KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMACEUTICS

IN – VIVO BIOAVAILABILITY COMPARISON BETWEEN GENERIC AND INNOVATOR BRANDS: THE CASE OF ARTEMETHER-LUMEFANTRINE TABLET FORMULATION

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY IN PHARMACEUTICS

By

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DECLARATION

I, Acquah Francis Asiedu, hereby declare that the experimental work carried out in this thesis for Master of Philosophy degree at the Department of Pharmaceutics under the supervision of Dr. Noble Kuntworbe has not been submitted for any degree except for portions where references have been duly cited. This dissertation is the result of my research.

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ABSTRACT

Background: With the introduction of the artemisinin-based combination therapies (ACTs) due to issues with treatment failure with monotherapies, the treatment outcomes of malaria have been improved significantly. Globally, from 2001 to 2015 there has been huge reduction in the incidence of malaria cases and deaths. This has been mainly attributed to prevention strategies and treatment interventions with effective and potent ACTs. These control measures have been estimated to have forestalled about more than 650 million cases in the last 15 years. Locally, the main ACT of choice for the treatment of uncomplicated malaria has been Artemether-Lumefantrine with a good number of less costly generic brands being good options for clients who cannot afford the relatively expensive innovator brand; Coartem®.

In the Sub-Saharan region of Africa, where generic substitution is encouraged and several studies have reported the presence of substandard ACTs on the various retail markets; this study sought to compare the in-vivo bioavailabilities of a locally manufactured generic artemetherlumefantrine tablet formulation to that of the innovator brand.

Methodology: A pilot survey was carried out to identify and sample the most available locally manufactured Artemether-lumefantrine generic tablet formulation on the market together with the innovator brand. The authenticity and genuineness of the samples were confirmed with colorimetric and melting point tests. The pharmaceutical equivalence of the innovator and generic

brands was determined using compendial tests such as uniformity of weight, friability, percentage content and disintegration tests. In addition, in-vitro dissolution profiles were determined using the USP-2 apparatus. The release profiles were compared based on their fit factors.

A reverse phase HPLC/UV detection method was developed for the in-vivo bioavailability study. The in-vivo bioavailability study on the two tablet formulations was in the form of single dose, two-period, cross-over design involving 20 healthy rabbits. The pharmacokinetic parameters AUC_{0-72}, $AUC_{0-\infty}$, and C_{max} for both brands derived from the study were analysed statistically to check if the Food and Drugs Authority bioequivalence criterion has been satisfied.

Results: Both the generic and innovator brands of Artemether-lumefantrine that were sampled passed the test and satisfied the requirements for identification, uniformity of weight, friability, disintegration and percentage content. The artemether component of both brands complied with the pharmacopoeia specification for dissolution testing while the lumefantrine did not. An accurate RP-HPLC/UV detection method was developed, validated and use to quantify the lumefantrine levels in the plasma in the bioequivalence study.

After subjecting the pharmacokinetic parameters to the FDA bioequivalence criterion, average bioequivalence was demonstrated with the geometric mean ratios and corresponding 90% confidence intervals falling within the acceptable limits of 0.80 – 1.25.

Conclusion: Based on the similarity demonstrated between the two brands, evidence have been shown to support substitution in favour of the generic brand; as it would likely produce similar plasma drug levels and by inference similar therapeutic response as the innovator.

DEDICATION

This work is dedicated to my father, James Allan Acquah, and to my brothers, Francis and Evans.

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*But by the grace of God I am what I am, and his grace to me was not without effect. No, I worked harder than all of them — **YET NOT I, but the grace of God that was with me -1 Cor 15:10**.* I would like to express the deepest appreciation to my supervisor, Dr. Noble Kuntworbe who has the attitude and the substance of a genius; he continually and convincingly conveyed a spirit of adventure with regards to research and scholarship. His suggestions, constructive criticisms, reminders, fatherly love throughout this project was invaluable. God bless you.

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1.1 Introduction

1.1.1 Background of the study

Malaria is a parasitic disease endemic in parts of the world where moisture and warmth permit the disease vector, mosquitoes of the genus Anopheles, to exist and multiply. Malaria poses as a serious life – threating disease with almost half of the world‟s population at risk of malaria. As at December 2015, there were 214 million cases with 438,000 deaths (World Health Organization, 2015b). The most vulnerable groups of persons include infants and children less than 5 years, pregnant women, non - immune migrants from malaria - free countries and immunocompromised patients such as people living with HIV/AIDS.

A disproportionately high number of malaria cases and deaths occur in the Sub – Saharan region of Africa. As of 2015, this region reported 88% of cases and 90% of the malaria deaths. From 2000 to 2015, even though there has been a steady decline in the malaria incidence (about 53%), that of the sub – Saharan Africa has lagged behind (32%) (World Health Organization, 2015b).

In Ghana, malaria is a major cause of morbidity and mortality among pregnant women and children. In 2012, malaria accounted for 38.9% of all out-patient department cases and 38.8% of all admissions with the Upper West region recording the highest prevalence. Malaria‟s impact on endemic countries is the imposition of a growth penalty of over 1.2% of the gross domestic product (Ghana Health Service, 2014).

Initial presentation of malaria is non-specific fever, chills, rigors, diaphoresis, malaise, and vomiting.

Case management of malaria involves basically initiating timely antimalarial therapy to eradicate the infection within 48 to 72 hours and to avoid complications such as hypoglycaemia, pulmonary oedema, and renal failure that are responsible for increased mortality in malaria (World Health Organization, 2015b).

In the prevention of malaria transmission, vector control remains a major strategy for the health sector. The World Health Organisation recommends two main control interventions to offer protection for all persons especially those at risk of malaria. These include:

- The use of long – lasting insecticidal nets especially for people falling into the vulnerable groups.
- In – doors residual spraying with effective insecticide.

Even though some of the success story in the control of malaria has been through the preventive measures, early diagnosis and treatment has been the main stay of reducing transmission and preventing deaths. The World Health Organization in 2001 recommended the use of combination therapy (based on the additive potential of two or more drug) for malaria treatment mainly to improve therapeutic efficacy and also delay the development of resistance. Artemisinin-based combination therapy, using Artemether-Lumefantrine (AL) or Artesunate-Amodiaquine (AS/AQ), is currently considered as the first choice treatment for *Plasmodium falciparum* malaria in endemic areas (World Health Organization, 2015b).

Artemether and Lumefantrine are markedly different when it comes to their rate of absorption and elimination. When these two medicines are administered as an oral combination, these differences act complementary to buttress the efficacy of the drug‟s therapy.

Artemether is absorbed quickly, reaching an ultimate concentration at almost two hours after dosing. It is hydrolysed to its main active metabolite, dihydroartemisinin (DHA), which also shows

a maximum concentration within two to three hours after dosing. Both DHA and Artemether are very active anti-malarial agents that produce a decrease in asexual parasite mass, which is accompanied by a rapid resolution of symptoms such as fever. Conversely, the Lumefantrine constituent of AL is absorbed and cleared more gradually, eradicating the residual parasites that may linger after Artemether and DHA have been eliminated from the body and thus prevent recurrence.

Coartem® (Artemether 20 mg and Lumefantrine 120 mg), is a fixed-dose combination of the two antimalarial agents. It is a very effective three-day malaria therapy with cure rates of more than 95% even in places with multi-drug resistance. It is the prominent artemisinin-based combination treatment (ACT) for malaria worldwide (Makanga and Krudsood, 2009). In 2001, Coartem® was the first fixed dose artemisinin-based combination therapy to satisfy the World Health Organization‟s (WHO) pre-qualification criteria for efficacy, safety and quality (Makanga and Krudsood, 2009). Thus it is considered as the innovator brand for fixed-dose combination therapy for malaria. Nonetheless, over the past decade, a lot of pharmaceutical manufacturing companies have ventured into the manufacturing of generics of this fixed – dose combination. This has been necessitated mainly due to the rising healthcare cost and the massive savings associated with the use of generic medications. Its introduction aims at improving access to life saving drugs to patients. Because generic drug products are meant to be interchanged with an innovator brand, regulatory authorities require that they are pharmaceutically and therapeutically equivalent (bioequivalent). Even though regulatory authorities ensure bioequivalence before these products are given marketing authorisation it is required that this equivalence is assured throughout the shelf-life of the product. This can go a long way to assure prescribers and pharmacists that they

can safely substitute the expensive innovator brand with a less costly and an equally effective generic product.

1.2 Justification

Ghana has since 2004 changed its drug policy from the use of Chloroquine to ArtesunateAmodiaquine and Artemether-Lumefantrine as the first line drug treatment for management of uncomplicated malaria.

Artemether-Lumefantrine offers a very effective and acceptable alternative to ArtesunateAmodiaquine among prescribers whose patient cannot tolerate adverse events of ArtesunateAmodiaquine.

There have been some efforts and support by the Ministry of Health to support the local pharmaceutical manufacturing industry to build capacity and meet internationally established requirements of Good Manufactuirng Practices with respect to production of quality artemisininbased combination medicines.

The flux of substandard locally manufactured generic antimalarials with low bioavailability has been a major setback in the rapid clearance of parasites from blood and the fight against drug resistance (Dondorp *et al.*, 2004).

El-Duah and Ofori – Kwakye, (2012) studied the authenticity and quality of 14 brands of artemisinin – based antimalarials in some licensed retail pharmaceutical outlet in Ghana. The study revealed that about 90% of samples contained either less or higher amount of the specified drug making them to be of substandard quality (El-Duah and Ofori-Kwakye, 2012). Rani and Pargal (2004). reviewed that a lot of marketed drug products having dissimilar amount (or even sometimes similar amount) of drug displayed marked difference in their therapeutic effects. This

difference in response has been well attributed to dissimilar plasma levels mainly due to impaired absorption (Rani and Pargal, 2004).

Thus prescribers and pharmacists are placed in a quandary as to which generic to substitute with the innovator brand when patients can‟t afford the innovator. Coupled with the extensive use of ACTs, there is the need to monitor and ascertain evidence of bioequivalence between generics and innovator brands on the market for the purposes of generic substitution.

1.3 Aim

This study seeks to compare in - vivo bioavailabilities of one locally manufactured generic tablet formulation of Artemether-Lumefantrine (fixed-dose combination) with the innovator product Coartem® from Novartis, Basel-Switzerland.

1.4 Specific Objectives

- To sample the most available locally manufactured artemether-lumefantrine fixed – dose combination formulation.
- To perform quality control tests on the brands of artemether-lumefantrine tablets sampled according to compendia and non-compendia methods.
- To develop a Reverse–Phase High Performance Liquid Chromatography with Ultra – Violet detection method to simultaneously identify and quantify artemether and lumefantrine in plasma.
- To assay the various samples of artemether-lumefantrine to determine the percentage content using the developed HPLC method.

- To perform comparative in-vitro dissolution study of the sampled brands and determine the similarity of the dissolution profiles of the brands
- To perform a standard single dose cross–over bioavailability study on the generic and innovator brand using animal models (rabbits) and the developed RP – HPLC method to determine the pharmacokinetic parameters; (AUC_{0-72}), ($AUC_{0-\infty}$), (C_{max}) and T_{max}.
- To perform statistical analysis on the various pharmacokinetic parameters to ascertain whether the FDA bioequivalence criterion has been satisfied.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

2.1 Malaria

2.1.1 Overview of Malaria

Malaria is a major source of mortality and morbidity in children and adults in the sub-Saharan region of Africa especially in children below the age of five. It is a disease transmissible to persons of all ages affecting the blood.

Malaria results from an infection by a Plasmodium species which can be one of the following *Plasmodium ovale, Plasmodium malariae, Plasmodium vivax* and *Plasmodium falciparum.* These Plasmodium species spread from one person to another through the bite of a female Anopheline mosquito; as such they are described as human malaria species. The *Plasmodium falciparum* is responsible for majority of the clinical cases and deaths. However recent reports have revealed that some malaria infections be attributed to *Plasmodium knowlesi,* a species known to cause the infection amongst monkeys in Asia (Cox-Singh and Singh, 2008, World Health Organization, 2014).

The parasites mature within the gut of the vector to become sporozoites and are transmitted every time it takes a blood meal from a human. The infection begins with a mosquito bite during which some sporozoites are carried by the blood to the liver where they attack the cells and multiply asexually. Subsequently 9–16 days after, the merozoites emerge from the liver and infect the red blood cells. These merozoites furthermore get attached to the endothelium of the blood vessels, where they multiply again, increasingly breaking down the red blood cells. The infection gets transmitted further as gametocytes gets back into mosquitoes when the next mosquito bites and

the whole cycle follows. In cerebral malaria, the infested red cells occlude the blood vessels in the brain (Tigano, 1980).

The clinical manifestations of the malaria infection include fever, chills, prostration and anaemia. Severe disease can manifest as delirium, metabolic acidosis, cerebral malaria and multi-organ system failure (Rang, 2007).

2.1.2 Malaria Epidemiology

Malaria ranks as one of the most severe public health issues in the world. It poses a critical obstacle in the economic growth and development of nations, especially developing countries.

The spread of malaria happens in all the six WHO regions (World Health Organization, 2014). Nevertheless the rate at which the transmission occurs differs sharply in the different regions and countries in those regions. It is hugely reliant on influences such as human proximity to mosquito breeding sites, rainfall patterns, temperature, and type of mosquito species prevalent in that zone.

Roughly 90% of all the malaria mortality worldwide ensues in sub-Saharan region of Africa. One major reason for this is the fact that the culprit parasite in many of the infections in this region is *P. falciparum; P. falciparum* is the most virulent and dangerous of the four human malaria parasites (Carpenter *et al.*, 1991).

Furthermore as implied from above, the vector found in Africa; *Anopheles gambiae* is most ubiquitous and the most challenging to control, making it an active vector (Samba, 1997). Together with the poor living conditions such as inaccessibility of portable drinking water, countries in the sub-region are considered malaria-prone all through the year.

The 2014 World Malaria report estimated about 198 million malaria cases in 2013 and put about 1.2 billion at high risk. The disease ultimately led to the demise of 584,000 people. 90% of which

occurred in the WHO African Region. Children below five years accounted for 78% of all deaths (World Health Organization, 2014).

As there are 300 to 500 million clinical cases reported every year it is estimated that a child dies every 40 seconds as a result of malaria ensuing in a day-to-day loss of over 2000 young lives worldwide.

2.1.3 The burden of Malaria

Malaria imposes an enormous economic burden on countries which are endemic. As such malaria and poverty seems to be directly related (Sachs and Malaney, 2002). The world is still facing a surge in the disease burden despite substantial successful programmes both at global and national levels to break transmission. This increase has been ascribed to factors such as shifting agricultural practices including the construction of irrigation systems and dams, deforestation, immigration into endemic regions, the failing public health systems in some poor countries and issues of global warming and climate change (Sachs and Malaney, 2002, World Health Organization, 2010b).

Universally, the incidence of malaria is unevenly distributed. Its transmission pattern indicates a disease concentrated in the tropics. The macroclimate in such zones allows the viability of the life-cycle of the parasite in the vector. As the temperature drops the life-cycle period of the parasite rises and transmission rates reduces as in the temperate zones (Bruce-Chwatt, 1980,

Cohen, 1992).

It has been said that "where malaria prospers most humanity has prospered least". In addition to malaria‟s concentration in subtropical regions, poverty is also centred in such areas worldwide (Gallup and Sachs, 2001). This seems to suggest a two-way direct relation between malaria and poverty. When the average Gross Domestic Product (GDP) of malarious countries were compared

to non-malarious countries, it showed a five-fold difference in favour of nonmalarious countries and a higher economic growth rate (Gallup and Sachs, 2001).

A study in 1989 on the suppression of malaria transmission through source reduction showed that, poverty can be fairly associated with the intense transmission of the disease in the poorer nations. Continual socio-economic development and programmes such as improved housing (breaking contact among humans and mosquitoes), clearing of swamplands and indoor residual spraying of insecticides effectively eliminated malaria from some countries in the temperate region (Kitron and Spielman, 1989).

Nonetheless, as it will be pointed out later in other reports, economic development only, may not always be adequate for complete elimination of the disease. People residing in comparatively well-to-do households in endemic countries also tend to suffer from malaria with incidence which do not differ significantly from other households (Filmer, 2000). Moreover relatively wealthier nations with high year-round temperatures such as Oman and United Arab Emirates haven‟t been able to eliminate malaria (Filmer, 2000).

The impact of malaria on economic growth and development is a substantial economic burden of the disease. In endemic countries, the malaria burden is estimated to be a growth penalty of more than 1.2% of their GDP. Malaria and related-illnesses and mortality alone cost the Africa economy $12 billion annually (Asante and Asenso-Okyere, 2003).

A cross country regression analysis showed that countries with a higher proportion of their population in malaria endemic regions, had their annual economic growth rate being 1.3% less than other countries over the period from 1965 to 1990 (Gallup and Sachs, 2001). Ways by which malaria inflicts economic burden include:

- A direct impact on the human resource; Children of school going age may suffer effects which may result in school absenteeism. As school absenteeism increases, the failure rates tend to increase ensuing in repetition of school years with high dropout rates (Brooker *et al.*, 2000).
- Malaria‟s impact on foetal growth and development results mostly in low-birth weight neonates, which sometime results in 2 to 4 times more likely to experience failure in school (Leighton and Foster, 1993).
- Malaria (both complicated and uncomplicated) is a major cause of anaemi;. Anaemia has been revealed to cause direct physical effect lowering lowering worker productivity and output (Scholz *et al.*, 1997); (Basta *et al.*, 1979).

With regards to the trade and foreign direct investments (FDI) into sub-Saharan African, malaria has repressed the economic relationships between endemic and non-endemic regions. Investors from non-endemic regions sometimes tend to avoid malarious regions because of fears of contracting the disease. This has affected the agriculture, mining, manufacturing and the tourism industry.

2.1.4 Malaria burden in Ghana

In Ghana, malaria is projected to be the cause of the loss of over 10.6% Disability Adjusted Life Years (DALYs) equivalent to 6% of GDP annually (Asante and Asenso-Okyere, 2003).

The disease during pregnancy causes maternal anaemia and placental parasitaemia both of which are responsible for stillbirths and low birth weight babies. Approximately 16.8% of pregnant women admissions in 2012 were as a result of malaria with 3.4% deaths being reported.

Malaria accounted for 38.9% of all outpatient illnesses and 38.8% of all admissions in 2012. The average malaria parasite prevalence among children aged 6-59 months was 27.5% with regional disparities from a high of 51% in the Upper West region to a low of 4% in the Greater Accra region (Ghana Health Service, 2014); (Ghana Statistical Service, 2011).

2.2 Antimalarials

In endemic areas particularly the tropics, the single effective technique of averting the mortality and decreasing the morbidity caused by malaria is via the usage of potent antimalarial medicines (Olaniyi, 2005). In Ghana currently, the main strategic intervention for the country on malaria is case management through the Test, Treat, and Track (T3) initiative proposed by the World Health Organisation (WHO, 2012).

Chemotherapy with potent antimalarials remains the cornerstone of management of malaria.

Ideally good antimalarial should possess the characteristics listed below;

- Promptly relieve signs and symptoms of the disease.
- Harmless to the patient with no untoward adverse effects.
- Preferably destroying all plasmodium species of at all developmental stages (including the gametocytes)
- Economically affordable and easy to administer.

No one available agent can reliably effect a radical cure, i.e. eliminate both hepatic and erythrocytic forms of the parasite. Few available agents are casual prophylactic drugs which are capable of preventing red blood cell infection. However, all chemo-prophylactic drugs kill erythrocytic parasites before they can sufficiently increase in numbers to cause clinical symptoms.

Several classes of antimalarial drugs are available. They are classified on the basis of their main action on the stage of the life - cycle of the plasmodium parasite or according to their chemical structure.

2.2.1 Classes of antimalarials based on type of antimalarial activity

- Tissue schizonticides: These act on the pre or post erythrocytic forms. These drugs eliminate developing or dormant liver forms of the malaria parasite.
  - for casual prophylaxis e.g. Pyrimethamine and Primaquine
  - for preventing relapse e.g. Primaquine

- Blood schizonticides: These act on the asexual erythrocytic forms of all species of the plasmodium parasite. Such drugs are used for treatment and sometimes for suppression or prevention of clinical symptoms. Examples include Chloroquine, Quinine, Mefloquine, Halofantrine, and Lumefantrine

- Gametocides: They are most active on sexual forms of all species of malaria parasite and prevent transmission to mosquitoes. Examples include Chloroquine and Quinine

- Sporontocides: These inhibit the sporogenic phase of development of the malaria parasite preventing the development of oocysts in the mosquito and abort transmission. Examples include Primaquine and Chloroguanide (Bruce-Chwatt, 1962, Warhurst, 1987).

2.2.2 Classes of antimalarials based on their chemical structure and their examples

- Aryl-Amino Alcohols -

  e.g. Mefloquine, Quinine, Halofantrine, Quinidine, Lumefantrine.

- 4--aminoquinolines-

  e.g. Chloroquine, and Amodiaquine

- Folate synthesis inhibitors-

  e.g. Sulphonamides, Proguanil and Chloroproguanil

- 8-aminoquinolines-

  e.g. Primaquine

- Peroxides-

  e.g. Artemisinin derivatives and analogues

- Antimicrobials-

  e.g. Tetracyclines, Clindamycin, Azithromycin, Fluoroquinolones

- Naphthoquinones-

  e.g. Atovaquone

- Iron chelating agents-

  e.g. Desferrioxamine (Olaniyi, 2005)

2.2.4 Resistance to antimalarial drugs

Antimalarial drug resistance is defined as "the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a medicine given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject, provided drug exposure at site of action is adequate" (WHO, 2010). Antimalarial drug resistance is a result of parasite mutations that occur spontaneously and affect the structure and activity, at the molecular level, of the drug"s target in the parasite. Mutant parasites are subsequently selected if drug levels

are adequate enough to inhibit the multiplication of only susceptible parasites and not mutants (Peters, 1970, White, 1998).

The major and cardinal reason for the fall in the efficacy in antimalarial drug response is the emergence and spread of *P. falciparum parasite* resistance. Together with other drugs, especially antibiotics, resistance of pathogenic organisms poses a serious global threat.

2.2.4.1 Factors affecting spread of resistance

The factors which influence development and spread of antimalarial resistance are associated or related to human - host interactions, the parasite and the drug itself.

- Some of the Parasite-related factors include :
  1. Intensity of transmission
  2. Relative risk of drug-resistant parasites transmitting viable gametocytes.
- Drugs with long elimination half-life when used in areas of high malaria transmission may tend to encourage development and spread of resistance (Kitua, 1999, Watkins and Mosobo, 1993).

2.2.5 Chloroquine

In the late 20th century, the antimalarial of choice was chloroquine. It was safe, inexpensive and highly effective against susceptible plasmodium parasites.

Through the mechanisms above, chloroquine resistance was reported in Southeast Asia, South America and in Africa which resulted in a gradual decline in the efficacy of chloroquine. This contributed significantly to the high mortality and morbidity from malaria in sub-Saharan Africa (Trape *et al.*, 1998).

In 1986, chloroquine resistance was first reported in Ghana and this increased gradually to a 50% parasitology response in 2003 in certain areas of the country (Neequaye *et al.*, 1986).

Malaria endemic countries, which are mostly poor, need inexpensive and efficacious drugs. To counter the threat of resistance of *P. falciparum* to monotherapies, and to improve treatment outcomes, combinations of antimalarials were recommended by the WHO for the treatment of *P. falciparum* malaria (WHO Fact Sheet, 2010). The most important of the combination of antimalarials were the artemisinin - based combination therapies (ACTs) which combine artemisinin based antimalarials with other antimalarials such as the aryl amino alcohol anitmalarials. These combinations offer a new and potentially highly effective way to counter drug resistance (Atemnkeng *et al.*, 2007).

The WHO in its guidelines for malaria treatment recommends the use of ACTs for the treatment of uncomplicated malaria. The artemisinins produce rapid clearance of parasites and rapid resolution of symptoms and its rapid reduction of parasite numbers is significantly more than any other available antimalarial (Reyburn, 2010). Because they are eliminated rapidly as well, they are combined with slowly eliminated class of antimalarials so as to shorten the treatment course and ensure complete clearance of all parasites (Reyburn, 2010).

An efficacy study subsequently demonstrated similarity between the various Artemisinin-based combination therapies (ACTs). This subsequently informed drug policy change from the use of chloroquine to the adoption of ACTs (Ghana Health Service, 2014).

2.2.6 Current management of uncomplicated malaria

Comparative studies conducted on safety and efficacies of Artesunate – amodiaquine, Artemether – Lumefantrine and Dihydroartemisin – Piperaquine did not show marked difference between the

three artemisinin-based combination therapies. These three artemisinin-based combination therapies therefore still remain the medicines of choice for managing uncomplicated malaria.

According to the 2014 Ghana Antimalarial Drug Policy, adherence to testing before treatment and using the appropriate dosing is essential to ensure prompt and effective treatment. Artesunate-Amodiaquine combination is the medicine of choice for the treatment of uncomplicated malaria. The alternative medicines for uncomplicated malaria treatment are the endorsed doses and dosage forms of:

- Artemether-Lumefantrine
- Dihydroartemisinin-Piperaquine.

These supplementary ACTs are used for patients who are unable to tolerate Artesunate – Amodiaquine (Ghana Health Service, 2014).

2.2.7 Artemisinin-based combination therapy

Artemisinin which is also known as Quinghaosu (in the Chinese translation) is a compound which was isolated from the leafy portion of sweet wormwood, *Artemisia annua* (Chinese antipyretic herb) by Chinese scientists in 1971 (Klayman, 1985). The discovery report of Quinghaosu even though the plant was being used over 2000 years ago was delayed due to reasons such as security and anti-western culture of not encouraging publishing scientific data in western journals by the Chinese researchers. Also there was a huge language barrier as a lot of research papers on artemisinins were published in Chinese journals (Jianfang, 2013).

Artemisinin as an antimalarial class is a sesquiterpene lactone possessing a vital endoperoxide bridge critical for antimalarial activity. The Artemisia plant as reported by Kuhn and Wang

represents the commercial source of artemisinin when compared to other chemical synthetic procedures involving bioengineered microbes (Kuhn and Wang, 2007). The parent drug itself has poor solubility properties in aqueous and oil medium thus the carbonyl group of the drug (artemisinin) is reduced to Dihydroartemisinin (DHA; a reduced lactol derivative) and its other products such as Artesunate (aqueous-soluble), Arteether and Artemether(lipid-soluble) (Meshnick *et al.*, 1996b).

The Artemisinins present as one of the most potent group of antimalarials which is very effective against almost all the stages (sexual and asexual) of the parasite (Kumar and Zheng, 1990, Skinner *et al.*, 1996). They have a rapid onset of action by killing parasites rapidly by a reduction ratio of almost 10,000 per erythrocyte cycle (Woodrow *et al.*, 2005).

Recent research and trials demonstrate artemisinin being superior to quinine in treating severe malaria and in the current antimalarial drug policy, Quinine and Artesunate are employed in severe malaria treatment. (Ghana Health Service, 2014, PrayGod *et al.*, 2008).

The endoperoxide ring present in the artemisinin imparts some properties on this group of antimalarials; example of such properties is the rapid clearance of parasites from the blood resulting in the prevention of selection of resistant parasites. Moreover there is little or no crossresistance with other class antimalarials (Meshnick *et al.*, 1996b).

One limitation to the use of artemisinin is their short elimination half-life which is responsible for the high rates of recrudescence when artemisinins are used alone for short periods (Woodrow *et al.*, 2005).

Consequently a longer duration regime (7 days) is required to achieve 80-90% cure rates with artemisinin monotherapy (Meshnick *et al.*, 1996b, Woodrow *et al.*, 2005). Thus the WHO recommends Artemisinin-based combination therapy whereby one artemisinin is combined (sometimes in fixed doses) with a long-acting antimalarial, for example, Lumefantrine or Amodiaquine as the mainstay of malaria pharmacotherapy. This is to tackle the challenges of recrudescence, drug resistance and non-compliance (World Health Organization, 2014).

2.2.7.1 Artemisinin and its derivatives

Artemisinin is a crystalline insoluble compound. Its derivatives are structural modifications at the C-10 position to generate artemether, arteether, artesunate, artelinic acid. These analogs have improved solubility properties and can be easily formulated for oral, parenteral and rectal administration. The artemisinins are tolerated relatively better than other antimalarial classes. Side effects reported with the use of artemisinins are mainly nausea, vomiting, and diarrhoea. Their use in pregnancy is avoided due to teratogenicity in animal studies (Bertram, 2007, Brunton *et al.*, 2006).

2.2.7.1.1 Mechanism of action

Notwithstanding the extensive research conducted on the artemisinins, there is some considerable debate with respect to its mechanism of action (Krishna *et al.*, 2004, Olliaro *et al.*, 2001). The endoperoxide bridge forms a significant part of its pharmacophore as it is essential for antimalarial activity. Derivation by substitution of the peroxide oxygen with a carbon results in a derivative completely devoid of antimalarial activity. Hence this serves as the basis for the design and development of the second generation of artemisinins (Meshnick *et al.*, 1996a, Ploypradith, 2004).

The antimalarial activity is largely attributed to the generation of free radicals through the opening of the peroxide ring. This bioactivation as some studies show are hugely dependent on iron complexation usually present in heme. Stocks et al demonstrated that the activation of the artemisinins were significantly antagonized by iron chelators (Stocks *et al.*, 2007). Artemisininderived free radicals especially reactive oxygen species cause damage via alkylation to cellular macromolecules.

Most importantly is the alkylation of heme (a toxic substance) which is generated from haemoglobin digestion in the food vacuole of the parasite. This alkylation as many studies suggest inhibits heme polymerization to hemozoin which is non-toxic to the parasite leading to accumulation of toxic heme in the parasite (Kannan *et al.*, 2002, Loup *et al.*, 2007). Alternate mode of action from other studies suggest inhibition of the parasite sarcoplasmic endoplasmic reticulum calcium ATPase; SERCA (Eckstein-Ludwig *et al.*, 2003)

2.2.7.2 Artemether

2.2.7.2.1 Antimalaria activity

Artemether is a semi-synthetic ether derivative of artemisinin used as part of the combination treatment of *P.falciparum* malaria. It is the most commonly used and widely accepted artemisinin derivative. It is the derivative with high lipid solubility than artesunate and the parent drug artemisinin. Nonetheless it is not as effective as artesunate in the management of severe malaria (Eckstein-Ludwig *et al.*, 2003).

Some studies and meta-analysis on the safety of artemether in pregnancy showed that it is safe in pregnancy with fewer side effects (when used in combination with lumefantrine) than quinine (Dellicour *et al.*, 2007, Piola *et al.*, 2010). This contradicts the data on animal studies showing artemether use with foetal loss and deformity.

2.2.7.2.2 Anthelminthic actions

In addition to its antimalarial activity, artemether (as discovered by Chinese scientists in the 1980s) possesses broad spectrum anthelminthic activity. Keiser and Utzinger. (2007), reports of significant activity of artemether against the juvenile stages of *S. japanicum*, *S. mansoni*, *Clonorchis scnensis* and *Fasciola hepatica* (Keiser and Utzinger, 2007, Shuhua *et al.*, 2002).

2.2.7.2.3 Chemistry and Properties

Chemically, artemether is a methyl-ether derivative of artemisinin. Its chemical name is (+)-(3alpha,5a-beta,6-beta,8a-beta, 9-alpha, 12-beta,12aR)-decahydro-10-methoxy-3,6,9-trimethyl3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin. It is insoluble in water, freely soluble in dehydrated alcohol and ethyl acetate and very soluble in acetone and dichloromethane. It is assayed by HPLC/UV spectrophotometry and should contain not less than 99.0% and not more than equivalent of 102.0% of $C_6H_{26}O_5$ calculated with reference to the dried substance (World Health Organization, 2015a)

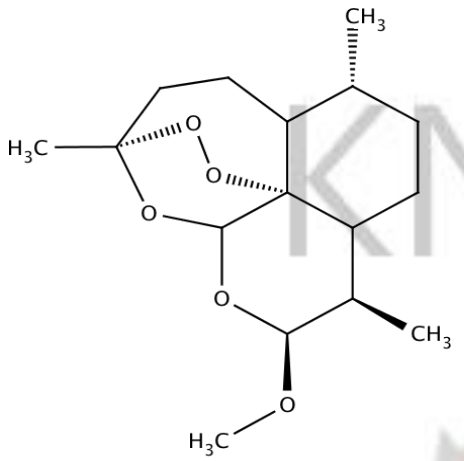

Figure 2.1 Chemical structure of Artemether

2.2.7.2.4 Pharmacokinetics of Artemether

Artemether after oral administration shows a peak plasma concentration around 2 - 3 hours and 6 hours with some erratic absorption after intramuscular injection (Ezzet *et al.*, 1998). It is metabolized via CYP_{450} to dihydroartemisinin, the active metabolite. Plasma protein binding is estimated to be around 95%, the elimination half-life following oral administration is approximately 1 hour and dose modifications are not necessary in renal and hepatic impairment.

2.2.8 Aryl-amino alcohol antimalarials

Aryl-amino alcohols are one of the most widely used groups of antimalarials since 1900s after the parent drug was obtained from the bark of *Cinchona spp*. They include quinine, quinidine, mefloquine, chloroquine, lumefantrine and halofantrine. Quinine, the major isolate from the

cinchona bark continues to be a main therapeutic alternative for management of severe and complicated *P. falciparum* malaria (Gilles, 1991).

Their antimalarial actions are hugely attributed to their interaction with heme produced as a result of haemoglobin (Pradines *et al.*, 1999).

2.2.8.1 Lumefantrine

Lumefantrine is also known as Benflumetol. It is a racemic fluorine derivative belonging to the aryl amino alcohol group of antimalarials as a result of its structure, physicochemical properties and mode of action (Pradines *et al.*, 1999) . It is used to treat acute uncomplicated malaria caused by chloroquine-resistant *P. falciparum.*

Chemically, lumefantrine is 2-(dibutylamino)-1-[(9Z)-2,7-dichloro-9-[(4-chlorophenyl) methylidene]fluoren-4-yl]ethanol. It is insoluble in water, soluble in dichloromethane; slightly soluble in methanol and freely soluble in ethyl acetate.

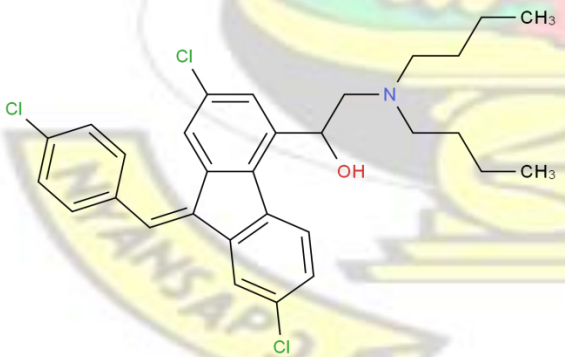

Figure 2.2 Chemical structure of Lumefantrine

According to the United States Pharmacopoeia (USP Salmous Standards), Lumefantrine can be assayed with non-aqueous titration and there should not be more than 102% of $C_6H_{13}C_{l3}NO$ calculated with reference to the dried substance (USP SALMOUS Standards, 2009).

Lumefantrine was developed and synthesized in Beijing, China in the 1970s by the Academy of Military medical Sciences. Studies by Basco et al suggested a high in-vitro activity of lumefantrine which is comparable to that of Mefloquine and Halofantrine (Basco *et al.*, 1998).

Lumefantrine oral bioavailability is dependent on its co-administration with food (fatty food) as it is increased 16 folds in the presence of the latter (Ezzet *et al.*, 1998). Nonetheless, lumefantrine exhibits variable absorption in malaria; slower onset of action and slowly eliminated with a halflife of 3 - 6 days with low recrudescence rate.

Lumefantrine is highly bound (99.7%) to serum protein in-vitro. After administration, peak plasma concentration occurs approximately after 6 hours. It is metabolized to desbutyllumefantrine which has a high antiparasitic effect (White *et al.*, 1999)

2.2.8.1.1 Toxicity

Lumefantrine is relatively safe and well tolerated. It does not share or possess the QT interval prolongation effect of halofantrine despite the pharmacokinetic and structural similarities. It does not show any significant serious adverse events (Van Vugt *et al.*, 1999). Most reported side effects are mild to moderate gastrointestinal tract effects and are quite indistinguishable from malaria

symptoms or other co-infections (Falade *et al.*, 2005, Vugt *et al.*, 1999). These include nausea, headaches, dizziness and abdominal discomfort.

2.2.9 Artemether-Lumefantrine combination treatment

According to the country‟s antimalarial drug policy, the first line treatment for uncomplicated malaria, is Artesunate-Amodiaquine yet more clinicians and patients prefer to opt for Artemether-Lumefantrine (2nd option) due to widely reported intolerable side effects which affect compliance. Consequently, artemether-lumefantrine predominate the Ghanaian market as the leading antimalarial with many generic formulations available (Ghana Health Service, 2014).

Artemether-Lumefantrine, a fixed- dose co-formulation is an artemisinin-based combination therapy with the two components complimenting each other. The combination is on the basis that Artemether is absorbed rapidly and its peak concentration as well as that for its active metabolite Dihydroartemesinin (DHA) occur at 2 hours after administration results in a quick reduction of parasitaemia and resolution of symptoms whiles Lumefantrine is absorbed and cleared slowly so tends to accumulate to prevent recrudescence by destroying any residual parasites post Artemether and DHA clearance.

Thus with successive 6-dose regimen, studies show a consistent efficacy of more than 95% with rapid parasite and symptoms clearance as well as preventing reinfections (Abdulla *et al.*, 2008, Makanga and Krudsood, 2009, Yeka *et al.*, 2008).

They are available as fixed dose conventional tablets, dispersible tablets or dry powers for reconstitution into suspension. The Artemether-Lumefantrine combination tablets was added to the Essential Medicines List of the World Health Organization in 2002 and this list guide most

procurement decisions by most developed and developing countries (World Health Organization, 2006a).

2.2.10 Coartem®

Coartem® is the brand name for Artemether /Lumefantrine fixed-dose combination drug manufactured by Novartis Pharmaceutical Corporation, Basel Switzerland. Coartem® received its international licensing approval since 1999 by the Swiss, but it became the first artemesinin/lumefantrine fixed- dose combination tablet to satisfy the WHO Pre-qualification criteria for safety, efficacy, and quality in 2004 (World Health Organization, 2010c). It subsequently received approval in 2009 by the USA Food and Drug Administration for the treatment of acute uncomplicated P. falciparum malaria infection. It was originally marketed as a 20mg/120mg fixed dose combination of Artemether-lumefantrine but due to issues of pill burden and patient compliance associated with it, Norvatis launched the first high strength artemisininbased combination therapy of 80/480mg, which has been pre-qualified by World Health Organisation.

2.2.10.1 Coartem® Dispersible

To address the challenge of inconvenience to patients and caregivers with respect to the administration of crushed standard Coartem® tablets to children, Novartis in partnership with Medicines for Malaria Venture developed a paediatric formulation specifically tailored to address the need of children, who form the most vulnerable group contributing significantly to the mortality and morbidity statistic due to malaria. In several randomised cross-over studies,

Abdulla et al showed no significant difference in the cure rates of groups receiving crushed Artemether/Lumether tablets and those receiving dispersible Artemether-Lumefantrine, which was well above 96%. Moreover coupled with its excellent safety, efficacy and tolerability, the dispersible Coartem® formulation possesses a sweet taste which makes them very palatable for easy administration and compliance (Center for Pharmaceutical Management, 2003).

2.2.10.2 Coartem® use as an innovator brand

The World Health Organization under its prequalification programme which ultimately seeks to make quality priority medicines available to those in need gives guidelines in the selection of an appropriate comparator product to be used to prove therapeutic bioequivalence of medicinal products.

The innovator product is ideally the first pharmaceutical product which first received marketing authorization. It is the most logical comparator product to be used by generic manufacturers to establish interchangeability and this utilization is based on the safety, efficacy and quality of the innovator product which has been fully assessed and documented in the pre-marketing studies as well as the post-marketing surveillance forms (World Health Organization, 2006b).

In the Prequalification of Medicines Program, WHO recommends some comparator products to be used to establish interchangeability. It recommends Coartem® or Riamet® by Novartis Pharmaceutical Company, as the comparator product to establish interchangeability with respect to Artemether-Lumefantrine fixed dose combination or co-packaged for paediatric formulation (World Health Organization, 2015c)

2.3 Generic Medicines

The WHO defines generic drug to be "a pharmaceutical product, usually intended to be interchanged with an innovator product that is manufactured without a license from the innovator company and marketed after the expiry date of the patent or other exclusive rights" (World Health Organization, 2010a). They are sometimes referred to as „multisource products" and are marketed under approved or non-proprietary names rather than a brand or proprietary name. Generics are comparable to an innovator or reference drug product in dosage form, route of administration, safety, strength, quality and intended use (Kanfer and Shargel, 2007).

After the expirations of the patent or exclusivity protection rights of innovator brands, generic brands which contain the same active pharmaceutical ingredients (API) as the innovator can be marketed upon approval by the regulatory bodies i.e. Food and Drug Authority (Meyer, 2000). The patent protection for a new drug is given to manufacturers to be sole marketers of the drug and recover the enormous cost incurred during drug development. The patent holders may waive their patent rights in some countries or jurisdiction so as to enable generic manufacturing.

2.3.1 Relevance of generics

Quality and approved generic medications are very important in the healthcare sector in every country. After the patent protection rights expire and the monopoly of the original manufacturers are removed, competition with respect to manufacturing and marketing is created. This obviously results in significant drop in the cost of the medicines in question. This competition in prices is not only as a result of competition between generic manufacturers and brand name companies but also among generic manufacturers themselves which drops the prices even further. This goes a long

way to make important life-saving drugs to be available to the patients who are unable to purchase innovator brands (Frank, 2007).

With the expiration of the patent of more than 100 drugs by 2010, of which contributed tens of billions of dollars to the healthcare budget, the introduction of their generic brands saved purchasers and customers of prescription medicines billions of dollars (Frank, 2007).

As of 2007, generic drugs accounted for 63% of all prescriptions for drugs in the United States, compared to 1984 when only 18.6% of the prescriptions were issued for generic drugs (Frank, 2007).

Approved generics deliver equivalent health benefits in terms of safety, quality and efficacy as innovator brand products at a cost significantly less to customers and purchasers.

In global campaigns for tackling infectious diseases especially HIV/AIDS, malaria and tuberculosis in developing countries funded by the Global fund and United Nations Children's Fund (UNICEF), generics by virtue of their affordability and availability offer a way out for the recent challenge of shrinking funding in these programs.

In assessing HIV/AIDS treatment programs, like United States Presidents Emergency Plan for AIDS Relief in several developing countries, Holmes et al showed that the use of generics resulted in saving over 300 million dollars of cost in just over 3 years through the use generic antiretroviral drugs. In such programs, the proportion of quality generics used has improved significantly in the last decade. This associated cost saving has allowed and improved access to antimalarials, anti-tuberculosis and antiretroviral therapy in sub-Saharan Africa. It has also allowed the program to shift funds from treatment budget and invest in other important activities such as training of healthcare workers and expansion of infrastructural and direct service provision to patients. The

savings from approved generic use and efficient programs have contributed to saving more lives at lower cost with no compromise in clinical outcomes (Holmes *et al.*, 2010).

Generics are cheaper principally because no research and development investments are involved in its manufacture as compared to innovator brands. Such manufacturers are not required to repeat the expensive clinical trials of the innovator drugs and pay less or nothing for advertisement, marketing and promotion. Moreover due to the competition among multiple generic manufacturers in the marketing of a single product, the prices of generics further drives down. Sometimes generics products can cost between 20%-80% less than that of the brand drug (Bera and Mukherjee, 2012).

2.3.2 Requirements for generics

Prior to its approval to be manufactured and marketed, a generic drug product is expected to meet some specific standards so as to provide a safe and effective low cost alternative to the innovator brand. These standards are established by the Food and Drugs Authority (Molzon, 1995). The whole approval process for generic products is captured under the Abbreviated New Drug Application (ANDA). This description is as a result of the fact that generic manufacturers are not usually required to present pre-clinical and clinical safety and efficacy data as part of the approval process. They just have to demonstrate empirically that the generic in question produces the same result as the already approved innovator brand (Molzon, 1995) i.e. they should be therapeutically equivalent. The specific requirements for generic approval include that the generic drug should:

i. be pharmaceutically equivalent to the innovator product or the approved effective and safe reference product such that it;

a. contains similar amounts of the active drug ingredients in the same dosage form and administration route

b. satisfy laid down and compendial standards in terms of purity, identity, strength and quality.

ii. bioequivalent to the innovator or reference product in that;

a. it will not present a potential bioequivalence issue and it meets an acceptable invitro dissolution testing or

b. it has shown to meet accepted and appropriate bioequivalence standards.

iii. Is appropriately and adequately labelled and iv. Manufactured in compliance with latest acceptable Good Manufacturing Practice.

(Food & Drug Administration, 2010).

2.4 Bioavailability studies

This is an important concept in bio-pharmaceutics. It describes the rate and extent to which an active ingredient in the dosage form is absorbed and becomes available at the site of action (Martin *et al.*, 2012). The amount of drug at the site of action i.e. tissues is assumed to be in equilibrium with the amount present in blood. Thus in determining the bioavailability of drug substances, the concentration of the drug is determined in blood or urine where most drug molecules are excreted. This equilibrium assumption rather than equal amount is very important in bioequivalent studies because even though the drug concentration may not be the equal at the tissue site and in blood, the concentration at the site is a proportion of the blood drug concentration (Martin *et al.*, 2012). Thus, because pharmacological activity is directly related to drug concentration at tissue site,

monitoring blood/plasma drug levels can give an indirect measure of the drug response or activity (Allen and Ansel, 2013). Ultimately, the efficacy of a drug is hugely dependent on how bioavailable it is.

2.4.1 Aspects of bioavailability

Bioavailability encompasses the onset, intensity and duration of the therapeutic response and efficacy of a drug substance, after a specified dose is administered. In assessing the bioavailability of a drug;

- The extent of absorption which is also the actual fraction of the drug reaching the blood stream (effective dose) is measured. It is generally a lesser amount of the administered dose of the drug.
- The rate of absorption is always important especially in acute conditions where a rather quick onset of therapeutic action of the drugs is required. Conversely, in circumstances in which adverse effects or prolonged duration of action is desired, a slower absorption rate may be optimum (Martin *et al.*, 2012).

2.4.2 Representation of bioavailability data

Bioavailability of an administered drug is graphically represented by a concentration-time curve of the drug in an appropriate tissue system e.g. Plasma, serum or urine. Data from such bioavailability representation include:

1. The fraction or proportion of the active drug absorbed from the dosage form and eventually reaching systemic circulation.
2. The rate (slow or fast) at which the drug was absorbed.

3. The duration or period of the drug‟s presence in the biological fluid or tissue, its correlation with patient response and the relationship between plasma drug levels and clinical efficacy of the drug and possible toxicity (Allen and Ansel, 2013).

2.4.3 Usefulness of bioavailability studies

Bioavailability assessment studies are quite important in several aspects of biopharmaceutics. These include the following:

- Comparison of different formulations of the same drug substance during product development stages of the drug. This is normally done so as to determine the formulation which shows desirable and optimal absorption characteristics.
- In pharmaceutical manufacturing, where different batches of the same drug are formulated, bioavailability studies can be used to check or compare the consistency of the availability of the drug substance across the batches.
- Amongst different dosage forms (capsules, suspensions and tablets) of the same drug substance, bioavailability studies can be used to compare availabilities of the drug substance.
- Comparison of bioavailability can also be useful in comparing drug substances in the same dosage form produced by different companies (Allen and Ansel, 2013).

2.4.4 Blood/Plasma concentration- time curves

Blood sampling at several pre-determined intervals following administration of solid oral dosage forms of a drug (example tablets) to determine drug concentration serves as the source for bioavailability studies. The data consequently obtained can be represented or plotted on a graph

sheet with the plasma drug concentration on the vertical axis and the time interval for sampling represented on the horizontal axis. This yields a curve shown in Fig 2.3 below.

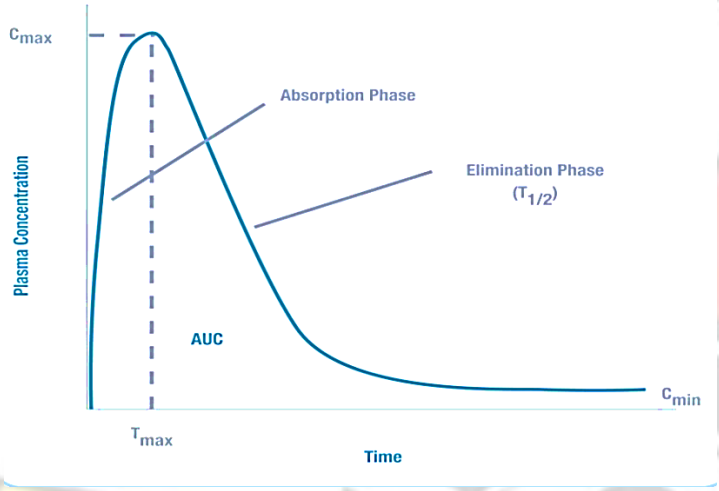

Figure 2.3.Typical Plasma – Concentration Time Curve following the oral administration of a single dose of a tablet (Allen and Ansel, 2013)

The first portion of the curve showing ascendency represents the absorption phase of the drug. Post administration of the dosage form (tablet), it has to disintegrate and dissolve before absorption starts. Thus at pre-dose time (zero time) the concentration of drug in plasma will be zero, then after administration, the plasma drug level rises steadily during this absorption phase. Even though distribution and elimination of the drug also takes place at this phase, they are superseded by the rate of absorption. At a specific period without successive doses, the highest concentration of the drug is obtained. This maximum concentration (C_{max}) signifies the end of the absorptive phase and represents equilibrium between the rate of the drug‟s appearance in plasma and the rate of its removal via distribution into tissues and elimination. After the C_{max} is attained, begins the descending arm of the plasma – concentration/time curve i.e. the elimination phase. Converse to

the absorptive phase the concentration of the drug in plasma declines because it rate of removal exceeds the rate of absorption. When the entire bioavailable dose has been absorbed, the absorption ceases and plasma drug concentration is controlled principally by the rate of elimination via metabolism and excretion (Allen and Ansel, 2013, Martin *et al.*, 2012)

2.4.4.1 Parameters for comparison and assessment of bioavailability

In bioequivalent studies, following the oral administration of a single dose of two or three different formulations of the same drug several parameters are utilized for comparison. They are C_{max}, T_{max} and Area Under the Curve (AUC).

1. Peak Height Concentration (C_{max}): This is the highest concentration observed in the plasma post dose. Ideally, for a conventional dosage form with single dosing, the Cmax usually occurs at a single point or time which also is the T_{max}. It is normally expressed in relation to a specific volume of the plasma. Example is mg/ml, µg/ml, µg/100ml, g/100ml. The value of the C_{max} is very important in that, it has to exceed the threshold concentration of the drug substance required for an adequate response to be exhibited. This threshold concentration is referred to as the minimum effective concentration (MEC). Moreover because toxicity is also related to the dose, the C_{max} should ideally not exceed the minimum toxic concentration (MTC) beyond which toxic effects are observed. Thus ultimately, the final objective in individual dosing is to achieve the MEC but not the MTC.

   One major factor affecting peak height concentration of a drug is the administered dose, thus for a drug of a particular dosage form, showing complete absorption and elimination, as the dose increases, the C_{max} increases proportionally however at the same T_{max} (Allen and Ansel, 2013).

2. Time of Peak Concentration (T_{max}) : This is the measurement of length of time necessary to attain the maximum concentration post drug administration. It gives an estimate or reflection of the rate of absorption of the drug substance from the dosage form. It is very important when in the comparison of different formulations, one is interested in the onset of action and duration of action of the formulation (Remington *et al.,* 2006). If the rate of absorption decreases, C_{max} would be lowered and will occur (T_{max}) at a later time.

3. Area Under the concentration-time Curve (AUC): This parameter is normally taken as the most important parameter in the comparison of bioavailabilities. This is hugely because the AUC value is considered representative of the total amount of the drug that is absorbed after administration of a single dose. It gives the total exposure of the body to the drug over a definite time period. The higher the AUC, the greater the drug absorbed and reaching circulation and vice versa. In bioavailability, the AUC represents the extent of drug absorbed systemically.

2.4.4.2 Usefulness of area under the concentration-time curve (AUC)

AUC is a critical parameter utilized in bioavailability or bioequivalence studies. The AUC of two different formulations of the same drug (administered in equivalent dose) can be compared to know whether they are equivalent. However other parameters should be included and factored in when establishing bioequivalence (Remington *et al.*, 2006).

AUC values can also be helpful in therapeutic drug monitoring of toxic medicines and it subsequent dosing. This is especially done for drugs with narrow therapeutic window.

It is also useful in determining the amount of drug eliminated. The area under the plasma concentration – time curve is calculated and reported in amount/volume time e.g. µg/ml×hr or g/100ml×hr.

2.4.4.3 Methods for calculating AUC

 Physical Methods

1. Cut and Weigh: With this method the curve is plotted, cut out and weighed on an electronic balance and the resultant weight is considered the AUC.
2. The use of an instrument, Planimeter to measure the graph and determine the AUC.

 Trapezoidal method: The trapezoidal rule is the most common used method for the determination of AUC. In this method, the curve plotted on an ordinary Cartesian graph is divided into several trapezoids at the times of sampling. The areas of the trapezoids are calculated and summed to obtain overall AUC;

$$\text{Area} = \frac{1}{2}(C_{n-1} + C_n)\cdot(t_n + t_{n-1})$$, where C is the concentration at nth sampling time t.

It assumes a linear function and the accuracy is enhanced as the number of appropriate sampling intervals and trapezoids increase (Allison *et al.*, 1995, Remington *et al.*, 2006).

2.4.5 Absolute and Relative bioavailability

Absolute bioavailability (represented by F) of a drug is the fraction of an administered dose that is absorbed intact and reaches systemic circulation. It is calculated by comparing the total amount of intact drug reaching systemic circulation after a known dose of a formulation is administered via an administration route to the total amount of drug reaching systemic circulation after an equivalent

dose of the same drug is administered via the intravenous (I.V) route. The IV route bolus injection is used as a reference which a drug"s bioavailability via a different route is compared with because with the IV route, there are no absorption barriers and the intact administered dose is introduced directly into systemic circulation and it becomes totally bioavailable.

Typical plasma concentration – time curves obtained by administering equivalent doses of the same drug by the IV route and per oral route are shown in fig 2.4

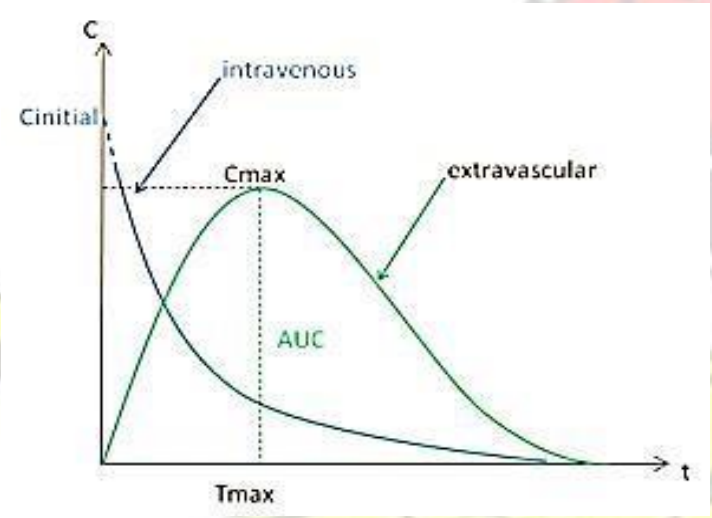

Figure 2.4. Typical Plasma – Concentration Time Curve following the oral administration of a single dose of the same drug by the IV route and per oral route (Aulton and Taylor, 2013)

Mathematically, for equivalent doses of administered drug;

$$\text{Absolute bioavailability} = \frac{[AUC]_{abs}}{[AUC]_{iv}} \quad \text{---------- Equation 1}$$

Where $[AUC]_{abs}$ is the total area under plasma concentration – time curve following administration of a single dose via an absorption site and $[AUC]_{iv}$ is the total area under the plasma concentration – time curve following administration by rapid IV injection.

Most often it becomes vital to use dissimilar dosages of drug via different routes e.g. a lower dose through the IV route so as to minimize toxic unwanted effects and also overcome formulation challenges. In such cases, when different doses are administered through both routes, the different doses are factored in the equation to correct the dose sizes i.e.

$$\text{Absolute bioavailability} = \frac{[AUC]_{abs} / D_{abs}}{[AUC]_{iv} / D_{iv}} \quad \text{---------- Equation 2}$$

D_{abs} = dose of drug administered via absorption site, D_{iv} = dose of drug administered as IV bolus.

2.4.5.1 Relevance of Absolute bioavailability

When a simple aqueous solution of a drug is formulated and administered by the oral and by the IV route, the absolute bioavailability is measured with respect to the oral route. This can provide a good insight into the effects that various factors which are associated with the oral route may have on bioavailability such as drug stability in the gastrointestinal fluid, pre – systemic metabolism by intestinal or liver enzymes, the formation of complexes between the drug and endogenous substances at the site of absorption.

Nonetheless, calculation, analysis and validity of absolute bioavailability values can only be assured for a drug in question if the kinetics of elimination and administration are independent of the route and time of administration and sizes of doses administered (Aulton and Taylor, 2013).

2.4.5.2 Relative bioavailability

For some drugs which cannot be administered via the intravenous route as a bolus injection, the absolute bioavailability cannot be determined using the equation 1. A comparative or relative bioavailability is determined instead of absolute bioavailability. This bioavailability of the drug in

the „test" dosage form is compared to that of the same drug administered in the „standard" dosage form. The standard dosage form in this context is ideally a recognized formulation of that drug that is clinically proven and effective or is known to be well absorbed. This standard dosage form is often an orally administered solution. Mathematically, the relative bioavailability of a given drug administered at equal doses of a test dosage form and a standard dosage form respectively by the same route of administration to the same subject in different occasions may be calculated as;

$$\text{Relative bioavailability} = \frac{[AUC]_{test}}{[AUC]_{standard}} \quad \text{---------- Equation 3}$$

Where $[AUC]_{test}$ and $[AUC]_{standard}$ are the total areas under the concentration – time curves following administration of a single dose of the test dosage form and the standard dosage form respectively. In cases when different doses are administered for both test and standard dosage forms the relation is corrected as follows:

$$\text{Relative bioavailability} = \frac{[AUC]_{test} / D_{test}}{[AUC]_{standard} / D_{standard}} \quad \text{---------- Equation 4}$$

Where $D_{standard}$ and D_{test} are the doses of the standard and test dosage forms respectively.

2.4.6 Urinary drug excretion curves in bioavailability studies

Under certain circumstances, blood or plasma samples cannot be collected because:

- It may be invasive to collect repeated blood samples from certain patient populations such as paediatrics.
- The apparent volume of distribution may be so large such that plasma concentrations are too low to be evaluated and quantified.
- There is lack of sufficiently sensitive analysis techniques for plasma drug concentration.

Such conditions may require the use of urinary excretion data for the bioavailability or pharmacokinetics studies. In such studies, data on the amount of unchanged drug excreted into urine is collected. However, to utilise this indirect approach, the drug must be excreted in significant quantifiable amount as unchanged in the urine. The cumulative urinary drug amount has direct relation to the drug absorbed as is shown in Figure 2.5 below.

As the plasma concentration rises and approaches zero, the drug is almost eliminated and the maximum amount of drug excreted in the urine is obtained. The data for amount of unchanged drug excreted is collected.

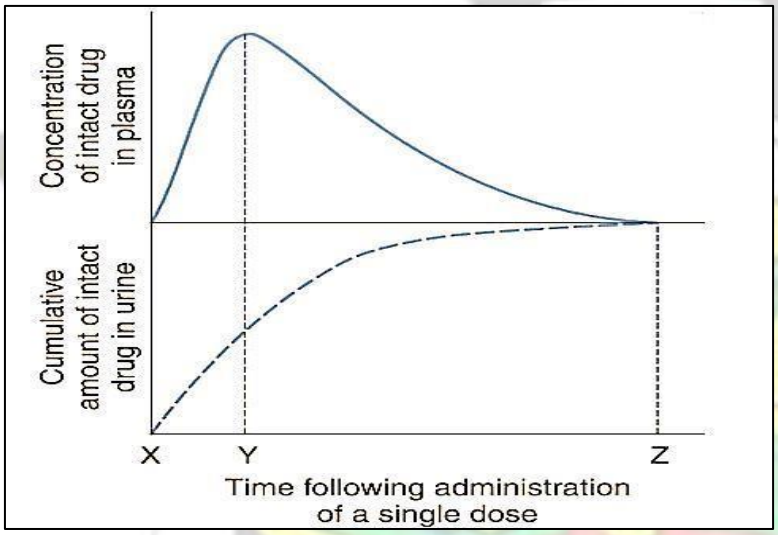

Figure 2.5 Corresponding plots showing the plasma concentration-time curve (upper curve) and the cumulative urinary excretion curve (lower curve) obtained following the administration of a single dose of a drug by the per-oral route.

Sometimes when a one – compartment model analysis is applied in this study, the overall elimination may take two parallel pathways. The fraction of the administered dose excreted in

1. unmetabolised form denoted by a rate constant, Ke and
2. metabolised form defined by Km.

Thus the overall elimination rate K_{el} encompasses K_e and Km; $K_{el} = K_e + K_m$.

The fraction of the administered dose excreted in unmetabolized form, fe is expressed as $\frac{Ke}{Kel}$.

Albeit, there are other routes of elimination which are considered shadow metabolism such as bile, sweat and not included in the analysis (Shargel *et al.*, 2007)

2.5 Bioequivalence

Another aspect of relative bioavailability comparisons is the comparison of drug products from different or competing manufacturers. When a particular drug is formulated into different formulations or dosage forms, they will exhibit different bioavailabilities (Remington *et al.*, 2006). This is due to the fact that the extent and rate of the drug‟s absorption from the various formulations and dosage forms hugely is dependent on the method of manufacture and the materials utilised in the formulation.

This concept of comparison whereby a test dosage form is compared to a standard dosage form is bioequivalence (Aulton and Taylor, 2013).

According to the United States, Food & Drug Authority Guidance for Industry, Bioequivalence is defined as „the absence of a significant difference in the rate and extent to which the active ingredient in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study (US Food and Drug Administration, 2010).

2.5.1 Definition of terms

There are different levels of equivalence that the FDA uses in comparing drug products.

2.5.1.1 Pharmaceutical Equivalents

These are drug products containing identical active pharmaceutical ingredient (API) (amount and chemical form, same salt or ester of the API) in identical dosage forms but not necessarily containing the same inactive ingredients. These pharmaceutical equivalents are required to meet the identical compendia and other applicable standards of identity, strength, quality, purity and potency. It should meet applicable content uniformity, disintegration times and dissolution rates (US Food and Drug Administration, 2010).

2.5.1.2 Pharmaceutical Alternatives

These are drug products containing an identical therapeutic molecule or a prodrug or precursor but not necessarily in the same amount or dosage form or as the same ester or salt. Such alternatives meet either their own compendial or identical applicable standard of purity, potency, content uniformity, disintegration times, and dissolution rates (US Food and Drug Administration, 2010).

Thus two products are considered bioequivalent when the rate and extent of absorption of the generic drug does not show a significant difference from the rate and extent of absorption of the brand drug, when administered at the same dose under similar experimental conditions.

2.5.2 Therapeutic Equivalence

Over the past decades, it has become somehow evident that drug products containing the same amount of the same drug molecule and marketed as generics exhibit marked differences in their responses therapeutically.

These marked differences have been in most cases as reviewed by Rani and Pargal. (2004), is largely related to dissimilar drug plasma levels as a result of impaired absorption (Rani and Pargal, 2004).

Prescribers thus in issuing prescriptions for generics are sometimes sceptical as to whether every one of the several available products will be equivalent therapeutically to the innovator brand.

The guidelines of the Committee for Proprietary Medicinal Products (CPMP) on bioavailability and bioequivalence describes a medicinal product as being therapeutically equivalent with another product if it contains the same active substance or molecules and clinically shows the same efficacy and safety (Committee for Medicinal Products for Human Use, 2010).

The practical studies that are necessary to have that confidence that a generic drug product will be therapeutically equi-effective to an innovator product is a standard bioequivalence study conducted in a cross – over design (Hauschke *et al.*, 2007).

2.5.3 Requirements for bioequivalence

Regulatory bodies such as the FDA and European Medicines Evaluation Agency (EMEA) prior to generic drug approval generally are required by regulations to ensure manufacturers submit data and evidence demonstrating bioequivalence between their product and the pioneer or innovator. Specific applications requiring bioequivalence studies include:

- Application for products containing approved active substances; these bioequivalence studies serve as bridging studies between pivotal clinical trial formulations and the to-be marketed medicinal product.
- Application for products containing approved active substances intended to be a generic. Such applications require in-vivo bioequivalence studies when there is some risk that possible bioavailability difference can result in therapeutic inequivalence.
- Application for products containing a known ingredient not intended to be generic requires bioequivalence studies. This is because when specification of the dosage form, its composition or manufacturing process is altered the new product must be demonstrated to be bioequivalent to the product with which the clinical trials were made and to which reference is given in terms of efficacy and safety.

  When the route of administration is changed, bioequivalence studies between a reference route must also be demonstrated (Food & Drug Administration, 2010)

2.5.4 Criteria to establish bioequivalence

Regulators of the pharmaceutical industry have laid down legislations and regulations that will allow for the approval of generic products.

This approval happens on the basis of the demonstration of bioequivalence with an innovator product. Generally, it is required that for bioequivalence of a generic drug product with an innovator product, any difference in the rate and extent to which the active moiety becomes available at the site of action is not clinically important. For systemically acting products, pharmacokinetic measurements are the most commonly and widely accepted approach for establishing bioequivalence (Nation and Sansom, 1994).

According to the Ghana Food and Drugs Authority guidelines for conducting bioequivalence study, the statistical analysis should be based on non-compartmental pharmacokinetic parameters, Area under the curve from 0 to last measurable time point (AUC_{0-t}), Area under the curve from 0 to infinity ($AUC_{0-\infty}$) and maximum concentration (C_{max}) derived from the drug concentration time curve (Food and Drugs Authority, 2013). These parameters are viewed as the variables which provide the most accurate or best estimation of the rate and extent of drug absorption and systemic exposure (Verbeeck and Musuamba, 2012).

The criteria for bioequivalence as stipulated by the regulatory authorities is that for these parameters mentioned above the 90% confidence interval for the ratio of the test and reference drug products, should fall within an acceptable interval of 0.80 – 1.25. To satisfy this confidence interval, the lower boundary should be ≥ 0.80 when rounded to 2 decimal places and the upper boundary should be ≤ 1.25 when rounded to 2 decimal places (Food and Drugs Authority, 2013).

This acceptance interval in spite of this, changes according to the type of drug. More stringent limits are normally required for drugs with narrow therapeutic index such as antiepileptics and cardiac glycosides (Besag, 2000). This is so because for such drugs a relatively small change in systemic concentration can go a long way to elicit profound or marked changes in pharmacodynamic response, such as reduced efficacy or the occurrence of serious adverse effects (Besag, 2000, Meredith, 2003). Bioequivalence assessment is based upon 90% confidence intervals for the ratio of the population geometric mean (test/reference) for the parameters under consideration.

This assessment and analysis is equivalent to two sided tests with the null hypothesis of bio - inequivalence at a 5% significance level (Food and Drugs Authority, 2013).

The 0.80 – 1.25 interval is based on the fact that according to the authorities a difference of up to 20% in the systemic drug exposure is not clinically significant, and because the pharmacokinetic parameters are log-normally distributed, a symmetric distribution ratios or differences around the 100% on natural log transformed ratio would be ± 0.223. Thus, the upper limit corresponds to 0.25 and the lower limit 0.80 as shown in the table below (Hauschke *et al.*, 2007).

Table 2.1 The various ratios and their corresponding log-transformed ratios

Test	Reference	Ratio	Percentage	ln(Ratio)
0.8	1.0	0.8	80%	-0.223
0.9	1.0	0.9	90%	-0.105
1.0	1.0	1.0	100%	0
1.1	1.0	1.1	110%	0.095
1.2	1.0	1.2	120%	0.182
1.25	1.0	1.25	125%	0.223

2.5.5 Various Study Designs

Generally in the comparison of the bioavailabilities of a test product and reference product, the study should be designed in a manner that the formulation effect can be distinguished from other effects.

2.5.5.1 Standard design

In this study, a randomised two-period, two-sequence single dose crossover design is recommended. The treatment period should be sufficient to ensure that drug concentrations are below the lower limit of bio-analytical quantification in all subjects at the start of the second period. To achieve this essential wash out period a minimum of five elimination half-lives is recommended (Food and Drugs Authority, 2013).

This standard study design is usually preferred over other designs since each subject receives both test and reference product leading to within- subject comparison.

2.5.5.2 Parallel design

There are alternate designs which may be considered under certain circumstances. They are accepted if the overall design and its statistical analysis are scientifically sound and validated. For drugs with extremely long half–lives, parallel designs are recommended. Each subject is administered a test or reference product.

2.5.5.2 Replicate design

Replicate study designs are considered for drug substances that are with highly variable pharmacokinetic characteristics (Food and Drugs Authority, 2013).

2.5.5.3 Single and Multiple – dose Studies

Generally most study designs are single-dose and such studies are sufficient to demonstrate bioequivalence. On the other hand, a multiple dose study in subjects is acceptable if a single dose

study cannot be conducted in healthy volunteers due to tolerability reasons. In situations where the sensitivity of the analytical procedures rules out precise plasma concentration measurements after a single dose administration, a multiple dose study can offer an alternative route if the concentration at steady state is sufficiently high to be reliably quantified (Hauschke *et al.*, 2007).

2.6 Review of analytical methods

Since its isolation, development and adoption by the WHO, artemether-lumefantrine has been of outstanding importance in the fight against malaria over the past decades (Haynes, 2001). Results from research all over the world especially those from China have provided some comprehensive understanding of the toxicology, chemistry, pharmacological profiles, metabolism and effects on the malaria parasite. There have been some documented methods for the assay of artemether and lumefantrine as separate products and as combination formulation.

Monographs for both artemether and lumefantrine are not present in the British Pharmacopoeia. The International Pharmacopoeia 2015 contains monographs of artemether injections, tablets, capsule and a recent addition of a comprehensive monograph for artemether-lumefantrine fixed combination tablets (World Health Organization, 2015a).

There have also been some papers published on the simultaneous assay of artemetherlumefantrine in tablets and also in plasma. Some of the methods have been reviewed in the next paragraph.

2.6.1 Ultra – violet (UV) spectrophotometric analysis of Artemether-Lumefantrine

Available published UV spectrophotometric assay methods make use of its hydrochloride degradation product, an α, β – unsaturated decalone which absorbs at a 254nm wavelength (Thomas *et al.*, 1992). The above procedure together with other assay methods developed later by

Shrivastava et al. (2008), and described in the International Pharmacopoeia (IP) requires vigorous conditions for its formation. It requires the addition of 1M ethanolic HCl solution to an aliquot of the artemether in ethanol solution followed by heating for long periods. This makes them very uneconomical and time-consuming (Shrivastava *et al.*, 2008).

Green et al also developed an assay method for artemether involving an hour long reaction of the acid – decomposition product from the artemisinin with a dye to yield a coloured product which absorbs at 420nm (Green *et al.*, 2001).

Lumefantrine chemically possesses strongly absorbing chromophores which makes it a very good candidate for assay by the UV spectrophometer. However it has not been well explored at least in the compendial. Its monograph in the pharmacopoeia does not make use of its chromophore for its analysis. da Costa Cesar et al described a UV spectrophotometry method for lumefantrine assay involving methanol as the solvent and a wavelength of 335nm (da Costa Cesar *et al.*, 2008).

2.6.2 HPLC analysis of Artemether-Lumefantrine

The USP Salmous Guidelines and International Pharmacopoeia prescribe procedures for the assay of artemether by reverse phase HPLC/UV detection. The various mobile phases have acetonitrile as a major component with wavelengths of analysis of 216nm and 210nm (USP SALMOUS Standards, 2009, World Health Organization, 2015a). Additionally, the USP Salmous describes methods for the assay of lumefantrine by HPLC/UV detection. da Costa et al in their study described an HPLC/UV method for lumefantrine (da Costa Cesar *et al.*, 2008).

For the simultaneous determination of artemether and lumefantrine in fixed dose combination formulations, methods are in the USP Salmous and the IP. They both employ gradient elution

sequence using acetonitrile and an ion – pairing reagent with a run time of about 55minutes. This method is not only expensive but also time consuming.

Several published papers also describe assay methods for the simultaneous determination of artemether-lumefantrine in fixed dose combination form. A good number of them utilise a mobile phase system of acetonitrile and an inorganic buffer (phosphate buffer) of pH less than 3 (Arun and Smith, 2011, Gupta *et al.*, 2013, Sridhar *et al.*, 2010, Suleman *et al.*, 2013, Sunil *et al.*, 2010).

Kalyankar and Kakde described a method consisting of methanol and trifluoroacetic acid with triethylamine buffer; pH of 2.8 (Kalyankar and Kakde, 2011).

2.6.3 Simultaneous determination of Artemether-Lumefantrine in plasma

Few methods have been reported for the simultaneous determination of artemether and lumefantrine in biological matrices. Ultra-violet detection in most cases is not adequate for artemether quantification in biological matrix due to its low sensitivity and selectivity. Hodel et al developed a method using HPLC-ESI-MS/MS for the quantification of 14 antimalarials in plasma including artemether and lumefantrine (Hodel *et al.*, 2009). An improvement was made to this method in César et al which involved the elimination of the drying step in sample preparation and a reduced chromatographic run time (César *et al.*, 2011). Sarma et al also described an efficient RP-HPLC method for the simultaneous detection of artemether and lumefantrine in human plasma using diode array detector (DAD) at 238nm (Sarma *et al.*, 2014). This method offers a relatively cost effective way to simultaneously quantify artemether and lumefantrine in plasma which is easily applicable in limited facilities as opposed to the expensive electrochemical or mass spectrometry detection.

The World Health Organisation in its prequalification of medicines programme proposes an LCMS/MS procedure to simultaneously quantify artemether and lumefantrine in plasma in bioequivalence studies.

2.6.4 Bioanalytical method validation

The analytical methods utilised in bioequivalence studies as required by the regulatory bodies should be performed in accordance with the principles of Good Laboratory Practice (GLP). The bioanalytical methods used must be well characterised, fully validated and documented to yield reliable and reproducible results that can be interpreted satisfactorily. Validation of the method remains one of the key measures universally recognised as a necessity for comprehensive system of quality assurance and also to ensure acceptability of the performance and the reliability of analytical results.

2.6.4.1 Method validation parameters

The International Conference of Harmonisation outlines several parameters in the validation process. They are explained below:

- Linearity: This tests the ability of the procedure within a given range to obtain test results which are directly proportional to the amount in the sample.
- Specificity: This parameter tests the method"s ability to assess unequivocally the analyte in question in the presence of components which may be expected to be present. Examples include matrix, degradants and impurities.
- Accuracy: This tests the agreement between a value which is accepted either as a conventional true value or an accepted reference value and the found value.

- Precision: Precision tests the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. It is considered at three levels of repeatability, intermediate precision and reproducibility. Precision is usually expressed as a variance, or standard deviation of a series of measurements.
- Range: This expresses the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has suitable level of linearity, accuracy and precision.
- Limit of Detection and Quanitification

  Limit of Detection of the method is the lowest amount of analyte in a sample which can be detected but may not be necessarily quantified. The quantification limit on the other hand is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

  These limits are based on:

- Signal – to - Noise
- Visual Evaluation
- The Standard deviation of the response and the slope

 Robustness: This parameter measures the capacity of the analytical method to remain unaffected by small, but deliberate variations in method parameters. This provides an indication of its reliability during normal usage. Typical variations with respect to HPLC/UV detection include:

- Different columns
- Temperature

- Flow rate (ICH harmonised tripartite Guidelines, 2005)

2.6.5 The use of internal standard in chromatographic analysis

In analytical chromatographic system, an internal standard may be employed to improve on the analysis. An internal standard in analytical chemistry is a chemical substance that is added in constant amount to samples, blank and calibration standards in a chemical analysis. In the use of the internal standard method, calibration involves plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentrations from a calibration curve.

The use of internal standard in HPLC analysis is normally employed basically to compensate for several types of random and systemic errors (Crouch and Skoog, 2007). It is very useful especially for analysis in which the quantity of samples analysed or the instrument response varies slightly from run –to – run for reasons that are difficult to control in the analytical determination.

During sample preparation especially with plasma matrices, internal standard is utilised to correct for the loss of analyte (Clarke and Moffat, 1986). A major challenge in this type of analysis is the finding of a suitable substance to serve as the standard.

An ideal internal standard should

- be chemically and physically similar to the analyte
- be eluted near to, but well resolved from the analyte of interest
- not be present in the original sample mixture
- be unreactive towards any of sample components
- be available in a highly pure form (Crouch and Skoog, 2007)

CHAPTER THREE

3.1 Materials and Methods

3.1.1 Equipment and Apparatus

HPLC System consisting of a Model-Spectra Series-P100 Isocratic pump. UV-Visible detector (PerkinElmer series 785A). eDAQ powerchrom series 280 integrator. A Shandon Hypersil ODS 5µm 4.6 x 250 mm column. Rheodyne injector with a 20 µl loop.

Adam-analytical weighing balance WA 210;210/0.0001g.

Hanna instruments pH 211 microprocessor pH meter.

FS 8H Fisher Scientific Sonicator.

Erweka Dissolution Apparatus (Type DT6-GmbH Heusenstamm, Germany Nr 68045).

Erweka Disintegrating Test Apparatus (Type ZT 3/1-GmbH Heusenstamm, Germany Nr 68318).

Erweka Friabilator (Type TA20, Germany).

Stuart melting point apparatus (SN: R000105350, Bibby Scientific Ltd., UK).

Whatman filter papers.

General purpose laboratory glassware.

3.1.2 Reagents and Samples

Methanol, Acetonitrile, Sodium Acetate, Hydrochloric acid, Potassium Iodide, Glacial acetic acid, Trifluoroacetic acid TFA (98%); they were all of HPLC grade.

Table 3.1 Profile of Pure Samples Used

Sample	Source	Batch No.	Manufacturing Date	Expiry Date
Lumefantrine	Ernest Chemists	LUM/BB/107/03/14	March 2014	February 2017
Artemether	Ernest Chemists	13/002	March 2014	December 2016
Artesunate	Guilin Pharma	ZA130908	August 2013	August 2016

3.2 Methodology

3.2.1 Sampling of the most available generic

The study was conducted between October 2015 and February 2016. A cross-sectional pilot survey to identify the most available and dispensed locally manufactured formulation containing a fixed combination of Artemether-Lumefantrine was carried out. An open – ended questionnaire was designed and taken to 25 pharmaceutical retail outlets in and around KNUST campus, Kumasi. They were answered by retail attendant (pharmacist, pharmacy technologist and medicine counter assistants) who were met at the time of the visit. There were 25 respondents in all. The most available locally manufactured generic from the study was selected, purchased and used for bioequivalence study with the Coartem as the innovator or reference brand. **Table 3.2 Brands of tablets used (20mg/120mg Artemether-Lumefantrine)**

Sample	Code	Source	Batch No.	Manufacturing Date	Expiry Date
Coartem	INN	Novartis Pharma	K0902	June 2014	May 2016
Malar – 2	LMG	Ernest Chemists	1005P	May 2014	May 2016

3.2.2 Identification test carried out on the samples

3.2.2.1 Colour test for the presence of artemether

A quantity of 50 mg Artemether powder was weighed and dissolved in 2ml of dehydrated ethanol.

About 0.2 g of KI was added to the mixture and heated on a water bath for about 2 minutes. A

similar procedure was employed in the test of artemether in the powered artemetherlumefantrine tablets. A yellow colouration indicated presence of artemether.

3.2.2.2 Colour test for the presence of Lumefantrine

10 ml of methanol was added to a quantity of powered artemethert/lumefantrine tablets equivalent to 20 mg of lumefantrine. 20 mg of $KMnO_4$ was added and boiled for about a minute.

The mixture was filtered and a few drops of Brady‟s reagent (2, 4-dinitrophenylhydrazine solution) was added and shaken and observed for a precipitate formation. A similar procedure was carried out on the pure lumefantrine powder.

3.2.2.3 Melting point determination

The open capillary method using Stuart melting point apparatus was utilised to determine the melting point of the artemether and lumefantrine reference samples. Small amount of the samples were packed into a sample capillary tube. The sample tube was inserted into the melting point apparatus and the temperature range over which the crystals melt in the tube was recorded as the melting point.

3.3.2 Quality Control Tests

3.3.2.1 Determination of Percentage Content

An aliquot of powdered artemether-lumefantrine tablets equivalent to the dose of drug in one tablet (20mg artemether /120mg lumefantrine) was weighed accurately and dissolved in a 100 ml volumetric flask already containing 25 ml of acetonitrile. It was made up to volume and sonicated for 20 minutes to achieve enough dissolution. The solution was filtered through whatman filter paper (No. 5) into a 100 ml volumetric flask. 1 ml aliquot of the filtrate was transferred into a 10 ml volumetric flask and an aliquot of the internal standard (artesunate) was added and made up to

volume with the mobile phase to yield a concentration of 250 µg/ml for artemether and 60 µg/ml for lumefantrine. 20 µl was analysed using the validated HPLC method. The injection was done in triplicates and the peak response of the analytes and internal standard recorded. The response factor and peak response were used to calculate the amount of drug present (Gupta *et al.*, 2013). The procedure was repeated for the other brand.

3.3.2.2 In-vitro dissolution study

Six vessels of the Erweka Dissolution USP Apparatus 2 were filled with 900 ml of 0.1 M HCl containing 1% Tween 80 and equilibrated to a temperature of 37^{o}C ± 0.5 (Umapathi *et al.*, 2011). The paddle was set at 50 rpm. One tablet was placed in each vessel and sampling was done at 5, 15, 30, 45, 60, 90 and 120 minutes. At each sampling period, 5 ml samples were withdrawn from a zone midway between the top of the paddle blade and the surface of the medium. Fresh medium (5ml) was withdrawn from the reservoir vessel and added to the vessel from which the volume was withdrawn. The samples were filtered and diluted 50 folds and analysed using the validated HPLC method in duplicates. The procedure was repeated for the other brand. The concentrations of artemether-lumefantrine in the samples were calculated and the percentage cumulative release values were then calculated. Mean percentage cumulative drug dissolved and their respective time points were plotted on a graph to obtain the release profiles of each formulation using Microsoft Excel. The similarity and difference factors of the release profiles of the two formulations were determined. Paired student t-test was used to statistically analyse the dissolution data obtained at the various time points. The dissolution efficiency (DE) was calculated for each brand according to the equation:

Dissolution efficiency (DE)=$\{(0\int^{t}Y.dt) / 100.(t_2 - t_1)\} \times 100$ where

$(0\int^{t}Y.dt)$ = area under the dissolution curve (AUC)

Y=the percentage dissolved at t_2 t_2=time for all active ingredient to dissolve t_1=time at which first sample was withdrawn

3.3.2.3 Disintegration Test

Six tablets from both brands were randomly selected and used for the test. They were placed separately into each of the six cylindrical tubes of the basket rack of the Disintegration apparatus. The test was carried out at 37^{o}C ± 0.5 and the bottom of the basket rack was positioned such that it was at least 15 mm below the surface of the distilled water. The time it took for complete disintegration, i.e. no granule of any tablet was left on the mesh, was recorded as the disintegration time. The procedure was performed in triplicates for both brands.

3.3.2.4 Friability Test

In this test, 10 tablets randomly selected from a particular brand, were de-dusted and weighed together. They were placed in the Friabilitor and operated at 25 revs/min for 4 minutes. After the revolutions the tablets were removed and re-weighed after de-dusting. The percentage weight loss was then calculated for each brand and represented as the percentage friability. The procedure was performed in triplicates for both brands.

3.3.2.5 Uniformity of Weight Test

Twenty tablets were selected randomly from each brand and collectively weighed to ascertain the total and average weight. The individual weights were then taken. The percentage deviation of each tablet from the mean was then determined. This procedure was repeated for the other brand. The percentage deviations of the tablets from the average weight were calculated using;

$$\text{Percentage deviation} = \frac{a - b}{b} \times 100\%$$

Where a = weight of tablet; b = average weight of tablets

3.3.3 Development and validation of HPLC method for determination of Artemether and Lumefantrine in plasma samples

3.3.3.1 Instrumentation

The HPLC system consisted of a model-Spectra P100Series Isocratic pump, rheodyne injector with a 20 µl loop, UV-Visible detector, eDAQ powerchrom series 280 integrator. Data acquisition and reporting was by eDAQ powerchrom software version 2.6.4.

A Shandon Hypersil ODS 5µm 4.6 x 250 mm column was used to achieve the separation of the analytes; Artemether (ART) and Lumefantrine (LUM) and the internal standard, Artesunate (AST). All HPLC measurements were done at 230nm

3.3.3.2 Selection of Mobile phase

Various mobile phases in several compositions (such as methanol-water, acetonitrile-water and acetonitrile-acetate buffer), were tested and screened (based on previous works and modifications)

(Minzi *et al.*, 2013, Sarma *et al.*, 2014). Based on the outcomes, Sodium Acetate Buffer, pH 2.5 (solution A) in combination with Acetonitrile (solution B) was chosen. An isocratic elution approach of 33% Buffer and 67% Acetonitrile was settled on after it produced good resolved peaks. The analysis was performed at a flow rate of 1.3 ml/min for all samples with an injection volume of 20 µl.

3.3.3.3 Sample Preparation

All samples were prepared by dissolving the appropriate weight of the pure artemether, lumfantrine and artesunate (internal standard) in the mobile phase solvent, was sonicated and filtered. Filtered solutions were diluted to the desired concentrations with the same mobile phase solvent. Samples were transferred into HPLC injection syringe and filtered through a 0.25µm filter before injection.

3.3.3.4 Validation of HPLC Method

The International Conference on harmonization (ICH) guidelines was used for the validation of the developed HPLC method. The validated parameter included linearity, limit of detection and quantification, accuracy and precision.

3.3.3.4.1 Linearity

A stock solution consisting of 10mg/ml Artemether and 100µg/ml Lumefantrine was prepared. Aliquots of the stock solution were diluted in mobile phase to six different concentration of 8, 4, 2, 1, 0.8, 0.4, mg/ml of ART and 80, 40, 20, 10, 8, 4, µg/ml of Lumefantrine.

A working standard solution of 1mg/ml of Artesunate (IS) was used throughout the work. Solutions containing the two analytes (ART/LUM) and internal standard (AST) were analysed in triplicate injections. All solutions were prepared just prior to injection. Calibration curves for concentration

versus mean response ratio (each analyte versus internal standard) were plotted for each analyte and the data obtained was subjected to regression analysis to determine the linearity of the method (Green, 1996).

3.3.3.4.2 Limit of Detection and Quantification

The limits of detection and quantification were established from standard deviation of the response and slope of the calibration curve (ICH harmonised tripartite Guidelines, 2005).

3.3.3.4.3 Recovery, Accuracy and Precision

Concentrations of 1.6, 2, 2.4 mg/ml and 16, 20, 24 µg/ml representing 80%, 100%, 120% of 2mg/ml/20µg/ml ART/LUM were analysed, 3 injections per sequence. The linear regression equations obtained earlier were used to determine the recovered concentration.

Inter-day variability of the method was investigated by analysing the samples for 3 consecutive days.

Accuracy was determined as the percentage recovery whiles the percentage relative standard deviation (%RSD) was taken as a measure of precision.

3.3.3.4.4 Determination of Response Factor for Plasma work

Concentrations of 1.6, 2, 2.4 mg/ml and 16, 20, 24 µg/ml of ART/LUM and Artesunate (1mg/ml) respectively were analysed in triplicate injections. The area under the peaks and corresponding concentrations were used to calculate the response factor.

3.3.3.4.5 Preparation of Spiked Blood samples to determine Accuracy and Precision of Extraction Procedure

Fresh blood was taken from rabbit without any drug history in EDTA tubes and stored in a freezer and used within 72 hours of collection. Blood was thawed for about 20 minutes before use.

Aliquots of thawed blood (1000 µl) were spiked with an equal volume of a working standard solution of artemether-lumefantrine mixture. It was mixed for about 20 seconds. 1 ml of the mobile phase was added to the samples, mixed thoroughly and centrifuged at 9000 rpm for 10 minutes. 1 ml of supernatant was carefully withdrawn and spiked with a volume of a standard solution of the internal standard (artesunate) to an effective concentration of 1mg/ml. It was injected via the rheodyne and analysed according to the HPLC conditions described earlier. The RF values were used to calculate the recovered concentrations in plasma. Accuracy of the extraction procedure was determined so as to reflect the intended application of the method for a bioequivalence study

3.3.4 Bioequivalence study using animal models

Twenty healthy rabbits were procured for the study. They were weighed and randomized into two groups and starved for 24 hours before the day of study. The study was a randomized, single dose, open label, two - period, two sequence crossover study design.

Each subject was administered both the test and innovator products at a dose of 4 mg/kg of artemether and 24 mg/kg of lumefantrine. After a 60–day wash–out period, the subjects was administered the alternate drug.

Table 3.3 Cross – Over study design

	PERIOD 1	WASHOUT PERIOD	PERIOD 2
GROUP A 10 Rabbits	Innovator Brand (INN)	≥5 HALF LIVES (60 days)	Test/Generic Brand (LMG)
GROUP B 10 Rabbits	Test/Generic Brand (LMG)		Innovator Brand (INN)

3.3.5 Blood Sampling and Plasma Analysis of Artemether-Lumefantrine

Xylene was applied to the ears of the rabbits to make their marginal ear veins more superficial. 1 ml of blood sample was then collected from the rabbit‟s marginal ear vein to serve as the baseline readings. The blood samples were collected in EDTA tubes, appropriately labelled with subject identification number and sampling times. Blood samples were kept in a cool box and transported to the laboratory for centrifugation to obtain plasma samples. The plasma samples obtained after centrifugation at 9000 rpm were transferred into eppendorf tubes, shaken with 1 ml of mobile phase to extract the drug. 500 µl of supernatant was withdrawn and a volume of the internal standard, Artesunate, was added to the sample to an effective concentration of 1 mg/ml. The sample with the internal standard was analysed using the developed HPLC method described earlier. The procedure was repeated for blood samples taken at 0.25, 0.5, 2, 6, 8, 12, 24, 72 hours after drug administration.

The concentrations of the samples were calculated using the response factor together with peak area of the analyte and internal standard.

3.3.6 Pharmacokinetic Assessment and Statistical Analysis

A plasma drug concentration - time curve was plotted using Microsoft Excel and GraphPad Prism version 6. The pharmacokinetic parameters AUC_{0-72} and $AUC_{0-\infty}$ of the reference and test drug were calculated using Non – compartmental Pharmacokinetic Data Analysis with PK Solutions 2.0 (Summit Research Services). The C_{max} and T_{max} were determined directly from the plasma concentration – time curve and the AUCs were calculated using the linear trapezoidal rule. $AUC_{0-\infty}$ was calculated by the summation of AUC_{0-72} and residual AUC.

The pharmacokinetics parameters were log – transferred and the geometric mean with standard deviation calculated according to the FDA guidelines. The geometric mean ratio of the test to the reference formulation and the 90% confidence interval around each mean ratio was determined. The intervals were compared to the FDA predefined limits of 0.80 to 1.25 on a forest plot.

CHAPTER FOUR

RESULTS

4.1 Analysis of Questionnaire Data

Table 4.1 Facility Identity and Demographics

Variable	Frequency (n)	Percentage (%)
Outlet		
Pharmacy	24	96
LCSs	1	4
Interviewee		
Pharmacist	12	48
LCS	1	4
DT	3	12
MCA	9	36

LCS=Licensed Chemical Seller DT= Dispensary Technician MCA=Medicine Counter Assistant

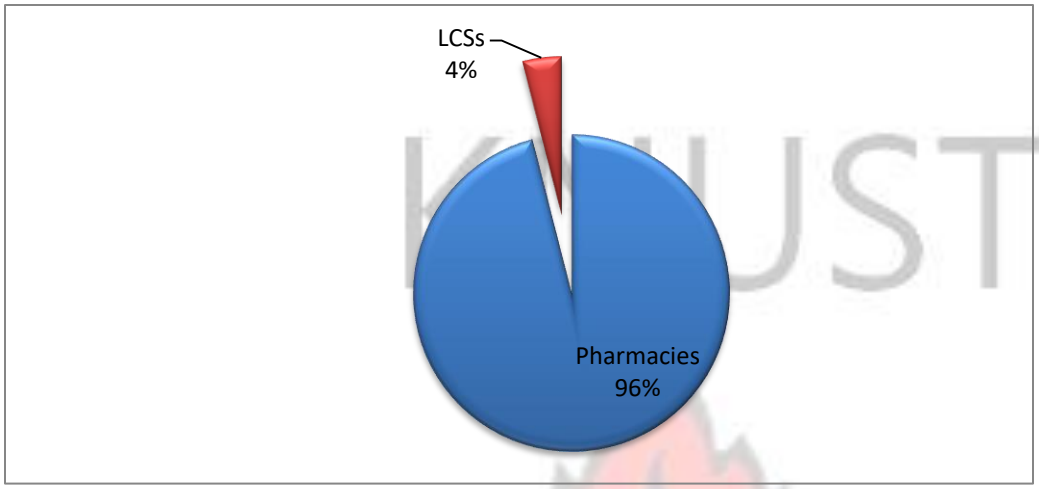

Figure 4.1a Pie Chart showing types of facilities engaged in survey.

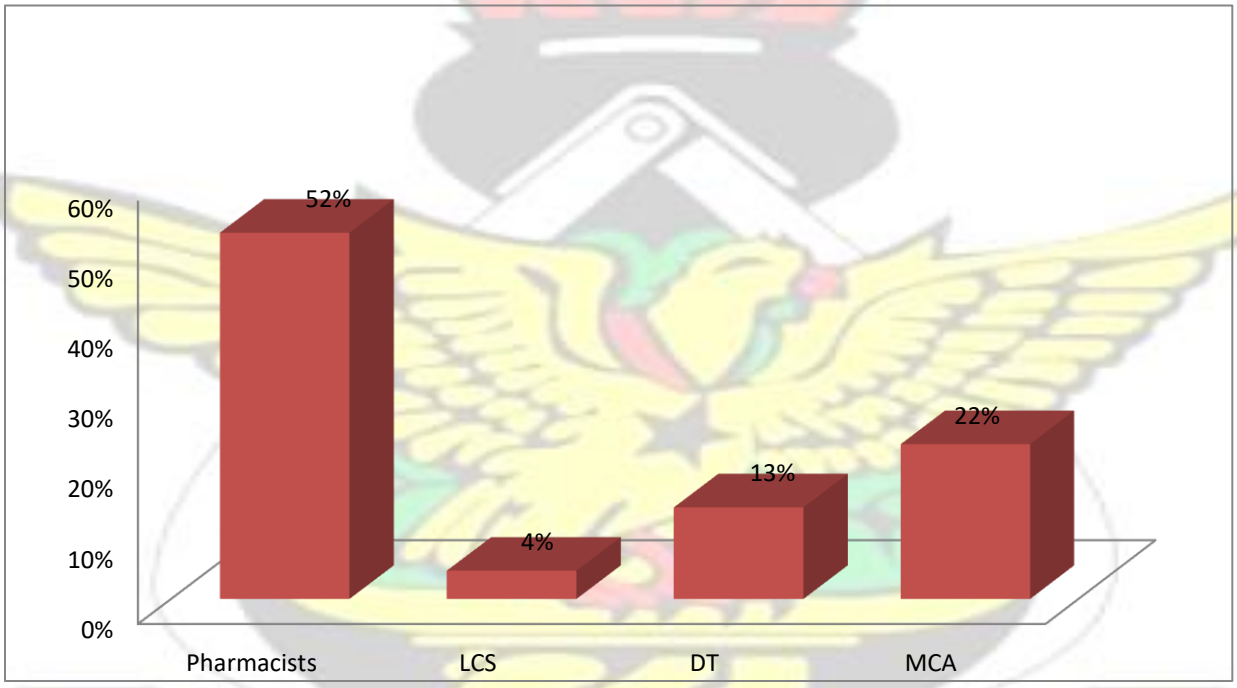

Figure 4.1b Bar Chart showing the type of respondent engaged in the survey **Table 4.2 Stocking preferences of Artemether / Lumefantrine**

Availability of Antimalarial Medications	Frequency (n =25)	Percentages (%)
Yes	25	100
No	0	0

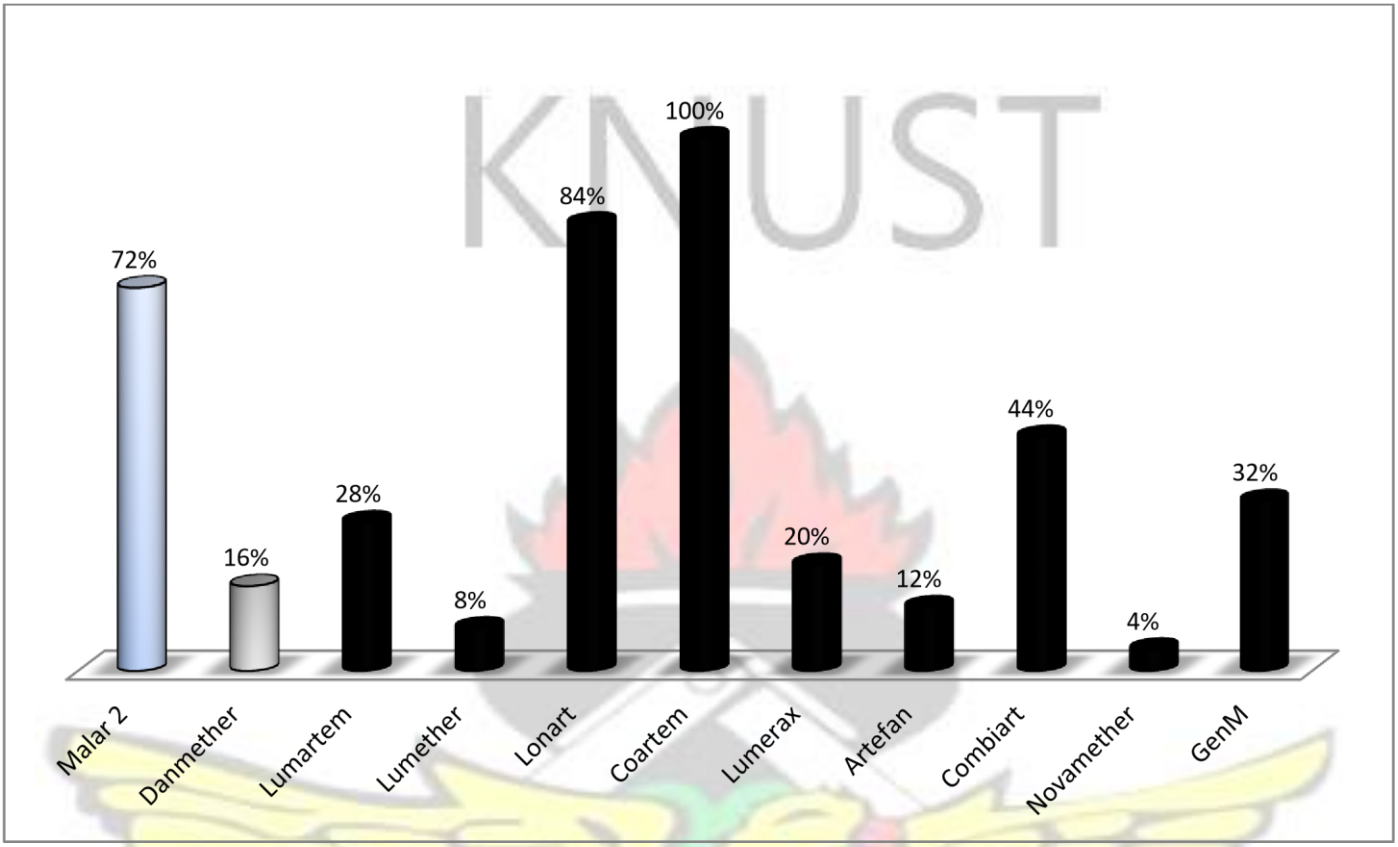

Fig 4.2 Availability of Various brands of Artemether-Lumefantrine

Table 4.3 Factors Influencing Client Choice for Innovator or Generic

Factors	Innovator	Generic
Affordability	30%	74%
Quality	65%	39%
Quantity	13%	13%
Popularity	9%	4%
Advertisement	4%	0%

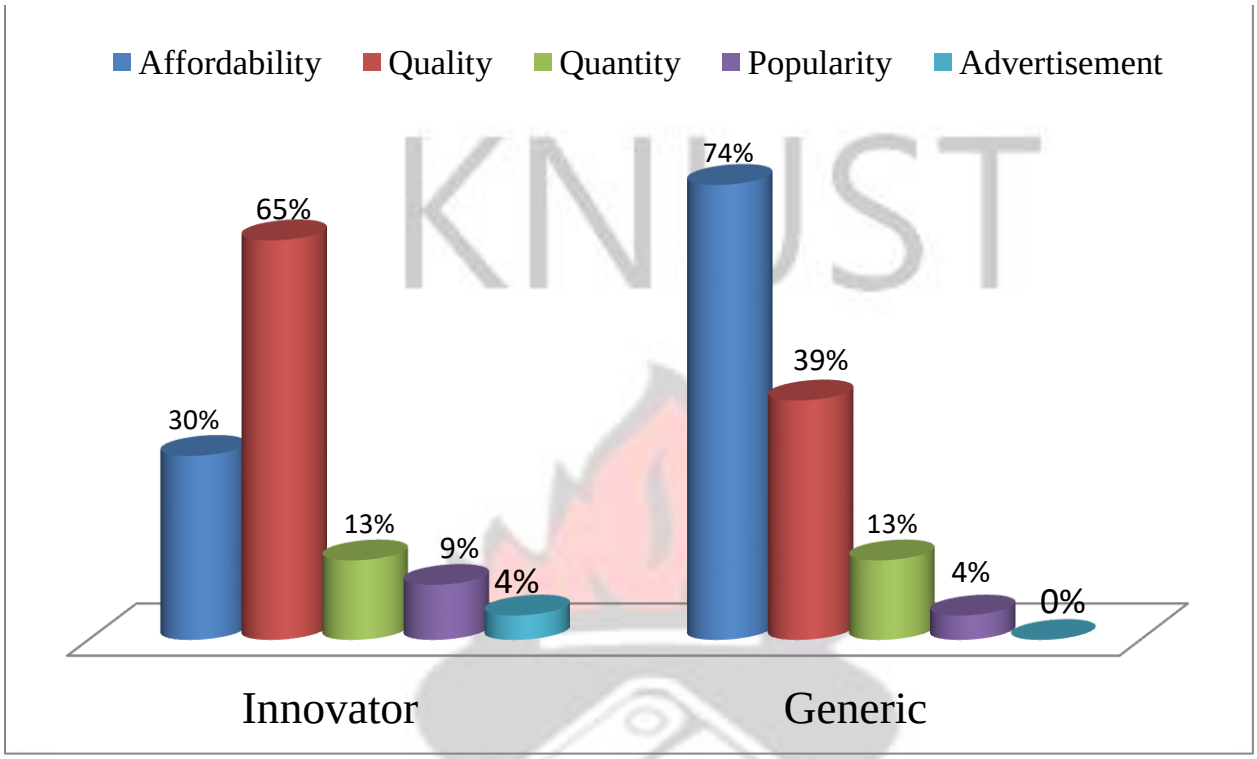

Figure 4.3 Factors that influence client's choice for innovator or generic brands of Artemether-Lumefantrine

4.2 Identification Test

4.2.1 Identification of Artemether and Lumefantrine

Table 4.4 Identification of Artemether by Colour test

Test	Observation	Inference
About 30mg of pure Artemether powder + 1ml dehydrated ethanol + 0.1g KI + Heat	A yellow colour was produced	Artemether present

About 30mg of powdered tablet + 1ml dehydrated ethanol + 0.1g KI + Heat	A yellow colour was produced	Artemether present

Table 4.5 Identification of Lumefantrine by Colour test

Test	Observation	Inference
About 30mg of pure Lumefantrine powder + 5ml methanol + 20mg $KMnO_4$ + Heat + Brady‟s reagent	An orange precipitate was produced	Lumefantrine present
About 30mg of powdered tablets + 5ml methanol + 20mg KMnO4 + Heat + Brady‟s reagent	An orange precipitate was produced	Lumefantrine present

Table 4.6 Melting Point Determination

Sample	Melting Point (ºC)		IP Reference Range (ºC)
	1st determination	2nd determination	
Artemether	86 – 89	86 – 88	86 – 90
Lumefantrine	128 - 130	128 - 131	128 – 132

4.3 Results of Quality Control tests conducted on the brands of tablets

4.3.1 Weight Uniformity test

Table 4.7 Uniformity of weight of the different brands of Artemether-Lumefantrine tablets

Code	Total Weight (g)	Mean Weight (g)	Number of tablets deviating by ± 7.5%	Number of tablets deviating by ± 15%	Inference
LMG	4.8580	0.2429 ± 0.004	nil	nil	Passed
INN	4.8000	0.2400 ± 0.002	nil	nil	Passed

4.3.2 Disintegration Test

Table 4.8 Disintegration time of the Artemether-Lumefantrine tablets

Code	1st Determination(min)	2nd Determination(min)	Average disintegration time (min)
LMG	2.39	2.27	2.33 ± 0.08
INN	3.37	3.26	3.325 ± 0.08

4.3.3 Friability test

Table 4.9 Friability test of the Artemether-Lumefantrine tablets

Code	Initial weight (g)	Final weight (g)	% Weight loss
LMG	2.483	2.461	0.886
INN	2.403	2.391	0.499

4.3.4 Assay of Artemether and Lumefantrine in the various tablets

Table 4.10 Determination of Percentage Content in Artemether-Lumefantrine tablets

Code	Assay (%)	
	Artemether	Lumefantrine
LMG	90.47 ± 10.85	94.94 ± 4.97
INN	107.37 ± 17.8	92.98 ± 4.78

(IP Range = 90.0% - 110.0%)

4.3.4 In-vitro dissolution studies

Table 4.11 Percentage Cumulative drug release of Artemether from the various brands of tablets

Time (minutes)	LMG (%)	INN(%)
5	38.19 ± 2.61	36.09 ± 1.83
15	55.74 ± 1.52	47.16 ± 1.94
30	69.50 ± 1.13	60.44 ± 2.02
45	70.85 ± 1.76	65.42 ± 2.57
60	80.12 ± 1.01	66.82 ± 1.48
90	86.38 ± 0.92	90.72 ± 0.92

120	86.40 ± 0.85	92.89 ± 1.44

Table 4.12 Percentage Cumulative drug release of Lumefantrine from the various brands of tablets

Time (minutes)	LMG(%)	INN(%)
5	20.13 ± 0.48	7.39 ± 0.46
15	39.26 ± 5.26	24.78 ± 1.08
30	46.90 ± 2.17	46.38 ± 0.56
45	56.78 ± 3.95	53.89 ± 4.049
60	60.25 ± 5.31	55.54 ± 2.52
90	82.76 ± 0.14	87.92 ± 0.07
120	92.25 ± 0.89	88.81 ± 1.37

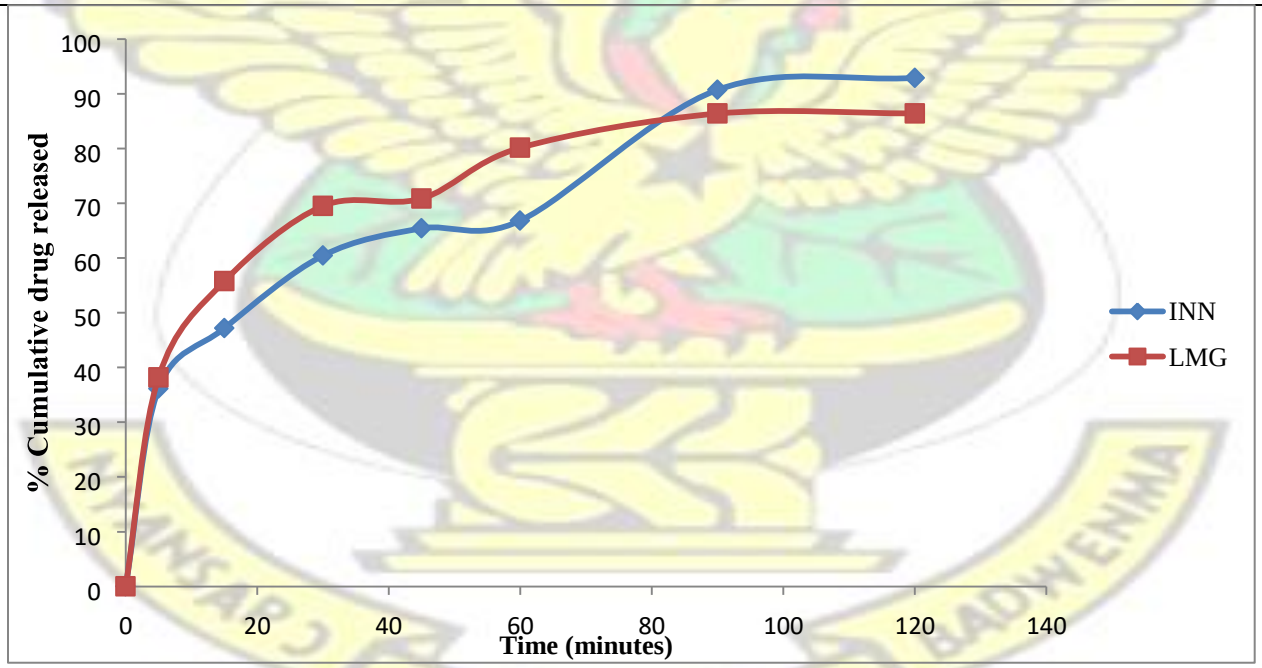

Figure 4.4 Dissolution profile of Artemether in 0.1M HCl containing 1% w/v Tween 80 from the two formulations

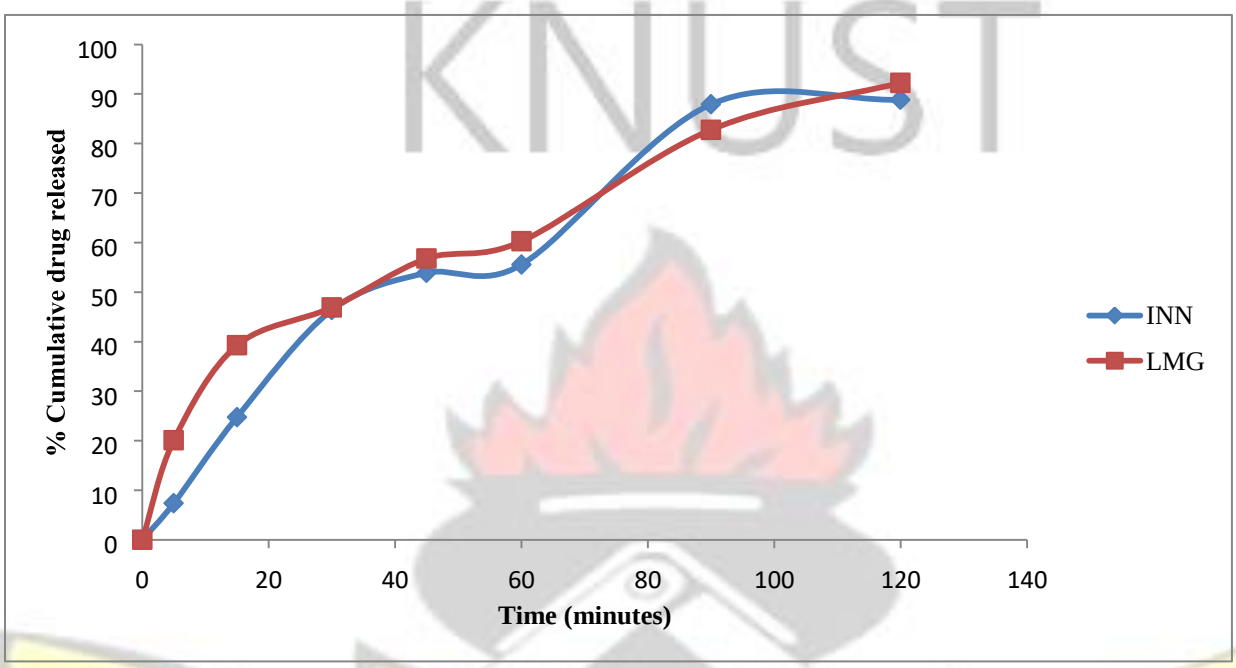

Figure 4.5 Dissolution profile of Lumefantrine in 0.1M HCl containing 1% w/v Tween 80 from the two formulations

Table 4.13 Difference (f_1) and Similarity factor (f_2) of Artemether-Lumefantrine tablets using INN as reference product

Fit factors	Artemether	Lumefantrine
Difference factor (f_1)	13	18*
Similarity factor (f_2)	91	99
Inference	SIMILARITY DEMONSTRATED	SIMILARITY DEMONSTRATED

Table 4.14 Dissolution efficiencies of the various brands of Artemether-Lumefantrine tablets

Code	Dissolution Efficiency (%)	
	Artemether	**Lumefantrine**
LMG	88.35	69.41
INN	78.32	69.94

4.4 HPLC method development and validation

The selected mobile phase system was composed of 33% Acetate buffer (pH=2.5): 67% Acetonitrile. This mobile phase system produced symmetric peaks with good resolution, distinct retention times and stable baseline.

A flow rate of 1.3ml/min was selected after different flow rates show peak tailing and prolong retention times.

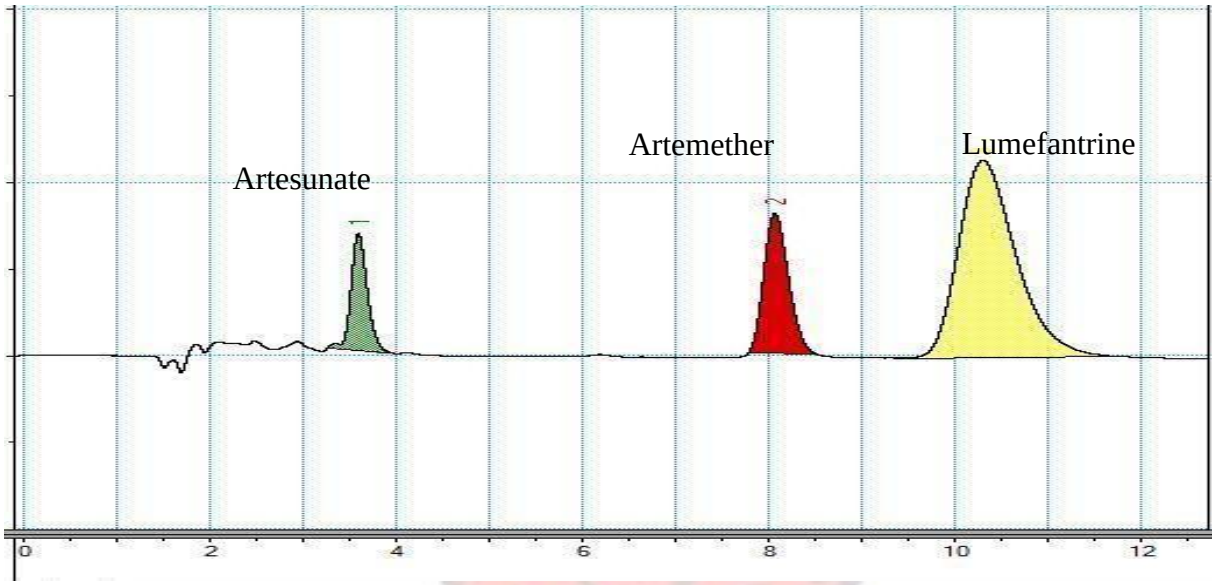

Figure 4.6. Chromatogram of Artesunate (internal standard), Artemether and Lumefantrine (analytes)

Table 4.15 Various analytes in the samples and their retention times

Substance	Retention time (minutes)
Artesunate	3.58
Artemether	8.06
Lumefantrine	10.29

4.4 .1 Linear Regression Analysis

Using the average peak areas of the internal standard and the two analytes, Artemether (ART) and Lumefantrine (LUM), the mean response ratios were calculated. A linear regression analysis was carried out on the concentrations of each analyte and it corresponding mean response ratio.

Table 4.16 Concentration of Artemether and Mean Response Ratio (n=3)

Concentration (mg/ml)	Mean response ratio ± SD
8	10.7909 ± 0.041
4	5.3010 ± 0.049
2	2.5454 ± 0.058
1	1.3450 ± 0.071
0.8	0.6454 ± 0.0345
0.4	0.2946 ± 0.041

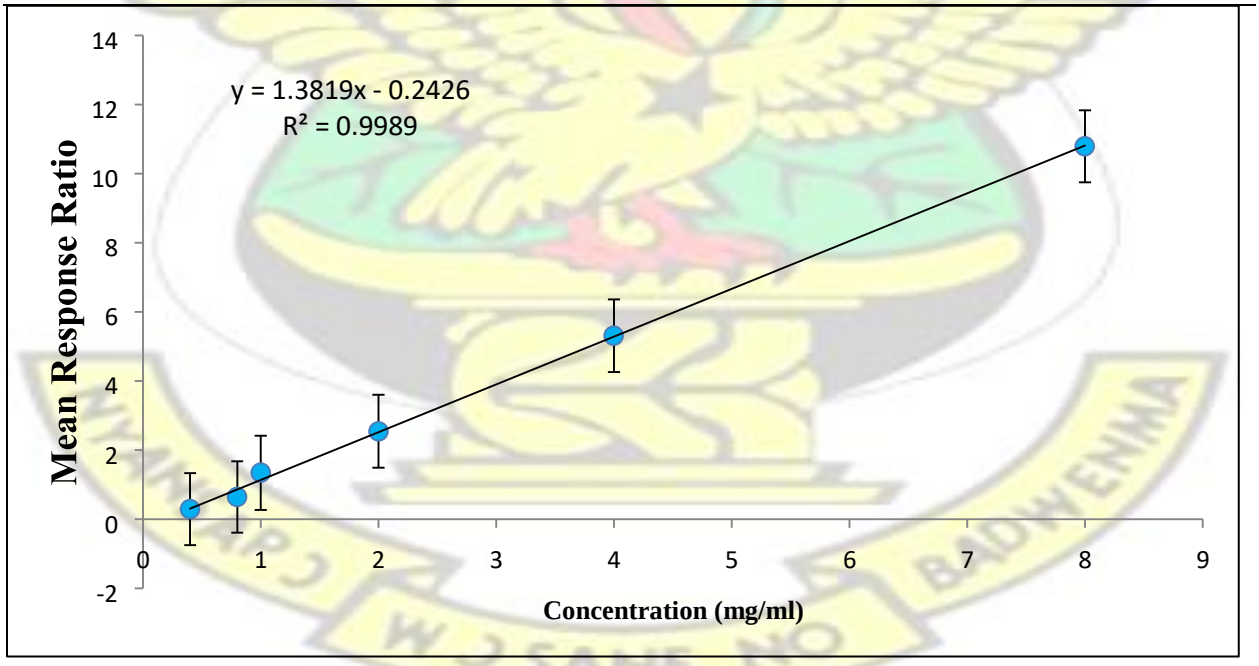

Figure 4.7 Linearity Curve of Concentration against Mean response ratio for Artemether
Table 4.17 Concentration of Lumefantrine and Mean Response Ratio (n=3)

Concentration (µg/ml)	Mean response ratio
40	16.9649 ± 0.035
20	8.4011 ± 0.3178
10	4.2048 ± 0.0570
8	2.0969 ± 0.1598
4	1.0324 ± 0.0285

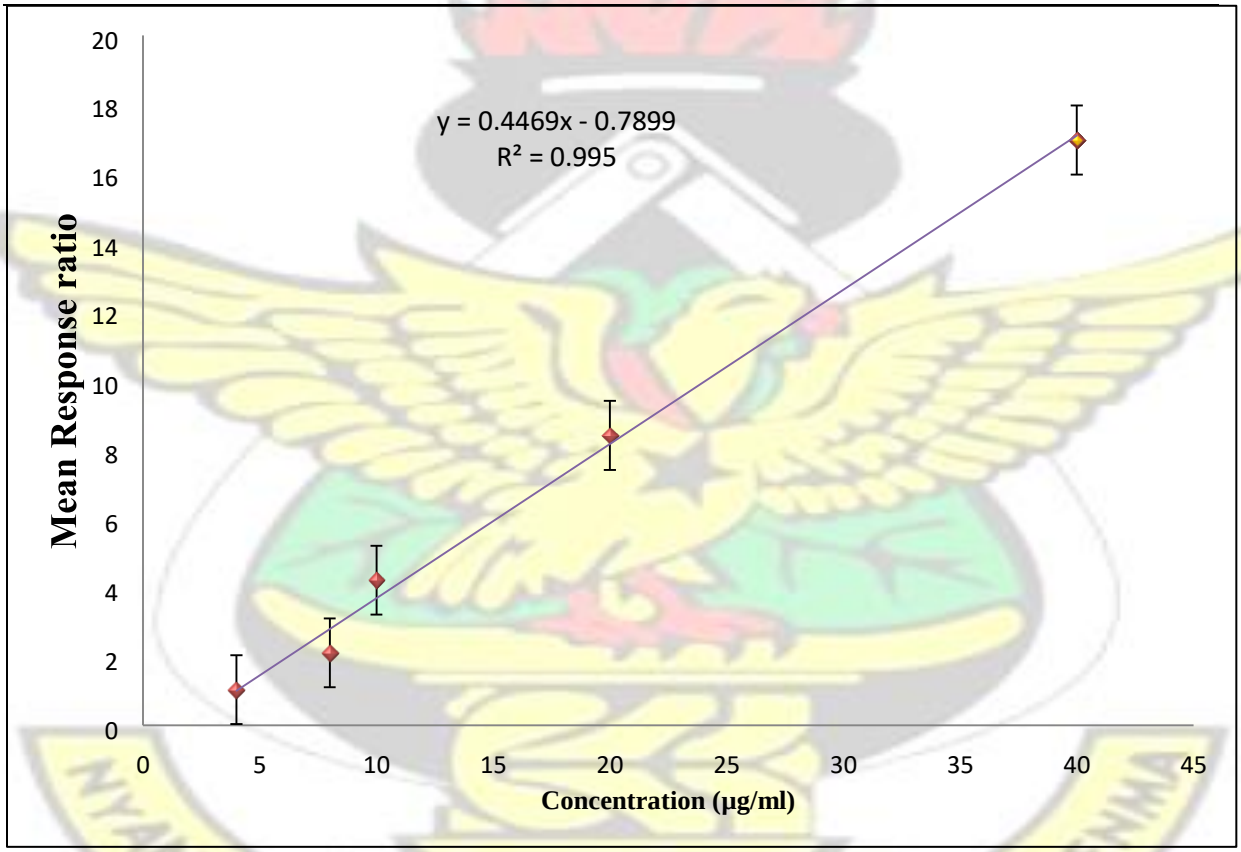

Figure 4.8 Linearity Curve of Concentration against Mean response ratio for Lumefantrine

.1.1 Limit of Detection and Quantification

Based on the standard deviation of the y-intercept and the slope of the linear curve the limit of detection (LOD) and limit of quantification (LOQ) according to the ICH guideline was

4.4

calculated.

LOD = $3.3\frac{\delta}{s}$ where $\delta$is the standard deviation and S is the slope of the linear curve.

LOQ = $10\frac{\delta}{s}$

Artemether:

δ = 0.088215499 S = 1.381894194

LOD = $3.3\ x\ \frac{0.088215499}{1.381894194}$

= 0.21mg/ml

LOQ = $10\ x\ \frac{0.088215499}{1.381894194}$

= 0.64mg/ml

Lumefantrine:

δ = 0.380066341 S = 0.446946

LOD = $3.3\ x\ \frac{0.3800663419}{0.446946}$

= 2.81µg /ml

LOQ = $10\ x\ \frac{0.3800663419}{0.446946}$

= 8.50µg/ml

4.4
.2 Precision of the HPLC Method

Table 4.18 Results for Interday Precision: Artemether

Interday (n=3)	Concentration (mg/ml)	Recovered Concentration (mg/ml)	% Recovery	% RSD
Day 1	2	1.976 ± 0.01	98.8371 ± 0.13	0.135
Day 2	2	1.977 ± 0.01	98.8500 ± 0.59	0.598
Day 3	2	2.003 ± 0.02	100.1463 ±1.08	1.083

Table 4.19 Results for Interday Precision: Lumefantrine

Interday (n=3)	Concentration (µg/ml)	Recovered Concentration (µg/ml)	% Recovery	% RSD
Day 1	20	19.804 ± 0.10	99.0196 ± 0.52	0.528
Day 2	20	19.870 ± 0.22	99.3520 ± 1.08	1.084
Day 3	20	20.028 ± 0.15	100.1379 ± 0.75	0.752

Table 4.20 Results for Intra-day Precision

Intra-day (analyte)	Concentration	Recovered Concentration	% Recovery	% RSD
Artemether **(n=6)**	2mg/ml	1.983 ± 0.02	99.1587 ± 0.997	1.005
Lumefantrine **(n=6)**	20 µg/ml	19.920 ± 0.19	99.6010 ± 0.941	0.944

4.4

.3 Accuracy of the HPLC Method

Table 4.21 Results for Accuracy: Artemether

	Concentration (mg/ml)	**Recovered Concentration (mg/ml)**	**% Recovery**	**% RSD**
	2.4	2.371 ± 0.03	98.7891 ± 1.35	1.364
(n=3)	2.0	1.977 ± 0.01	98.8500 ± 0.59	0.598
	1.6	1.571 ± 0.01	98.1856 ± 0.91	0.928

Table 4.22 Results for Accuracy: Lumefantrine

	Concentration (µg/ml)	Recovered Concentration (µg/ml)	% Recovery	% RSD
	24	23.880 ± 0.21	99.5011 ± 0.91	0.919
(n=3)	20	19.870 ± 0.22	99.3520 ± 1.07	1.084
	16	15.846 ± 0.22	99.0405 ± 1.39	1.407

4.4

.4 Robustness of the HPLC Method

Table 4.23 Effect of Flow rate on Recovery

		Artemether (2mg/ml)		Lumefantrine (20µg/ml)	
	Flow Rate (ml/min)	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**
1.2 99.2851 ± 0.43 0.437 98.1600 ± 1.54 1.572 **(n=3)** 1.3 98.8371 ± 0.13 0.135 99..0196 ± 0.52 0.528					
	1.4	99.9941 ± 0.67	0.668	98.8505 ± 0.41	0.417

Table 4.24 Effect of Wavelength on Recovery

		Artemether (2mg/ml)		Lumefantrine (20µg/ml)	
	Wavelength (nm)	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**
	229	100.900 ± 1.45	1.442	99.3889 ± 0.76	0.766
(n=3)	230	99.2851 ± 0.43	0.437	98.1600 ± 1.54	1.572
	231	97.798 ± 0.994	1.016	97.5575 ± 0.35	0.361

.5 Stability Studies over the Analysis Period

4.4

Table 4.25 Results of Stability Studies over 6 – hour period

		Artemether (2mg/ml)		Lumefantrine (20µg/ml)	
	Time (hours)	% Recovery	% RSD	% Recovery	% RSD
(n=3)	I	99.28 ± 0.87	0.876	105.33 ± 1.05	0.997
	2	98.42 ± 0.28	0.284	99.53 ± 0.49	0.493
	3	95.58 ± 1.03	1.078	99.09 ± 1.43	1.443
	4	98.71 ± 0.12	0.122	98.19 ± 0.52	0.529
	5	97.86 ± 0.15	0.153	97.12 ± 0.32	0.329
	6	97.28 ± 0.82	0.842	97.03 ± 0.39	0.402

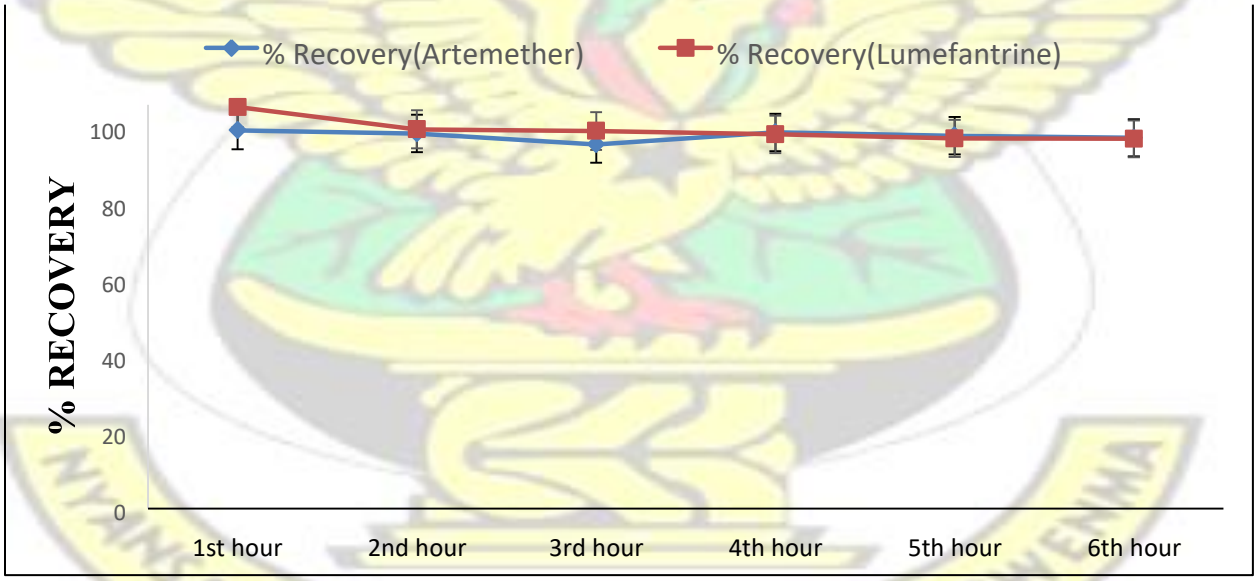

Figure 4.9 Stability curves of samples over analysis period

4.4

4.4.6 Determination of Artemether / Lumefantrine in plasma

Quantification of the two analytes, Artemether and Lumefantrine with the Artesunate as internal standard is based on the observation that the ratio of the respective peak areas of the IS and analytes is directly proportional to their respective concentration ratio. The constant of proportionality .i.e. the response factor *RF* is calculated using the equation below:

$$\frac{\text{AUC}_{is}}{\text{AUC}_{al}} = \text{RF}\,\frac{[IS]}{[al]}$$

Where AUC_{is} and AUC_{al} are the respective areas under the peak of internal standard and analyte and [*IS*] and [*al*] are their respective concentrations and *RF* is the response factor.

By analysing standard solutions of known concentrations of pure samples of the analytes, artemether and lumefantrine and internal standard, artesunate.

The obtained RF was used to calculate the concentration of the analyte in the plasma. **Table 4.26 Determination of Response factor to quantify Artemether in plasma**

Concentration of Analyte Concentration of Internal Standard	Mean Area under the peak	Response Factor
Artemether (2mg/ml)	3.1733 ±0.0208	0.7920 ± 0.0019
Artesunate (1mg/ml)	1.2567 ± 0.0058	
Artemether (2.4mg/ml)	3.7433 ± 0.0551	0.7802 ± 0.0197
Artesunate (1mg/ml)	1.2167 ± 0.0153	
Artemether (1.6mg/ml)	2.4133 ± 0.0252	0.8177 ± 0.0069
Artesunate (1mg/ml)	1.2333 ± 0.0153	
Average Respose Factor of Artemether		0.7966 ± 0.0192

Table 4.27 Determination of Response factor to quantify Lumefantrine in plasma

Concentration of Analyte Concentration of Internal Standard	Mean Area under the peak	Response Factor
Lumefantrine (20µg/ml)	10.1667 ± 0.1209	0.0025 ± 0.000018
Artesunate (1mg/ml)	1.2567 ± 0.0058	
Lumefantrine (2.4µg/ml)	12.0233 ± 0.1193	0.0024 ± 0.000048
Artesunate (1mg/ml)	1.2167 ± 0.0153	
Lumefantrine (1.6µg/ml)	7.7600 ± 0.1229	0.0025 ± 0.000011
Artesunate (1mg/ml)	1.2333 ± 0.0153	
Average Respose Factor of Lumefantrine		0.0025 ± 0.000057

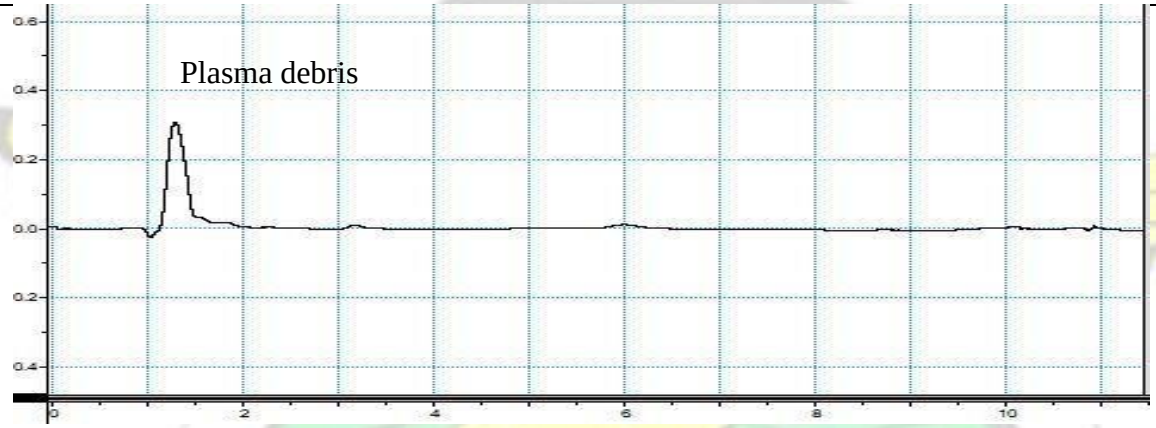

Figure 4.10 Chromatogram of blank plasma without any drug or analyte.

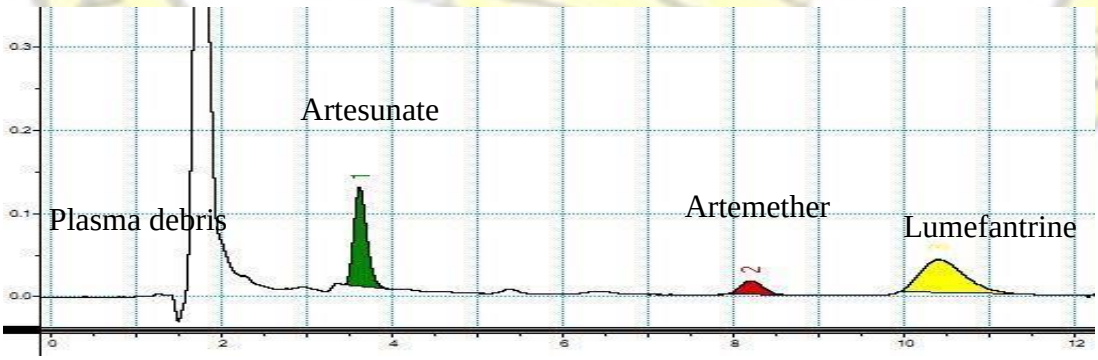

Figure 4.11 Sample chromatogram of artemether, lumefantrine and artesunte in plasma sample.

Table 4.28 Recovery of artemether / lumefantrine from plasma samples

Analyte	Spiked Concentration	Recovered Concentration (mg/ml)	% Recovery	% RSD
Artemether (n = 3)	0.33mg/ml	0.3201 ± 0.064	96.99 ± 1.95	2.008
	0.25mg/ml	0.2515 ± 0.191	100.58 ± 7.65	7.609
Lumefantrine (n = 3)	3.3µg/ml	3.2681 ± 0.206	99.03 ± 6.25	6.310
	2.5µg/ml	2.4154 ± 0.074	96.62 ± 2.99	3.101

4.5 Bioavailability studies using animal models

Table 4.29 Profiles of the animal subjects utilised in the study

Subjects	Identity	Body Weight (kg)	Gender
1 I 1.95 F 2 II 2.2 M 3 III 2.2 F 4 IV 2.5 M			
5	V	2.4	F
6	VI	1.95	F 7 VII 2.5 M
8	VIII	2.2	F
9	IX	2.6	F
10	X	2.00	F
11	XI	2.30	M
12	XII	2.15	F

13	XIII	2.25	M
14	XIV	2.5	M
15	XV	2.4	F
16	XVI	2.20	F
17	XVII	2.6	F
18	XVIII	2.2	M
19	XIX	2.6	M
20	XX	1.95	F

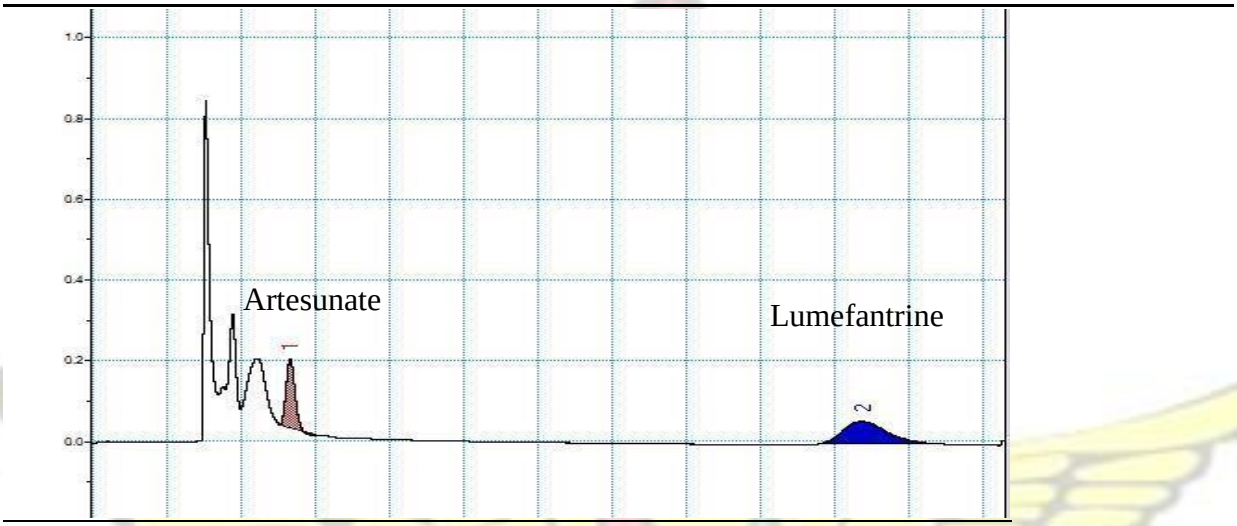

Figure 4.12 Typical Chromatogram of a plasma sample showing Artesunate (Internal Standard) and Lumefantrine

From the chromatogram above, the Lumefantrine component of the combination formulation was the only analyte to be adequately detected and quantified by the HPLC method developed.

Thus the results and analysis herein are based on the plasma concentrations of Lumefantrine only.

Table 4.30 Average plasma concentrations of Lumefantrine obtained from subjects

	Average plasma concentration (µg/ml)	
Time(hrs)	INN	LMG
0	0	0
0.25	0	0
0.5	0.383679	0.398099

2	1.563027	1.388195
6	5.711252	5.854583
8	6.536101	5.927733
12	4.551837	4.118235
24	2.552344	2.874740
72	0.742345	0.598186

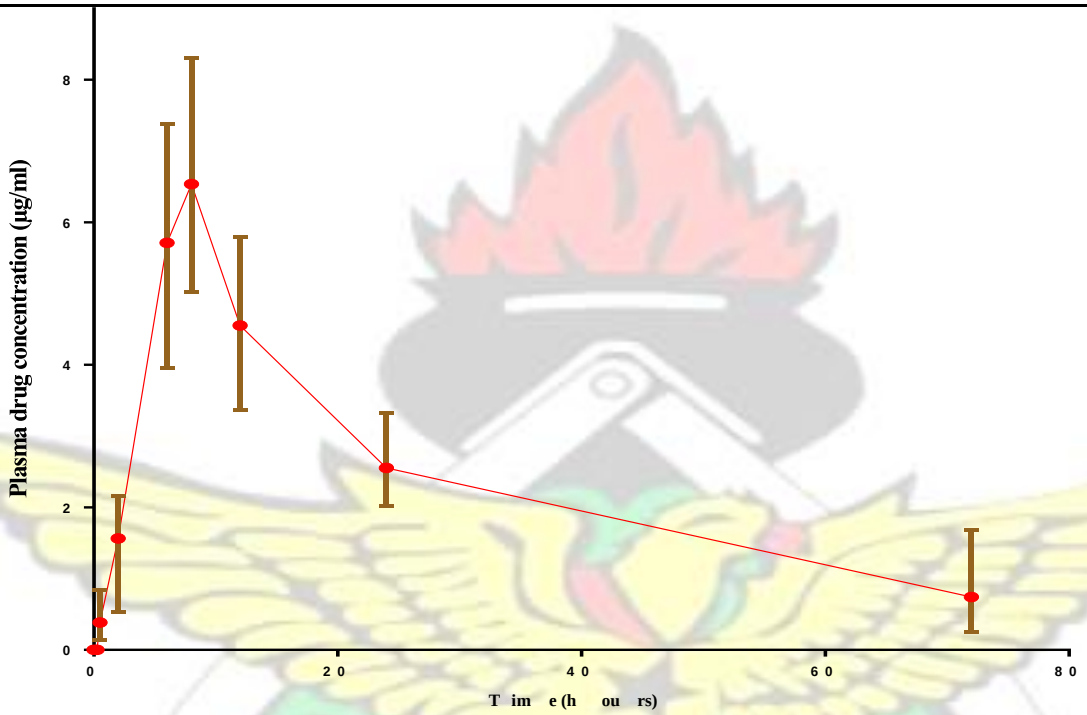

Figure 4.13 Mean plasma Lumefantrine concentration – time profiles obtained for INN

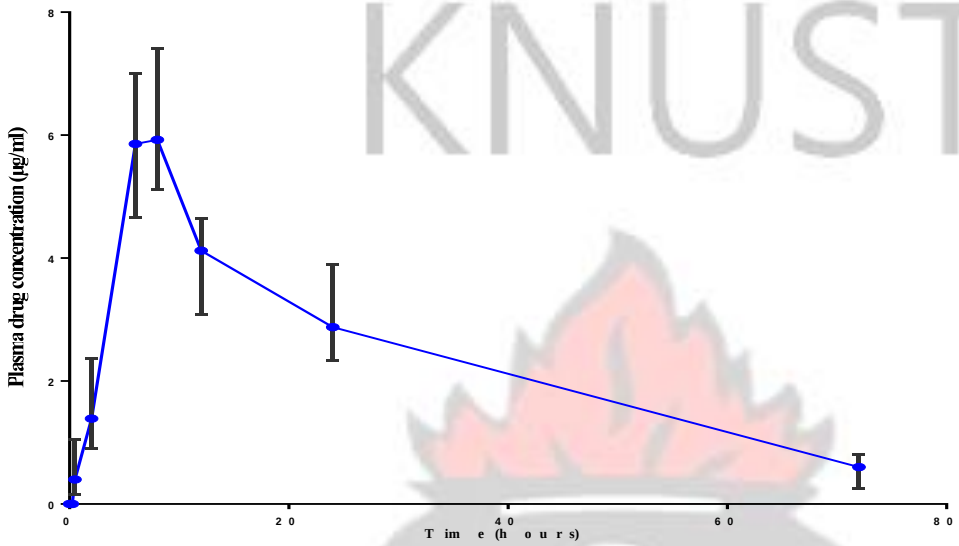

Figure 4.14 Mean plasma Lumefantrine concentration – time profiles obtained for LMG

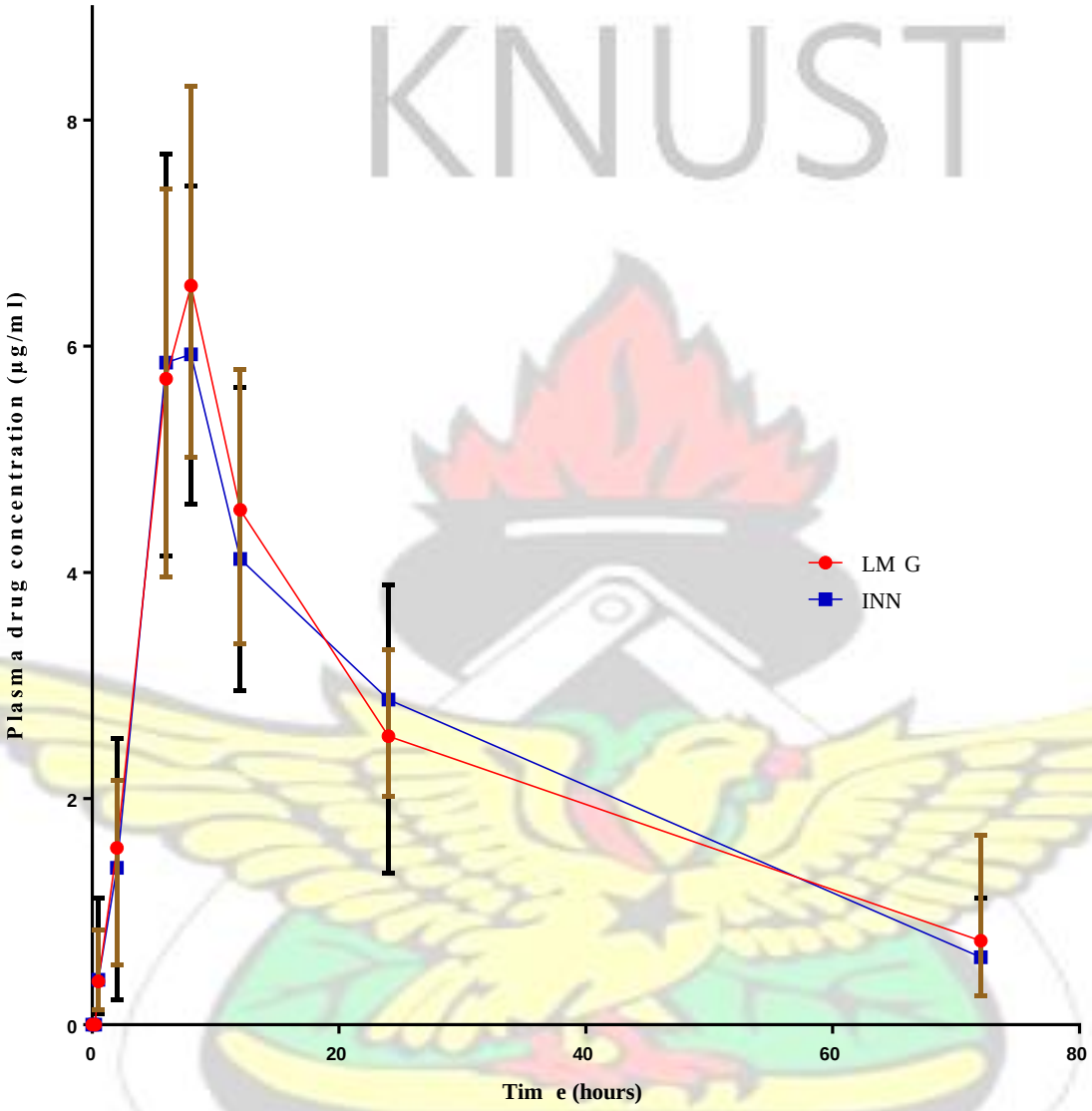

Figure 4.15 Mean plasma concentration – time profiles of Lumefantrine in subjects after oral administration of both INN and LMG.

4.6 Pharmacokinetic Assessment and Statistical Analysis

The results of the pharmacokinetic parameters are presented as mean ± standard deviation with reference to Lumefantrine.

Table 4.31 Ratio of average untransformed data and data obtained by Norvatis Pharma.

Pharmacokinetic Parameters	LMG	INN	P value	% Ratio of test to reference	Norvatis data
(AUC_{0-72}) (µg·hr/ml)	173.150 ± 18.12	172.336 ± 16.14	0.9086	100.47	--
($AUC_{0-\infty}$) (µg·hr/ml)	193.209 ± 19.56	207.493 ± 47.57	0.3141	93.12	108.0- 243.0
(C_{max}) (µg/ml)	6.173 ± 0.63	6.531 ± 0.84	0.2057	94.52	5.1 - 9.8
T_{max} (hr)	6.6 ± 0.97	8 ± 0.00	0.0013	82.50	6 – 8

[*Significantly different = (P < 0.10)*]

Table 4.32 Confidence interval for log – transformed data for Bioequivalence Assessment

Pharmacokinetic Parameters	Ratio of LMG to INN	90% Confidence Interval of the Geometric mean	Acceptable Range	P-value
$lnAUC_{0-72}$	100.36	93.2 - 108.0	80 – 125	0.9340
$lnAUC_{0-\infty}$	94.42	83.9 - 106.3	80 – 125	0.1024
lnC_{max}	96.76	86.8 - 103.4	80 – 125	0.1712

[*Significantly different = (P < 0.10)*]

Forest Plot showing the 90% confidence interval limits was plotted for the ratios of AUC_{0-72}, $AUC_{0-\infty}$ and C_{max} obtained from the study and the FDA requirements

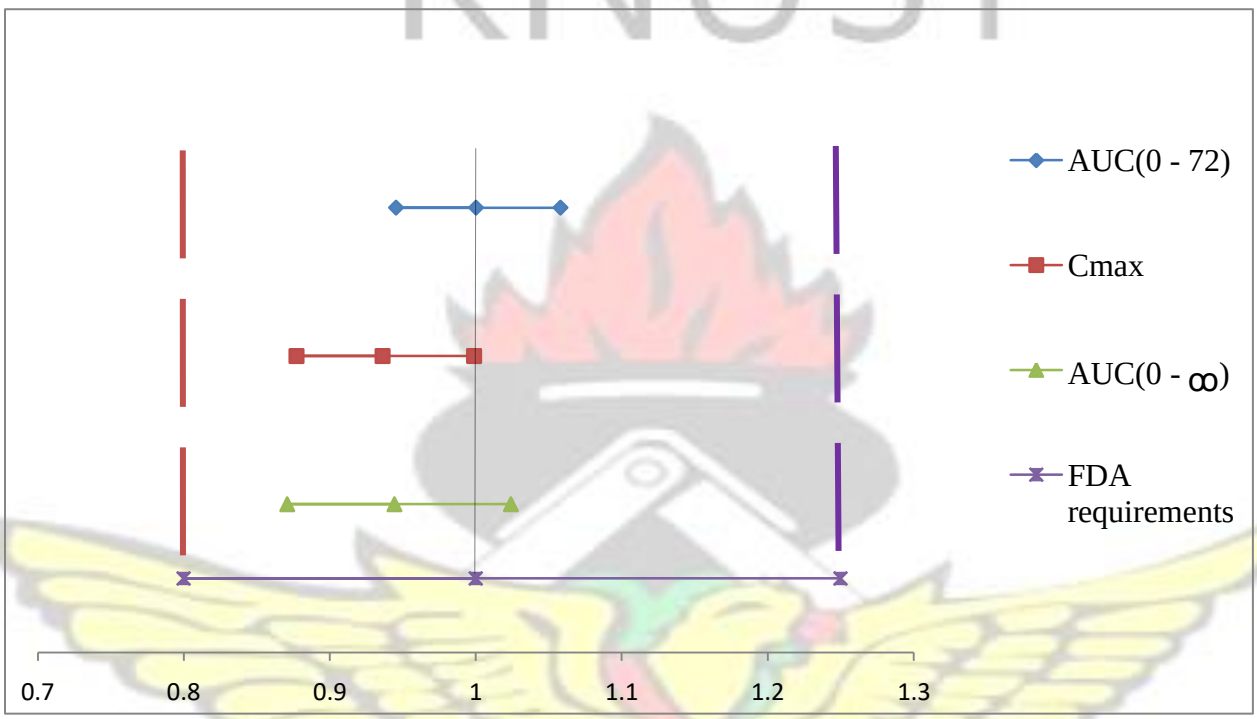

Figure 4.16 A forest plot showing the 90% confidence interval of the pharmacokinetic parameters plotted over the FDA requirement boundaries (blue and red dotted lines showing upper and lower limits respectively)

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

DISCUSSION

5.1 Analysis of Questionnaire

Sampling of the most available locally manufactured generic was done through the use of a questionnaire. A lot of generics of artemether-lumefantrine are seen on the market and the pilot survey sought to reveal how available each of them is on the market. Table 4.1 and 4.2 show the type of pharmaceutical retail outlet visited and how available antimalarial medications are present. It was observed that all the facilities visited had antimalarial medication on the shelves. This 100% availability can be attributed to the fact that malaria is very endemic in these parts of the world (World Health Organization, 2014) and to make medicines available to affected patients, the Ministry of Health has reclassified antimalarial therapies from prescription-only medications to over-the-counter medications. This reclassification meant that they no longer have to be prescribed by a clinician and dispensed by a pharmacist but permissible to be dispensed at all levels of healthcare to ensure ready availability to the general public. Thus all the outlets visited (both pharmacies and licensed chemical shops) had antimalarial medications.

Figure 4.2 shows the availability of the different brands of artemether-lumefantrine. The figure reveals that most of the generic brands of artemether-lumefantrine are imported mainly from India manufacturers with only two of the brands being locally manufactured. This observation coupled with the 100% availability of artemether-lumefantrine medicines at all the facilities visited shows that most of the artemether-lumefantrine tablet formulations used is imported mainly from India. High importation vis-à-vis low local production is not only present in the Ghana pharmaceutical

sector but also in sectors like food, textiles and the general consumer market (Seiter and Gyansa-Lutterodt, 2009). This high influx of foreign brands even though makes drug available to patients, does not encourage the local manufacturing industry. The high cost of production in terms of energy, inputs and capital can be said to be the main reason for most pharmaceutical companies opting to import artemether-lumefantrine tablets and repackage them rather than manufacture them locally. These challenges are not peculiar only to Ghana but most African countries. In his review, Sarkar pointed out that India accounted for 17.7% (up from 8.5% in 2002) of African pharmaceutical imports in 2011. In addition to the reasons mentioned earlier domestic manufacturers most often struggle to implement good manufacturing practices (GMP) and ensure quality production (Sarkar, 2014).

From Figure 4.2 Malar -2 showing 72% availability was the most available locally manufactured generic. On this basis it was selected as the generic to be compared to the innovator brand. Table 3.2 displays the information gathered on the various selected brands of artemether-lumefantrine. They were coded so as to eliminate any bias during the various analysis carried out on them. The samples of the brand purchased for the study were obtained from recognised outlets and had more than 6 months left on the shelf-life.

5.2 Identification of Artemether-Lumefantrine reference samples and tablets

Before reference and test samples were used, their identities were verified using preliminary identification tests. The samples were subjected to colorimetric tests and melting point determination and the results compared to those stated in literature. From the results obtained as shown in Table 4.4 and Table 4.5, the pure samples gave positive identification results for the colorimetric test. The melting point obtained for the pure samples were similar and complied with the melting point stated in the International Pharmacopoiea (World Health Organization, 2015a).

It can thus be inferred that the pure samples are of appropriate standards to be used as reference samples. The two sampled brands of artemether-lumefantrine combination tablets also showed positive results for the presence of artemether and lumefantrine.

5.3 Quality Control Assessment of Sampled Artemether-Lumefantrine

The assurance of quality medications requires a robust and stringent drug regulatory authority which has the capacity to adequately identify substandard products in circulation. This assurance is possible through the use of quality evaluation tests not only to determine the drug contents but also pinpoint or identifying the source (if any) of the poor quality of the drug. As pointed out by studies in 2010, the physicochemical properties of the active ingredients, excipients and the formulation of the drug product can have pronounce effect on the quality parameters of the dosage form. These include its hardness, friability, weight variation, disintegration time, dissolution and release profiles (Kalakuntla *et al.*, 2010, Ofori-Kwakye *et al.*, 2010). These parameters no matter how different they may seem are closely related to each other and all contribute to have an effect on the drug‟s bioavailability and ultimately it‟s absorption (Awofisayo *et al.*, 2010).

5.3.1 Uniformity of Weight Test on Artemether-Lumefantrine tablets

For a batch of solid oral dosage forms, a fundamental quality feature is a constant dose of the active ingredients among the individual dosage forms. Practically, there exist variations in the individual weight; nonetheless specifications in the pharmacopoeias establish acceptable limits.

These established limits tend to ensure that the variations with respect to weight and eventually the dose is reduced to a minimum as well as maintaining a consistency of dosage units during compression.

The brands of artemether-lumefantrine weighed less than 250 mg and thus to pass the uniformity of weight test, not more than two of the individual weights should deviate from the average weight by more than a percentage deviation of ± 7.5%. Furthermore, none should deviate by more than twice of the permissible range (British Pharmacopoeia, 2013). From the results obtained, the sampled brands passed the uniformity of weight test (Table 4.7). This can be attributed to factors such as good amount of fill placed in the die, good flow properties of granules and uniform compression force employed during the tableting process.

Since the variation of each tablet weight is a valid indication of the corresponding drug content variation, it can be deduced that the sampled brands are likely to have a low variation in their respective drug content (Rawlins, 1979).

5.3.2 Disintegration Test on Artemether-Lumefantrine

Disintegration test as applied to solid dosage forms (example tablets) is to evaluate the disintegration capability of such tablets which is very crucial when it comes to the immediate release of the drug from the tablets. Disintegration is said to be complete when any residue of the dosage unit except fragments of insoluble coating or capsule shell remaining on the screen of the test apparatus is a soft mass with no palpable firm core. According to the BP 2013, the specification for disintegration test stipulates that for uncoated tablets the disintegration time should be less than 15 minutes and within 30 minutes for film coated tablets (British Pharmacopoeia, 2013). Table 4.8 which is the results of the disintegration test showed that all the brands passed the test. Optimal force of compression, content of disintegrants and binder can be said to be the reasons the brands passed the disintegration test.

5.3.3 Friability Test on Artemether-Lumefantrine

For uncoated tablets, there exists some tendency for them to break, crumble and drop after compression. This tendency is termed friability. The friability test tends to assess the tablet‟s ability to withstand this tendency and maintain its integrity. In this test the BP 2013 specifies a weight loss of not more than 1% of the weight of tablet as satisfactory (British Pharmacopoeia, 2013). Table 4.9 shows that all the sampled brands (both generic and innovator) passed the friability test with the percentage weight loss of less than 1%. This can be attributed to appropriate compression force and sufficient binder used in the tablet manufacture (British Pharmacopoeia, 2013).

5.3.4 Assay of Artemether-Lumefantrine

A study by Awofisayo *et al*. showed that only two brands (out of six) of ArtemetherLumefantrine tablets on the Nigeria market complied with the specifications according to the International Pharmacopoeia (Awofisayo *et al.*, 2010). Another study in 2012 showed that only one brand of Artemether-Lumefantrine tablet on the Ghanaian pharmaceutical retail market actually contained the correct amount of the drugs (El-Duah and Ofori-Kwakye, 2012). These studies together with others give rise to concern with respect to how cheap substandard antimalarial flood the African market (Esimone *et al.*, 2008, Ofori-Kwakye *et al.*, 2008, OseiSafo *et al.*, 2010, Tipke *et al.*, 2008).

The IP specifies that Artemether-Lumefantrine co-formulation tablet should contain not less than 90.0% and not more than 110.0% of the labelled amount of artemether and lumefantrine respectively. The results obtained from the HPLC analysis as shown in Table 4.10 showed that all the brands fell within the monograph specification for both active ingredients. This shows that the sampled brands are of the right quality in terms of drug content. Strong regulations and strong

compliance of the pharmaceutical industry with the WHO good manufacturing practices might have contributed to this success.

5.3.5 Dissolution Testing

Solid immediate release dosage forms for oral administration are designed to disintegrate in the gastric fluid after which dissolution of the API follows. Dissolution is described as „the rate of mass transfer of the API from the solid surface into the medium under standardized conditions of liquid/solid interface, temperature and solvent composition" (Singhvi and Singh, 2011). By virtue of the process leading to absorption, disintegration is a subset of dissolution and the latter is very essential for systemic absorption of active ingredients. Dissolution testing is used to predict the in-vivo release of some solid dosage forms (Siewert *et al.*, 2003). Additionally dissolution testing is useful in distinguishing the influence of manufacturing variables such as binder effect and mixing effect on the overall release of the active medicaments.

The IP monograph for artemether-lumefantrine does not include specifications for dissolution test. Nonetheless there are some separate methods in the USP Salmous Standards 2009 for each drug. Due to different solubility characteristics of the two substances, getting a common dissolution medium is a huge challenge. A study in 2011 developed and validated a method to be used as a single dissolution test for both artemether and lumefantrine (Umapathi *et al.*, 2011). This method was adopted for the study of the release characteristics of artemether and lumefantrine in the sampled brands with slight modification (1% Tween 80 replacing 2% Myrj

52).

The USP Salmous Standards specifies that for artemether, not less than 45% of the labelled amount is dissolved in 60 minutes whiles for lumefantrine not less than 60% of the labelled amount is

dissolved in 45 minutes. From the results obtained in the dissolution study the release of artemether from both brands passed the tolerance standard (Table 4.11).

Conversely when the specifications for lumefantrine was juxtaposed with the results obtained in Table 4.12, at 45 minutes the percentage release was less than the 60% threshold. Thus both the innovator and generic brands failed the dissolution test with respect to the release of lumefantrine. The slight modification in the dissolution medium could have accounted for the slight deviation from the acceptance criterion as dissolution medium significantly affect
dissolution rate of drug.

5.3.6 Dissolution Profile Comparison

In the absence of in-vivo bioequivalence testing, comparison of dissolution profiles using f_1 and f_2, a model-independent mathematical method developed by Moore and Flanner can help in assuring similarities between products (Moore and Flanner, 1996). The f_2 which is the similarity factor measures the closeness of the two dissolution profiles whiles f_1, the difference factor is proportional to the average difference between the two profiles. For comparison of dissolution profile of different products the regulatory bodies tend to focus more on the f_2 comparison as it is tilted more towards knowing how similar the profiles are and to know a measure which is more sensitive to large difference at each time point (Costa, 2001). Normally, f_2 from 50 - 100 indicates similarity between two profiles. From the results, comparing the dissolution profiles of artemether, similarity factor was 91 and that for lumefantrine is 99 (Table 4.13). f_1 values, (which is a measure of the relative error between the two curves) up to 15 indicates minor difference. Table 4.13 shows f_1 values of 13 and 18 for the curves of artemether and lumefantrine respectively. Thus with the dissolution of artemether, there was only a minor difference in the release from the generic relative to the innovator. However the difference factor for the dissolution curves for lumefantrine fell

slightly outside the 0 – 15 range. Combining the f_1 and f_2 analysis conducted, it can be inferred that when it comes to the release of the two ingredients the dissolution profiles are similar even due to a very high similarity factor for both ingredients.

The dissolution efficiency (DE) as suggested by Khan and Rhodes is the area under the dissolution curve up to a certain time t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. It is determined so as to ascertain the extent to which a brand or batch will be effective in releasing the active ingredient (Khan and Rhodes, 1972). Generally, the higher the DE the more efficient a brand is in releasing the active ingredient. From the Table 4.14 LMG was more efficient in the release of artemether than the innovator brand with a higher DE of 88.35%. The DE of both brands in the case of lumefantrine was low indicating the release of that drug was not efficient. This relatively low DE value can be attributed to formulation factors such as amount and type of excipients e.g. disintegrants, granule size and its distribution. The dissolution medium could have also contributed to the low DE observed (Ghayas *et al.*, 2013).

5.4 HPLC Method Development and Validation

The simultaneous quantification of both artemether and lumefantrine in the sampled brands and in plasma was done with a developed HPLC method which was validated. The method was a modification of two previous studies (César *et al.*, 2011, Sarma *et al.*, 2014). It employed a mobile phase system of acetonitrile (67%) and acetate buffer pH=2.5 (33%) in a isocratic elution program using artesunate as the internal standard. The flow rate selected was 1.3 ml/min after different rates showed peak tailing and prolonged retention times. A run time of about 12 minutes was optimal for analytes to be observed on the chromatogram as shown in Figure 4.6. The ICH guidelines was utilised to validate the method developed. Linearity was observed with concentration over the range of 0.4 mg/ml to 8 mg/ml for artemether and 4 µg/ml to 40 µg/ml for lumefantrine. The

coefficient of correlation R^2 for both linearity curves were close to unity establishing good linearity between response and concentration. The LOD and LOQ of artemether were higher than that of lumefantrine. These parameters that test the sensitivity of the method showed that the method was very sensitive in detecting and quantifying lumefantrine than artemether (Armbruster and Pry, 2008). This can be attributed to the lack of strong absorbing chromophores in the artemether chemical structure vis-à-vis that of lumefantrine (Figure 2.1 and Figure 2.2).

A good precision was observed with the method. From Table 4.18, 4.19 and 4.20, it was observed that the relative standard deviation of the response fell below 2%. This means for both intra-day and inter-day the method would be precise in its response.

The method was shown to be robust as the response remain unaffected by small intentional changes in chromatographic conditions (such as wavelength and flow rate) resulted in a relative standard deviation of less than 2%.

In the quantification of both artemether and lumefantrine in the plasma, the response factor approach was utilised. In that approach the ratio of the respective response of the analyte and internal standard is directly proportional to the respective concentration ratio, and if the constant of proportionality is determined, it can be utilised to estimate the unknown concentration of subsequent samples. The import of the response factor approach is that in plasma analysis, there can be slight variation in analytical conditions as well as in runs and because the internal standard and analyte will be affected to the same extent, the proportionality factor is constant.

From Table 4.26 and 4.27, the average response factor of artemether and lumefantrine against artesunate showed low standard deviations indicating reliability of the factors.

In Table 4.28, when the response factor was utilised to estimate the recovery of spiked plasma samples with the two analytes, the lowest percentage recovery obtained was 96.62%. This showed good accuracy (high recovery) of the response factor approach in predicting the concentration of an unknown analyte.

5.5 In-vivo bioavailability studies using animal models

In the in-vivo bioavailability study, out of the analytes, lumefantrine was the only analyte to be adequately detected and quantified by the method as observed in Figure 4.12. A study in 2013 which also compared bioavailabilities of different artemether-lumefantrine formulations in Tanzania also reported that lumefantrine was the only analyte quantified in-vivo. It was subsequently used as the active ingredient to assess the bioequivalence between the formulations (Minzi *et al.*, 2013).

Two reasons might have contributed to this observation. The quantity of artemether per dose in the combination formulation is lower relative to lumefantrine (ratio of 1:6). Thus a low amount coupled with first pass effect and lack of absorbing chromophore might have contributed to the non - detection of the artemether from the extracted plasma by the Ultra-Violet detector.

Figure 4.15 shows comparable plasma concentration – time curves of lumefantrine obtained after the administration of a single dose of the two samples. The mean pharmacokinetic parameters obtained from the concentration – time profiles showed no statistical difference ($p > 0.05$) between both brands except T_{max} (Table 4.31). This implies the AUCs and C_{max} which measure the extent and rate of absorption of drug from both brands were similar even though the T_{max} is dissimilar. This is because most regulatory authorities rely on C_{max} rather than T_{max} to estimate the rate of absorption in bioavailability studies (Chen *et al.*, 2001).

The pharmacokinetic parameters obtained were all within the range of values by Novartis Pharma (manufacturers of the innovator brand), thus giving a strong indication of both brands achieving comparable plasma levels of lumefantrine (Minzi *et al.*, 2013). In the bioequivalence assessment of the two brands, the geometric mean ratios of all the various pharmacokinetics parameters (AUC_{0-72}, $AUC_{0-\infty}$, and C_{max}) were within the FDA criterion of bioequivalence of 0.80 – 1.25. In comparing the 90% confidence interval obtained for the pharmacokinetic parameters to the criterion range, all the intervals fell within the range as shown in Table 4.32. The confidence intervals of the ratios of AUC_{0-72}, $AUC_{0-\infty}$, and C_{max} on a forest plot showed the intervals to be within the boundary of the FDA criterion range (Figure. 4.16). Accordingly it can be inferred that based on this study the two tablet formulations meet the FDA bioequivalence criteria.

Thus the innovator can adequately be interchanged with the generic brand. In recent times, a number of studies in the post marketing setting have highlighted some lack of equivalence between generic and innovator brands (Del Tacca *et al.*, 2009, Elkoshi *et al.*, 2002, Minzi *et al.*, 2013). Nonetheless the similarity that has been exhibited in this study can be taken as evidence in support of therapeutic effectiveness and safety of the generic product LMG.

CONCLUSIONS

From the experiments conducted and subsequent deductions made in the discussion, the following conclusions were drawn;

- Even though artemether-lumefantrine coformulation tablets are widely distributed in the pharmaceutical retail outlets, most of them are imported brands.

- The brands of artemether-lumefantrine sampled for this study satisfied the specification for identification, disintegration test, uniformity of weight test, friability test and percentage content.
- The sampled brands satisfied the specification for dissolution testing with respect to artemether but failed that of lumefantrine. Statistical analysis showed similar dissolution profiles for artemether but not for lumefantrine.
- An accurate, robust RP-HPLC method was developed, validated and utilised to estimate the lumefantrine levels in plasma for the bioavailability studies.
- Average bioequivalence between LMG and INN was demonstrated due to compliance with the FDA 90% confidence intervals. This makes them bioequivalent and thus LMG can be interchanged with INN as it is likely to produce a similar therapeutic response.

RECOMMENDATIONS

- Further in-vivo bioequivalence studies involving healthy human volunteers could be performed on the sampled brands.
- An even more sensitive analytical technique (HPLC-MS/MS) could be used in subsequent invivo bioavailability studies so as to also quantify the artemether component of the formulations.

- Similar bioequivalent studies can be performed on the imported brands of artemetherlumefantrine so as to get more evidence to support interchangability decisions or otherwise.
- Regulatory bodies after initial approval should actively perform bioequivalent studies in the post-market setting so as to ensure as well as boost adequate monitoring of the quality of generic drugs.

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