KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

ASSESSING THE QUALITY OF SOME ARTESUNATE-CONTAINING TABLETS IN KUMASI MARKET

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT

FOR THE DEGREE OF

MASTER OF PHILOSOPHY IN

PHARMACEUTICAL ANALYSIS AND QUALITY CONTROL

BY

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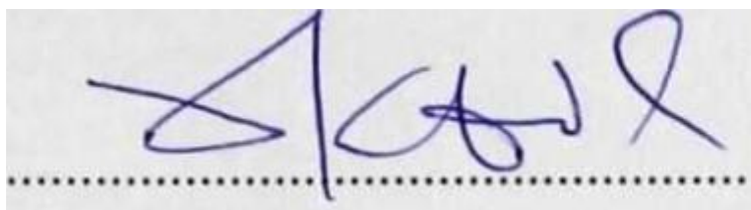
DECLARATION

It is hereby declared that this thesis is the outcome of research work undertaken by the author. Any assistance obtained has been duly acknowledged. The work is neither in part nor in whole been presented for another degree elsewhere.

HEAD OF DEPARTMENT



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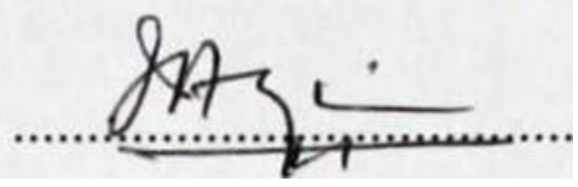

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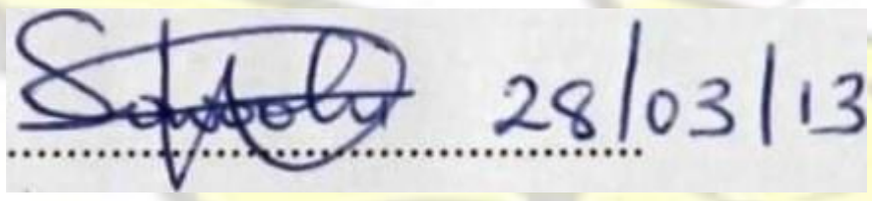

SOWOOLU-COATES TITILAYO THERESA

DEDICATION

I dedicate this research work to my parents, siblings, my loving husband and our adorable son Joel.

ACKNOWLEDGEMENT

I give all glory, honour, power, and majesty to God Almighty for being my pillar of strength all through this research work.

My sincere gratitude goes to my supervisor Prof J.S.K Ayim, for his support towards the success of this research work, inspiring and motivating me at all times to put in my best.

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To all those who have contributed in one way or the other to the success of this research work I say thank you God bless you.

ABSTRACT

Artesunate (ART) is a semi-synthetic artemisinin derivative synthesised by esterifying Dihydroartemisinin (DHA) with succinic anhydride under alkaline conditions. It is used in combination with non-artemisinin based drug in the treatment of malaria. ART is hydrolysed slowly to DHA when exposed to moisture, hence affecting the quality of the drug.

As a step towards the assessment of the quality of ART-containing tablets in the Kumasi market, a simple, rapid and accurate isocratic reverse-phase HPLC method with UV detection was developed and validated to detect and quantify ART and DHA simultaneously in five brands of ART tablets obtained from the Kumasi market. The analysis was performed using methanol: 1% Trifluoroacetic acid (TFA) v/v at a flow rate of 1.5ml/min on an ODS C-18 Phenomenex Bondclone 10,300 x 3.90mm micron column. The mean retention time for Artesunate was 2.54±0.15min (n=3), that of Dihydroartemisinin was 4.34±0.13 min (n=3). Calibration curves for Artesunate and Dihydroartemisinin were plotted using concentrations 0.1-0.5%w/v, and they were found to be linear according to the International Conference on Harmonisation (ICH) with R^2 value of 0.993 and 0.994 respectively, and their limit of detection (LOD) and limit of quantification (LOQ) were 0.00048823%w/v, 0.0014795%w/v and 0.00026080%w/v respectively. The HPLC percentage content of ART in the five tablet brands FA-FE were found to be 95.51±0.69, 100.50± 2.13, 85.50± 1.09, 85.85±2.39, and 130.75±4.53 respectively. The HPLC percentage content of DHA in the five tablet brands FAFE were found to be 18.33± 0.39, 6.11± 0.58, 18.05± 0.98, 29.44±0.78, and 33.88±0.38 respectively.

Four of the five brands of tablets assayed were found to have lower ART content than that recommended by the International Pharmacopoeia (IP); all the five ART brands contained the breakdown product of DHA.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Infectious diseases generally accounts for about 14-17 million deaths of people worldwide each year, and nearly all these people live in developing countries (WHO, 2007).

Malaria is widespread in tropical and subtropical regions of the world, having an incidence of about 515 million cases yearly, killing It03 million people, majority of who are pregnant women and children in sub-Saharan Africa (Snow et al, 2005). In Ghana, Malaria accounts for over 44% of reported outpatient visits at Hospitals and an estimated 22% mortality of children under 5 years old (WHO, 2005).

Malaria control in Ghana as in many developing countries is being hampered by the poor quality of Antimalarials available, which in turn contributes to the growing resistance of the major parasite plasmodium falciparum to cheap and affordable antimalarial therapies. Effective Antimalarials play a major role in the control of malaria, however, it has been severally hampered due to the persistent increase in the prevalence of drug-resistant malaria parasite (White N.J, 2004).

Several significant incidents of substandard antimalarial medicines within the Ghana pharmaceutical distribution chain was identified by studies conducted by the World Health Organisation-(WHO, 2003). Evident by a high percentage of content failure and poor dissolution test results. Low bioavailability results in under -dosage which promotes the development of resistance to the drug inevitably.

Due to the danger of resistance development, definite measures should be taken to strengthen the quality control of antimalarial medicines in the country.

1.1.1 MALARIA

Malaria is a disease caused by the infection of red blood cells with a parasitic protozoan of the genus plasmodium. The parasites are inoculated into the human host by a feeding female anopheles mosquito, though transmission may occur occasionally by organ transplant, blood transfusion, needle sharing or congenitally from mother to foetus.

1.1.2 SPECIES OF PLASMODIUM

It has been shown that over 100 species of plasmodium cause malaria in a wide variety of vertebrate host, however, four plasmodium species are known to affect humans, and they are:

l. Plasmodium viva : This parasite causes vivax or benign tertian malaria.
2. Plasmodium ovale: This parasite causes ovale malaria.
3. Plasmodium malariae: This parasite causes quartan malaria.
4. Plasmodiumfalciparum: This parasite causes severe malaria.

1.1.3 LIFE CYCLE OF PLASMODIUM

The plasmodium lifecycle is divided between two hosts, and they are the vertebrates and the mosquito which serves as a vector. In humans, the infection is initiated by the inoculation of sporozoites into the blood stream by the bite of infected female mosquito. The sporozoites migrate to the liver within two hours and penetrate the hepatocytes, and then they undergo nuclear division after one to two weeks producing thousands of merozoites that are released by the rupture of the cell. This phase of the life cycle of plasmodium is called the exoerythrocytic or liver schizogonic phase. The merozoites invade the erythrocytes and progress through a series of developmental forms into mature erythrocytic schizonts. Each

merozoite matures into schizont which then undergoes asexual multiplication forming twenty-four merozoites. The merozoites invade other erythrocytes when the red blood cell ruptures, this part of the lifecycle is called the erythrocytic phase and it takes about 24 to 72 hours. The rupture of the infected red blood cells result in fever. Within the erythrocytes, some merozoites mature into male and female gametocytes, which occurs once the infected blood is ingested by the mosquito, within the vector, the gamete undergoes fertilization and forms a zygote, the zygote then elongates in about 12 to 48 hours to form an ookinete, ookinetes invade the stomach of the mosquito and develops into oocysts. In about a week after the formation of the oocyst, it enlarges forming over 10,000 sporozoites. The rupture of the oocyst results in the release of sporozoites which migrates to the salivary gland of the mosquito and are inoculated into humans the next time the mosquito feeds.

1.1.4 OCCURENCE OF MALARIA

Malaria transmission occurs in large areas of south and central America, the island of Hispaniola (The Dominican Republic and Haiti), Africa, Asia (including the Indian subcontinent, southeast Asia, and the middle east), eastern Europe, and the south pacific, however 90% of global occurrence of clinical malaria as well as 90% of global malaria mortality occur in sub-Saharan Africa (WHO, 2000).

In Ghana, malaria accounts for an average of 13.2% of all mortality cases and 22% of deaths in children below 5 years.13.8% of all the pregnant women that report in the hospital suffer from malaria, and 9.4% of them actually die from the disease (Asante, Asenso-Okyere et al, 2003).

1.1.5 CLINICAL MANISFESTATION OF MALARIA

Clinical symptoms of malaria are non-specific and varied but they commonly include fever, headache, fatigue, and sweating. A common complication due to haemolysis is anaemia and

serious complication in falciparum malaria such as acute renal failure, pulmonary oedema and cerebral dysfunction.

1.1.6 PREVENTION AND TREATMENT OF MALARIA

Malaria can be prevented by controlling the vector i.e. the female anopheles mosquito using three practical ways and they are the elimination of the vector, drug therapy and vaccination. The primary option over the years in the fight against malaria has been antimalarial treatment, many of which has been developed and used in the treatment of the disease over the years.

Partly, due to the development of multi-drug resistant plasmodium falciparum strains, the burden of malaria is still heavy (WHO, 1993).

1.1.7 ANTIMALARIALS

Antimalarials are drugs that are designed for the prevention and cure of malaria. They are used in the prevention of persons with no immunity visiting a malaria-endemic region (prophylaxis), they are also used for the routine intermittent treatment of certain groups in endemic regions (intermittent preventive therapy), and in the treatment of malaria in individuals with suspected or confirmed infection (WHO, 2010).

1.1.8 CLASSES OF ANTIMALARIALS

The cinchöffóalkaloids an-aúïeKisinins are two classes of Antimalarials. They are both __Äant origin, the cinchona alkaloids e.g quinine is extracted from the bark of the cinchona tree, while artemisinins also known as quinghaosu are extracted from the herb Artemisia annua (sweet worm wood).Artemisinins are the most important class of antimalarial presently in use , and this is particularly because of their effectiveness against parasites that are resistant to almost all other classes of Antimalarials.

1.1.9 COMBINATION THERAPY

Malaria is being treated presently using the concept of combination therapy. Combination therapy with antimalarial drugs is the simultaneous use of two or more blood schizontocidal drugs with different biological targets in the parasite and independent mode of action.

The use of combination therapy has several advantages among which are reduced risks of developing resistance ,reduced risk of treatment failure, enhanced convenience and reduced side-effects. The emergence and spread of chloroquine resistance has hampered the efforts on malaria control, which was first recorded in 1979 in East Africa, but has now been reported in almost all malaria endemic countries of Africa (WHO, 2000).

1.1.10 CHALLENGES WITH THE USE OF COMBINATION THERAPY

The challenges with the use of combination therapy particularly in Africa are as follows:

- The choice of drug combinations best suited for the different epidemiological situations.
- The cost of combination therapy
- The timing ofthe introduction of combination therapy.

1.1.11 TYPES OF COMBINATION THERAPY

There are two types of cotíiúçtçerapy, namely

1. Non-artemisinin based combinations
2. Artemisinin-based combination (ACT) therapy. Examples of Non-artemisinin based combination therapy are:

a) Sulfadoxine- pyrimethamine (SP) (Fansidar).
b) SP plus chloroquine.

c) SP plus amodiaquine.

d) SP plus mefloquine (Fansimef).

e) Quinine plus tetracycline or doxycycline.

Sulfadoxine-pyrimethamine (SP) was, until recently, seen as the replacement to chloroquine; however there has been the development of resistance even with its current use thereby reducing its therapeutic activity.

Artemisinin-based combination therapies should be used in preference to amodiaquine plus Sulfadoxine-pyrimethamine for the treatment of uncomplicated P.falciparum malaria (WHO, 2010). It has shown to be more effective and contains drug resistance in South-East Asia.

Examples of artemisinin-based combination drugs are:

a. Artesunate plus chloroquine.

b. Artesunate plus amodiaquine.

c. Artesunate plus Sulfadoxine-pyrimethamine.

d. Artesunate plus mefloquine.

e. Artemether plus lumefantrine.

f. Dihydroartemisinin plus piperaquine

1.1.12 ADVANTAGES OF ARTEMISININ-BASED COMBINATION THERAPY OVER NON-ARTEMISININ BASED THERAPY

- It gives a large reduction of the biomass of the parasite.
- It is effective against multidrug-resistant P.falciparum.
- It gives a rapid resolution ofclinical symptoms.

- It results in the reduction of gametocyte carriage, which may reduce the transmiof resistant alleles (in areas with low or moderate malaria transmission).

- There have been few adverse clinical effects.

1.2 PROBLEM STATEMENT

The purpose of all quality assurance programmes is to design and implement systems and procedures that provide a high probability that each package of a pharmaceutical product will have the same characteristics and properties, within acceptable limits, to ensure both clinical safety and efficacy of the formulation.

In recent times, it's been observed that stability poses serious problem for many manufactured products, particularly those distributed in countries with adverse climatic conditions and those entering international commerce. Improper storage and distribution of pharmaceutical products results in physical deterioration and chemical decomposition, which result in reduced bioactivity, formation of harmful degradation products or unstable and unusable products particularly under tropical conditions of high temperature and humidity.

Artesunate is a hemisuccinate derivative of the active metabolite dihydroartemisinin synthesised by the esterification of dihydroartemisinin with succinic anhydride under alkaline conditions (Presser, 2009). Artemisinins generally have the problem of chemical instability due to the presence of the endo-peroxide bridge in its structure; Artesunate however has a greater problem of chemical and molecular stability due to the presence of the hemi-ester linkage which makes it prone to hydrolysis slowly in the presence of moisture.

Dry or wet granulation is involved in the industrial conversion of Artesunate powder to tablets, wet granulation involves mixing the powder with water, drying it at 50-55^{0}C and compressing it with excipients such as disintegrants. lubricants, binders, colorants and preservatives, water from residual moisture during wet granulation as well as moisture

absorbed by excipients such as lactose could contribute to hydrolysis of Artesunate (WeinQin, 2006).

The lack of chromophore responsible for fluorescent and ultraviolet detection in artemisinins generally including Artesunate has made analytical detection difficult.

1.3 GENERAL OBJECTIVE

The main aim of this research work is to assess the quality of some Artesunate-containing tablets in the Kumasi market, in order to achieve this aim, the objectives are stated below:

- To develop and validate an HPLC method to simultaneously quantify Dihydroartemisin and Artesunate.
- To use the method developed in determining quantitatively the extent of breakdown of Artesunate in some combination tablets in the Kumasi market.

1.4 JUSTIFICATION OF WORK

There has been an increasing report of product recall of Artesunate containing tablets by the drug regulatory agencies in recent times, this is due to the presence of the degradation products dihydroartemisinin and succinic acid above its specified limit in the tablet, however dihydroartemisinin remains the major breakdown product of the hydrolysis of Artesunate. For a drug to be eúsidered stable it must be able to retain its physical, chemical, microbiological

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and biopharmaceutical properties within the specified limits throughout its shelf-life.

Artesunate is chemically and molecularly unstable due to the presence of the endoperoxide linkage as well as the hemi-ester linkage in its structure which is responsible for its susceptibility to hydrolysis in the presence of moisture, Artesunate-amodiaquine being the

World Health Organisation recommended first-line treatment of malaria in sub-Saharan Africa makes it necessary that reliable and cost effective analytical methods that can detect the presence of degradation products, ensuring the quality of Artesunate tablets in the market are developed.

1.5 SCOPE OF STUDY

Five brands of Artesunate-amodiaquine tablets available in Kumasi market were considered for this research work. Artesunate-amodiaquine combination tablets were used for this research because it is the only Artesunate combination drug approved by the World Health Organisation as the first- line treatment of malaria in Africa.

1.6 LIMITATION OF WORK

The main limitation to this research work was the low wavelength of detecting Artesunate and its breakdown product Dihydroartemisinin which is due to the lack of chromophore in their structures.

1.7 ORGANISATION OF STUDY

Pure Artesunate and Dihydroartemisinin powders will be obtained and identification tests will be carried out on them based on the international pharmacopoeia (IP) 2006 edition, then five brands of Artesunate combination tablets will be obtained from pharmacies in Kumasi market, pharmacopoeia tests such as uniformity of weight and disintegration tests will be carried the tablets_jbeR-assays determining the percentage purity and percentage content will be carried out on the pure powders and the tablets based on the international pharmacopeia respectively.

Thin layer chromatography will be carried out on the tablets as well as the pure powders to detect the presence or absence of breakdown product in the tablets as well as to detect the presence of Artesunate in the tablets.

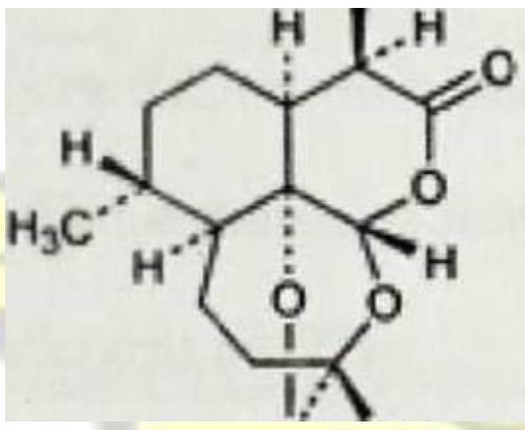
An HPLC method that can quantify Artesunate and Amodiaquine simultaneously will be developed and calibration curve for pure Artesunate and Dihydroartemisinin will be obtained to determine the linearity of the method, then the tablets obtained will be assayed for their percentage content using the HPLC method developed, the method will then be validated.

CHAPTER TWO -LITERATURE REVIEW

2.0 ARTEMISININ

Artemisinin also known as quinghaosu is a sesquiterpene trioxane lactone, extracted from the herb of sweet wormwood plant (klayman et.al 1984), it has a chemically rare peroxide bridge linkage, which is thought to be responsible for majority of its antimalarial activity although the target within the parasite remains controversial. The lactone can be reduced easily with sodium borohydride which results in the formation of Dihydroartemisinin, having even more antimalarial activity than artemisinin itself. Artemisinin is present at 0.01-0.8% dry weight ofthe leaves and flowers of the herb of sweet or annual wormwood.

CH3



0' CHs Fig 1 Structure of Artemisinin

Artemisinin appears as a white or colourless crystalline powder, practically insoluble in water, very soluble in dichloromethane, glacial acetic acid, methanol and ethanol; it is also freely soluble in ethyl acetate and acetone (IP, 2006). It has a molecular formula of C15H2205, molecular weight of 282.322g/mol, its systematic name is (3R, 5aS, 6R, 8As, 9r, 12s, 12aR)-

Octahydro-3, 60-trimethyl-3, 12-epoxy-12H-pyrano[4, 3-j]-l, 2-benzodioxepin-10(3H)-one.

Not less than seven carbon atoms of artemisinin are asymmetric; this feature makes it stereochemically unique. The presence of a peroxide bridge on top of oxygen containing seven membered ring is chemically quite remarkable, however, these heterocyclic ring systems are known to be chemically rather unstable (O'Neill, et.al, 2010). It has a three cyclic

ring system with a peroxide bridge built over a seven membered heterocyclic ring, with the peroxide in a configuration called a tri-oxane, also it carries two other oxygen atoms which gives it a lactone function.

2.1 HISTORICAL DEVELOPMENT OF ARTEMISININ AND ITS DERIVATIVES

The traditional Chinese pharmacopoeia has documented Artemisinin (quinghaosu) extracted from the herb quinghao for the treatment of fevers over two thousand years ago, to have a longer history of use. It is derived from the plant Artemisia annua, with the first documentation as a successful therapeutic agent in the treatment of malaria in 340 AD by Ge Hong in his book Zhou Hou Bei Ji Fang (A Handbook ofPrescriptions for Emergencies).

In 1967, the government of the People's Republic of China began a programme to identify Antimalarial principles in plants used in Chinese herbal medicines (klayman, 1985) Ge Hong extracted the artemesinin using a simple macerate. The active principle quinghaosu, now called artemisinins from quinghao was identified in 1972, but was first isolated by Chinese scientists in 1971.

The structure of Artemisinin was known in 1977, modifications were later introduced to improve its solubility in both oil and water producing active derivatives of Artemisinins which are today very potent Antimalarials.

2.2 MODE OF ACTION

It has a very raTid action and the vast majority of acute patients treated with artemisinin show

significant improvement within 1—3 days of receiving treatment. It has demonstrated the _fistest clearance of all the anti-malarias currently in use acting primarily on the trophozite phase, thus preventing progression of the disease.

Semi-synthetic artemisinin derivatives (e.g. Artesunate, Artemether) are easier to use than the parent compound and are converted rapidly once in the body to the active compound Dihydroartemisinin. Few side effects are associated with artemesinin use; however, headaches, nausea, vomiting, abnormal bleeding, dark urine, itching and some drug fever have been reported by a small number of patients.

2.3 USES OF ARTEMISININ

Artemisinin is increasingly being used in the treatment of malaria caused by plasmodium vivar; however, the use of the drug as a monotheraphy is explicitly discouraged by the World Health Organisation due to signs that resistance to the drug are being developed by malaria parasites.

2.4 DERIVATIVES OF ARTEMISININS

In order to improve oral bioavailability as well as oil or water solubility of artemisinin, several artemisinin derivatives was synthesised (Gary, 1999) Artemisinin does not dissolve in oil or water due to its high crystalline nature. (Woodrow, Haynes et al, 2005).

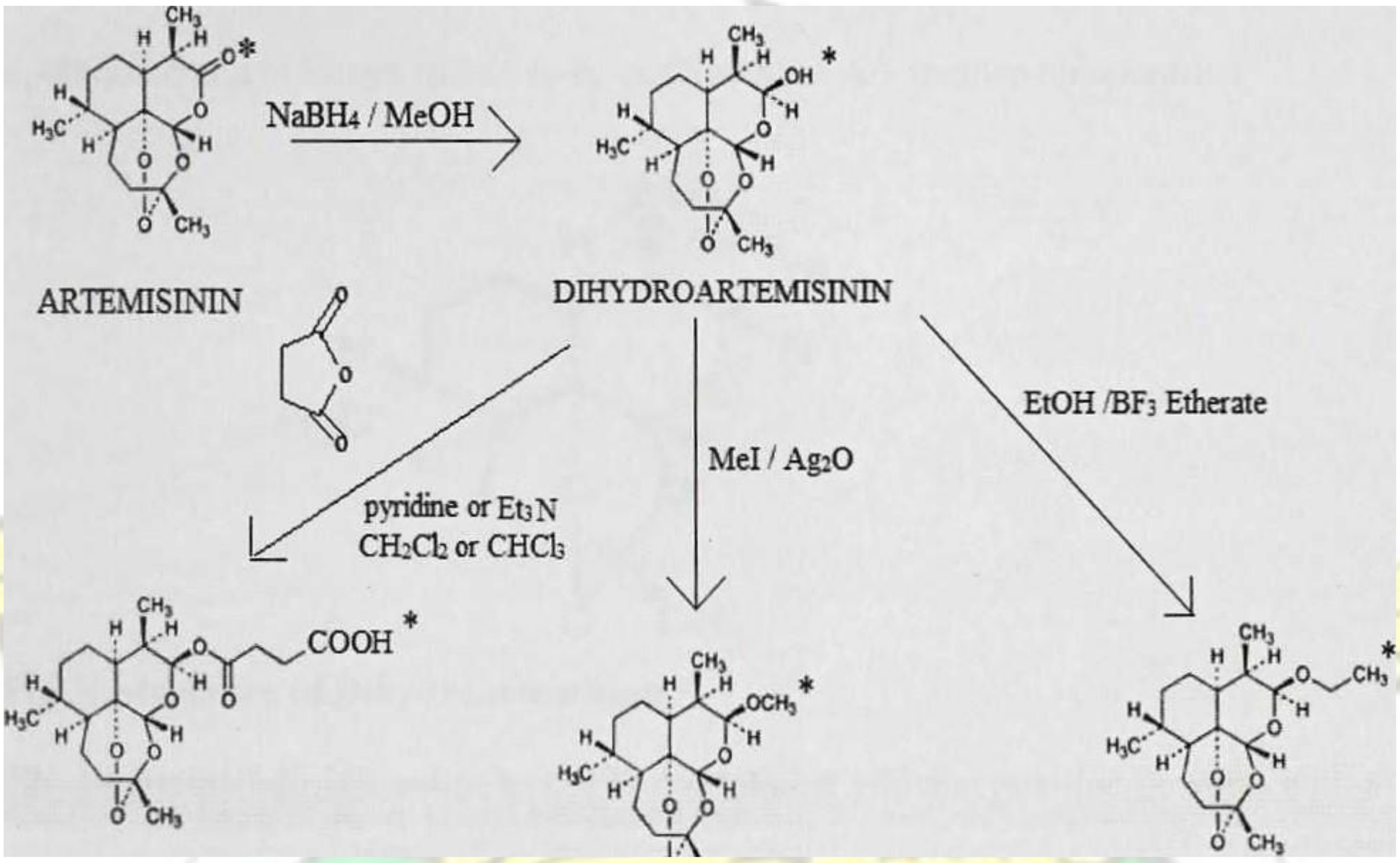
The derivatives of artemisinin are as follows:

- Artesunate: it is a semi-synthetic derivative of artemisinin, soluble in water which has encouraged its use intramuscularly, orally, intravenously and even intrarectally (krishina et al.,2001; Hien et al.,1991; Hien, 1994).

- Arteether: it is a so known as Artemotil, it is an ethyl ether derivative of _____ dihydroartemisinin, used specifically in the treatment of chloroquine-resistant cerebral malaria and plasmodiumfalciparum malaria, and it is soluble in oil.

- Dihydroartemisinin: it is-the active metabolite of all artemisinin compounds, and it is also available as a drug in itself.
- Artemether: it is also known as dihydroartemisinin methyl ether, it is the methyl ether derivative of artemisinin, and it is soluble in oil.

2.5 SYNTHESIS OF ARTEMISININ DERIVATIVES



* represents position ofchemical mo&ation

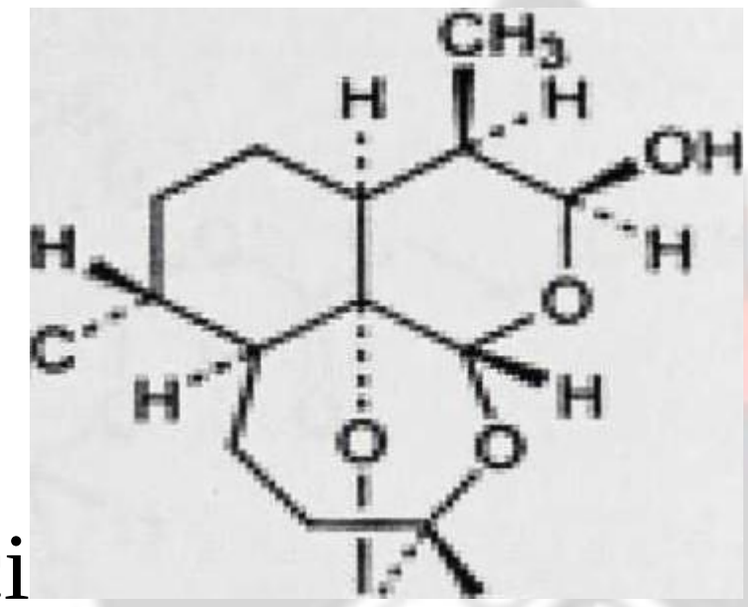
Fig 2: synthesis of Artemisinin Derivatives

Dihydroartemisinin is synthesised as a mixture of epimers by reducing artemisinin with sodium borohydride (Olaniyi, A.A, 2005). Artesunate is synthesised by esterifying dihydroartemisinin with sueciãäñhýðride under alkaline conditions (Presser A, Buzzi S., 2009). Artemether is produced by treating dihydroartemisinin with methanol and an acid catalyst (Hayes and Vonwiller, 1994). Arteether is synthesised from dihydroartemisinin using boron trifluoride.

2.6 DERIVATIVES UNDERSTUDY

2.6.1 DIHYDROARTEMISININ

Dihydroartemisinin also called dihyroquinghaosu or artenimol or artenimolum is a drug used in the treatment of malaria, it is the active metabolite of all artemisinin derivatives such as Artesunate, Artemether and Arteether. It is sold commercially in combination with piperaquine and has been shown to be equivalent to Artemether-lumefantrine.

Haci 

O caH3

Fig 3: Structure of Dihydroartemisinin

Dihydroartemisinin is a colourless, white or almost white crystalline powder, with a relative molecular mass of 284.4g/mol, and the molecular formula of C15H2405, it is practically insoluble in water, slightly soluble in Acetonitrile, ethanol and dichloromethane, and it has a melting range of 158-160 0 C, Its chemical name is(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-I ,2-benzodioxepin-10-01.

DHA is the most effective Artemisinin compound and the least stable. It has a strong blood schizonticidayaction and -redTëëÇ@õmetocyte transmission. It is used for therapeutic treatment of cases of resistant and uncomplicated P. falciparum.

Dihydroartemisinin is synthesised from artemisinin by its reduction with sodium borohydride in ethanol or methanol at about OOC to 50C, it can also be synthesised by the reduction of artemisinin with DIBAL-H in dichloromethane at -780C, however the process has a

disadvantage of giving a smaller yield and the high cost of the means of reduction and the high price of the solvent (Buzzi, Presser et al., 2007).

2.6.2 ARTESUNATE

It is a hemisuccinate derivative of the active metabolite dihydroartemisin used in the treatment of malaria.

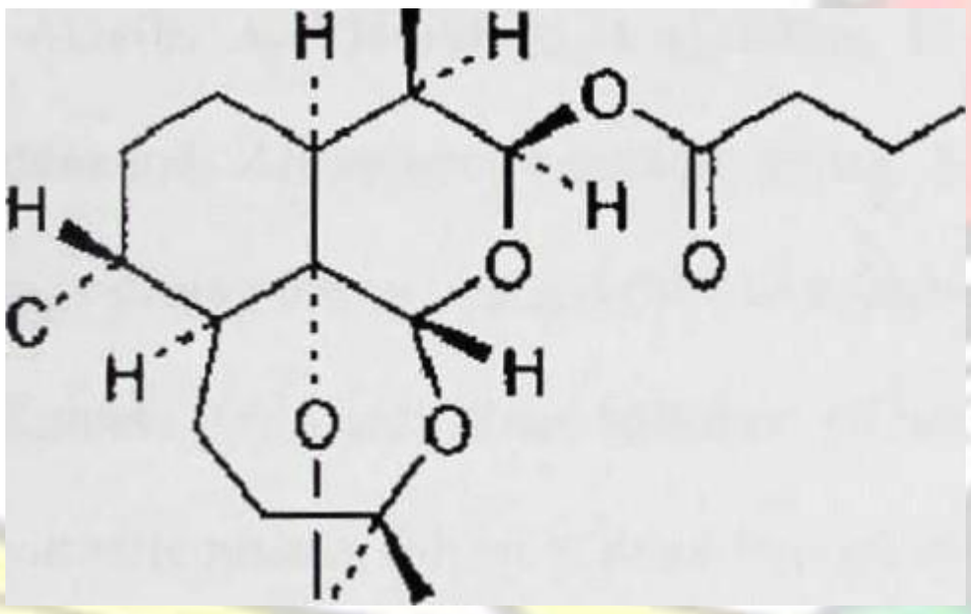


Fig 4: structure of Artesunate

It exist as a fine white crystalline powder, its relative atomic mass is 384g/mol, it has a molecular formula of C19H2808, its chemical name is(3R,5aS,6R,8aS,9R,10S,12R,12aR)Decahydro-3,6, 9-trimethyl-3, 2-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin- 10-01, hydrogen succinate. It is very slightly soluble in water, very soluble in dichloromethane, freely soluble in ethanol and acetone with a melting point range of 132-135^{0}C (IP, 2006).

Artesunate should be stored in a well-closed container, a cool place protected from light.

•x-ffé̌šûnate is synthesised from dihydroartemisinin (DHA) by reacting it with succinic acid anhydride in basic medium sodium bicarbonate in chloroform, pyridine as solvent or base and catalyst DMAP (N,N-dimethylaminopyridine) and triethylamine with yields of up to

100% have also been used in the synthesis of Artesunate. Alpha-Artesunate can be exclusively synthesised through a large scale process which involves treating DHA in dichloromethane with a catalytic amount of DMAP, a mixture of pyridine, and succinic anhydride, stirring the mixture for six to nine hours to obtain Artesunate in quantitative yield.

In order to prevent the development of resistance, Artesunate must always be given with another antimalarial such as Mefloquine (Nosten F, van Vugt M, Price R, et al, 2000) or Amodiaquine (Adjuik M, Agnamey P, Babiker A, et al, 2002). The combination of Artesunate and amodiaquine has been found to be equivalent to co-Artemether (Meremikwu M, Alaribe A, Ejemot R, et al, 2006). For severe malaria during pregnancy, the WHO recommends Artesunate or quinine during the first trimester and Artesunate as the first-line therapy during the second and third trimesters (WHO, 2007).

Artesunate has a very short half-time of less than 10 minutes; it is metabolised in vivo to dihydroartemisinin, which is more important in exerting antimalarial effects because it has a longer elimination half-time of approximately one hour. The antimalarial Artesunate, dihydroartemisinin and ethers have the problems of chemical and metabolic instability and neurotoxicity. Due to the high instability of Artesunate as a result of its hemi ester linkage which makes it susceptible to hydrolysis, the limits of its impurities are higher than those of other artemisinin derivatives UP, 2006).

2.7 REVIEW OF ANALYTICAL METHODS

2.7.1 IDENTIFICATION TEST

Identification tests are very important in verifying the quality and identity of drug or products; it is usually achieved by a combining simple chemical tests and measurement of appropriate physical constants. Identification tests consist of spectroscopy which includes infrared absorption spectroscopy which forms the basis for most identification methods, due to its high structural specificity. chromatography such as thin layer chromatography and Gas chromatography, physical constants such as refractive index, melting point, boiling point, and solubility characteristics which are distinctive properties that are useful for maintenance of standards of purity as well as in identifying the product(Beckett and Stenlake, 1997).

2.7.1.1MELTING POINT OR RANGE

Melting point is a physical constant that indicates the identity and purity of a material; it is defined as the temperature at which the solid, liquid and gaseous phases of the substance are in equilibrium in an evacuated closed system. It is one of the oldest identification methods and it can also be said to be the range between the corrected temperatures at which the substance begins to collapse or form droplets on the wall of a capillary tube and the corrected temperature at which it completely melts (IP, 2006).

Factors such as sample size, capillary dimensions, temperature of insertion and rate of temperature ri§e makes it diffãõGsure reproducibility, which serves as a disadvantage of the data obtained from determining the melting point hence, Melting range is therefore a more practical criterion of identity and purity. (Beckett and Stenlake, 1997). A more or less pronounced lowering of the melting point of a substance is due to the presence of impurities in the substance UP, 2006).

2.7.1.2 THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is a universal analytical method involving the movement of a mobile phase across a uniform, thin layer of finely divided stationery phase by capillary action. Examples of stationery phase are silica gel, alumina, cellulose, e.t.c, binding agents are added to ensure the adherence of the stationery phase to the chromatographic plate .TLC is a very commonly used technique for identifying compounds, determining their purity and following the progress of a reaction. TLC can be both preparative and analytical and the separation is based on adsorption, partition, ion-exchange or on combinations of these mechanisms (BP, 2007).

TLC involves the preparation of the plate, applying the sample, running the plate and locating the spots. The sample is dissolved in a suitable solvent and the solution is spotted on the plate at about 1.5cm from the end of the plate with the aid of a melting point tube, micropipette or microsyringe, the solvent system is placed in the development chamber to a depth of about

0.5cm-1cm., the solvent is then allowed to rise about 10-15cm up the plate, the plate is then removed and air-dried, and the spot located by visual inspection and examination in ultraviolet light within the range of 254nm and 366nm (Olaniyi, 2000).

The basic chromatographic measurement of a substance in TLC is referred to as the Rf value

Rf= Distance the substance travels from origin

Distance the solvent travels from igin Rf

value varies from 0 to I(Olaniyi, 2000).

2.7.1.2.1 THIN LAYER CHROMATOGRAPHY FOR ARTESUNATE

According to the International Pharmacopoeia (IP), silica gel was used as the coating substance and a mixture of 5 volumes of ethyl acetate and 95 volumes of toluene as the mobile phase. 2k11 of the following solutions in toluene containing (a) 0.10mg of Artesunate per ml, and (b) 0.10mg of Artesunate per ml were applied separately to the chromatographic plate. After removing the

plate from the chromatographic tank, it is allowed to air-dry; sprayed with methanol, and heated to 120^0C for five minutes. On examining the chromatogram in ultraviolet light (254nm), the principal spot obtained with solution (a) should correspond in position, appearance and intensity with that obtained with solution (b)

2.7.1.3 SOLUBILITY

Solubilities of pharmacopoeial substances are provided mainly for information, and are being expressed in descriptive terms increasingly.

2.7.1.3.1 SOLUBILITY OF ARTESUNATE

The solubility of Artesunate in water has enabled its administration orally, intramuscularly, intravenously and even intrarectally (Krishina et.al, 2001; Hien et al, 1991; Hien, 1994). Injectable Artesunate results in a more rapid systemic availability of Artesunate This pharmacokinetic advantage may provide a clinical advantage in the treatments of severe and complicated malaria, however when the Artesunate breaks down to Dihydroartemisinin which is insoluble in water poses a problem to its use and can be dangerous when injected.

2.7.2ASSAY METHODS

2.7.2.1 ACID BASE TITRATION

Titrimetry is an absolute method of analysis in which the purity of a substance is determined without a reference standard. Titrimetry is generally simple and inexpensive, it involves the use of volumetric flasks, burettes and pipettes. Several titrimetric methods exist but the choice of the method depends on the sensitivity required, presence of interfering substances and alternate methods of analysis. The accuracy and reproducibility of a titrimetric method depends on weighing accurately and the preparation of solutions, the ability to locate the end point of a reaction accurately (Olaniyi, 2000).

Acid-base titrations may be a direct titration or a back titration. Direct titration involves the accurate determination of the strength of a solution using a standard solution of known purity

and strength. Salts are usually formed, and they are not hydrolysed in aqueous solution. Back titration consists of the addition of a definite excess of a standard volumetric solution to a weighed amount of the sample and the determination of the excess not used up by the sample (Olaniyi, 2000).

2.7.2.1.1 TITRATION OF ARTESUNATE

Artesunate is assayed by direct acid-base titrimetry in the IP, the method involves the reaction between the hydroxyl end of Artesunate and sodium hydroxide, this method is not a reliable method because when Artesunate breaks down to Dihydroartemisinin and succinic acid, the hydroxyl ends of the degradation products still reacts with NaOH resulting in a higher percentage content, hence the need for an accurate method of assay i.e the HPLC method.

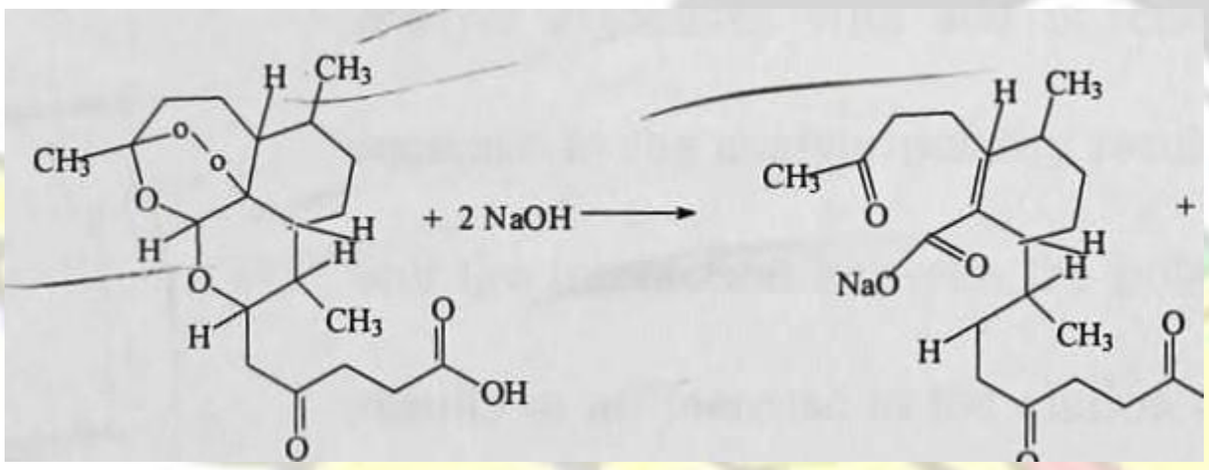


Fig 5: Reaction between Artesunate and sodium hydroxide.

2.7.2.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The technique of high performance liquid chromatography (HPLC) was developed in the late 1960s and early 1970s from knowledge of the theoretical principles of column chromatography in particular, as well as advances made in column packing materials and in the design of chromatographic equipment. HPLC is based on the following modes of separation namely adsorption, partition, ion exchange and gel permeation and they are the same mode of separations involved in classical chromatography (Beckett and Stenlake, 1997). HPLC is a form of column chromatography that is used regularly in biochemistry and analytical chemistry to identify, separate, and quantify compounds based on their differences in polarities and interactions with the column's stationary phase.

2.7.2.2.1 TYPES OF HPLC

There are two types of HPLC, and they are normal phase chromatography and reversed phase chromatography.

- Normal phase Chromatography: This method was one of the first kinds of HPLC that was developed by chemist, it is also known as adsorption chromatography, the method separates analytes based on polarity as well as adsorption to a stationary phase. The method employs the use of a polar stationary phase and a non-polar, non-aqueous mobile phase and is very effective for separating analytes readily soluble in non-polar solvents; the analyte associates with and is retained by the polar stationery phase. An increase in t e analyte polarity results in an increase in adsorption strengths, and the interaction between the polar stationery phase and the polar analyte results in an increase in the elution time. A decrease in the retention time of analyte is achieved by the use of more polar solvents in the mobile phase.

  In the 1970s with the development of reversed-phase chromatography, normal phase chromatography fell out of favour due to its lack of reproducible retention times as a result of the change in the hydration state of silica or alumina chromatographic media caused by water or protic organic solvent.

- Reversed phase chromatography (RPC): This method has a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC is the most commonly used type of HPLC method . RPC plays a major role in all processes in life science, and it operates on the principle of hydrophobic forces, which originates from the high symmetry in the dipolar water structure.

2.7.2.2.2 HPLC Instrumentation

The HPLC chromatograph consists of the following: the pump, injection system, column, detector, and data analysing system as well as the mobile phase reservoirs.

Pump

Pumps are needed to deliver a constant flow of mobile phase from the enclosed solvent reservoir to the column at pressures ranging from 0.1 to 55Mpa. Three types of pumps are used in HPLC and they are the reciprocating pump (which is the most popular), the displacement type of pump and the pneumatic or constant pressure pump. The pumping system must be able to provide pressure of up to 6000psi, pulse-free output, flow rates of within 0.1 to 10ml/min, flow control and flow reproducibility of 0.5% relative or better

(Olaniyi, 2000).

Injection Systems

•here-are two basic types of injection ports; they are those in which the sample is deposited before the column inlet and then carried by a valving action into the column by the mobile phase and those in which the sample is injected directly into the column (Beckett and Stenlake).

The constant-volume loop is the most popular design of HPLC injector; it allows the introduction of samples at pressures up to 7000 psi and provides sample sizes from 5 t0500gl. Overloading of columns must be avoided to prevent band broadening also the introduction of samples unto the column packing must be reproducible so as not to affect the precision of liquid chromatographic measurement (Olaniyi, 2000).

Column

Chromatographic separation takes place in the column. HPLC columns are usually made of high quality stainless steel in order to cope with the high pressure; they contain the packing material and are often at ambient temperature but sometimes may need to be maintained at high temperature. The internal diameter of Standard analytical columns is 4-5mm and

1030cm in length, shorter columns are 3-6cm in length producing better efficiencies, microbore columns have an internal diameter of 1-2mm and are 10-25cm in length and they have the advantage of consuming less volume of solvent and giving a lower detection limit. The type and nature of the material to be separated determines the choice of the column, whether it is a normal column or a reversed column. A normal phased column is that which the coating contains polar functional groups such as amino and diol while a reversed phased column is that which the bonded coating is non-polar in nature. The octadecylsilane (ODS) bonded columns are the_most widely used reverse phased column.

Detector

Thedetector is a critical component in the performance of any given HPLC system; it is a device that functions in continuous monitoring at the column exit. A good HPLC detector should have low dead volume so as to reduce zone broadening, it should also be highly sensitive and of good stability, it should not be responsive over as great a temperature range. Ultraviolet-visible (UV-Vis) spectrophotometers, including diode array detectors are the most commonly employed detectors.

Data analysing systems

The data handling system primarily interprets the detectors output. Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store, and reprocess chromatographic data. Modern data stations are computer based and have a large storage capacity to collect, process and store data for possible subsequent reprocessing (IP, 2006).

Mobile phase reservoir

They are made of glass or stainless steel equipped with a means of removing dissolved gases such as oxygen, for example degassers, and a means of filtering off dust and particulate matter from solvents. The solvent used in HPLC must be pure, particulate matter are filtered

off from solvents by the use of a Millipore filter under vacuum, a process known as ultrafiltration or ultrasonication.

2.7.3 ANALYTICAL METHOD VALIDATION

Analytical method validation is the process that is utilised in order to confirm or demonstrate that a particular method for the quantitative determination of an analyte or for a specific test is reliable for the intended purpose or is suitable for its intended use. It helps in establishing through laboratory ory studies that the analytical method provide results that are used in evaluating the quality of the pharmaceutical product, it also provides confidence that meaningful and acceptable data are generated during routine analysis.

Analytical methods need to be validated or revalidated:

- Whenever there is a change in the method is changed, and the change is outside the original scope of the initial method.
- Before the method is used routinely.
- Whenever there is a change in the conditions for which the method had been earlier validated.

The acceptability of the performance of an analytical method are ensured by the following essential parameters, namely, precision, accuracy, ruggedness, robustness, specificity, limit of quantification, limit of detection, and linearity and range.

Precision

Precision of an analytical method is a measure of the extent or degree of agreement among individual test results of multiple injections when applied to separate or identical samples of the same homogenous batch of material (Olaniyi, 2000). It is usually expressed as Standard Deviation and it measures the degree of reproducibility of the analytical method, giving the

degree of confidence that be placed on the test result provided it was carried out under normal operating circumstances.

The Relative Standard Deviation (RSD) should not be more than 2% for an analytical method to be precise consideration ofprecision should be at three categories as shown below:

- Repeatability (Intra-assay precision)

Repeatability is obtained when an operator carries out the analysis in a laboratory using the Säõðperating conditions over a short interval of time. It is recommended that a minimum of three determinations at each of three concentrations across the intended range, or a minimum of six determinations at the test concentration is carried

out.

- Intermediate Precision

it is the long-term variability of the measurement process, determined by comparing the results of a method run in a laboratory over a period of time. Intermediate precision expresses within-laboratory variations.

- Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology). Reproducibility is determined in order to ascertain that the method will give the same result in different laboratories.

Accuracy

The accuracy of an analytical method is the extent to which the test result obtained and the true value agree. One of the ways in which the true value of accuracy can be assessed is by comparing the result obtained from the method with that of an established reference method,

it can also be determined by applying the method to samples of material to be examined after adding known amount of analyte. Percentage recovery should be between 98% and 102% for an analytical method to be accurate.

Ruggedness

Ruggedness is a measure of the degree of reproducibility of test results under a variety of conditions suchas different ~~analyst, laboratories,~~ instrument, environmental condition, e.t.c

Rgþustness

It examines the effect that operational parameters such as the flow rate, pH, column temperature, injection volume, detection wavelength or mobile phase composition have on the result of the analysis. It involves the varying of the operational parameters within a realistic range and determining the quantitative effects of these changes.

Specificity/ Selectivity

it is the ability of an analytical method to measure the analyte in the presence of other components that may be expected to be present in the matrix, specifically and accurately.

Limit of Quantification (LOQ)

The limit of quantification of an analytical method is the lowest concentration of analyte in a sample that can be determined with suitable precision and accuracy under the stated experimental conditions. It is usually expressed as the concentration of analyte in the sample, which can be in percentage or parts per billion.

Detection Limit (LOD)

The detection limit of an analytical procedure is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value under stated

experimental conditions. The detection limit is usually expressed as the concentration of analyte (e.g., percentage, parts per billion) in the sample. LOQ can be determined by various methods depending on the procedure, whether it is instrumental or non-instrumental.

Linearity

The linearity of an analyticaúÃiGïWs its ability to obtain test results that are directly proportional to the concentration of analyte in the sample within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte; it may also be demonstrated directly on the test substance. The accepted coefficient of correlation (R^2) of any good analytical method should be between 0.995and l.

Range

The range of an analytical method is the interval between the upper and lower concentration of analyte for which it has been demonstrated by the analytical method to have a suitable level of precision, accuracy and linearity. Range is usually demonstrated by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range. It is usually expressed in the same unit as the test results obtained by the method.

2.8 STABILITY OF PHARMACEUTICALS

The stability of a drug product is defined as the ability of a particular drug formulation in a specific container to maintain its chemical, physical, and microbiological and biopharmaceutical properties within specified limits throughout its shelf-life. Such a product must:

a. Contain at least 90% of its stated concentration.

b. Exhibit not less than 90% of its stated therapeutic activity.

c. Develop no toxicity or irritancy.

d. Exhibit no-visible change, e.g discoloration, precipitation or development of offodours.

ontainan effective concentration of added preservative.

2.8.1 FACTORS AFFECTING THE STABILITY OF PHARMACEUTICALS

The factors affecting the stability of pharmaceuticals can be grouped into two namely environmental factors and product-related factors.

2.8.1.1 ENVIRONMENTAL FACTORS

There are a large number of possible reactions that can lead to drug degradation; however, majority of those that occur can be classified as either oxidation or hydrolysis, this is due to the nearly ubiquitous occurrence of oxygen and water. The environmental factors include temperature (heat), oxygen, moisture, light and pH changes.

2.8.1.1.1 TEMPERATURE (HEAT)

The stability of both solid and liquid dosage forms is affected by ambient temperature and is of particular importance for drugs which are used in the tropics or those that are subjected to heat sterilisation. Acceleration of chemical reaction as well as breathing of the container which leads to transport of air and moisture into the drug are caused by temperature fluctuations in the storage facilities. For every IOOC rise in temperature, the speed of many reactions increases about two or three times, hence, an increase in temperature often causes an appreciable increase of the decomposition rate, which makes it essential that drug products stored under defined conditions requires appropriate storage conditions. The maintenance of quality, safety and efficacy throughout the shelf-life of a product should be

guaranteed by the storage conditions recommended by the manufacturer on the basis of stability studies.

2.8.1.1.2 OXYGEN

A major cause of drug instability is oxidative degradation; the presence of 20% oxygen in the atmosphere obvious potential stability problems for most drugs because they exist in reduced form and are converted to a more oxidised state. Oxidation of many p_rmaceutical products, especially light-sensitive formulations are caused by oxygen in the presence of light or heat. Usually the presence of one or more of the following functional groups namely olefinics, phenolics, thioesters, amines and miscellaneous such as the

antibiotics, steroids, ascorbic acid, folic acid and riboflavine causes pharmaceutical products to decompose in the presence of oxygen, light or heat.

2.8.1.1.3 MOISTURE

Storage of pharmaceutical products in an environment of high humidity accelerates decomposition arising from hydrolysis, hence the need and importance of excluding moisture from them; high humidity also affects the kinetics of decomposition. pH varies the rate of hydrolytic reactions catalysed by 1-1+ and OH- ions considerably, however, at intermediate pH range, the rate can be catalysed by both and OH- ions or independent of pH. Compounds containing esters, amide, glycosides or lactam linkages are prone to hydrolysis.

Limits of water content of some substances are given in the British pharmacopoeia because many pharmacopoeial substances contain water in absorbed form (Olaniyi and Ogungbamila, 1991).

Certain measures can be taken to protect pharmaceutical substances from hydrolysis, since hydrolysis of drugs in solution may be catalysed by taking the following measures:

- Formulating the drug, where possible, in non-aqueous solutions, e.g ethanol, glycerol or propylene glycol. Due to the dielectric constant of non-aqueous solvents it is often successful in reducing hydrolysis

- The correct selection of packaging can help in minimising the access of moisture during storage of drugs. This can be achieved by the use of blister packages for tablets and capsyles, and the use of moisture-resistant packages for drugs in the tropics because of high humidity and frequent rains which make it difficult to prevent ——— —moisture from entering them.

- Ensuring the compliance of drugs with the pharmacopoeial limit of moisture content.
- Supplying drugs in dry form for reconstitution by adding a specified quantity of water just before dispensing, when an aqueous preparation of an unstable drug such as ampicillin or erythromycin is needed.

- Adjusting the pH of the solution to a value at which the compound is found by kinetic experimentation to exhibit the lowest rate constant by using buffers.

2.8.1.1.4 LIGHT

Due to the complex chemical structure of many new drugs, consideration of the decomposition of pharmaceutical compounds resulting from the absorption of radiant energy in the form of light has become more imperative in recent years. Exposure to light especially at shorter wavelengths of light results in degradative reactions such as oxidation-reduction, ring rearrangement or modification and polymerization.

The stability of light-sensitive pharmaceuticals and drug products such as the phenothiazine tranquillizers, ascorbic acid, amyl nirite, ergotamine, furosemide, folic acid, hydrocortisone, nitrofurantoin, prednisolone, riboflavine, tetracycline, e.t.c are ensured by taking extra care, these products when administered orally, topically or by

parenteral route, are liable to react photochemically by inducing a photosensitizing response leading to a reduced shelf-life of the product, phototoxic response or to a photoallergic reaction. The mechanisms of photodegradation are of such complexity that they have only been fully elucidated in only a few cases.

The use of coloured (opaque) glass container as well as the storage of drugs in dark places and coating tablets with a polymer film containing UV absorbers adequately protects —pharmaceutical products from photo- induced decomposition.

2.8.1.1.5 pH CHANGES

Since, different isomers of a drug exhibit different bioactivity, the conversion of a drug into its geometrical and optical isomer is regarded as a form of degradation, because it often results in a serious loss of the therapeutic activity of the drug converted. For example, cis-trans isomerisation may be a cause of loss of potency of a drug if the two geometric isomers have different therapeutic activities.

In order to avoid degradation due to isomerisation and epimerisation caused by pH changes, changes in the pH of the product solution should be avoided by making use of a buffer.

2.8.1.2 PRODUCT-RELATED FACTORS

The degree and rate of deterioration of pharmaceutical products have been found to be influenced by a number of product-related factors which are explained below:

The method of production: Product stability is influenced by contamination caused by atmospheric pollutants and technological procedures due to the manufacturing process used, as well as the environmental conditions.

- The dosage form and its composition: Positive or negative influences on the stability of the active substance in drugs are being exerted by the formulation of the dosage form. Thus the active ingredients of sugar or polymer film-coated tablets are protected from interaction with oxygen, light, and moisture from the

atmosphere. The coating must however be such that does not absorb moisture up to the relative humidity of 90-110% and be based on a formula that is not freely hygroscopic or deliquescent, especially if the product is to be used in the tropics.

- The actiVe drug substance and excipients: The stability of drugs could be influenced by the chemical and physical properties of the active drug substance as well as the excipients present in the drug formulation.

- The nature and properties of the packaging material: The stability of pharmaceutical products-is influenced by the nature of the packaging material. When the packaging material is not a barrier enough to the environmental barrier it results in deterioration of the product, when active material interacts with the container, it results in its decomposition.

2.8.2 STABILITY OF ARTESUNATE

The antimalarial Artesunate, dihydroartemisinin and ethers have the problems of chemical and metabolic instability. The stability of Artesunate is affected by the presence of moisture in the environment, as a result of its hemi-ester linkage which makes it prone to degradation by slowly hydrolysing it to Dihydroartemisinin and succinic acid. Temperature also affects the stability of Artesunate. According to the rule of thumb, the rate of a reaction doubles for every 10^0 rise in temperature. High temperature fluctuations lead to a condition known as "breathing" of packaging materials such as plastics, leading to transport of moisture and air into the product to initiate hydrolysis. The effects oftemperature can be minimised by storing Artesunate- containing formulations in cool dry conditions of about 25^0C.

CHAPTER THREE MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 EQUIPMENT

- ADAM analytical weighing balance.
- Gallenkamp regulator hotplate.
- Stuart melting point apparatus.
- Eutech instruments pH 510 pH meter.
- Buchi R-210 water bath.
- Fisher scientific FS 28H sonicator.
- Erweka tablet disintegration machine.
- HPLC chromatograph:
- Kontron instruments HPLC pump.
- Applied Biosystems Powerchrome 280. • Perkin Elmer 785A UV/VIS Detector.
- Bibby quick fit thermometer.
- Melting point capillary tubes.
- Pre-coated TLC plates (whatman UV 254nm, 250gm layer).

3.1.2 GLASSWARE •

Conical flasks.

- Measuring-beakers.
- Volumetric flasks (10ml,25ml,50ml,100ml,200ml,500ml).
- Graduated pipettes (5ml, 10ml).
- Measuring cylinders.

3.1.3 CHEMICALS, REAGENTS AND DRUG SAMPLES

- Dichloromethane.
- Ethanol.
- Acetone.
- Acetonitrile (BDH).
- Potassium iodide.
- Sulphuric acid (BDH).
- Starch.
- Sodium hydroxide (BDH).
- Trifluoroacetic acid TFA98%
- Phenolphthalein.
- Distilled Methanol
- Toluene.
- Ammonia.
- Anisaldehyde/ methanol solution.

Table 1: Table showing the drug sample

Drug sample	Batch number	Manufacturing date	Expiry date
Artesunate	I-IJ 011009	October, 2009	October, 2012
Dihydroartemisinin (DHA)	006D KRI	September, 2007	August, 2011

Table 2: Table showing the tablet brands used

Brand	Code	Country of production	Dosage (mg)	Batch number	Manufacturing date	Expiry date
Camosunate		Ghana	100	1010230	October, 2010	October, 2012
Combicure		Ghana	100	1370061	June, 2011	November, 2012
Arsumod		Ghana	100	P07277	June, 2011	June, 2013
Arsuamoon		China	50	LQ110601A	May, 2011	May, 2013
Letasunate		Ghana	50	1100019	April, 2009	March, 201 1

3.2 METHOD

3.2.1 IDENTIFICATION TESTS

3.2.1.1 COLOUR REACTION TEST

40ml dehydrated ethanol was added to 0.1g of pure Artesunate powder, the solution was shaken then filtered, half of the filtrate was evaporated on a water bath to a volume of about 5ml, a few drops ofthe filtrate was placed on a white porcelain dish and a drop of sulphuric acid was added to it, and the observation was recorded.

0.5ml dehydrated ethanol was added to 0.005g of pure dihydroartemisinin (DHA), then Iml of KI solution was added after which 2.5ml of sulphuric acid was also added, then four drops of starõ§ðlution was added.

3.2.1.2 SOLUBILITY TEST

0.010g of pure Artesunate powder was dissolved in 20ml of the following solvents; water, dichloromethane, ethanol and acetone and the observation was recorded.
0.010g of pure dihydroartemisinin (DHA) was dissolved in 20ml of the following solvents;

water, Acetonitrile, dichloromethane, and ethanol and the observation recorded.

3.2.1.3 MELTING POINT

Melting point tubes were filled to about lcm with pure Artesunate sample and placed in the melting point apparatus and the melting point was determined, the method was repeated using pure DHA samples.

3.2.1.4 THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was carried out so as to identify the pure Artesunate sample and the pure dihydroartemisinin sample, and also detect or identify the presence of Artesunate and DHA in the commercial tablets collected for this research work.

Mobile phase: The mobile phase system was made up of toluene, ethanol and ammonia in the ratio 5:5:0.15.

Stationary phase: precoated TLC plates.

Procedure: 50mg of dihydroartemisinin, 50mg of pure Artesunate and an equivalent of 50mg Artesunate in tablet were individually dissolved in 10ml ethanol, in a petridish the ethanol was evaporated using a water bath, after which 5ml toluene was added and then spotted on a preheated precoated TLC plate and placed in a chromatographic chamber containing the mobile phase, after the run, the plate is sprayed with anisaldehyde/methanol solution and allowed to air dry then it is placed in an oven for some few seconds, then the spots become visible; and the number of spots were noted as well as their Rf values

calculated. The same procedure was repeated for all the brands of tablets used for this research.

3.2.2 PHARMACOPOEIAL TEST

3.2.2.1 DISINTEGRATION TEST

Six tablets of each brand of Artesunate tablets were taken, and each tablet was placed in the cylindrical tube of the disintegration basket, ensuring that the base of the disintegration

basket was at least 15mm below the surface of the water and the temperature of the water was maintained at a constant of 37±2ºc while the apparatus was operated for 30 minutes. The disintegration time for each tablet was recorded as well as the average disintegration time per brand, if the disintegration time was 15minutes the tablet passed the test, however if it is greater than 15 minutes the procedure has to be repeated with an additional 12 tablets.

3.2.2.2 UNIFORMITY OF WEIGHT TEST

Random selection of twenty tablets from each brand was done, they were then weighed individually, after which they were weighed collectively, and the average weight of the tablets determined, the deviation of each tablet from the average weight was calculated as well as their percentage deviation, and the result obtained was compared to the standards in the British Pharmacopoeia to ascertain if the brand passed the uniformity of weight test or not.

3.2.3 ASSAY OF PURE ARTESUNATE BY TITRATION

3.2.3.1 Preparation of 0.05M sulphamic acid

1.2138g of sulphamic acid was weighed accurately and placed in a 250ml volumetric flask and made to mark with distilled water.

3.2.3.2 Preparation of 0.05M sodium hydroxide

2.0867-g-ofsodium hydroxide pellet was weighed accurately and transferred into a 1000ml volumetric flask and made to mark with distilled water.

3.2.3.3 Standardisation of 0.05M NaOH using 0.05M sulphamic acid

To 25ml of sulphamic acid, two drops of phenolphthalein/ethanol indicator was added, and then the solution was titrated against 0.05M NaOH till a faint pink colour was observed. The volume ofNaOH used was recorded and the procedure done in replicate.

3.2.3.4 Preparation of phenolphthalein/ethanol indicator

0.5g of phenolphthalein powder was weighed and transferred to a 50ml volumetric flask and made to mark with ethanol.

3.2.3.5 Preparation of neutralised ethanol

To 100ml dehydrated ethanol, 0.5ml phenolphthalein/ethanol indicator was added and titrated with 0.1M NaOH until a faint pink colour was observed.

3.2.3.6 Assay of pure Artesunate

To a quantity of powder containing about 0.25g of Artesunate, 25ml of neutralised ethanol was added, and then titrated with 0.05M NaOH using two drops of phenolphthalein/ethanol as indicator. A blank titration was also done, the difference in the volume ofNaOH between the test titration and the blank titration represents the volume ofNaOH that reacted with the Artesunate.

Each ml of (0.05mol/l) Vs NaOH is equivalent to 19.22mg ofC19H2808.

3.2.4 HPLC METHOD DEVELOPMENT AND VALIDATION

3.2.4.1 Chromatographic Conditions

Column: Phenomenex BondcloneTÚõSð0 X 3.90mm micron.

Mobilgphase: Methanol: 0.1% TFA (90:10)

Flow rate: 1.5ml/min

Wavelength of detection: 220nm

AUFS: 1.0000

Mode of elution: Isocratic

Mode of HPLC: Reverse Phase Liquid Chromatography (RPLC)

3.2.4.2 Assay of Artesunate

Preparation of mobile phase

1% v/v Trifluoroacetic acid (TFA) was prepared by pipetting Iml TFA into a 100ml volumetric flask and making it up to volume with distilled water. 1000ml of the mobile phase was prepared by measuring 900ml of methanol into a litre volumetric flask and topping it up with 1% TFA to mark.

Calibration curve of Artesunate

Artesunate solution was prepared by weighing 250mg of Artesunate pure powder dissolving it in methanol and making it up to 50ml. The solution was sonicated and filtered. Serial dilutions were done with methanol to obtain the following concentrations, 0.4, 0.3, 0.2and 0.1 % ^{w}/v. The solutions were filtered injected and the results were recorded accordingly.

Validation of analytical method

The linearity of the method developed was demonstrated by weighing accurately 250mg of Artesunate pure powder and dissolving it in methanol to give a 50ml solution. Concentrations of 0.4, 0.3, 0.2 and 0.1%w/v were prepared by serial dilution from the stock solution. The solutions were filtered and injected and results were recorded accordingly. A graph of absorbance against concentration was plotted with the readings obtained. The LOD and LOQ were calculated from the plotted graph.

The precision of the method developed was determined by weighing 20mg pure Artesunate powder and dissolving it in sufficient methanol to obtain 10ml, filtering the solution and then injecting it six times and recording the result.

The accuracy of the method developed was also demonstrated by weighing 20mg of pure Artesunate and adding starch, the mixture was dissolved in sufficient methanol to obtain 10ml. the resulting solution was filtered and injected and the results were recorded.

To demonstrate the robustness of the developed method several parameters such as the mobile phase composition, flow rate and wavelength were varied each at a time while keeping other parameters constant

Reproducibility of the developed method was determined by weighing three different concentrations three times, injecting and recording the result.

Assay of Artesunate in tablets

An equivalent of 20mg of Artesunate was weighed and diluted with sufficient methanol to make up to 10ml. The solution was shaken thoroughly and filtered. The solution was filtered injected and results recorded. This was done for the five brands of Artesunate tablets used for this research.

3.2.4.3 HPLC method development for dihydroartemisinin

Preparation of mobile phase

1% v/v Trifluoroacetic acid (TFA) was prepared by pipetting Iml TFA into a 100ml volumetric flask and making it up to volume with distilled water. 1000ml of the mobile phase

was prepared by measuring 900ml-ef-methäñOl into a litre volumetric flask and topping it up with TFA to mark.

Calibration curve of Dihydroartemisinin

Dihydroartemisinin solution was prepared by weighing 250mg of Dihydroartemisinin pure powder, dissolving it in methanol and making it up to 50ml. The solution was sonicated and

filtered. Serial dilutions were done with methanol to obtain the following concentrations, 0.4, 0.3, 0.2and 0.1 % ^{W}/v. The solutions were filtered injected and the results were recorded accordingly.

Validation of analytical method

The linearity of the method developed was demonstrated by weighing accurately 250mg of dihydroartemisinin pure powder and dissolving it in methanol to give a 50ml solution. Concentrations of 0.4, 0.3, 0.2 and 0.1%w/v were prepared by serial dilution from the stock solution. The solutions were filtered and injected and results were recorded accordingly. A graph of absorbance against concentration was plotted with the readings obtained. The LOD and LOQ were calculated from the plotted graph.

The precision of the method developed was determined by weighing 20mg pure Dihydroartemisinin powder and dissolving it in sufficient methanol to obtain 10ml, filtering the solution and then injecting it six times and recording the result.

The accuracy of the method developed was also demonstrated by weighing 20mg of pure Dihydroartemisinin and adding starch, the mixture was dissolved in sufficient methanol to obtain 10ml. the resulting solution was filtered and injected and the results were recorded.

To demonstrate the robustness of the developed method several parameters such as the mobile phase composition, flow rate and wavelength were varied each at a time while keeping other parameters constant

ReprOúÍÉility of the developed method was determined by weighing three different concentrations three times, injecting and recording the result.

Determination of content of Dihydroartemisinin in Artesunate tablets.

An equivalent of 20mg of Artesunate was weighed and diluted with sufficient methanol to make up to 10ml. The solution was shaken thoroughly and filtered. The solution was filtered injected and results recorded. This was done for the five brands of Artesunate tablets used for this research.

CHAPTER FOUR RESULTS

4.1 IDENTIFICATION TESTS

4.1.1 COLOUR REACTION TEST RESULTS FOR ARTESUNATE (ART) AND DHA

TEST	OBSERVATION	EXPECTED FROM OBSERVATION
0.1g ART + 10ml water	Slightly soluble	Slightly soluble.

o.lg ART + 10ml		

Table 3: Table showing the colour reaction test result for ART and DHA

TEST	OBSERVATION	INFERENCE
To 0. lg of Artesunate + 40ml ethanol, shake and filter, to filtrate + few drops of sulphuric acid.	Reddish brown colouration	Artesunate is present.
To 5mg DHA + 0.5ml ethanol, + Iml KI solution, + 2.5ml sulphuric acid Four drops of starch solution was added.	Yellow colouration Violet-blue colouration	DHA suspected. DHA is present.

4.1.2 SOLUBILITY TEST RESULTS FOR ART AND DHA

Table 4: Table showing the solubility test result of ART and DHA

dichloromethane	Very soluble	Very soluble.
0.1g ART + 10ml ethanol	Freely soluble	Freely soluble.
0. lg ART + 1 Oml acetone	Freely soluble	Freely soluble.
0.1g DHA +1 Oml water	insoluble	Insoluble.
o.lg DHA + 10ml Acetonitrile	Slightly soluble	Slightly soluble.
o.lg DHA + 1 Omi ethanol	Slightly soluble	Slightly soluble.

4.1.3 MELTING POINT DETERMINATION RESULT FOR ART AND DHA

Table 5: Table showing the melting point determination result of ART and DHA

SAMPLE	MELTING POINTOC 1 st and 2n determinations	REFERENCE RANGEOC
Artesunate (ART)	130-1320C & 130-1320C	132-1350C
Dihydroartemisinin (DHA)	140-1430C & 140-1430C	143-1450C

4.1.4 TLC CHROMATOGRAM OF PURE ART, DHA AND ART TABLET

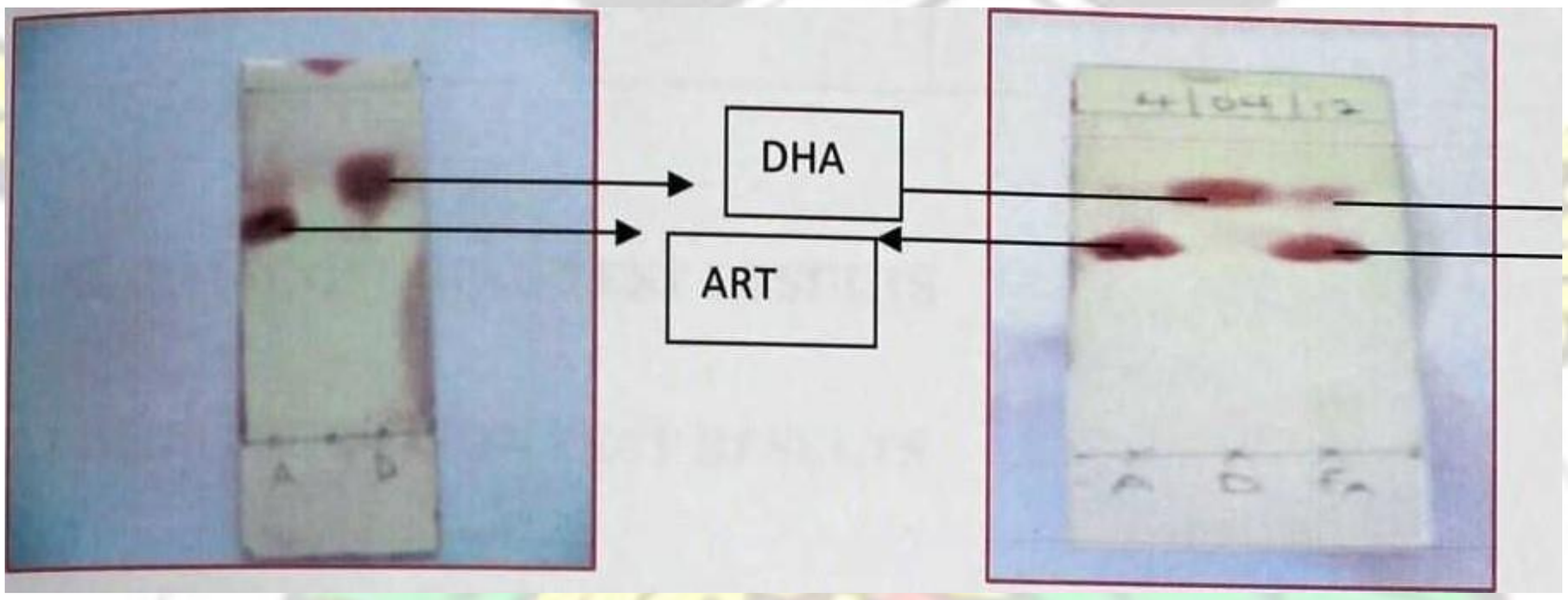


Fig 6: TLC of pure Artesunate (ART) and Dihydroartemisinin (DHA)

Fig. 7: TLC of pure Artesunate (ART) and Dihydroartemisinin (DHA) and ART tablet FA

4.1.4.1 CALCULATION OF Rfvalue

Rf= Distance moved by solute

Distance moved by solvent font

Table 6: Table showing the Rf values of pure ART, DHA and formulations FA -FE

Sample	Rf value of ART reference sample	Rf value of DHA reference sample	Rf value(s) of ART tablets
Pure ART	0.60	----------	----------

Pure DHA	----------	0.72	----------
FA (2 visiblespots were seen)		0.75	0.58 0.75
FB (2 visiblespots were seen)	0.62	0.75	0.58 0.75
(2 visible spots were seen).	0.64 0.66 0.64	0.84 0.72 0.78	0.64 0.62 0.64 0.78

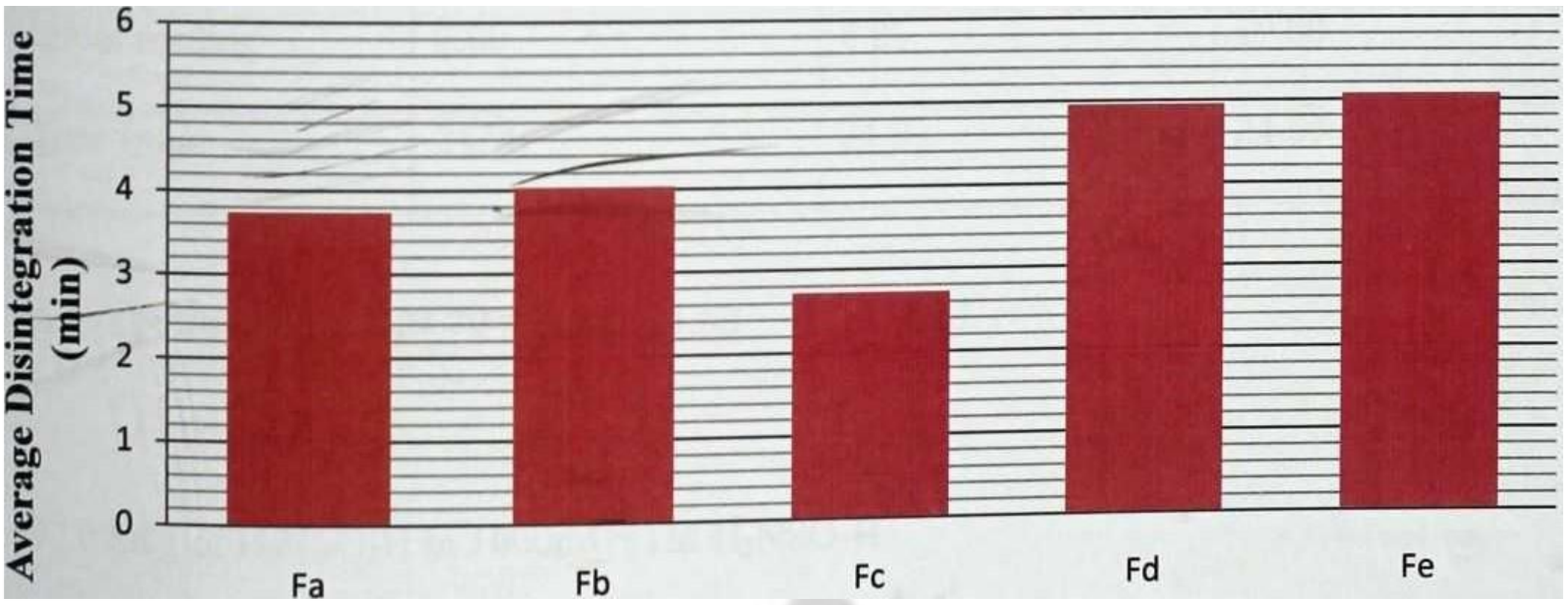
4.2 PHARMACOPOEIAL TEST RESULTS

4.2.1 DISINTEGRATION TEST RESULTS

Table 7: Table showing the disintegration test results of ART tablets.

Brand of tablet	Average disintegration time (min)	Disintegration test result
	3.73	PASSED
FB	4.02	PASSED
	2.71	PASSED
	4.94	PASSED
FE	5.06	PASSED

Fig. 8: Graph showing the average disintegration time of Artesunate tablets



BrandsofArtesunate Tablet

4.2.2 UNIFORMITY OF WEIGHT TEST RESULT

Table 8: Table showing the uniformity of weight result

Brand of tablet	result
	PASSED
FB	PASSED
	PASSED
FD	PASSED
FE	PASSED

Burette reading (ml)	I^S determination	2^n determination	3 determination
Final reading	24.70	24.80	43.60
Initial reading	0.00	0.00	19.00
Titre value	24.70	24.80	24.60

4.3 ASSAY OF PURE ART POWDER BY TITRATION

4.3.1 STANDARDISATION OF 0.05M NaOH USING 0.05M SULPHAMICACID

Table 9: Result of the titration of 0.05M NaOH with 0.05M sulphamic acid

itrevalue = 24.70 +24.80+24.60=24.70ml.

3

97.0900g ofH2NS03H in IOOOm1 1M H2NS03H

9.7090g ofH2NS03H in IOOOm1 0.1M H2NS03H
4.8545g ofH2NS03H in IOOOm1 0.05M H2NS03H

Burette reading (ml)	1 determination	211 determination	3 determination	Blank determination
Final reading	13.10	26.10	39.00	39.10

Initial reading	0.00	13.10	26.10	39.00
Titre value	13,10		12.90	0.10

1.2136g ofH2NS03H in IOOOml 0.05M H2NS03H

Norminal weight of sulphamic acid = 1.2136g

Actual weight taken = ——————— 1.2138g.

Factor of H2NS03H = actual weight taken

——— Norminal weight

=1.2138g = 1.0001

1.2136g

Factor ofNaOH = 1.0001 X 25.00 1.0122.

24.70

4.3.2 DETERMINATION OF 0/0 PURITY OF ART PURE POWDER

Table 10: Result for the titration of ART pure powder with 0.05M NaOH

For first determination

Amount of C19H2809 weighed = 0.2502g.

Volume of 0.05M NaOH used = 13.10 - 0.10 -- 13.00ml.
Actual volume ofO.05M NaOH = 13.00 X 1.0122 = 13.16ml.

If Iml of 0.05M NaOH is equivalent to 19.22mg of C19H2208, the amount of C19H2208 equivalent to 13.16m1 ofO.05MNaOH= 13.16mlX 0.01922

= 0.2529g.

% content of Artesunate = 02529 . x 100
0.2502

= 101.08%w/w.

For second determination

Amount of C19H2809 weighed = 0.2506g.

Volume of 0.05M NaOH used = 13.00 - 0.10 = 12.90m1.

Actual volume ofO.05M NaOH = 12.90 X 1.0122 = 13.06m1.

If Iml of 0.05M NaOH is equivalent to 19.22mg of C19H2208, the amount of C19H2208 equivalent to 13.16ml ofO.05M NaOH= 13.06mlX 0.01922

= 0.2510g.

% content of 0.2506 Artesunate= 0.2510 x 100

= 100.16%w/w.

For third determination

Amount of C19H2809 weighed = 0.2508g.

Volume of 0.05M NaOH used = 12.90 - 0.10 = 12.80ml.

Actual volume ofO.05M NaOH = 12.80 X 1.0122 = 12.96ml.

If Iml of 0.05M NaOH is equivalent to 19.22mg of C19H2208, the amount of C19H2208 equivalent to 13.16ml of 0.05M NaOH= 12.96m1X 0.01922

= 0.249 lg.

% content of Artesunate = 02491 . x 100

0.2508

= 99.32%w/w.

Average percentage purity = 101.08 + 100.16 + 99.32

3

= 100.18% w/w.

Table 11: Average percentage purity of ART pure powder

Average percentage purity (% w/w)	Reference range % w/w (IP)
100.18	99-101

4.3.3 ASSAY OF ARTESUNATE TABLETS BY TITRATION

Table 12: Table showing theÞyntage-content of ART in tablets.

Brand	% content (O/ow/w)	Reference range O/ow/w (IP)
	100.11 ± 0.44	90.0 - 110.0
FB	109.67 ± 1.09	90.0 - 110.0
	107.50 ± 0.29	90.0 - 110.0
	99.05 ± 0.94	90.0 - 110.0

F_E	135.67 ± 0.44	90.0 - 110.0

4.4 HPLC METHOD DEVELOPMENT AND VALIDATION

4.4.1 PURE ARTESUNATE (ART)

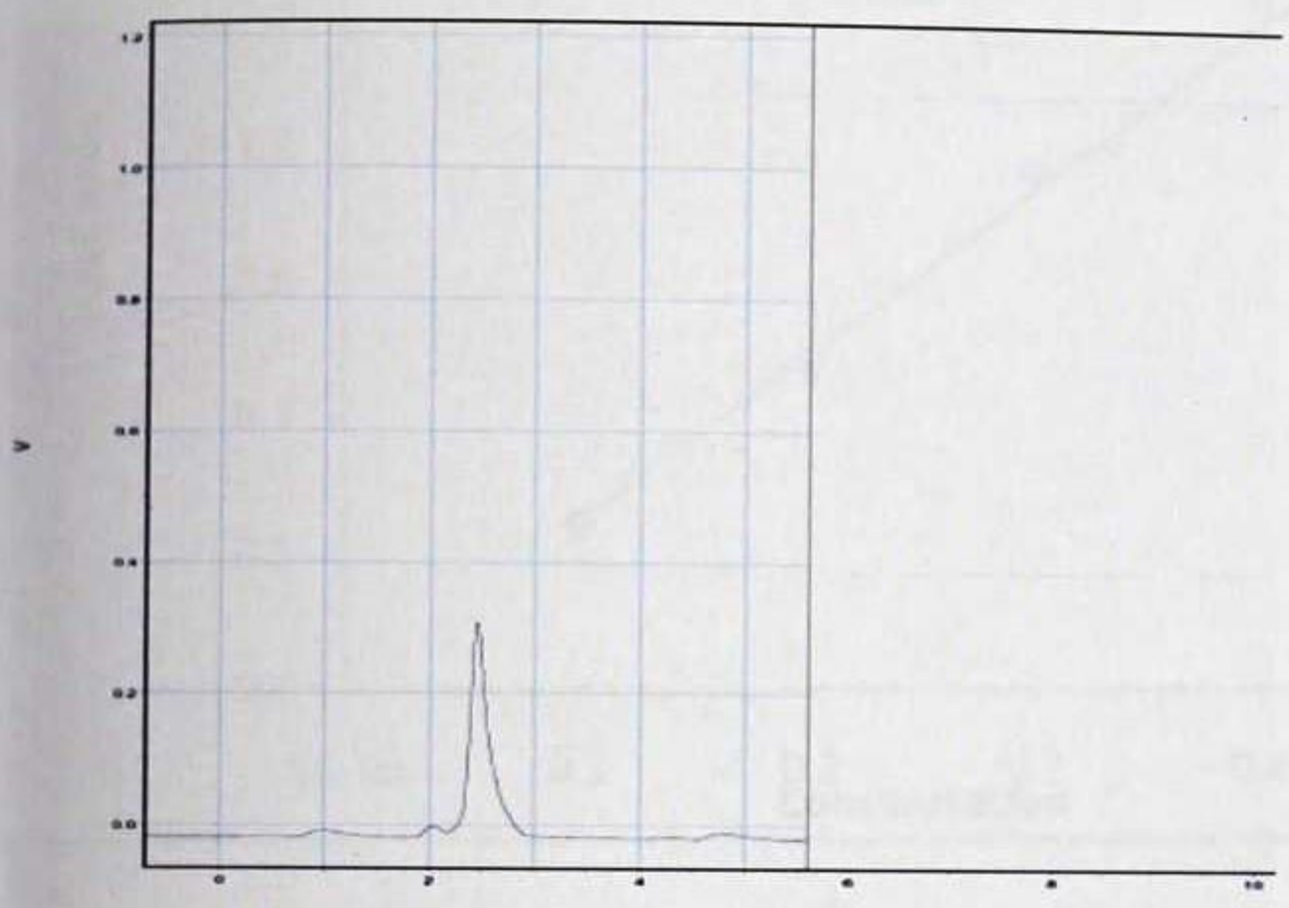


Fig. 9: HPLC of ART

Table 13 Retention time of ART

PARAMETER	VALUE (min)
Retention time	2.54±0.15

Table 14: calibration£urve result for pure ART

Concentration %w/v	Average Peak Area Ratio
0.1	0.54
0.2	0.78
0.3	1.07
0.4	1.32
0.5	1.49

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1.7Calibration Curve for Pure Artesunate

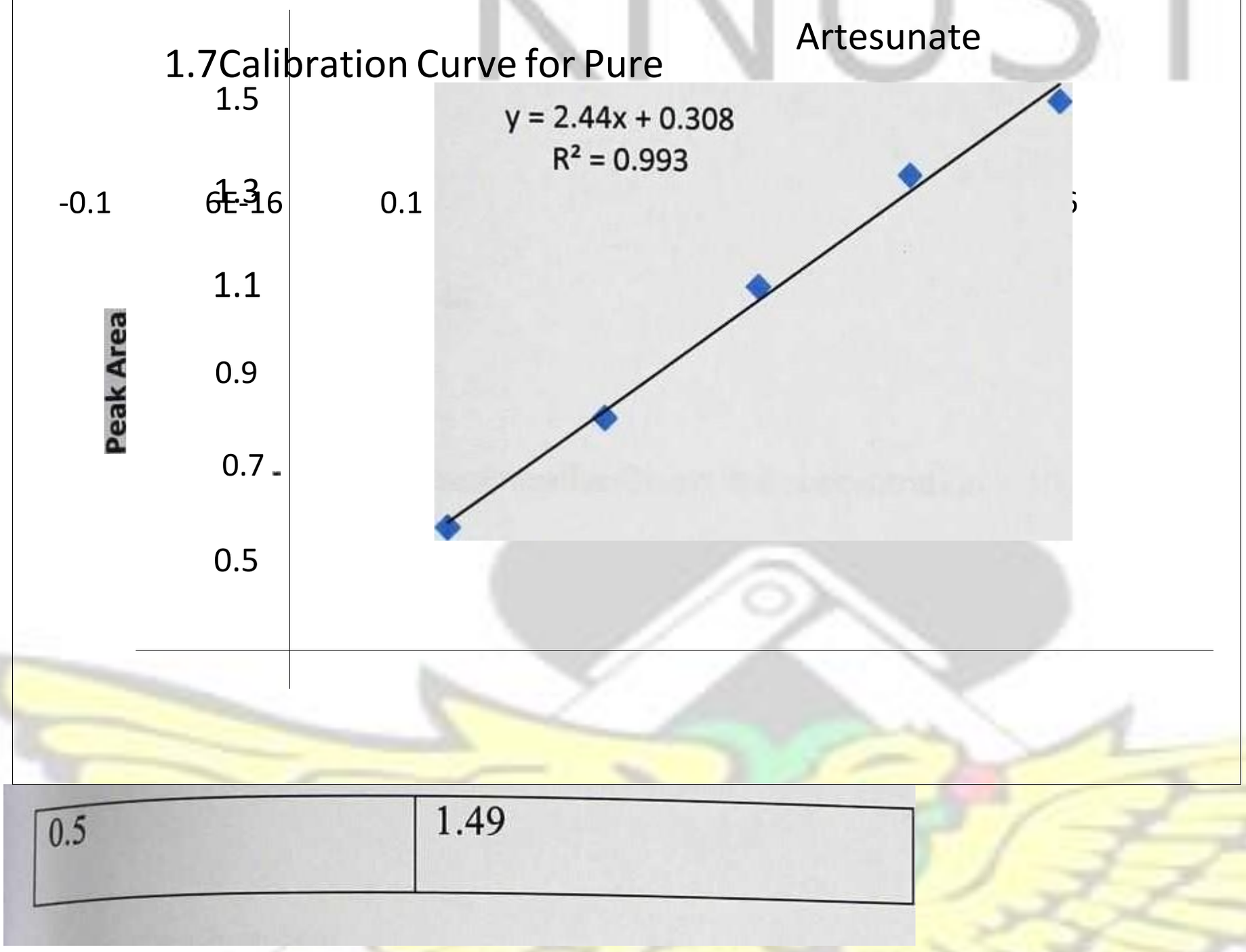

Fig 10: calibration curve of Artesunate

Table 15:

Parameter	Value
	0.993
Intercept	0.308
Slope	2.4
Range	0.1%^{W}/v- 0.5%^{W}/v

Parameters of the calibration curve

Percentage purity calculation of pure ART sample

The equation of a line is given as, y = mx + c,

Where; y = Peak area, m = Slope of Calibration Curve, x — —

Concentration,

c = y - intercept

From the graph,

2.44x +0.308 y =

0.925 x = y -

0.308/2.44 x = 0.925-

0.308/ 2.44 x-0.2529

% purity = Actual concentration/Norminal concentration x 100

Nominal concentration = 0.2500

% purity = 0.2529/0.2500 X 100

= 101.16%w/v.

4.4.1.1 Calculation of Limit of Detection (LOD) and Limit of Quantification (LOQ) for pure ART sample

Equation of the line from the calibration curve is given as, y = 2.44x +0.308

Table 16: Calculation of LOD and LOQ for pure ART sample

Concentration (x)	Peak Area (Y)		
0.1	0.54	0.552	0.0120
0.2	0.78	0.7960	0.0160
0.3	1.07	1.0400	0.0300
0.4	1.32	1.2840	0.0360
0.5	1.49	1.5280	0.0380
			- vest) = 0.0760

Number of injections (n) = 5

Degrees of freedom (5 — 1) = 4

σ_{res}= {E(Y -Yest) / n-1}^{2}

= $(0.0760/4)^2$

= $(0.019)^2$

= 0.000361

LOD= 3.30/ s

Where o = standard deviation of slope

S = slope of the calibration curve.

= (3.3 x 0.000361)/2.44

LOD = 0.000488237%w/v

LOQ = / S

= (10 x 0.000361)/2.44

LOQ = 0.0014795%w/v.

4.4.1.2 Precision

Repeatability (fñti•aday)

Table 17: Repeatability for HPLC method of pure ART powder

Concentration (O/ow/v)	Peak Area						Mean Peak Area	Standard Deviation	RSD (0/0)
	1	2	3	4	5	6			
0.2	0.78	0.82	0.79	0.81	0.80	0.80	0.80	0.014142	1.76

4.4.1.3 Accuracy

Table 18: Accuracy for HPLC method of ART

DETERMINATION	0/0 RECOVERED

	102.50%
2	100.82%
3	98.77%
Average	100.69%

4.4.1.4 Robustness

Table 19: Robustness- (HPLC assay of ART)

Flow rate (ml/min)	0/0Recovery
1.40	98.77
1.30	96.50

4.4.1.5 Reproducibility

Table 20: Reproducibility (HPLC assay of ART)

Concentration O/ow/v	% Recovery	RSD
	104.09	1.31
2	105.46	
3	102.73	
4	103.69	0.99
5	101.64	
6	102.66	
7	102.50	1.85
8	100.82	
9	98.77	

RSD = 1.38.

4.2 DIHYDROARTEMISIN

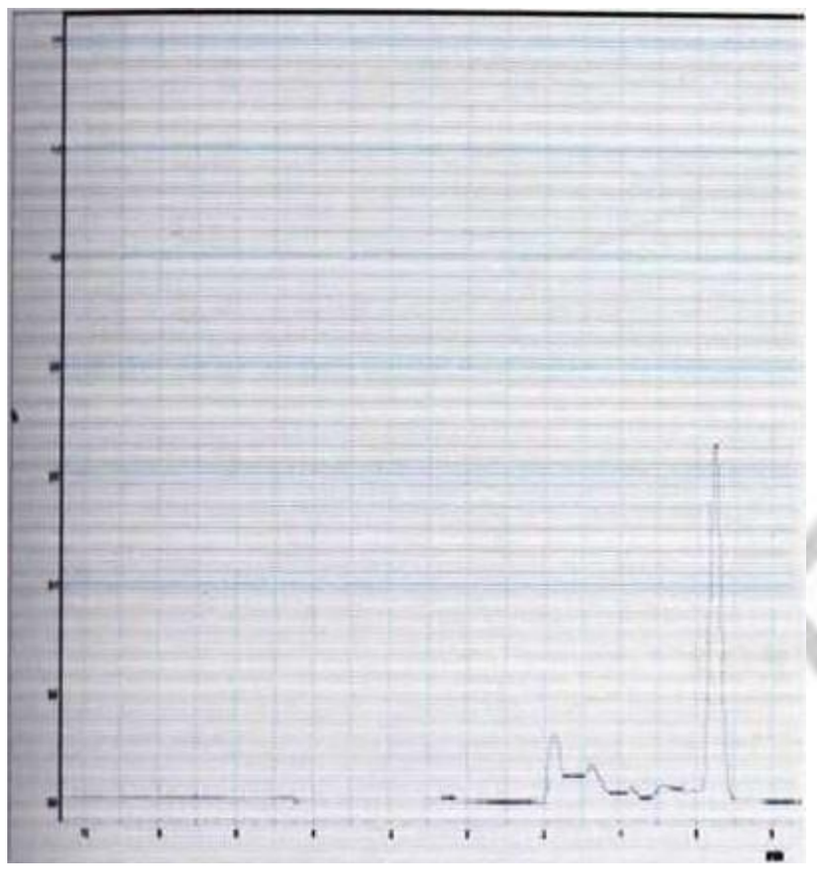

Fig 11: HPLC of pure Dihydroartemisinin (DHA)

Table 21: Retention time of Pure DHA

PARAMETER	VALUE (min)
Retention time	4.34 ± 0.13

Table 22*Calibration Curve Result of Pure DHA

Concentration % w/v	Average Peak Area Ratio
0.1	0.50
0.2	0.72
0.3	1.02
0.4	1.28
0.5	1.46

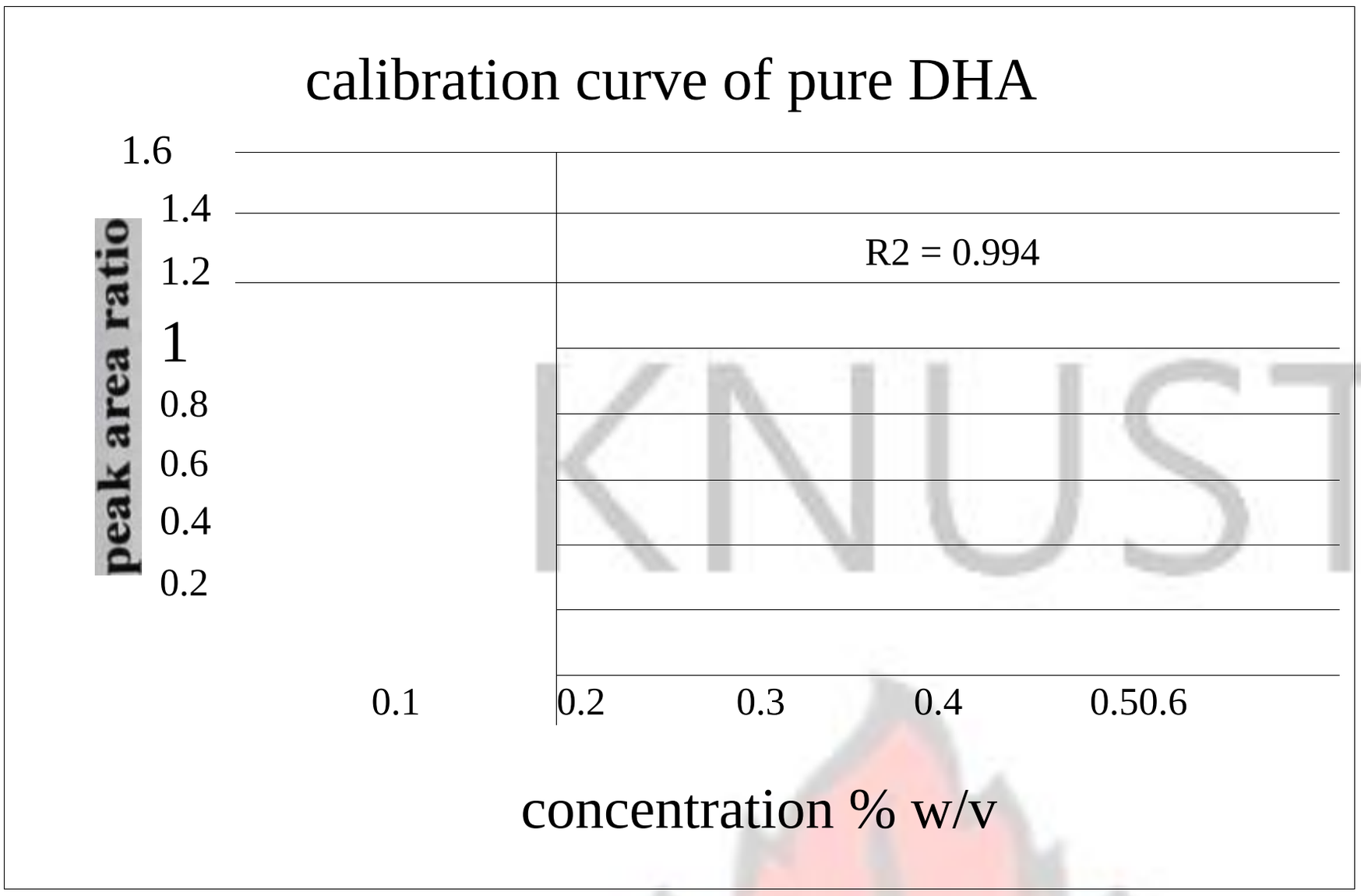

Fig 12.• Calibration curve of Dihydroartemisinin

Table 23: Parameters of the calibration curve

Parameter	Value
	0.994
Intercept	0.252
Slope	2.48
Range—---—	0.1%^{W}/v- 0.5%^{W}/v

LOD = 0.00026080%w/v.

LOQ = 0.0007903%w/v.

Table 24: Repeatability

Determination	% Recovery
	100.00
2	104.00
3	100.00
4	98.40
5	102.40

6	100.40
7	101.88
8	97.83
9	100.00
RSD	1.92

4.4.3 ASSAY OF TABLETS BY HPLC

Table 25: Table showing the Assay result of Brands of ART Tablets.

BRAND	ART	DHA
	95.51 ± 0.69	18.33 ± 0.39
	100.50 ±2.13	6.11±0.58
	83.50 ± 1.09	18.05±0.98
	85.85 ± 2.39	29.44±0.78
	130.75 ± 4.53	33.88 ± 0.38

CHAPTER FIVE DISCUSSIONS, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSIONS

5.1.1 Identification test

5.1.1.1 Colour reaction test

Artesunate

No colour was observed when 40ml of ethanol was added to 0.1000g of pure Artesunate powder, however when a drop of concentrated sulphuric acid was added to a few drops of the solution after half of the filtrate had been evaporated on a water bath to a volume of 5ml, a reddish brown colour was observed, this confirmed the presence of pure Artesunate powder UP, 2006).

Dihydroartemisinin

A yellow colouration was observed when 0.5ml ethanol was added to 0.0050g of Dihydroartemisinin, followed by Iml of potassium iodide solution, after which 2.5ml sulphuric

acid was added, a violet-blue colour was observed when four drops of starch solution was added to the resulting solution, confirming the presence of Dihydroartemisinin

UP, 2006).

5.1.1.2 Solubility test

ArtëšûKate

The sample was very soluble in dichloromethane, freely soluble in acetone and ethanol and sparingly soluble in water, this is a positive result indicating that the sample is Artesunate.

Dihydroartemisinin

The sample was insoluble in water, slightly soluble in Acetonitrile, dichloromethane and ethanol this conformed to the international pharmacopoeia standard on solubility of Dihydroartemisinin.

5.1.1.3 Melting Point Determination

Artesunate

The reference melting range given by the International Pharmacopoeia for Artesunate is 132^0C to 135 ^{0}C, the melting point obtained for two determinations using pure Artesunate powder were both 1300C to 132^0C, the value obtained agrees with the reference range.

Dihydroartemisinin

The reference melting range stated by the International Pharmacopoeia for Dihydroartemisinin is 1430C to 145^0C. For two determinations using pure

Dihydroartemisinin powder, the melting range obtained were both 1400C to 143^0C.

5.1.1.4 Thin layer Chromatography

Artesunate

A single spot waSobserved for Artesunate, indicating that the Artesunate powder was pure, the Rfvalue was 0.60.

Dihydroartemisinin

A single spot was also observed for Dihydroartemisinin, indicating that the Dihydroartemisinin powder was pure, the Rfvalue was 0.72.

5.1.2 Pharmacopoeial Tests

5.1.2.1 Disintegration test

It is required that all the six tablets used in the disintegration test must disintegrate within 15 minutes at a temperature of 37^0C ± 2. The test should be repeated on 12 additional dosage units, if one or two ofthe dosage units fail to disintegrate within 15 minutes (BP, 2007).

The average disintegration times for the Artesunate brands FA, FB, Fc, FD and FE were 3.73,

4.02, 2.71, 4.94, and 5.06 minutes respectively, this means that all the five brands used in this analysis passed the tablet disintegration test. Variation in the excipients used in their manufacture as well as different manufacturing conditions, is responsible for the difference in their disintegration times.

5.1.2.2 Uniformity of weight test

After weighing twenty tablets individually and determining their average mass, according to the British Pharmacopoeia, not more than two of the individual masses should deviate from the average mass by more than 10% for an average mass of 80mg and less, and 7.5% for an average mass greater than 80mg but less than 250mg, based on this, all the Artesunate brands used in this analysis passed the uniformity ofweight test.

5.1.3 Determination of % Purity of Pure Artesunate by Titration

101.08%w/w, 100.16%w/w, and 99.32%w/w are the percentage purity obtained for the Artesunate pure sample after three determinations. The mean percentage purity of the

Artesunate pure powder was 100.18%w/w. Artesunate pure powder assayed by the titration method is required by the international pharmacopoeia to be between 99 and 101%w/w for it to pass the test UP, 2006), this implies that the Artesunate pure sample used for this analysis was 100.18% pure, complying with the stated requirement.

5.1.4 ASSAY OF TABLETS BY TITRATION

100.11, 109.67, 107.50, 99.05,and 135.67 are the average percentage content obtained for the five brands of Artesunate tablets FA, FB, Fc, FD, and FE respectively. According to the International Pharmacopoeia, Artesunate tablets should contain not less than 90.0% and not more than 110.0%. From the result obtained above, brands FA, FB, Fc, and FD, passed the assay, since their average percentage content were not less than 90.0% and not more than 110.0%. However brand FE failed to meet the assay requirement, because its average percentage content was higher than 1 10.0%, hence, it failed the test, and this could be due to the breakdown of Artesunate to succinic acid and Dihydroartemisinin, with the hydroxyl ends of the succinic acid reacting with NaOH resulting in higher percentage content.

5.1.5 THIN LAYER CHROMATOGRAPHY OF ARTESUNATE TABLETS

In order to ascertain whether the brands of Artesunate tablets to be used for the analysis actually contained Artesunate, thin layer chromatography was carried out. The mobile phase system used was toluene, ethanol and ammonia in the ratio (5: 5: 0.15). The brands FC and FD showed only one principal spot corresponding in appearance and Rf value to that of pure Artesunate reference sample, their Rfvalues were 0.64 and 0.62 respectively. The Artesunate tablet brands FA, FB and FE showed two principal spots 0.58 and 0.75, 0.58 and 0.75, 0.64 and 0.78 respectively, one comparable in appearance and Rfvalue to Artesunate and the other to DHA. The appearance of two principal spots in the brands FA, FB and FE could be due to the fact-that the Artesunate in the tablets might have hydrolyse and therefore contain DHA which was responsible for the appearance of the second spot.

5.1.6 HPLC Method Development

Several mobile phase systems were tried, however that of methanol and 0.1% Trifluoroacetic acid in the ratio 90: 10 was observed to elute Artesunate at a UV wavelength of 220nm. A good chromatographic method must be cost effective; because methanol is relatively cheap when compared to most chromatographic solvent, this mobile phase system was chosen. The main aim of the method development was to develop a method that will simultaneously elute Artesunate and its major breakdown product such that when a solution of Artesunate tablet is injected under the chromatographic condition stated above, Artesunate and its major breakdown product Dihydroartemisinin can be easily assayed simultaneously if present, giving one an indication of the extent of breakdown of Artesunate to Dihydroartemisinin if present at all.

Due to the fact that Artesunate and Dihydroartemisinin are both soluble in methanol and absorb at the same wavelength, it has made it possible for the HPLC chromatographic method developed to elute them simultaneously.

Trifluoroacetic acid was chosen as the additive for the mobile phase due to its excellent solvating and ion pairing property enhancing resolution and the appearance of sharper peaks. Quite a number of concentrations ofTFA was prepared and tried, however that of 1% TFA in a ratio of 10:90 with methanol at a flow rate of 1.5ml/min was settled for because it gave desirable peaks for Artesunate and DHA. The C-18 column was used for this analysis at a wasælength of absorption of 220nm.

5.1.6.1 HPLC Analysis of Artesunate

Artesunate was analysed under the conditions stated above, a desirable peak was obtained at a retention time of 2.54 ± 0.15 min.

The stated HPLC conditions were employed in the analysis of Artesunate. The Artesunate pure sample was dissolved in methanol throughout the analysis. The retention time for Artesunate was found to be 3.74 ± 0.06 min.

The calibration graph of pure Artesunate was plotted, and the linearity of the graph was determined, this was done by considering the R^2 value obtained from the calibration graph, the R^2 value was 0.9930, indicating that within the range of 0.05%w/v -0.4%w/v the method was of a good linearity.

The limit of detection (LOL)) for Artesunate was 0.00048823%w/v while the limit of quantification (LOQ) was 0.0014795%w/v.

The RSD of the intra-day precision was 1.76%, according to the ICH guideline on validation requirement, for a method to be acceptable, its RSD should be less than 2%, since the value is less than 2%, the method can be said to give precise results.

When the Artesunate pure powder was analysed for accuracy by mixing it with the tablet excipients before analysis, the average percentage recovery was calculated as 100.69%. This value falls within the ICH requirement range of 98-102%.

There was no significant difference in the result obtained when the flow rate was varied from 1.30ml to 1.40ml demonstrating the robustness of the method.

The inter day precision of the method developed for Artesunate reference sample was 1.89, since-twe value is less than 2%, the method can be said to be reproducible.

5.1.6.2 HPLC analysis of Dihydroartemisinin

Dihydroartemisinin was analysed using the condition developed earlier in the analysis of Artesunate with a mobile phase comprising of methanol and 1% TFA at a ratio of 90:10 at a flow rate of 1.5ml/min and the wavelength of absorption of 220nm. The retention time of Dihydroartemisinin was 4.34±0.13 minutes.

The calibration graph of Dihydroartemisinin was plotted in order to determine the linearity of the method developed, the R^2 was 0.9940, within the range of 0.1%w/v-0.5%w/v indicating that the method was linear.

The Limit of detection (LOD) for Dihydroartemisinin was 0.00026%w/v, while its Limit of quantification is 0.0007903%w/v.

The RSD value of the repeatability demonstration of the method developed for DHA was 1.92, since this value is less than 2%, the method can be said to be precise.

5.1.6.3 HPLC analysis of Artesunate tablets

The five Artesunate brands obtained from the market was analysed using the HPLC method developed, the percentage content of Artesunate and DHA if present was determined. All the Artesunate tablet brands contained Artesunate, the percentage content of Artesunate for the brands FA, FB, Fc, FD, and FE were 95.51±0.69, 100.50±2.13, 83.50±1.09, 85.85±2.39, and 130.75±4.53 respectively, from the result above it can be inferred that brand FC,FD and FE failed the test, since they didn't fall within the International pharmacopoeia standard of 96102%.

All the five brands of Artesunate tablets showed the presence of DHA, with the percentage f18.33±0.39, 6.11±0.59, 18.05±0.98, 29.44±0.78, and 33.88±0.38 for the brands FA, FB, Fc, FD, and FE respectively. Brand FE had the highest DHA content; this could be due to the fact that it had expired before the analysis was carried out, hence the breakdown of Artesunate to DHA.

5.2 CONCLUSION

5.2.1 IDENTIFICATION TEST

The identification tests carried out on the pure samples used for this analysis confirm that they are indeed pure Artesunate and Dihydroartemisinin powder.

5.2.2 THIN LAYER CHROMATOGRAPHY

From the results of the thin layer chromatography carried out on the five brands of Artesunate tablets used for this analysis, it can be concluded that all the five brands contained Artesunate; however three of the brands namely FA, FB, and FE also contained DHA.

5.2.3 PHARMACOPOEIAL TEST

After pharmacopoeial test was carried out on the five brands of the Artesunate tablets used for this analysis, it can be concluded from the result that all the five brands passed the disintegration test as well as the uniformity of weight test.

5.2.4 DETERMINATION OF 0/0PURITY OF PURE ARTESUNATE POWDER

The percentage purity of the pure Artesunate powder used for the analysis was determined, based on the result obtained, it can be concluded that the powder was 100.18% pure, hence it passed the test because the value fall within the IP range of 99-1010/0.

5.2.5 ASSAY OF THE ARTESUNATE TABLETS BY TITRATION

The five Artesunate tablet brands namely FA, FB, Fc, FD and FE had the percentage content 100.11, 109.67, 107.50, 99.05, and 135.67% respectively. This result implies that tablet FE failed to meet the standard set by the International pharmacopoeia, which states that the percentage content must be between 90% and 110%. The four other tablet brands FA, FB, Fc,

and FD passed the test.

5.2.6 HPLC METHOD DEVELOPMENT FOR ARTESUNATE AND DIHYDROARTEMISININ

The HPLC method developed is one that can be used in the simultaneous assay of Artesunate and Dihydroartemisinin; this will facilitate its use in the detection of breakdown products in Artesunate tablets as well as its analysis. The mobile phase developed was methanol and 1% Trifluoroacetic acid in the ratio 90: 10. The calibration curve of Artesunate and Dihydroartemisinin was plotted; the LOD and LOQ for Artesunate were 0.00048823%w/v and 0.0014795%w/v, while that of Dihydroartemisinin was 0.00026%w/v and 0.0007903 respectively. The R^2 value obtained from the calibration curve for Artesunate and Dihydroartemisinin was 0.993 and 0.994 respectively.

5.2.6.1 Assay of Artesunate Tablets by HPLC method

All the five brands Artesunate tablets contained Artesunate, however three of the brands namely Fc, FD. and FE, failed the test. All the five brands of Artesunate tablets gave peaks for Dihydroartemisinin; however that of FE was the highest. From this result it can be concluded that Artesunate can breakdown to Dihydroartemisinin during its shelf life, due to hydrolysis of its ester linkage caused by the presence of moisture. Hence, Artesunate tablets should be properly packaged to prevent the inflow of moisture.

5.3 RECOMMENDATION

The stability of more Artesunate tablet brands should be monitored by ensuring that researches are carried out on more of the Artesunate tablet brands available in the local market.

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Table UNIFORMITY OF WEIGHT RESULT FOR ARTESUNATE

APPENDIX

26: TEST

TABLET FA Average weight (t) = 0.2892g.

	Weight (g) (t)	Deviation (x-t)	% deviation (x-t/t x 100
1	0.2835		1.97
2	0.2938	0.0046	1.59
3	0.3057	0.0165	5.70
4	0.2866	-0.0026	0.89
5	0.2902	0.0001	0.03
6	0.3034	0.0142	4.91
7	0.2896	0.0004	0.14
8	0.2854	-0.0038	1.31
9	0.2817	-0.0075	2.59
10	0.2920	0.0028	0.97
11	0.2849	-0.0043	1.49
12	0.2967	0.0075	2.59
13	0.2922	0.0003	1.04
14	0.2944	0.0052	1.79
15	0.2866	-0.0026	0.90
16	0.2589	-0.0303	4.47
17	0.2997	0.0105	3.63

Table UNIFORMITY OF WEIGHT TEST RESULT FOR ARTESUNATE

			(t) =
18	0.2864	-0.0028	0.97
19	0.2957	0.0065	225
20	0.2888	-0.0004	0.14

27:

TABLET FB

Average weight 0.3516g.

	Weight (g) (t)	Deviation (x-t)	% deviation x-t/t x 100
1	0.3507	-0.0009	0.26
2	0.3525	0.0009	0.26
3	0.3560	0.0044	1.25
4	0.3505	-0.0011	0.31
5	0.3498	-0.0018	0.51
6	0.3538	0.0022	0.66
7	0.3534	0.0018	0.51
8	0.3564	0.0048	1.36
9	0.3560		1.25
10	0.3505	-0.0011	0.31
11	0.3505	0.0011	0.31
12	0.3491	-0.0025	0.71

Table UNIFORMITY OF WEIGHT TEST RESULT FOR ARTESUNATE

			(t) =
13	0.3550	0.0034	0.97
14	0.3536	0.0002	0.57
15	.3554	0.0038	1.08
16	0.3497	-0.0019	0.54
17	0.3546	0.0003	0.09
18	0.3517	0.0001	0.03
19	0.3610	0.0094	2.67
20	0.3538	0.0022	0.63

28:

TABLET FC Average weight oftablets 0.3448g.

	Weight (g) (t)	Deviation (x-t)	% deviation x-t/t x 100
1	0.3464	0.0016	
2	0.3448	0	
3	0.3475	0.0027	0.78
4	0.3515	0.0067	1.94
5	0.3495	0.0047	1.36
6	0.3421	-0.0027	0.78
7	0.3434	-0.0014	
8	0.3408	-0.0004	1.16
9	0.3468	0.0002	0.58
10	0.3470	0.0022	0.64
11	0.3448		

Table UNIFORMITY OF WEIGHT TEST RESULT FOR ARTESUNATE

			(t) =
12	0.3464	0.0016	0.46
13	0.3515	0.0067	1.94
	0.3475	0.0027	0.78
15	0.34	-0.0027	0.78
16	0.3495	0.0047	1.36
17	0.3408	-0.0004	0.12
18	0.3434	-0.0014	0.41
19	0.3470	0.0022	
20	0.3468	0.0002	0.06

UNIFORMITY OF WEIGHT TEST RESULT FOR ATRESUNATE

(t) =

Table29:

TABLET FD Average weight of tablet 0.2692g.

	Weight (g) (t)	Deviation x-t	% Deviation x-t/t x 100
1	0.2714	0.0022	0.82
2	0.2712	0.0002	0.07
3	0.2682	-0.0010	0.37
4	0.2726	0.0034	1.26
5	0.2998	0.0306	1.37
6	0.3619	0.0927	4.44
7	0.2661	-0.0031	1.15
8	0.2613	-0.0079	2.9
9	0.2716	0.0024	0.89
10	0.2701	0.0009	0.33
11	0.2680	-0.0012	0.45
12	0.2730	0.0038	1.41
13	0.2714	0.0022	0.82
14	0.2700	0.0008	0.30
15	.2671	-0.0021	0.78
16	0.2699	0.0007	0.26
17	0.2718	0.0026	0.97
18	0.2736	0.0044	1.63
19	0.2633	-0.0059	2.19
20	0.2773	0.0081	3.01

Table 30:

TABLETS FE

Average weight 0.3088g.

	Weight (g) (x)	Deviation (x-t)	% deviation x-t/t x100
1	0.3346	0.0258	8.35
2	0.3279	0.0191	6.19
3	0.3138	0.0050	1.62
4	0.2960	-0.0128	4.15
5	0.3073	-0.0015	0.49
6	0.3108	0.0020	0.65
7	0.3074	-0.0014	0.45
8	0.3271	0.0183	5.92
9	0.2949	0.0139	4.50
10	0.3040	-0.0048	1.55
11	0.3145	0.0057	1.85
12	0.2966	-0.0122	3.95
13	0.3095	0.0007	0.23
14	0.3028	-0.0006	1.94
15	.3049	-0.0039	1.26
16	0.3024	-0.0064	2.07
17	0.3244	0.0156	5.05
18	0.2965	-0.0123	3.98
19	0.3258	0.0170	5.51
20	0.2953	-0.0135	4.37

HPLC Chromatogram of the five Artesunate tablet brands

UNIFORMITY OF WEIGHT TEST RESULT FOR ATRESUNATE

(t) =

Fig 13: HPLC Chromatogram of Brand FA.

Fig 14: HPLC Chromatogram of FB

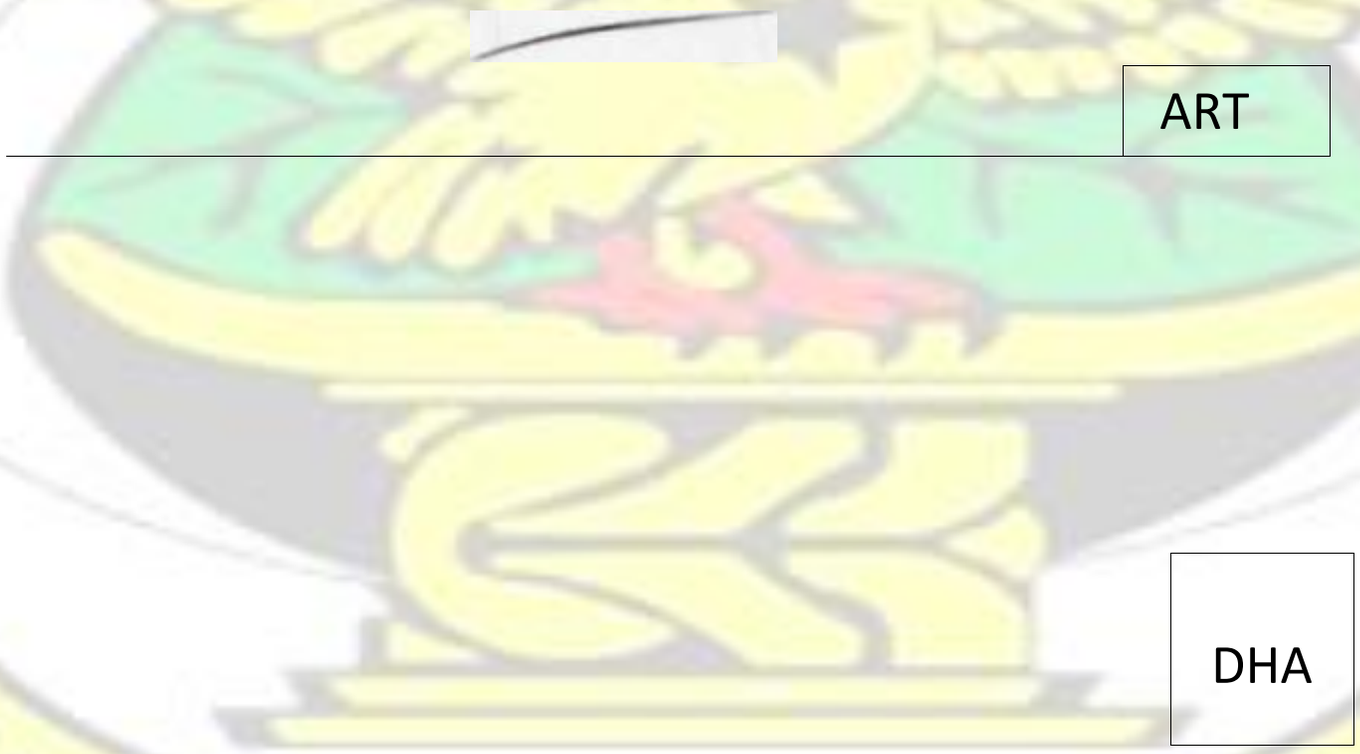

Fig 15: HPLC Chromatogram of FC

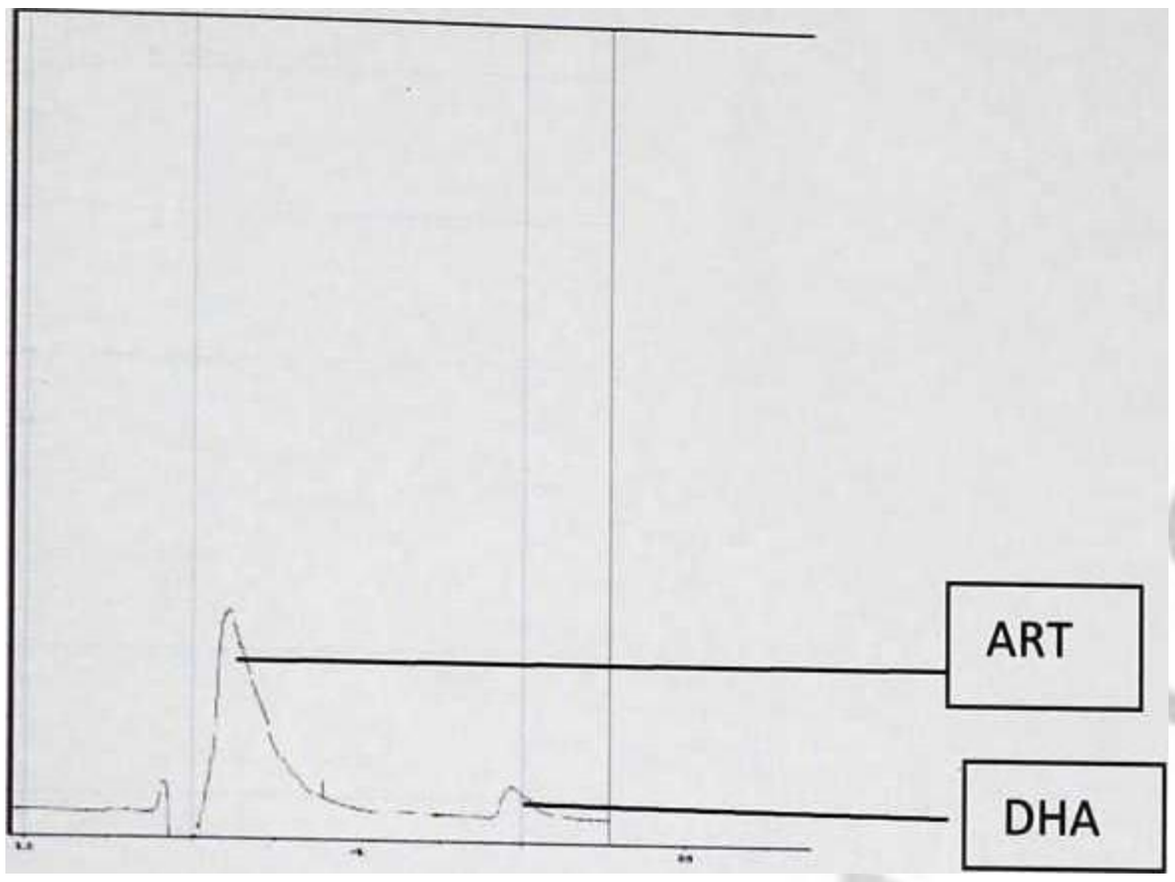

Fig 16: HPLC Chromatogram of FD.

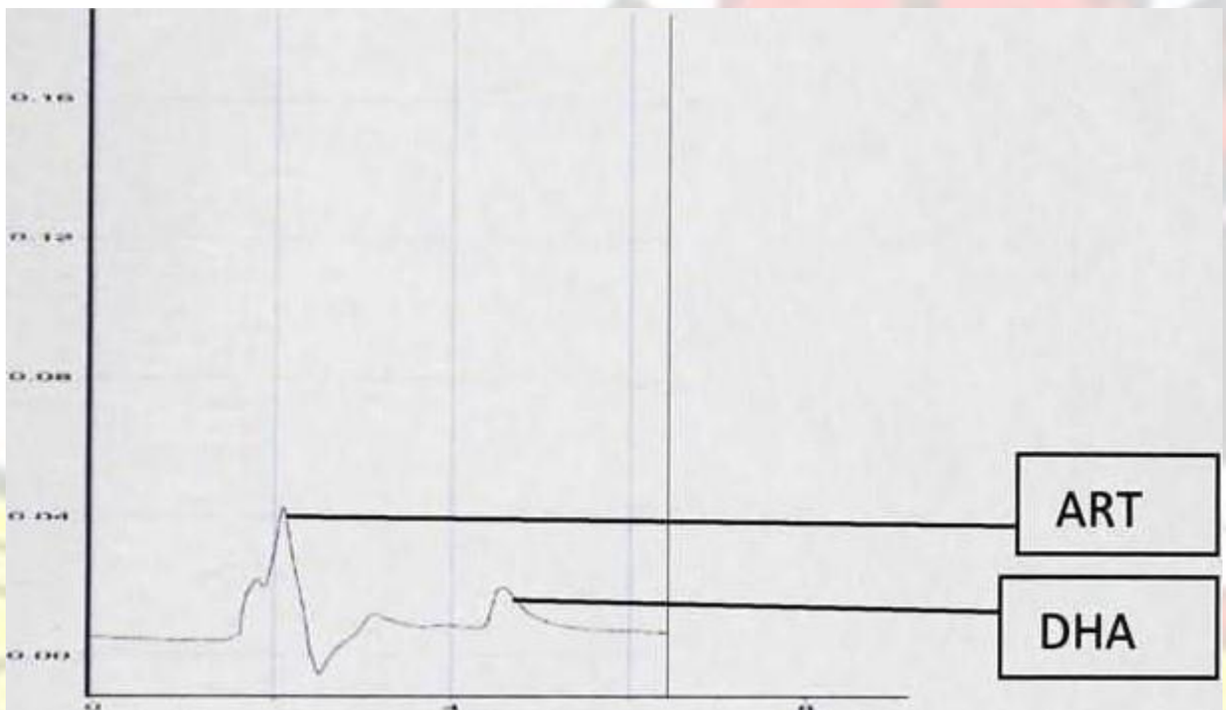

Fig 17: HPLC Chromatogram of FE

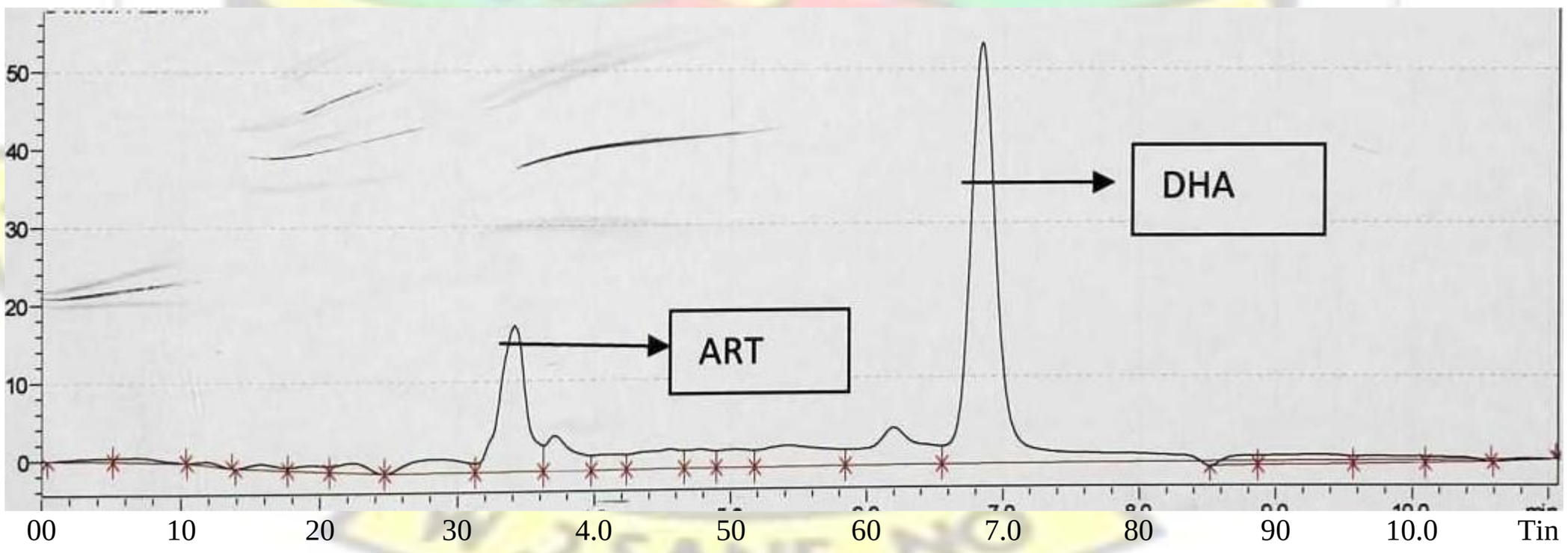

Fig 18: HPLC chromatogram of a mixture of pure ART and DHA