**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI**

**COLLEGE OF HEALTH SCIENCES**
**FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES**
**DEPARTMENT OF PHARMACEUTICAL CHEMISTRY**

# DESIGN OF SIMPLE UV SPECTROPHOTOMETRIC AND HPLC METHODS FOR THE ASSAY OF ARTEMETHER AND LUMEFANTRINE IN FIXED-DOSE COMBINATION TABLETS

BY

DANIEL AFOSAH

MAY, 2010

# DESIGN OF SIMPLE UV SPECTROPHOTOMETRIC AND HPLC METHODS FOR THE ASSAY OF ARTEMETHER AND LUMEFANTRINE IN FIXED-DOSE COMBINATION TABLETS

A THESIS SUBMITTED IN PARTIAL FULFILMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN PHARMACEUTICAL

ANALYSIS AND QUALITY CONTROL

In the

Department of Pharmaceutical Chemistry,

Faculty of Pharmacy and Pharmaceutical Sciences

BY

**DANIEL AFOSAH**

**KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,**

**KUMASI**

MAY, 2010

## DECLARATION

The experimental work described in this thesis was carried out at the Department of Pharmaceutical Chemistry, KNUST. Any assistance obtained has been duly acknowledged. This work has not been submitted for any other degree.

HEAD OF DEPARTMENT

……..

DR. R. K. ADOSRAKU

SUPERVISOR

……

PROF. J. S. K. AYIM

CANDIDATE

……

DANIEL AFOSAH

## ABSTRACT

**Artemether-Lumefantrine** combinations have become very important in the treatment of uncomplicated malaria. However, there are very few methods to assay these fixed-dose combination formulations. To help in ensuring the quality of these medications, simple and sensitive Ultra-Violet (UV) Spectrophotometric and High Performance Liquid Chromatographic (HPLC) methods of assaying **Artemether** and **Lumefantrine** in fixed-dose combination tablets were developed and validated.

The UV Spectrophotometric method for the assay of **Lumefantrine** made use of a 0.1M methanolic HCl solution of **Lumefantrine**, with the analysis being carried out at 335nm whilst the UV assay of **Artemether** made use of its HCl decomposition product, with the analysis being carried out at 254nm. In the fixed-dose combination tablets, the UV methods proved suitable for the assay of only **Lumefantrine**, as **Lumefantrine** interfered with the analysis of **Artemether**. The assay of **Artemether** by the UV Spectrophotometric method was however possible in **Artemether**-only tablet formulations.

The HPLC method required the separate analysis of **Artemether** and **Lumefantrine** as a simultaneous analysis of the two was not possible. The assay of **Lumefantrine** employed a C18 reverse phase column (Ultracarb 3μ ODS (20)) with an isocratic mixture of methanol and 0.1% Trifluoroacetic acid in water (90:10) as the mobile phase. The flow rate was 2.5ml/min and detection was by means of a UV detector set to 335nm. The retention time of **Lumefantrine** was 6.0 ± 0.19 minutes. The assay of **Artemether** employed the same C18 reverse phase column with an isocratic mixture of methanol and 0.04% Trifluoroacetic acid (90:10) as the mobile phase, pumped at a rate of 2.5ml/min. Detection was carried out with a UV detector set to 235nm. The retention time of **Artemether** was 5.8 ± 0.15 minutes.

Six brands of **Artemether-Lumefantrine** fixed-dose combination tablets on the Ghanaian market (one local and five foreign) were analyzed. All the brands passed the Uniformity of Weight Test and the Tablet Disintegration Test. Of the Six brands of **Artemether-Lumefantrine** fixed-dose combination tablets analyzed, all passed with respect to their **Lumefantrine** content using both UV and HPLC. Two of the foreign brands however failed with respect to their **Artemether** content.

# DEDICATION

I dedicate this work to my parents, Ebenezer Daniel Afosah and Comfort Somuah and my brother, Richard Holland

## ACKNOWLEDGEMENT

I would like to thank the Almighty God for seeing me through this course successfully.

My heartfelt gratitude goes to Prof. J. S. K. Ayim (my project supervisor) for his unflinching support and kindness; his patience and guidance helped me greatly in carrying out this work.

My appreciation goes to my brothers (especially Richard Holland) and also to my mother Comfort Somuah for the enormous faith they have in me.

My appreciation to my friends: Joy, Kenneth and Kevin for the assistance they gave me in carrying out this work.

My gratitude also extends to all my colleagues, to the teaching and non-teaching staff of the Department of Pharmaceutical Chemistry at the Kwame Nkrumah University of Science and Technology.

May God bless all those who supported and encouraged me throughout my programme of study.

# TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## ABBREVIATIONS

ACT: Artemisinin-based Combination Therapy

AM – LM: Artemether-Lumefantrine

AM: Artemether

BP: British Pharmacopoeia

DHA: Dihydroartemisinin

HPLC: High Performance Liquid Chromatography

ICH: International Conference on Harmonization

IP: International Pharmacopoeia

LM: Lumefantrine

LOD: Limit of Detection

LOQ: Limit of Quantitation

ODS: Octadecylsilane

$R_f$: Retardation factor

RP-HPLC: Reverse Phase HPLC

RSD: Relative Standard Deviation

SALMOUS: Standards for Articles Legally Marketed Outside the U.S

SD: Standard Deviation

TLC: Thin Layer Chromatography

USP: United States Pharmacopoeia

UV: Ultra-Violet

WHO: World Health Organization

# CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

## 1.1 INTRODUCTION

Malaria is an important cause of morbidity and mortality in children and adults in tropical countries. Mortality, currently estimated at over a million people per year, has risen in recent years. Malaria control requires an integrated approach made up of prevention including vector control and treatment with effective antimalarials (WHO Guidelines for Treatment of Malaria, 2010).

Drug quality assurance is a very important in the fight against malaria. However, quality assurance of antimalarial drugs in the third world countries where malaria is endemic is often a neglected issue. Lack of quality drugs for malaria treatment can result in many dire consequences which can range from failure of therapy to death of patients. Another serious consequence is the development of resistance to antimalarial medication by Plasmodium species which has led to the ineffectiveness of some important antimalarial medications, an example of which is chloroquine. To counter the threat of resistance of *P. falciparum* to monotherapies, and to improve treatment outcomes, combinations of antimalarials are now recommended by the WHO for the treatment of falciparum malaria (WHO Fact Sheet, 2010).

The **Artemisnin-based Combination Therapies (ACTs)**, which are recommended by the WHO, have become the main-stay of malaria treatment. **Artemether-Lumefantrine** is the first fixed-dose ACT regimen to be manufactured under Good Manufacturing Practice conditions, and is the most widely adopted ACT regimen used in malaria control programs (Adjei, Goka et al. 2009). It is necessary to prevent the development of resistance by plasmodium to the ACTs including **Artemether** and **Lumefantrine**. To achieve this, there is the need to ensure the quality of these medications as substandard drugs can lead to the development of resistance amongst other consequences. There are however a few methods to assess the quality of **Artemether** and **Lumefantrine** products and thus, this research work aims to make a contribution to the assessment of these products and also assess some of these products on the Ghanaian market.

## 1.2 JUSTIFICATION

The increasing use of **Artemether–Lumefantrine** combination as an effective treatment for resistant malaria demands the need of analytical methods to quantify these formulations in order to evaluate their quality.

Previous works have described the analysis of **Artemether** in plasma, using HPLC with electrochemical or mass spectrometry detection. Previous studies have also reported the determination of **Lumefantrine**, using HPLC with UV detection at 335nm and focusing mainly in its quantitation in plasma or blood. However, methods for **Artemether** and **Lumefantrine** determination in pharmaceutical products, such as tablets are very few. These methods available require expensive reagents and equipment, are time consuming and are cumbersome to perform.

There is no UV method for the assay of **Lumefantrine** in the official monographs. The UV method described in the IP for the assay of **Artemether** requires heating at 60˚C for 5 hours. The time and energy requirements of this method of assay are very high making it less favourable as an analytical method. The HPLC methods of assay of **Artemether** and **Lumefantrine** in fixed-dose combination tablets in the USP SALMOUS Standard and the IP utilize gradient elution. Also, a long run time of up to 55 minutes per injection is required. Acetonitrile is also the major component of the mobile phases in these methods of analysis. Acetonitrile is very expensive and thus the development of a method which utilizes a cheaper mobile phase would be appreciated.

In the tropical countries where the disease burden is high, it is important to develop analytical techniques that are simple, fast and less expensive

## 1.3 OBJECTIVES

The objectives of the research work are

- To develop and validate simple UV/Vis Spectrophotometric methods for the assay of Artemether and Lumefantrine in fixed-dose combination tablets
- To develop and validate HPLC methods for the assay of Artemether and Lumefantrine in fixed dose combination tablets
- To use the developed methods to assay brands of fixed-dose Artemether and Lumefantrine combination tablets on the Ghanaian market

## 1.4 LITERATURE REVIEW

### 1.4.1 MALARIA

Malaria is an important cause of death and illness in children and adults in tropical countries. Human malaria results from infection with *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* or *Plasmodium malariae*. Occasional infections with monkey malaria parasites, such as *Plasmodium knowlesi*, also occur (WHO guidelines for treatment of malaria, 2010). Malaria parasites are transmitted from one person to another by the female anopheline mosquitoes. The parasites develop in the gut of the mosquito and are transmitted each time it takes blood from a human being. The male mosquitoes do not transmit the disease, as they feed only on plant juices. *Plasmodium falciparum* causes a large majority of the clinical cases and mortalities (Bozdech et al., 2003).

The infection starts with a mosquito bite and the sporozoites are then carried by the blood to the liver where they invade the cells and multiply asexually. After 9–16 days, the merozoites emerge from the liver and infect the red blood cells. The merozoites also get attached to the endothelium of the blood vessels, where they multiply again, progressively breaking down the red cells. The infection gets further transmitted via gametocytes back to the mosquitoes when the next mosquito bites and the whole cycle follows. In cerebral malaria, the infected red cells obstruct the blood vessels in the brain.

Clinical manifestations can include fever, chills, prostration and anaemia. Severe disease can include *delirium, metabolic acidosis, cerebral malaria and multi-organ system failure*. Coma and death may ensue.

#### 1.4.1.1 Prevalence/Epidemiology of Malaria

Malaria is a barrier to national economic growth and poses a constant threat to health, well-being and economic stability to millions of poor people worldwide. The level of malaria transmission varies in different regions, countries and also within countries. Malaria transmission rates can differ depending on local factors such as, rainfall patterns, temperature, the proximity of mosquito breeding sites to people, and types of mosquito species in the area. In the malaria endemic countries, living conditions are often poor, including scarce access to clean portable drinking water. Some regions have a fairly constant

number of cases throughout the year and these regions are termed "malaria endemic". In other areas there are "malaria seasons" usually coinciding with the rainy season.

There were 247 million cases of malaria in 2006, causing nearly one million deaths, mostly among African children (WHO fact sheet, 2008). According to recent publication, Africa has the highest endemicity of malaria where 0.35 billion people are at a high level of risk of getting malaria (Hay, Guerra et al. 2009). It is well established that in areas of moderate or high malaria transmission, where adults usually have a high level of immunity to malaria, falciparum malaria is more common. Malaria is also more common in pregnant than non-pregnant women. The pattern of infection in pregnancy is comparable to that observed in infants and children (Brabin 1983). In Africa, malaria in pregnancy is usually caused by strains of *Plasmodium falciparum* that express unique variant surface antigens which allow the parasite to sequester in the placenta by binding to chondroitin sulphate A (Bozdech, Llinas et al. 2003).

### 1.4.2 ANTIMALARIAL DRUGS

In many parts of the malaria endemic areas, particularly the African region, the only effective method of preventing the mortality and reducing the morbidity caused by the disease is through the use of antimalarial drugs (Olaniyi A. A., 2005). An ideal antimalarial drug should have the following characteristics.

- Rapidly relieve symptoms of the disease.
- It should be harmless to the patient and have no unpleasant side-effects
- It should preferably destroy all the stages of development of plasmodium species including the gametocytes
- It should be economically cheap and easy to administer

#### 1.4.2.1 Classification of antimalarial drugs

Antimalarial drugs can be classified according to

1. **Type of antimalarial activity and**
    - Tissue schizonticides
        - for causal prophylaxis e.g. Pyrimethamine and Primaquine
        - for preventing relapse e.g. Primaquine:

- Blood schizonticides e.g. Chloroquine, Quinine, Mefloquine, Halofantrine, and Lumefantrine
- Gametocytocides e.g. Chloroquine and Quinine
- Sporontocides e.g. Primaquine and Chloroguanide.

## 2. Structure of the compound

- Aryl Amino Alcohols – Quinine, Quinidine, Mefloquine, Halofantrine, Lumefantrine.
- 4-aminoquinolines – Chloroquine, Amodiaquine
- Folate synthesis inhibitors – Sulphonamides, Biguanides like Proguanil and Chloroproguanil
- 8-aminoquinolines – Primaquine
- Peroxides – Artemisinin derivatives and analogues
- Antimicrobials – Tetracyclines, Clindamycin, Azithromycin, Fluoroquinolones
- Naphthoquinones – Atovaquone
- Iron chelating agents – Desferrioxamine

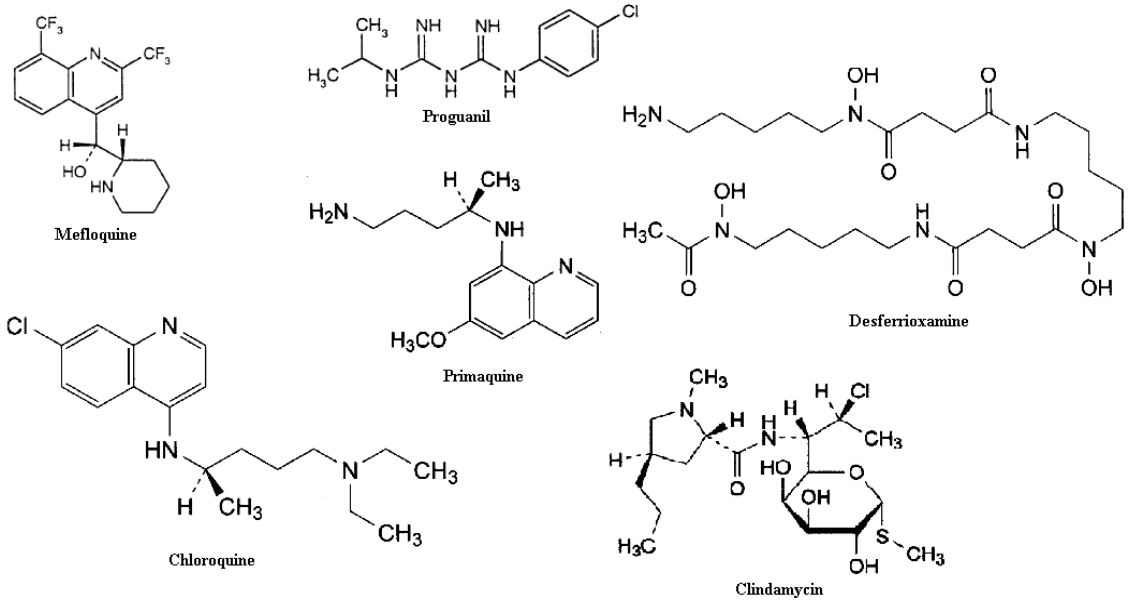



**Fig. 1 Structures of Some Antimalarial Drugs**

### 1.4.2.2 Antimalaria Drugs Currently in Use

Antimalarial drug resistance has now become a serious global challenge and it is the principal reason for the decline in antimalarial drug efficacy (Taylor, Rigal et al. 2003). Several drugs are effective but the emergence of parasite resistance limits the choice in various parts of the world. Resistance to **mefloquine** and even to **quinine** has been reported in Southeast Asia (Pickard, Wongsrichanalai et al. 2003).

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 outcome, combinations of antimalarials are now recommended by the WHO for the treatment of falciparum malaria (WHO Fact Sheet, 2010). The most important of the combination of antimalarials are the ACTs which combine artemisinin based antimalarials with other antimalarials such as the aryl amino alcohol anitmalarials. Artemisinin-based combinations offer a new and potentially highly effective way to counter drug resistance (Atemnkeng, De Cock et al. 2007).

Currently, the WHO recommends the following ACTs in the treatment of uncomplicated malaria.

- Artemether + Lumefantrine,
- Artesunate + Amodiaquine,
- Artesunate + Mefloquine,
- Artesunate + Sulfadoxine–Pyrimethamine.

### 1.4.2.3 Artemisinin-Based Antimalarial Drugs

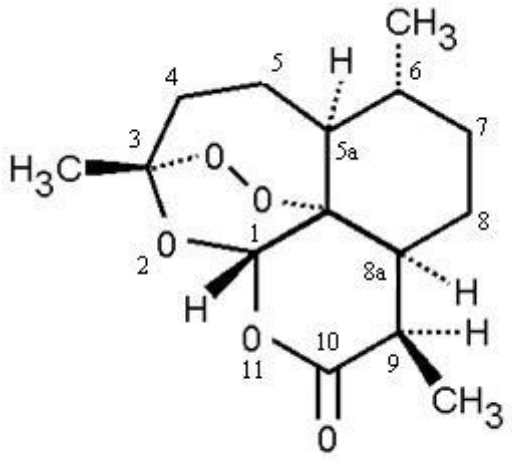



**Fig. 2 Structure of Artemisinin**

Artemisinins form the most important class of antimalarials currently available, particularly because they are effective against parasites resistant to almost all the other classes (Krishna, Uhlemann et al. 2004). Artemisinin is a natural product extracted from *Artemisia annua* or sweet wormwood (*qinghao*) which has been used for many centuries in Chinese traditional medicine as a treatment for fever and

malaria. (Klayman 1985; Woodrow, Haynes et al. 2005). Artemisinin is a sesquiterpene lactone. Its structure, which includes an endoperoxide bridge (C-O-O-C), is unique among antimalarial drugs. Dihydroartemisinin is the reduced lactol derivative of artemisinin, and the semisynthetic derivatives artemether, arteether, artesunate and artelinate are ethers or esters of the lactol (Meshnick, Taylor et al. 1996).

In general, the endoperoxides have several advantages over existing antimalarial drugs. Firstly, there is little or no cross-resistance with other antimalarial drugs. Secondly, the endoperoxides clear the peripheral blood of parasites more rapidly than other available drugs do. Finally, resistance to the endoperoxides has not yet developed despite widespread clinical use (Meshnick, Taylor et al. 1996). A disadvantage however with the endoperoxides is that, they have short half-lives, and effective levels in plasma are sustained for only relatively brief periods (Meshnick, Taylor et al. 1996). Therefore, the WHO recommends their use in combination with long acting antimalarial drugs such as **lumefantrine** or **mefloquine** to manage drug resistance, recrudescence, and non compliance (Gautam, Ahmed et al. 2009). These combination products are recommended over monotherapies because they help to prevent the development of resistant parasites. Over 40 countries in sub-Saharan Africa have adopted artemisinin-based combination therapy.

#### 1.4.2.3.1 Artemisinin Derivatives

Artemisinin itself is a highly crystalline compound that does not dissolve in oil or water and so can only be given by the enteral route (Woodrow, Haynes et al. 2005). This resulted in the need to synthesize derivatives with better properties for administration. The parent compound has been chemically modified at the C10 position to produce artesunate, artemether, arteether, dihydroartemisinin, and artelinic acid. These compounds have variously been formulated for oral, rectal, and parenteral administration.

In the synthesis of artemisnin derivatives, Artemisinin is reduced with sodium borohydride to produce dihydroartemisinin as a mixture of epimers (Olaniyi A. A., 2005). To produce Artemether, the mixture is treated with methanol and an acid catalyst (Haynes and Vonwiller 1994). Artemether can also be prepared from dihydroartemisinin using boron trifluoride. Artesunate is produced by esterification of dihydroartemisinin using succinic anhydride under basic conditions (Chekem and Wierucki 2006).

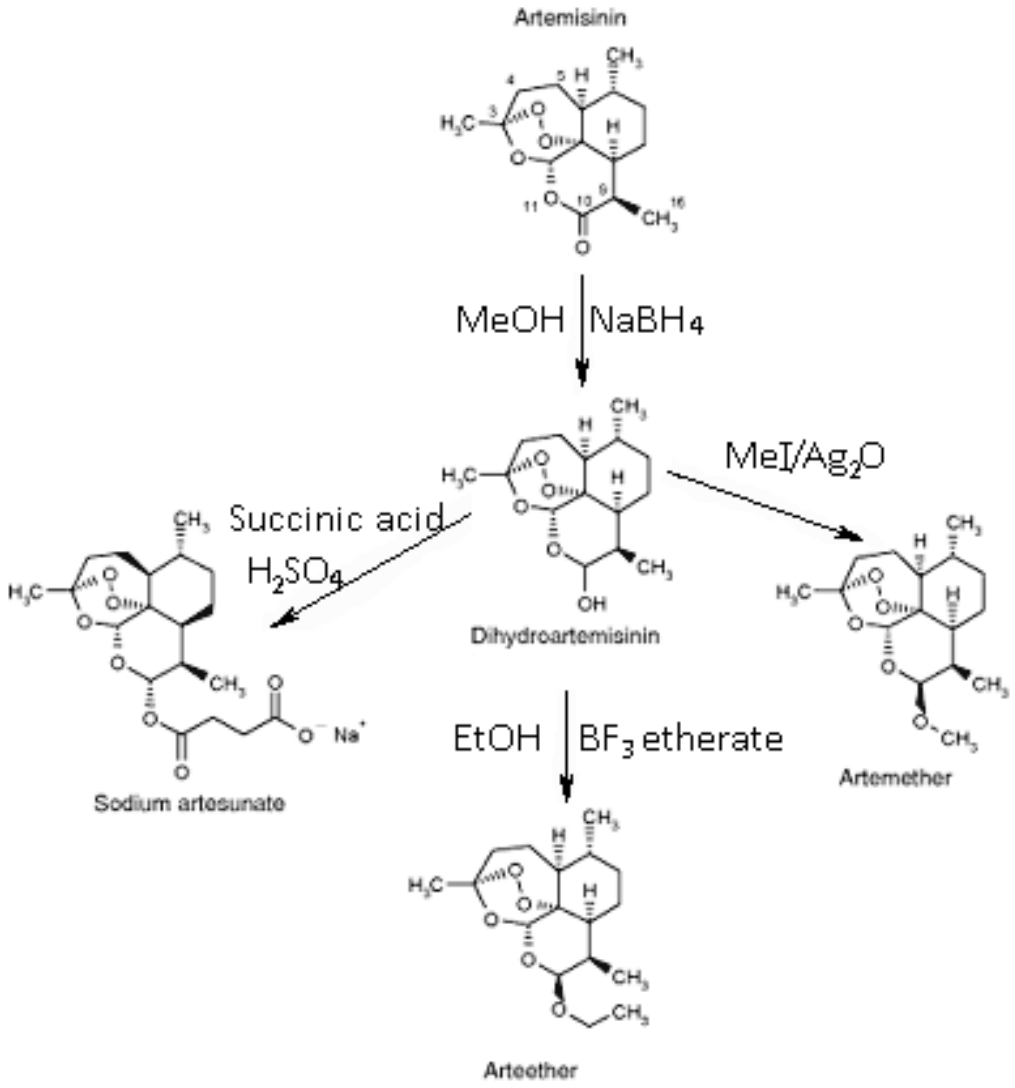



**Fig. 3 Synthesis of Artemisinin Derivatives**

#### 1.4.2.3.2 Mechanism of Action of Artemisinins

The peroxide structure of the artemisinins is essential for activity but their mechanism of action is controversial (Krishna, Uhlemann et al. 2004). For several decades, the antimalarial action of artemisinins has been attributed to their chemical capability to generate free radicals. This mechanism of action has been suggested partly on the grounds that well recognized sources of free radicals (such as tert-butylperoxide) can themselves kill malaria parasites, albeit in comparatively high concentrations (Clark, Hunt et al. 1984).

In the presence of intra-parasitic iron, these drugs are converted into free radicals and other electrophilic intermediates which then alkylate specific malaria target proteins (Meshnick, Taylor et al. 1996). An alternative mechanism of action for artemisinins based on inhibition of the malarial parasite's calcium ATPase (sarcoplasmic endoplasmic reticulum calcium ATPase, SERCA) has also been suggested. Their potency has been proven to be similar to thapsigargin which is another sesquiterpene lactone, a highly specific SERCA inhibitor (Eckstein-Ludwig, Webb et al. 2003)

### 1.4.2.4 Arylamino Alcohol Antimalarials

The aryl amino alcohol group of antimalarials include, quinine, quinidine, mefloquine, halofantrine, lumefanrine and chloroquine. Their use as antimalarials has been of great importance. Biochemical studies suggest that their antimalarial effect involves lysosomal trapping of the drug in the intra-erythrocytic parasite, followed by binding to toxic haemin that is produced in the course of haemoglobin digestion. This binding prevents the polymerization of haemin to non-toxic malaria pigment (WHO, 2001).

### 1.4.2.5 Quality of Antimalarial Agents

There are several reports of sub-standard and counterfeit antimalarial drugs circulating in the markets of developing countries. A review of literature published up to, and including 26$^{th}$ February 2007, on the quality of antimalarial drugs indicated that:

(i) most antimalarial products pass the basic tests for pharmaceutical dosage forms, such as the uniformity of weight for tablets,

(ii) most antimalarial drugs pass the content test and,

(iii) in-vitro product dissolution is the main problem area where most drugs fail to meet required pharmacopoeial specifications, especially with regard to sulfadoxine-pyrimethamine products (Amin and Kokwaro 2007).

Recently in Ghana, there was a report of fake Coartem® tablets on the market. The fake drug found in Ghana did not contain the active pharmaceutical ingredients of the Novartis Coartem® product it was being sold as, posing a significant health threat to patients relying on the medication (Eurekalert, 2009).

Also, a study on the Quality of active ingredients in artemisinin-derivative antimalarials within Kenya and DR Congo gave the following findings; nine of the 24 drug samples analyzed did not comply with the pharmacopoeial requirements of 95–105%: seven samples were under dosed and two were slightly overdosed. DHA was the active ingredient in 57% of the under dosed samples. Arteether injections had the lowest drug content (77%). Two-thirds of the dry powder suspensions were either substandard or fake. Tablets were up to 23% out of range (Atemnkeng, De Cock et al. 2007).

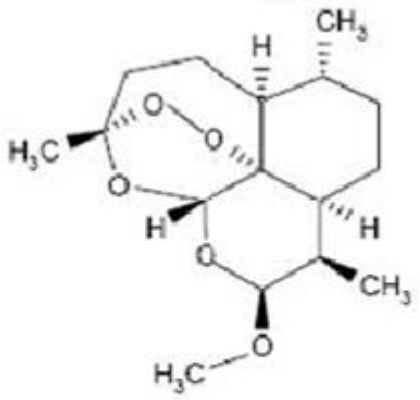
Recently, partial artemisinin-resistant *P. falciparum* malaria has emerged on the Cambodia–Thailand border. Exposure of the parasite population to artemisinin monotherapies in subtherapeutic doses for over 30 years, and the availability of substandard artemisinins, have probably been the main driving force in the selection of the resistant phenotype in the region (Dondorp, Yeung et al.)

#### 1.4.2.6 Artemether-Lumefantrine Combinations

**Artemether** and **Lumefantrine** exhibit complementary pharmacokinetic profiles. **Artemether** is absorbed quickly. Peak concentrations of **Artemether** and its main active metabolite, dihydroartemisinin (DHA) occur at approximately two hours post-dose, leading to a rapid reduction in asexual parasite mass and a prompt resolution of symptoms. **Lumefantrine** is absorbed and cleared more slowly (terminal elimination half-life 3–4 days in malaria patients), and accumulates with successive doses, acting to prevent recrudescence by destroying any residual parasites that remain after **Artemether** and DHA have been cleared from the body. **Artemether-Lumefantrine** combination formulations are available as fixed dosed tablets. Dispersible tablets and powders for reconstitution into suspensions are also available for infants and young children.

### 1.4.3 PROFILE OF ACTIVE INGREDIENTS UNDER STUDY

#### 1.4.3.1 Artemether



**Artemether** is an ether derivative of artemisinin used for the treatment of multi-drug resistant strains of *falciparum* malaria. is now the most widely used artemisinin derivative in the treatment of malaria. It is more lipid soluble than artemisinin or artesunate and is remarkably well tolerated.

**Fig. 4 Structure of Artemether**

- **Chemistry**

**Artemether** is available as White crystals or a white, crystalline powder which melts at 86-90˚C. Its chemical name is *(3R,5aS,6R,8aS,9R,10S,12R,12aR)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin* and it has a molecular mass of 298.4. The compound is practically insoluble in water; very soluble in dichloromethane, chloroform and acetone; freely soluble in ethyl acetate, dehydrated ethanol and methanol.

Its Specific optical rotation using a 10 mg/ml solution in dehydrated ethanol is

$[\alpha]_D^{20°C} = +166°$ to $+173°$.

**Artemether** is assayed by HPLC and UV Spectrophotometry and should contain not less than 99.0% and not more than the equivalent of 102.0% of $C_{16}H_{26}O_5$ calculated with reference to the dried substance (IP, 2008).

- **Formulations**
  - Capsules containing 40 mg of artemether.
  - Tablets containing 50 mg of artemether.
  - Ampoules of injectable solution for intramuscular injection containing 80 mg of artemether in 1 ml for adults or 40 mg of artemether in 1 ml for paediatric use.
  - In a co-formulation with Lumefantrine:
    - Tablets containing 20 mg of **Artemether** and 120 mg of **Lumefantrine**.

- **Pharmacokinetics**

Peak plasma concentrations occur around 2–3 hours after oral administration (Ezzet, Mull et al. 1998). Following intramuscular injection, absorption is very variable, especially in children with poor peripheral perfusion. Peak plasma concentrations generally occur after around 6 hours but absorption is slow and erratic and times to peak can be 18 hours or longer in some cases (Hien, Davis et al. 2004). Artemether is metabolized to DHA, the active metabolite. After intramuscular administration, Artemether predominates, whereas after oral administration DHA predominates. Biotransformation is mediated via the cytochrome P450 enzyme CYP3A4. Autoinduction of metabolism is less than with artemisinin. Artemether is 95% bound to plasma proteins. The elimination half-life is approximately 1 hour, but following intramuscular administration the elimination phase is prolonged because of continued absorption. No dose modifications are necessary in renal or hepatic impairment.

- **Toxicity**

In all species of animals tested, intramuscular **Artemether** and **Arteether** cause an unusual selective pattern of neuronal damage to certain brain stem nuclei. Neurotoxicity in experimental animals is related to the sustained blood concentrations that follow intramuscular administration, since it is much less frequent when the same doses are given orally, or with similar doses of water-soluble drugs such as artesunate. Clinical, neurophysiological and pathological studies in humans have not shown similar findings with therapeutic use of these compounds (Hien, Davis et al. 2004). Toxicity is otherwise similar to that of artemisinin.

### 1.4.3.2 Lumefantrine

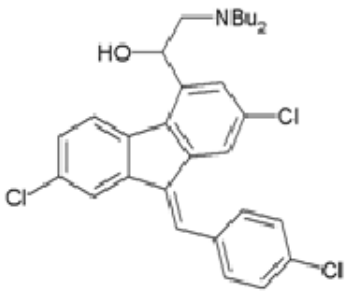



**Fig. 5 Structure of Lumefantrine**

**Lumefantrine** (previously called **benflumetol**) was synthesized originally in the 1970s by the Academy of Military Medical Sciences in Beijing, China (WHO, 1990). It conforms structurally, physicochemically and in mode of action to the aryl amino alcohol group of antimalarial agents including quinine, mefloquine and halofantrine (Pradines, Tall et al. 1999). The precise mode of the efficacy of lumefantrine on plasmodia is not fully understood. It results,

most likely, from the interaction of heme, a degradation product of the hemoglobin metabolism, with the active ingredients in the digestive vacuole of the malaria parasite. It may, however, also interfere with the synthesis of nucleic acids and proteins (Nosten and White 2007)

- **Chemistry**

**Lumefantrine** is available as a yellow crystalline powder which melts at 128 - 132˚C.
Its Chemical name is *2-Dibutylamino-1-[2, 7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]-ethanol* and it has a molecular weight of 528.9. It is practically insoluble in water; freely soluble in ethyl acetate; soluble in dichloromethane; slightly soluble in ethanol and methanol. Lumefantrine can be assayed by non aqueous titration and should contain not less than 98.0% and not more than 102.0% of $C_{30}H_{32}Cl_3NO$, calculated with reference to the dried substance (USP SALMOUS Standard).

- **Formulations**

Available only in an oral preparation co-formulated with Artemether

- **Pharmacokinetics**

The pharmacokinetic properties of Lumefantrine are reminiscent of characteristics of halofantrine (Nosten and White 2007). Oral bioavailability is variable and is highly dependent on administration with fatty foods (Ezzet, Mull et al. 1998). Absorption increases by 108% after a meal and is lower in patients with acute malaria than in convalescing patients. Peak plasma levels occur approximately 10 h after administration. The terminal elimination half-life is around 2 to 3 days in healthy volunteers and 4 to 6 days in patients with clinically relevant *P. falciparum* infections.

- **Toxicity**

Despite similarities with the structure and pharmacokinetic properties of halofantrine, Lumefantrine does not significantly prolong the electrocardiographic QT interval, and has no other significant toxicity (van Vugt, Ezzet et al. 1999). In fact the drug seems to be remarkably well tolerated. Reported side effects are generally mild – nausea, abdominal discomfort, headache and dizziness – and cannot be distinguished from symptoms of acute malaria.

- **Drug interactions**

The manufacturers of **Artemether-Lumefantrine** recommend that the patient should avoid the following: grapefruit juice; antiarrhythmics, such as amiodarone, disopyramide, flecainide, procainamide and quinidine; antibacterials, such as macrolides and quinolones; all antidepressants; antifungals such as imidazoles and triazoles; terfenadine; other antimalarials; all antipsychotic drugs; and beta blockers, such as metoprolol and sotalol. However, there is no evidence that co-administration with these drugs would be harmful.

### 1.4.3.3 Indometacin (Standard)

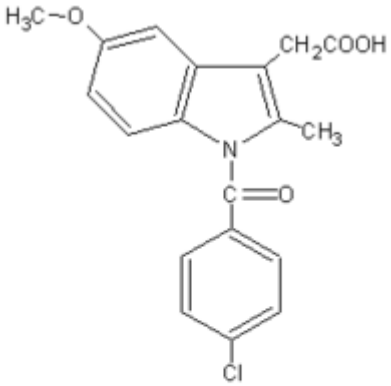



**Fig. 6 Structure of Indometacin**

Indometacin is an analgesic and anti-inflammatory drug. It is available as a white or yellow, crystalline powder and melts at 158-162°C. The chemical name of indometacin is *[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl] acetic acid* and it has a molecular weight of 357.8. It is practically insoluble in water and sparingly soluble in alcohol

Indometacin is assayed by acid base titration and it should contain not less than 98.5% and not more than the equivalent of 100.5% of *[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid*. Indometacin is not stable in alkaline solutions. Indometacin solutions below pH 7.4 are stable. Solutions at pH 7.4 show no changes up to 24 hours, but decomposition is rapid in alkaline solutions.

### 1.4.4 THEORY AND INSTRUMENTATION OF ANALYTICAL PROCEDURES

#### 1.4.4.1 Thin Layer Chromatography

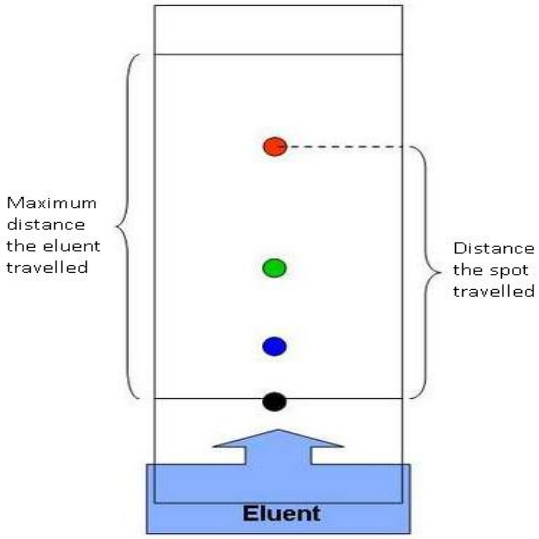



**Fig. 7 TLC Chromatogram**

Thin layer chromatography (TLC) is a chromatographic technique used to separate mixtures. TLC can be both analytical and preparative. TLC is performed on a sheet of glass, plastic, or aluminum foil, coated with a thin layer of adsorbent material. The substances frequently used as coating materials are silica gel, alumina and cellulose. To give stable layers they often contain binders such as calcium sulphate or starch (Beckett A. H. et. al. 1988). The coating material may also contain an organic fluorescent indicator e.g. zinc silicate which fluoresces upon irradiation at a suitable wavelength. Though adsorption is the most frequently used mechanism in TLC, partition, ion exchange and molecular exclusion may also play a part. Though pre-coated TLC plates are readily available, they can be easily prepared in the laboratory at a cost cheaper than those obtained commercially.

The sample under examination is dissolved in a suitable solvent and applied as spots or bands on one side of the coated plate. An eluent is allowed to flow through the sorbent by capillary action starting at a point below the applied samples. As the eluent front migrates through the sorbent material, the components of the sample migrate at different rates and are thus separated. When the solvent front reaches the top of the sorbent material, the plate or sheet is removed and dried. The spots or bands on the developed plate are visualized, if required under UV light, by derivatization or by chemical treatment (Wall P. E., 2005). Some of the most commonly used reagents for location of spots in TLC are iodine vapour, Dragendorff's reagent, bromocresol green and ninhydrin.

The basic chromatographic measurement of a substance in TLC is the $R_f$ value. $R_f$ is defined as

$$R_f = \frac{\text{distance the substance travel from origin}}{\text{distance the solvent travels from origin}}$$

TLC is primarily used as a qualitative analytical tool for the identification of organic and inorganic compounds by the comparison of samples with standard compounds.

For quantitative measurements in TLC, the spots can be assessed by

- Photodensitometric methods
- Elution of sample spots into a suitable solvent and determination by an appropriate technique
- Measurement of spot areas.

#### 1.4.4.2 Non-Aqueous Titration

Non-aqueous titration is the titration of substances dissolved in nonaqueous solvents. It is the most common titrimetric procedure used in pharmacopoeial assays and serves a double purpose:

1. It is suitable for the titration of very weak acids and very weak bases, and
2. It provides a solvent in which organic compounds are soluble.

The most commonly used procedure is the titration of organic bases with perchloric acid in anhydrous acetic acid. The end point of most titrations is detected by the use of visual indicator but the method can be inaccurate in very dilute or colored solutions. However under the same conditions, a potentiometric method for the detection of the equivalence point can yield accurate results without difficulty.

#### 1.4.4.3 Ultra-Violet Visible Spectroscopy

Analytical spectroscopy is the science of determining how much of a substance is present in a sample by accurately measuring how much light is absorbed or emitted by atoms or molecules within it. Different types of spectroscopy are available, depending on the wavelength of electromagnetic radiation absorbed or emitted by the atom or molecule. Although spectroscopy can be carried out on different types of compounds, with different electronic configurations, most quantitative work will involve compounds with ‘pi’ electron systems. The p electrons are the electrons found in multiple bonds. These p electrons are easily excited and promoted to a high-energy anti-bonding orbital. When the electron falls back to the ground state, the energy released can be measured by a spectrophotometer. The part of the molecule that is responsible for the absorption of light is called the chromophore

and consists of a region of double or triple bonds, especially if the multiple bonds are conjugated (Cairns D., 2008).

The use of the UV/Visible spectrophotometer for quantitative work follows the **Beer-Lambert's law**. It states that the proportion of light absorbed by a solute in a transparent solvent is independent of the intensity of the incident light and is proportional to the number of absorbing molecules in the light path. Mathematically the Beer-Lambert's Law is given by the equation below.

$$\log_{10} (I_o/I) = A = \varepsilon c \ell$$

Where; $I_o$ = intensity of incident light

$I_t$ = intensity of transmitted light

$\varepsilon$ = molar absorptivity or molar extinction coefficient

c = concentration of solute in moles per litre

$\ell$ = cell (path) length (cm)

A = absorbance

#### 1.4.4.4 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography is basically a highly improved form of column chromatography. It is the most widely used form of chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through the column under high pressures and this improves separation. The improvements of HPLC have enabled liquid chromatography to match the great success of Gas chromatography (Jeffery G.H et. al., 1989). The separation principles involved may include

- Adsorption
- Partition
- Ion exchange
- Gel permeation
- Affinity

HPLC can be used for both qualitative and quantitative analysis. In Qualitative analysis, the retention time of the compounds are made use of. In quantitative analysis on the other hand, the area under the peak, which is proportional to the concentration of the compound is used.

#### 1.4.4.4.1 HPLC Instrumentation

An **HPLC** apparatus consists of a **pumping system**, an **injector**, a **chromatographic column**, a **detector** and a **data acquisition system**. The mobile phase is supplied from one or several reservoirs and flows through the column, usually at a constant rate, and then through the detector.

- **Pumps**

In HPLC, there is the need to deliver a constant flow of the mobile phase. Pumping systems are thus required to deliver the mobile phase at a constant flow rate. Pressure fluctuations are to be minimized as they affect the separation efficiency. The pump must be able to provide pressure of up to 6000psi, pulse free output, flow rate ranging from 0.1 – 10ml/min, flow control and flow reproducibility. Microprocessor controlled systems are capable of accurately delivering a mobile phase of either constant (isocratic elution) or varying composition (gradient elution), according to a defined programme (BP, 2007). The delivered flow rate must be independent of the back pressure, even if this changes during a separation, which is usually the case with gradient elution. Moreover, the flow should be pulseless, especially when a refractive index, conductivity or electrochemical detector is used (Meyer V. R., 2004)

- **Injection Systems**

Injection ports are of two basic types

  - Those in which the sample is injected directly into the column and
  - Those in which the sample is deposited before the column inlet and then swept by a valve action into the column by the mobile phase.

**On-column** injection systems are not as reproducible as the valve injectors and generally are used in older or simple HPLC apparatus. (Beckett A. H. et. al. 1988)

Injectors are further grouped into manual and auto injectors (auto samplers). The manual injectors require the analyst to inject the sample into the valve by a syringe whereas in the

auto injectors the injection of the sample is done automatically. Auto samplers eliminate variations due to the analyst, improving reproducibility. Auto samplers can be controlled by computers and are of value where large numbers of samples are to be analyzed or unattended operation is required. Samples and standards loaded into racks or turntables can be run in a predetermined sequence and under different operating conditions. Such devices can also be used for single samples to improve injection precision (Kealey D. and Haines P. J., 2002)

- **Columns and Stationary Phases**

The column is where the separation process occurs. It is the central component of HPLC. There are many types of stationary phases in HPLC depending on the particular separation technique being employed. *Heavy wall glass, stainless steel* and *plastic* are among materials that can withstand high pressures and are used to construct HPLC columns. Columns must not chemically interfere with the mobile phase. Usually a short guard column is placed before the column and this serves to prolong the life of the column by removing particulate matter and contaminants in the solvent.

- **Detectors**

The detector for an HPLC is the component that emits a response due to the eluting samples and subsequently signals a peak on the chromatogram. The detection of the separated components from the column is based upon the bulk property of the eluate or the solute property of the individual components (Beckett A. H. et. al. 1988). There are six main types of detectors used for HPLC: **refractive index (RI)**, **ultraviolet (UV)**, **fluorescence (FL)**, **electrochemical (EC)**, **conductivity (CD)**, and mass **spectrometric (MS)**. Infrared and nuclear magnetic resonance detectors have been used, but they suffer from solvent limitations (McMaster C. M., 2007)

#### 1.4.4.4.2 Reverse-Phase (RP) Chromatography

Reverse phase chromatography is the most widely used form of HPLC. In reverse phase partition chromatography, the stationary phases are non-polar and thus polar mobile phases are required.

- **Stationary Phase**

The stationary phase is silica, chemically bonded through a siloxane linkage to low polar functional group. The surface of the support, e.g. the silanol groups of silica, is reacted with various silane reagents to produce covalently bound silyl derivatives covering a varying number of active sites on the surface of the support. The nature of the bonded phase is an important parameter for determining the separation properties of the chromatographic system. Common reverse phase materials include octadecylsilane (ODS or $C_{18}$) and octylsilane ($C_8$).

- **Mobile Phase**

The mobile phase generally comprises water and a less polar organic solvent modifier such as methanol or acetonitrile. The solutes in reverse phase chromatography are eluted in order of their decreasing polarities. Separations in these systems are considered to be due to different degrees of hydrophobicity of the solute, the less polar solute partitioning to a greater extent into the non-polar stationary phase and consequently being retained on the column longer than the more polar solute. The rate of elution of the components is controlled by the polarity of the organic modifier and its proportion in the mobile phase. The rate of elution is increased by reducing polarity e.g. by increasing the proportion of the organic solvent or by using acetonitrile instead of methanol (Beckett A. H. et al. 1988)

#### 1.4.4.4.3 Solvent Parameters Relating to HPLC

The following are some of the solvent parameters that need to be considered when choosing a suitable mobile phase for HPLC analysis.

- **UV Transparency and UV cut-off**

Most solvents are more transparent to UV down to a certain wavelength and below that they totally absorb UV. To be useful with UV detection, the solvent has to have a lower UV cut off than the absorption of any of the sample components. In general, reverse phase eluents have much lower UV cut-off points than normal phase eluents.

- **Solvent Miscibility**

Some solvents such as alkanes (e.g. hexane, pentane etc) are very non-polar and will not mix with others (such as water) which are very polar. Since solvents are mixed in HPLC to fine

tune the polarity, thereby controlling their eluent strength, it is essential that solvents chosen are totally miscible.

- **Viscosity**

HPLC operates in dynamic equilibrium. Almost 90% of the surface area of a 5micron packing material is inside the pores. So the lower the viscosity of a solvent, the lower the back pressure and the better the mass transfer in and out of the pores. This in turn gives better separation efficiency, i.e. sharper peaks.

- **Purity**

For HPLC, solvents used require a higher level of purity. For this purpose, HPLC grade solvents are required. If these are not available, Analytical (AR) grade solvents can be distilled at least once and filtered. The importance of solvent purity is that when analyzing small quantities of sample (20ul), impurities in the 20-30ml of solvent used during a run can be quite significant.

- **Eluent Strength**

For Reverse Phase HPLC, water is the weakest eluent. Its eluent strength is then modified by adding a less polar but miscible solvent such as methanol. The less polar the solvent, the greater the eluent strength.

For Normal Phase HPLC, hexane (or heptane) is the weakest eluent and a more polar solvent is added to modify eluent strength. These include Chloroform, Dichloromethane, Ethyl Acetate, Acetone, Ether etc.

If a change in eluent composition is made for a selectivity reasons e.g. from Methanol to Acetonitrile in RP-HPLC, the ratios must be changed to maintain the same eluent strength.

- **Toxicity**

Some solvents are more hazardous than others. Toxicity, flammability, carcinogenicity amongst others are therefore very essential in selecting solvents for HPLC. Some have a very unpleasant odour. Some have a low flash point. It is important to be aware of the hazards.

- **Cost**

Some solvents are very much more expensive than others. For example, for an analysis where methanol or acetonitrile could be used, methanol would be preferred when cost is taken into consideration.

#### 1.4.4.4.4 Acetonitrile and Alternatives to Its Use in RP-HPLC

Acetonitrile is the chemical compound with formula $CH_3CN$. This colourless liquid is the simplest organic nitrile. It is produced mainly as a by-product of acrylonitrile manufacture. It is mainly used as a polar aprotic solvent in purification of butadiene. In the laboratory, it is used as a medium-polarity solvent that is miscible with water. Though it is a very expensive solvent, acetonitrile is very useful in mobile phases in RP-HPLC. Acetonitrile's usefulness in reverse phase HPLC is due to its superior spectroscopic characteristics and solubilising properties which are unmatched among other solvents. Acetonitrile has a low UV cut-off point of 190 (Moffat et al. 1986). This ensures a very low background absorbance at wavelengths as low as 200 nm (< 0.05 Absorbance Unit (AU)). This nearly ideal spectroscopic quality, coupled with excellent solubilising capabilities and unique chromatographic properties lead to acetonitrile being the most commonly used solvent in RP separations.

The recent global acetonitrile shortage has driven many industries and laboratories to try and find suitable alternatives for acetonitrile and also, ways to minimize the use of acetonitrile especially in HPLC analysis. Solvents which have been considered as alternatives for acetonitrile include the alkyl alcohols such as methanol or isopropanol and tetrahydrofuran (THF) (Fisher Scientific, 2010). Though none of these solvents can match acetonitrile in Reverse Phase HPLC, methanol is the solvent that is most commonly used as an alternative to acetonitrile. Methanol has a UV cut-off of 205nm (Moffat et. al. 1986) and an absorbance at 215 nm greater than 0.3 AU. Closer examination of the methanol leads to the conclusion that achieving a background absorbance contribution from methanol of less than 0.05 AU requires either working at wavelength of greater than 235 nm or limiting the methanol level in the mobile phase to less than 15% at wavelength of 215 nm (Fisher Scientific, 2009)

#### 1.4.4.4.5 Internal Standards in HPLC

An internal standard in analytical chemistry is a chemical substance that is added in a constant amount to samples, the blank and calibration standards in a chemical analysis. This substance can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards. Internal standards are especially useful for analyses in which the quantity of sample analyzed or the instrument response varies slightly from run to run for reasons that are difficult to control.

Internal standards are also used to correct for the loss of analyte during sample preparation. To use an internal standard, a known mixture of standard and analyte are prepared and the relative response of the detector to the two species measured. This gives the response ratio of the analyte to that of the standard and these are used for quantitative analysis (Moffat et al. 1986).A suitable internal standard for HPLC analysis should satisfy the following criteria

- **Stability**

The internal standard should be sufficiently stable in the sample dissolving solvent to prevent the formation of degradation products, which would interfere with the integration results. It should also be chemically stable in the solid state to allow suitable storage

- **Solubility**

The internal standards should be freely soluble in the sample solvent.

- **Commercial availability**

The material selected should be cheap and readily available in a high-purity form from commercial suppliers so that the method can be readily reproduced elsewhere.

- **Toxicity**

The toxicity of the internal standard should be minimal to reduce any handling precautions that may be required.

- **Suitability**

The compound should have a good UV response at the detection wavelength so that a high signal can be obtained to reduce any integration-related variability generated with small peaks.

#### 1.4.4.4.6 External Standards in HPLC

The external standard is used when a suitable internal standard that can be separated from the components of the mixture cannot be selected. In this case the external standard is run as a separate chromatogram under exactly the same conditions. The properties of the standard from the separate chromatogram are then compared with the properties of the solutes in the chromatogram of the mixture. In general, analyses obtained by employing an internal standard provide more accurate results than those employing an external standard.

### 1.4.5 REVIEW OF ANALYTICAL METHODS

As Artemether-Lumefantrine combination formulations have gained prominence in the treatment of malaria, one would expect that there would be several methods of assay for such formulations. However, there are very few documented methods for the assay of Artemether and Lumefantrine as individual products and also as combination formulations. In the tropical countries where malaria is endemic, it is important to ensure the quality of antimalarial drugs.

There are no monographs for both Artemether and Lumefantrine in both the B.P and the USP. The international pharmacopoeia (IP, 2008) contains monographs of Artemether pure sample, the injection formulation as well as tablet and capsule formulations but no monographs on the combination formulation with Lumefantrine. Monographs of Artemether tablets can be found in the USP SALMOUS standard. Monographs of Lumefantrine and its tablet formulations are available only in the USP SALMOUS Standard and are now being drafted for inclusion in the IP. A monograph of the Artemether-Lumefantrine fixed-dose tablet is only available in the USP SALMOUS Standard and is now being drafted for inclusion in the IP. There are also a few published papers on the assay of Artemether and Lumefantrine. The available methods are reviewed below.

#### 1.4.5.1 UV Spectrophotometric Analysis of Artemether

The artemisinins lack strongly absorbing chromophores and Artemether is no exception. Due to its lack of such chromophore groups, artemisinin and its derivates absorb weakly in the low wavelength region and this makes their quantification difficult. The available UV Spectrophotometric methods for the analysis of Artemether make use of its HCl decomposition product. This acid decomposition product of Artemether has been described as an α β unsaturated decalone and absorbs at a wavelength of 254nm (Thomas, Ward et al. 1992). Though this product absorbs strongly at the said wavelength, it requires very vigorous conditions for its formation. The IP method for the assay of Artemether (both as the pure sample and in formulations) requires the addition of 1M ethanolic HCl solution to an aliquote of Artemether in ethanol solution followed by heating at 55˚C for five hours

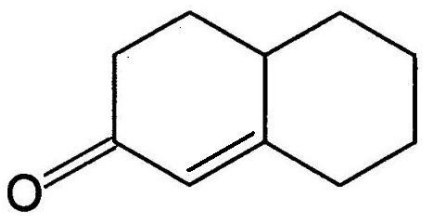



**Fig. 8 Structure of α β Unsaturated Decalone**

(IP, 2008). Another method developed by Shrivastava A. et al requires heating at 60 ˚C for three hours (Shrivasatava et al. 2008). The time demands as well as the heating required by these methods make them uneconomical. Green, Mount et. al. have also described a method for the assay of Artemether and other artemisinins by the reaction of the acid decomposition product with a dye to yield a coloured derivative which absorbs at 420nm. This method requires a period of one hour for the formation of the product prior to reaction with the dye (Green, Mount et al. 2001).

#### 1.4.5.2 UV Spectrophotometric Analysis of Lumefantrine

Lumefantrine possesses strongly absorbing chromophores but the assay of Lumefantrine by UV spectrophotometric analysis has not been well explored. This can be attested to by the fact that UV spectorphotometric methods for the assay of Lumefantrine are virtually non-existent. The monographs in the pharmacopoeias do not make use of its chromophores for analysis. One method by da Costa Cesar, Nogueira et al uses UV Spectrophotometry to assay Lumefantrine (da Costa Cesar, Nogueira et al. 2008). This method employs methanol as the solvent for the analysis but since Lumefantrine is slightly soluble in methanol, it was initially dissolved in dichloromethane to ensure complete dissolution. The wavelength employed for the analysis was 335nm.

#### 1.4.5.3 HPLC Analysis of Artemether

The international pharmacopoeia and the USP SALMOUS edition describe methods for the assay of Artemether by Reverse phase HPLC with UV detection. The wavelengths of analysis in the above methods are 216 and 210nm respectively. Acetonitrile is the major component of the mobile phases as it has a low cut-off point.

Some papers have also described the analysis of Artemether in plasma, based on HPLC with electrochemical (Navaratnam, Mansor et al. 1995; Karbwang, Na-Bangchang et al. 1997) or mass spectrometric detection (Souppart, Gauducheau et al. 2002). Thomas, Ward et al described the analysis of Artemether in plasma by HPLC with UV detection both with pre-column derivatization of the Artemether (Thomas, Ward et al. 1992).

#### 1.4.5.4 HPLC Analysis of Lumefantrine

Methods for assay of Lumefantrine by HPLC with UV detection are described in the USP SALMOUS edition. Several papers also describe the determination of Lumefantrine in blood

plasma using HPLC with UV detection (Zeng, Lu et al. 1996; Ntale, Ogwal-Okeng et al. 2008). There are however few papers that describe the assay of Lumefantrine in formulations by HPLC with UV detection (da Costa Cesar, Nogueira et al. 2008).

#### 1.4.5.5 Determination of Artemether and Lumefantrine in Fixed Dose Combination Formulations

Methods for the simultaneous determination of Artemether and Lumefantrine are described in the USP SALMOUS edition. The method employs gradient elution using acetonitrile and an ion-pairing reagent. Cesar Ida, Andrade Nogueira et al. also described a method for the simultaneous determination of Artemether and Lumefantrine in fixed dose combination formulations (Cesar Ida, Andrade Nogueira et al. 2008). All the above methods require the use of acetonitrile as the major component of the mobile phase.

### 1.4.6 METHOD VALIDATION

Laboratories have a professional obligation to provide accurate and reliable analytical results to customers. The Laboratory should justify the customer's trust by providing the correct answer to the analytical part of the problem, in other words, provide results that have demonstrable 'fitness for purpose'. Analytical method validation is one of the measure universally recognized by laboratory as a necessity for a comprehensive system of quality assurance.

Analytical method validation is a process of performing several tests designed to verify that an analytical test system is suitable for its intended purpose and is capable of providing useful and valid analytical data. A validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely.

#### 1.4.6.1 Method Validation Parameters

There are several parameters that are considered in the method validation process. The parameters outlined in the International Conference of Harmonization (ICH) guidelines are explained below.

- **Specificity**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

- **Linearity**

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

- **Range**

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity

- **Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

- **Precision**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

- **Limit of Detection (LOD)**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of detection can be found based on

1. **Visual Evaluation**
2. **Signal-to-Noise**
3. **The Standard Deviation of the Response and the Slope**

- **Limit of Quantitation (LOQ)**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The limit of quantitation may be determined by

1. **Visual Evaluation**
2. **Signal-to-Noise**
3. **The Standard Deviation of the Response and the Slope**

- **Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

In the case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in a mobile phase
- Influence of variations in mobile phase composition
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

- **System Suitability**

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. Efficiency, capacity factor, resolution factor, and symmetry factor are parameters that are normally used in assessing the column performance.

# CHAPTER 2 – EXPERIMENTAL

## 2.1 INSTRUMENTS AND MATERIALS

- Hanna instruments pH 211 microprocessor pH meter
- Erweka Tablet Disintegration Apparatus
- Cecil CE 2041 2000 Series-UV Spectrophotometer
- FS 8H Fisher Scientific Sonicator
- Adam-analytical weighing balance, WA 210 ; 210/0.0001g
- Büchi Water bath
- Büchi rotary evaporator
- HPLC Chromatograph
    - Shimadzu LC-6A Liquid Chromatograph pump
    - Applied Biosystems 783 programmable Absorbance Detector
    - Shimadzu CR 501 Chromatopac Integrator
    - Ultracarb 3µ ODS(20), 200×3.20mm Column
- Volumetric flasks (200ml, 1000ml, 50ml, 25ml)
- Conical flasks
- Measuring beakers (25ml)
- Transfer pipettes (0.5ml, 1ml, 2ml, 5ml, 10ml)
- Graduated pipettes (1ml, 5ml, 10ml)
- No. 1 sintered glass crucible
- No. 1 whatman filter paper
- Glass funnel
- Quick-fit test tubes with stoppers
- Melting point capillary tubes
- Pre-coated TLC plates (Gf 254, 0.25mm Merck W.)

## 2.2 REAGENTS AND SAMPLES

- Hydrochloric acid (36%w/w) (BDH)
- Perchloric Acid (70%)
- Acetic Anhydride
- Trifluoro acetic acid TFA (98%)
- Glacial acetic acid (BDH)
- Cyclohexane
- Sulphuric acid (98%w/w) (BDH)
- Ethyl acetate (BDH)
- Dichloromethane
- Chloroform
- Methanol (Re-distilled Analar Grade)
- Ethanol
- Vanillin
- Tablet excepients
    - Talc
    - Methyl cellulose
    - Starch

**Table 1 Pure Samples Used**

| Sample | Source | Batch No. | Man. Date | Exp. Date |
|---|---|---|---|---|
| Lumefantrine | IPCA Labs. | 8019LU3R11 | Jun - 08 | May - 11 |
| Artemether | IPCA Labs. | 8004AR3R11 | Jan - 08 | Dec - 11 |
| Indometacin | Ernest Chemists | X061210 | Dec - 07 | Nov - 10 |

**Table 2 Brands of Tablets Used (20mgAM/120mg LM)**

| Brand | Code | Batch No. | Man. Date | Exp. Date | Country of Origin |
|---|---|---|---|---|---|
| Artefan | A | AM0359F | Jun-09 | May-11 | India |
| Coartem | B | X1417 | Aug-09 | May-11 | China |
| Co-Malagon | C | T8059 | May-08 | Apr-10 | India |
| Lonart | D | LN-167 | Sep-09 | Aug-11 | India |
| Lumerax | E | DOV8001F | Oct-08 | Sep-10 | India |
| Lumether | F | 002 | Feb-09 | Nov-10 | Ghana |

## 2.3 METHODS

### 2.3.1 PREPARATION OF REAGENTS

- **Preparation of Vanillin/Sulphuric Acid (TS1)**

5g of vanillin was weighed and added to 100ml of concentrated $H_2SO_4$. It was stirred to ensure adequate mixing.

- **Preparation and Standardization of 0.1M Perchloric Acid**
    - **Preparation**

    900 ml of Glacial acetic acid was measured into a 1L volumetric flask. 8.5ml of 70% Perchloric acid was slowly added with continuous and efficient mixing. 30 ml acetic anhydride was slowly added and the volume adjusted to 1L with glacial acetic acid. The solution was left to stand for 24 hours.

    - **Standardization**

    0.5g of potassium hydrogen phthalate was accurately weighed into a 250 ml conical flask and 25ml of glacial acetic acid was added. The solution was warmed to dissolve the salt. It was then cooled and titrated with the 0.1M perchloric acid using oracet blue as the indicator.

### 2.3.2 IDENTIFICATION TESTS

#### 2.3.2.1 Identification of Artemether (IP)

i) About 30mg of Artemether was taken and 1ml of dehydrated ethanol added to it. About 0.1 g of potassium iodide was added and the mixture heated on a water-bath.

ii) About 30 mg of Artemether was dissolved in 6.0 ml of dehydrated ethanol. A few drops of the mixture were placed on a white porcelain dish. 1 drop of vanillin/sulphuric acid TS1 was added.

iii) Melting point determination

### 2.3.1.2 Identification and Assay of Lumefantrine

- **Identification**

i) About 20 mg of Lumefantrine was accurately weighed and dissolved in 200 ml of methanol by sonication for about 15 minutes. The solution was allowed to cool to room temperature and diluted five times with methanol. The absorbance reading at the maximum of 302nm was taken with methanol as blank and the specific absorbance at that wavelength calculated

ii) Melting point determination

- **Assay of Lumefantrine Pure Sample by Non-Aqueous Titration**

About 0.45g of Lumefantrine was accurately weighed and dissolved in 50 ml of glacial acetic acid by stirring for about 15 minutes. The solution was titrated with 0.1M perchloric acid (0.1 mol/l) VS and the end-point determined potentiometrically.

**Each ml of 0.1M perchloric acid VS is equivalent to 52.89 mg of Lumefantrine.**

### 2.3.1.3 Identification of Artemether and Lumefantrine in Tablets by TLC (USP)

- **Chromatographic Conditions**

Pre-coated TLC plates were used.

The developing solvent system was a made up of a combination of cyclohexane, ethyl acetate, and glacial acetic acid (20 : 5 : 2.5).

The developing reagent was 20% sulphuric acid in methanol.

- **Sample Preparation, Application and Development**

A solvent consisting of a mixture of chloroform, methanol, ethyl acetate, and water (11:10:2:2) was prepared and used in preparing solutions of the pure samples.

For the standard solutions of Artemether and Lumefantrine, an amount of Artemether and Lumefantrine pure samples were weighed and dissolved in the solvent to obtain solutions with concentrations of about 0.8 mg/ml and 4.8 mg/ml respectively. This solution was used in spotting the plates.

For the test solution, a portion of powdered Tablets, equivalent to 20 mg of Artemether and 120 mg of Lumefantrine was weighed and transferred to a measuring beaker. 2 ml of

water, 2 ml of ethyl acetate, 10 ml of methanol, and 11 ml of chloroform were added to it. The resulting solution was sonicated for 15 minutes, the solution filtered and the clear filtrate used in the spotting of the plates.

After spotting, the plates were developed in a chromatank containing the mobile phase. The plates were air-dried and sprayed with 20% sulphuric acid in methanol. They were then placed in an oven at 140˚C for about 10 minutes and the spots examined under daylight and under UV light at 366 nm. The $R_f$ values of the spots obtained from the tablets were calculated and compared to those of the standards.

### 2.3.2 PHARMACOPOEIAL TESTS

#### 2.3.2.1 Uniformity of Weight Test

Twenty tablets from each brand of the tablets were selected at random. The tablets were weighed together and the average weight of a tablet determined. The tablets were weighed individually and the deviations of the weights of each tablet from the average weight of a tablet were calculated. The percentage deviation of each tablet from the average tablet weight was calculated and the results compared to the standards in the BP.

#### 2.3.2.1 Tablet disintegration test

Six tablets were taken from each brand and a tablet placed in each of the cylindrical tubes in the disintegration basket. The bottom of the disintegration basket was at least 15mm below the surface of the water and apparatus was made to operate. The time taken for each tablet to disintegrate was recorded.

### 2.3.3 UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT

#### 2.3.3.1 Assay of Lumefantrine

- **Preparation of 0.1M Methanolic HCl**

4.3ml of conc. HCl was accurately measured and transferred into a measuring beaker containing about 50ml of methanol. The solution was mixed adequately and allowed to cool. It was then transferred into a 500ml volumetric flask and made up to the mark with more methanol. Adequate mixing of the solution was ensured by shaking the volumetric flask gently.

- **Plotting of Calibration Curve**

Approximately 20mg of Lumefantrine was accurately weighed and dissolved with 0.1M methanolic HCl to the 200ml mark in a volumetric flask. From this stock solution, solutions with concentrations of 0.0008, 0.0012, 0.0016, 0.0020, 0.0024 and 0.0028%w/v were prepared by serial dilution. The absorbances of these solutions at 335nm were obtained with 0.1M methanolic HCl as blank and used in the plotting of a calibration curve.

- **Assay of Lumefantrine in Tablets**

An amount of the powdered tablets equivalent to 20mg of Lumefantrine was weighed and dissolved with 0.1M methanolic HCl to 200ml in a volumetric flask. The solution was filtered with a sintered glass crucible and the first 20ml discarded. From the above solution, a solution with concentration of 0.0016%w/v was prepared. The absorbance of this solution at 335nm was taken using 0.1M methanolic HCl as blank and the content of Lumefantrine calculated from the calibration curve.

- **Method validation**

Linearity was observed with concentrations ranging from 0.0004%w/v to 0.004%w/v and five different concentrations approximately 0.0008, 0.0012, 0.0016, 0.0020, 0.0024 and 0.0028%w/v were selected for plotting calibration curves.

The intra-day precision was evaluated by analyzing six weighed samples ($n$ = 6), at 100% of the test concentrations using the method. The inter-day precision was evaluated in three consecutive days also analyzing six different samples ($n$ = 18). The relative standard deviations (RSD) of the percentage content of Lumefantrine were calculated.

To demonstrate the accuracy of the method, Lumefantrine pure sample was accurately weighed and added to a mixture of the tablet excepients. From this mixture, three different concentrations corresponding to 0.0012, 0.0016 and 0.0020%w/v of Lumefantrine in 0.1M methanolic HCl were prepared. Their absorbances at 335nm were found and the percentage recovery of Lumefantrine was determined at these concentrations.

To demonstrate robustness of the method, 0.075M and 0.15M methanolic HCl was used in place of 0.1M methanolic HCl. Also the prepared solutions were allowed to stand for periods of up to 24 hours and their absorbance measured for any deviations from their initial readings to be noted.

To demonstrate specificity, a solution containing a mixture of the tablet excepients (methyl cellulose, starch and talc) was prepared using the sample preparation procedure and the UV spectrum of this solution was recorded in the range of 200–400 nm. This was done to evaluate the presence of possible interfering bands at 335 nm. Also, a sample of Artemether was weighed and taken through the same procedure and the spectrum analyzed for any interfering bands. The LOD and LOQ were calculated from the calibration curve.

#### 2.3.3.2 ASSAY OF ARTEMETHER

- **Calibration curve**

About 400mg of Artemether was accurately weighed and dissolved in sufficient methanol to give a 100ml solution. The solution was filtered using a sintered glass crucible, discarding the first 10mls. Concentrations of approximately 0.016, 0.024, 0.032, 0.040, 0.048, 0.056 and 0.064%w/v were prepared by serial dilution from the stock solution. 2ml of each of the resulting solutions was pipetted into a quick-fit test tube and 2ml of concentrated HCl added. The test tubes were stoppered and allowed to stand in a water bath set to 30 ˚C (or room temp.) for 25 minutes. Each of the resulting solutions was diluted with sufficient methanol to 50ml. The absorbance readings were taken at a maximum of 254nm against a blank solution made up of 2ml of HCl made up to 50ml with methanol. A calibration curve was plotted with the readings.

- **Assay of Artemether in pharmaceutical preparations**

An amount of the powdered sample containing about 40mg of was weighed and dissolved in sufficient methanol to produce 100ml. The resulting solution was filtered using a sintered glass crucible, discarding the first 10mls. 2ml of the resulting solution was pipetted into a quick-fit test tube and 2ml of concentrated HCl added. The test tube was stoppered and allowed to stand in a water bath set to 30 ˚C (or room temp.) for 25 minutes. The resulting solution was diluted with sufficient methanol to 50ml. The absorbance reading at a maximum of 254nm was taken against a blank solution made up of 2ml of HCl made up to 50ml with methanol. The content of Artemether was calculated from the calibration curve.

- **Method validation**

To establish linearity, 400mg of Artemether was weighed and dissolved in methanol to produce 100ml of solution. Concentrations of 0.016, 0.024, 0.032, 0.040, 0.048, 0.056 and 0.064%w/v of Artemether in methanol were prepared from the stock solution above. 2ml of these solutions were pipetted into quick fit test tubes and 2ml of conc. HCl was added to each. The test tubes were stoppered and allowed stand for a period of 25 minutes. Each of the resulting solutions was diluted to 50ml and their absorbance readings at a maximum of 254nm were taken. A graph of absorbance against concentration was plotted and analyzed

To demonstrate the accuracy of the method, an amount Artemether pure sample was accurately weighed and added to a mixture of the tablet excepients. From this mixture, a concentration of approximately 0.0016%w/w corresponding to Artemether in methanol was prepared. 2ml of the solution was pipetted into quick fit test tubes and 2ml of conc. HCl was added to it. The test tube was stoppered and allowed stand for a period of 25 minutes. The resulting solution was diluted to 50ml and the absorbance reading at a maximum of 254nm was taken. The percentage recovery of Artemether was calculated from the calibration curve.

To demonstrate the robustness of the method, the following were checked:

- The stability of the complex formed before further dilution with methanol.
- The ability of the final solution to maintain the same absorbance values for periods up to 24hours.

To demonstrate specificity, a solution containing a mixture of the tablet excepients (methyl cellulose, starch and talc) was prepared using the sample preparation procedure and the UV spectrum of this solution was recorded in the range of 200–300nm for any interferences. The LOD and LOQ were calculated from the calibration curve.

### 2.3.4 HPLC METHOD DEVELOPMENT

#### 2.3.4.1 Assay of Lumefantrine

- **Chromatographic Conditions**
  - Column: Ultracarb 3µ ODS(20), 200×3.20mm
  - Mobile phase: Methanol: 0.1% TFA (90:10)
  - Flow rate: 2.5ml/min
  - Wavelength of detection: 335nm
  - AUFS: 0.200

- **Preparation of Mobile Phase**

The mobile phase is composed of Methanol and 0.1% TFA (90:10)

To prepare 0.1%TFA, 1%v/v TFA was prepared by dilution with distilled water and 10ml made up to 100ml with distilled water.

To prepare 500ml of the mobile phase, 450ml of Methanol and 50ml of 0.1%TFA were measured and mixed in a suitable measuring cylinder. The mobile phase was sonicated to expel gases and the solution was filtered.

- **Preparation of internal standard**

Approximately 20mg of indometacin was weighed and dissolved in methanol to 100ml. The solution was filtered and the first 10ml discarded. Serial dilution was performed using this solution to obtain a solution with concentration 0.002%w/v.

- **Plotting Calibration curve**

40mg of Lumefantrine was accurately weighed and 2ml of dichloromethane added to ensure total dissolution. The solution was transferred quantitatively to a 100ml volumetric flask and made up to the mark with methanol. The solution was filtered, discarding the first 10mls. From this stock solution, concentrations of 0.00032, 0.00048, 0.00064, 0.00080 and 0.00096%w/v were prepared and injected using indometacin at a final concentration of 0.00040%w/v as the internal standard. The peak area ratios of Lumefantrine to indometacin obtained were used in the plotting of a calibration curve calibration curve.

- **Assay of Tablets**

An amount of powdered tablets containing 40mg of Lumefantrine was accurately weighed. 2ml of dichloromethane was added to ensure total dissolution. The solution was transferred quantitatively into a 100ml volumetric flask and made up to the mark with methanol. The solution was filtered discarding the first 10mls. From this stock solution, a concentration of 0.00064%w/v of Lumefantrine was prepared and injected also employing indometacin at a final concentration of 0.00040%w/v as internal standard. The peak area ratio of Lumefantrine to indometacin was used in calculating the amount of Lumefantrine in the tablet from the calibration curve.

- **Method validation**

To demonstrate linearity, eight different concentrations of Lumefantrine ranging from 0.00016%w/v to 0.00128%w/v were prepared and injected. A calibration curve of peak area ratio against concentration was plotted and analyzed.

To demonstrate the accuracy of the method, an amount of Lumefantrine pure sample was accurately weighed and added to a mixture of the tablet excepients. From this mixture, three different concentrations corresponding to 0.00048, 0.00064 and 0.00080%w/v of Lumefantrine were prepared. The solutions were injected onto the column and the percentage recovery calculated at these concentration levels.

To demonstrate the intra-day precision, six different Lumefantrine samples were analyzed at 100% of the test concentration (i.e. 0.00064%w/v). The relative standard deviations of the percentage contents were calculated. The inter-day precision was evaluated by analyzing six different samples on three consecutive days. The relative standard deviation of their percentage contents was calculated.

To demonstrate robustness, the parameters of the HPLC method for the quantification of Lumefantrine were sequentially varied but keeping all other chromatograph parameters constant. The parameters varied were: mobile phase composition, flow rate, wavelength of detection. The LOD and LOQ were calculated from the calibration curve.

### 2.3.4.2 Assay of Artemether

- **Chromatographic Conditions**
  - Column: Ultracarb 3µ ODS(20), 200×3.20mm
  - Mobile phase: Methanol: 0.04% TFA (90:10)
  - Flow rate: 2.5ml/min
  - Wavelength of detection: 235nm
  - AUFS: 0.200

- **Preparation of Mobile phase**

The mobile phase is composed of Methanol and 0.04% TFA (90:10)

To prepare 0.04%TFA, 1%v/v TFA was prepared by dilution with distilled water and 4ml of the solution made up to 100ml with distilled water.

To prepare 500ml of the mobile phase, 450ml of Methanol and 50ml of 0.04% TFA were measured and mixed in a measuring cylinder and sonicated to expel gases. The solution was filtered.

- **Preparation of external standard for assay of Artemether**

Approximately 20mg of indometacin was weighed and dissolved in methanol to 100ml. The above solution was diluted to obtain a solution with final concentration of 0.00008%w/v.

- **Preparation of Artemether stock solution**

A stock solution of Artemether was prepared by weighing 200mg of Artemether and dissolving it in methanol. The solution was made up to the100 ml mark in a 100ml volumetric flask.

- **Calibration curve**

50mg of pure Artemether was weighed and made up to 100ml with methanol. 5ml of the Artemether stock solution above was added to corresponding volumes of the prepared Artemether solution and made up to the 25ml mark in a 25ml volumetric flask with the mobile phase giving concentrations of 0.044, 0.048, 0.052, 0.056 and 0.060%w/v. The solutions were injected using indometacin at a concentration of 0.00008%w/v as an external standard.

- **Assay of Artemether in tablets**

An amount of the powdered tablets corresponding to 50mg of Artemether was weighed and dissolved in methanol to 100ml in a volumetric flask. The solution was shaken to ensure complete dissolution. The resulting solution was filtered using whatman filter paper discarding the first 10ml. 6ml of the solution was pipetted into a 25ml volumetric flask. 5ml of the Artemether stock solution was added and the solution was made up to the 25ml mark with mobile phase. A final concentration of Artemether of 0.052%w/v was expected. The solution was injected also employing indometacin of concentration 0.00008%w/v as an external standard.

- **Method validation**

To demonstrate linearity, eight different concentrations of Artemether ranging from 0.042 to 0.064% of Artemether were prepared and injected. A calibration curve of peak area ratio against concentration was plotted and analyzed.

To demonstrate the accuracy of the method, an amount of Artemether pure sample was accurately weighed and added to a mixture of the tablet excepients. From this mixture, three different concentrations corresponding to 0.048, 0.052, and 0.056%w/v of Artemether were prepared. The solutions were injected onto the column and the percentage recovery calculated at these concentration levels.

To demonstrate the intra-day precision, six weighed samples were analyzed at 100% of the test concentration. The relative standard deviations of the percentage contents of the tablets were calculated. The inter-day precision was evaluated by analyzing six different samples on three consecutive days. The relative standard deviation of their percentage contents was calculated.

To demonstrate robustness, the parameters of the HPLC method for the assay of Artemether were sequentially varied but keeping all other chromatograph parameters constant. The parameters varied were: mobile phase composition and flow rate.

The Limits of detection and quantitation were calculated from the formula as with the other procedures.

# CHAPTER 3 – RESULTS

## 3.1 IDENTIFICATION TESTS

### 3.1.1 IDENTIFICATION OF ARTEMETHER (AM)

**Table 3 Identification of AM by Colour Tests**

| Test | Observation | Expected Observation |
|---|---|---|
| About 30mg of A.M + 1ml dehydrated ethanol + 0.1g KI + Heat | A yellow colour was produced | Yellow Colouration |
| About 30mg of A.M + 6ml dehydrated ethanol + Vanillin sulphuric acid reagent (TS1) | A pink colour was produced | Pink Colouration |

**Table 4 Melting Point Determination of AM**

| Sample | Melting point (˚C) | | Reference Range (˚C) (IP) |
|---|---|---|---|
| | 1st determination | 2nd determination | |
| Artemether | 86-88 | 86-89 | 86 – 90 |

### 3.1.2 IDENTIFICATION AND ASSAY OF LUMEFANTRINE (LM)

#### 3.1.2.1 Identification of Lumefantrine

- **UV/Vis Analysis**

  A = abc

  A = Absorbance = 0.674

  a = Specific absorbance

  b = Path length = 1

  c = Concentration of solution = 0.00208%w/v

  a = A/bc

  = 0.674/1×0.00208

  = 324

**Table 5 Melting Point Determination of LM**

| Sample | Melting point (˚C) | | Reference Range (˚C) (IP Draft) |
|---|---|---|---|
| | 1st determination | 2nd determination | |
| Lumefantrine | 128 - 130 | 128 - 131 | 128 - 132 |

### 3.1.2.2 Assay of Lumefantrine

**Table 6 Standardization of 0.1M $HClO_4$ using Potassium Hydrogen Phthalate**

| Burette reading | 1st determination (0.5004g) | 2nd determination (0.5008g) | Blank Determination |
|---|---|---|---|
| Final reading (ml) | 31.40 | 31.50 | 0.10 |
| Initial reading (ml) | 0.00 | 0.00 | 0.00 |
| Titre (ml) | 31.40 | 31.50 | 0.10 |

204.2g of $C_8H_5O_4K$ in 1000ml ≡ 1M $HClO_4$

0.02042g of $C_8H_5O_4K$ ≡ 1ml of 0.1M $HClO_4$

For first determination

Amount of $C_8H_5O_4K$ weighed = 0.5004g

Volume of 0.1M $HClO_4$ ≡ 0.5004g of $C_8H_5O_4K$ = 31.40 – 0.10

= 31.30

Amount of $C_8H_5O_4K$ equivalent to 31.30ml of 1M $HClO_4$ $= \frac{31.30 \times 0.02042}{1ml}$

= 0.6391g

Factor of $HClO_4 = \frac{0.5004}{0.6391}$

= 0.7830

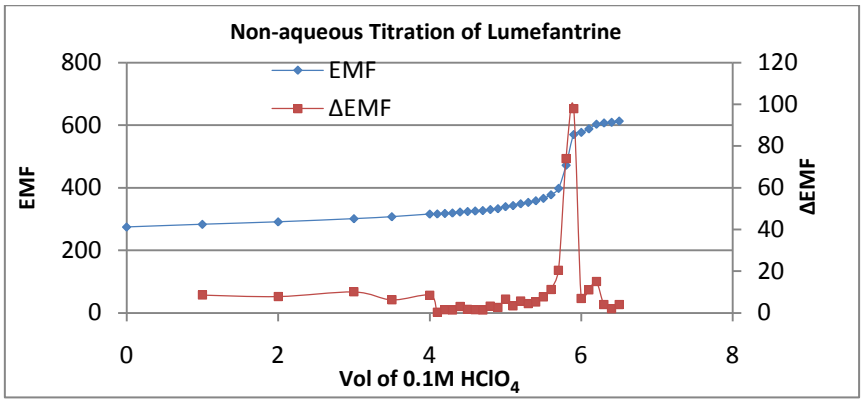



**Fig. 9 Titration Curve of LM with 0.1M $HClO_4$**

**Table 7 Results of Titration of LM with 0.1M $HClO_4$**

| Burette reading | 1st determination (0.2393) | 2nd determination (0.2403g) | Blank Determination |
|---|---|---|---|
| Final reading (ml) | 5.95 | 5.95 | 0.10 |
| Initial reading (ml) | 0.00 | 0.00 | 0.00 |
| Titre (ml) | 5.95 | 5.95 | 0.10 |

From Milliequivalent calculations, each ml of perchloric acid (0.1M) VS is equivalent to 52.89 mg of $C_{30}H_{32}Cl_3NO$

**First determination**

Actual vol. of $HClO_4$ = (5.95-0.10) × F ($HClO_4$)

= 5.85 × 0.7830

= 4.5806

Amount of Lumefantrine = 4.5806 ×52.89mg

= 242.3mg

Percentage Purity of Lumefantrine = 0.2423/0.2393 × 100

= 101.25%w/w

**Table 8 Purity of LM sample**

| Percentage Purity (%w/w) | Reference range (%w/w) (USP SALMOUS) |
|---|---|
| 101.04 ± 0.30 | 98.00 - 102.00 |

### 3.1.3 TLC OF ARTEMETHER AND LUMEFANTRINE

A B C D E F

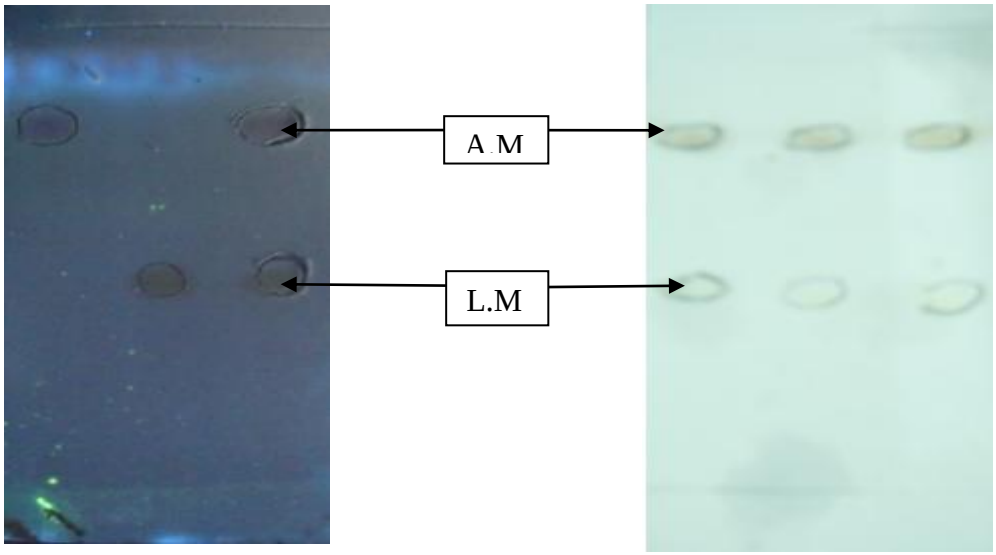



**Fig. 11 TLC of AM and LM under UV** **Fig. 10 TLC of AM and LM in Day-light**

**Table 9 Interpretation of TLC Chromatogram**

| | |
|---|---|
| A | Spot of pure Artemether |
| B | Spot of pure Lumefantrine |
| C | Spots of pure Artemether and Lumefantrine |
| D | Spots of pure Artemether and Lumefantrine |
| E | Spot of tablet brand B |
| F | Spot of tablet brand D |

#### 3.1.3.1 Calculation of $R_f$ Values

**Rf = distance the substance travel from origin**
**distance the solvent travels from origin**

- **For Artemether pure sample**
  - Distance sample moved from origin = 4.0cm
  - Distance solvent travelled from origin = 5.2cm

$$\mathbf{Rf = \frac{4.0}{5.2} = 0.77}$$

- **For Lumefantrine pure sample**
  - Distance sample moved from origin = 2.2cm
  - Distance solvent travelled from origin = 5.2cm

$$\mathbf{Rf = \frac{2.2}{5.2} = 0.42}$$

**Table 10 RF Values of AM and LM**

| **Sample** | **RF of Artemether** | **RF of Lumefantrine** |
|---|---|---|
| A | 0.77 | 0.41 |
| B | 0.77 | 0.42 |
| C | 0.78 | 0.41 |
| D | 0.77 | 0.42 |
| E | 0.78 | 0.43 |
| F | 0.78 | 0.43 |
| Pure samples | 0.77 | 0.42 |

### 3.2 PHARMACOPOEIAL TESTS

**Table 11 Results of Uniformity of Weight Test of Tablets**

| Brand of Tablet | Result |
|---|---|
| A | Passed |
| B | Passed |
| C | Passed |
| D | Passed |
| E | Passed |
| F | Passed |

**Table 12 Results of Tablet Disintegration Test**

| Brand of Tablet | Average Disintegration Time (min.) | Result |
|---|---|---|
| A | 0.5 | Passed |
| B | 4.2 | Passed |
| C | 9.2 | Passed |
| D | 2.2 | Passed |
| E | 5.0 | Passed |
| F | 2.4 | Passed |

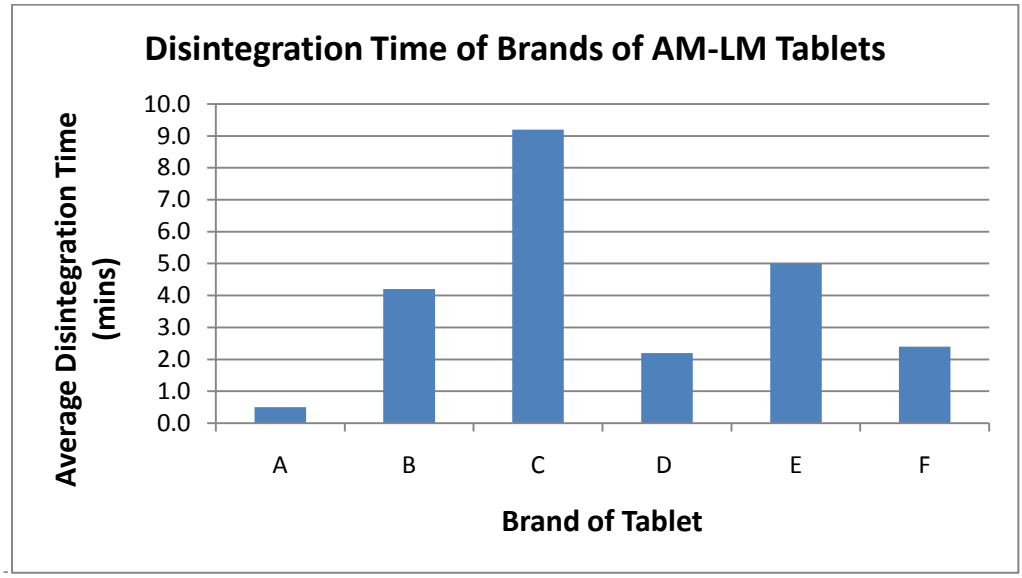



**Fig. 12 Graphical Representation of Tablet Disintegration Test**

## 3.3 UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION

### 3.3.1 LUMEFANTRINE

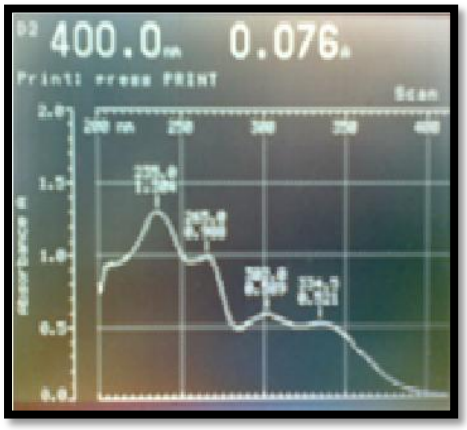



**Fig. 13 UV Spectrum of LM**

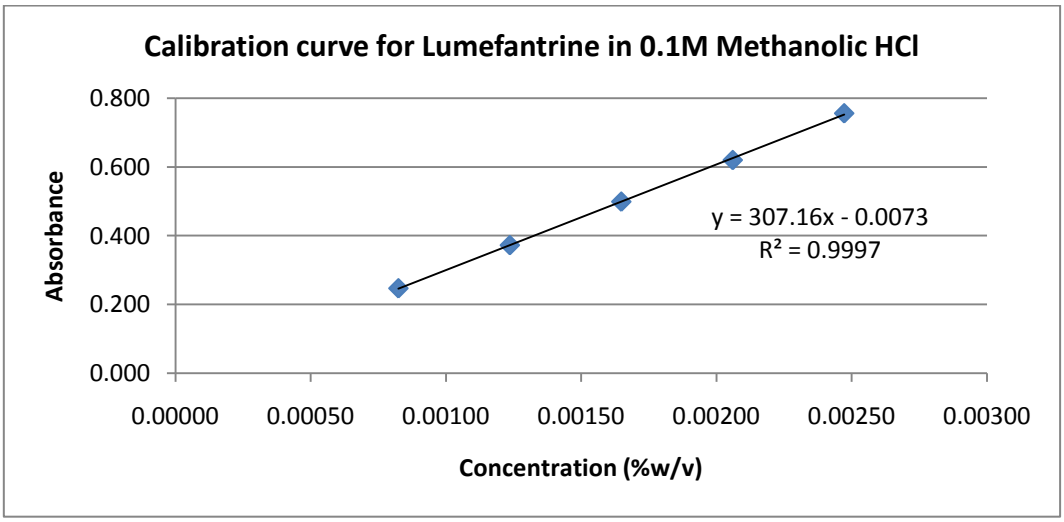



**Fig. 14 Calibration Curve for LM (UV Method)**

**Table 13 Calibration Curve Parameters (UV Assay of LM)**

| **Parameter** | **Value** |
|---|---|
| Range | 0.0008%w/v – 0.0028%$^{w}/_{v}$ |
| Slope | 307.86 ± 2.90 |
| Intercept | 0.0073 ± 0.0054 |
| $R^2$ | 0.9997 |

- **Sample Calculation**

From Beer-Lambert's law

**A = abc, Where**

- A = Absorbance at 335nm = 0.360
- a = a(1%, 1cm)
- b = path length
- c = concentration

From graph, equation of curve: **y = 307.16x - 0.0073**

**For y = 0.360, a = 307.16, b = 1, c = x,**

**0.360 = 307.16x - 0.0073**

**(0.360 + 0.0073)/307.16 = x**

**x = 0.00119579%w/v**

**% Content = (0.00116375/0.00117900) × 100**

**= 101.42%w/w**

This was repeated for all the other concentrations and determinations.

- **Accuracy**

Mixture of tablets and excepients was made up of

- Lumefantrine pure sample
- 40% of Methyl Cellulose
- 20% of Starch
- 1% of talc

**Table 14 Accuracy (UV Assay of LM)**

| Concentration (%w/v) | % Recovered | RSD |
|---|---|---|
| 0.001179 | 101.95 | 0.26 |
| 0.001572 | 100.58 | 0.41 |
| 0.001965 | 99.89 | 0.54 |

- **Precision**

**Table 15 Intra-day Precision (UV Assay of LM)**

| Determination | % Content | |
|---|---|---|
| 1 | 98.66 | |
| 2 | 99.29 | |
| 3 | 99.57 | |
| 4 | 100.40 | |
| 5 | 101.56 | Average = 100.14 |
| 6 | 101.36 | RSD = 1.17 |

**Table 16 Inter-day Precision (UV Assay of LM)**

| Determination | % Content | |
|---|---|---|
| 1 | 98.66 | |
| 2 | 99.29 | |
| 3 | 99.57 | |
| 4 | 100.40 | |
| 5 | 101.56 | |
| 6 | 101.36 | |
| 7 | 102.68 | |
| 8 | 102.88 | |
| 9 | 99.64 | |
| 10 | 100.24 | |
| 11 | 101.56 | |
| 12 | 101.36 | |
| 13 | 102.68 | |
| 14 | 102.88 | |
| 15 | 99.64 | |
| 16 | 100.24 | |
| 17 | 101.56 | Average = 100.98 |
| 18 | 101.36 | RSD = 1.32 |

- **LIMIT OF DETECTION (LOD)**

**LOD = $\frac{3.3\sigma}{S}$**

**Where,**

- σ = the standard deviation of y-intercepts of regression lines
- S = the slope of the calibration curve

From calibration curves,

σ = 0.005481

S = 307.16

$LOD = \frac{3.3 \times 0.005481}{307.16}$

= 0.00006%w/v

- **LIMIT OF QUANTITATION (LOQ)**

**LOQ = $\frac{10\sigma}{S}$**

**Where,**

- σ = the standard deviation of y-intercepts of regression lines
- S = the slope of the calibration curve

From calibration curves,

σ = 0.005481

S = 307.16

$LOD = \frac{10 \times 0.005481}{307.16}$

= 0.00018%w/v

- **Robustness**

**Table 17 Robustness (UV Assay of LM)**

| Parameter | Variation | Average % Recovered |
|---|---|---|
| Using laboratory reagent Grade methanol | | 98.74±0.41 |
| Allowing solutions to Stand for specific time periods | 2 hours | 100.15±0.40 |
| | 12 hours | 101.21±0.39 |
| Varying concentration of Methanolic HCl | 0.75M | 97.84±1.03 |
| | 1.5M | 98.34±0.15 |

- **SPECIFICITY**

The tablet excepients when taken through the same procedure showed no absorbance at 335nm.

A sample of Artemether when taken through the same procedure showed no absorbance at 335nm.

### 3.3.2 ARTEMETHER

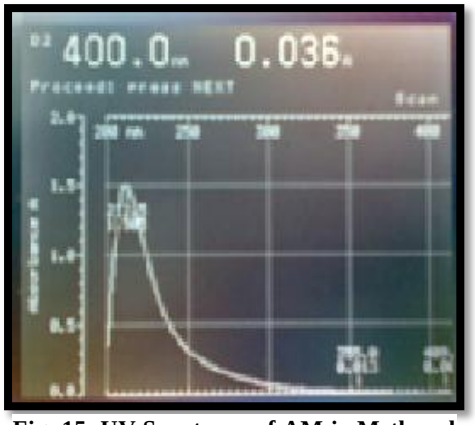



**Fig. 15 UV Spectrum of AM in Methanol (0.2%w/v)**

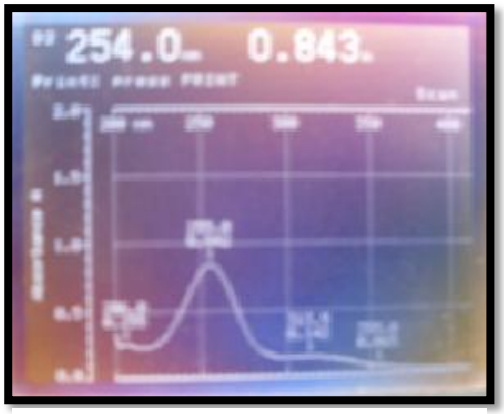



**Fig. 16 UV Spectrum of AM HCl Decomposition Product (0.0025%w/v)**

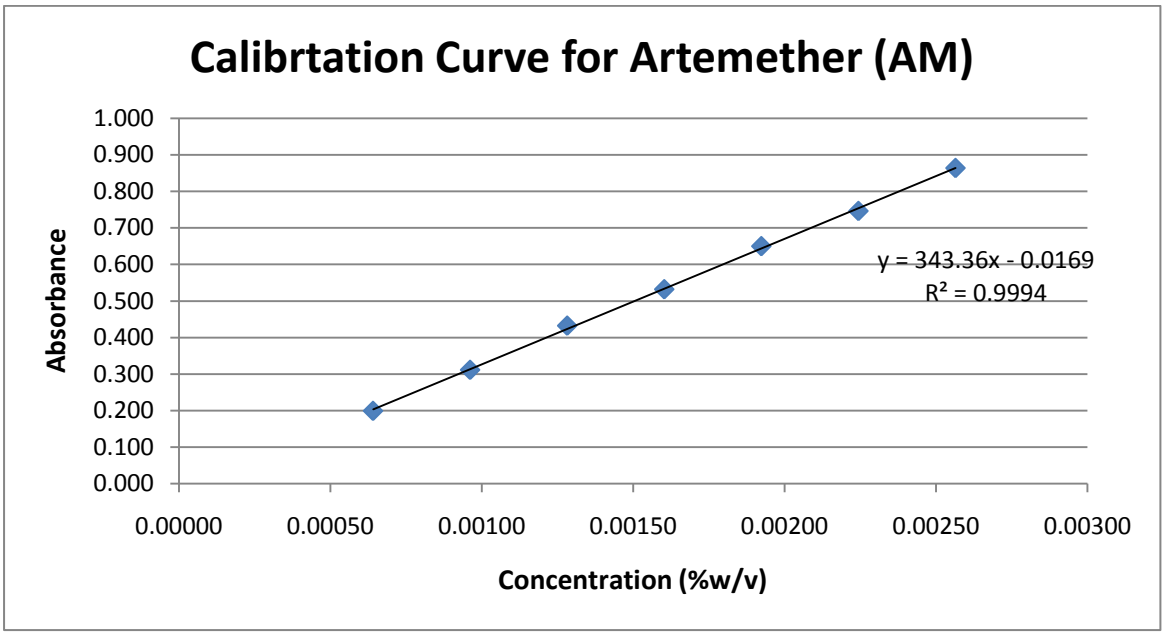



**Fig. 17 Calibration Curve for AM (UV Method)**

**Table 18 Parameters of Calibration Curve (UV Assay of AM)**

| **Parameter** | **Value** |
|---|---|
| Range | 0.00064%w/v – 0.00256%$^{w}/_{v}$ |
| Slope | 343.36 ± 1.70 |
| Intercept | -0.0169 ± 0.0033 |
| $R^2$ | 0.9994 |

- **Accuracy**

**Table 19 Accuracy (UV Assay of AM)**

| Concentration | % Recovered | RSD |
|---|---|---|
| 0.00125 | 100.68 | 0.88 |
| 0.00156 | 100.42 | 1.67 |
| 0.00187 | 99.59 | 0.98 |

- **Precision**

**Table 20 Intra-day Precision (UV Assay of AM)**

| Determination | % Content | |
|---|---|---|
| 1 | 101.92 | |
| 2 | 102.29 | |
| 3 | 100.23 | |
| 4 | 100.98 | |
| 5 | 101.17 | Average = 101.26 |
| 6 | 100.98 | RSD = 0.73 |

**Table 21 Inter-day Precision (UV Assay of AM)**

| Determination | % Content | |
|---|---|---|
| 1 | 101.92 | |
| 2 | 102.29 | |
| 3 | 100.23 | |
| 4 | 100.98 | |
| 5 | 101.17 | |
| 6 | 100.98 | |
| 7 | 98.74 | |
| 8 | 98.93 | |
| 9 | 99.30 | |
| 10 | 98.37 | |

| | | |
|---|---|---|
| 11 | 99.49 | |
| 12 | 98.74 | |
| 13 | 100.26 | |
| 14 | 99.35 | |
| 15 | 99.90 | |
| 16 | 100.08 | |
| 17 | 99.71 | Average = 99.98 |
| 18 | 99.17 | RSD = 1.12 |

- **Limit of Detection (LOD) = 0.000032%w/v**
- **Limit of Quantitation (LOQ) = 0.000096%w/v**

- **Robustness**

**Table 22 Robustness (UV Assay of AM)**

| Parameter | Variation | Average % Recovered |
|---|---|---|
| Allowing solutions to Stand for specific time periods | 2 hours | 100.81±1.43 |
| | 20 hours | 97.29 ±2.87 |
| Varying vol. of HCl used in derivatization | 1.5ml | 99.31±0.55 |
| | 4ml | 100.31±0.40 |

- **SPECIFICITY**

Tablet excepients when taken through the same procedure gave no absorbance at 254nm. Lumefantrine however interfered with the determination at 254nm.

## 3.4 HPLC METHOD DEVELOPMENT AND VALIDATION

### 3.4.1 LUMEFANTRINE

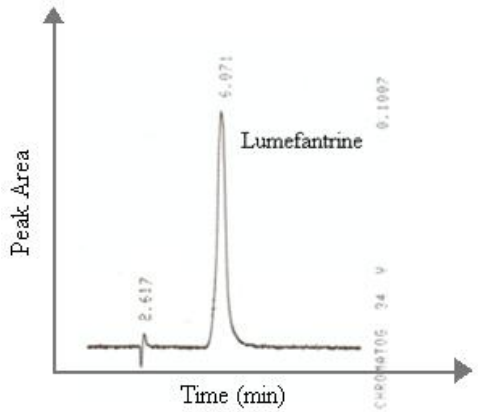



**Fig. 19 Chromatogram of LM**

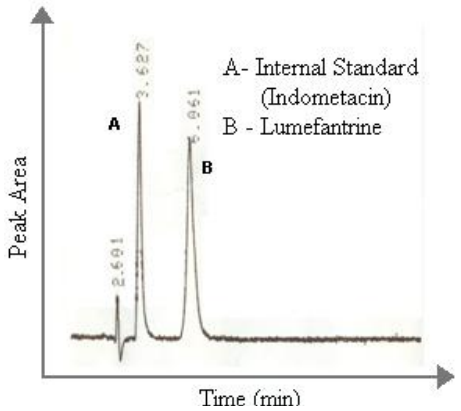



**Fig. 18 Chromatogram of LM with Internal Standard**

**Table 23 Parameters of Chromatograms (HPLC Assay of LM)**

| Parameter | Value |
|---|---|
| Retention Time (min) | 6.0 ± 0.19 |
| Tailing Factor | 1.05 |

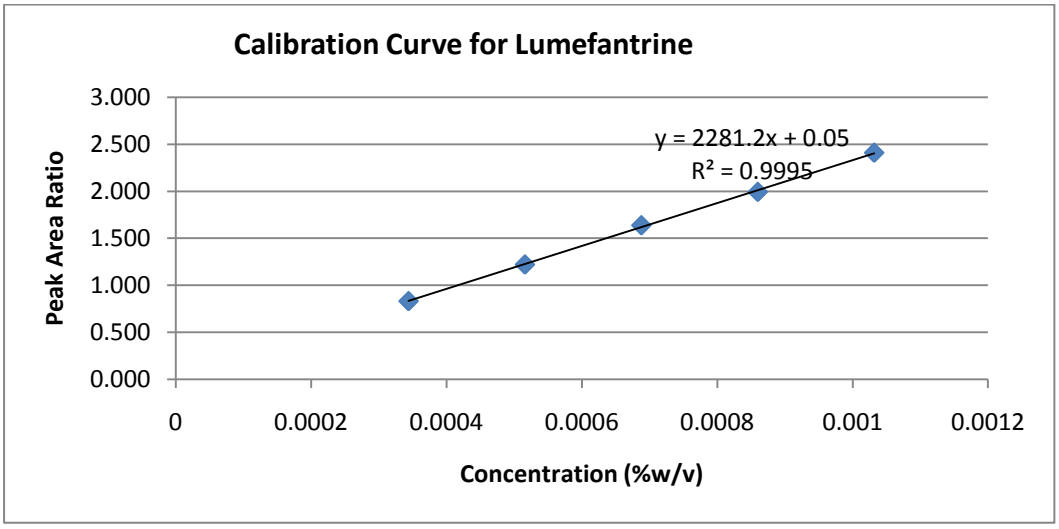



**Fig. 20 Calibration Curve for LM (HPLC Method)**

**Table 24 Parameters of Calibration Curve (HPLC Assay of LM)**

| **Parameter** | **Value** |
|---|---|
| **Range** | 0.00032%w/v – 0.00096%$^{w}/_{v}$ |
| Slope | 2281.25 ± 5.70 |
| Intercept | 0.05 ± .0053 |
| $R^2$ | 0.9994 |

- **Sample Calculation**

**y = mx + c, Where**

- y = Peak Area Ratio
- m = Slope of Calibration Curve
- x = Concentration
- c = y - intercept

From graph, equation of curve: **y = 2281.2x + 0.05**

**For y = 1.6117**

**1.6117 = 2281.2x + 0.05**

**(1.6117 - 0.05)/2281.2= x**

**x = 0.00068460%w/v**

**% Content = (0.00068460/0.0006890) × 100**

**= 99.36%w/w**

- **Accuracy**

**Table 25 Accuracy (HPLC Assay of LM)**

| Concentration (%w/v) | % Recovered | RSD |
|---|---|---|
| 0.0004720 | 98.80 | 1.32 |
| 0.0006294 | 99.86 | 0.49 |
| 0.0007867 | 98.92 | 0.39 |

- **Precision**

**Table 26 Intra-day Precision (HPLC Assay of LM)**

| Determination | % Recovered | |
|---|---|---|
| 1 | 100.43 | |
| 2 | 100.19 | |
| 3 | 101.37 | |
| 4 | 101.65 | |
| 5 | 100.84 | Average = 100.93 |
| 6 | 101.12 | RSD = 0.55 |

**Table 27 Inter-day Precision (HPLC Assay of LM)**

| Determination | % Content | |
|---|---|---|
| 1 | 100.43 | |
| 2 | 100.19 | |
| 3 | 101.37 | |
| 4 | 101.65 | |
| 5 | 100.84 | |
| 6 | 101.12 | |
| 7 | 102.33 | |
| 8 | 101.65 | |
| 9 | 102.72 | |
| 10 | 101.65 | |
| 11 | 100.84 | |
| 12 | 103.54 | |

| | | |
|---|---|---|
| 13 | 100.45 | |
| 14 | 99.49 | |
| 15 | 101.37 | |
| 16 | 102.34 | |
| 17 | 100.98 | Average = 101.41 |
| 18 | 102.48 | RSD = 0.99 |

- **Limit of Detection (LOD) = 0.000040%w/v**
- **Limit of Quantitation (LOQ) = 0.00012%w/v**

- **Robustness**

**Table 28 Robustness - Flow rate (HPLC Assay of LM)**

| Flow rate (ml/min) | %Recovery |
|---|---|
| 2.0 | 97.60 |
| 3.0 | 95.56 |

**Table 29 Robustness - Wavelength of Detection (HPLC Assay of LM)**

| Wavelength of Detection | %Recovery |
|---|---|
| 340 | 98.33 |
| 330 | 98.05 |

**Table 30 Robustness - MP Composition (HPLC Assay of LM-MP)**

| Flow rate (ml/min) | %Recovery |
|---|---|
| Methanol: TFA (95/5) | 97.20 |
| Methanol: TFA (87:13) | 96.45 |

- **Specificity**

Tablet excepients when taken through the procedure gave no peak. Artemether did not interfere with the analysis as it had a different retention time under the analytical chromatographic conditions. Other compounds also gave different retention times under the chromatographic conditions

### 3.4.2 ARTEMETHER

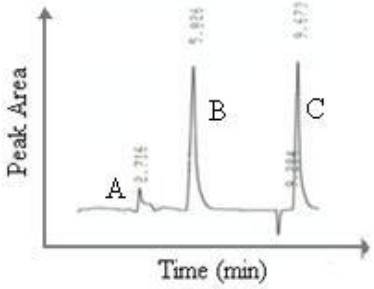



**Fig. 22 Chromatogram of AM with External Standard**

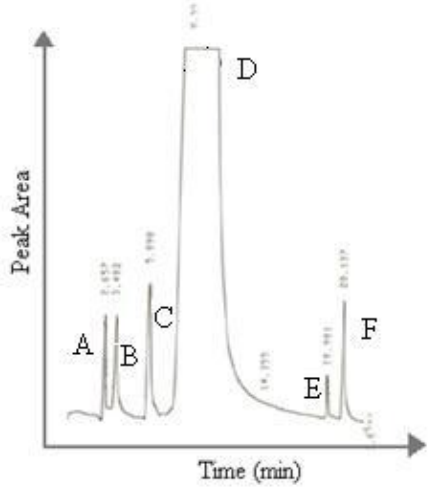



**Fig. 21 Chromatogram of AM Tablet with External Standard**

A – Solvent Peak

B – AM

C – External Standard (Indometacin)

A – Solvent

B – Tablet Excepient

C – AM

D – Lumefantrine

E – Solvent

F – External Standard

**Table 31 Parameters of Chromatograms (AM)**

| Parameter | Value |
|---|---|
| Retention Time of Artemether (min.) | 5.8 ± 0.15 |
| Tailing Factor | 1.2 |
| Retention Time of Lumefantrine (min.) | 8.6 ± 0.21 |

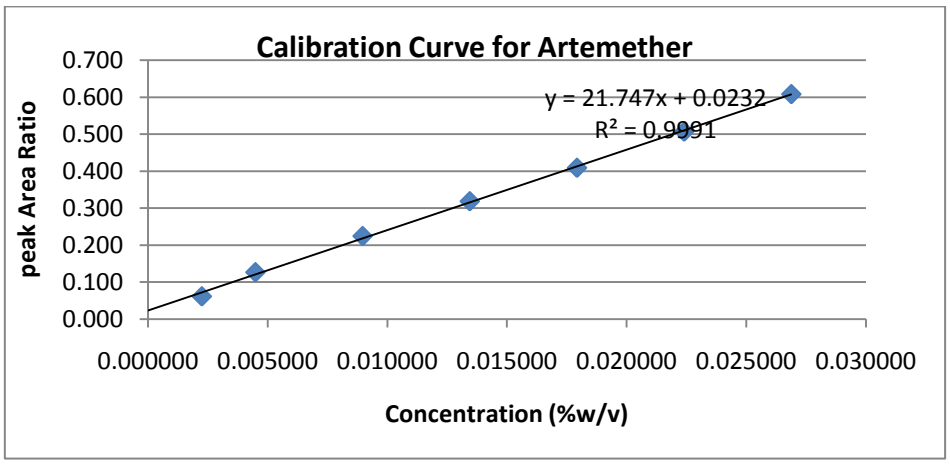



**Fig. 23 Calibration Curve for AM (HPLC Method)**

**Table 32 Parameters of Calibration Curve (HPLC Assay of AM)**

| **Parameter** | **Value** |
|---|---|
| Range | 0.004480%w/v - 0.026880%w/v |
| Slope | 21.589 ± 0.271 |
| Intercept | 0.0200 ± 0.0050 |
| $R^2$ | 0.9991 |

- **Accuracy**

**Table 33 Accuracy (HPLC Assay of AM)**

| Concentration (%w/v) | % Recovered | RSD |
|---|---|---|
| 0.008512 | 98.55 | 0.61 |
| 0.012768 | 98.08 | 0.67 |
| 0.017088 | 97.77 | 0.70 |

- **Precision**

**Table 34 Intra-day Precision (HPLC Assay of AM)**

| Determination | % Recovery | |
|---|---|---|
| 1 | 99.33 | |
| 2 | 98.15 | |
| 3 | 98.05 | |
| 4 | 97.85 | |
| 5 | 96.30 | Average =98.20 |
| 6 | 99.50 | RSD = 1.18 |

**Table 35 Inter-day Precision (HPLC Assay of AM)**

| Determination | % Recovery | |
|---|---|---|
| 1 | 99.33 | |
| 2 | 98.15 | |
| 3 | 98.05 | |

| | | |
|---|---|---|
| 4 | 97.85 | |
| 5 | 96.30 | |
| 6 | 99.50 | |
| 7 | 98.83 | |
| 8 | 97.64 | |
| 9 | 98.87 | |
| 10 | 97.19 | |
| 11 | 97.47 | |
| 12 | 99.74 | |
| 13 | 99.09 | |
| 14 | 97.39 | |
| 15 | 99.01 | |
| 16 | 97.19 | |
| 17 | 96.78 | Average = 98.10 |
| 18 | 97.46 | RSD = 1.01 |

- **Limit of Detection (LOD) = 0.00085%w/v**
- **Limit of Quantitation (LOQ) = = 0.0026%w/v**

- **Robustness**

**Table 36 Robustness- Flow rate (HPLC Assay of AM)**

| Flow rate (ml/min) | %Recovery |
|---|---|
| 2.0 | 96.80 |
| 3.0 | 95.56 |

**Table 37 Robustness- Flow rate (HPLC Assay of AM)**

| Flow rate (ml/min) | %Recovery |
|---|---|
| Methanol: TFA (95/5) | 98.10 |
| Methanol: TFA (87:13) | 96.76 |

- **Specificity**

Tablet excepients when taken through the procedure gave no peak. Lumefantrine did not interfere with the analysis as it had a different retention time under the analytical chromatographic conditions. Other compounds also gave different retention times using the chromatographic conditions.

## 3.5 ASSAY OF TABLETS

**Table 38 Assay of Brands of AM-LM Tablets**

| Brand | LM (UV analysis) | LM (HPLC analysis) | AM (HPLC analysis) |
|---|---|---|---|
| A | 101.23 ± 0.95 | 100.17 ± 0.70 | 83.50 ± 0.65 |
| B | 102.85 ± 0.89 | 101.77 ± 0.22 | 99.20 ±1.03 |
| C | 94.15 ± 4.21 | 93.71 ± 0.88 | 74.62 ± 0.88 |
| D | 104.35 ± 0.68 | 103.45 ± 0.49 | 95.82 ± 1.06 |
| E | 97.35 ± 1.43 | 95.26 ± 1.35 | 105.27 ± 3.98 |
| F | 94.83 ± 1.08 | 96.22 ± 0.70 | 92.05 ± 1.24 |

# CHAPTER 4 – DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 4.1 DISCUSSION

### 4.1.1 IDENTIFICATION AND ASSAY OF PURE SAMPLES

Reference samples of drugs are very important in analysis. This is because they serve as a reference to which other drugs and formulations are compared to both for qualitative and quantitative analysis. To ensure their authenticity, they have to be identified and assayed prior to their use.

#### 4.1.1.1 Artemether

The Artemether was identified by colour tests stated in the IP and also by its melting point.

A yellow colour was produced when potassium iodide was added to the ethanolic solution of the sample and heated. Adding a drop of vanillin/sulphuric acid TS1 to the ethanolic solution of the sample also produced a pink colour. These tests indicate that the sample was Artemether. The melting point of the sample agreed with the literature value of 86-90˚C as stated in the IP, further confirming the sample as Artemether and also ascertaining its purity.

The purity of the Artemether sample was further ascertained from its TLC and HPLC Chromatograms. The TLC chromatogram when observed both in day-light and UV light at 366nm showed only one spot. The HPLC chromatogram also gave only one peak using different chromatographic conditions.

#### 4.1.1.2 Lumefantrine

The sample was identified as stated in the IP (Draft). The methanolic solution of the Lumefantrine gave a specific absorbance of 324 which falls within the reference range of 314–348 as stated in the IP Draft. The melting range of the sample was 128–130˚C and this agreed with the literature value of 128–132˚C. An assay of the sample by non-aqueous titration gave the percentage purity as 101.04 ± 0.30%w/w which falls within the range of 98-102%w/w as stated in the USP SALMOUS Standard.

The purity of the Lumefantrine sample was further ascertained from its TLC and HPLC Chromatograms. The TLC chromatogram when observed both in day-light and UV showed only one spot. The HPLC chromatogram also gave only one peak using different chromatographic conditions.

### 4.1.2 IDENTIFICATION OF ARTEMETHER AND LUMEFANTRINE IN TABLETS

TLC was used to confirm that the tablets contained Artemether and Lumefantrine, using a mobile phase made up of cyclohexane, ethyl acetate and glacial acetic acid (20 : 5 : 2.5). Extracts of the tablets after spotting on the plates and observing in both day-light and under UV, gave spots that corresponded to those of Artemether and Lumefantrine pure samples. Artemether was identified as a greyish-purple spot on a white background in daylight and as a light yellow fluorescent spot on a blue background under UV light at 366 nm. Lumefantrine appeared as a greyish-yellow spot on a white background in daylight and as a dark spot on a blue fluorescent background under UV light at 366 nm. The $R_f$ values of the spotted tablets were the same as those of Artemether and Lumefantrine pure samples. Artemether gave an $R_f$ value of 0.77 and Lumefantrine gave an $R_f$ value of 0.42.

### 4.1.3 PHARMACOPOEIAL TESTS

#### 4.1.3.1 Uniformity of Weight Test

According to the British Pharmacopoeia, when 20 tablets or capsules are selected for the uniformity of weight test, then not more than 2 of the individual masses should deviate from the average mass by more than the percentage deviation shown in Table 39 and none should deviate by more than twice that percentage.

**Table 39 BP Reference for uniformity of weight test**

| Pharmaceutical form | Average mass | Percentage deviation |
|---|---|---|
| Tablets (uncoated and film coated) | 80mg or less | 10 |
| | More than 80mg and less than 250mg | 7.5 |
| | 250mg or more | 5 |
| Capsules | Less than 300mg | 10 |
| | 300mg or more | 7.5 |

From the results as shown from Tables 40- 45, all the brands of the tablets passed the uniformity of weight test. The tablets had average weights ranging from 0.24 to 0.36g.

#### 4.1.3.2 Tablet Disintegration Test

For tablets to pass the tablet disintegration test, six tablets selected at random from a batch of tablets should disintegrate within 15 minutes. As can be seen from Table 12, all the brands of tablets disintegrated in less than 15 minutes and thus passed the test. There was however a marked difference in the disintegration time of the tablets from the different brands. Tablets from Brand A had the shortest disintegration time of 0.5 minutes and those from Brand C had the longest disintegration time of 9.2 minutes. The differences could be attributed to differences in excepients used in the manufacture of the tablets as well as differences in the manufacturing process. Though there is no direct correlation between disintegration and dissolution, the tablet disintegration test is very useful and should not be ignored.

### 4.1.4 METHOD DEVELOPMENT AND VALIDATION

The methods were validated according to ICH guidelines.

#### 4.1.4.1 UV Analysis

##### 4.1.4.1.1 Lumefantrine

- **Method Development**

Lumefantrine is sparingly insoluble in methanol. However acidifying methanol ensured the complete dissolution of Lumefantrine and this was employed in its assay. 0.1M methanolic HCl was thus used as the solvent. The UV spectrum of Lumefantrine in 0.1M Methanolic HCl gave λ max values at 235, 265, 303 and 335nm. 335nm was selected for the analysis as it had the least interference from other compounds.

- **Method Validation**

Linearity was observed with concentrations of Artemether over the range of 0.0008%w/v – 0.0028%w/v. Within this range, graphs of absorbance against concentration of Lumefantrine gave $R^2$ values of above 0.99 in all instances. This shows that there is linear relationship between the Lumefanatrine concentrations and the absorbance values at 335nm

Also, with respect to accuracy, there was a percentage recovery close to 100% when Lumefantrine reference sample was mixed with tablet excepients and analyzed. The average percentage recovered was 100.81%w/w.

The method proved to be precise. In both the intra-day and inter-day precision, the method presented RSD values lower than 2.0%, assuring a good precision. The intra-day and inter-day precision assessment yielded RSD values of 1.17 and 1.32 respectively.

Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of some parameters were introduced. Thus, the method showed to be robust for changes in concentration of Methanolic HCl from 0.075 - 0.15M. Also a solution for analysis after standing for 12 hours gave a percentage recovery of 101.21%.

The method proved to be specific for Lumefantrine as tablet excepients an Artemether did not interfere with the analysis.

The LOD and LOQ values obtained from the calibration curve were 0.00006%w/v and 0.00018%w/v respectively.

#### 4.1.4.1.2 Artemether

- **Method Development**

As earlier discussed, the acid decomposition product of Artemether is made use of in its assay by UV Spectrophotometry as the compound itself lacks strongly absorbing chromophores. This product is achieved by treatment with HCl. As can be seen from the results, there was a drastic decrease in the analysis time when concentrated HCl was used in the assay as compared to the existing methods that used lower concentrations of HCl.

The results indicated that a volume of 2ml concentrated HCl, room temperature (or a temperature of 30˚C) and a reaction time of 25 minutes were the most appropriate conditions for the formation of the HCl decomposition product and hence these were selected. It can be seen that using 1ml of HCl at room temperature gave sub maximal absorbance readings after the solutions were allowed to stand for up to 1 hour. Also using volumes of HCl above 2ml at room also gave absorbance readings that were slightly lower as compared to those obtained when 2ml of HCl was used for the times the solutions were allowed to stand for. In the case of using volumes of HCl higher than 2ml, the absorbance readings peaked at an earlier time as compared to when using 2ml. When using a 3ml HCl, the absorbance reading reached its maximum after standing for a period of 15 minutes whereas in the case of using 4ml HCl, the maximum was reached at about 10 minutes. Using the 2ml of HCl, gave a maximum reading

between 15 and 20 minutes. Using a volume of 1.5 ml of HCl gave results that were similar to those obtained when using 3ml HCl at 15 and 25minutes of standing.

Also, from the results it can be seen that the use of elevated temperatures resulted in irregularities in the absorbance values obtained. When volumes of HCl of 2ml or more were used and a temperature of 40˚C was employed, there was a gradual decrease in absorbance values. It can thus be inferred that the elevated temperatures broke down the derivative and hence the decreased absorbance readings. Using 1ml of concentrated HCl and a reaction temperature of 40˚C showed an initial increase in absorbance values up to 15 minutes followed by a decrease with increasing reaction time up to 50 minutes. The volume of the Artemether solution was maintained at 2ml to ensure uniformity in the procedure.

- **Method Validation**

Linearity was observed with concentrations of Artemether over the range of 0.00064%w/v – 0.00256%w/v. Within this range, graphs of absorbance against concentration values of Artemether gave straight lines with $R^2$ values of above 0.99 in all instances. This shows that there is linear relationship between the Artemether concentrations and the absorbance values at 254nm.

Also, with respect to accuracy, there was an average percentage recovery of 100.29% of Artemether when Artemether reference sample was mixed with tablet excepients and analyzed.

A good precision was observed with the method. The intra-day and inter-day precision assessment yielded RSD values of 0.73 and 1.12 respectively.

The method proved to be robust as there was no significant difference in absorbance readings of the prepared solutions when they were left standing for periods of up to 24 hours. The amounts of Artemether recovered from the solutions after standing for 2 hours and 12 hours were 100.81 and 97.29% respectively. Also varying the volumes of HCl used for the analysis did not show any significant difference. Employing 1.5 ml and 4ml of HCl for the assay gave percentage recoveries of 99.31 and 100.31 respectively.

Calculating the LOD and LOQ from the calibration curve gave their values as 0.000032%w/v and 0.000096%w/v respectively.

The major limitation with this method of analysis is that, it can only be used in the analysis of pure Artemether samples or Artemether-only tablets. This is because Lumefantrine interferes with the analysis at the wavelength of 254nm when fixed-dose Artemether-Lumefantrine combination tablets were analyzed using this method. A bid to achieve separation of Artemether and Lumefantrine by liquid-liquid extraction proved futile.

#### 4.1.4.2 HPLC Analysis

##### 4.1.4.2.1 Chromatographic Conditions

As indicated earlier, there are a few HPLC methods of analysis for fixed-dose Artemether-Lumefantrine combination formulations. These methods also require working at very low wavelengths of detection and hence employ acetonitrile as the main solvent in the mobile phase. Also, some of the methods employ gradient elution for analysis.

The price of acetonitrile coupled with its recent shortage created a major problem in analytical work involving the use of acetonitrile. Methanol, though it has some limitations is purported to be a suitable alternative to acetonitrile. Hence for this analysis, methanol was studied as an alternative to acetonitrile in the assay of Artemether and Lumefantrine in fixed-dose combination tablets. However since methanol has a higher cut off point compared to acetonitrile, there was the need to shift to a higher wavelength as compared to the existing methods. This made the simultaneous determination of the two impossible, hence the need to assay them separately.

Though the two components were to be assayed using different chromatographic conditions, there was the need for the Mobile phases selected to effectively elute both compounds. This would ensure a shorter washing period between runs. To ensure this, slight variations were made in the mobile phases for the analysis of the two components. Different wavelengths of detection were also employed due to the differences in the UV Spectra of the two compounds.

Also, two different concentrations of the test solutions of the tablets were prepared for analysis. This is because, it was realized that at the concentration where Artemether could be determined, Lumefantrine was off-scale. The higher concentration was thus used to determine Artemether and the lower concentration for the determination of Lumefantrine. In the analysis of Artemether, the concentrations of the solutions for analysis were augmented

with a fixed amount of a prepared artemether stock solution to facilitate the calculation of the peak areas.

**Indometacin** was employed as an internal standard in the analysis of Lumefantrine. However, in the analysis of Artemether it was used as an external standard due to interference from other compounds at the working wavelength of 235nm.

#### 4.1.4.2.2 Lumefantrine

- **Method Development**

Methanol was the major component of the mobile phase. To improve separation, there was the need to acidify the mobile phase to ensure complete ionization of Lumefantrine. Glacial acetic acid was tried but it did not suit the purpose well as there was considerable tailing with Lumefantrine at concentrations up to 5%v/v in the mobile phase. There was thus the need to adopt a stronger acid and TFA proved suitable for this purpose. A mobile phase of Methanol and 0.1%TFA was employed for the assay of Lumefantrine. From the UV spectrum, a wavelength of 335nm was selected for analysis. This wavelength was selected because there is no interference from Artemether and many other compounds. A good retention time of 6.0 ± 0.19 minutes was also achieved. Decreasing the concentration of the TFA resulted in increasing the Retention time of the compound.

- **Method Validation**

Linearity was observed with concentrations of Artemether over the range of 0.00032%w/v – 0.00096%w/v. Within this range, graphs of absorbance against concentration of Lumefantrine gave straight lines with $R^2$ values of above 0.99 in all instances. This shows that there is linear relationship between the Artemether concentrations and the peak areas in chromatogram.

There was a percentage recovery of 99.53 when Lumefantrine reference sample was mixed with tablet excepients and analyzed.

The Intra-day and inter-day precision values were 0.55 and 0.99 respectively indicating a good precision.

Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of some parameters were introduced. Thus, the method showed to be robust

for changes in mobile phase flow rate from 2.0 to 3.0 ml/min and Methanol proportion in the mobile phase from 87% to 95%.

The method proved to be specific for Lumefantrine as tablet excepients an Artemether did not interfere with the analysis. The LOD and LOQ values from the calibration curve were 0.000040%w/v and 0.00012%w/v respectively.

#### 4.1.4.2.3 Artemether

- **Method Development**

For the assay of Artemether a mobile phase of Methanol and 0.04% TFA (90:10) was employed. The concentration of the TFA did not affect the separation of the Artemether significantly because as can be seen from its structure, the acid does not modify the compound. 235nm was selected for the assay because as can be seen from its UV Spectrum, Artemether absorbs considerably at this wavelength. At this wavelength however, Lumefantrine also absorbs strongly hence there was the need to choose a mobile phase that would considerably increase the retention time of Lumefantrine and thus prevent it from interfering with the separation of Artemether. This led to led to the need to decrease the concentration of TFA as compared to that in the assay of Lumefantrine. Using this mobile phase, Artemether was eluted in 5.8 ± 0.15 minutes and Lumefantrine in 8.6 ± 0.21 minutes ensuring a good separation.

- **Method Validation**

Linearity was observed with concentrations of Artemether over the range of 0.004480%w/v - 0.026880%w/v. Within this range, $R^2$ values of calibration curves were above 0.99 in all instances. This shows that there is linear relationship between the Artemether concentrations and peak areas in the chromatograms.

There was an average percentage recovery of 98.13 Lumefantrine reference sample was mixed with tablet excepients and analyzed.

In both the intra-day and inter-day precision, the method presented RSD values lower than 2.0%, assuring a good precision. The intra-day precision was 1.18 and the Inter-day precision was 1.01.

Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of some parameters were introduced. Thus, the method showed to be robust

for changes in mobile phase flow rate from 2.0 to 3.0 ml/min and Methanol proportion in the mobile phase from 87% to 95%.

The method proved to be specific for Artemether as tablet excepients and Lumefantrine did not interfere with the analysis. Other tested compound did not also interfere with the analysis. Calculating the LOD and LOQ from the calibration curve gave their values as 0.00006%w/v and 0.00018%w/v respectively.

### 4.1.5 ASSAY OF TABLETS

According to the USP SALMOUS standard, Artemether-Lumefantrine tablets should contain not less than 90.0 percent and not more than 110.0% of the labelled amounts of Artemether and of Lumefantrine.

From the HPLC method of assay, the mean contents of Artemether in the brands of tablets assayed ranged from 74.62 to 105.27%w/w of the labelled amount with standard deviations from 0.65 to 3.98. The mean contents of Lumefantrine in the tablets from the HPLC method of assay ranged from 93.71 to 103.45%w/w with standard deviations from 0.22 to 1.35.

With regards to the UV Assay of Lumefantrine, mean contents of Lumefantrine in the brands of tablets assayed, ranged from 94.15 to 104.35% of the labelled amount with standard deviations from 0.68 to 4.21.

Results show that all the brands of Artemether-Lumefantrine fixed dose combination tablets contained amounts of Lumefantrine that conformed to the required amounts as stated in the USP SALMOUS standard. Two of the brands, A and C, however had their contents of Artemether being lower than required. These tablets had percentage contents of Artemether of 83.50 %± 0.65 and 74.62% ± 0.88.

Statistical analysis showed that the UV and HPLC methods for the analysis of Lumefantrine did not differ significantly. The $t_{exp}$ values calculated were less than the critical value of 3.17 at the 99% confidence interval, indicating that the methods gave results that were not significantly different level.

## 4.2 CONCLUSION

### 4.2.1 IDENTIFICATION OF SAMPLES

The identification tests confirmed that the samples were Artemether and Lumefantrine and also of good purity.TLC also confirmed that all the tablets contained Artemether and Lumefantrine.

### 4.2.2 PHARMACOPOEIAL TESTS

All the tablets passed the Uniformity of Weight test and the Tablet Disintegration Test.

### 4.2.3 MEHOD DEVELOPMENT AND VALIDATION

The proposed UV and HPLC methods proved to be suitable for the assay of Artemether and Lumefantrine in fixed-dose combination tablets. The UV method for the assay of Artemether however cannot be used in the assay of Artemether in fixed-dose combination tablets due to interference from Lumefantrine.

### 4.2.4 ASSAY OF TABLETS

All the brands of tablets contained the required amount of Lumefantrine when assayed by both UV and HPLC methods according to the USP SALMOUS edition. Two of the foreign brands contained less than the required amounts of Artemether when assayed by the HPLC method.

## 4.3 RECOMMENDATIONS

Studies should be conducted on the different brands of the Artemether-Lumefantrine tablets to determine their dissolution profiles.

# APPENDIX

- **PHARMACOPOEIAL TESTS**

**Table 40 Uniformity of Weight Test for Tablet Brand A**

| Tab. No. | Wt. Of tab. | Deviation | % Deviation |
|---|---|---|---|
| 1 | 0.2418 | -0.0005 | -0.2064 |
| 2 | 0.2405 | -0.0018 | -0.7429 |
| 3 | 0.2399 | -0.0024 | -0.9905 |
| 4 | 0.2405 | -0.0018 | -0.7429 |
| 5 | 0.2429 | 0.0006 | 0.2476 |
| 6 | 0.2424 | 0.0001 | 0.0413 |
| 7 | 0.2435 | 0.0012 | 0.4953 |
| 8 | 0.2400 | -0.0023 | -0.9492 |
| 9 | 0.2388 | -0.0035 | -1.4445 |
| 10 | 0.2435 | 0.0012 | 0.4953 |
| 11 | 0.2467 | 0.0044 | 1.8159 |
| 12 | 0.2403 | -0.0020 | -0.8254 |
| 13 | 0.2413 | -0.0010 | -0.4127 |
| 14 | 0.2430 | 0.0007 | 0.2889 |
| 15 | 0.2440 | 0.0017 | 0.7016 |
| 16 | 0.2410 | -0.0013 | -0.5365 |
| 17 | 0.2428 | 0.0005 | 0.2064 |
| 18 | 0.2407 | -0.0016 | -0.6603 |
| 19 | 0.2436 | 0.0013 | 0.5365 |
| 20 | 0.2432 | 0.0009 | 0.3714 |

| |
|---|
| Wt. Of 20 tabs = 4.8455g |
| Average Wt. Of 1 tab = 0.2423g |

**Table 41 Uniformity of Weight Test for Tablet Brand B**

| Tab. No. | Wt. Of tab. | Deviation | % Deviation |
|---|---|---|---|
| 1 | 0.2448 | 0.0025 | 1.0318 |
| 2 | 0.2433 | 0.0010 | 0.4127 |
| 3 | 0.2435 | 0.0012 | 0.4953 |
| 4 | 0.2418 | -0.0005 | -0.2064 |
| 5 | 0.2439 | 0.0016 | 0.6603 |
| 6 | 0.2434 | 0.0011 | 0.4540 |
| 7 | 0.2394 | -0.0029 | -1.1969 |
| 8 | 0.2421 | -0.0002 | -0.0825 |
| 9 | 0.2418 | -0.0005 | -0.2064 |
| 10 | 0.2398 | -0.0025 | -1.0318 |
| 11 | 0.2427 | 0.0004 | 0.1651 |
| 12 | 0.2427 | 0.0004 | 0.1651 |
| 13 | 0.2433 | 0.0010 | 0.4127 |
| 14 | 0.2429 | 0.0006 | 0.2476 |
| 15 | 0.2425 | 0.0002 | 0.0825 |
| 16 | 0.2404 | -0.0019 | -0.7842 |
| 17 | 0.2416 | -0.0007 | -0.2889 |
| 18 | 0.2439 | 0.0016 | 0.6603 |
| 19 | 0.2421 | -0.0002 | -0.0825 |
| 20 | 0.2401 | -0.0022 | -0.9080 |

| |
|---|
| Wt. Of 20 tabs = 4.8457g |
| Average Wt. Of 1 tab = 0.2423g |

**Table 42 Uniformity of Weight Test for Tablet Brand C**

| Tab. No. | Wt. Of tab. | Deviation | % Deviation |
|---|---|---|---|
| 1 | 0.3412 | 0.0071 | 2.1251 |
| 2 | 0.3337 | -0.0004 | -0.1197 |
| 3 | 0.3402 | 0.0061 | 1.8258 |
| 4 | 0.3258 | -0.0083 | -2.4843 |
| 5 | 0.3444 | 0.0103 | 3.0829 |
| 6 | 0.3360 | 0.0019 | 0.5687 |
| 7 | 0.3305 | -0.0036 | -1.0775 |
| 8 | 0.3356 | 0.0015 | 0.4490 |
| 9 | 0.3332 | -0.0009 | -0.2694 |
| 10 | 0.3347 | 0.0006 | 0.1796 |
| 11 | 0.3232 | -0.0109 | -3.2625 |
| 12 | 0.3317 | -0.0024 | -0.7183 |
| 13 | 0.3245 | -0.0096 | -2.8734 |
| 14 | 0.3341 | 0.0000 | 0.0000 |
| 15 | 0.3280 | -0.0061 | -1.8258 |
| 16 | 0.3479 | 0.0138 | 4.1305 |
| 17 | 0.3448 | 0.0107 | 3.2026 |
| 18 | 0.3366 | 0.0025 | 0.7483 |
| 19 | 0.3262 | -0.0079 | -2.3646 |
| 20 | 0.3236 | -0.0105 | -3.1428 |

| |
|---|
| Wt. Of 20 tabs = 6.6822g |
| Average Wt. Of 1 tab = 0.3341g |

**Table 43 Uniformity of Weight Test for Tablet Brand D**

| Tab. No. | Wt. Of tab. | Deviation | % Deviation |
|---|---|---|---|
| 1 | 0.3419 | 0.0060 | 1.7862 |
| 2 | 0.3325 | -0.0034 | -1.0122 |
| 3 | 0.3347 | -0.0012 | -0.3572 |
| 4 | 0.3376 | 0.0017 | 0.5061 |
| 5 | 0.3347 | -0.0012 | -0.3572 |
| 6 | 0.3342 | -0.0017 | -0.5061 |
| 7 | 0.3355 | -0.0004 | -0.1191 |
| 8 | 0.3383 | 0.0024 | 0.7145 |
| 9 | 0.3361 | 0.0002 | 0.0595 |
| 10 | 0.3370 | 0.0011 | 0.3275 |
| 11 | 0.3412 | 0.0053 | 1.5779 |
| 12 | 0.3355 | -0.0004 | -0.1191 |
| 13 | 0.3302 | -0.0057 | -1.6969 |
| 14 | 0.3384 | 0.0025 | 0.7443 |
| 15 | 0.3354 | -0.0005 | -0.1489 |
| 16 | 0.3422 | 0.0063 | 1.8756 |
| 17 | 0.3375 | 0.0016 | 0.4763 |
| 18 | 0.3309 | -0.0050 | -1.4885 |
| 19 | 0.3334 | -0.0025 | -0.7443 |
| 20 | 0.3300 | -0.0059 | -1.7565 |

| |
|---|
| Wt. Of 20 tabs = 6.7186g |
| Average Wt. Of 1 tab = 0.3359g |

**Table 44 Uniformity of Weight Test for Tablet Brand E**

| Tab. No. | Wt. Of tab. | Deviation | % Deviation |
|---|---|---|---|
| 1 | 0.2467 | -0.0003 | -0.1215 |
| 2 | 0.2433 | -0.0037 | -1.4980 |
| 3 | 0.2493 | 0.0023 | 0.9312 |
| 4 | 0.2448 | -0.0022 | -0.8907 |
| 5 | 0.2509 | 0.0039 | 1.5789 |
| 6 | 0.2512 | 0.0042 | 1.7004 |
| 7 | 0.2411 | -0.0059 | -2.3887 |
| 8 | 0.2467 | -0.0003 | -0.1215 |
| 9 | 0.2447 | -0.0023 | -0.9312 |
| 10 | 0.2485 | 0.0015 | 0.6073 |
| 11 | 0.2434 | -0.0036 | -1.4575 |
| 12 | 0.2466 | -0.0004 | -0.1619 |
| 13 | 0.2491 | 0.0021 | 0.8502 |
| 14 | 0.2450 | -0.0020 | -0.8097 |
| 15 | 0.2500 | 0.0030 | 1.2146 |
| 16 | 0.2515 | 0.0045 | 1.8219 |
| 17 | 0.2417 | -0.0053 | -2.1457 |
| 18 | 0.2467 | -0.0003 | -0.1215 |
| 19 | 0.2447 | -0.0023 | -0.9312 |
| 20 | 0.2483 | 0.0013 | 0.5263 |

| |
|---|
| Wt. Of 20 tabs = 4.94396g |
| Average Wt. Of 1 tab = 0.2470g |

**Table 45 Uniformity of Weight Test for Tablet Brand F**

| Tab. No. | Wt. Of tab. | Deviation | % Deviation |
|---|---|---|---|
| 1 | 0.3570 | -0.0060 | -1.6529 |
| 2 | 0.3712 | 0.0082 | 2.2590 |
| 3 | 0.3746 | 0.0116 | 3.1956 |
| 4 | 0.3537 | -0.0093 | -2.5620 |
| 5 | 0.3607 | -0.0023 | -0.6336 |
| 6 | 0.3657 | 0.0027 | 0.7438 |
| 7 | 0.3659 | 0.0029 | 0.7989 |
| 8 | 0.3673 | 0.0043 | 1.1846 |
| 9 | 0.3522 | -0.0108 | -2.9752 |
| 10 | 0.3626 | -0.0004 | -0.1102 |
| 11 | 0.3628 | -0.0002 | -0.0551 |
| 12 | 0.3572 | -0.0058 | -1.5978 |
| 13 | 0.3710 | 0.0080 | 2.2039 |
| 14 | 0.3600 | -0.0030 | -0.8264 |
| 15 | 0.3740 | 0.0110 | 3.0303 |
| 16 | 0.3539 | -0.0091 | -2.5069 |
| 17 | 0.3659 | 0.0029 | 0.7989 |
| 18 | 0.3658 | 0.0028 | 0.7713 |
| 19 | 0.3672 | 0.0042 | 1.1570 |
| 20 | 0.3520 | -0.0110 | -3.0303 |

| |
|---|
| Wt. Of 20 tabs = 7.2812g |
| Average Wt. Of 1 tab = 0.3641g |

**Table 46 Tablet Disintegration Test**

| Brand of Tablet | Disintegration Time (min.) | | | | | | Average Disintegration Time |
|---|---|---|---|---|---|---|---|
| A | 0.4 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 ± 0.04 |
| B | 4.3 | 4.3 | 4.0 | 4.3 | 4.3 | 4.3 | 4.2 ± 0.12 |
| C | 8.0 | 8.5 | 9.0 | 9.0 | 10.0 | 10.5 | 9.2 ± 0.93 |
| D | 2.0 | 2.0 | 2.2 | 2.3 | 2.3 | 2.3 | 2.2 ± 0.15 |
| E | 4.8 | 5.0 | 5.0 | 5.0 | 5.2 | 5.3 | 5.0 ± 0.18 |
| F | 2.2 | 2.3 | 2.5 | 2.5 | 2.5 | 2.5 | 2.4 ± 0.13 |

- **UV METHOD DEVELOPMENT (AM)**

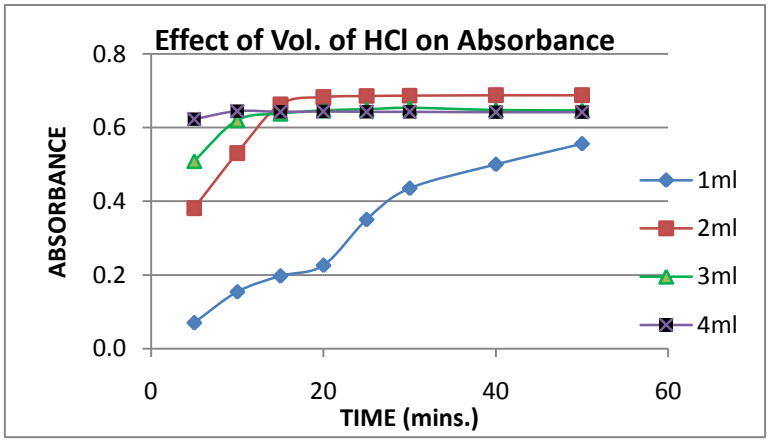



**Fig. 24 Effect of Vol. of HCl and Reaction Time on Absorbance of AM**

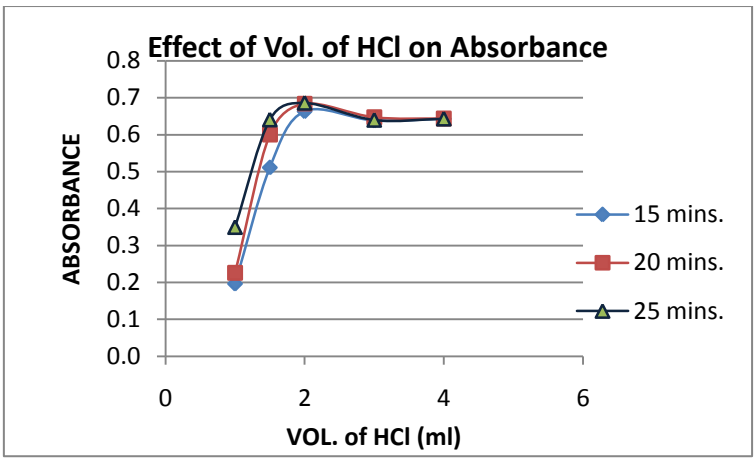



**Fig. 25 Effect of Vol. of HCl and Reaction Time on Absorbance of AM**

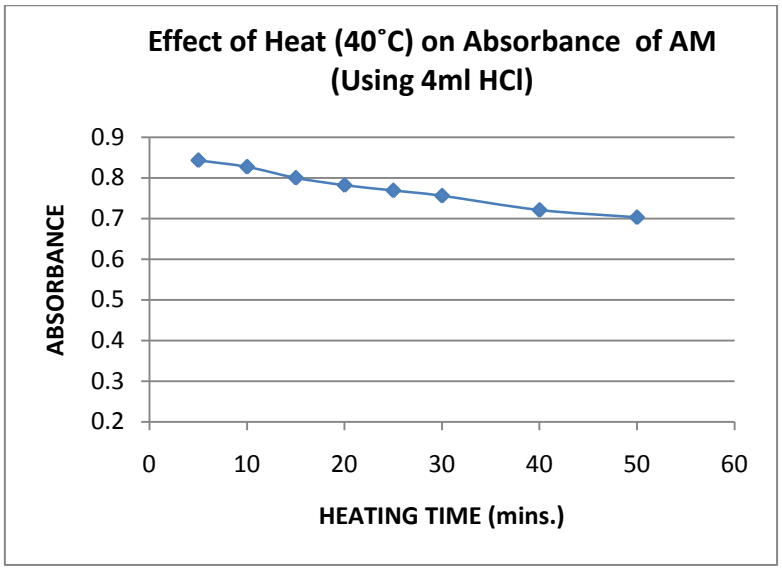



**Fig. 26 Effect of Heat on Absorbance of AM (4ml HCl)**

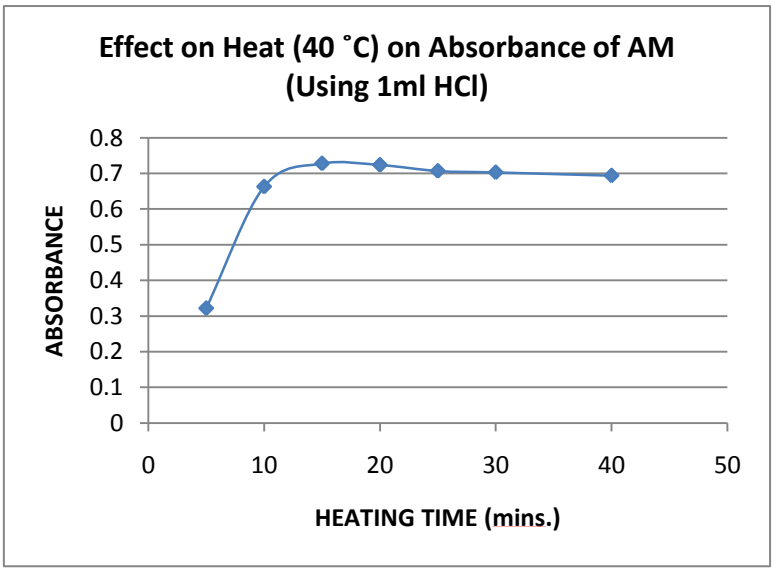



**Fig. 27 Effect of Heat on Absorbance of AM (1ml HCl)**

- **HPLC METHOD DEVELOPMENT**

**Table 47 Summary of Mobile Phase Systems Tried**

| Mobile Phase Composition | Flow Rate (ml/min) | Solvent Peak $R_t$ (min) | Artemether $R_t$ (min) | Lumefantrine $R_t$ (min) | Comments |
|---|---|---|---|---|---|
| 3% Acetic acid (Methanolic)/Water (95/5) | 2.5 | 1.8 | | 9.7 | Tailing observed with L.M |
| 5% Acetic acid (Methanolic)/Water (95/5) | 2.5 | 1.6 | | 9.0 | Tailing observed with L.M |
| Methanol/0.05%TFA (90/10) | 3.0 | 1.9 | 4.4 | 6.3 | A.M and L.M Peaks well defined and well spaced |
| Methanol/0.05%TFA (90/10) | 2.5 | 2.5 | 5.7 | 8.1 | A.M and L.M Peaks well defined and well spaced |
| Methanol/0.05% TFA (90/10) | 2.0 | 3.46 | 8.0 | 11.0 | A.M and L.M Peaks well defined and well spaced |
| Methanol/0.04% TFA (90/10) | 2.5 | 3.49 | 5.90 | 8.60 | A.M and L.M Peaks well defined and well spaced |
| Methanol/0.05%TFA (80/20) | 2.5 | 3.1 | | | No peaks observed as at 12 mins. |
| Acetonitrile/0.05%TFA (90/10) | 2.5 | 1.3 | 2.4 | 5.0 | A.M and L.M Peaks well defined and well spaced. |
| Acetonitrile/0.05%TFA (90/10) | 1.5 | 2.8 | 5 | 11.8 | Artemether Peak well defined but tailing observed with Lumefantrine peak. Peaks well spaced |
| Acetonitrile/0.05%TFA (80/20) | 1.5 | 2.7 | | 11.6 | Tailing observe d with L.M peak |

| | | | | | |
|---|---|---|---|---|---|
| Acetonitrile/0.05%TFA (80/20) | 2.5 | 1.4 | | 6.5 | Well defined L.M peak |
| Methanol/0.05%TFA (95/5) | 2.5 | 2.0 | 3.7 | 5.6 | A.M and L.M Peaks well defined and well spaced. |
| Methanol/0.1%TFA (90/10) | 2.5 | 2.6 | 5.3 | 5.9 | A.M and L.M elute too closely to each other. Peaks merge when samples are injected together. |
| Methanol/0.1%TFA (90/10) | 2.0 | 3.4 | 7.9 | 8.5 | A.M and L.M Peaks well defined but elute too closely to each other. Peaks merge when samples are injected together. |
| Methanol/Acetonitrile/ 0.05%TFA (45/45/10) | 2.5 | 1.6 | 3.0 | 5.0 | A.M and L.M peaks well defined and well spaced. |
| Methanol/Acetonitrile/ 0.05%TFA (60/30/10) | 2.5 | 1.9 | 3.7 | 5.7 | A.M and L.M peaks well defined and well spaced. |

**Table 48 Retention times of Some Compounds Using AM Chromatographic Conditions**

| Compound | Retention time (min.) |
|---|---|
| Ibuprofen | 3.80 |
| Metronidazole | 2.80 |
| Caffeine | 3.10 |
| Naproxen | 3.30 |
| Ciprofloxacin | 3.70 |
| Benzoic Acid | 3.00 |
| Prednisolone | 3.00 |
| Indometacin | 3.46 |
| Piroxicam | 3.60 |

- **CALI BRATION CURVES**
- **UV Analysis of Lumefantrine**

**Table 49 Calibration Curve (UV Assay of LM - Ap. 1)**

| Conc. (%w/v) | Abs. at 335nm | | Average Abs. |
|---|---|---|---|
| | 1 | 2 | |
| 0.000856 | 0.264 | 0.266 | 0.265 |
| 0.001284 | 0.390 | 0.394 | 0.392 |
| 0.001712 | 0.537 | 0.534 | 0.536 |
| 0.002140 | 0.664 | 0.665 | 0.665 |
| 0.002568 | 0.789 | 0.791 | 0.790 |

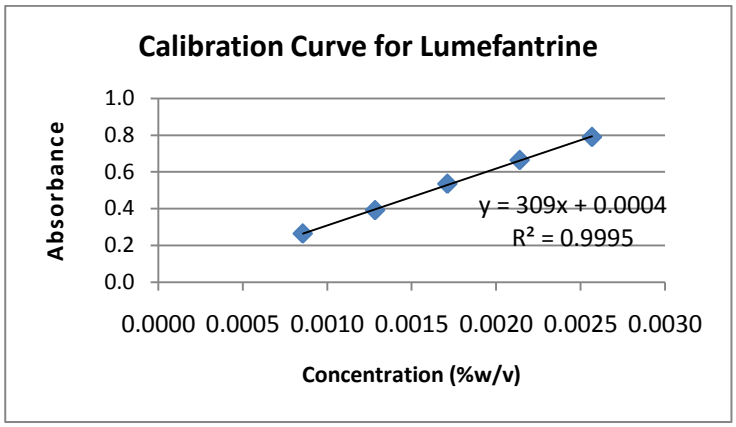
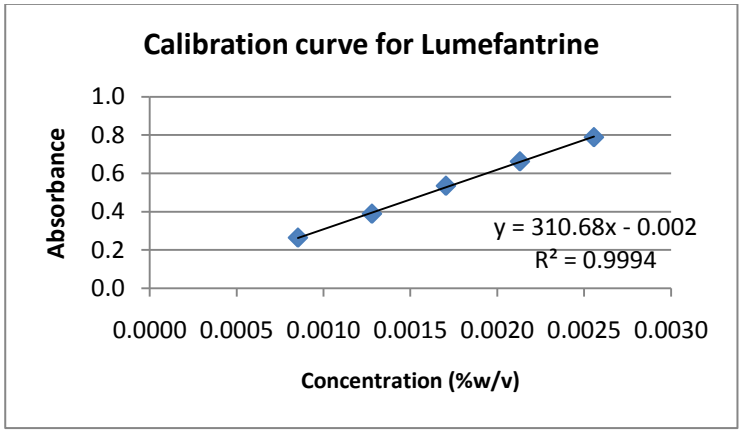



**Table 50 Calibration Curve (UV Assay of LM - Ap. 2)**

| Conc. (%w/v) | Abs. at 335nm | | Average Abs. |
|---|---|---|---|
| | 1 | 2 | |
| 0.000852 | 0.264 | 0.263 | 0.264 |
| 0.001278 | 0.387 | 0.39 | 0.389 |
| 0.001704 | 0.534 | 0.535 | 0.535 |
| 0.002130 | 0.660 | 0.664 | 0.662 |
| 0.002556 | 0.789 | 0.788 | 0.789 |



- **UV Analysis of Artemether**

**Table 51 Calibration Curve (UV Assay of AM - 2ml HCl)**

| Conc. (%w/v) | Abs. at 254nm | | Average Abs. |
|---|---|---|---|
| | 1 | 2 | |
| 0.000641 | 0.199 | 0.200 | 0.200 |
| 0.000962 | 0.310 | 0.313 | 0.312 |
| 0.001283 | 0.430 | 0.433 | 0.432 |
| 0.001603 | 0.531 | 0.532 | 0.532 |
| 0.001924 | 0.649 | 0.651 | 0.650 |
| 0.002244 | 0.745 | 0.749 | 0.747 |
| 0.002565 | 0.863 | 0.864 | 0.864 |

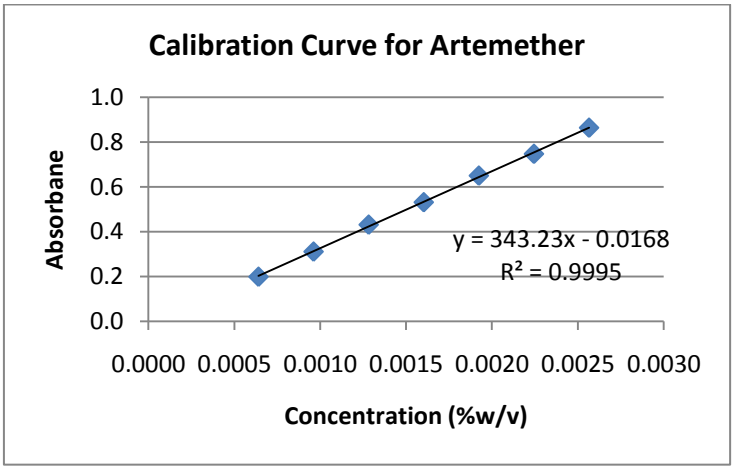



**Table 52 Calibration Curve (UV Assay of AM - 3ml HCl)**

| Conc. (%w/v) | Abs. At 254nm | | Average. Abs. |
|---|---|---|---|
| | 1 | 2 | |
| 0.000652 | 0.229 | 0.222 | 0.226 |
| 0.000978 | 0.330 | 0.326 | 0.328 |
| 0.001304 | 0.438 | 0.444 | 0.441 |
| 0.001630 | 0.539 | 0.542 | 0.541 |
| 0.001956 | 0.651 | 0.656 | 0.654 |
| 0.002283 | 0.749 | 0.751 | 0.750 |
| 0.002609 | 0.864 | 0.862 | 0.863 |

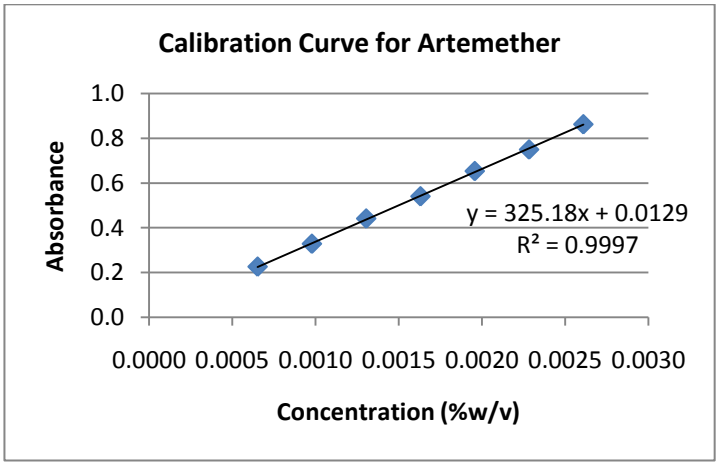
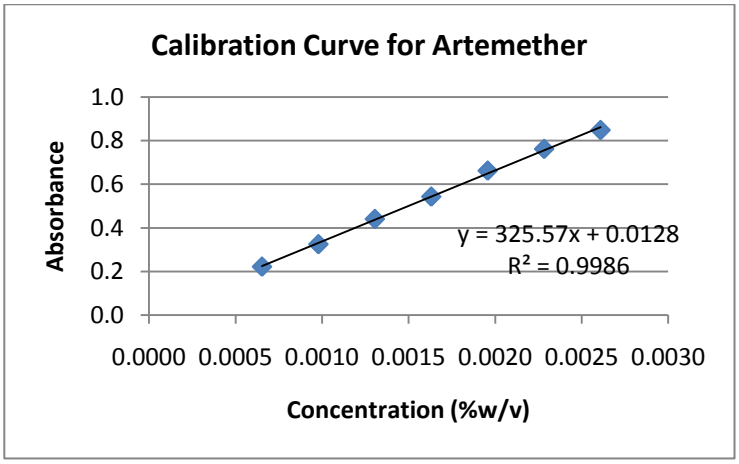


**Table 53 Calibration Curve (UV Assay of AM - 4ml HCl)**

| Conc. | Abs at 254 | | Average abs. |
|---|---|---|---|
| | 1 | 2 | |
| 0.000652 | 0.218 | 0.227 | 0.223 |
| 0.000978 | 0.326 | 0.325 | 0.326 |
| 0.001304 | 0.439 | 0.443 | 0.441 |
| 0.001630 | 0.544 | 0.543 | 0.544 |
| 0.001956 | 0.659 | 0.666 | 0.663 |
| 0.002283 | 0.759 | 0.765 | 0.762 |
| 0.002609 | 0.849 | 0.848 | 0.849 |




- **HPLC Analysis of Lumefantrine**

**Table 54 Calibration Curve (HPLC Assay of LM - Ap. 1)**

| Conc. (%w/v) | Average Peak Area Ratio |
|---|---|
| 0.000344 | 0.8333 |
| 0.000516 | 1.2222 |
| 0.000688 | 1.6389 |
| 0.000860 | 1.9931 |
| 0.001032 | 2.4097 |

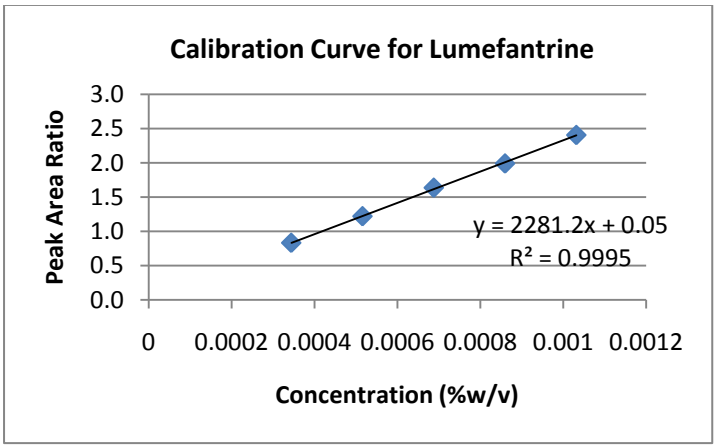
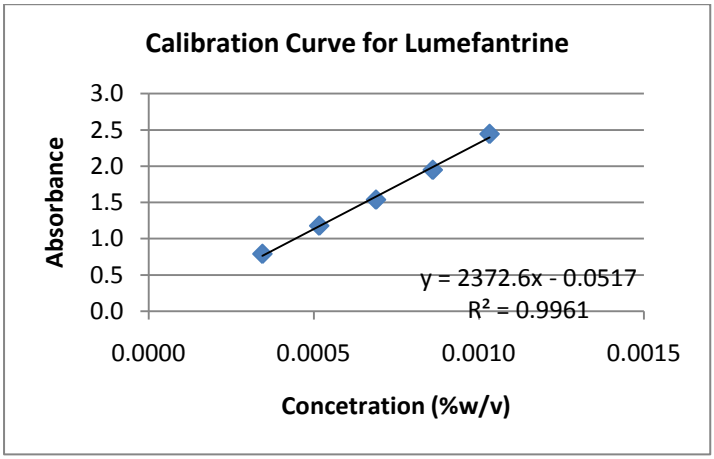



**Table 55 Calibration Curve (HPLC Assay of LM - Ap. 2)**

| Conc. (%w/v) | Average Peak Area Ratio |
|---|---|
| 0.000344 | 0.7906 |
| 0.000516 | 1.1788 |
| 0.000688 | 1.5385 |
| 0.000860 | 1.9494 |
| 0.001032 | 2.4457 |



- **HPLC Analysis of Artemether**

**Table 56 Calibration Curve (HPLC Assay of AM - Ap. 1)**

| Conc.(%w/v) | Average Peak Area Ratio |
|---|---|
| 0.002240 | 0.0616 |
| 0.004480 | 0.1268 |
| 0.008960 | 0.2246 |
| 0.013440 | 0.3188 |
| 0.017920 | 0.4094 |
| 0.022400 | 0.5072 |
| 0.026880 | 0.6087 |

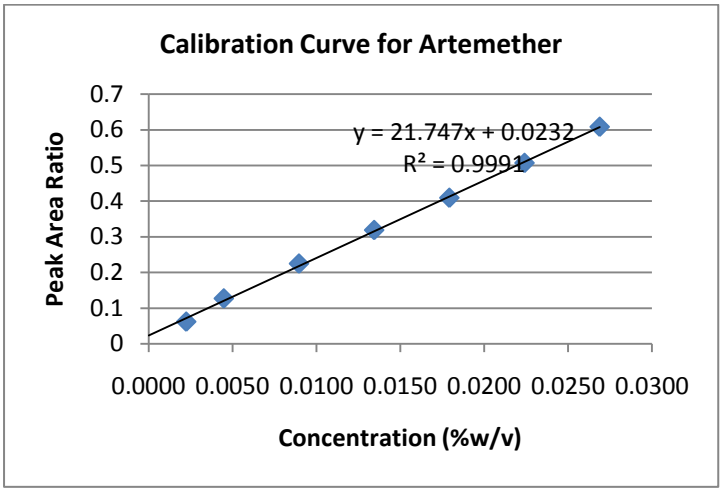
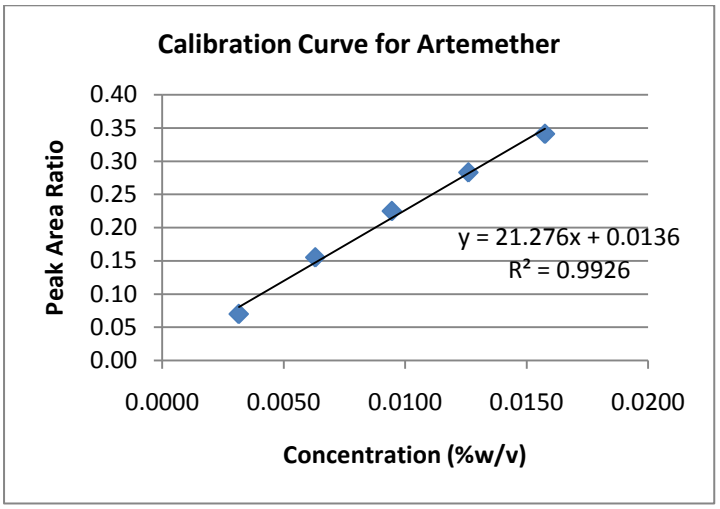



**Table 57 Calibration Curve (HPLC Assay of AM - Ap. 2)**

| Conc.(%w/v) | Average Peak Area Ratio |
|---|---|
| 0.003152 | 0.0698 |
| 0.006304 | 0.1550 |
| 0.009456 | 0.2248 |
| 0.012608 | 0.2830 |
| 0.015760 | 0.3411 |



## METHOD VALIDATION PARAMETERS

- **ACCURACY**

- **HPLC Analysis of Lumefantrine**

**Table 58 Accuracy (UV Assay of LM - Ap.)**

| Concentration | Determination | % Recovered | |
|---|---|---|---|
| 0.001179 | 1 | 101.95 | |
| | 2 | 101.68 | Average = 101.95 |
| | 3 | 102.22 | RSD = 0.26 |
| 0.001572 | 1 | 100.17 | |
| | 2 | 100.99 | Average = 100.58 |
| | 3 | 100.58 | RSD = 0.41 |
| 0.001965 | 1 | 99.43 | |
| | 2 | 100.48 | Average = 99.89 |
| | 3 | 99.76 | RSD = 0.54 |

- **UV analysis of Artemether**

**Table 59 Accuracy (UV Assay of AM - Ap.)**

| Concentration | Determination | % Recovered | |
|---|---|---|---|
| 0.00125 | 1 | 99.65 | |
| | 2 | 101.18 | Average = 100.68 |
| | 3 | 101.22 | RSD = 0.88 |
| 0.00156 | 1 | 102.10 | |
| | 2 | 100.42 | Average = 100.42 |
| | 3 | 98.74 | RSD = 1.67 |
| 0.00187 | 1 | 99.33 | |
| | 2 | 100.68 | Average = 99.59 |
| | 3 | 98.76 | RSD = 0.98 |

- **HPLC Analysis of Lumefantrine**

**Table 60 Accuracy (HPLC Assay of LM - Ap.)**

| Conc. | % Recovered | |
|---|---|---|
| 0.0004720 | 97.29 | |
| | 99.56 | Average = 98.80 |
| | 99.56 | RSD = 1.32 |
| 0.0006294 | 99.29 | |
| | 100.14 | Average = 99.86 |
| | 100.14 | RSD = 0.49 |
| 0.0007867 | 99.14 | |
| | 98.47 | Average = 98.92 |
| | 99.14 | RSD = 0.39 |

- **HPLC Analysis of Artemether**

**Table 61 Accuracy (HPLC Assay of AM - Ap.)**

| Conc. | Determination | % Recovered | |
|---|---|---|---|
| 0.008512 | 1 | 97.88 | |
| | 2 | 99.07 | Average = 98.55 |
| | 3 | 98.7 | RSD = 0.61 |
| 0.012768 | 1 | 98.64 | |
| | 2 | 97.34 | Average = 98.08 |
| | 3 | 98.25 | RSD = 0.67 |
| 0.017088 | 1 | 97.72 | |
| | 2 | 98.49 | Average = 97.77 |
| | 3 | 97.09 | RSD = 0.70 |

- **PRECISION**

- **UV analysis of Lumefantrine**

**Table 62 Precision (UV Assay of LM - Day 1)**

| Determination | Nom. Conc. (%w/v) | Average Abs. | Act. Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.00156 | 0.476 | 0.00154 | 98.66 | |
| 2 | 0.00156 | 0.479 | 0.00155 | 99.29 | |
| 3 | 0.00156 | 0.481 | 0.00156 | 99.57 | |
| 4 | 0.00156 | 0.485 | 0.00157 | 100.40 | |
| 5 | 0.00159 | 0.500 | 0.00162 | 101.56 | Average =100.14 |
| 6 | 0.00159 | 0.499 | 0.00161 | 101.36 | RSD = 1.16 |

**Table 63 Precision (UV Assay of LM - Day 2)**

| Determination | Nom. Conc. (%w/v) | Average Abs. | Act. Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.00160 | 0.509 | 0.00165 | 102.68 | |
| 2 | 0.00160 | 0.510 | 0.00165 | 102.88 | |
| 3 | 0.00160 | 0.493 | 0.00159 | 99.64 | |
| 4 | 0.00160 | 0.496 | 0.00160 | 100.24 | |
| 5 | 0.00159 | 0.500 | 0.00162 | 101.56 | Average =101.39 |
| 6 | 0.00159 | 0.499 | 0.00161 | 101.36 | RSD = 1.29 |

**Table 64 Precision (UV Assay of LM - Day 3)**

| Determination | Nom. Conc. (%w/v) | Average Abs. | Act. Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.00190 | 0.592 | 0.00191 | 100.98 | |
| 2 | 0.00190 | 0.591 | 0.00191 | 100.81 | |
| 3 | 0.00161 | 0.497 | 0.00161 | 100.07 | |
| 4 | 0.00161 | 0.500 | 0.00162 | 100.67 | |
| 5 | 0.00159 | 0.500 | 0.00162 | 101.56 | Average = 100.91 |
| 6 | 0.00159 | 0.499 | 0.00161 | 101.36 | RSD = 0.53 |

- **UV analysis of Artemether**

**Table 65 Precision (UV Assay of AM - Day 1)**

| Determination | Nom. Conc. (%w/v) | Average Abs. | Act. Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.00156 | 0.529 | 0.00159 | 101.92 | |
| 2 | 0.00156 | 0.531 | 0.00160 | 102.29 | |
| 3 | 0.00156 | 0.52 | 0.00156 | 100.23 | |
| 4 | 0.00156 | 0.524 | 0.00158 | 100.98 | |
| 5 | 0.00156 | 0.525 | 0.00158 | 101.17 | Average = 101.26 |
| 6 | 0.00156 | 0.524 | 0.00158 | 100.98 | RSD = 0.73 |

**Table 66 Precision (UV Assay of AM - Day 2)**

| Determination | Nom. Conc. (%w/v) | Average Abs. | Act. Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.00156 | 0.512 | 0.00154 | 98.74 | |
| 2 | 0.00156 | 0.517 | 0.00155 | 99.67 | |
| 3 | 0.00156 | 0.515 | 0.00155 | 99.30 | |
| 4 | 0.00156 | 0.51 | 0.00153 | 98.37 | |
| 5 | 0.00156 | 0.516 | 0.00155 | 99.49 | Average = 99.05 |
| 6 | 0.00156 | 0.512 | 0.00154 | 98.74 | RSD = 0.51 |

**Table 67 Precision (UV Assay of AM - Day 3)**

| Determination | Nom. Conc. (%w/v) | Average Abs. | Act. Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.00160 | 0.535 | 0.00161 | 100.26 | |
| 2 | 0.00160 | 0.53 | 0.00159 | 99.35 | |
| 3 | 0.00160 | 0.533 | 0.00160 | 99.90 | |
| 4 | 0.00160 | 0.534 | 0.00160 | 100.08 | |
| 5 | 0.00160 | 0.532 | 0.00160 | 99.71 | Average = 99.68 |
| 6 | 0.00160 | 0.527 | 0.00158 | 98.81 | RSD = 0.53 |

- **HPLC analysis of Lumefantrine**

**Table 68 Precision (HPLC Assay of LM -Day 1)**

| Determination | Nom. Conc. (%w/v) | Peak Area Ratio | Actual Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.000638 | 1.5117 | 0.000641 | 100.43 | |
| 2 | 0.000649 | 1.5333 | 0.000650 | 100.19 | |
| 3 | 0.000637 | 1.5230 | 0.000646 | 101.37 | |
| 4 | 0.000629 | 1.5085 | 0.000639 | 101.65 | |
| 5 | 0.000662 | 1.5729 | 0.000668 | 100.84 | Average = 100.93 |
| 6 | 0.000645 | 1.5378 | 0.000652 | 101.12 | RSD = 0.56 |

**Table 69 Precision (HPLC Assay of LM -Day 2)**

| Determination | Nom. Conc. (%w/v) | Peak Area Ratio | Actual Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.000665 | 1.6024 | 0.000681 | 102.33 | |
| 2 | 0.000629 | 1.5085 | 0.000639 | 101.65 | |
| 3 | 0.000637 | 1.5426 | 0.000654 | 102.72 | |
| 4 | 0.000629 | 1.5085 | 0.000639 | 101.65 | |
| 5 | 0.000662 | 1.5729 | 0.000667 | 100.84 | Average = 102.12 |
| 6 | 0.000635 | 1.5498 | 0.000657 | 103.54 | RSD = 0.93 |

**Table 70 Precision (HPLC Assay of LM -Day 3)**

| Determination | Nom. Conc. (%w/v) | Peak Area Ratio | Actual Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.000638 | 1.5120 | 0.000641 | 100.45 | |
| 2 | 0.000649 | 1.5229 | 0.000646 | 99.49 | |
| 3 | 0.000637 | 1.5230 | 0.000646 | 101.37 | |
| 4 | 0.000629 | 1.5185 | 0.000644 | 102.34 | |
| 5 | 0.000662 | 1.5749 | 0.000668 | 100.98 | Average = 101.18 |
| 6 | 0.000645 | 1.5578 | 0.000661 | 102.48 | RSD = 1.13 |

- **HPLC analysis of Artemether**

**Table 71 Precision (HPLC Assay of AM -Day 1)**

| Determination | Nom. Conc. (%w/v) | Peak Area Ratio | Actual Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.012012 | 0.2827 | 0.011932 | 99.33 | |
| 2 | 0.011984 | 0.2790 | 0.011762 | 98.15 | |
| 3 | 0.011996 | 0.2790 | 0.011762 | 98.05 | |
| 4 | 0.012020 | 0.2790 | 0.011762 | 97.85 | |
| 5 | 0.012040 | 0.2754 | 0.011595 | 96.30 | Average = 98.20 |
| 6 | 0.011992 | 0.2827 | 0.011932 | 99.50 | RSD = 1.18 |

**Table 72 Precision (HPLC Assay of AM -Day 2)**

| Determination | Nom. Conc. (%w/v) | Peak Area Ratio | Actual Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.009676 | 0.2170 | 0.009562 | 98.83 | |
| 2 | 0.009600 | 0.2130 | 0.009373 | 97.64 | |
| 3 | 0.009668 | 0.2170 | 0.009559 | 98.87 | |
| 4 | 0.009644 | 0.2130 | 0.009373 | 97.19 | |
| 5 | 0.009624 | 0.2132 | 0.009381 | 97.47 | Average = 98.29 |
| 6 | 0.009584 | 0.2170 | 0.009559 | 99.74 | RSD = 1.00 |

**Table 73 Precision (HPLC Assay of AM -Day 3)**

| Determination | Nom. Conc. (%w/v) | Peak Area Ratio | Actual Conc. (%w/v) | % Content | |
|---|---|---|---|---|---|
| 1 | 0.009650 | 0.2170 | 0.009562 | 99.09 | |
| 2 | 0.009624 | 0.2130 | 0.009373 | 97.39 | |
| 3 | 0.009658 | 0.2170 | 0.009562 | 99.01 | |
| 4 | 0.009644 | 0.2130 | 0.009373 | 97.19 | |
| 5 | 0.009504 | 0.2093 | 0.009198 | 96.78 | Average = 97.82 |
| 6 | 0.009584 | 0.2123 | 0.009340 | 97.46 | RSD = 0.98 |

- **ROBUSTNESS**

- **UV Analysis of Artemether**

**Table 74 Allowing Solutions to stand for Specified Time Periods**

| Time | Determination | % Recovered | |
|---|---|---|---|
| 2 hours | 1 | 101.82 | Average = 100.81 |
| | 2 | 99.80 | |
| 20 hours | 1 | 99.32 | Average = 97.29 |
| | 2 | 95.26 | |

**Table 75 Varying Volume of HCl Used In Derivatization of AM**

| Volume of HCl (ml) | Determination | % Recovered | |
|---|---|---|---|
| 1.5 | 1 | 98.92 | Average = 99.31 |
| | 2 | 99.70 | |
| 4.0 | 1 | 100.59 | Average = 100.31 |
| | 2 | 100.03 | |

- **UV Analysis of Lumefantrine**

**Table 76 Using Undistilled Methanol**

| Determination | % Recovered (%w/w) | |
|---|---|---|
| 1 | 98.45 | Average = 98.74 |
| 2 | 99.03 | |

**Table 77 Varying Concentration of Methanolic HCl**

| Conc. of Methanolic HCl | Determination | % Recovered | |
|---|---|---|---|
| 0.75M | 1 | 97.11 | Average = 97.84 |
| | 2 | 98.56 | |
| 1.5M | 1 | 98.43 | Average = 98.34 |
| | 2 | 98.22 | |

**Table 78 Allowing Solutions to Stand for Specified Time Periods**

| Time | Determination | % Recovered | |
|---|---|---|---|
| 2 hours | 1 | 99.87 | Average = 100.15 |
| | 2 | 100.43 | |
| 12 hours | 1 | 101.48 | Average = 101.21 |
| | 2 | 100.93 | |

- **ASSAY OF TABLETS**

**Table 79 Assay of LM in Tablets Using HPLC Method**

| Brand | Determination | % Content of tab. | |
|---|---|---|---|
| A | 1 | 99.47 | |
| | 2 | 100.86 | Average = 100.17 |
| | 3 | 100.17 | SD = 0.70 |
| B | 1 | 101.65 | |
| | 2 | 102.03 | Average = 101.77 |
| | 3 | 101.65 | SD = 0.22 |
| C | 1 | 93.23 | |
| | 2 | 94.66 | Average = 93.71 |
| | 3 | 93.23 | SD = 0.88 |
| D | 1 | 102.97 | |
| | 2 | 103.39 | Average = 103.45 |
| | 3 | 103.97 | SD = 0.49 |
| E | 1 | 94.51 | |
| | 2 | 94.51 | Average = 95.26 |
| | 3 | 96.76 | SD = 1.35 |
| F | 1 | 96.26 | |
| | 2 | 96.87 | Average = 96.22 |
| | 3 | 95.52 | SD = 0.70 |

**Table 80 Assay of AM in Tablets Using HPLC Method**

| Brand | % Content | SD |
|---|---|---|
| A | 83.50 | 0.65 |
| B | 99.20 | 1.03 |
| C | 74.62 | 0.88 |
| D | 95.82 | 1.06 |
| E | 105.27 | 3.98 |
| F | 92.05 | 1.24 |

**Table 81 Assay of LM in Tablets Using UV Method**

| Brand | % Content | SD |
|---|---|---|
| A | 101.23 | 0.95 |
| B | 102.85 | 0.89 |
| C | 94.15 | 4.21 |
| D | 104.35 | 0.68 |
| E | 97.35 | 1.43 |
| F | 94.83 | 1.08 |

## REFERENCES

### 1. BOOKS


Beckett A. H. and Stenlake J. B., (1988), Practical Pharmaceutical Chemistry, Fourth Edition, Part Two, The Athlone Press, 44 Bedford Row, London, WC1R 4LY, UK, Pages 158-167, 117 – 125.

British Pharmacopoeia 2007 CD ROM, Incorporating the requirements of the 5th Edition of the European Pharmacopoeia 2004 as amended by Supplements 5.1 and 5.5, Version 110.

Cairns D., (2008), Essentials of Pharmaceutical Chemistry, Third Edition, Pharmaceutical Press, 1 Lambeth High Street, London SE1 7JN, UK, Page 162

Jeffery G. H et. al. (1989), Vogel's Textbook of Quantitative Chemical Analysis, Fifth Edition, Longman Scientific and Technical, Longman House, Burnt Mill, Harlow, Essex, CM20, 2JE, England, Page 216

Kealey D., and Haines P. J. (2002), Principles and Practice of Analytical Chemistry, Fifth Edition, Bios scientific publishers, Pages 119- 129

McMaster C. M., (2007), HPLC- A Practical User's Guide, Second Edition, John Wiley & Sons, Inc., Hoboken, New Jersey, Pages 113-120

Meyer V. R. (2004), Practical High Performance Liquid Chromatography, Fourth Edition, John Wiley & Sons Inc, Page 52

Moffat A.C. (1986), Clarke's Isolation and Identification of Drugs, Second Edition, The Pharmaceutical Press London.

Olaniyi A. A. (2005), Essential Medicinal Chemistry, Third Edition, Hope Publications, Ibadan, Nigeria, Pages 404 - 409

Wall P. E. (2005) Thin Layer Chromatography - A Modern Practical Approach, Royal Society of Chemistry, Thomas Graham House (290), Science Park, Milton Road, Cambridge CB4 0WF, Pages 1-2


### 2. DOCUMENTS


Eurekalert, http://www.eurekalert.org/pub_releases/2009-07/up-cad072209.php (23rd April 2010, 19:35)

Fisher Scientific, http://www.fishersci.com/wps/downloads/segment/Scientific/pdf/acn_facts_ideas.pdf (28th April 2010, 13:40)

Fisher Scientific, http://www.fr.fishersci.com/home/products/media/Alternatives%20for%20Acetonitrile.doc (20th September 2009 14:50)

ICH (1996), Guidance for Industry, Q2B Validation of Analytical Procedures: Methodology

The International Pharmacopoeia (2008), Fourth Edition (incl. First Supplement), http://www.who.int/phint/ 19th September 2009

USP SALMOUS Standards

- http://www.usp.org/pdf/EN/nonUSStandards/artemether.pdf 19th September 2009
- http://www.usp.org/pdf/EN/nonUSStandards/lumefantrine.pdf 19th September 2009
- http://www.usp.org/pdf/EN/nonUSStandards/lumefantrineArtemetherTablets.pdf 19th September 2009

WHO fact sheet (2008), www.net4nets.net/pdf/Malaria_Facts_Detailed.pdf 27th March 2010, 14:30

WHO fact sheet (2010), www.who.int/mediacentre/factsheets/fs094/en/ 27th March 2010, 15:10

WHO guidelines for treatment of malaria, 2010 WHO Press, World Health Organization, 20, avenue Appia, 1211 Geneva 27, Switzerland

WHO, Practical Chemotherapy of Malaria, W.H.O Technical Report, Series No. 805. Geneva. 1990.

WHO, The Use of Antimalarial Drugs-Report of an Informal Consultation, World Health Organization, Geneva, 2001 http://www.rollbackmalaria.org/cmc_upload/0/000/014/923/use_of_antimalarials.pdf

## 3. JOURNALS

Adjei, G. O., B. Q. Goka, et al. (2009). "Artemether-lumefantrine: an oral antimalarial for uncomplicated malaria in children." Expert Rev Anti Infect Ther **7**(6): 669-81.

Amin, A. A. and G. O. Kokwaro (2007). "Antimalarial drug quality in Africa." J Clin Pharm Ther **32**(5): 429-40.

Atemnkeng, M. A., K. De Cock, et al. (2007). "Quality control of active ingredients in artemisinin-derivative antimalarials within Kenya and DR Congo." Trop Med Int Health **12**(1): 68-74.

Bozdech, Z., M. Llinas, et al. (2003). "The transcriptome of the intraerythrocytic developmental cycle of Plasmodium falciparum." PLoS Biol **1**(1): E5.

Brabin, B. J. (1983). "An analysis of malaria in pregnancy in Africa." Bull World Health Organ **61**(6): 1005-16.

Cesar Ida, C., F. H. Andrade Nogueira, et al. (2008). "Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection." J Pharm Biomed Anal **48**(3): 951-4.

Chekem, L. and S. Wierucki (2006). "[Extraction of artemisinin and synthesis of its derivates artesunate and artemether]." Med Trop (Mars) **66**(6): 602-5.

Clark, I. A., N. H. Hunt, et al. (1984). "Radical-mediated damage to parasites and erythrocytes in Plasmodium vinckei infected mice after injection of t-butyl hydroperoxide." Clin Exp Immunol **56**(3): 524-30.

da Costa Cesar, I., F. H. Nogueira, et al. (2008). "Comparison of HPLC, UV spectrophotometry and potentiometric titration methods for the determination of lumefantrine in pharmaceutical products." J Pharm Biomed Anal **48**(1): 223-6.

Dondorp, A. M., S. Yeung, et al. "Artemisinin resistance: current status and scenarios for containment." Nat Rev Microbiol.

Eckstein-Ludwig, U., R. J. Webb, et al. (2003). "Artemisinins target the SERCA of Plasmodium falciparum." Nature **424**(6951): 957-61.

Ezzet, F., R. Mull, et al. (1998). "Population pharmacokinetics and therapeutic response of CGP 56697 (artemether + benflumetol) in malaria patients." Br J Clin Pharmacol **46**(6): 553-61.

Gautam, A., T. Ahmed, et al. (2009). "Pharmacokinetics and pharmacodynamics of endoperoxide antimalarials." Curr Drug Metab **10**(3): 289-306.

Green, M. D., D. L. Mount, et al. (2001). "Authentication of artemether, artesunate and dihydroartemisinin antimalarial tablets using a simple colorimetric method." Trop Med Int Health **6**(12): 980-2.

Hay, S. I., C. A. Guerra, et al. (2009). "A world malaria map: Plasmodium falciparum endemicity in 2007." PLoS Med **6**(3): e1000048.

Haynes, R. K. and S. C. Vonwiller (1994). "Extraction of artemisinin and artemisinic acid: preparation of artemether and new analogues." Trans R Soc Trop Med Hyg **88 Suppl 1**: S23-6.

Hien, T. T., T. M. Davis, et al. (2004). "Comparative pharmacokinetics of intramuscular artesunate and artemether in patients with severe falciparum malaria." Antimicrob Agents Chemother **48**(11): 4234-9.

Karbwang, J., K. Na-Bangchang, et al. (1997). "Determination of artemether and its major metabolite, dihydroartemisinin, in plasma using high-performance liquid chromatography with electrochemical detection." J Chromatogr B Biomed Sci Appl **690**(1-2): 259-65.

Klayman, D. L. (1985). "Qinghaosu (artemisinin): an antimalarial drug from China." Science **228**(4703): 1049-55.

Krishna, S., A. C. Uhlemann, et al. (2004). "Artemisinins: mechanisms of action and potential for resistance." Drug Resist Updat **7**(4-5): 233-44.

Meshnick, S. R., T. E. Taylor, et al. (1996). "Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy." Microbiol Rev **60**(2): 301-15.

Navaratnam, V., S. M. Mansor, et al. (1995). "Determination of artemether and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies." J Chromatogr B Biomed Appl **669**(2): 289-94.

Nosten, F. and N. J. White (2007). "Artemisinin-based combination treatment of falciparum malaria." Am J Trop Med Hyg **77**(6 Suppl): 181-92.

Ntale, M., J. W. Ogwal-Okeng, et al. (2008). "A field-adapted sampling and HPLC quantification method for lumefantrine and its desbutyl metabolite in whole blood spotted on filter paper." J Chromatogr B Analyt Technol Biomed Life Sci **876**(2): 261-5.

Olliaro, P., W. R. Taylor, et al. (2001). "Controlling malaria: challenges and solutions." Trop Med Int Health **6**(11): 922-7.

Pickard, A. L., C. Wongsrichanalai, et al. (2003). "Resistance to antimalarials in Southeast Asia and genetic polymorphisms in pfmdr1." Antimicrob Agents Chemother **47**(8): 2418-23.

Pradines, B., A. Tall, et al. (1999). "In vitro activities of benflumetol against 158 Senegalese isolates of Plasmodium falciparum in comparison with those of standard antimalarial drugs." Antimicrob Agents Chemother **43**(2): 418-20.

Shretta, R., J. Omumbo, et al. (2000). "Using evidence to change antimalarial drug policy in Kenya." Trop Med Int Health **5**(11): 755-64.

Shrivastava, A., B. P. Nagori, et al. (2008)." New Simple and Economical Spectrophotometric Method for Estimation of Artemether in Pharmaceutical Dosage Forms " Asian J. Research Chem **1**(1): 19 – 21.

Sipilanyambe, N., J. L. Simon, et al. (2008). "From chloroquine to artemether-lumefantrine: the process of drug policy change in Zambia." Malar J **7**: 25.

Souppart, C., N. Gauducheau, et al. (2002). "Development and validation of a high-performance liquid chromatography-mass spectrometry assay for the determination of artemether and its metabolite dihydroartemisinin in human plasma." J Chromatogr B Analyt Technol Biomed Life Sci **774**(2): 195-203.

Taylor, W. R., J. Rigal, et al. (2003). "Drug resistant falciparum malaria and the use of artesunate-based combinations: focus on clinical trials sponsored by TDR." J Vector Borne Dis **40**(3-4): 65-72.

Thomas, C. G., S. A. Ward, et al. (1992). "Selective determination, in plasma, of artemether and its major metabolite, dihydroartemisinin, by high-performance liquid chromatography with ultraviolet detection." J Chromatogr **583**(1): 131-6.

van Vugt, M., F. Ezzet, et al. (1999). "No evidence of cardiotoxicity during antimalarial treatment with artemether-lumefantrine." Am J Trop Med Hyg **61**(6): 964-967.

Woodrow, C. J., R. K. Haynes, et al. (2005). "Artemisinins." Postgrad Med J **81**(952): 71-8.

Zeng, M. Y., Z. L. Lu, et al. (1996). "Determination of benflumetol in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection." J Chromatogr B Biomed Appl **681**(2): 299-306.