2	81.7	76.5	76.7	84.9	85.3	87.6
15.0	79.2	90.7	93.7	64.8	68.8	41.1	72.5	95.1	44.3	44.4	36.1	37.4	48.9
30.0	50.9	40.4	42.3	40.6	47.4	31.5	55.4	70.7	36.3	36.2	35.1	36.4	38.0
45.0	32.5	34.5	30.6	31.6	36.8	18.4	50.0	61.3	34.8	35.0	34.4	35.0	36.2
50.0	31.8	34.4	30.1	31.0	35.6	18.2	49.3	48.7	33.4	33.4	33.5	34.1	34.8
60.0	30.6	34.2	29.7	30.4	34.1	18.1	48.4	48.2	32.4	32.5	32.8	33.0	33.5
75.0	21.1	30.1	28.7	21.6	32.7	17.3	35.5	37.9	31.5	31.5	31.9	31.8	31.8

Table 6.14: Comparative dissolution profiles of various brands of amodiaquine, n=6

Time (mins)	Percentage drug release				
	AA	BB	CC	DD	EE
0	0.0	0.0	0.0	0.0	0.0
5	72.0	72.0	53.4	71.2	67.3
10	80.5	82.5	58.0	80.8	79.6
15	77.3	75.8	65.1	73.6	75.8
30	67.0	60.0	75.0	67.2	63.8
45	57.6	49.3	70.1	61.9	56.9
50	47.3	47.9	62.2	57.2	41.4
60	43.9	44.2	54.9	52.5	38.9
75	39.8	39.7	46.9	45.4	35.1

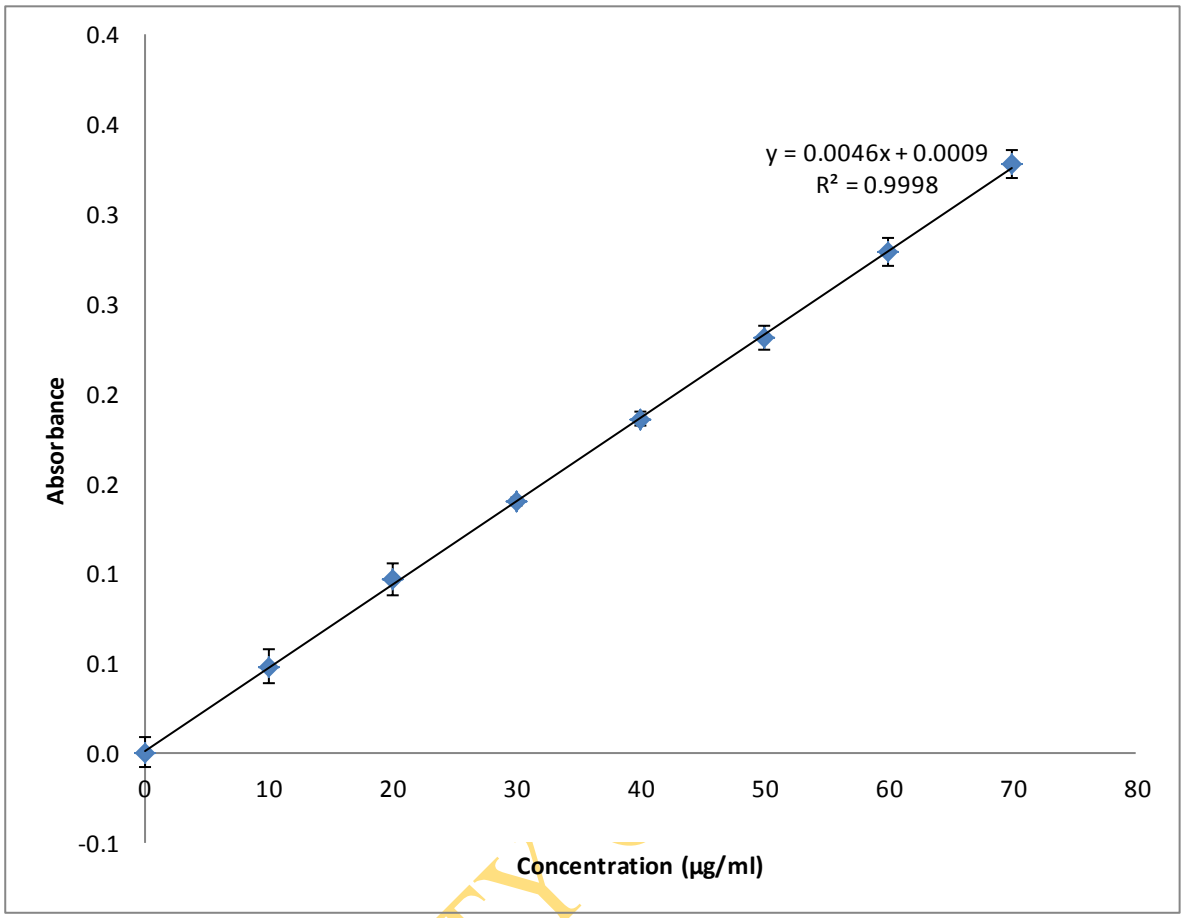


Figure 6.2: Calibration curve for dissolution of artesunate in 0.1M HCl (Mean±S.E.M, n=3)

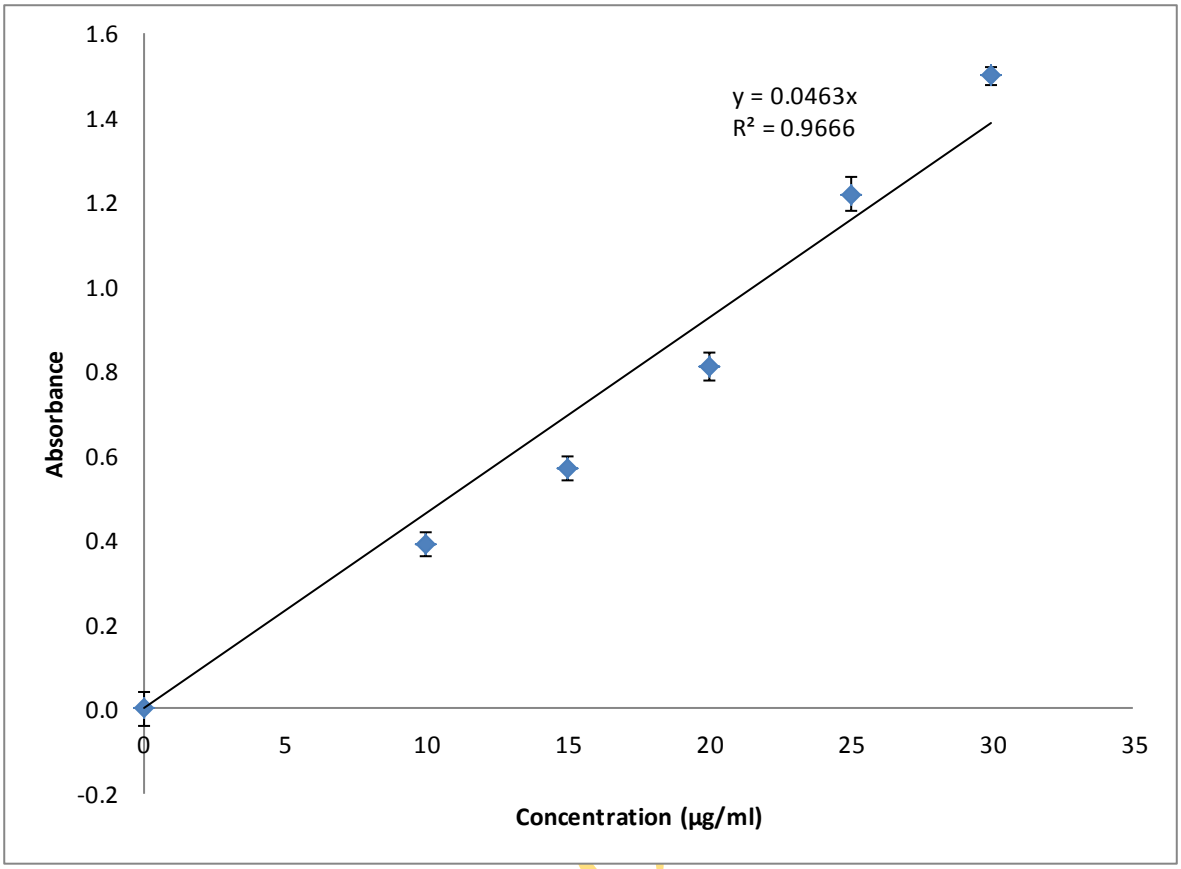


Figure 6.3: Calibration curve for dissolution of amodiaquine in water (Mean±S.E.M, n=3)

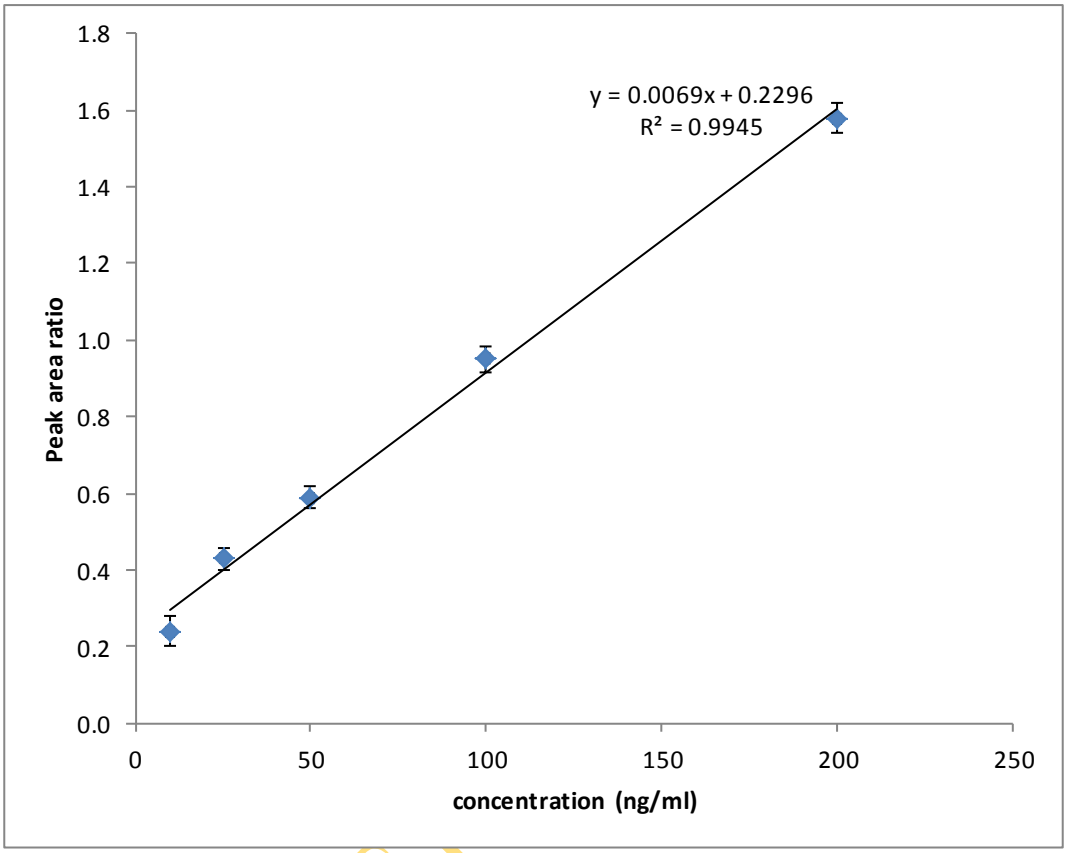


Figure 6.4: Calibration curve for artesunate in buffer (Mean±S.E.M, n=6)

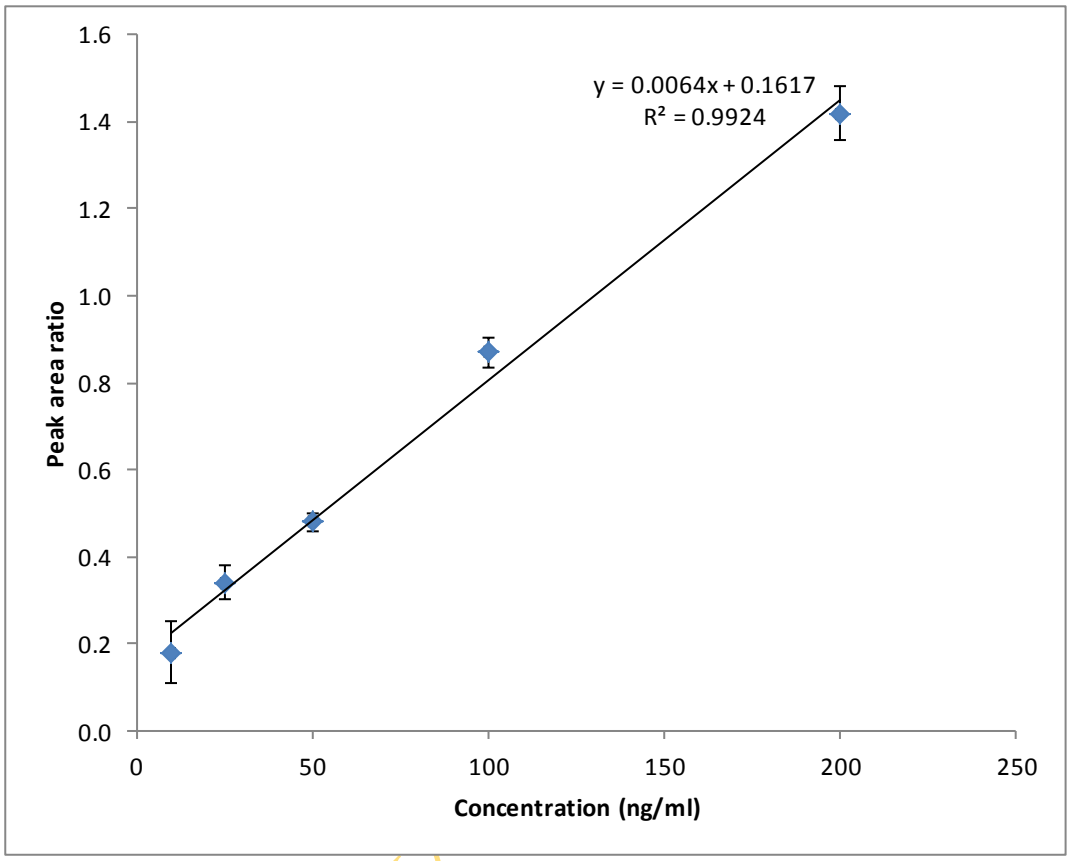


Figure 6.5: Calibration curve for artesunate in plasma (Mean±S.E.M, n=6)

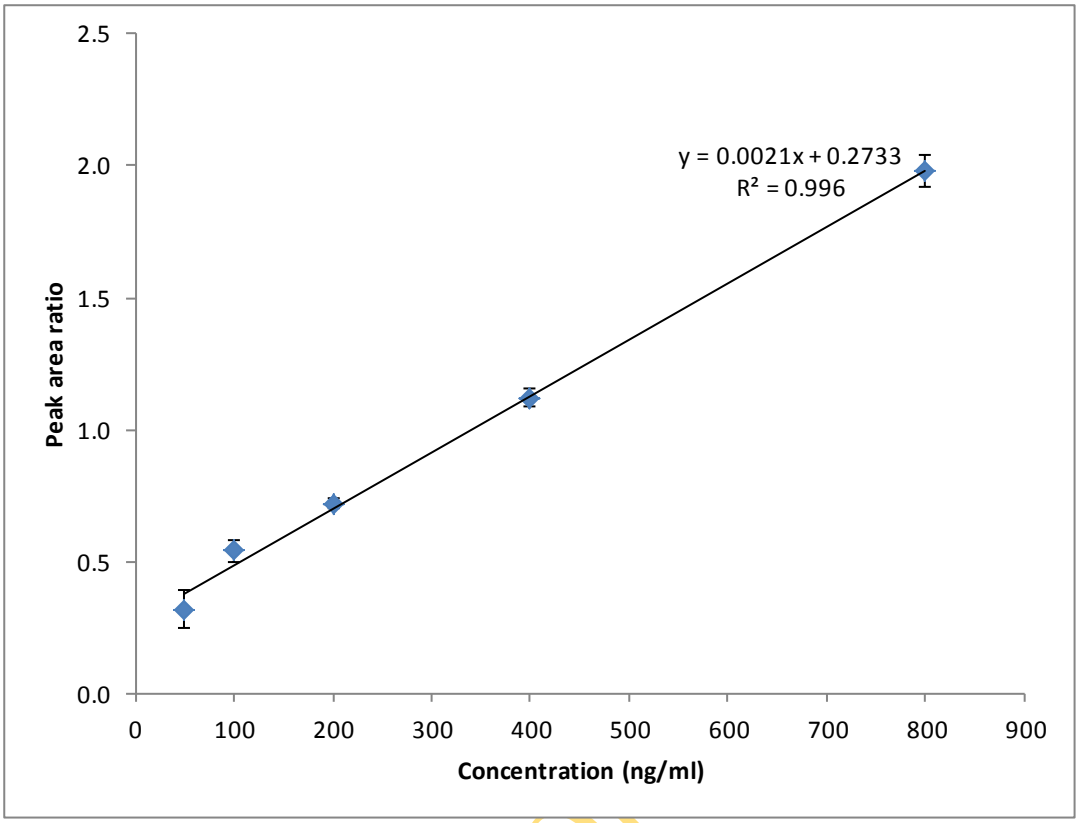


Figure 6.6: Calibration curve for dihydroartemisinin in buffer (Mean±S.E.M, n=6)

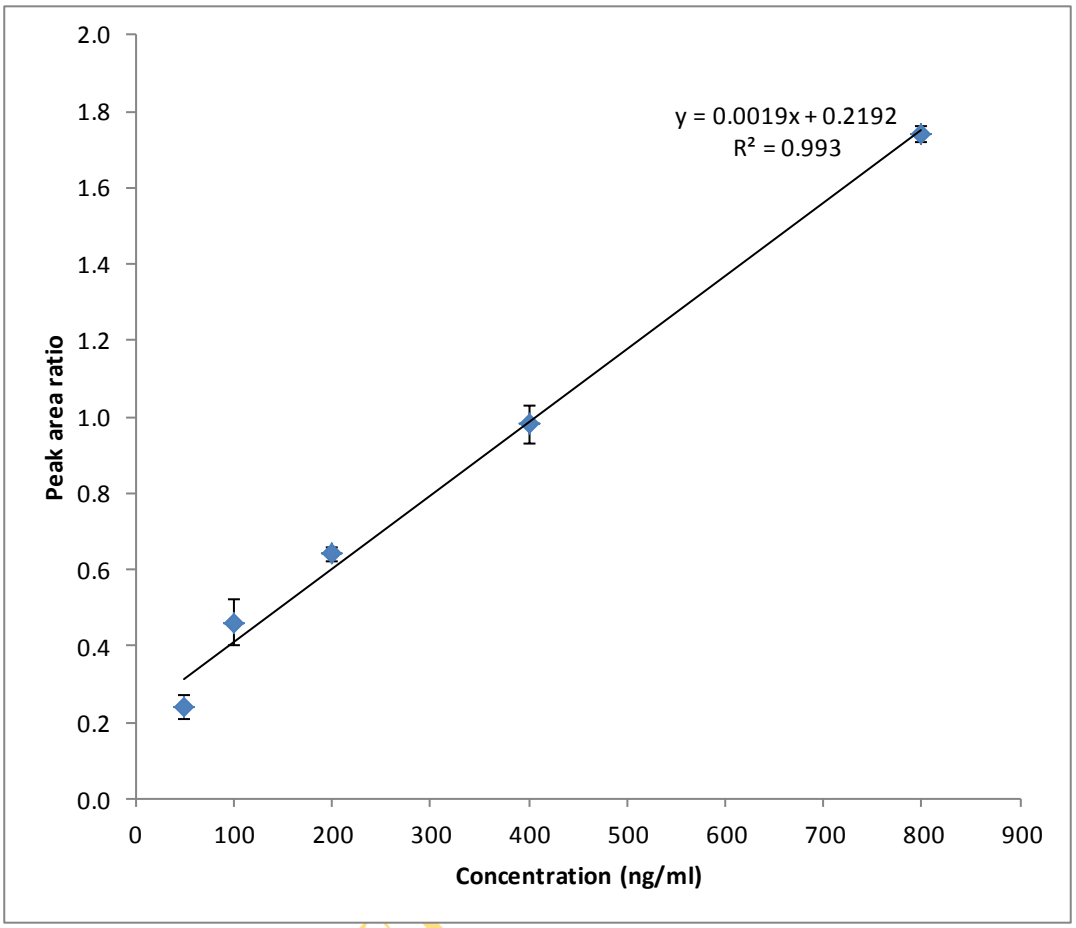


Figure 6.7: Calibration curve for dihydroartemisinin in plasma (Mean±S.E.M, n=6)

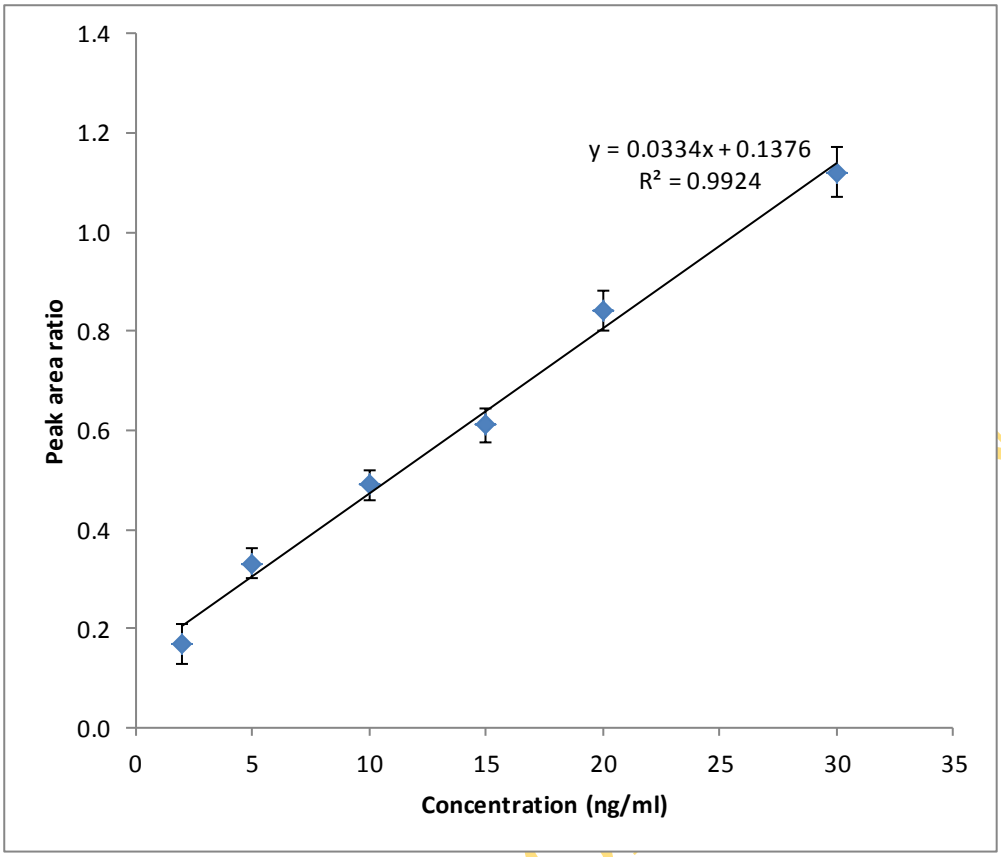


Figure 6.8: Calibration curve for amodiaquine in buffer (Mean±S.E.M, n=6)

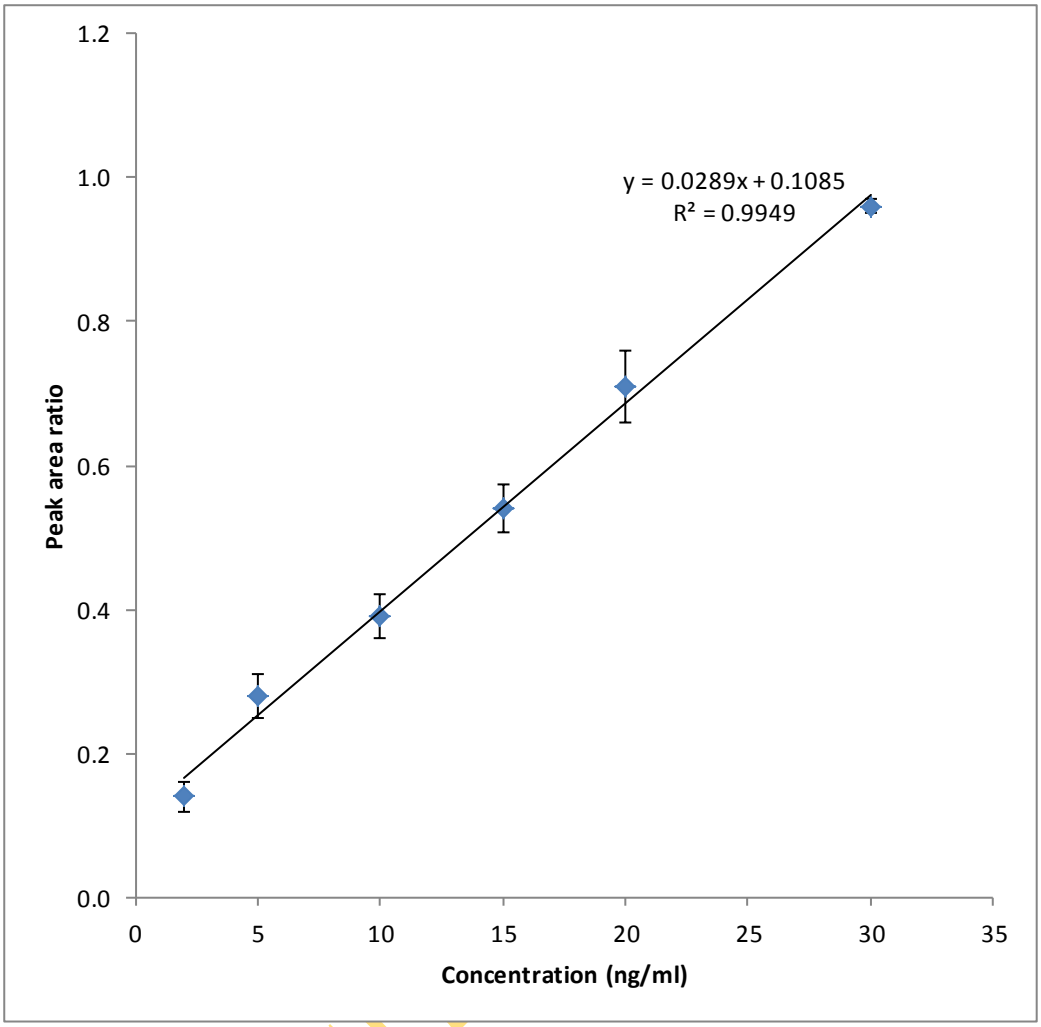


Figure 6.9: Calibration curve for amodiaquine in plasma (Mean±S.E.M, n=6)

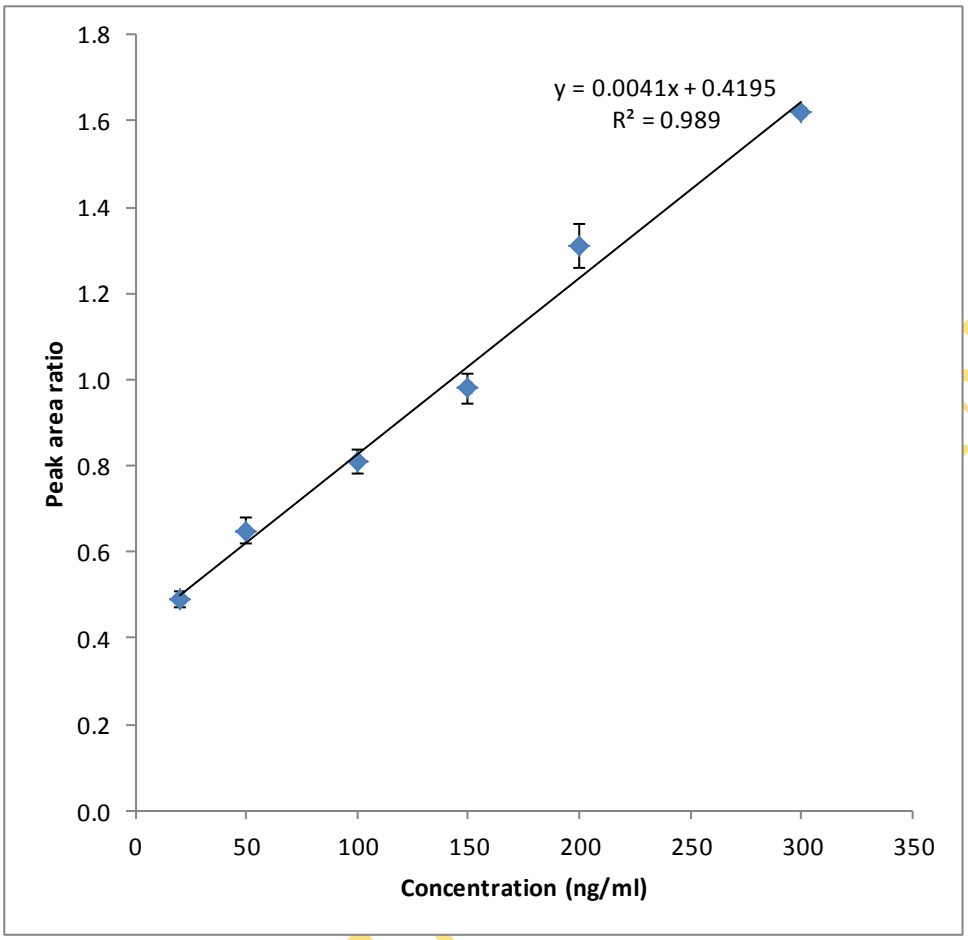


Figure 6.10: Calibration curve for desethylamodiaquine in buffer (Mean±S.E.M, n=6)

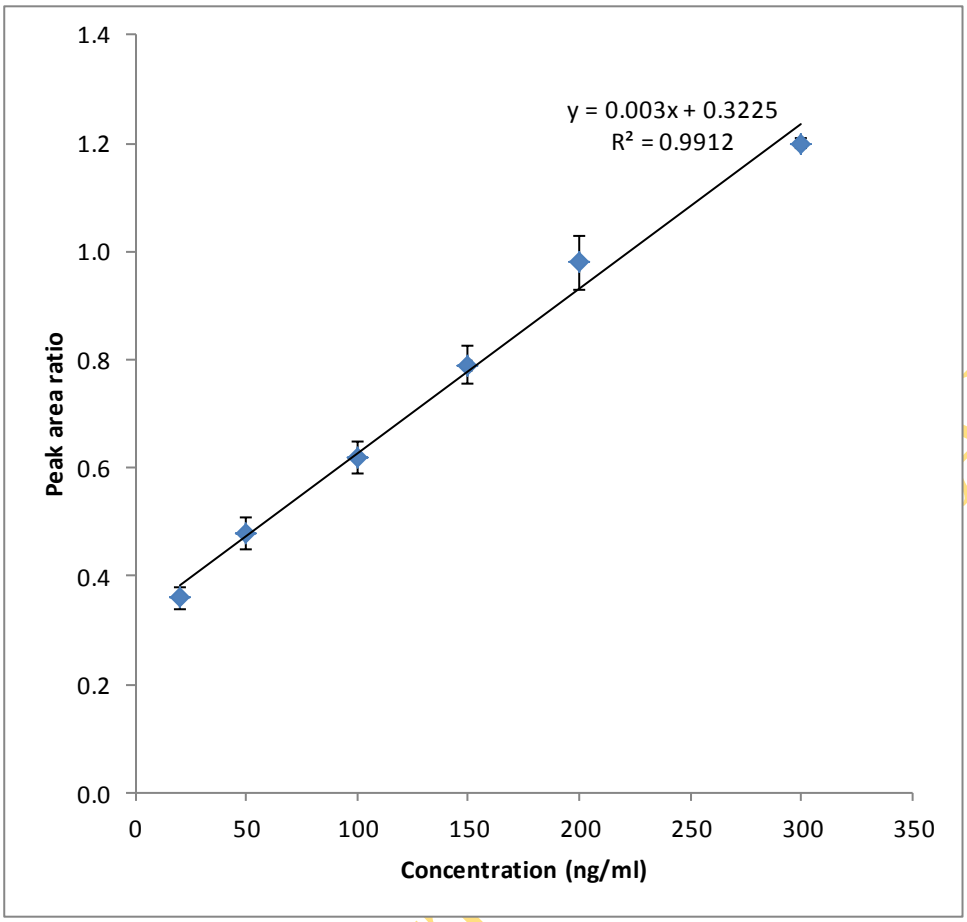


Figure 6.11: Calibration curve for desethylamodiaquine in plasma (Mean±S.E.M, n=6)

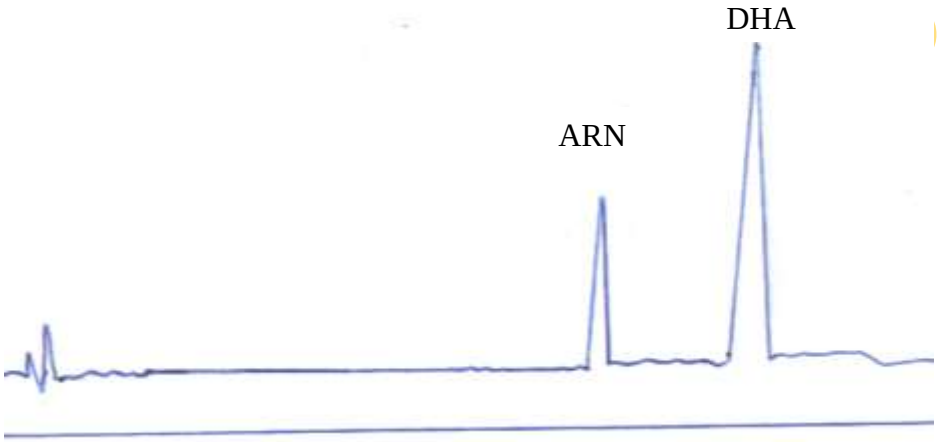


Figure 6.12: HPLC chromatograms of Dihydroartemisinin (DHA) (pure sample in extracted spiked plasma) and internal standard (Artemisinin)-P.H adjusted to 4.0

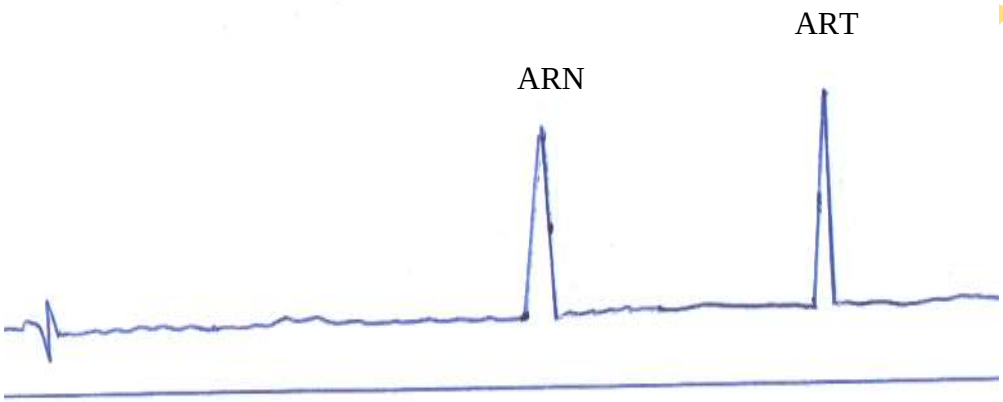


Figure 6.13: HPLC chromatograms of artesunate (ART) (pure sample in extracted spiked plasma) and internal standard (Artemisinin)- P.H adjusted to 4.0

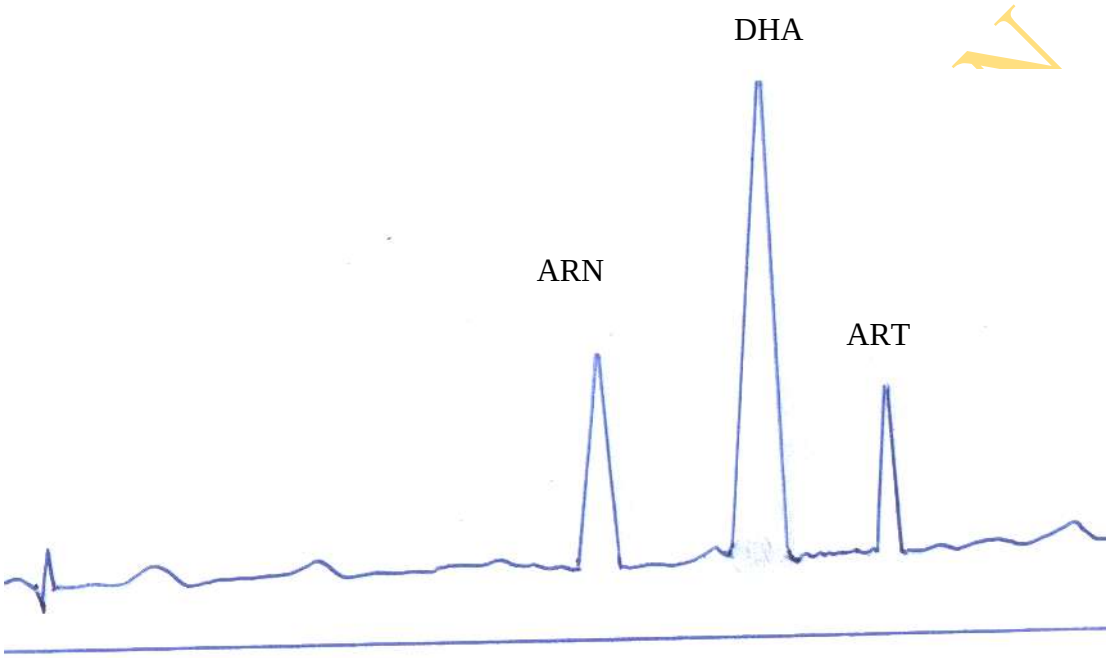


Figure 6.14: HPLC chromatograms of artesunate (ART) and Dihydroartemisinin (DHA) (experimental sample in extracted spiked plasma) and internal standard (Artemisinin)- P.H adjusted to 4.0

DEAQ

QUIN

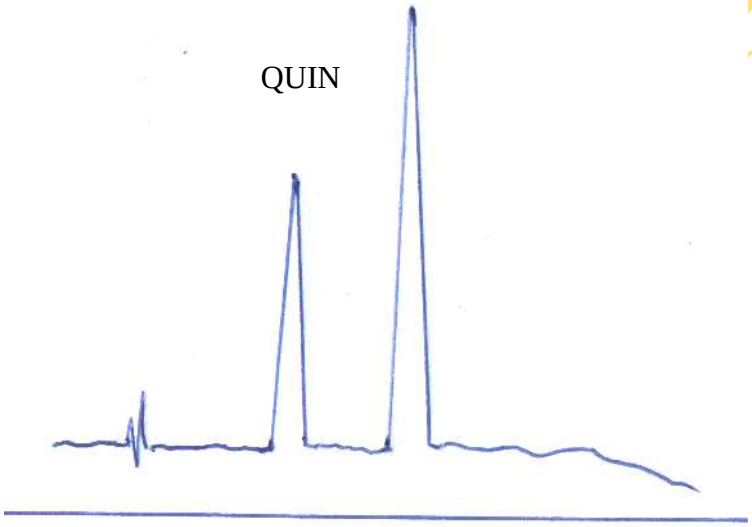


Figure 6.15: HPLC chromatograms of desethylamodiaquine (DEAQ) (pure sample in extracted spiked plasma) and internal standard (Quinidine)- P.H adjusted to 4.0

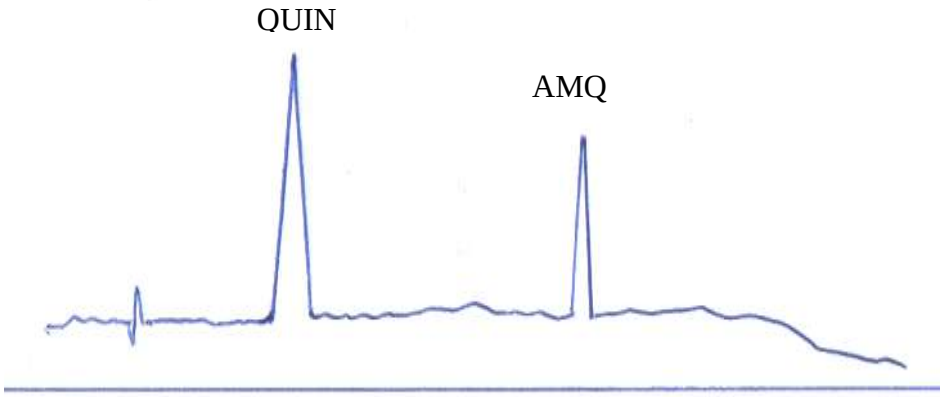


Figure 6.16: HPLC chromatograms of amodiaquine (AMQ) (pure sample in extracted spiked plasma) and internal standard (Quinidine)- P.H adjusted to 4.0

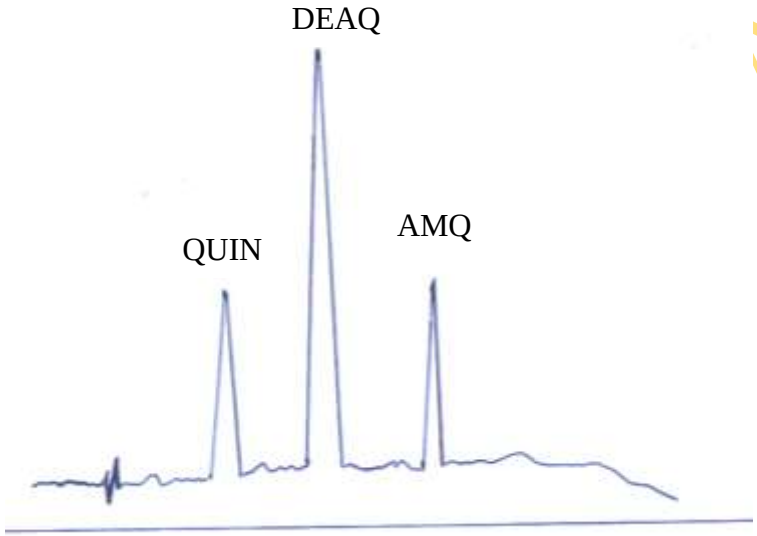


Figure 6.17: HPLC chromatograms of amodiaquine (AMQ) and desethylamodiaquine (DEAQ) (experimental sample in extracted spiked plasma) and internal standard (Quinidine)- P.H adjusted to 4.0

APPENDIX 3

Certificate of Consent

This form should be filled in duplicates. A copy of the signed form should be retained by the subject. If any subject who freely agrees to take part in this study gives verbal consent, the project staff who provided the information about this study may sign the form provided that he/she ensures that the subject has understood the information provided.

Every information you provide and data collected from you will be treated with absolute confidentiality. If you voluntarily agree to take part in this study, please sign below.

'I have read the foregoing information or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study and understand that I have the right to withdraw from the study at any time without in any way affecting my further medical care'

YES……. NO……..

A. Person Providing Information

Name: _____

Date: _____ Signature (or thumb print): _____

B. Witness of person providing information

Name: _____

Date: _____ Signature (or thumb print): _____

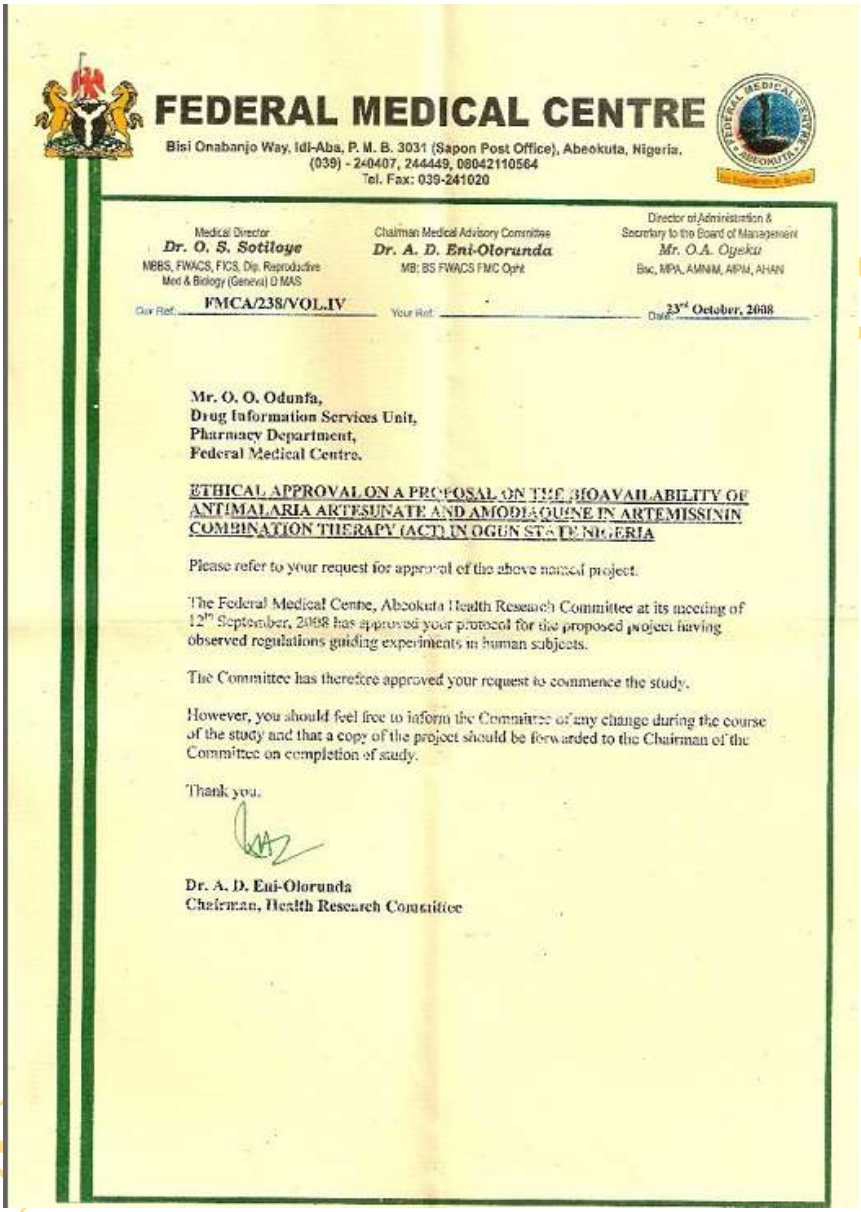
For Official Use Only

Name of project staff: _____

Study Site: _____

Assigned subject code: _____

APPENDIX 4



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Our Ref: FMCA/238/VOL.IV Your Ref: Date: 23rd October, 2008

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Drug Information Services Unit,
Pharmacy Department,
Federal Medical Centre.

ETHICAL APPROVAL ON A PROPOSAL ON THE BIOAVAILABILITY OF ANTIMALARIA ARTESUNATE AND AMODIAQUINE IN ARTEMISSININ COMBINATION THERAPY (ACT) IN OGUN STATE NIGERIA

Please refer to your request for approval of the above named project.

The Federal Medical Centre, Abeokuta Health Research Committee at its meeting of 12th September, 2008 has approved your protocol for the proposed project having observed regulations guiding experiments in human subjects.

The Committee has therefore approved your request to commence the study.

However, you should feel free to inform the Committee of any change during the course of the study and that a copy of the project should be forwarded to the Chairman of the Committee on completion of study.

Thank you.

Dr. A. D. Eni-Olorunda
Chairman, Health Research Committee