SPECTROPHOTOMETRIC DETERMINATION AND STABILITY STUDIES OF ARTEMETHER IN ARTEMETHER-LUMEFANTRINE SUSPENSIONS MARKETTED IN ZARIA, NIGERIA.

BY

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DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY, FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

OCTOBER 2015

DECLARATION

I declare that the work in this dissertation entitled **"**Spectrophotometric Determination And Stability Studies Of Artemether In Artemether-Lumefantrine Suspensions Marketted In Zaria, Nigeria" has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any othe Institution.

Yusuf Maryam Talatu

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(Signature) (Date)

CERTIFICATION

This dissertation entitled "SPECTROPHOTOMETRIC DETERMINATION AND STABILITY STUDIES OF ARTEMETHER IN ARTEMETHER LUMEFANTRINE SUSPENSIONS MARKETTED IN ZARIA, NIGERIA" by MARYAM TALATU YUSUF meets the regulations governing the award of the degree of MSc. Pharmaceutical and Medicinal Chemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literature presentation.

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My colleagues and coursemates.

DEDICATION

Dedicated to my Children Farida, Ibrahim and Yusuf Yusuf Lawal.

ABSTRACT

The increasing use of artemether-lumefantrine combination as an effective treatment for resistant malaria demands the need for analytical methods for the quality control of these drugs in tablets and suspensions. Though some UV Spectrophotometric methods have been developed for quantification of artemether in various biological fluids and formulations, they require strainous heating conditions which is a limitation. This limitation coupled with non-availability of HPLC hence the need to develop and validate a simple method for the quantification of artemether in peadiatric suspensions. In this work, we report the method developed by reacting artemether solution in methanol with concentrated HCl for 30 minutes to obtain an α,β-unsaturated ketone which was scanned with a UV Spectrophotometer. The method developed obeyed Beers law in the range 20 – 120 µg/ml, slope (y); 0.01 0, intercept (x) 0.193, correlation coefficient (r) 0.9987, λmax 260 nm, precision (% CV); 2, Accuracy (% Er); 2.67 and a recovery of 97%. The detection and quantification Limit (µg/ml) are 0.14 and 0.58 respectively. The developed method was successfully applied in the assay of five brands of artemether-lumefantrine suspensions with 98-101.6% content, and comparism of the means of the assay results of the method and the IP (2008) method showed no statistically significant difference ($P<0.05$). Stability studies of the standard artemether suspension prepared and five different brands of artemether/lumefantrine powders for suspension was carried out by extracting artemether with methanol from the suspensions and analyzing it using the developed method. The content of artemether over 14 days study period ranged between 98.5-102% and this showed that the suspensions are stable under ambient conditions for upto 14 days after reconstitution with bottled table water. The results of the study suggested that the developed method could be used interchangeably in

analysis, and that co-formulation of artemether with lumefantrine has no effect on the stability of artemether.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.2 General Introduction

Malaria is a public health problem (Loset and Kaur, 2009; Karuna *et al.*, 2014). It is an important cause of morbidity and mortality in children and adults in tropical countries. About half of the world's population is at risk of this mosquito borne parasitic disease (WHO, 2010).

Malaria is caused by the protozoan parasite Plasmodium and transmitted by mosquitoes (Shah and Patel, 2012). Five species of the parasite have been shown to infect humans: *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*. While they share a basic life-cycle, certain distinctive features relate to the virulence of each species. P. falciparum causes the most severe manifestations of malaria including coma, anaemia and multi organ failure. The severity of P. falciparum infection has been attributed to the relatively high parasitemias during infection and to the adherence of P. falciparum infected erythrocytes to the endothelium of capillaries and venules, a process known as sequestration (Dondorp, 2008)

The main treatments for malaria were inexpensive "monotherapies" such as chloroquine (Price and Douglas, 2009). Unfortunately, the malaria parasite quickly developed resistance to many of these monotherapies, including amodiaquine, chloroquine, mefloquine, quinine sulphadoxine and pyrimethamine (WHO, 2006). One of the cornerstones of control programs today is the early diagnosis of malaria and its treatment with highly effective drugs. New combination therapies containing artemisinin derivatives are central to this approach, providing practical treatment regimens with high

cure rates and transmission-blocking potential (Dondorp, 2008; Price and Douglas, 2009).

Artemisinin based combination therapy (ACT) is increasingly being advocated as promising treatment (Arun and Smith, 2011). ACT is based on the use of two drugs with different modes of action: The rationale is that the short-acting but highly potent artemisinin derivative delivers a rapid and effective reduction in parasite biomass, with the remaining parasites being removed by the intrinsically less active but more slowly eliminated partner drug (Price and Douglas, 2009; Arun and Smith, 2011). The most common combinations are artemether-lumefantrine, artesunate-amodiaquine, artesunate-sulfadoxine-pyrimethamine, artesunate-mefloquine, and dihydroartemisinin-piperaquine. World Health Organization (WHO) recommends the ACTs as first line therapy for falciparum malaria in endemic areas (WHO, 2010).

The drug distribution network in Nigeria is in a state of chaos because it consists of open markets that act as major source of purchase to pharmacy stores, hospitals, wholesalers, retailers, medicine stores and pharmaceutical manufacturers (Erhun *et al.*, 2001). Most importers supply drugs to open drug markets because they make more profit from there. The lack of strict monitoring and regulatory mechanisms allows for easy access to legitimate channels of distribution, making counterfeiting an appealing source of illicit revenue (Finlay, 2011).

Olugbenga, (2014) demonstrated that profits accruing to pharmaceutical industries are so huge that it attracts a lot of prospective investors, both genuine and fake, a situation that renders it very susceptible to fraud and corruption, especially through the distribution

network as products are transported across international boundries and distributed in many countries in order to reach the final consumer.

The WHO defines counterfeit drugs as drugs that have been deliberately or fraudulently mislabeled with respect to identity and or source (WHO, 2011). The products could include incorrect ingredients, may misstate the amount of the active ingredients, or are manufactured under circumstances that lack quality control. Counterfeit drugs in Nigeria include preparations without active ingredients, toxic preparations, expired drugs that are relabelled, drugs issued without complete manufacturing information and drugs that are unregistered with the National Agency for Food and Drug Administration and Control (NAFDAC) (Akinyandenu, 2013). Current estimate suggests that 10% of prescription drugs sold worldwide are counterfeits, fake or contaminated, and in parts of Africa and Asia, the figures exceed 50% (Erhun *et al.*, 2001).

The loose control system in the Nigerian economy has contributed to the circulation of fake and counterfeit drugs in the country. A major function of NAFDAC is the regulation and control of imported products. This is done by having inspectors at various airports and seaports. Registration of pharmaceuticals is a criterion that must be passed before any drug is released into the Nigerian market. A condition for registration is the analysis and testing of the drug to ensure quality and safety. Unfortunately, the forensic laboratory, which is the major public laboratory for the purpose of quality control analysis, is not adequately equipped to cope with the volume of work, particularly for analysis of imported/smuggled drugs. These loose control systems are exploited by counterfeiters to manufacture, import and distribute fake and adulterated products. (Chinwendu, 2008)

The high cost of drugs allows for the proliferation of counterfeit drugs in Nigeria and poses a major challenge to public health. Most genuine drugs are expensive and counterfeiters take advantage to supply cheap fake drugs to consumers, especially those who cannot afford the high priced good quality version in the legal sector (Chinwendu, 2008).

The proliferation of fake drug has led to treatment failures, organ dysfunction or damage, worsening of chronic disease conditions and death of many Nigerians (Bate *et al.,* 2009). In 1947, fourteen children were reported dead after being administered chloroquine phosphate injections and in 1990, 109 children died after being administered fake paracetamol (Aluko, 1994; Bate *et al.,* 2009). In 1995, the Nigerian supply of 88,000 Pasteur Merieux and SmithKline Beecham meningitis vaccines to Niger republic during an epidemic resulted in about 2,500 deaths after vaccination. In 2004, three Nigerian hospitals reported cases of adverse reactions from the use of contaminated infusions produced by four Nigerian companies. It was established that the infusions were heavily contaminated with microorganisms and 147 of the 149 brands of screened water for injection were found to be unsterile (Akinyandenu, 2013). In November 2008, 34 Nigerian children, aged 4 months to 3 years died and more than 50 were hospitalised with severe kidney damage after taking the drug "My Pikin" ("my child" in local pidgin), a teething mixture containing paracetamol (Aluko, 1994; Akinyandenu, 2013). The outbreak was due to the use of diethylene glycol (DEG) as a solvent for the paracetamol. DEG was present because of inadvertent or deliberate substitution of propylene glycol, a less toxic compound than DEG, widely used in the pharmaceutical industry (Akinyandenu, 2013).

In Nigeria today, drugs are still sold in open markets, car parks, unlicensed chemists and shops, on buses, ferries and almost in any gathering of people. Most of the products sold in such and similar places are exposed to adverse weather conditions that can affect their quality, apart from the fact that they are mostly adulterated or substandard (Olugbenga, 2014).

The increasing use of artemether-lumefantrine combination as an effective treatment for resistant malaria demands the need for simpler and accurate analytical methods for the quality control of these drugs in tablets and suspension dosage formulations to complement the existing methods used for determining either artemether or lumefantrine in various pharmaceutical and biological matrices (Arun and Smith, 2011).

UV-visible Spectrophotometric methods are the instrumental methods which are commonly used in industrial and research laboratories because of their simplicity, accuracy, precision and low cost (Raza *et al*., 2003; Raza *et al*., 2005). The act of identifying materials based on their color was probably one of the earliest examples of qualitative molecular absorption spectrophotometry. Also, the first recognition that color intensity can be the indicator of concentration was probably the earliest application of employing molecular absorption spectroscopy for quantitative determination. The first measurements were made by using the human eye as the detector and undispersed sunlight or artificial light as the light source. Later it was found that the accuracy and the precision could be improved by isolating specific frequencies of light using optical filters. Further improvement of the measurement came with the use of prism and grating monochromators for wavelength isolation. Photoelectric detectors were soon developed, but were quickly replaced with phototubes and photomultiplier tubes. The development

of solid state microelectronics has now made available a wide range of detector type which if coupled with the computers; provide highly sophisticated readout electronic systems (Marczenko, 2000).

The artemisinins lack strongly absorbing chromophores as a result, artemisinin and its derivatives 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 *et al.,* 1992; Arun and Smith 2011). Though this product absorbs strongly at the said wavelength, it requires very vigorous conditions for its formation.

The International Pharmacopoeial (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 (IP, 2008).

Another method developed by Shrivastava *et al.,* (2008) requires heating at 60 ˚C for three hours. Green *et al*., (2001) 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.

1.2 Statement of Reseach Problem

Evidence abounds on the circulation of poor quality drugs in tropical areas of the world, (Nigeria inclusive) and counterfeiting of drugs is also a major concern in these parts of the world (Awofisayo *et al.,* 2010). Lack of readily availabile and accessible

sophisticated equipments (e.g HPLC) required for analysis of drugs is also a major concern. Thus, simpler accessible analytical methods are needed to monitor the quality of diverse brands of ACTs in the market.

1.3 Justification for the Study

Proliferation of various brands of ACTs have led to the presence of fake and substandard products in several underdeveloped countries such as Nigeria. Although some UV Spectrophotometric methods have been developed for quantification of artemether in various biological fluids, the strainous heating conditions required make them uneconomical and may limit their application in routine analysis.

Also very few studies have been carried out on the stability of artemether/ lumefantrine FDC suspensions to check for the quality and stability over the period of use after reconstitution of the powder with water, thus there is need to determine the quality and stability of the drugs especially the suspensions.

1.4 Aim of the Research

The aim of this work is to develop a simple, accurate cost effective and sensitive UV spectrophotometric method of analysis for the determination of artemether in pure and combination dosage formulations and to apply it in the assay and determination of the stability profile of some selected brands of the suspension.

1.5 Specific Objectives

1. Development and validation of UV spectrophotometric method for the determination of artemether by using the acid decomposition product of the drug.
2. Apply the method in assaying different brands of artemether/lumefantrine powders suspension.
3. Assay different brands of artemether/lumefantrine powders for suspension using the IP method and compare the results with the developed method.
4. Determine the stability of the reconstituted suspensions over a period of 14 days.

1.6 Research Hypothesis

The developed UV method can be used to assay and determine the stability profile of the suspensions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Artemether as Drug of Analysis

Artemether is chemically, (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin; it is described as White crystals or a white, crystalline powder, it is practically insoluble in water; very soluble in dichloromethane and acetone ; freely soluble in ethyl acetate and dehydrated ethanol. Artemether should be kept in a tightly closed container and protected from light. Artemether is an antimalarial drug (IP 2008).

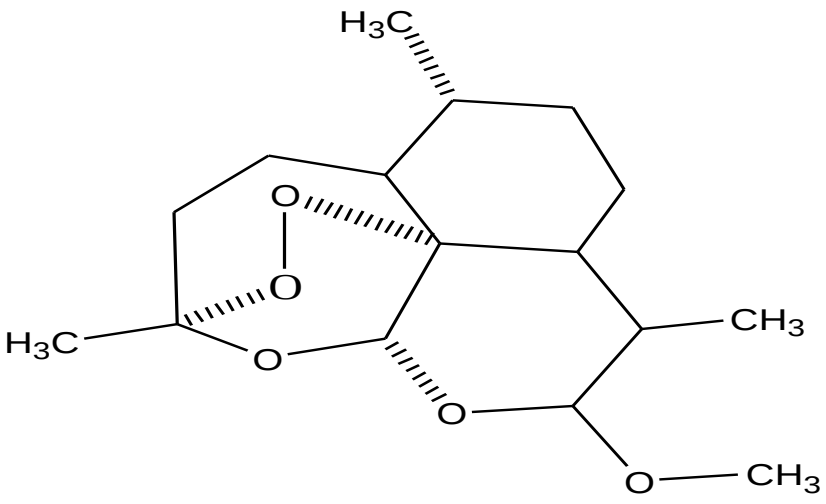


Fig 2.1; Structure of Artemether

Artemether is a semisynthetic polyoxygenated amorphene containing a peroxide bridge that confers potent antimalarial activity (Awofisayo *et al.,* 2010). It is the o-methyl ether pro-drug of dihydroartemisinin and a derivative of artemisinin (quinghaosu), the principal antimalarial constituent of the chinese herb Artemisia annua. (Awofisayo *et al.,* 2010)

Artemether has a relative molecular mass of 298.4 and melting point in the range; 86.0 - 90.0 °C, for specific optical rotation; Use a 10 mg/ml solution in dehydrated ethanol R; $[\alpha]_D^{20\,°C}$ = +166° to +173°. Sulfated ash should not be more than 1.0 mg/g. Artemether is assayed by HPLC and UV and should contain not less than 97.0% 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).

Uses of artemether; Artemether is highly effective against the blood schizonts of both malarial parasites *P. falciparum* and *P. vivax*. It is available as mono-therapy but usually applied in combination with lumefantrine in clinical treatments of malaria. World Health Organization guidelines for the treatment of uncomplicated falciparum malaria recommend the use of this artemisinin-based combination as first-line therapy in national malaria treatment guidelines in 2002. The efficacy of the six-dose regimen of Coartem has been confirmed in many different patient populations around the world, rapidly clearing parasitaemia and fever, and demonstrating a significant gametocidal effect, even in areas of widespread parasite resistance to other antimalarials (WHO, 2010).

Other uses; The full drug potential of artemether was underestimated in the Western World for many years until very recently, when it has been proven that it possess an anti-parasitic activity in other neglected infections, such as schistosomiasis and leishmaniasis, as well as an anti-viral activity (Utzinger *et al.,* 2010). It has also been confirmed that it possesses an important potential as anti-cancer drugs by inducing apoptosis and by inhibiting angiogenesis in different types of tumour (Vandercruyssen *et al.*, 2013). In addition to anti malarial action Artemether has analgesic–antipyretic effects on the body by relieving pain and reducing fever (Vandercruyssen *et al.*, 2013).

Special precautions for storage; Artemether/lumefantrine suspension vials should be stored at room temperature. In a closed vial the powders are stable for 3 years and once the suspension has been made up, it is stable for at least 14 days. Longer conservation is not recommended (WHO, 2010).

2.1.1 IR Spectra of artemether

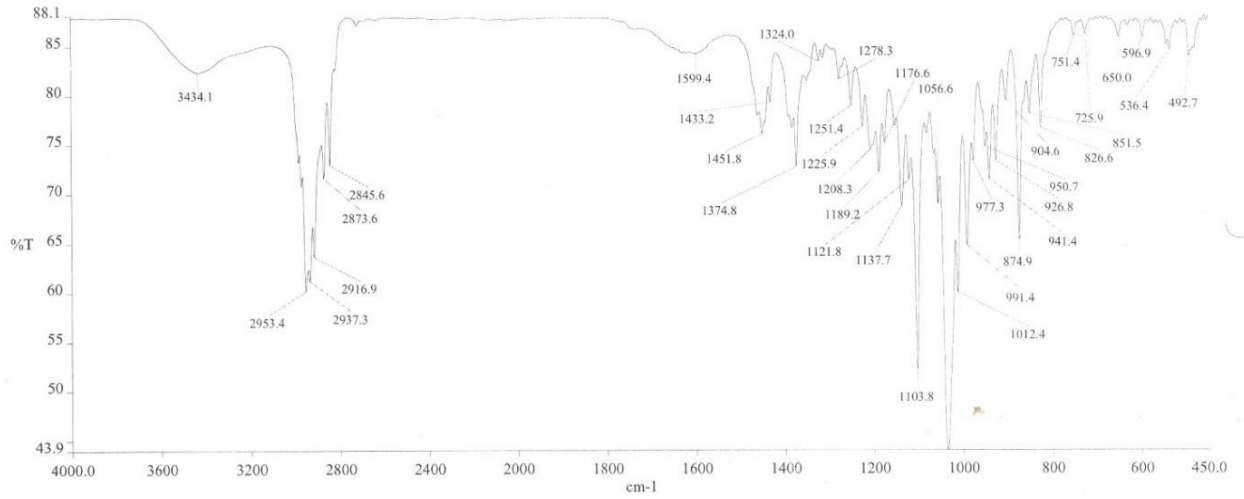


Fig. 2.2; Infra red Spectra of Artemether (Adopted from Karuna *et al*., (2014)

From the IR spectra of artemether, it shows the following peaks: 2952 cm^{-1} assigned to CH_3 stretch,1480 cm^{-1} assigned to (O-H) bending, 1262 cm^{-1} assigned to (O-C) stretch, 1099 cm^{-1} (C-O) stretch and 808 cm^{-1} for (C-H) bending.

2.1.2 Synthesis of artemether

Artemether is synthesed by reducing artemisinin with sodium borohydride to produce dihydroartemisinin as a mixture of epimers (Olaniyi, 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).

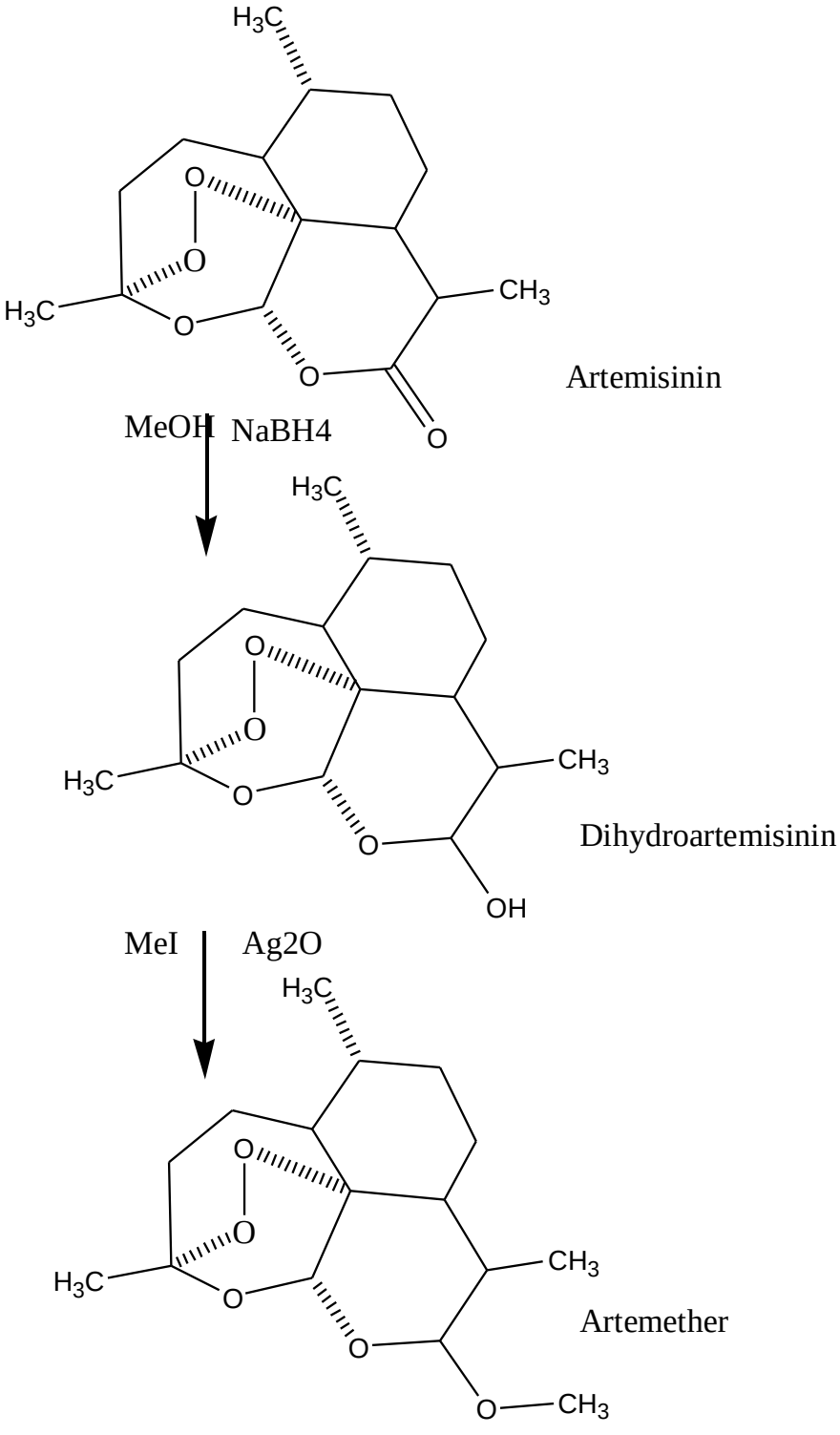


Fig. 2.3; Synthesis of artemether

2.1.3 Mechanism of action of artemether

The peroxide structure of the artemisinins is essential for activity but their mechanism of action is controversial (Krishna *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 and Hunt, 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 and Taylor, 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.

2.1.4 Pharmacodynamics of artemether

The presence of the endoperoxide bridge in artemether, generating singlet oxygen and free radicals which are very cytotoxic to the plasmodia, appears to be essential for antimalarial activity. Morphological changes of the parasitic membranes induced by artemether have been described as being the result of free-radical action. Other in vitro tests suggest that it causes a marked diminution of nucleic acid synthesis. Inhibition of protein synthesis as the basic mechanism of action is suggested in studies which showed morphological changes in ribosomes as well as in the endoplasmic reticulum. Although, artemether acts essentially as a blood schizonticide, artemether/lumefantrine suspension did clear gametocytes in comparative clinical trials (WHO 2010).

2.1.5 Pharmacokinetics of artemether

Orally administered artemether is rapidly absorbed reaching therapeutic levels within 60-90 minutes (Ezzet *et al*., 1998). Artemether is metabolized in the liver to the demethylated derivative dihydroartemisinin (DHA). The elimination is rapid, with a $t_{1/2}$ of 2-4 hours. DHA, being a potent anitmalarial itself, has a $t_{1/2}$ of about 2-4 hours. The degree of binding to plasma proteins varied markedly according to the species studied. After intramuscular administration, artemether predominates, whereas after oral administration dihydroartemisinin 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 one hour, but following intramuscular administration the elimination phase is prolonged because of continued absorption. No dose modifications are necessary in renal or hepatic impairment. Radioacivity distribution of artemether was found to be equal between cells and plasma. (WHO 2010).

2.1.6 Drug Interactions of artemether

Artemether may reduce the effectiveness of hormonal contraceptives. CYP3A4 inducers May result in decreased concentrations of artemether and loss of anti-malarial efficacy. CYP3A4 inhibitors (eg, grapefruit juice, ketoconazole) may result in increased concentrations of artemether. CYP2D6 metabolized drugs (eg, amitriptyline, clomipramine, flecainide, imipramine) coadministration may significantly increase plasma concentrations and increase the risk for adverse effects. Mefloquine; If mefloquine is administered immediately prior to artemether/lumefantrine, there may be decreased exposure to lumefantrine. Monitor patients for decreased efficacy and

encourage food consumption. Avoid coadministration with non sedating antihistaminics (eg, terfenadine, astemizole) (Drug Bank).

2.1.7 Toxicity

In all species of animals tested, intramuscular artemether 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 and Davis, 2004; WHO 2010).

2.1.8 Formulations, Dosage and Administration of Artemether

Artemether can be given as an oil-based intramuscular injection or orally. It is also coformulated with lumefantrine for combination therapy in form of:

1. 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.
2. Tablets containing 20 mg of artemether and 120 mg of lumefantrine
3. Tablets containing 80 mg of artemether and 480 mg of lumefantrine
4. suspensions containing 180 mg of artemether and 1080 mg of lumefantrine.

The dose depends on the severity of the case and the clinical situation of the patient. In general; 4 mg artemether/kg body weight in combination with a 6 fold of that that dose for lumefantrine per day, administered as a single dose.

For each patient, the dose to be administered will be calculated in millilitres. It is recommended to round off the dosage to the nearest subdivision as shown below in table 2.1.

Table 2.1; Dose of artemether/lumefantrine suspension(mls) with the corresponding body weight

Body weight	**Number of ml**		
	Day 1	Day 2	Day 3
5 kg	7 ml	7 ml	7 ml
7.5 kg	10 ml	10 ml	10 ml
10kg	14 ml	14 ml	14 ml
15kg	20ml	20ml	20ml

A 3-day treatment schedule with a total of 6 doses is recommended for adult patients with a body weight of 35 kg and above. Per oral (PO) 4 tablets as a single initial dose, 4 tablets again after 8 h, and then 4 tablets twice daily (morning and evening) for the following 2 days (total course of 24 tablets each containing 20mg artemether and 120 mg lumefantrine).

2.2 Methods for determination of artemether

As artemether-lumefantrine combination formulations are increasingly being advocated as standard treatment for malaria infections, it would be expected that there would be several methods for the assay of 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 (Nigeria inclusive) where

malaria is endemic, it is important to ensure the quality and stability of antimalarial drugs.

There are no monographs for artemether in both the B.P and the USP. The international pharmacopoeia (IP, 2008) contains monographs of pure artemether 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.

2.2.1 UV Spectrophotometric methods for analysis of artemether

Artemether lacks strongly absorbing chromophores and as such it absorbs weakly in the low wavelength region and this makes its 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 *et al.*, 1992). Though this product absorbs strongly at the said wavelength, it requires very vigorous conditions for its formation.

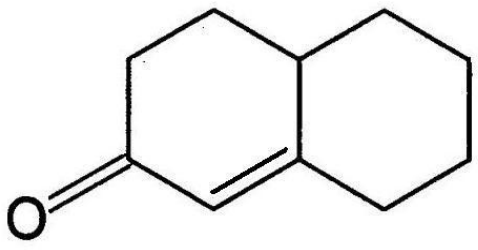


Fig. 2.4 Structure of α, β Unsaturated Decalone

The IP method for the assay of artemether (both as the pure sample and in formulations) requires the dissolution of a weighed amount of artemether, in sufficient dehydrated

ethanol R, diluting a portion of this solution to 100ml with hydrochloric acid/ethanol (1 mol/l) VS and heating in a water-bath at 55 °C for 5 hours before taking the absorbance in a UV at 254 nm (IP, 2008).

Another method developed by Shrivastava *et al.,* requires the addition of methanolic hydrochloric acid, shaking and heating at 60 ˚C for three hours before taking the absorbance at 254 nm (Shrivasatava *et al*. 2008). The time requirement as well as the heating condition required by these methods make them uneconomical.

Green *et al*., (2001) have also described a colorimetric method for the assay of artemether and other artemisinins by the reaction of the acid decomposition product (the α, β unsaturated decalone) with a dye to yield a coloured derivative which absorbs at 420nm. This method requires a period of at least one hour for the formation of the product prior to reaction with the dye.

Another UV-Spectrophotometric method was developed and validated for quantitative determination of artemether in capsules. The method employed lN HCl as a solvent and used to derivatize the drug at 80°C for 20 minutes and water was used as a diluting solvent. The wavelength of maximum absorbance of Artemether was found at 254 nm. The method obeyed the Beer's law in the concentration range of 5-40 μg/ml. The linear regression showed good linear relationship with r^2 = 0.998, slope and intercept were 0.0182 and 0.006622 respectively. Method was validated statistically where SD and RSD were found to be satisfactorily low. Percentage recovery of the drug for the method was found in the range of 99.5-100.86% indicating no interference of the capsule excipients.

LOD and LOQ for artemether were found to be 2.30 μg/ml and 4.08 μg/ml respectively the method though simple, also requires heating at 80°C (Pawar *et al.*, 2011).

2.2.2 HPLC methods for artemether determinations

Vandercruyssen *et al*., (2013) developed a stability-indicating HPLC method, with appropriate sample preparation steps, for β-artemether assay and profiling of related impurities, including possible degradants, such as α β dihydroartemisinin, artemisinic acid and in a powder for oral suspension. Quantitative UV-detection was performed at 210 nm. Acetonitrile was used as extraction solvent for sample preparation.

Arun and Smith, (2011) developed and validated a HPLC-UV method for the simultaneous estimation of artemether and lumefantrine in fixed-dose combination tablets. The HPLC analyses were carried out with UV detector, at 254nm. A linear correlation was found between the concentration range of 32-192 (μg/mL) for artemether.

Another Reverse Phase-HPLC method was developed for simultaneous estimation of lumefantrine and artemetherwith UV detection at 210nm by Shah *et al.*, (2013).

Though the HPLC methods described are highly selective and sensitive, the cost of chemicals required, the unavailability of HPLC machine and lack of competent hands to operate it where available may limit the application of the methods.

2.3 Absorption Spectroscopy

The quantitative method used in this study is UV/VIS spectroscopy which is based on absorption spectroscopy. In absorption spectroscopy a beam of radiation passes through a sample. Much of the radiation is transmitted without loss in intensity. At selected frequencies however, the radiation's intensity is attenuated. This process of attenuation is called absorption. Two general requirements must be met if an analyte is to absorb electromagnetic radiation. The first requirement is that there must be a mechanism by which the radiation's electric field or magnetic field interacts with the analyte. For ultraviolet and visible radiation, this interaction involves the electronic energy of valence electrons. The second requirement is that the energy of the radiation must exactly equal the difference in energy, ΔE, between two of the analytes quantized energy states (Harvey, 2000).

The attenuation of radiation as it passes through a sample is described quantitatively by two separate, but related terms: transmittance and absorbance. Transmittance is defined as the ratio of the radiation's power exiting the sample, P_T, to that incident on the sample from the source, P_0.

$$T = P_T/P_O$$

Attenuation of radiation as it passes through the sample leads to a transmittance of less than 1. Besides absorption by the analyte, several additional phenomena contribute to the net attenuation of radiation, including reflection and absorption by the sample container, absorption by components of the sample matrix other than the analyte and the scattering of radiation. To compensate for this loss of radiation's power, a method blank is used.

The radiation's power exiting from the method blank is taken to be P_0. An alternative method for expressing the attenuation of radiation is absorbance, A, which is defined as;

$$A = -\log_{T} = -\log P_T/P_O = \log P_O/P_T$$

Absorbance is the more common unit for expressing the attenuation of radiation because it is a linear function of the analyte's concentration. When monochromatic radiation passes through an infinitesimally thin layer of sample, of thickness b, it experiences a decrease in intensity. The fractional decrease in intensity is proportional to the sample's thickness and analyte's concentration, C; thus

$$A = abC$$

Where *a*, is the analyte's absoptivity with units of cm^{-1} $conc^{-1}$. This equation is based on Beer's law. When concentration is expressed using molarity, the absoptivity is replaced by the molar absoptivity, ε with (units of cm^{-1} M^{-1})

$$A = \varepsilon bC$$

The absoptivity and molar absoptivity give, in effect, the probability that the analyte will absorb a photon of given energy. As a result, values for both A and ε depend on the wavelength of radiation (Harvey, 2000).

In pharmaceutical products, concentrations and amounts are usually expressed in grams or milligrams rather than in moles and thus for the purposes of analysis of these products, the Beer's equation is written in the following form:

$$A = A\ (1\%, 1\ cm)\ bC$$

A is the measured absorbance; A (1%, 1cm) is the absorbance of a 1% w/v (1 g/100ml) solution in a 1 cm cell; b is the path length in cm (usually 1 cm); and *C* is the concentration of the sample in g/100ml. Since measurements are usually made in a 1 cm cell, the equation can be written:

$$C = A/A(1\%, 1\ cm)$$

This gives the concentration of the analyte in g/100 ml (Watson, 2005).

2.3.1 UV/VIS spectroscopy

When a molecule or ion absorbs ultraviolet or visible radiation it undergoes a change in its valence electron configuration. The valence electrons in organic molecules, and inorganic anions, occupy quantized sigma bonding (σ), pi bonding (π), and non bonding (η) molecular orbitals. Unoccupied sigma anti-bonding (σ □) and pi anti-bonding (π □) molecular orbitals often lie close enough that the transition of an electron from an occupied to an unoccupied orbital is possible. Four types of transitions between quantized energy levels accounts for molecular UV/Vis spectra (Table 2.2). Of these transitions, the most important are the η → π □ and π → π □ ,because they involve functional groups that are characteristic of the analyte and the wavelengths that are easily accessible. The bonds and functional groups that give rise to the absorption of ultraviolet and visible radiation are called chromophores (Harvey, 2000).

Table 2.2 Electronic transitions involving η, σ and π molecular orbitals

Transition	Wavelength range	Examples

$\sigma \rightarrow \sigma^*$	<200	C-C, C-H
$\eta \rightarrow \sigma^*$	160-260	H_2O, CH_3OH, CH_3Cl
$\pi \rightarrow \pi^*$	200-500	C=C, C=O, C=N
$\eta \rightarrow \pi^*$	250-600	C=O, C=N, N=N, N=O

Source: adapted from Harvey, 2000

The act of identifying materials based on their color was probably one of the earliest examples of qualitative molecular absorption spectrophotometry. Also, the first recognition that color intensity can be the indicator of concentration was probably the earliest application of employing molecular absorption spectroscopy for quantitative determination. The first measurements were made by using the human eye as the detector and undispersed sunlight or artificial light as the light source. Later it was found that the accuracy and the precision could be improved by isolating specific frequencies of light using optical filters. Further improvement of the measurement came with the use of prism and grating monochromators for wavelength isolation. Photoelectric detectors were soon developed, but were quickly replaced with phototubes and photomultiplier tubes. The development of solid state microelectronics has now made available a wide range of detector type which coupled with the computers, provide highly sophisticated readout electronic systems. Two or more chromophoric groups in the molecule often enhance one another's effect, to deepen the color by displacing the absorption maximum (λ max) towards longer wavelengths (from the ultraviolet towards the red). This is called bathochromic shift. The displacement of the absorption maximum from the red towards the ultraviolet is known as a hypsochromic shift. The color of a molecule may be intensified by substituents called auxochromic groups. These groups may also affect

bathochromic shifts. The color-determining factor in a number of molecules is the introduction of conjugation of double bonds by means of electron donor and electron acceptor groups. The quantitative applicability of the UV absorption method is based on the fact that the number of photons absorbed is directly proportional to the number or concentration of atoms, ions or molecules (Blaedel and Meloche, 2001).

2.4 Stability studies

Formal stability studies is a Long term, accelerated (and intermediate) studies undertaken on primary and/or concormittant batches according to a prescribed stability protocol to establish or confirm the re-test period of an Active Pharmaceutical Ingredient (API) or the shelf life of a Finished Pharmceutical Product (FPP). An API or FPP is considered as stable if it is within the defined/regulatory specifications (WHO 2006).

2.4.1 Potential instability issues of FPPs

1. Loss/increase in concentration of API
2. Formation of (toxic) degradation products
3. Modification of any attribute of functional relevance
4. Alteration of dissolution time/profile or bioavailability
5. Decline of microbiological status
6. Loss of package integrity
7. Reduction of label quality
8. Loss of pharmaceutical elegance and patient acceptability

2.4.2 Stability-indicating quality parameters

Stability studies should include testing of those attributes of the FPP that are susceptible to change during storage and are likely to influence quality, safety and/or efficacy. They

include: appearance, hardness, friability, moisture content, dissolution time, degradants, assay, microbial purirty etc. (WHO, 2006).

Nayyar *et al*., (2012) have classified poor quality drugs into three types. Falsified drugs are fraudulently manufactured, with fake packaging and usually either contain no active ingredient or one other than that specified on the label. Substandard drugs result from poor manufacturing processes. They may contain too much or too little active ingredient, but are not manufactured with the intention to deceive. Degraded drugs were of good quality at the time of their manufacture, but have deteriorated, often, due to poor storage conditions. The first two classifications may be considered to be problems of genuinely poor quality, while the last classification is a problem of drug stability. Proper patient care requires any drugs dispensed to be both of good quality and adequate stability (Gitua *et al*., 2014).

2.4.3 Some reported stability studies

A number of studies have evaluated anti-malarial drug quality and stability under conditions typically found in Africa. Studies used variety of methods to determine drug quality and stability including storage under simulated tropical conditions, determining the amount of drug released after dissolution of a solid dosage form but there are few studies reported on antimalarial suspensions.

John *et al*., (2014) developed stability and quantification studies on amoxicillin-clavulanic acid oral powder for suspension by HPLC method. The studies were carried out on three different brands of the powder and they found that the powders were stable after seven days of reconstitution irrespective of the type of water used for reconstitution;

i.e mineral water, distilled water or treated tap water. Both compounds were considered stable if the degradation was not more than 10% of their initial concentration.

Bate *et al*., (2009) obtained samples of artemether/lumefantrine that were 1 – 58 months past their labeled expiry date. They found that 97% of expired products passed tests for tablet integrity and the presence and amount of active ingredients using thin layer chromatography, tablet disintegration and infrared (IR) spectroscopy. They concluded that the usual two year labelled shelf life for stability could probably be safely extended.

A volunteer group from USA on a two weeks medical mission to Mali carried out a stability studies of a brand of artemether/lumefantrine tablets manufactured by IPCA laboratories in reference to another product (coartem) by Novartis pharmaceuticals. They stored a blister pack in Mali and another identical pack under controlled conditions in a laboratory in USA. Experimental analysis of the drug samples revealed there was no difference in drug content between the products stored under ambient conditions in Mali or temperature and humidity controlled conditions in the USA compared to the reference product. This indicates that any drug degradation in the Malian warehouse was not different than any degradation seen in the product stored under temperature and humidity controlled conditions or the reference product (Gitua *et al*., 2014).

Karuna *et al.,* (2014) carried out stability studies for artemether lumefantrine (FDC) tablets formulation at 25±2ºC/60 ± 5%RH and 40±2ºC/75 ± 5% RH for a period of 3 months and observed no significant change in the drug content, physical properties and dissolution rate of these tablets after the 3 months storage period in various conditions according to ICH guidelines. The samples were analyzed for drug content by HPLC using UV detector at 210 nm.

Dafra Pharma, a pharmaceutical company carried out stability studies of coartesiane their brand of artemether lumefantrine powder for suspension at time 0hr, 14 days and 30 days after reconstitution with water. The suspension (in which the water insoluble lumefantrine and Artemether are freely floating) was kept at 30°C in a humidity room of 65%. The samples collected were analysed for drug content using HPLC and the suspensions were found to be stable for the period under study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

The chemicals used include the following: Concentrated HCl, Ethanol, Potassium iodide and Methanol.

3.1.2 Drug samples and drug reference standard

Reference standard of Artemether powder was generously provided by Green Life pharmaceuticals limited. Five different brands of artemether/lumefantrine suspensions were collected from pharmacy shops in Zaria, taking note of the expiry date. The samples collected were stored appropriately before conducting the research work.

Table 3.1; Drug Samples used in the study

Code	S	A	B	C	D	E
M. Date	01/10/2013	03/2014	02/2014	11/2013	12/2013	03/2014
E. Date	30/09/2017	02/2016	02/2016	10/2016	11/2016	02/2016
B. No.	ARM015/13	LG-313	586T	0151	ASX1301	DS4004
Naf. No		04-8970	A4-1717	A4-4044	A4-3821	A4-1980

3.1.3 Materials

1. Mettler AE 240 digital analytical balance, Mettler Instruments Limited, Switzerland.
2. Mcdonald HH-S digital thermostatic water bath
3. Beakers, 50, 100, 250ml
4. Measuring cylinders, 10, 50, 100ml
5. Pipettes, 1, 5, 10ml
6. Volumetric flasks, 5, 10, 100, 500ml
7. Whatman filter paper no. 41
8. Test tubes and test tube holders
9. Spatula
10. Hand gloves

3.1.4 Instrumentation

A double scanning UV/VIS spectrophotometer (Helios Zeta, Model 164617) was used to monitor the drug content throughout the analysis.

3.2 Methods

3.2.1 Preparation of solutions and Reagents

3.2.1.1 Preparation of hydrochloric acid/ethanol (1 mol/l)

Hydrochloric acid/ethanol (1 mol/l) was prepared by adding 86mls of concentrated HCl to a 1 litre measuring cylinder containing about half its content of ethanol and making up the volume to 1 litre with ethanol.

3.2.1.2 Preparation of standard stock solution of artemether in methanol

A standard solution of Artemether was prepared by accurately weighing and dissolving 20mg of pure Artemether powder in 20ml of methanol to obtain a concentration 1000µg/ml solution.

3.2.2 Identification and assay of artemether (Official methods)

3.2.2.1 Identification of artemether

Artemether was identified in both the reference standard and powder for suspension sampled using official methods (IP, 2008).

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 waterbath. The procedure was repeated for the different brands sampled.

ii) Melting point determination was carried out for artemether reference standard using the melting point apparatus with a thermomether.

iii)FTIR Analysis was performed in NARICT Bassawa Zaria. The sample was prepared using potassium bromide (KBr) discs. Before taking the spectrum of the sample, a blank spectrum of air background was taken. The sample was taken and compressed under 10-ton pressure in a hydraulic press to form a transport pellet. The Fourier transform infrared (FTIR) spectrums of the samples were recorded in KBr Phase in the frequency region 500 – 4500 $cm^{-1.}$

3.2.2.2 Assay of artemether

International Pharmacopoeial method for assay of artemether

In this method, an amount of the powder of brands A, B, C, D and E containing 0.050 g of Artemether were accurately weighed, and dissolved in five separate 100 ml volumetric flasks containing sufficient dehydrated ethanol R to produce 100 ml. 2 ml of each solution was diluted to 100ml with hydrochloric acid/ethanol (1 mol/l). Each flask was stoppered and placed in a water-bath at 55 °C for 5 hours. They were allowed to cool to room temperature. The absorbance of each solution was measured in a 1-cm layer at the maximum of 254 nm. The percentage content of $C_{16}H_{26}O_5$ was calculated by comparison with artemether RS, similarly and concurrently examined, and with reference to the dried substance.

3.2.3 Method development

The method is based on preparing a solution of artemether in methanol followed by hydrolysis with concentrated HCl to obtain the decomposition product, an α,β-unsaturated ketone.

3.2.3.1 Determination of λ max

From the stock solution, 1 ml was taken and diluted to 10 ml with methanol in a volumetric flask to get 100 µg/ml solution and 1 ml of the solution was extracted using a pipette and transferred to a test tube. 1 ml of concentrated HCl acid was added and stoppered. The solution was left to stand for 30 mins and then scanned on

spectrophotometer in the range 200 to 600nm and the maximum absorption was observed at 260nm.

3.2.3.2 Preparation of calibration curve

From the stock solution, different aliquots in the range 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml were transferred into series of 10 ml volumetric flask and the volume made up to the mark with methanol to obtain serial dilutions of the concentrations 20, 40, 60, 80, 100 and 120 µg/ml. 1 ml of each solution was taken and transferred into series of test tubes and 1 ml of conc HCl acid added to each test tube and stoppered. The solutions were left to stand for 30 mins and their respective absorbances were determined at 260 nm against the reagent blank. A plot of absorbance against the corresponding concentration gave the calibration curve.

3.2.3.3 Validation of method

The method was checked for its precision, accuracy, recovery, limit of detection and limit of quantitation.

Precision: The precision of this method was checked by replicate analysis of the calibration curve responses determined. This was done by taking five replicates of three concentrations within the range of the calibration curve. . The percentage coefficient of variation (% CV) for the replicate analysis of each determination was taken as a measure of precision. With good technique and reliable methodology the precision should be < 15 % CV. Calculation of % CV is done as follows:

$$\% CV = S/X \times 100 \ldots\ldots\ldots \text{ Formula 1}$$

Where S is the standard deviation and X is the mean

Accuracy: The accuracy of this method was checked by preparing five samples of same concentration 40 µg/ml separately and their absorbances measured. The mean of the value obtained was used to calculate the percentage relative error (accuracy).

Accuracy is usually expressed as percentage relative error (%Er) and is calculated as follows:

$$\% \text{ Er} = X - \mu / \mu \times 100 \text{…….. Formula 2}$$

Where X is the mean and µ is the expected value

Recovery: The recovery of this method was checked by having four 5 ml volumetric flasks each containing 2ml of 55 µg/ml solution of artemether. Flask 1 was left untouched but 0.19, 0.27, and 0.29 ml of the standard stock solution of artemether were added in flask 2, 3, and 4 respectively and made up to the volume with methanol to obtain concentrations of 60, 70, and 80 µg/ml respectively. The absorbance of each solution was measured and the measured concentrations extrapolated from the calibration curve by subtracting the absorbance of flask 1 from that found in each of the respective flasks, thus the percentage recovery was computed using the formula:

$$\% \text{ Recovery} = \text{measured concentration} / \text{added concentration} \times 100 \text{…. Formula 3}$$

The average of these determinations was taken as the percentage recovery. Simple dilution method was adopted in determining the actual quantities that were taken from the stock solution. The dilution formulas that were used are as follows:

$$C_1V_1 = C_2V_2$$

$$C_1V_1 + C_2V_2 = C_3V_3 \text{ ..... Formula 4}$$

Detection limit: The detection limit (DL) was determined by studying the calibration curve using samples in the range of DL containing the drug in the concentrations of 20, 40, 60, 80, 100 and 120 µg/ml. The standard deviation of y-intercepts of the regression lines was used as standard deviation. DL is expressed as:

$$DL = 3.3\ \sigma / S \text{…….. Formula 5}$$

Where σ is the standard deviation of y-intercepts of the regression lines and S is the slope of the calibration curve.

Quantitation limit: The quantitation limit (QL) was determined using the expression:

$$QL = 10\ \sigma / S \text{…….. Formula 6}$$

Where σ is the standard deviation of y-intercepts of the regression lines and S is the slope of the calibration curve.

3.2.3.4 Application of the developed method in the assay of Artemether lumefantrine powders for suspension

For the assay of artemether/lumefantrine powders for suspension, each of Brand A, B, C, D and E were accurately weighed and the weights recorded. An amount of powder equivalent to 45 mg artemether from each sample was weighed. These were quantitatively transferred to a 100 ml calibrated volumetric flask containing about 30 ml

of methanol. The volume was made up to the mark with methanol and shaken for 10 minutes. The solutions were filtered through a Whatman filter paper no. 41. From the filtrate 1 ml each was transferred to five 10 ml volumetric flasks and each was made up to the mark with methanol to obtain a concentration of 45 µg/ml. A quantity (1 ml) of each of the solutions were transferred separately into five different test tubes and 1 ml of concentrated HCl acid added and left to stand for 30mins. The absorbance of the contents of each test tube was measured at 260 nm.

The assay results of the proposed method were statistically compared with that of the International Pharmacopoeial method.

3.3 Stability Studies

3.3.1 Preparation of suspensions

3.3.1.1 Preparation of standard artemether suspension

180 mg of artemether standard powder was weighed and transferred into a clean and dried mortar. 600mg of microcrystalline cellulose (as suspending agent) was weighed and added and the powders mixed thoroughly. About 30 ml of water was added and the mixture stirred gently and transferred into a pre calibrated 60 ml bottle. The mortar was rinsed with water and the water transferred into the bottle and the volume made up to the mark with more water.

3.3.1.2 Preparation of different brands of artemether/lumefantrine suspensions

Each of the five brands (A, B, C, D and E) of powders for suspension were reconstituted with water according to the directions on the label. After opening the vial (breaking the seal), drinking water was added and carefully brought to the mark point indicating 60 ml level. After adding the water the mixture was vigorously shaken until all powder had

disappeared from the bottom and a suspension was formed. More water was added to readjust the volume to the 60 ml mark.

3.3.2 Protocol for stability studies

2 ml of each brand (except 1.5 ml for Brand A) of the suspensions and the standard artemether suspension prepared were measured and dissolved in methanol and shaken for 10 minutes. These were transferred to series of 100 ml calibrated volumetric flask, and the volumes were made up to the mark with methanol and shaken for 10 minutes. The solutions were filtered through a Whatman filter paper no. 41. From each of the filtrates, 1 ml was transferred to a test tube and 1 ml of conc HCl acid added, stoppered and left to stand for 30minutes. The absorbances of each solution was measured at 260 nm. The remaining suspensions were stored in a cupboard at room temperature and the assay was repeated at day 1, 3, 5, 7, 10 and 14.The various concentrations obtained from these subsequent days were compared to the concentrations obtained immediately after reconstitution to check the stability of the suspensions after a period of 14days.

CHAPTER FOUR

RESULTS

4.1 Identification Tests for Artemether Reference Standard and Powders for Suspension

4.1.1 Colour test

The standard artemether powder and all the five brands produced the expected yellow colour.

4.1.2 Melting point

The melting point of the artemether reference standard was found to be 86-89 ^{o}C.

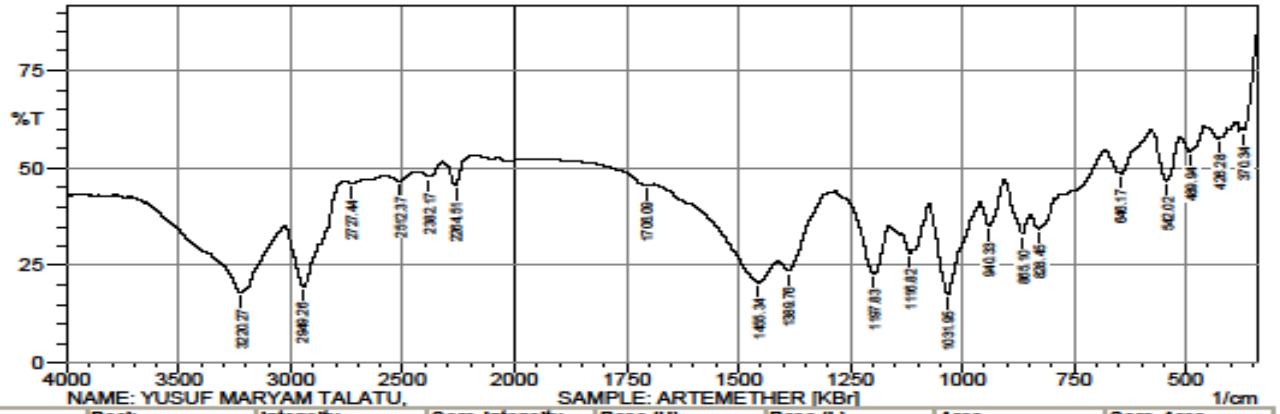
4.1.3 FTIR analysis

The Fourier transform infrared (FTIR) analysis results for standard powder of artemether RS, samples A, B, C, D and E and the decomposition product formed are presented in figure 4.1a, 4.1b, 4.1c, 4.1d, 4.1e, 4.1f and 4.1g respectively.

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR-8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



NAME: YUSUF MARYAM TALATU, SAMPLE: ARTEMETHER [KBr]

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	370.34	59.696	4.437	375.17	339.48	5.719	0.816
2	426.28	57.608	0.957	448.46	419.53	6.733	0.15
3	489.94	54.332	4.904	513.08	459.07	13.417	1.202
4	542.02	46.836	12.039	577.7	513.08	18.09	3.262
5	646.17	48.65	7.672	680.89	577.7	28.122	3.022
6	828.45	34.513	4.825	845.81	794.7	22.266	1.912
7	865.1	33.169	7.626	906.57	845.81	25.283	2.536
8	940.33	35.115	8.425	959.62	906.57	21.488	2.688
9	1031.95	17.607	23.473	1073.42	959.62	60.705	16.842
10	1116.82	28.228	7.462	1137.07	1073.42	31.328	3.746
11	1197.83	22.995	14.6	1258.59	1164.08	48.194	8.97
12	1389.76	23.677	5.162	1408.08	1292.35	56.169	1.599
13	1455.34	20.667	8.629	1593.25	1414.83	97.762	10.375
14	1706.09	45.619	0.09	1708.02	1703.2	1.642	0.002
15	2264.51	45.573	6.719	2316.58	2179.63	41.029	2.712
16	2382.17	47.815	0.077	2433.28	2380.24	16.589	-0.076
17	2512.37	46.757	1.813	2573.13	2437.14	43.581	0.999
18	2727.44	46.144	0.094	2754.44	2723.58	10.306	0.026
19	2949.26	19.668	0.191	3028.34	2948.29	44.838	-1.508
20	3220.27	18.123	0.165	3223.16	3216.41	4.993	0.012

Comment;
NAME: YUSUF MARYAM TALATU,
SAMPLE: ARTEMETHER [KBr]

User; Administrator

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Resolution;

Fig 4.1a: FTIR spectra for artemether RS

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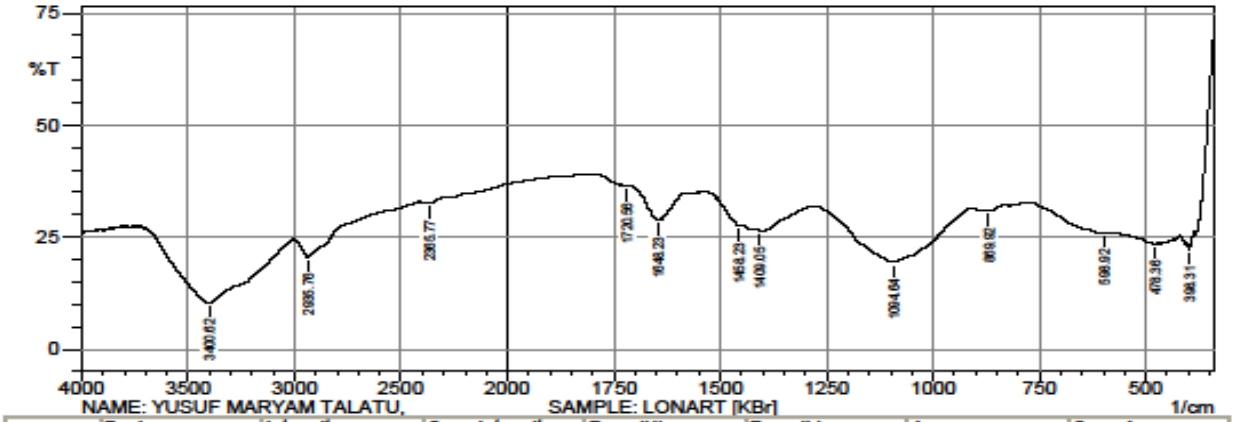
FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



NAME: YUSUF MARYAM TALATU, SAMPLE: LONART [KBr]

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	398.31	22.104	2.279	402.17	392.53	6.14	0.248
2	478.36	23.569	0.016	480.29	477.4	1.815	0
3	598.92	25.969	0.011	609.53	597.95	6.768	0.001
4	869.92	30.891	0.531	884.39	829.42	27.648	0.219
5	1094.64	19.443	12.275	1279.81	915.25	221.19	39.399
6	1409.05	26.426	0.092	1415.8	1406.15	5.559	0.003
7	1458.23	27.543	0.469	1509.35	1455.34	27.95	0.149
8	1648.23	28.83	0.267	1651.12	1638.58	6.746	0.025
9	1720.56	36.375	0.131	1722.49	1716.7	2.536	0.004
10	2365.77	32.533	0.01	2366.74	2364.81	0.941	0
11	2935.76	20.574	4.905	3000.37	2411.1	330.539	8.648
12	3400.62	10.411	0.034	3401.58	3000.37	316.241	-3.151

Comment;
NAME: YUSUF MARYAM TALATU,
SAMPLE: LONART [KBr]

User; Administrator

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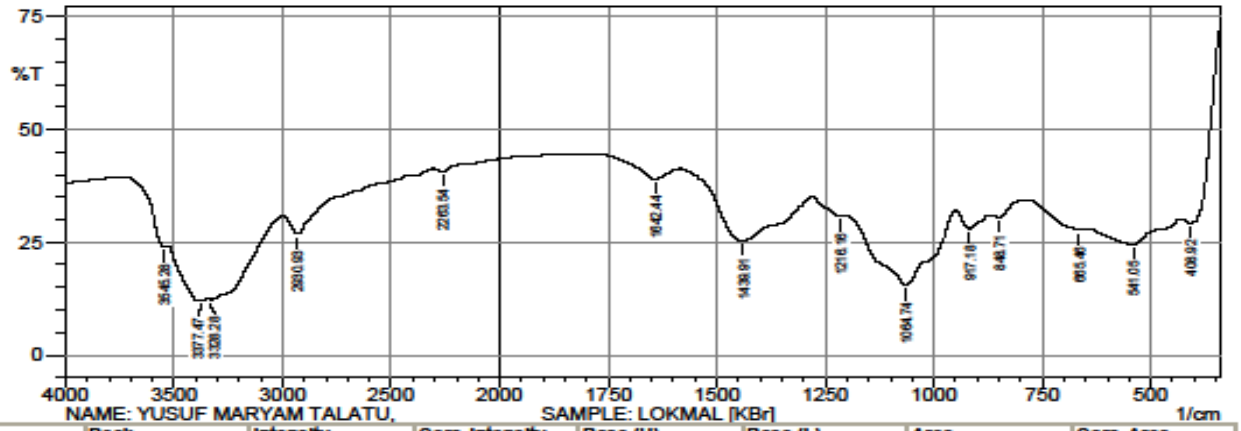
Fig. 4.1b; FTIR spectra for A

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



NAME: YUSUF MARYAM TALATU, SAMPLE: LOKMAL [KBr]

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	408.92	29.207	11.199	430.14	339.48	37.231	7.694
2	541.05	24.645	0.29	546.84	430.14	65.887	0.027
3	665.46	27.743	1.247	794.7	643.28	78.006	1.184
4	848.71	30.481	1.561	868.96	794.7	36.529	0.534
5	917.18	28.055	3.627	949.97	868.96	42.745	2.203
6	1064.74	15.563	15.995	1202.66	949.97	165.563	38.807
7	1216.16	30.766	0.916	1279.81	1202.66	37.797	0.599
8	1439.91	25.311	12.999	1586.5	1279.81	153.832	25.119
9	1642.44	39.053	3.145	1795.79	1586.5	78.884	2.098
10	2263.54	40.741	0.867	2304.05	2150.7	58.448	0.472
11	2930.93	26.925	4.756	2995.55	2412.06	269.671	3.532
12	3328.28	12.322	0.89	3343.71	2995.55	250.703	3.511
13	3377.47	12.04	2.526	3528.89	3343.71	149.437	8.57
14	3545.28	24.015	1.552	3719.65	3528.89	92.962	-4.353

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SAMPLE: LOKMAL [KBr]

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Fig. 4.1c; FTIR spectra for B

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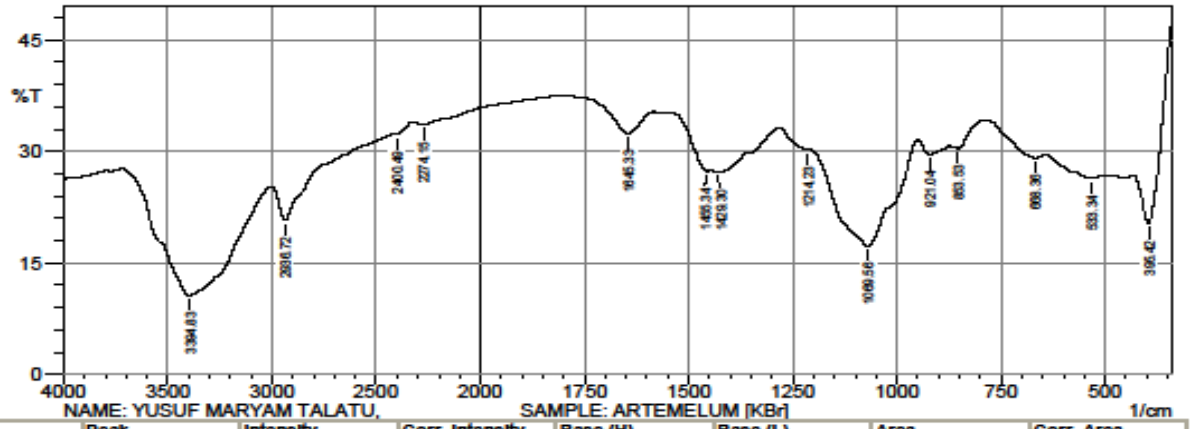
FTIR-8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



NAME: YUSUF MARYAM TALATU, SAMPLE: ARTEMELUM [KBr]

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	395.42	20.312	14.729	432.07	339.48	51.293	9.906
2	533.34	26.343	0.946	643.28	509.22	75.161	1.299
3	668.36	29.102	1.201	790.84	643.28	74.643	1.157
4	853.53	30.406	1.123	872.82	790.84	40.477	0.362
5	921.04	29.569	1.736	949.01	872.82	39.574	1.007
6	1069.56	17.307	13.678	1209.41	949.01	166.818	34.088
7	1214.23	30.203	0.204	1281.74	1209.41	36.465	0.339
8	1429.3	27.224	0.706	1443.77	1281.74	85.714	1.376
9	1455.34	27.332	1.036	1537.32	1443.77	47.693	0.149
10	1645.33	32.35	3.661	1761.07	1583.61	81.228	3.118
11	2274.15	33.607	0.448	2305.98	1815.08	221.608	1.315
12	2400.49	32.232	0.36	2414.96	2327.19	42.384	0.263
13	2936.72	20.715	5.363	3004.23	2414.96	329.013	8.499
14	3394.83	10.659	15.927	3716.95	3004.23	544.474	132.744

Comment;
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User; Administrator

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Resolution;

Fig. 4.1d; FTIR spectra for C

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FTIR ANALYSIS RESULT NARICT,ZARIA

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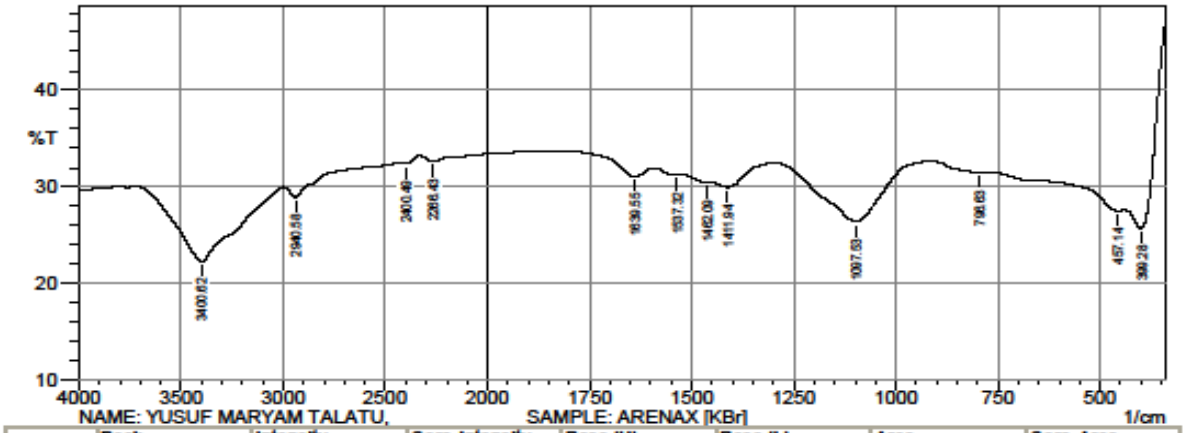

NAME: YUSUF MARYAM TALATU, SAMPLE: ARENAX [KBr]

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	399.28	25.741	10.065	441.71	339.48	52.316	7.152
2	457.14	27.499	0.286	771.55	441.71	172.111	-2.756
3	796.63	31.501	0.238	913.32	771.55	70.337	0.392
4	1097.53	26.405	6.21	1297.17	913.32	201.341	14.547
5	1411.94	29.966	1.121	1461.13	1297.17	83.098	0.8
6	1462.09	30.472	0.012	1524.78	1461.13	32.498	0.009
7	1537.32	31.272	0.023	1592.29	1535.39	28.514	0.038
8	1639.55	31.096	1.201	1813.15	1592.29	107.496	0.578
9	2266.43	32.642	0.674	2332.02	1926.95	194.13	1.371
10	2400.49	32.422	0.292	2429.42	2332.02	47.277	0.209
11	2940.58	28.931	1.317	2998.44	2429.42	287.478	-0.247
12	3400.62	22.324	7.762	3719.85	2998.44	417.123	40.716

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Fig. 4.1e; FTIR spectra for D

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FTIR ANALYSIS RESULT NARICT,ZARIA

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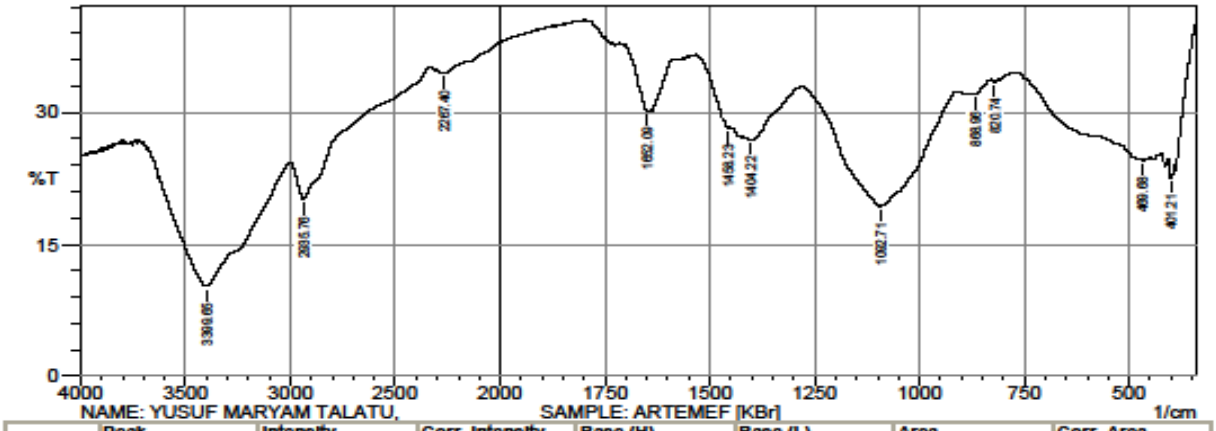

NAME: YUSUF MARYAM TALATU, SAMPLE: ARTEMEF [KBr]

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	401.21	22.6327	0.6327	406.99	399.28	4.869	0.0473
2	469.68	24.5852	0.0707	475.47	467.75	4.6954	0.0062
3	820.74	33.5338	0.2346	829.42	810.13	9.12	0.0253
4	868.96	32.0359	0.5753	883.43	829.42	26.2366	0.2116
5	1092.71	19.3288	13.345	1279.81	914.29	219.7938	42.2467
6	1404.22	26.9124	0.0181	1406.15	1402.3	2.1985	0.0005
7	1458.23	28.1865	0.5391	1508.38	1455.34	26.7852	0.181
8	1652.09	30.2232	0.2696	1684.88	1651.12	16.2642	0.0499
9	2267.4	34.4473	0.0102	2268.36	2266.43	0.8926	0.0001
10	2935.76	20.0707	5.2522	2999.41	2413.03	330.3128	9.981
11	3399.65	10.2293	10.6774	3579.04	2999.41	470.0119	85.6101

Comment;
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SAMPLE: ARTEMEF [KBr]

User; Administrator

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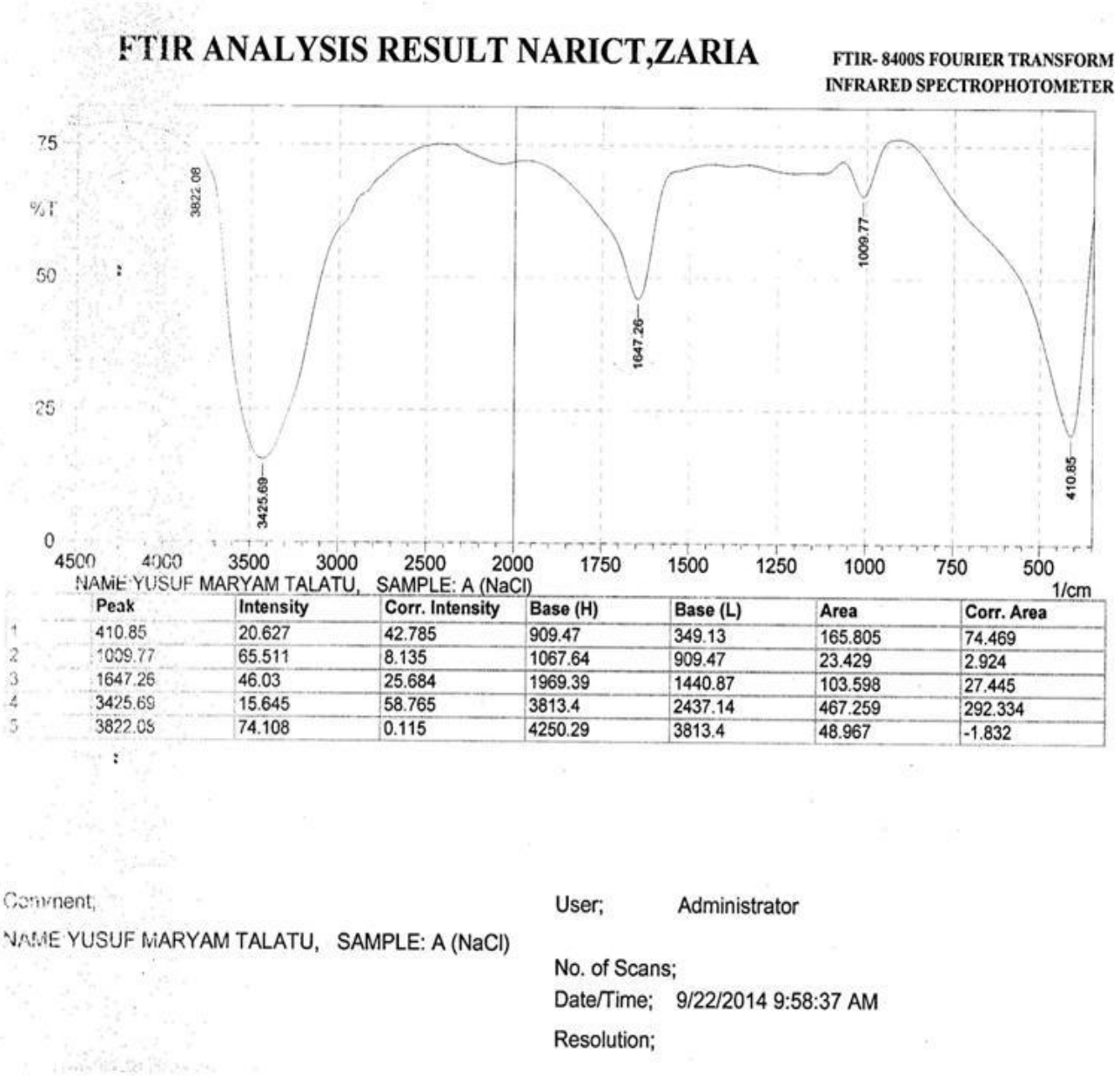
Fig. 4.1f; FTIR spectra for E



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	410.85	20.627	42.785	909.47	349.13	165.805	74.469
2	1009.77	65.511	8.135	1067.64	909.47	23.429	2.924
3	1647.26	46.03	25.684	1969.39	1440.87	103.598	27.445
4	3425.69	15.645	58.765	3813.4	2437.14	467.259	292.334
5	3822.08	74.108	0.115	4250.29	3813.4	48.967	-1.832

Fig. 4.1g; FTIR spectra for the decomposition product

4.2 Method Development

4.2.1 Wavelenght of maximum absorption

The wavelength of maximum absorption for the determination of artemether using the proposed method was found to be 260nm and the UV spectra is shown below in figure 4.2.

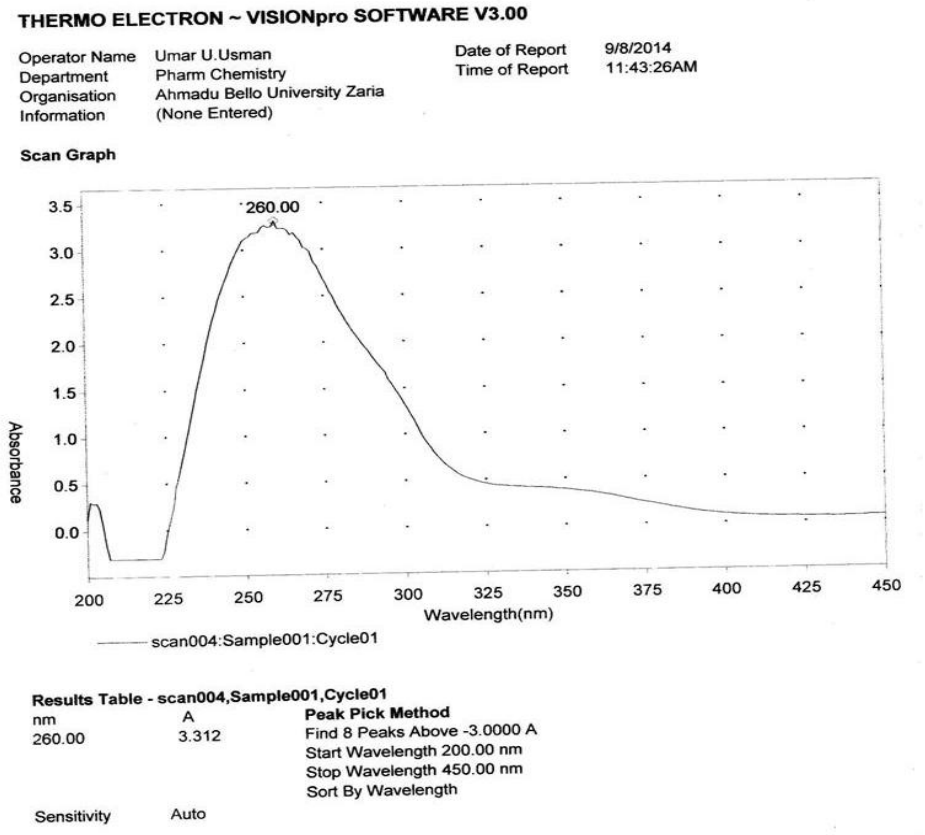


Fig 4.2; Wavelenght of Maximum Absorption

4.2.2 Calibration Curve

The calibration curve for the quantitative determination of artemether using the developed method obeyed the Beer-Lambert's law in the range of 20 - 120 µg/mls. The linear relationship between absorbance (A) and concentration (C in µg/ml) gives

the regression equation A = Cy + x, and the coefficients of correlation (r) are shown in table 4.2 and the calibration curve is presented in figure 4.3.

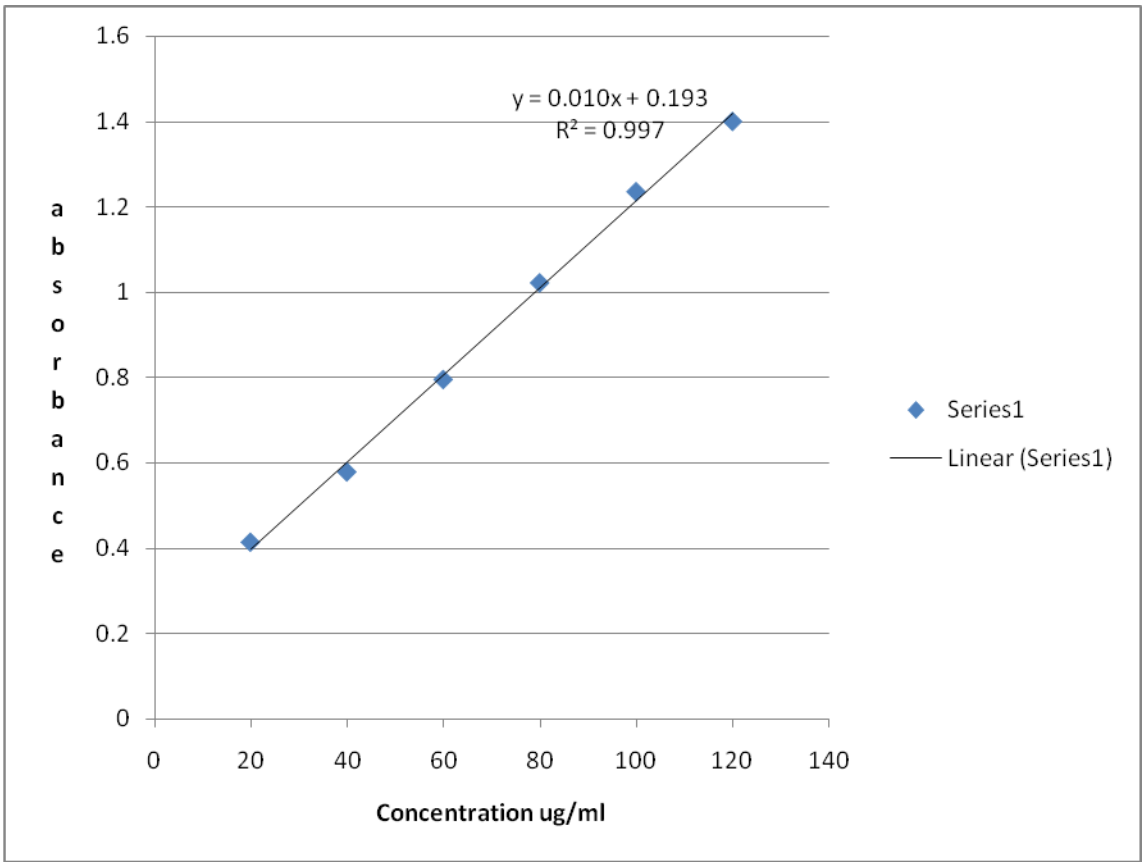


Figure 4.3: Calibration curve of developed method

Table 4.1; Calibration parameters of the developed method

Parameter	Values
Beers law range (µg/ml)	20 – 120
Regression equation	A = Cy + x
Slope (y)	0.01 0
Intercept (x)	0.193
Correlation coefficient (r)	0.9987

A = absorbance, C = concentration

4.2.3 Validation of method

The accuracy, precision, percentage recovery, detection limit and quantitation limit for the developed method are shown in table 4.2. The relatively low percentage coefficient of variation (% CV) is within the acceptable limit of < 15 % CV and shows the precision of the method. The percentage relative error (% Er) obtained is within the range (1 – 5 %) for moderately accurate procedure (Harvey, 2000). Satisfactory percentage recovery, detection and quantitation limits were achieved for artemether using the developed method.

Table 4.2 Validation parameters of the developed method

Parameter	Values
λmax (nm)	**260**
Precision (% CV)	**2**
Accuracy (% Er)	**2.67**
Percentage recovery (%)	**97**
Detection Limit (µg/ml)	**0.14**
Quantitation Limit (µg/ml)	**0.58**

Note :*CV = coefficient of variation, Er = relative error, λmax= wavelength of maximum absorbtion*

4.2.4 Assay

The assay results of the five different brands of artemether/lumefantrine suspensions were within the official limits of 98 – 102.0 % of the stated amount in IP, 2008. The assay results of the artemether/lumefantrine suspensions for both the developed method and the International Pharmacopoeial method are shown in table 4.3.

Table 4.3 Comparative assay of artemether/lumefantrine suspensions using the developed method and IP method

Brands	Percentage (%±SEM)Drug Content Assayed IP Method (n = 3)	Developed Method (n = 3)
A	**101.6±0.35**	**101±0.35**
B	**101.6±0.25**	**99±0.05**
E	**99.0±0.01**	**98.4±0.22**
C	**98±0.8**	**98±0.21**
D	**99±0.22**	**99.8±0.01**
S	**100±0**	**100.03±0.0**

No statistically significant difference between the means ($P < 0.05$)

4.3 Stability Studies

The result of the stability studies of the standard artemether suspension prepared and the reconstituted suspension of the different brand samples shows that the products were all stable over the fourteen days period of the study as none of them degraded beyond 10% of their content. The results are presented in table 4.4 below.

Table 4.4 Content of artemether remaining in suspension of the standard (S) and the samples (A, B, C, D and E) over 14 days

Content (%) of artemether remaining in suspension

Brands	0hr	day1	Day3	Day5	Day7	day10	day14
S	**101.1**	**101.1**	**101**	**100.8**	**100.6**	**100.6**	**100.5**
A	**102**	**102**	**101.8**	**101.8**	**101.5**	**101.3**	**101.5**
B	**101.8**	**101.8**	**101.8**	**101.8**	**101.3**	**101.2**	**101.3**
C	**100.8**	**100.6**	**100.3**	**99.8**	**99.8**	**99.3**	**98.5**
D	**101.2**	**101**	**100.8**	**100.6**	**100.2**	**100**	**100**
E	**100.8**	**100.8**	**100.6**	**100.6**	**100.5**	**100.6**	**100**

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Identification Tests

The colour test performed showed the presence of artemether in the reference standard and the five brands A, B, C, D and E of the powders for suspension analysed.

The result of the melting point of artemether RS was found to be 86-89oC which is within the official range of 86-90 ^{o}C (IP, 2008). This confirms that the sample collected is artemether and the sharp melting point obtained further ascertain the purity of the sample.

The FTIR results indicated peaks at 2952 cm^{-1} for (C-H_3) stretch, 1480 cm^{-1} for (O-H) bending, 1262 cm^{-1} for (O-C) stretch, 1099 cm^{-1} for (C-O) stretch and 808 cm^{-1} for (C-H) bending, for the reference sample and the five different brands of powders for suspension. This is in agreement with those obtained by Karuna *et al*.,(2014) and Gitua *et al*., (2014) and further established the presence of artemether in all the samples.

The FTIR analysis results revealed a spectral difference between the spectrum of artemether RS, and the decomposition product formed after hydrolysis with conc. HCl, suggesting that a reaction has taken place. In the spectra of the decomposition product, the peak at 2952 cm-^{1} for (C-H_3) disappeared, the peak also at 1480 cm-^{1} indicating (O-H) bending also α, β unsaturation disappeared and a strong peak at 1647 cm-^{1} which is indicative of (C=O) stretch in was observed. The peak at 1009 cm^{-1} was assigned to (C=C) stretch also showing unsaturation and the (C-H) stretch at 3425 cm^{-1}.

5.2 Method Development

The method developed makes use of the HCl decomposition product of the drug, an α, β unsaturated ketone. Concentrated HCl was used for the hydrolysis of the drug. A waiting period of 30 minutes was observed to ensure complete reaction. No heating was required and this shows how easy and simple the method developed is.

5.2.1 Calibration curve

The calibration curves for quantitative determination of Artemether in the developed method obeyed Beer-Lamberts law with coefficient of determination (r) of 0.9987. This clearly showed the direct proportional relationship and high correlation between the absorbance (A) and the respective concentrations (C) used for these determinations which gives the regression equations for the developed method (Table 4.1). Vandercruyssen *et al.,* (2013) reported an r value of 0.997 for a LC-UV MS for determination of artemether. Shah *et al*., (2013) reported an r Value of 0.998 for a RP-HPLC method for artemether. Pawar *et al.,* (2011) reported an r value of 0.998 for a UV method of determination of artemether.

5.2.2 Validation parameters

The accuracy of the developed method was computed to be 2.67% and is within the range (1 – 5 %) for moderately accurate procedure (Harvey, 2000). Precision of the method was 2% expressed as percentage of coefficient of variation (% CV). The relatively low % CV which was within the acceptable limit of < 15 % CV and showed the method has high precision. Mohamed *et al.,* (1999) reported % CV of 3-10.4% for a GC MS selected ion monitoring method; Shah *et al.,* (2013) also reported a precision of 2% for a RP-HPLC

method for artemether. This showed that the precision of the developed spectrophotometric method is satisfactory. The percentage recovery determined for the developed method was 97 %. Mohamed *et al*., (1999) reported 94.9% recovery for artemether, suggesting the percentage recovery of the developed method was better. Detection limit (DL) and quantitation limit (QL) for the developed method was 0.14 and 0.58 µg/ml respectively. Pawar *et al.,* (2011) reported a DL and QL of 2.3 and 4.08 respectively for a UV method of determination of artemether. Vandercruyssen *et al.,* (2013) reported a QL of 5.5 for artemether.

5.2.3 Assay results and statistical analysis

The assay results for the five different Brands (A, B, C, D, and E) of Artemether/ lumefantrine suspensions sampled were (98-101.6%) which is within the official limits of 98 – 102 % of the stated amount in IP, 2008. The values obtained are in line with those obtained by Shah *et al.,* (2013) for artemether which is 99.9% of the label claim. Also, the assay carried out by Gitua *et al.*, (2014) of artemether in artemether/ lumefantrine tablets by IPCA lab and also coartem tablets by Novartis revealed that the products contained the amount of artemether as on their package label. The study is also in line with the assay results of artemether capsules (98.05-99%) carried out by Pawar *et al.*, (2011). Statistical analysis revealed no statistically significant difference ($P < 0.05$) between the means of content of artemether assayed by the developed method and the International Pharmacopoeial method. This shows that the method can be used interchangeably with the IP method in routine analysis of the drug.

The development of simple and reliable method is essential to assure the identification and quantitative determination of antimalarial drugs, since the problem of counterfeit or substandard antimalarial is well established all over the world. The use of these poor quality drugs might contribute to the development of plasmodium resistance in endemic areas due to the exposure to antiinfective sub therapeutic doses (Arun and Smith, 2011). The quality control of the antimalarial pharmaceutical preparations marketed nowadays may help to assure the treatment efficacy and avoid the development of resistance to antimalarial drugs.

5.3 Stability Studies

Bottled table water was used in the reconstitution of all the different brands of the powders for suspension and in the preparation of the standard artemether suspension. Artemether was considered to be stable if it retained ≥ 90% of its initial drug (John *et al.*, 2014). Physical observations of brands A, B, C, D, E and the standard artemether suspension prepared did not reveal any visible change in all the six samples throughout the storage period of 14 days. From the results of the stability studies conducted for the five different brands and the standard artemether suspension prepared showed that the suspensions are stable under ambient conditions for upto 14 days after reconstitution as none of the suspensions degraded upto 10%. This suggested that the product is safe for use for upto 14 days of reconstitution and the maximum benefit can be achieved during that period. Patients can also be advised to use bottled water in the reconstitution of the suspension. Also, stability of the different brands indicates that co-formulation of artemether with lumefantrine has no effect on the stability of the artemether content.

This study is in line with the earlier stability studies of the artemether/ lumefantrine suspension carried out by Dafra Pharmaceuticals limited. The suspension at day 0 after reconstitution contained 100% artemether, at 14 days it contained 96% and after 30 days of reconstitution contained 92% which showed that the suspensions was stable 30 days after reconstitution with water. Stability studies carried out by Gitua *et al.,* (2014) of artemether/lumefantrine tablets showed that the tablets were stable for upto a year stored under ambient conditions. The study by Karuna *et al.,* (2014) on some fdc tablets of artemether/lumefantrine tablets also indicated that the tablets were stable in the blister packs as no significant changes were observed in the drug content and physicochemical parameters. The content of artemether ranged from 97.22-99.76 during the 3 months study period.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the results obtained, it can be concluded that a new spectrophotometric method for artemether determination in pure form and dosage forms was developed and validated. The method was successfully applied in the assay of fixed dose combination powder for suspension, with no statistically significant difference ($P < 0.05$) between the means of the assayed results of the developed method and that of the International Pharmacopoeial method, this shows that the developed method can be used interchangeably with IP method in analysis.

The result of the stability studies showed that the standard artemether suspension and all the proprietory brands were stable 14 days after reconstitution as none of the suspension degraded upto 10% of the labeled concentration. This study also shows that co-formulation of artemether with lumefantrine has no effect on the stability of the artemether even after reconstitution for 14 days.

6.2 Recommendations

The present study developed a new spectrophotometric method for artemether determination in pure and dosage forms, and recommends the utilization/adoption of the method in routine quality control and analysis of artemether and lumefantrine combination suspension formulation.

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