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POST MARKETING SURVEILLANCE OF ACTIVE PHARMACEUTICAL INGREDIENTS (API) IN ANTIMALARIAL DRUGS USED IN MALAWI

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IBRAHIM CHIKOWE, BSc (Ed).

(10361119)

DEPARTMENT OF CHEMISTRY

UNIVERSITY OF GHANA,

LEGON

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DECLARATION

I, **Ibrahim Chikowe**, declare that the work contained in this thesis has been undertaken at the University of Ghana-Legon campus in the Chemistry Department under supervision and has never been presented for any other degree elsewhere.

…………………………………………

Ibrahim Chikowe

(Student)

……………………………..

Prof. Ivan Addae-Mensah

(Supervisor)

………………………………...

Dr. (Mrs.) Dorcas Osei-Safo

(Supervisor)

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May Almighty God Bless You All.

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DEDICATION

This project is dedicated to Me, My Father and My Mother.

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ABSTRACT

The use of poor quality antimalarials causes low bioavailability of the active pharmaceutical ingredients (APIs) to the drug targets resulting in treatment ineffectiveness or failure and parasite resistance over a short period of drug usage. Resistance has rendered the hitherto cheap and effective drugs like chloroquine and sulphadoxine/pyrimethamine ineffective, resulting in their replacement with the more expensive artemisinin-based combination therapy (ACT) for malaria treatment. The high cost of the ACTs has made them attractive to counterfeiters leading to the proliferation of poor quality antimalarials on the drug markets. One hundred and twelve (112) samples of antimalarial drugs were purchased from licensed and unlicensed markets in all parts of Malawi. Samples were subjected to visual inspection of dosage form and packaging, registration verification with the Pharmacy, Medicines and Poisons Board of Malawi, basic tests and semi-quantitative thin layer chromatography (SQ-TLC) and HPLC tests to quantify the APIs in the samples and compare with pharmacopoeial specifications and the manufacturers' claims. The results showed an 85 % registration status with all samples purported to be imported and 100% compliance with visual inspection requirements and basic tests confirming the presence of requisite APIs. The results of the SQ-TLC showed that 4.88% of artemether/lumefantrine (Atm/Lum) FDC samples were compliant with pharmacopoeial specifications, 2.44% were borderline compliant and a further 92.68% were non-compliant (48.15% of Atm component was overdose and 51.85% under dose; 57.14% of Lum component was overdose and the remaining 42.86% under dose). The HPLC results confirmed this result with 4.88% found to be compliant and 95.12% non-compliant (48.15% overdose and 51.85% under dose for Atm component; 50% overdose and 50% under dose for Lum component). SQ-TLC tests on artesunate/sulphadoxine/pyrimethamine (Ats/SP) samples showed that 77.78% were non-

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compliant all of which were under dose, while 22.22% were borderline compliant. However, the HPLC results showed a compliant percentage of 11.10% and 88.90% non-compliance (under dose). The dihydroartemisinin/piperaquine phosphate (Dha/Pp) samples recorded SQ-TLC percentage of 42.86% being compliant, 14.29% borderline compliant and 42.86% non-compliant with all the non-compliant quantities of the components found under dose. With HPLC, they were found to be 28.57% compliant, 7.14% borderline compliant and 64.29% non-compliant (under dosed components). Dihydroartemisinin/sulphadoxine/pyrimethamine (Dha/SP) samples were found to be 100% non-compliant for both SQ-TLC and HPLC tests. The quantities of the APIs in the non-compliant Dha/S/P samples fell within the range of 51% and 84%. Samples containing sulphadoxine/pyrimethamine (SP) were found to be 4.35% compliant, 8.70% borderline compliant and 86.95% non-compliant using SQ-TLC method (25.00% of the non-compliant pyrimethamine component overdose, 75.00% under dose and 100.00% of sulphadoxine under dose). With HPLC, half (50.00%) of the samples that were borderline compliant were found to be non-compliant with the remaining half being compliant giving rise to the following results: 8.70% and 91.30% compliant and non-compliant respectively with all non-compliant sulphadoxine and 72.73% of pyrimethamine being under dose and 27.27% of the quantities of pyrimethamine being overdose. None of the quinine samples were compliant with SQ-TLC (28.57% borderline compliant, 71.43% non-compliant and overdose). HPLC analysis found 30.77%, 15.38% and 53.85% to be compliant, borderline compliant and non-compliant (85.71% overdosed) respectively. Generally, 85.71% failure rate was found arising from Atm/Lum (95.12%), Dha/P (64.30%), Dha/SP (100.00%), SP (91.30%), Ats/SP (88.90%) and quinine (53.80%) failure rates indicating wide spread circulation of poor quality antimalarial drugs in Malawi.

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LIST OF ABBREVIATIONS

AA	Artesunate-Amodiaquine
ABC	Artemisinin-Based Combination
ACTs	Artemisinin-Based Combination Therapies
AD	Anno Domini
API	Active Pharmaceutical Ingredient
ATM	Artemether
ATS	Artesunate
CHAM	Christian Health Association of Malawi
DESI	Desorption Electro Spray Ionization
DHA	Dihydroartemisinin
2, 4-DNPH	2, 4-Dinitrophenylhydrazine
EDCTP	European and Developing Countries Clinical Trials Partnership
FDC	Fixed dose combination
GMP	Good Manufacturing Practice
GPHF	Global Pharma Health Fund
HCl	Hydrochloric acid
HIV	Human Immunodeficiency Virus
HoD	Head of Department
HPLC	High Pressure Liquid Chromatography
INN	International Non-proprietary Name
IPTp	Intermittent Preventive Treatment of Pregnant women
IRS	Indoor Residual Spraying

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ITN	Insecticide Treated Net
KI	Potassium Iodide
LC-MS	Liquid Chromatography-Mass Spectroscopy
LUM	Lumefantrine
NABC	Non-Artemisinin -Based Combination
NMCP	National Malaria Control Programme
Ph. Int.	International Pharmacopoeia
PPQ/PP	Piperaquine
QAMSA	Quality of selected Antimalarial Medicines circulating in Six countries of Sub-saharan Africa
Rf	Retardation Factor
RS	Reference Standard
SP	Sulphadoxine-Pyrimethamine
SQ-TLC	Semi-Quantitative Thin-Layer Chromatography
TLC	Thin-Layer Chromatography
TS	Test Solution
USAID	United States Agency for International Development
USP	United States Pharmacopoeia
UV	Ultraviolet
VS	Volumetric Solution
WHO	World Health Organization

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CHAPTER ONE

1 INTRODUCTION

1.1 WHAT IS MALARIA?

Malaria was alleged to be caused by bad air (*mal'aria* in Italian) from which its name was derived. This allegation was held until the 1880s and 1890s when its cause was associated with a parasite and its transmission linked to mosquitoes by Alphonse Laveran, Battista, Ronald Ross and others [1].

Malaria is an infectious disease caused by a protozoon of the subphylum sporozoa of the genus *Plasmodium* and the species *falciparum, malariae, vivax* and *ovale* [2]. Molecular evidence has further shown two subspecies in *P. ovale* and one of them, *P. knowlesi*, is also capable of infecting humans through zoonosis, but it is mostly misdiagnosed as *P. malariae* [3, 4]. The *Plasmodia* are spread by an infected female anopheles mosquito that bites late at night and early morning [4]. Nevertheless, most malaria associated deaths are caused by *P. falciparum* and *P. vivax*, with the former accounting for 1 million deaths per annum; about 90% of all malarial deaths and approximately 250 million morbidity rates per year [3].

Malaria infection has several devastating physiological effects like anaemia and jaundice. *P. falciparum* infection causes many dangerous malaria cases and may further cause kidney failure, acidosis, cerebral malaria, kidney damage, multifunction failure, severe anaemia, spleen rupture, coma and death if not treated quickly and effectively [4, 5].

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1.2 OVERVIEW OF THE MALARIA SITUATION

Malaria is a disease that is still claiming millions of lives worldwide. Approximately 3.3 billion people were affected by the disease worldwide in 2010. Out of 216 million clinical episodes due to malaria reported in that year, 655,000 resulted in deaths. More deaths were recorded in Africa (~91%) compared to South–East Asia region (6%) and the East Mediterranean region (3%). Children accounted for up to 86% of these deaths globally, most probably due to their relatively low level of immunity which makes them easily overwhelmed by the infection if not promptly treated [3, 6]. These results indicate that the disease remains the main killer in Africa and its children are the most vulnerable [4].

This stunning death rate has been attributed mainly to the rapid development of resistance by malaria parasites to the conventional antimalarial drugs such as chloroquine, quinine and sulphadoxine/pyrimethamine FDC [7]. Currently, a new drug regimen called the artemisinin-based antimalarials has been developed, introduced and deployed to counterattack this resistance. That has seen the subsequent introduction of the new, more potent and prevalent artemisinin-based combination therapies (ACTs) in which an artemisinin-based compound is formulated in combination with a non-artemisinin antimalarial. These newly WHO-recommended first line therapy antimalarial drugs for uncomplicated and unconfirmed malaria have given such a good hope to the fight against malaria due to their ability to affect the *Plasmodia* at several stages of their life cycle, which affects their gametocyte presence and infection [8]. Since the ACTs are mainly aimed at uncomplicated malaria, the other drug types are still used for other serious and special cases of malaria [6]. However, the effective use of these newly introduced drugs is mainly hampered by the presence and use of substandard and fake drugs such that even this therapeutic

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line is equally threatened by parasite resistance. It is disappointing and frightening to note that some of these substandard drugs are produced even by the certified companies believed to be committed to good manufacturing practices (GMP) [9].

1.3 MALARIA IN MALAWI

1.3.1 Malaria Situation

In the Malawi Ministry of Health World Malaria Day communiqué of 2012, it was reported that for every 1000 people, 325 have a medical condition of malaria [10]. An estimated 30% of the outpatients treated at health facilities have malaria-related infections and it is estimated that 40% of children die of diseases related to malaria [11, 12]. Malaria suspected cases in Malawi have been on the increase since 2005. As a matter of illustration, 3.7 million cases were registered in 2005 and the number almost doubled to 6.1 million cases in 2009 of which 50% of these cases were reported in children alone [13].

In a 2010 Malawi National Malaria Indicator Survey in which Slide microscopy was used, a national parasite occurrence rate of 43.3% was found with vulnerability to parasite being found to be increasing with aging. In addition, severe anaemia study conducted simultaneously showed that it was prevalent in 12.3% of children under 5 years with an average HB concentration of less than 8g/dL, with vulnerability to severe anaemia found to be diminishing with aging. In addition, the study found that children who did not sleep under the ITN were more prone to severe anaemia and malaria parasite, with the poor contributing much to the parasite and anaemia prevalence [14]. In a related study, Harms and Feldmeier in Kabuluzi *et al.* reported that 66% of pregnant women were found anaemic and this condition was mainly attributed to malaria.

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Furthermore, the results showed that malaria parasitaemia contributed significantly to the rampant vitamin A deficiency in 44% and 70% of pregnant women and pre-school children respectively (MOH/CDC/UNICEF, 2001 in Kabuluzi *et al.,* 2004) [15].

Malaria in Malawi is controlled by chemotherapy for infection prevention and treatment, with artemether-lumefantrine being the first line treatment for uncomplicated and unconfirmed cases since 2007 after replacing SP that also replaced chloroquine due to parasite resistance. However, antimalarial drugs like quinine, SP and others are still being used for special cases. Sleeping under insecticide treated net (ITN) or long lasting insecticide-treated nets (LLITN) and indoor residual spraying (IRS) are used for vector control [13, 16]. However, failure to significantly adhere to treatment and policies has been an area of concern in an effort to implement malaria interventions effectively [14].

1.3.2 Distribution of Malaria

Inhabitants are infected throughout the year in all parts of the country with highest transmission cases recorded in the low-lying areas during the rainy season. The areas with repeated high infection rates are those with high temperatures notably the lower Shire valley and lakeshore areas except mountainous parts of the North and South [13]. Malaria is amongst the diseases that are commonest amongst the poor people [14], who constitute 54% of the population and 65% of the population living on less than a dollar per day [13] and this makes most Malawians not able to access prompt malaria treatment within 24 hours of observing the infection symptoms as reported by UNICEF, which worsens the burden of this preventable and treatable disease [10].

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1.4 PREVENTION OF ANTIMALARIAL DRUG RESISTANCE

After a thorough understanding of available malaria treatment, most of the drugs have faced significant rates of resistance and the only hope for future malaria treatment currently rests on artemisinins even though other regimens are still in use. Therefore, it is imperative to protect these drugs from any impending drug resistance and this can be achieved by among others, proper use of good and effective quality ACTs and a relentless combat against the use of poor quality drugs as well as strict patient compliance to treatment regimen [17].

1.5 STATEMENT OF THE PROBLEM

No other disease has killed more human kind than malaria [18]. With the widespread development of resistant malarial parasites to widely used antimalarial drugs, various efforts have been suggested by WHO and its partners for malaria control. These recommendations have been duly adopted by governments and one such measure is the use of artemisinin-based combination drugs as first-line prescription for the treatment of malaria. However, most countries including Malawi import most of these drugs. With the high demand, cost of production and prices of these drugs, a significant number of fake and sub-standard drugs have been imported and/or produced locally. This has called for routine quality control activities to check this malpractice.

However, national drug regulatory authorities in most developing countries like Malawi have not been able to conduct these crucial routine quality assessment activities to curb this rapid inflow of these unwanted drugs, due to the lack of the necessary state-of-the-art tools and qualified personnel. The National Malaria Control Programme (NMCP) points out that the quality control system for laboratories in Malawi is weak and it is an area of concern that needs a prompt

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attention. Therefore, this research was aimed at fulfilling and supplementing the drug regulatory efforts as outlined by the WHO Expert Committee on Quality Assurance of Medicines [13].

1.6 AIM AND OBJECTIVES OF THE PROJECT

The research was aimed at finding out if the antimalarial drugs used in Malawi comply with the WHO accepted standards for active pharmaceutical ingredients (APIs) in ACTs and Non-ACTs. To achieve this aim, the following specific objectives were set:

i. To find out the registration status of the antimalarial drugs available on the markets.

ii. To visually inspect dosage forms and packaging using the guidelines outlined in the WHO pharmacopoeia and the literature or otherwise.

iii. To carry out a qualitative determination to establish the presence or otherwise of the active pharmaceutical ingredients using the authenticated rapid tests outlined in WHO publications.

iv. To carry out a quantitative determination of the API content using SQ-TLC and HPLC.

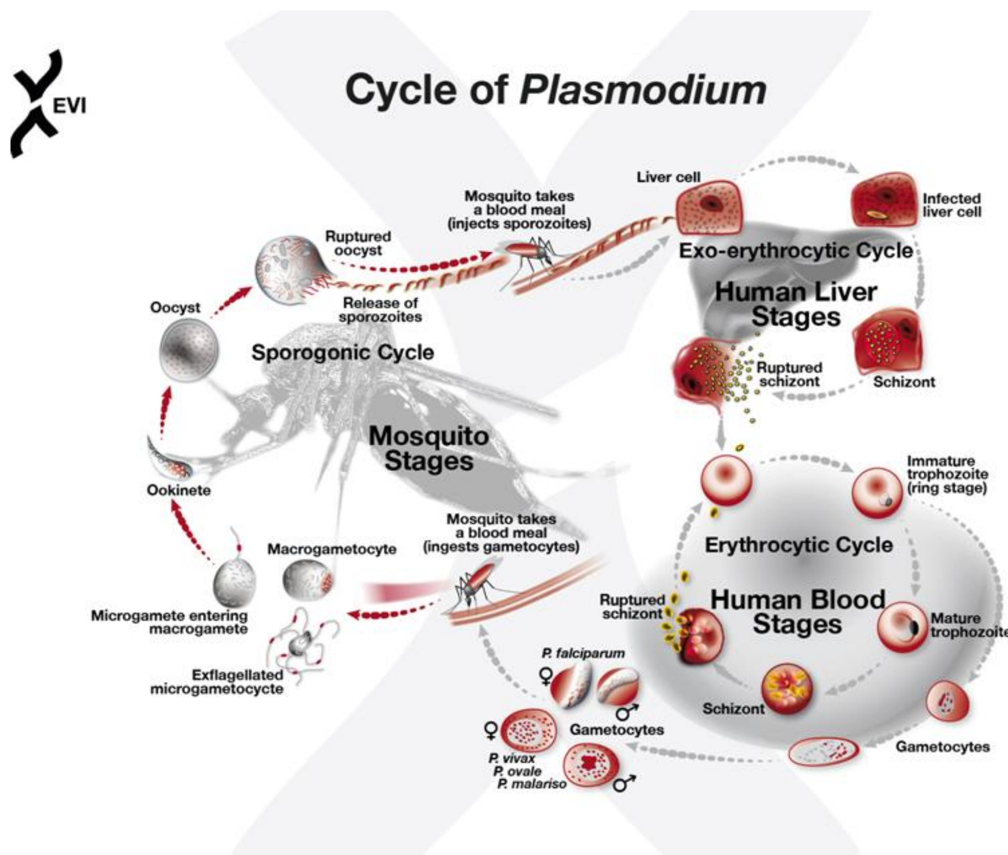
v. To further validate the newly developed methods and their adoption for analyzing antimalarial drugs used in Malawi.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 LIFE CYCLE OF THE MALARIA PARASITE - *PLASMODIUM*

The life cycle of malaria parasites is hosted by female anopheles mosquito (primary host) that harbours the sexual cycle, and humans (secondary host) that host the asexual cycle (Figure 1) [19].



Source: European Vaccine Initiative (EVI) [20]

Figure 1: **Life cycle of *plasmodium***

Malaria infection in humans starts with an exo-erythrocytic stage where an infected adult female *anopheles* mosquito bites in search of food and in the process, inoculates some sporozoites into the bloodstream of humans and some other mammals [2]. The sporozoites then invade, penetrate

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and localize in the liver tissues and subsequently infect the liver cells. The sporozoites start growing in the liver followed by asexual reproduction, budding into schizonts (tissue schizonts) that contain merozoites. Upon maturity, the schizonts burst open releasing the matured merozoites [19]. Some merozoites enter the bloodstream to start the erythrocytic stage while others may re-infect the liver initiating the secondary tissue stage [2], where they evolve to dormant hypnozoites in the liver especially in infections by *P. vivax* and *P. ovale* species leading to long incubations and late relapses [3, 4].

The merozoites in the bloodstream attack the erythrocytes (red blood cells) to consume some of the haemoglobin and develop into immature trophozoites. When the trophozoites mature, they form blood schizonts within which merozoites develop through asexual reproduction. A burst of the schizonts releases more merozoites into the bloodstream repeating the infection and multiplication cycle. Recurrence of the cycle persists unless it is sorted out by either the immune system or an antimalarial drug [21] and this is responsible for the malarial symptoms like fever and chills [2, 3]. This process also accounts for anaemia and HB concentration decrease. While some merozoites repeat the erythrocyte invasions, others differentiate to male and female gametocytes. When a female anopheles mosquito bites, it is infected with the gametocytes from the bloodstream that evolve into sporozoites which discharge into the salivary glands of the mosquito; a subsequent bite by the mosquito re-starts the cycle [2, 4].

2.2 BRIEF HISTORY OF ANTIMALARIAL DRUGS

Cinchona bark and Wormwood (Qinghao) had been used effectively as herbs in treating fevers several hundred years prior to the discovery of the mosquito cycle in the early 20th century. The

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mosquito cycle breakthrough brought novel methods in vector control. Later, pure chemical compounds and subsequent synthetic drugs overshadowed herbal materials. These two ancient natural treasures still live today as quinine and artemisinin extracted from cinchona bark and qinghao respectively and are still the prime antimalarials in use today [1].

2.2.1 Quinine and Its Derivatives

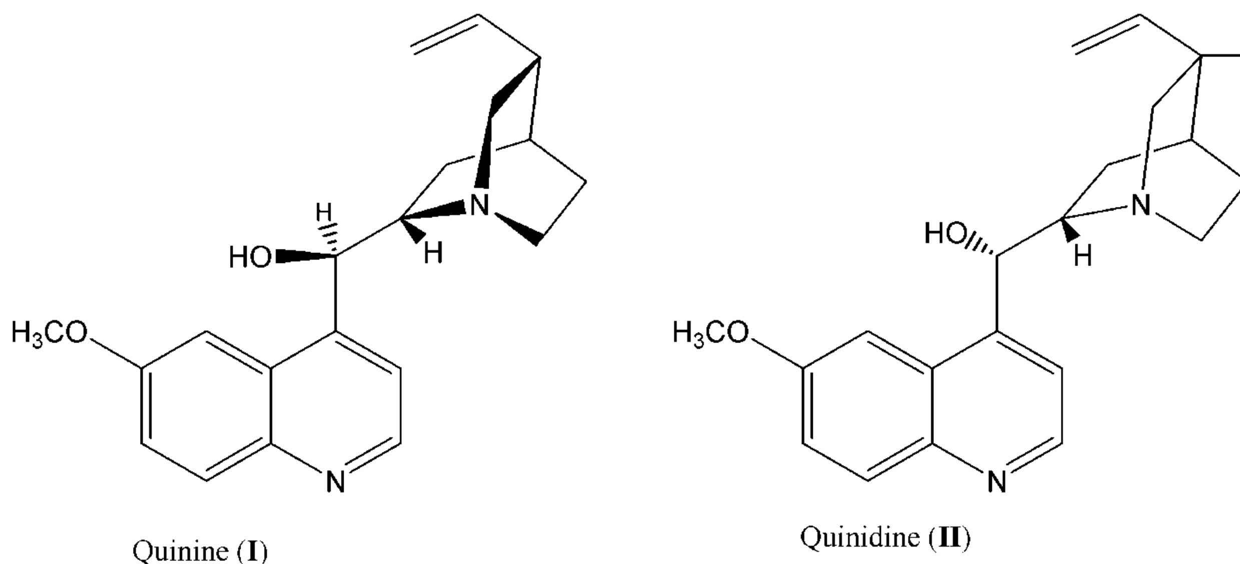

Figure 2: Structures of the first pure compounds for malaria treatment

The alkaloids quinine and cinchonine were isolated from Cinchona bark in 1820 by two French chemists, Pierre Pelletier and Joseph Caventou [1]. The name quinine was derived from quina-quina (bark of barks), an Inca word [3]. French physicians started administering pure quinine for intermittent fever patients, which turned out successful, thus putting malaria amongst the first diseases to be cured by a pure chemical compound.

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William Henry Perkins, an English chemist aged 18 had the first but unsuccessful attempt to synthesize quinine and the successful synthesis of quinine was eventually achieved in 1944, though not commercially viable. Perkins' failed attempt to synthesize quinine led to yet another important product, the first artificial textile dye called Mauveine (Methylene blue) that could not be washed by water, which later promoted the development of medicine. It had research application in microbiology and enabled microbiologists see microbial pathogens under microscope after staining them with the dye, a phenomenon that was not possible prior to the dye discovery [1].

Later, a German scientist, Paul Ehrlich found that malaria parasites were also successfully stained by the dye, also known as methylene blue and reasoned that it would eventually poison the parasites *in vivo*. In 1891, Ehrlich used synthetic drugs in humans for the first time in history when he used methylene blue to cure two patients of malaria based on his reasoning, making malaria the first disease to be treated by a synthetic compound. Methylene blue was later used as a prototype for the development of novel synthetic antimalarial drugs [1]. Mepacrine was produced for *P. falciparum* in 1932 by the Germans for their combat soldiers who were affected by malaria in earlier battles in Europe. Later, mepacrine (atebrine) manufacturing was extended to the USA, with chemical intermediates from Germany [18]. In 1925, the first 8-aminoquinoline plasmoquine (pamaquine) was developed for the prevention of *P. vivax* malaria relapses [1]. In 1934, chloroquine synthesis began indirectly by H. Andersag and by 1946, US clinical trials proved the superiority of chloroquine to atebrine (mepacrine). Eventually, chloroquine was recognized as a powerful antimalarial drug and was extensively used worldwide. In the 1950s and 1960s, WHO recommended chloroquine as the main choice for its Global Eradication

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Programme. Below is Figure 3 illustrating the development of various antimalarial drugs from the prototype methylene blue [22].

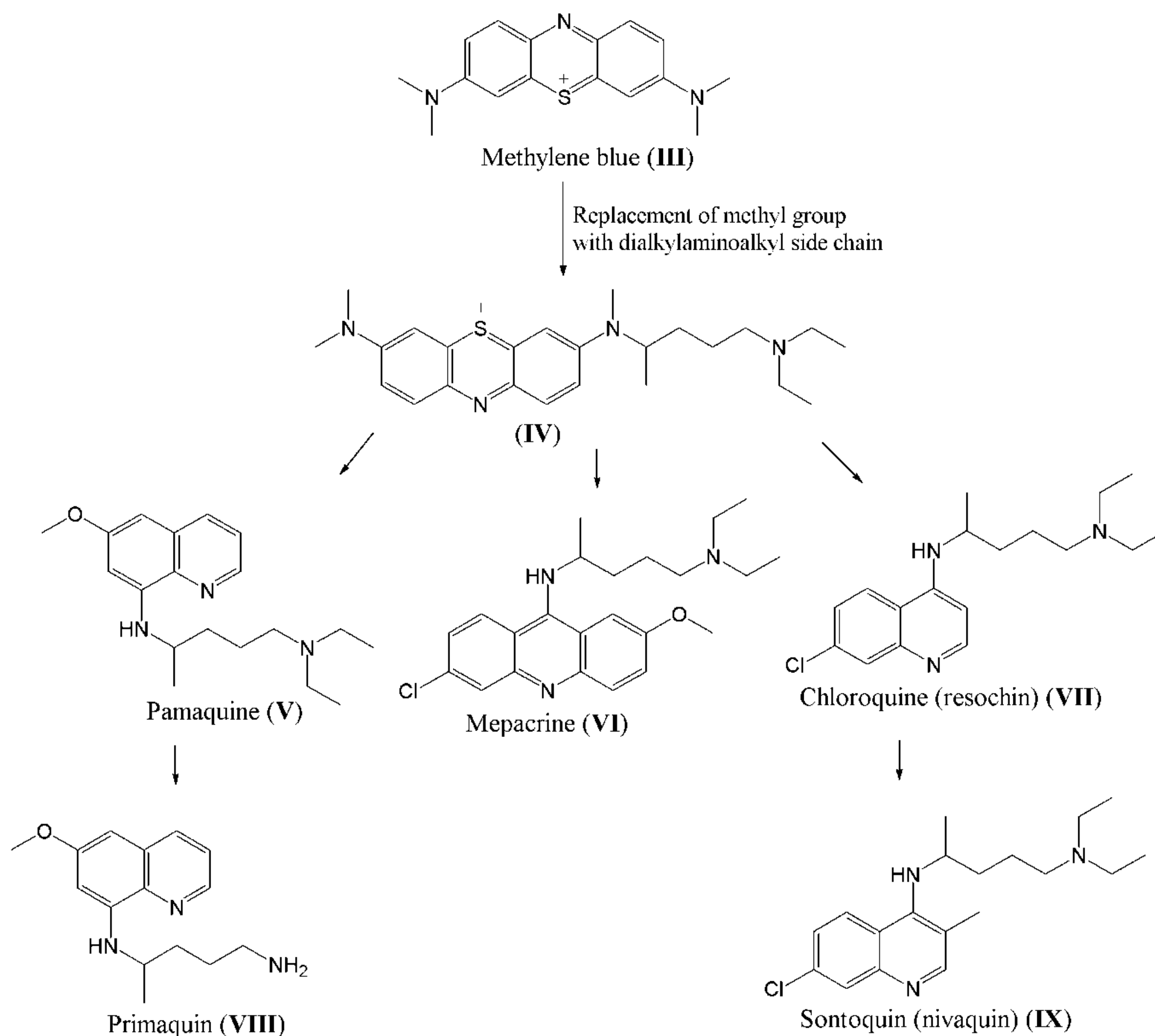

Figure 3: Development of various antimalarial drugs from methylene blue.

Antifolates were developed after the 1940s, following detailed studies of bacterial systems and this breakthrough helped in the development of drugs such as proguanil, a prodrug that metabolizes to active cycloguanil *in vivo*, pyrimethamine, sulphonamides such as sulphadoxine and sulphones like dapsone [3]. In the 1970s, pyrimethamine was combined with sulphadoxine

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for synergistic purpose, commercially available as Fansidar and it is still in use today in some parts of Africa [1].

Post-war efforts by American scientists have led to improvement of plasmoquine to the more effective primaquine as a standard drug for *P. vivax* malaria relapse prevention. Some drugs have been used as prototypes, such as SN10275 for mefloquine and 2-hydroxynaphthoquinones for atovaquone. Other drugs include WR 238605 and tafenoquine [1]. Since 1980, several drugs have been developed, while others are still underway all for purposes of malaria control and most notable ones are dapsone, mefloquine, halofantrine, doxycycline, primaquine and the artemisinins [23].

2.2.2 Artemisinin and Derivatives

The Chinese herbal medicine practitioners originally used *Artemisia annua* (Qinghao) for treating haemorrhoid over at least 2000 years [1]. Qinghao therapy dates back to two prominent ancient physicians; Ge Hong and Li Shizhen who used it separately within 283-343 AD and 1518-1593 AD periods respectively [24, 25]. Ge Hong assembled and studied various herbal prescriptions and went further to author a book titled *Zhou Hou Bei Ji Fang (Handy Therapies for Emergencies)* where his prescriptions were coded. Li Shizhen also authored a book that had monographs of each herb or medicinal matter called *Bencao Gangmu (Great Compendium of Herbs)* that was published after his death [24].

In 1967, the Mao-led Chinese government decided to professionalize traditional medicine in a multi-disciplinary programme called Project 523. In the early 1970s, colourless pure crystals that

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were active against plasmodia in animal models were extracted from *A. annua*. The extract was called Qinghaosu or artemisinin [18]. Later, clinical trials of artemisinin showed that artemisinin was effective against chloroquine-resistant *P. falciparum* coupled with quick action and low toxicity. Structure elucidation of artemisinin revealed that it was a sesquiterpene lactone with the formula $C_{15}H_{23}O_5$ and the chemical structure was found to have features uncommon to natural products, i.e. three fused rings; one ring having seven atoms and one ring spanned by a peroxide bridge (-O-O-) that could be broken easily (Figure 4) [24].

Artemisinin has rapid action, short half-life (1-3hrs), low toxicity and effective against antifolate and quinoline-resistant *P. falciparum* [24]. It is this rapid action that causes a prompt treatment for severe infections [26]. However, it has low solubility in water and oil coupled with poor availability and these properties limit its therapeutic value along with effectiveness [24]. Thus, artemisinin extract has poor pharmacological properties and this is improved by modifying the sesquiterpene lactone endoperoxide, which increases cost of production [3]. In this process, artemisinin is chemically converted to dihydroartemisinin, followed by partly synthetic derivatives that are more effective such as artesunate, artemether and arteether, also known as first generation derivatives of artemisinin [24]. Firstly, artemisinin is reduced with sodium borohydride ($NaBH_4$) in the presence of methanol to produce dihydroartemisinin. Secondly, the derivatives are produced by other reactions as illustrated in Figure 4 below.

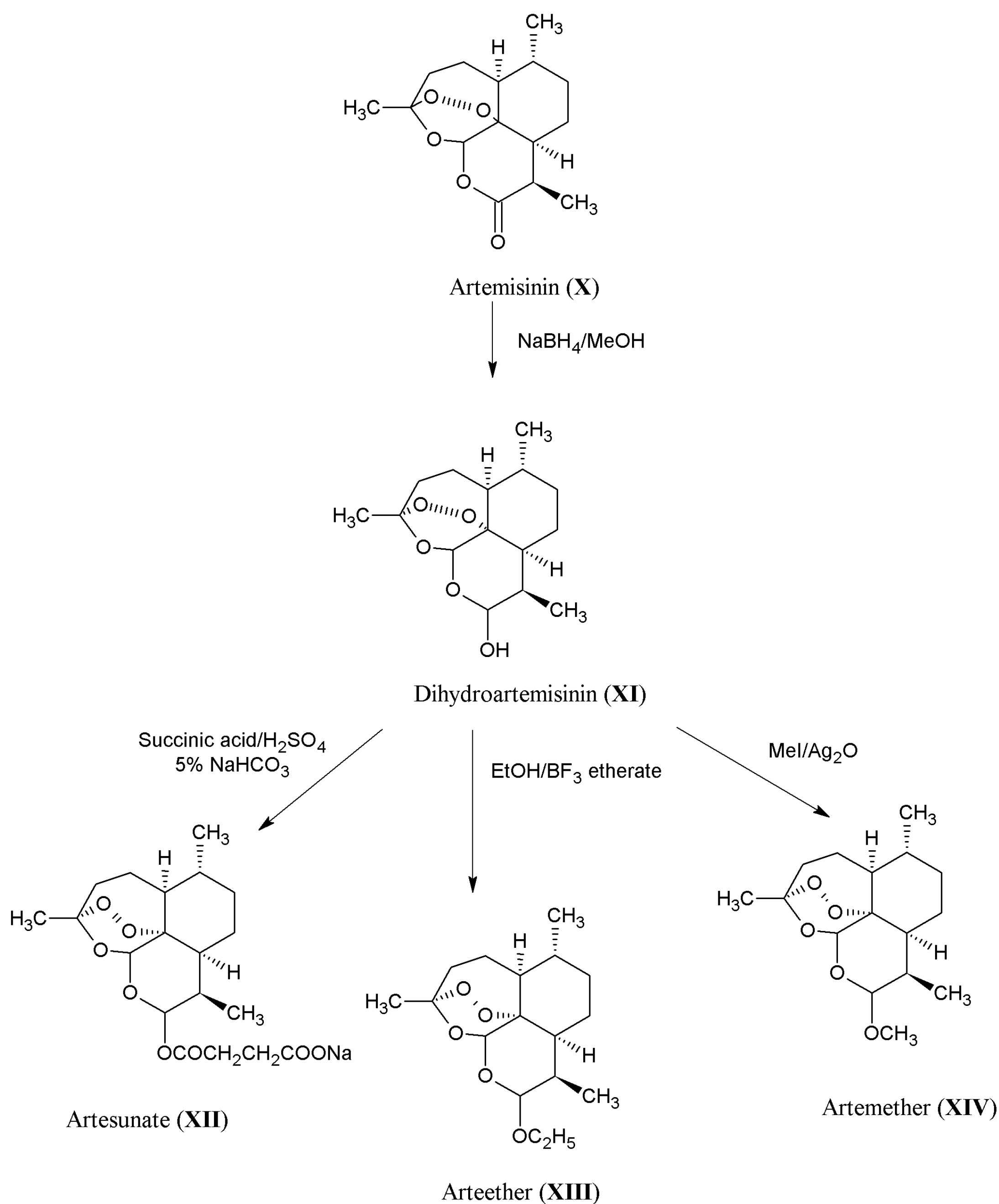

Figure 4: Conversion of artemisinin to its derivatives

Currently, the discovery, design and development of antimalarials have stalled due to the dwindling interest in antimalarial drug development by the pharmaceutical industry because of

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the high risk and low investment returns associated with antimalarials. Any persistence in the current *P. falciparum* resistance development will see malaria incurable in some parts of the malarious areas [27]. Hence, there is a great need to safeguard the present drugs against resistance through proper drug use, handling and checking against poor quality drugs.

2.3 FORMS OF ANTIMALARIAL DRUG COMBINATIONS

There are two forms of antimalarial drugs: artemisinin-based combination therapies (ACTs) and Non-artemisinin based combination therapies [28]. Table 1 below shows few examples of antimalarial drug combinations.

Table 1: Examples of ACTs and non-ACTs

Artemisinin-based combination therapy (ACTs)	**Non-ACTS**
Artesunate-amodiaquine	Sulphadoxine-pyrimethamine (SP)
Artesunate-mefloquine	SP-chloroquine
Artemether-lumefantrine	SP-amodiaquine
Artesunate-sulphadoxine-pyrimethamine	SP-mefloquine
Dihydroartemisinin-piperaquine+derivatives	Quinine-tetracycline-doxycycline
Dihydroartemisinin-sulphadoxine-pyrimethamine	
Artesunate-sulphamethoxypyridazine-pyrimethamine	

2.4 MEDICINAL CHEMISTRY OF ANTIMALARIAL DRUGS

2.4.1 Quinolines and Related Compounds

These compounds have a basic quinoline ring system and several compounds such as chloroquine, amodiaquine, primaquine, piperaquine, quinine, quinidine and mefloquine have been synthesized from the basic quinoline moiety of quinine (see Figure 5 for chemical

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structures of some quinolines). Related to the under listed drugs are halofantrine derived from phenanthrene ring system and lumefantrine from the fluorene ring [3].

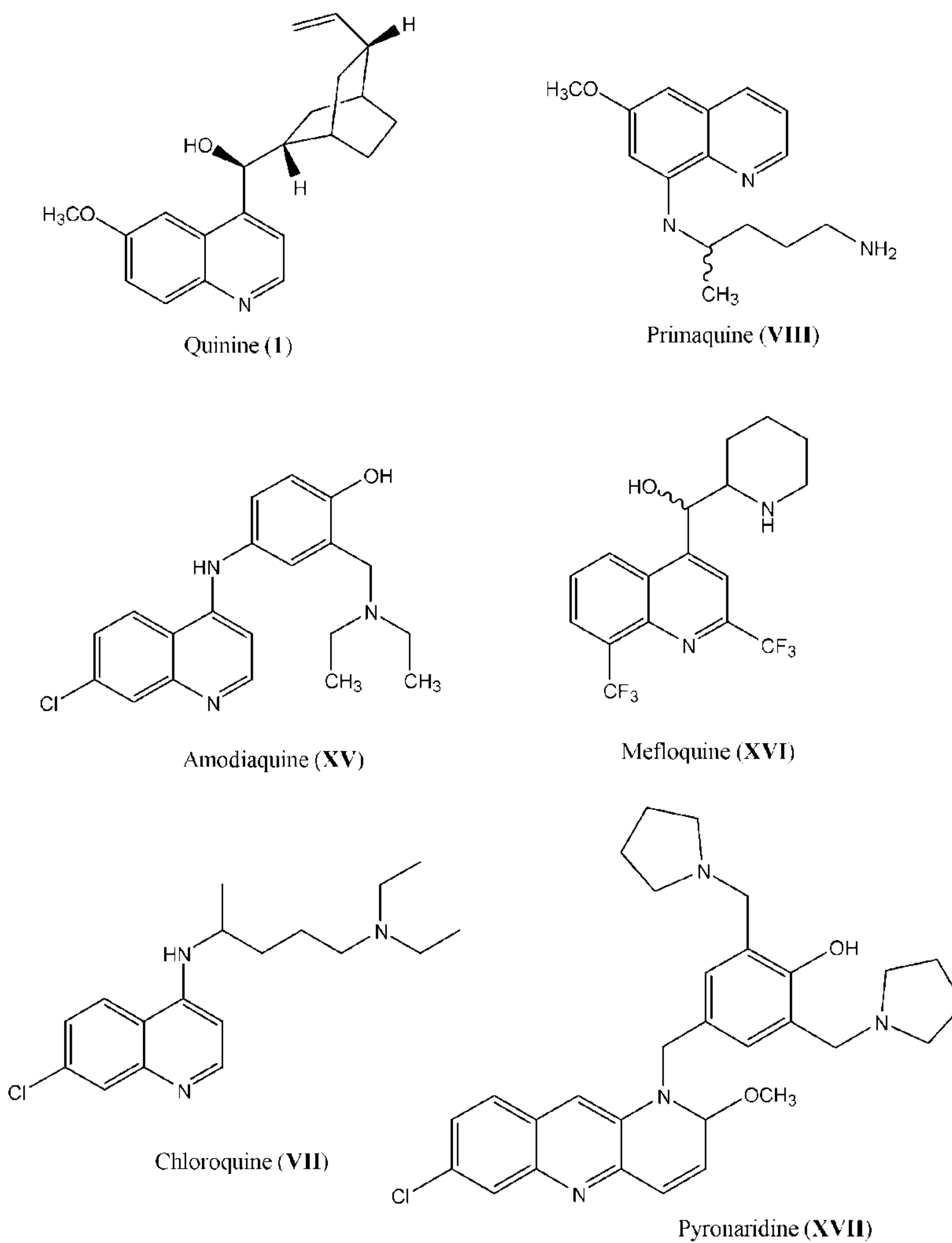

Figure 5: Chemical structures of some quinolines

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2.4.1.1 Chloroquine

It is a synthetic derivative of 4-aminoquinoline, a blood schizonticide and one of the longest serving synthetic antimalarials. The exact mode of action of chloroquine is not known, but several mechanisms have been proposed. *Plasmodium* survives on amino acids obtained from the digestion of haemoglobin and this process produces β-hematin dimer haeme that is toxic to the parasite. So a bio-mineralization process is conducted by the parasite to form a non-toxic complex called Hemozoin. Chloroquine and other quinolines are used to block this haeme elimination process by producing complexes with the haeme, thus depriving the parasites of dimerization and crystallization processes for detoxification, thereby causing accumulation of toxic haeme within them and subsequently poisoning to death. In addition, the creation of drug-haeme complex hampers the formation of peptides and this also reduces necessary amino acid supply to the parasites, threatening their capability [3]. Moreover, chloroquine interferes with the biosynthesis of nucleic acids thereby inhibiting the DNA and the RNA of the parasite [29].

However, chloroquine has faced widespread *P. falciparum* parasite resistance due to the mutation of its transporter (PfCRT), with only North Africa, Carribean region and Central America still using it. This resistance dates back to 1957 when it was first discovered along the Venezuelan-Colombian border and spread across sub-saharan Africa in the late 1970s and 1980s [3]. In Africa, the resistance started in the Eastern African region (e.g. Kenya, Tanzania, Uganda, Rwanda), but took quite a long time to spread to the West Coast. Chloroquine resistance in West Africa therefore became a major problem only during the latter years of the 20th century [30, 31, 32].

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2.4.2 Antifolates

These are antimalarial chemotherapeutic agents that act by competing with a natural substrate to suppress unpleasant body chemical reactions and they are called competitive (reversible) inhibitors. They are mostly structural analogues of their substrates. The Figure 6 below shows structures of some of these drugs. As competitive inhibitors, pyrimethamine and chloroguanil inhibit dihydrofolate reductase (DHFR) responsible for critical activities of bifunctional DHFR-thymidylate synthatase (TS) protein. This averts the biosynthesis of purines plus pyrimidines and eventually DNA synthesis, cell division as well as reproduction [3].

Sulphonamides like sulphadoxine and sulphamethoxypyridazine as well as sulpha drugs inhibit dihydropteroate synthase (DHPS) that is involved in the folate synthesis pathway and it is also crucial for the synthetic pathway of amino acids needed for parasite growth, thus preventing *p*-aminobenzoic acid (PABA) from converting to dihydrofolic acid and eventually tetrahydrofolate synthesis. For optimum efficacy against susceptible strains of malaria, sulphadoxine is combined with pyrimethamine in a product called Fansidar and many others for synergistic effect. However, the effectiveness of antifolates has been compromised by the emergence of the point mutation of the essential genes of the parasites; a form of mutation that occurs when a single base nucleotide is replaced by another nucleotide of a genetic matter, RNA or DNA [3].

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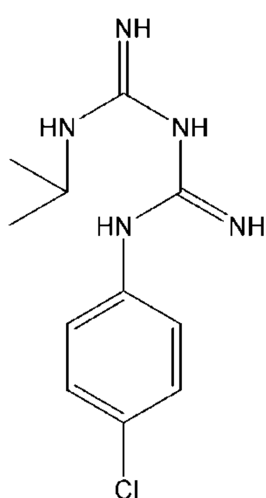
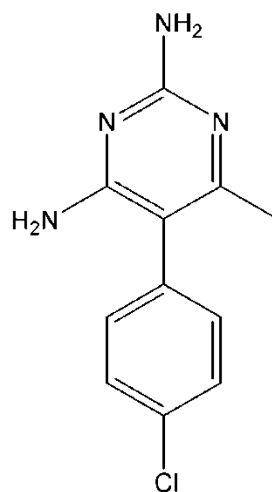
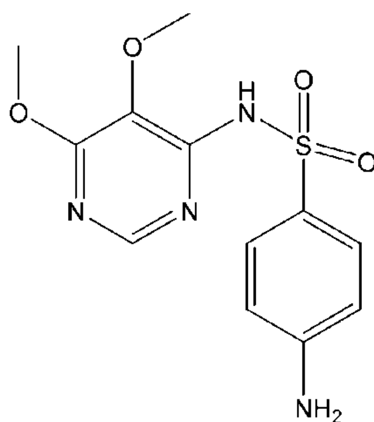


Proguanil (**XVIII**)



Pyrimethamine (**XIX**)



Sulfadoxine (**XX**)

Figure 6: Chemical structures of some selected examples of antifolates

2.4.3 Mechanism of Action of Artemisinins

A clear mechanism of action of these compounds is yet to be fully determined. However, various studies have proposed that the activity comes from the endoperoxide bridge through a two-step process of haemoglobin iron activation and alkylation [3, 33].

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The haemoglobin iron activation starts when the haeme moiety containing a reduced iron, ferrous iron (Fe^{2+}) (derived from the food vacuole's haemoglobin digestion) interacts with the endoperoxide bridge [3]. The ferrous iron (Fe^{2+}) is oxidised to ferric iron (Fe^{3+}), releasing an electron to the endoperoxide bridge of the artemisinins, causing the drug activation; but the Fe^{2+} cation acts as a catalyst because it is regenerated after the process. The peroxide bond breaks forming carbon-centred alkoxyl radicals, which are believed to be the source of the drug activity, because all the derivatives that cannot produce this radical (those without endoperoxide bridge like deoxyartemisinin) are devoid of biological activity [34]. This proposed mechanism of artemisinin degradation by iron is shown in Figure 7 below. Apart from acting as a catalyst for artemisinin breakdown, haeme also forms complexes with artemisinin producing specific radicals that are neither in artemisinin nor haemin products alone as shown in Figure 7 below, which are believed to act at a specific target that has not yet been discovered [36].

Furthermore, the artemisinin derivatives are alleged to act by alkylating particular proteins comprising iron-sulphur protein, transporters and translationally controlled tumour protein (TCTP) homologue [3]. Figure 8 shows a proposed reaction between the generated radicals and the bio molecules and it also shows a ferrous haeme (Fe (II) protoporphyrin IX) activating an artemisinin to form a seco C-4 radical (**XXVI**) and alkylated adduct (**XXX**) through pathway **a** (second row).

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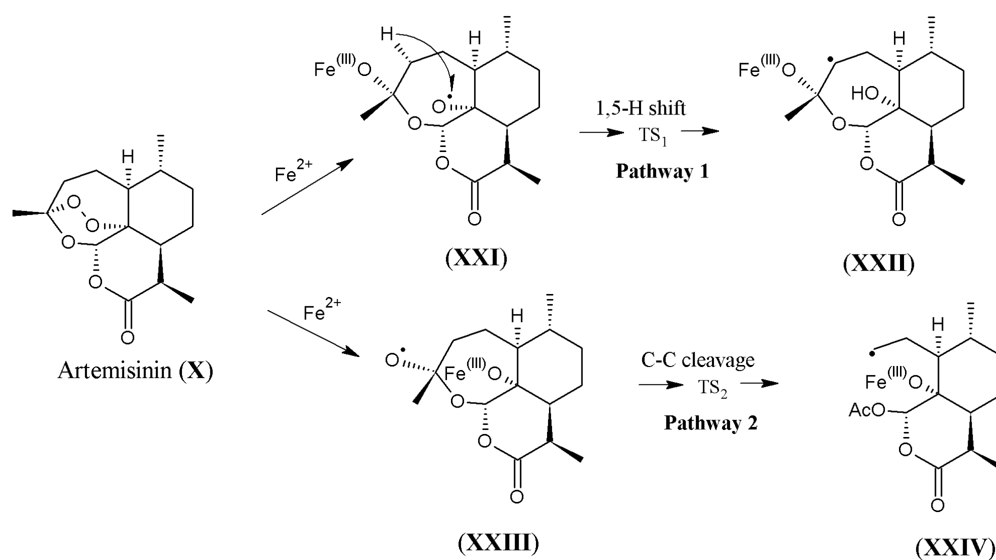

Source: Scafat et al. [35]

Figure 7: Proposed mechanism of artemisinin degradation by iron to form two radicals

Another possibility in which the radical exerts its activity is its loss of presumed water molecule from the haeme (**XXXI**) to alkylate the necessary parasite protein site in pathway **b** (first row) [37]. In contrast to other alkylating agents, artemisinin does not react with the DNA [38]. Therefore, the two processes of iron activation and alkylation deprive the parasite of its normal activities as follows: parasites consume part of the haeme of an erythrocyte they have invaded for their metalloenzymes and the rest is converted to hemozoin because the haeme is toxic to them [39, 40].

Alkylation prevents haemoglobin digestion by inhibiting proteases that facilitate the digestion. The process, therefore, deprives the parasite of vital amino acids. Alkylation also inactivates the

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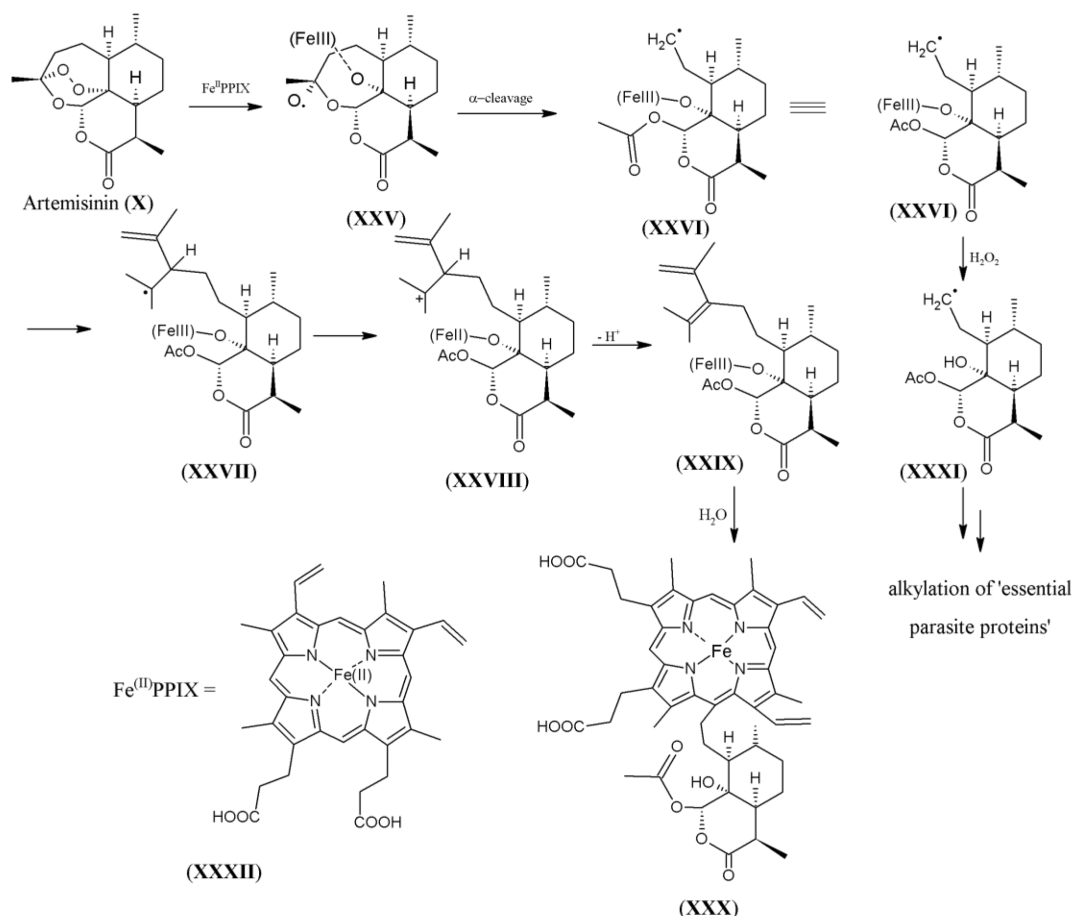
histidine-rich protein involved in the polymerization of haeme to hemozoin and the poisonous haeme molecules accumulate, eventually destroying the parasite [41].



Source: Krishnaa et al. [37]

Figure 8: Reaction mechanism of the ferrous haeme with the endoperoxide bridge

These presumed actions of inhibition of histidine-rich protein (HRP) catalyst for hemozoin formation are believed to be caused by haeme-artemisinin adduct and C-4 radical alkylation respectively. Both actions lead to the subsequent accumulation of toxic haeme-Fe^{IV}. This toxic haeme-iron complex is formed when Fe^{2+} is changing to Fe^{3+} as it goes through a stage where

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Fe^{IV} is formed. This paralyses parasite activities through interaction with its proteins of the food vacuole and pro-oxidant activities [37]. However, evidence-based results have shown some traces of resistance reported to have been caused by the ability of the parasite to resist artemisinin, refuting earlier suggestions that it might be due to the host and pharmacokinetic factors [3].

2.5 PLASMODIA RESISTANCE TO ANTIMALARIAL DRUGS

Drug resistance of a parasite is confirmed when the effectiveness of a drug declines in either disease cure or symptoms recovery in a patient. The word 'drug resistance' is suitably used when referring to pathogen-caused diseases, whereby the drug is intended to destroy the pathogens. However, a drug is not always aimed at destroying or inhibiting pathogens; in such other case, drug ineffectiveness (resistance) is suitably called drug tolerance or dosage failure. Some pathogens are tagged as multidrug resistant when they can defend themselves against more than one type of drug and/or approaches concomitantly administered [42].

Bruce-Chwatt *et al.* (in Bloland, 2001: 12) define resistance to antimalarials as the *"ability of a parasite to survive and/or multiply despite administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within tolerance of the subject. The drug in question must gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action."* Recent studies have established that resistance is caused by host and parasite factors. The host factors include prophylaxis drugs over use, use of poor quality drugs and non-compliance as well as haphazard use of drugs by patients leading to unfinished therapeutic treatments, which in turn, cause a decrease in drug build up to a recommended level necessary for an effective physiological response . The parasite factors

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include change-flexibility in target genes and metabolic pathway, increase in concentration of target, abnormal rates of reproduction, sexual reproduction (in mosquitoes); which in turn cause the multiplication of resistant genes and drug deactivation [3, 43].

2.5.1 Remedy for Drug Resistance

There are basically three ways of dealing with drug resistance and these are; prevention of drug resistance emergence, containment of the emerged resistance and drug efficacy scrutiny. Firstly, drug resistance can be prevented by controlling the parasite transmission, use of good quality antimalarials and adoption of combination therapy. Transmission of parasite can be prevented by the use of vaccines, vector control and decrease in infection reservoir; which has been blamed for spread of resistance and hence its decrease can be achieved by early diagnosis, efficient treatment and using gametocytocidal drugs. Furthermore, drug resistance emergence can be prevented by the use of combination therapy like ACTs and non-ACTs [17].

Besides, good quality ACTs are predictive of a successful parasite clearance as well as fight against resistance and this can be reinforced by easy access to ACTs, improved disease diagnosis, correct use of drugs especially in the private sector. This can be achieved by proper and updated education for the practitioners, increased compliance and supervising drug administration. Good quality drugs administration can also be achieved by routine quality assessment of drugs to fish out poor quality drugs from the market. Secondly, recommended drugs in the treatment guidelines are supposed to be routinely monitored for efficacy to detect any occurrence of resistance of the parasite quickly. This would help in establishing the extent of

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resistance and call for a prompt change of drug policy if necessary to prevent multi-drug resistance [17].

2.5.2 EFFECTS OF ANTIMALARIAL DRUG RESISTANCE

Parasite resistance to malaria treatment has had several devastating effects in the fight against malaria disease. This has led to an increase in morbidity and mortality rate (inclusive of anaemia and low birth weight), parasite transmission, rate and severity of pandemic, change in malaria distribution. In turn, this has caused pressure on the economy (government and individuals) due to increase in health services cost arising from prevalent treatment failures and death. These effects have made people resort to the informal private sector, thereby exposing themselves to poor quality drugs mostly blamed for escalation of drug resistance [17]. Specifically, some pockets of parasite resistance to artemisinin resistance have been attributed to the sub-therapeutic doses derived from fake and substandard drugs [8].

2.6 POOR QUALITY DRUGS

These are drugs that have either wrong ingredient, incorrectly formulated APIs, insufficient amount of APIs or dosage forms contaminated with other exogenous substances that may render the drugs harmful, ineffective or cause death. Poor quality drugs are classified into three types: counterfeit, substandard and degraded [44]. WHO classifies monotherapy of artemisinins as substandard even when they have enough requisite API [45].

Drugs are classified as counterfeit if they are fraudulently and deliberately mislabelled to depict one ingredient when they contain another (harmless or toxic). The ingredient might be either

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active or inactive against the disease they are intended for or contain the correct requisite API, but manufacturer or packaging is faked or mislabelled. The drug might also contain insufficient active ingredients, or without active ingredient. These drugs might be generic products, prescription medicines, over the counter medication or traditional remedies. In addition, it includes drugs that contain misleading information with respect to name, composition, strength, and manufacturer, country of manufacturing, and country of origin, marketing authorization holder or steps of distribution [46]. Small amounts of active ingredients are added sometimes aimed at just passing the rapid tests at entry points [47].

Substandard drugs are those drugs that are manufactured by well-known and established licensed companies with all the requirements, but deviate significantly from the accepted limits of API and other chemical additives' contents as well as falling outside the recommended range of dissolution times. This is attributed to poor manufacturing practices, lack of technical know-how and unsatisfactory infrastructure. Degraded drugs on the other hand are those that contain inadequate amount of API and other unwanted compounds resulting from decomposition of the well manufactured drugs that have been exposed to adverse conditions such as humidity, light and heat. Nevertheless, it is challenging to know whether a drug falls outside accepted limits due to poor manufacturing practices or exposure to adverse effects. Besides, it is also challenging to decide between counterfeit and substandard drugs as the latter might be due to poor manufacturing practices or deliberate move by a manufacturer [48]. However, Bate *et al.* state that determination of a drug as counterfeit or substandard requires a forensic examination of the trademarks, product designs and holograms [45].

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Research has shown that poor quality drugs are more widely found in Asia than Africa but the situation is rapidly deteriorating in the latter. This has been attributed to poor regulatory capabilities for manufacturing and importation activities [45]. It is estimated that 15% of the drugs used globally are counterfeit and 50% of the drugs used in some parts of Africa as well as Asia are counterfeit [49]. The fake drugs look almost the same as the original ones in both the packing materials and the barcodes, to a layman [50]. This situation has increased rapidly recently especially in Asia because most of these new drugs are very expensive as compared to the old ones. Market prices have shown that the current cost of ACTs is 10 to 20 times higher than the cost of SP and chloroquine. However, there is an expectation that the prices will decrease when the forces of demand and supply play their part [44]. An estimated US$20 billion, representing 7% of the pharmaceutical industry's total revenue, is lost to counterfeiters annually [51].

Most counterfeit drugs have been found to contain incorrect ingredients and this can cause adverse effects especially on HIV treated patients due to a possible drug-drug interaction [47]. It is also alleged that since other ingredients in the counterfeited drugs are sometimes not known, they create a toxicity threat due to unilateral activity, by-products effects or combined effects with other body contents [52]. In addition, some of these drugs contain zero active ingredients and others contain insufficient doses of the active ingredients that are too low to eradicate the available pathogens, while others contain too much of active ingredients that might have various adverse effects especially for the drugs that have low therapeutic indices [48].

These poor quality drugs result in low bioavailability of drugs; hence sub-therapeutic blood concentrations [48]. This, in turn, can cause preferential selection for the drug sensitive parasites,

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leaving the drug resistant parasites unaffected. When using a long half-life drug that stays in the body for a long time like SP, it is later encountered with new infections that are then exposed to sub-therapeutic residual drug that is below the least required inhibitory concentration [53, 54]. The result of these sub-therapeutic concentrations is cross-resistance; a situation in which microorganisms are conditioned to tolerate toxic levels of a drug due to the previous experience to similar medicine or mode of action. This can arise by either deoxyribonucleic acid (DNA) shift or spontaneous metamorphosis [42]. These factors contribute greatly to drug resistance either collectively or autonomously [48]. Serious cases lead to treatment failure and death especially for vulnerable groups like children who have a rapid evolution of malaria from mild to severe infection [49].

Therefore, artemisinin-based treatment is equally threatened by the proliferation of resistant strains and no other hope is foreseen to surpass this remedy in case of full resistance in the near future. It is, therefore, recommended that the best way is to safeguard good manufacturing practices to minimize or eradicate the inflow of substandard drugs by strengthening the pharmacovigilance tools specifically in this case, post-marketing surveillance of the drugs already on the market to flush out such dangerous drugs and perpetrators [55]. There is also a need for careful implementation of universal medicine availability whilst safeguarding the quality of the drugs at the same time, because any compromise means resistance and subsequent change of treatment regimen to newer and more costly drugs [49], which are not yet even available.

Thus, it is recommended that regulatory authorities in various countries should invest in modern facilities to enable them carry out their routine quality control activities well so that people can

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access best quality drugs. This would help in fighting against the inflow of these unwanted drugs that are already threatening to escalate the spread of resistance in Africa. This is very important as any ability of this resistance to infiltrate Africa would roll back malarial control initiative to zero [47].

In view of this widespread distribution of poor quality drugs most of which are counterfeit and/or substandard and the consequent growing public health crisis, an official World Health Organisation (WHO) body called the International Medical Products Anti-Counterfeiting Taskforce (IMPACT) was set up in 2006 by WHO and other stakeholders to coordinate the fight against the production and distribution of counterfeit drugs worldwide [56].

2.7 QUALITY CONTROL PARAMETERS IN POST-MARKETING SURVEILLANCE (PMS)

A drug product is mostly tested for safety, efficacy and quality to qualify it as suitable for human treatment or consumption. Quality control is the oldest parameter that has been in place for assessment of drug products, while safety and efficacy came into force towards the end of the 1950s after the Thalidomide tragedy. Drug product quality continues to be a very important assessment criterion and the procedures are performed in a wide range of infrastructure from simple to state-of-the-art facilities. For antimalarials, quality control involves physical methods done on liquids, solids and semi-solids. It entails chemical methods analyzing the API content, excipients and impurities, *in vitro* disintegration and dissolution tests as well as *in vivo* bioavailability tests [49].

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Post marketing surveillance (PMS) involves the assessment of drug products when they are ready for market or at the market. Lucas *et al.* explain that a drug quality may involve assessment of the drug for stability and shelf life under specific moisture and temperature conditions. A resolution was passed by the International Conference on Harmonization (ICH), to standardize methods for medicine stability tests. In addition, stability of drugs was divided into three operational classes of long term, intermediate and accelerated stability studies [57]. Other quality control tests reported in the literature are residue analysis, excipients and binding materials status, degradation products and unidentifiable materials analysis, expiry status and content analysis, uniformity of weight, friability, tablet hardness, product shelf life under specific conditions, bioavailability and bioequivalence for generic drugs especially those with limited solubility and development of new analytical procedures that are cost effective and rapid [49].

2.8 CASE STUDIES ON QUALITY CONTROL

Several studies have been conducted in malaria endemic areas of the world on poor quality drugs. Such poor quality drugs have contributed to the abandonment of once effective antimalarial drugs and such poor quality drugs still pose a threat to the new artemisinin-based combination therapies that have been recommended and widely used as first line treatment for uncomplicated Malaria cases. Various studies have confirmed the widespread existence of these substandard and counterfeit antimalarial drugs. A few of these case studies are discussed.

2.8.1 Reports from Outside Africa

In eastern Burma, following the death of a male patient diagnosed of uncomplicated hyperparasitaemic *P. falciparum* malaria and treated with an artesunate drug, quality analysis

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was conducted on the purported artesunate antimalarial drugs. The drugs were labelled as manufactured in China by Guilin pharmaceuticals. Tests (or analyses) showed that the drugs contained only 10mg out of the required 50 mg indicated on the packaging material and mainly contained paracetamol. The paracetamol addition may have been aimed at fever reduction to give a false impression of the drug's efficacy, to cover up its failure to cure malaria [58].

In the Mekong region covering Cambodia, Burma (Myanmar), Laos, Thailand and Vietnam, a study conducted in 2001 to assess the quality of drugs using 104 samples collected from shops showed that 38% of the samples had zero artesunate. In 2004, a follow up study assessed 188 artesunate and 44 mefloquine tablet samples and results demonstrated a deteriorating situation as 53% had zero artesunate. In addition, out of the 44 mefloquine samples, 9% had amounts less than 10% of the claimed amount labelled on the pack [59].

Abdo-Rabbo *et al.* reported a study conducted in Yemen. Samples of antimalarials containing chloroquine and SP were investigated for content and dissolution compliance using validated methods (HPLC) in the pharmacopoeia. Chloroquine syrup samples registered a 6.7% failure rate, while tablets had 20% failure rate on content analysis. For dissolution analysis, 8% of the chloroquine tablets failed. In addition, 100% of the SP tablets passed the content analysis, but 80% failed the dissolution tests, with pyrimethamine having extremely poor results [60].

In 2007, a study carried out in Vientiane, Laos using visual and chemical analysis showed that 53% of the samples were falsified. Most of the fake artesunate pills had a deceptive visual look for an ordinary eye due to their sophiscated packaging, holograms and logos that could be

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detected as fake only when a UV light was used. Some of the samples were found with inappropriate materials such as flour, starch, chalk, acetaminophen (paracetamol) or chloroquine as well as fatal sulpha drugs for those allergic to them. Some samples also had a trace of artemisinin that showed positive result for Fast-Red dye test, yet insufficient to cure malaria [61].

Newton *et al.* analyzed 391 artesunate samples collected from the Thai-Myanmar border (16), Cambodia (48), Vietnam (75), Lao PDR (115) and Myanmar (137). Results showed that there were 16 different fake holograms within the samples. In addition, some samples had wrong and banned ingredients, carcinogens and raw materials of the narcotic drug, Ecstasy. Overall, 195 of 391 drugs had little or no artesunate representing 49.9% failure rate; a genuine drug was supposed to have 50mg of the API, but instead, majority of the samples had only 12mg. Those samples that were counterfeit were also found with some pollen, calcite as well as charcoal and further investigations traced these face medicines to South-East China [62].

Nayyar *et al.* reviewed published and unpublished study data from 7 countries of Southeast Asia and sub-Saharan African regions to establish the extent of distribution of poor quality antimalarials between 1999 and 2010. Data for five classes of antimalarial drugs consisting of artemether, artesunate, chloroquine, mefloquine, quinine, sulphadoxine–pyrimethamine and tetracycline were collected from Southeast Asia. For southeast Asia, multinational surveys of seven countries showed that while 497 out of 1437 (35%) of the drug samples had failed chemical tests, 46% (423/919) of the samples had failed packaging analyses and assessments, while 36% (450/1260) were found to be counterfeit. However, due to the lack of enough data, they could not establish the frequency of substandard antimalarials [63].

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2.8.2 Reports from Africa

In Tanzania, a sample of an antimalarial drug purported to contain dihydroartemisinin (60 mg/tablet, cotecxin) was found on analysis using both TLC and HPLC to contain none of the active ingredients [64]. In another study also conducted in the same country in 2005, 1080 samples consisting of 679 antifolates (SP, 394 and sulphamethoxypyrazine/pyrimethamine, 285), 260 amodiaquine, 63 quinine and 51 artemisinin derivatives were collected from retail shops in 21 districts. Out of the total number of the samples, 304 samples were selected for the laboratory analysis during which US pharmacopoeia monographs were used when available for dissolution and HPLC tests. The HPLC and dissolution test results showed that 13% of the antifolate samples had poor quality, while 23.8% and 7.5% of quinine and amodiaquine samples respectively were found to be of poor quality, with all the artemisinin derivative samples being found compliant [65].

In Cameroun, a study conducted in 2004 to investigate the quality of 284 samples comprising chloroquine, quinine and sulphadoxine-pyrimethamine components collected from 132 different unauthorised retailers and distributors of the rural and urban areas, in which simple colour reaction tests and SQ-TLC methods were used reported that 38% (50/133), 74% (52/70) and 12% (10/81) of the drug tablets had failed the quality tests due to the presence of either unsatisfactory, wrong, unknown or inactive pharmaceutical ingredient(s) [66].

In Ghana, a study was conducted by Osei-Safo *et al.* in 2009 on various samples of antimalarial drugs collected from selected parts of Accra. The results showed that out of 49 samples comprising single tablet artemisinin-based fixed-dose combination drugs, separately formulated

artemisinin-based combination drugs packaged on the same blister to be taken concomitantly (ACTs) and artemisinin-based monotherapy formulations, only 28.6% (14/49) fulfilled the pharmacopoeia standards based on SQ-TLC. When these samples were further subjected to HPLC test, only 8.0% (4/49) of the samples complied with pharmacopoeia standards [67]. In 2012, a related study was conducted in Ghana by Addae-Mensah *et al*. as part of a joint Ghana-Togo study. 86 antimalarial drug samples consisting of 50 ACTs, 8 artemisinin monotherapy and 28 non-ACTs were collected from the major cities and border towns. The samples were analysed using basic tests, SQ-TLC and HPLC tests. The results showed that there was a widespread circulation of poor quality drugs, whereby 6 out of 7 zones of the collection exercise had samples that failed the tests, with one zone recording an 85% failure rate and more than 60% failure rate by the others. Out of the 50 ACTs, 8 (17%) were compliant with the Ph. Int. requirements, with 39 (83%) being non-compliant. In addition, out of the 8 artemisinin monotherapy samples, 1 (12.5%) was compliant, whereas a further 1 (12.5%) and 6 (75%) were marginally compliant and non-compliant respectively. While 12 (43%) of the non-ACTs passed the tests, 16 (57%) failed the tests. Majority of the samples failed due to the inadequacy of API amounts [68]

In Kenya and DR Congo, a survey on the quality of artemisinin-based drugs conducted on 24 samples collected and analyzed using European Pharmacopoeia guidelines showed that 9 did not comply with the quantity requirement of 95-105% of the labelled content, but all the failed samples had some traces of the API [69]. Separately, another study was conducted in East DR Congo using European Pharmacopoeia guidelines on chloroquine syrup and injection batches, quinine injection batches, SP tablet batches and proguanil batches. Outcomes showed that samples from one batch out of the two chloroquine injection batches investigated using UV-

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spectrophotometry and HPLC-UV were overdose by 14% and samples from one out of the four selected quinine injection batches were overdose by 8%, whereas chloroquine syrup was compliant with the European Pharmacopoeia limits of 95-105%. Samples containing sulphadoxine-pyrimethamine components that were analysed using HPLC-UV only showed that sulphadoxine component of the samples were under dosed (91-94%) with respect to European Pharmacopoeia (95-105%), but within the compliance limits according to the United States Pharmacopoeia (90-110%) requirements in all the batches. However, samples from two of the sulphadoxine-pyrimethamine batches had pyrimethamine components that were overdose by 106% and 108% respectively and one batch had its samples found without any of the requisite ingredients. In addition, proguanil also passed after registering 98.7% of the label claim [70].

In Nigeria, a survey was conducted in 2001, in which 581 drug samples were collected from pharmacies. Two hundred and eighty-six (286) of the drug samples were antimalarials while the rest were antibacterial, antifungal and antituberculosis drugs. They collected drug samples that were on the WHO model list of essential drugs only. The samples were analyzed for API content using a corroborated HPLC method in accordance with British Pharmacopoeia (BP) specifications. The validated HPLC methods were used in this survey and the results compared with pharmacopoeia requirements. Out of the 286 antimalarial drug samples were 82 chloroquine phosphate (29 capsules, 20 syrups, 18 tablets and 15 injections), 31 chloroquine sulphate (11 syrups, 19 tablets and 1 capsule), 19 proguanil hydrochloride tablets, 10 quinine hydrochloride injection, 18 quinine sulphate (1 syrup and 17 tablets) and 126 sulphadoxine-pyrimethamine (SP) (100 SP tablets and 26 syrups). The results of chloroquine phosphate showed that 70% (20), 100% (20), 94% (17) and 93% (14) of the capsules, syrups, tablets and injections respectively

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fell outside the BP limits, while the failure rates in chloroquine sulphate samples were as follows; syrups (73%; 8/11), tablets (79%; 15/19) and 0% for the capsules. Out of the 17 quinine sulphate tablets, 24% (4) failed the tests. Of the 100 SP tablets and the 26 syrup samples, 13% (13) and 30.79% (8) respectively failed the tests. All proguanil hydrochloride, quinine hydrochloride injections and quinine sulphate syrup registered compliance with the BP specifications [71].

In 2003, WHO carried out a pilot study on samples of SP, chloroquine tablets and syrups collected from Ghana, Sudan, Gabon, Zimbabwe, Mali, Mozambique, Kenya and Tanzania. The samples were analyzed for quality in terms of API content and dissolution. Table 2 below shows the combined percentage results from all countries with the results of the samples failing pharmacopoeial standards out of the total number of samples analysed. Samples from Tanzania were not analyzed because their collection was delayed and samples from Gabon were not analysed for dissolution tests because they were too few. Therefore, the results showed a widespread circulation of poor quality antimalarial drugs throughout the study countries. The report also showed that most samples failed the tests due to insufficient amount of the APIs for chloroquine samples, but for SP samples, dissolution rather than API content accounted for an unusually high failure rate. Such poor results were attributed to poverty which in turn gave rise to poorly equipped laboratories, under-funded regulatory bodies and poor handling and manufacturing practices [72, 73]. However, the undesirable quality of the drugs might also have been caused by greed on the part of certain manufacturers.

In Uganda, a market survey in May, 2007 identified a quinine BP 300mg tablet as fake. The general public was then notified of this drug by the National Drug Authority. The drugs were identified as having batch number 0908, manufactured in 2006 with 2009 as expiry date. It was found that the drugs were not manufactured by the sole licensed pharmaceutical producer of quinine in Uganda, the Kampala Pharmaceutical Industries Ltd as claimed on the package [74].

Table 2: Combined country results: percentage failure of samples

Country	Chloroquine syrups	Chloroquine tablets		Sulphadoxine/pyrimethamine Tablets	
	API Content (%)	API Content (%)	Dissolution (%)	API Content (%)	Dissolution (%)
Gabon	0, (0/8)	29.0, (5/17)	14.29, (1/7)	0, (0/10)	-
Ghana	5.0, (1/20)	66.7, (12/18)	20.0, (3/15)	37.5, (3/8)	75.0, (3/4)
Mali	66.7, (4/6)	47.3, (9/19)	5.2, (1/19)	0, (0/7)	100, (7/7)
Kenya	25.0, (2/8)	42.8, (3/7)	28.6, (2/7)	0, (0/12)	91.7, (11/12)
Mozambique	25.0, (3/12)	20.0, (3/15)	6.7, (1/15)	5.5, (1/18)	100, (18/18)
Sudan	26.6, (4/15)	5.2, (1/19)	12.5, (2/16)	0, (0/20)	80.0, (12/15)
Zimbabwe	13.3, (2/15)	57.1, (8/14)	7.1, (1/14)	10.0, (1/10)	100, (10/10)
Average failure	23.0	38.3	12.2	7.6	91.1, (n= 6)
Range	0-66.7%	20-66.7%	5.2-28.6%	0-37.5%	75-100%,(n= 6)

In Kumasi (Ghana), a study was also conducted on artesunate containing samples to assess the content of the API and the uniformity test on tablets in accordance with European Pharmacopoeia and International Pharmacopoeia specifications. Results showed that content analysis gave API content range of 47.9-99.9% for all samples. Specifically, 11 (47.1%) failed the content uniformity test, while 6 (35.3%) passed the tests with respect to International Pharmacopoeia specifications. Additionally, 3 samples (17.6%) conformed to European Pharmacopoeia conditions for API content [75]. In 2009, fake Coartem tablets were reported to be

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circulating on the Ghanaian market. Analysis of the samples found them to contain none of the requisite APIs labelled on the pack [76].

In 2008, another study was carried out in 6 countries from the most malarious regions of Africa and the selected countries were Kenya, Rwanda, Uganda, Tanzania, Ghana and Nigeria. A total of 210 antimalarial drugs consisting of artemether-lumefantrine, artesunate, artemether, dihydroartemisinin, mefloquine and amodiaquine were selected and the semi-quantitative thin layer chromatography method coupled with dissolution tests were used for analysis. It was reported that dihydroartemisinin had the highest failure rate of 55% followed by amodiaquine (48%), SP (38%), artesunate (31%), artemether (27%), mefloquine (24%) and artemether-lumefantrine (19%). Furthermore, the country drug failure rates were Ghana (35%), Kenya (38%), Nigeria (32%), Rwanda (33%), Tanzania (32%) and Uganda (35%) [45].

In South East Nigeria, Onwujekwe *et al.* (2009) conducted a survey on artesunate, dihydroartemisinin, SP, quinine and chloroquine to assess their dissolution efficiency and content in some 4 artesunate samples as well as some 24 dihydroartemisinin samples using an uncertified HPLC method. All the Artesunate samples passed the tests, while 46% of the quinine as well as 39% of SP failed and were found to be substandard. Generally, 37% of the samples did not comply with the pharmacopoeia requirements [77].

In Tanzania, following the detection of a fake Metakelfin by the Tanzania Food and Drug Authority (TFDA) in 2009 on the markets through an inspection of some 40 pharmacies, the Drugs Board suspended the drugs' importation, distribution, sale and use. The study revealed

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that the fake drugs had batch numbers that were not approved at the time of importation and some had low API content inconsistent with the 90-110% pharmacopoeia requirement [78]. A separate survey in the same country found some wheat flour instead of the labelled APIs [79].

Between 2001 and 2005, Thoithi *et al.,* sampled 41 packs of antimalarials in Kenya comprising Artemisinin derivatives, SP, Quinine and Amodiaquine to assess them for content and dissolution compliance using pharmacopoeia guidelines. Many SP drugs failed especially the dissolution tests. In addition, half (50%) of the 20 SP samples and one-third (33.33%) of artemisinin-based drugs did not comply with the pharmacopoeial requirements [80].

A WHO (2011) report outlined the status of the quality for antimalarials in six sub-saharan countries. The six selected countries were Cameroon, Tanzania, Ghana, Kenya, Nigeria and Ethiopia and the samples collected were ACTs and SP. Out of the six countries, Nigeria had the highest failure rate of 64%, followed by Ghana (39%), Cameroon (37%), Tanzania (11%), Kenya (5%), and Ethiopia (0%). However, Ethiopia registered 41% of non-registered drugs, making the country vulnerable to fake drugs. Most of the poor drug results were attributed to low API content, degradation products and poor dissolution. For example, 62.5% of ACTs failed due to the low API content and dissolution failure. Samples containing SP also had a failure rate of 66.7%. The overall assessment showed that 33% of the drugs were non-compliant. Most of the countries with high failure rates had their antimalarial products sourced from many different manufacturers. Imported drugs had similar failure rate results for both registered and non-registered. The results analysis led to the speculation that the non-compliance might have come from poor manufacturing practices [55].

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In Malawi, a national survey was conducted in 2007 to assess the quality of antimalarial drugs upon request from USAID-funded Strengthening Pharmaceutical System (SPS) programme, after being neglected in several WHO QAMSA multinational surveys. A total of 199 antimalarial drug samples consisting of 57.8% and 42.2% of ACTs and SP respectively were collected throughout the country from all the pharmaceutical outlets. After rejecting 26 (13%) monotherapies the remaining 173 samples were analysed. The samples were analyzed using the Minilab physical and basic tests in Malawi and confirmed later in Kenya. Results showed that all samples tested positive for disintegration and basic tests and 4% (8) failed the physical examination. In addition, 16% (8/50) of the samples failed confirmatory tests and out of the 8 samples that failed the confirmatory tests, 37.5% (3/8) were SP and 62.5% were ACTs (branded and generic); 12.5% (1/8) was not compliant with uniformity tests and a further 87.5% (7/8) failed dissolution test. This study helped the country to have an insight into establishing a national antimalarial drug monitoring system [81].

Several programmes and studies have been conducted in Malawi and some are still ongoing to kick out malaria. The options being employed include treatment modalities and diagnostic techniques, epidemiology, community and public health education, pharmacological and clinical efficacy and genetic diversity, intermittent preventive treatment of pregnant women (IPTp), insecticide treated net (ITNs) and indoor residual spraying (IRS) [82], with little or no attention being paid to quality control studies. In addition, reported cases of the surveillance of drug quality by the national drug regulatory authority on the required capacity, scale and frequency or their occurrence is not well documented in the published literature. This declaration of the capacity of the regulatory body is made following an ACT Consortium report by Staedke in 2009

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[83] which recommended the establishment of drug safety register and systematic surveillance to monitor the quality, influx of poor quality drugs, their sources and the extent of the effects of transportation and storage on the quality of genuine drugs in Malawi. In addition, SPS in its 2011 report following a survey also categorised Malawi as one of the countries that had no or minimal capacity for pharmacovigilance (PV) due to the lack of legal or structural frameworks for PV systems, no coordinated passive/active surveillance and lack of national coordination in any ongoing PV activity [84]. Unconfirmed reports from the regulatory authority in 2012 revealed that such activities are being undertaken with specific activities and extent not mentioned. However, the Ministry of Health and the Drug Regulatory Board issued a statement that all drugs that are distributed in government and Christian Health Association of Malawi (CHAM) health centres are tested for efficacy, safety and quality [85], with no mention of drugs accessed from the private drug stores or pharmacies as well as the parameters assessed.

In a nutshell, there has been widespread circulation of poor quality drugs in some parts of Asia and Africa. Most of them contain sub-therapeutic amounts of the APIs or no API at all or even toxic compounds and if this is not checked, it can lead to emergence of drug resistance.

2.9 QUALITY ASSESSMENT METHODS

There is ample evidence that circulation of poor quality drugs in Africa is increasing at an alarming rate due to poor regulatory capabilities in most regulatory authorities in terms of manufacturing and import regulations [45]. As one of the ways of moving towards a counterfeit drugs-free society, the WHO Roll Back Malaria Initiative urges all those that have adopted its

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initiative into malaria programmes to implement one of its goals that stipulates that at least 80% of antimalarial products in a country should satisfy acceptable international quality standards [49].

So many mitigating factors have been employed to solve this problem of substandard and bogus drugs inflow. These include consistent monitoring of drugs quality to ensure the purchase, supply, import, manufacture and distribution of quality drugs. Guidelines and methodologies for the quality assurance surveillance are outlined in the pharmacopoeias for this exercise [86].

2.9.1 Pharmacopoeia Methods

There are several pharmacopoeias by individual countries, territories, regions and many more. However, few have been adopted, then widely used and these are United States Pharmacopoeia (USP), British Pharmacopoeia (BP), Japanese Pharmacopoeia (JP), European Pharmacopoeia (Ph. Eur.), Pharmacopoeia of the People's Republic of China (PPRC) and International Pharmacopoeia (Ph. Int.). These pharmacopoeias have common features outlining recommended methods from published monographs with information such as procedures for analysis, determination of active pharmaceutical ingredients (APIs), excipients and dosage forms.

Most of the pharmacopoeia guidelines are very expensive and difficult to organize for many programmes in developing countries. Therefore, to minimize this cost burden, the Directorate of Quality Assurance and Safety: Medicines of the WHO introduced a programme aimed at developing cost effective reliable methods of chemical analysis to be adopted by national drug regulatory authorities in standard noncompliance screening prior to drug’s use. Therefore, cost effective methods have been developed suitable for the screening of artesunate, artemether and

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other derivatives' quality [87]. Some of these innovated methods are used together with those pharmacopoeia methods that are cheaper and easy to use. Indeed, the WHO developed the International Pharmacopoeia as a means of providing simple inexpensive but accurate methods that can be used in any less well-endowed analytical laboratory.

2.9.2 Case Studies on Method Developments

Green *et al.* developed and validated colorimetric [ARTS-Fast red TR (FRTR)] method. It was initially developed for the detection of fake artesunate, which was later extended to artemisinin, dihydroartemisinin and other derivatives. For artesunate analysis, artesunate is decomposed to an alkaline product which reacts with fast red TR salt giving a yellow colour. The absorbance of the yellow colour is then measured. Furthermore, Green *et al.* went on to find out the efficiency of using colorimetric and refractometric methods alone and together by comparing them with the HPLC assay results. Effectiveness of refractometry and colorimetry combined was observed in the assessment of artesunate, chloroquine injection, quinine and sulphadoxine with an accuracy of 0.96-1.00, while the accuracy was lower for enteric-coated chloroquine with an accuracy of 0.78 [88].

In 2004, German pharmaceutical companies involved in research set up a charitable trust called German Pharma Health Fund (GPHF), which developed a mini-laboratory tool called GPHF-Minilab containing consistent, cheap and simple methods for the prompt verification of drug quality for tuberculosis, malaria and HIV/AIDS, antibiotics and many more important drugs in low income or developing countries. This was aimed at detecting fake and substandard drugs [89].

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An easy thin-layer chromatography (TLC) method was also developed and reported by Ioset and Kaur in 2009. The method was developed for artemisinin and its derivatives detection in antimalarial drugs, using 2,4-dinitrophenylhydrazine or 4-benzoylamino-2, 5-dimethoxybenzenediazonium chloride hemi (zinc chloride) salt as a spraying agent. The presence of artemisinin and derivatives is shown by a pink colour for the former reagent and blue colour for the latter reagent. However, the method cannot detect artemisinin and derivatives of contents 10% or less [90].

At the request of the WHO, Addae-Mensah and Osei-Safo in 2006 also developed and validated a user-friendly semi-quantitative thin layer chromatography method for artemisinin and non-artemisinin antimalarials with two suitable solvent systems for each API and anisaldehyde/methanol as a spraying reagent for the artemisinins. This method gives a valid estimation of the API in a drug which can then be more accurately verified by an HPLC method. The method is useful for rapid field estimation of the quality of all antimalarial drugs [91]. This method has subsequently been successfully used in two major World Health Organisation (WHO) and West African Health Organisation (WAHO)-sponsored surveys [67, 68, 92]. This method has also been used in the current study and the procedure has been articulated in the subsequent chapters.

2.9.3 Other Analytical Methods

Most of the methods developed above are mostly for rapid tests in areas that are economically challenged and they are partially accurate, although some accurate but expensive methods have also been developed the same way. Therefore, it is recommended that confirmatory tests using

other more accurate, specific and precise though expensive expertise-technology dependent methods such as High Performance Liquid Chromatography (HPLC) and Dissolution tests should be used to complement the TLC and basic disintegration results respectively. TLC and basic disintegration tests are tentative or approximate hence can be easily challenged [48, 55]. Other tools that have been used in this course include GPHF Minilab, HPLC (coupled with either of the following; fluorescence, single wavelength ultraviolet/visible absorbance, photodiode array (PDA), electrochemical or refractive index), HPLC-Mass spectroscopy hyphenated method (LC-MS), Open air ionization-mass spectroscopy, desorption electro spray ionization (DESI)-mass spectroscopy and many more [48].

2.9.4 Selected Quality Control Tools for the Present Study

Following the recommendations by WHO and other players reported in the literature for post-market surveillance of antimalarial drugs, the following methods have been selected for the current study:

2.9.4.1 Visual Inspection

A drug sample can be subjected to visual inspection which among others involves the verification of tablets' mass uniformity, size, colour, crimping, weight, printing, bar codes and holograms in comparison to the authentic genuine drugs. However, this method has suffered a lot due to an overwhelming response by counterfeiting entities such that counter-mechanisms have been applied to beat this trap. The features used under this method are also difficult to determine, i.e. difficult to differentiate between a genuine and counterfeit drug. However, it is still suitable and widely used in surveillance in resource constrained poor developing countries [52].

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2.9.4.2 Basic Tests

These are simple and readily applicable methods used to confirm the identity of APIs [93]. Due to the success and challenges the visual inspection method has met, there are some methods that are supposed to be done in addition to it such as basic or colorimetric tests. The literature and International pharmacopeia (Ph. Int.) have reported several methods of basic tests that are mainly based on the functional group of a drug [93, 94, 95, 96].

For example, artemisinins have been analyzed as follows: heating of a drug sample extract with potassium iodide producing a yellow colour; reaction of potassium iodide (KI) / starch with sample extract to produce a violet colour; reaction of Hydroxylamine HCl with a sample producing reddish violet colour. The colour change in each case indicates the presence of an API. The Figures 9 and 10 below show the reaction mechanisms of artemisinins reacting with KI / starch and vanillin / sulphuric acid (H_2SO_4) as examples [94].

Firstly, Figure 9 below shows an artemisinin derivative reacting at room temperature in the presence of glacial acetic acid and concentrated sulphuric acid mixture (10:1), producing a combination of α, β-unsaturated ketones (ketone-lactones). These products, therefore, react with an aromatic aldehydic vanillin whose product gives the observed corresponding pink colour in artemisinin derivatives basic tests [94]. Secondly, Figure 10 shows an alternative path a reaction can take; after an artemisinin derivative has reacted in the presence of an acid to produce many products, amongst them hydrogen peroxide (H_2O_2), i.e. from step **XXXIII** to **XXXIV** in Figure 9; the peroxide can be involved in a chemical reaction instead. Addition of KI at that stage results in the oxidation of iodide to iodine. Addition of starch reagent gives a colour reaction [94].

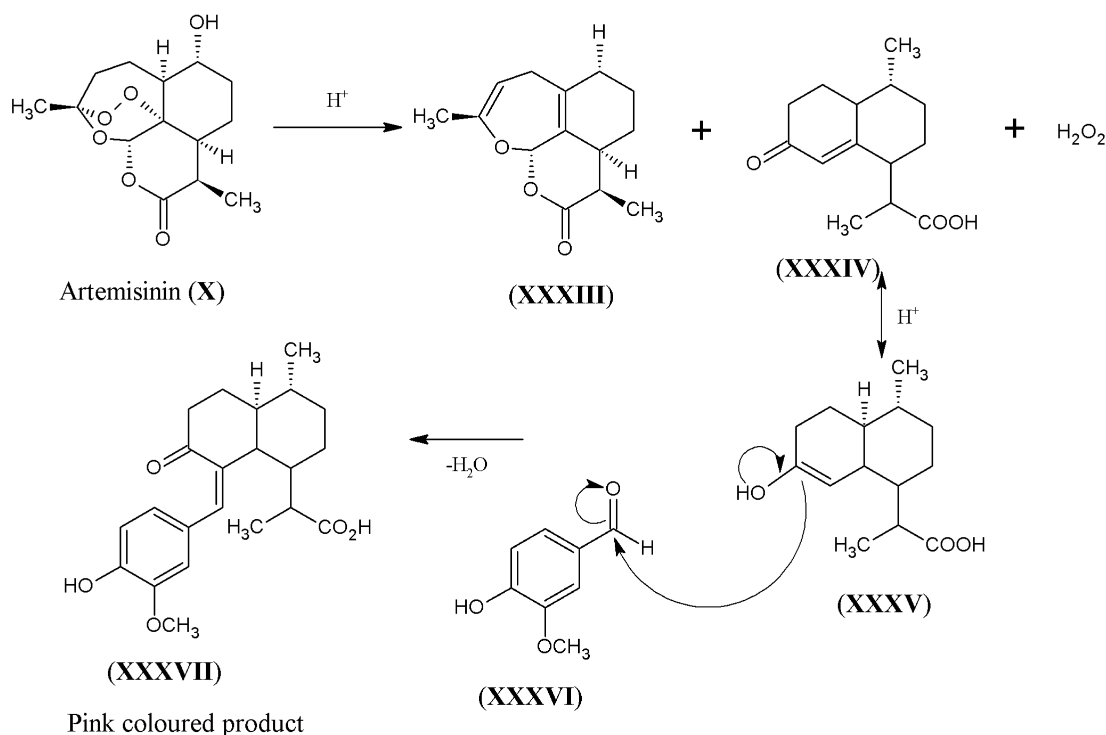

Figure 9: Basic test proposed reaction of artemisinin decomposition products with vanillin

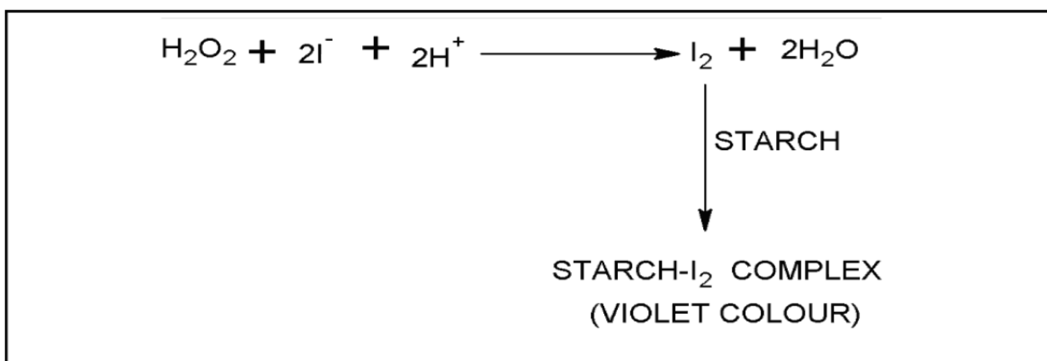

Figure 10. Reaction of hydrogen peroxide with potassium iodide

Furthermore, alkaloidal antimalarials also have their basic tests outlined in the WHO literature and International Pharmacopoeia. These alkaloidal antimalarials react similarly with suitable reagents and chemicals using their distinctive chromophores in preferably colour and precipitate

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producing reactions. For example, lumefantrine tablets and suppositories are analyzed using such a test as shown in a proposed reaction mechanism shown in Figure 11 below.

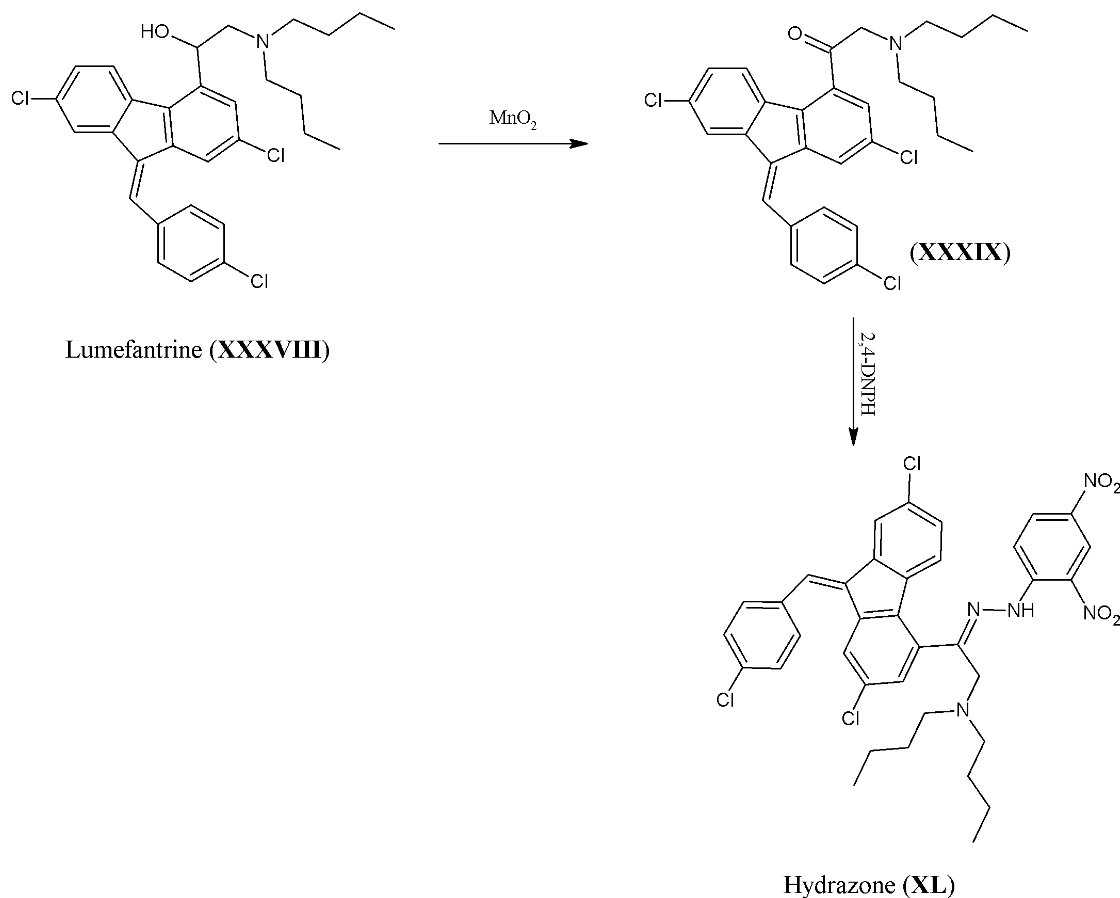

Figure 11: Basic test proposed reaction for lumefantrine.

In this reaction, lumefantrine having allylic alcohol functional group is oxidised with manganese dioxide (MnO_2) to an analogous ketone. The ketone then reacts with 2, 4-DNPH to produce an orange coloured precipitate of a hydrazone [92].

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Basic tests are simple and readily available methods to confirm the presence of API using easily available reagents. It would also serve to inform if significant degradation has occurred in substances under unfavourable conditions [95]. However, this method cannot give quantity of an API and compounds with functional group similar to the required API can react similarly giving a false impression. For example, any compound with a similar allylic alcohol in Figure 11 can react similarly [92].

2.9.4.3 Semi-Quantitative Thin Layer Chromatography (SQ-TLC)

There has been a wide call to use semi quantitative TLC, and basic disintegration test in addition to the basic tests in pharmaceutical analysis, whereby the latter serves as a basic predictor of the time taken for the drug to break up for body absorption [49]. A TLC method, apart from its primary purpose, can also expose the impurities and substances resulting from degradation if they are in significant proportions [48]. This method is relatively easy, inexpensive, selective and cost effective. Results based on this method can be used to qualify a product as satisfying label specifications and legal for use [97]. In addition, it uses inexpensive and affordable technology, hence suitable for resource-limited communities and institutions [98]. Nevertheless, this method cannot detect the counterfeits containing incorrect inactive or active ingredients if they cannot be eluted by the solvent system and visually seen by the reagents employed for the correct API [97].

2.9.4.4 High Performance Liquid Chromatography (HPLC)

It is a chromatographic technique that separates a mixture of compounds for further activities such as identification, purification or quantification of compounds in a mixture. It is used for medical, legal, research and manufacturing purposes [99]. For example, it is generally used for the

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analysis of impurities of volatile and non-volatile compounds. It is also used for isolation, separation and purification of compounds, determination of ionic, zwitterionic and neutral molecules, preparative and process-scale separation ultra-tracing as well as qualitative and quantitative purposes [100]. It is a non-destructive method and can separate closely related compounds and a large variety of compounds such as organic, inorganic, biological, chiral and thermally labile compounds, polymers, small ions as well as macromolecules. HPLC has had a wide variety of applications such as measuring levels of active drugs, synthetic by-products, degradation products in medicines, hazardous compounds (like pesticides) as well as some compounds (amino acids, nucleic acids, and proteins) in physiological samples. In addition, it is used for monitoring environmental samples, purification of compounds, separation of polymers and determination of their weight distribution in mixtures, tracking synthetic reactions and quality control [100].

It works based on the basic principles of chromatography, where the components carried by a mobile phase interact with a stationary phase (sorbent) subsequently causing separation [100, 101]. The principle is the same as TLC, with the difference being the use of a pump in HPLC to pass the mobile phase and sample mixture with an operational pressure through a column, as opposed to TLC which depends on gravitational force for the mobile phase mobility. In addition, the sorbent materials in HPLC are smaller (average 2-5 micrometer), giving it a better resolving power. The typical solvents used are water, acetonitrile and/or methanol. The separation process is controlled by the temperature and composition of mobile phase, which influence the interaction between sample components and sorbent, which could be in the form of dipole-dipole, ionic and dispersive (hydrophobic) forces [99].

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A typical HPLC system has a mobile phase reservoir, degasser, pump (50-350 bars), injector, column compartment, detector, data processor and waste collector. Using an HPLC requires suitable column, mobile phase solvents/reagents, standard solvent, analytical instruments (pH meter and balance), reference standard (for quantification), glassware and many more [102]. There are different kinds of detectors such as UV/Vis absorbance detector, fluorescence detector, electrochemical detector, conductivity detector, refractive index detectors, mass spectrometer [100], and photo diode array (PDA) [99].

There are different kinds of columns with different modes of separation as shown in Table 3 below [101].

Table 3: Kinds of chromatographic columns and their modes of separation

Type of Column	**Mode of Separation**
Normal / reversed phase	Hydrophobicity (polarity) differences
Gel filtration	Size (molecular weight) differences
Ion exchange	Charge difference at particular pH
(Bio-) affinity	Interaction difference with ligand

HPLC is widely used for pharmaceutical analysis in drug discovery, development, manufacturing and post-marketing quality control routine tests due to its sensitivity and accuracy. In pharmaceutical analysis, the widely used columns are reversed and normal phase columns [102], with the former preferentially used often [101]. The two columns differ due to the polarity of mobile and stationary phases as illustrated in Table 4 below.

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Table 4: Columns and their respective stationary and mobile phases as functions of polarity

Column	Stationary Phase	Mobile Phase
Normal phase	Polar	Non-polar
Reversed phase	Non-polar	Polar

In a normal phase chromatography, non-polar molecules are eluted first because the polar molecules associate with the polar stationary phase retaining them in the process. When polarity of the mobile phase is increased, elution time of the polar molecules increases, at the same time increasing the rate at which non-polar molecules are eluted. Due to the lack of reproducibility, it became unpopular until the development of the Hydrophilic Interaction Liquid Chromatography (HILIC) bonded phase, when it became useful again. In reversed phase chromatography, the polar silica gel in the stationary phase is covered with a non-polar layer of alkanes, after reaction of silica gel with long chain hydrocarbon-substituted Silane. This process reverses the polarity phase. Here, polar molecules are eluted first because it is the non-polar molecules that associate with the non-polar stationary phase and get retained. This allows polar molecules to elute first and quickly. Hence, polar compounds are used as mobile phases and water and acetonitrile are commonly used [103].

HPLC analysis can be run using either Isocratic or Gradient elution. Isocratic elution involves the use of a mobile phase with constant composition while in gradient elution, the solvent strength is adjusted during the separation and this is suitable for samples that are complex in nature [103]. Detection is usually done by UV method and/or mass spectrometer for analytes that

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do not show UV absorption. Therefore, to get a desirable well resolved peak, detector, column type and mobile phase are manipulated [103].

When used for quantification, the amount/concentration of the analyte is equivalent to the peak area of the chromatogram or area under the curve (AUC). The first step involves the creation of a calibration curve that is produced by generating various chromatographs from different known concentrations of a reference standard. The peak or curve areas are calculated and equated to their respective known concentrations and this data is used to plot a graph of area under the curve/peak (AUC) against the known concentration of the reference standard, to obtain a calibration curve. The second step involves the determination of the unknown analyte concentration. A measured amount of the sample of unknown concentration is injected into the HPLC system and the peak area of its chromatograph is calculated and its concentration is interpolated from the calibration curve automatically by a computer connected to the HPLC system.

Like most analytical methods, HPLC also has some limitations like difficult resolution for complex samples, one sample analysis at a time, training needed for optimum separation, relatively long analysis times, sample preparation and need for complementary techniques like MS, NMR and IRS sometimes [100] as well as the high cost of columns, reference samples and the HPLC grade solvents.

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CHAPTER THREE

3 CURRENT INVESTIGATION

3.1 SAMPLING

3.1.1 Study Area

The selection of study areas was based on the conventional random sampling in all the country's 3 regions; north, central and south. The country was divided into 4 zones with each zone consisting of 5-7 districts based on the National Malaria Control Programme (NMCP) strategy partitions; giving the south west (1), south east (2), central (3) and north (4) zones. In each zone, the chosen districts were randomly selected based on the prevalence of Malaria, geographical position and economic activities. These are factors that are deemed to influence the demand and inflow of drugs. Therefore, the districts that were selected for the survey were Lilongwe, Blantyre, Zomba, Mzuzu (in Mzimba), Karonga, Dedza, Ntcheu, Mangochi, Mulanje, Thyolo, Mwanza, Kasungu, Mchinji and Nkhota-kota out of the 28 districts. Figure 12 below is a map of Malawi showing the zones and the districts in which sampling took place and Table 5 shows the number of samples that were collected in each zone.

3.1.2 Sample Size

A total of 112 samples was purchased in all the zones based on their availability regardless of size, company name, brand, product name, dosage form and strength, though not more than one sample of the same name, batch number and characteristics was bought at one outlet. Some strategically designated areas were left out due to the lack of antimalarial drug outlets.

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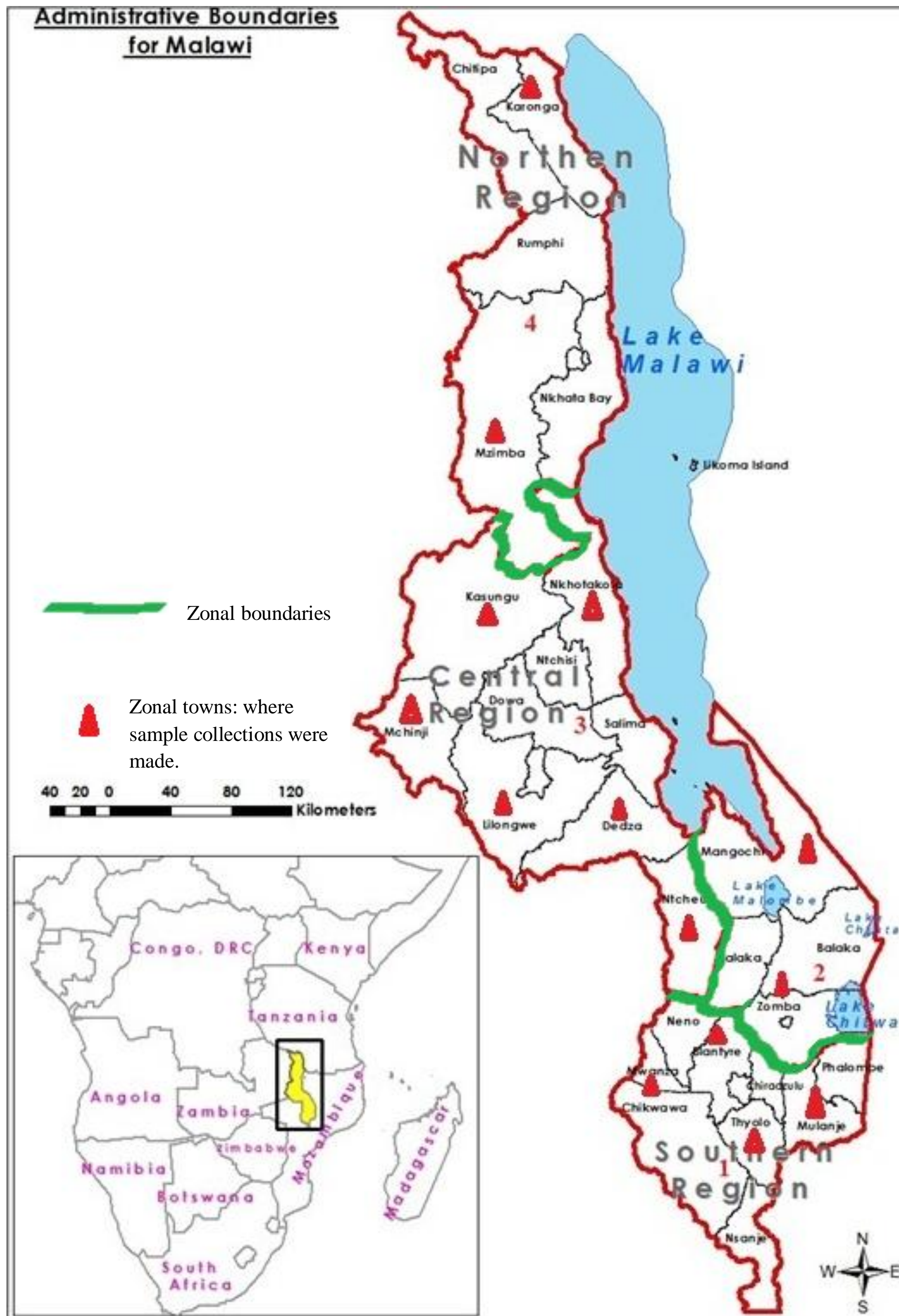

Figure 12: Map of Malawi showing sampling sites

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Table 5: Number of samples by zone of collection

Zone of Collection	Place of Collection	Number of Collected Samples
1	South west	41
2	South east	17
3	Central	25
4	North	29
Total		**112**

The samples consisted of 76 ACTs and 36 non-ACTs representing 67.86% and 32.14% of the total samples respectively. The ACTs were both in fixed dose and single dose co-packed on the same blister combinations, whereas all non-ACTs were fixed dose combinations. The large percentage of ACTs over the non-ACTs suggests that the implementation of ACT use in the country has been on the right track. The largest component of the ACT samples was the artemether-lumefantrine formulation (53.95%) and this might be attributed to the fact that it is a first line treatment for malaria in Malawi and has been found to be more tolerated with minimal side effects. Amongst the non-ACTs, sulphadoxine-pyrimethamine formulation had the largest quantity (63.89%) and this might also be attributed to the fact that it was the first-line treatment prior to the introduction of the artemether-lumefantrine formulation and that people are still using it because it is still being prescribed for special cases of malaria. However, the number of artemether-lumefantrine formulations is larger than sulphadoxine-pyrimethamine. Table 6 below summarizes the categories of drugs that were collected.

The production, sale and use of blister co-packed ACTs was reported to be encouraging artemisinin monotherapy as people preferred using them alone, leaving the non-artemisinin drugs due to either bitterness or saving for another time [104].

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Table 6: Categories of collected antimalarial drug samples

Fixed dose non-ACTs		Doses co-packed on one blister		Fixed dose ACTs	
API	**No.**	**API**	**No.**	**API**	**No.**
Quinine sulphate	6	Ats co-packed with SP	4	Atm/Lum	41
Quinine hydrochloride	3			Dha/Pp	14
Quinine bisulphate	4			Dha/SP	12
SP	23			Ats/SmP	5
TOTAL	**36**		**4**		**72**

Atm; artemether, **Ats**; artesunate, **Lum**; lumefantrine, **Dha**; dihydroartemisinin, **S**; sulphadoxine, **P**; pyrimethamine, **Pp**; piperaquine phosphate, **Sm**; sulphamethoxypyridazine.

Hence, it was recommended that the co-packed blisters should be phased out [104].

However, the results show that there were 4 samples of single dose co-packed on the same blister drugs, a sign that this category of drugs is still in circulation. Considering the fact that the mostly affected people in Malawi are poor and coincidentally also illiterate, there is a high possibility of such occurrence of drug abuse.

3.1.3 Sampling Strategy

Sampling was conducted between December and January, within the rainy season in Malawi. Malawi records peak malarial transmission in the rainy season that is experienced mostly between November and April. This agrees with the fact that during this period, there are so many stagnant water points, which are favourable breeding grounds for the malaria vector, the mosquito. The antimalarial drugs were purchased from every available outlet due to limited number of drug outlets in designated places. Included were both licensed and unlicensed markets `such as private pharmacies and hospitals, street vendors and shops. The samples were in the form of tablets, suspensions, injections and mixtures. Additionally, the samples were composed of APIs such as sulphadoxine, pyrimethamine, quinine, lumefantrine, piperaquine, sulphamethoxypyridazine, artemether, artesunate and dihydroartemisinin. The samples were

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labelled for identification as well as recording and kept in containers that protected them from light, moisture, crushing, heat and mechanical shock.

3.2 PRE-LABORATORY ANALYSIS

3.2.1 Registration Status of Samples

The companies and individuals that manufacture, prepare, circulate and process drug products as well as biological products for human and veterinary use are required by law to register the products with a regulatory body to validate their safety, efficacy and quality. Therefore, the drugs were subjected to registration verification with the drug regulatory authority - the Pharmacy, Medicine and Poisons Board of Malawi as soon as the collection exercise was completed. There were 25 different brands of drugs purchased in all the regions and all of them were presumed to be imported as there were no samples labelled to have been manufactured or packed in Malawi. As of 31st December, 2011, 92% (23/25) of the brands and 86.61% (97/112) of the collected samples as well as their formulation types were registered with the regulatory board, according to their annual registration publication. Table 7 below demonstrates the drug registration status with respect to the zones of collection.

Table 7: Number of samples collected in each zone and their registration status

Zone of Collection	Number of Samples per Zone	Registered Samples per Zone	Unregistered samples per Zone
1	41	35	6
2	17	17	0
3	25	20	5
4	29	25	4

Table 7 demonstrates that zone 2 (central) had best registration standing with 100% (17/17) registration rate, followed by zone 4 with 86.21% (25/29), zone 1 with 85.57% (35/41) and zone

3 with 80% (20/25). On the whole, the registration status of antimalarials in Malawi was found to be quite satisfactory, compared to countries such as Ghana and Togo. The most recent studies indicated 55% and 78% unregistered antimalarials in Ghana in 2008 and 2012 respectively and 17% unregistered antimalarials in Togo in 2012 [67, 68]

3.2.2 Origin of the Collected Drug Samples

None of the drug samples was manufactured locally and an inventory confirmed that they were imported from Asia, Africa and North America. Figure 13 below shows the countries and regions where the drugs were manufactured and imported into Malawi.

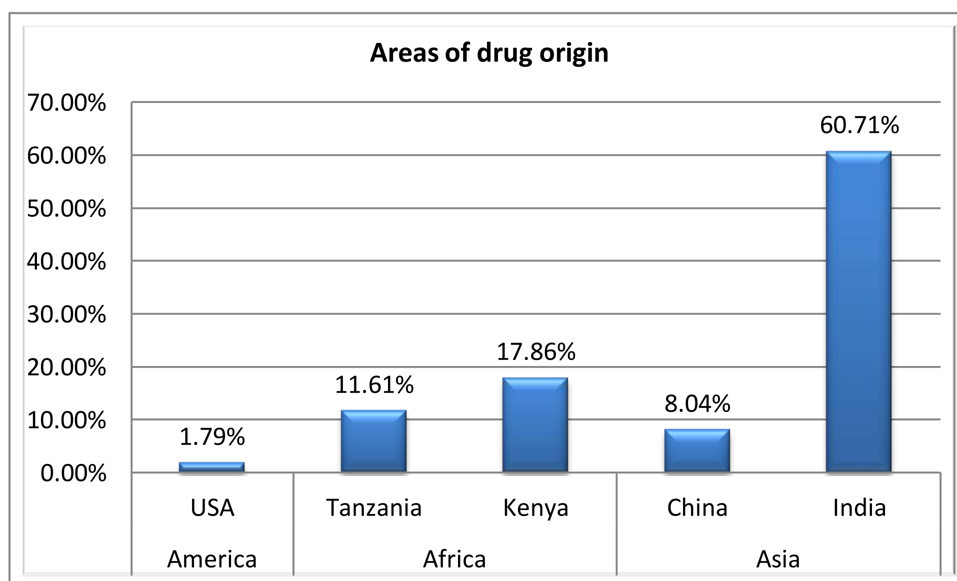

Figure 13: Sources of the collected drug samples

3.2.3 Visual Inspection of Dosage Form and Packaging

This was done with respect to guidelines outlined in the WHO International Pharmacopoeia (2006, Version 2). It is stated that *"Every pharmaceutical preparation must comply with the*

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labelling requirements established under Good Manufacturing Practice." Below are the requirements as stipulated in the pharmacopoeia:

"(1) the name of the pharmaceutical product;

(2) the name(s) of the active ingredient(s); International Non-proprietary Names (INN) should be used wherever possible;

(3) the amount of the active ingredient(s) in each tablet and the number of tablets in a container;

(4) the batch (lot) number assigned by the manufacturer;

(5) the expiry date and, when required, the date of manufacture;

(6) any special storage conditions or handling precautions that may be necessary;

(7) directions for use, warnings, and precautions that may be necessary; and

(8) the name and address of the manufacturer or the person responsible for placing the product on the market."

A sample was deemed to have passed the visual inspection if it contained all the pharmaceutical product information outlined above and the results showed that the manufacturers of all the collected drug samples complied with the packaging material labelling requirements.

3.3 LABORATORY ANALYSIS

3.3.1 Qualitative Colour Reactions

Drugs consist of active pharmaceutical ingredients (APIs) that are responsible for pharmacological effect and excipients, which are inactive pharmaceutical ingredients added together with the APIs for the former's biopharmaceutical, stability and technical purposes. These are basically chemical compounds and they have some distinctive functional groups,

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which are mostly identical to a specific drug API or group of APIs. Reactions and suitable reagents are chosen to distinguish a particular API from the rest. The reactions that exhibit a colour change or produce a precipitate are preferable; a colour change or precipitate formation depicts the presence of the compound in question. This method has been widely used in API identification because it is rapid, cheap, simple and easily applicable; hence suitable for the poorly equipped laboratories in less privileged communities, especially in developing countries. It is mostly the first chemical test for drug products verification of the labelled ingredients after visual inspection. For each API, at least two methods of identification were used because there are some compounds that can mimic API reactions and produce false results [95].

The present drug samples had different APIs, formulations, excipients as well as dosage forms and each API had its distinct methodology and apparatus as specified in pharmacopoeias and the literature. Therefore, all samples were subjected to colorimetric tests to determine if they contained the APIs claimed by the manufacturers and below are the details of the methods and procedures that were used.

3.3.1.1 General Procedure

Drug samples in the form of tablets were prepared for analysis by weighing them using analytical balance individually and all together. The individual and total masses were recorded and later averages derived from them, then compared for consistency. The tablets were ground using a pestle and a mortar to powder. The powdered samples were treated according to the procedures below.

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3.3.1.2 Specific Procedure

3.3.1.2.1 Artesunate containing drug samples

Description of the artesunate containing samples:

- Tablets of artesunate co-packed on the same blister with sulphadoxine/pyrimethamine combination tablets; artesunate/sulphadoxine/pyrimethamine: 100mg / 500mg / 25mg
- Fixed dose combination tablets of artesunate/sulphamethoxypyridazine/pyrimethamine: 200mg / 500mg / 25mg and 100mg / 500mg / 12.5mg formulations

3.3.1.2.1.1 Artesunate

Colour and other reactions [96, 105]

a) *A quantity of the powdered tablets equivalent to 100mg of artesunate was weighed into a clean dry beaker and 40ml of dehydrated ethanol added. The mixture was shaken together to dissolve the active ingredients. This was then filtered and the filtrate divided into approximately 2 equal parts, 20ml each. To one half of the filtrate was added 0.5ml of hydroxylamine hydrochloride TS 2 and 0.25ml of 2M sodium hydroxide solution. This mixture was then heated on a water bath to boiling. The solution was then allowed to cool and two drops of 2M HCl solution added followed by 2 drops of iron (III) chloride (50 g/l).*

   Expected observation: production of a light-red violet colour

b) *The other half of the filtrate from (a) above was evaporated on a water bath to a volume of about 5 ml. A few drops of the mixture were placed on a white porcelain dish and one drop of vanillin/sulphuric acid TS1 added.*

   Expected observation: Production of a reddish-brown colour.

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3.3.1.2.1.2 Sulphadoxine:

Colour and other reactions [105]

a) *A quantity of the pulverized tablet equivalent to 50mg of the fixed-dose combination was dissolved in 3ml of sodium hydroxide (0.1 mol/l) VS and heated gently. The mixture was then cooled and 1.0 ml of copper (II) sulphate (80 g/l) TS was added.*

Expected observation: production of a greenish yellow precipitate, the colour of which changes gradually to blue.

b) *A quantity of the powdered tablets equivalent to 50mg of the fixed-dose combination was dissolved in 2ml hydrochloric acid (~70 g/l) TS. The resulting solution was cooled in ice, treated with 4ml of sodium nitrite (10 g/l) TS and poured into 2ml of 2-naphthol TS1 containing 1g of sodium acetate R.*

Expected observation: production of an orange-red precipitate.

3.3.1.2.1.3 *Pyrimethamine*:

Colour and other reactions [93]

a) A quantity of the powdered tablet equivalent to 0.25g was shaken and dissolved in 50ml of ethanol (~750g/L) TS and heated to 60 ^oC. The solution was then filtered and heated to dryness with constant weight (test substance). 0.05g of the test substance was then dissolved in 5ml of sulphuric acid (~100g/L) TS. Freshly prepared potassio-mercuric iodide TS was then added to the solution.

Expected observation: Formation of a creamy white precipitate.

b) A mixture of 5mL of water and 2mL of ethyl acetate R was added to 1 mL of methyl orange/ethanol TS and shaken; ethyl acetate remained colourless. A solution prepared by

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dissolving 5mg of test substance in 5ml of sulphuric acid (~5g/L) TS was added, shaken well and allowed to settle (about 30 minutes).

Expected observation: Formation of a yellow colour in the ethyl acetate layer.

3.3.1.2.1.4 *Sulphamethoxypyridazine*:

Colour and other reactions [96, 105]

a) *A quantity of the powdered tablet equivalent to 20mg of sulphamethoxypyridazine was added to 10ml of sulphuric acid (100 g/l) TS. This was then shaken to dissolve and 0.1ml of potassium bromate (50 g/l) TS was added to the mixture.*

Expected observation: Production of a yellow colour that changes to amber and brown precipitate gradually forms.

b) *A quantity of the powdered tablets equivalent to 50mg of the fixed-dose combination was dissolved in 2ml hydrochloric acid (~70 g/l) TS. The resulting solution was cooled in ice, treated with 4ml of sodium nitrite (10 g/l) TS and poured into 2ml of 2-naphthol TS1 containing 1g of sodium acetate R.*

Expected observation: Production of a bright orange-red precipitate.

3.3.1.2.1.5 Results for the basic tests

Table 8: Results for basic tests of artesunate containing drug samples

Identity	Artesunate		Sulphadoxine		Pyrimethamine		Sulphamethoxypyridazine	
	Test a	Test b	Test a	Test b	Test a	Test b	Test a	Test b
*2_4Y_{13}	+	+	+	+	+	+		
*$4Y_{13}$	+	+	+	+	+	+		
*3_4Y_{13}	+	+	+	+	+	+		
*3_2Y_{13}	+	+	+	+	+	+		
4_4Y_{12}	+	+			+	+	+	+
4_2Y_{12}	+	+			+	+	+	+
4_3Y_{12}	+	+			+	+	+	+

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4_1Y_{12}	+	+			+	+	+	+
3_1Y_{12}	+	+			+	+	+	+

* stands for samples co-packed on the same blister, + means API present.

3.3.1.2.2 Artemether containing drug samples

Description of the artemether containing drug samples:

- Fixed dose combination tablets and suspension powder of artemether / lumefantrine: 80mg/480mg; 20mg/120mg; 40mg/240mg; 180mg/1080mg formulations.

3.3.1.2.2.1 *Artemether*:

Colour and other reactions for artemether tablets [96, 105]

a) *To a quantity of the powdered tablets equivalent to about 80 mg of Artemether was added 40 ml of dehydrated ethanol. The solution was shaken well to dissolve, and filtered. Half of the filtrate was evaporated to about 1 ml and 100 mg of potassium iodide was added and heated on a water bath for about five minutes.*

   Expected observation: Production of a yellow colour.

b) *The remaining filtrate from test (a) above was evaporated to about 5 ml. A few drops of this solution was placed on a white porcelain dish and 1 drop of vanillin/sulphuric acid TS1 added.*

   Expected observation: Production of a pink colour.

3.3.1.2.2.2 *Lumefantrine*:

Colour and other reactions [92]

a) *To a quantity of the powdered tablets equivalent to 10mg of lumefantrine was added 5ml of ethanol and shaken well to dissolve the active ingredient. 20 mg of MnO_2 was added to the*

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solution and boiled on a water bath for about a minute. The solution was filtered and a few drops of 2, 4-dinitrophenylhydrazine (2, 4-DNPH) solution were added and shaken.

Expected observation: Appearance of an orange precipitate of hydrazone within a minute.

b) *To a quantity of the powdered tablets equivalent to 10 mg Lumefantrine in a test tube was added 5 ml of ethyl acetate. A few drops of 1M HCl solution were added. The solution was stirred, warmed and filtered. To a portion of the test solution was added a few drops of Dragendorff's reagent.*

Expected observation: formation of a brown to orange precipitate within 5 minutes.

3.3.1.2.2.3 Results for the basic tests

Table 9: Results for basic tests of artemether containing drug samples

Identity	Artemether		Lumefantrine	
	Test a	**Test b**	**Test a**	**Test b**
$2X_{15}$	+	+	+	+
$4X_{20}$	+	+	+	+
1_1X_1	+	+	+	+
1_1X_{11}	+	+	+	+
1_1X_{14}	+	+	+	+
1_1X_{17}	+	+	+	+
1_1X_{18}	+	+	+	+
1_1X_{20}	+	+	+	+
1_2X_1	+	+	+	+
1_2X_{11}	+	+	+	+
1_2X_{14}	+	+	+	+
1_3X_1	+	+	+	+
1_3X_{11}	+	+	+	+
1_4X_1	+	+	+	+
1_4X_{11}	+	+	+	+
1_5X_1	+	+	+	+
1_5X_{11}	+	+	+	+
1_6X_1	+	+	+	+
1_7X_1	+	+	+	+
1_8X_1	+	+	+	+
1_9X_1	+	+	+	+
2_1X_1	+	+	+	+

2_2X_1	+	+	+	+
2_3X_1	+	+	+	+
2_4X_1	+	+	+	+
2_5X_1	+	+	+	+
3_1X_1	+	+	+	+
3_2X_1	+	+	+	+
3_1X_{11}	+	+	+	+
3_3X_1	+	+	+	+
3_4X_1	+	+	+	+
3_6X_1	+	+	+	+
4_1X_{11}	+	+	+	+
4_2X_{11}	+	+	+	+
4_3X_1	+	+	+	+
4_4X_1	+	+	+	+
4_5X_{12}	+	+	+	+
$1_{10}X_1$	+	+	+	+
1_6X_{11}	+	+	+	+
$1_{12}X_1$	+	+	+	+
$1_{13}X_1$	+	+	+	+

3.3.1.2.3 *Dihydroartemisinin (artenimol) containing drug samples*:

Description of the dihydroartemisinin containing samples:

- Fixed dose combination tablets of artenimol / piperaquine phosphate : 40mg / 320mg
- Fixed dose combination tablets of dihydroartemisinin / sulphadoxine / pyrimethamine: 60mg / 500mg / 25mg.

3.3.1.2.3.1 Dihydroartemisinin (Artenimol)

Colour and other reactions [96, 105]

a) *To a quantity of the powdered tablets equivalent to 10mg of artenimol, 20ml of dehydrated ethanol was added, shaken to dissolve, filtered and evaporated to dryness. The following reagents were added to half of the residue: 0.1ml of dehydrated ethanol, 1ml of potassium iodide (80g/l) TS, 2.5ml of sulfuric acid (~100g/l) TS, and 4 drops of starch TS.*

Expected observation: production of a violet colour

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b) *The remaining residue from test (a) above was dissolved in 0.5ml of dehydrated ethanol R, and about 0.5ml of hydroxylamine hydrochloride TS2 and 0.25ml of sodium hydroxide (~80 g/l) TS were added. The mixture was heated in a water-bath to boiling and cooled. Two drops of hydrochloric acid (~70g/1) TS and 2 drops of ferric chloride (50g/l) TS were added.*

Expected observation: immediate production of a deep violet colour.

3.3.1.2.3.2 *Piperaquine*:

Colour and other reactions for piperaquine [92]

To a quantity of the powdered tablets equivalent to 10 mg piperaquine in a test tube was added 5cm^3 of distilled water. A few drops of 1M HCl solution were added. The solution was stirred, warmed and filtered. To the filtrate was added a few drops of Dragendorff's reagent.

Expected observation: Formation of a copious brownish red precipitate.

3.3.1.2.3.3 Results for the basic tests

Table 10: Results for basic tests of dihydroartemisinin containing drug samples

Identity	Dihydroartemisinin		Sulphadoxine		Pyrimethamine		Piperaquine
	Test a	Test b	Test a	Test b	Test a	Test b	Test
1_1Z_3	+	+					+
1_2Z_3	+	+					+
1_4Z_3	+	+					+
1_6Z_1	+	+					+
1_7Z_1	+	+					+
2_6Z_1	+	+					+
2_7Z_1	+	+					+
3_2Z_3	+	+					+
3_3Z_3	+	+					+
3_7Z_1	+	+					+
4_1Z_3	+	+					+
4_2Z_5	+	+					+
4_3Z_3	+	+					+
4_4Z_3	+	+					+
1_5Z_1	+	+	+	+	+	+	
1_3Z_1	+	+	+	+	+	+	

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1_2Z_1	+	+	+	+	+	+	
1_4Z_1	+	+	+	+	+	+	
2_1Z_1	+	+	+	+	+	+	
2_3Z_1	+	+	+	+	+	+	
2_4Z_1	+	+	+	+	+	+	
2_5Z_1	+	+	+	+	+	+	
3_4Z_1	+	+	+	+	+	+	
3_5Z_1	+	+	+	+	+	+	
4_5Z_1	+	+	+	+	+	+	
4_6Z_1	+	+	+	+	+	+	

3.3.1.2.4 *Quinine containing drug samples*:

Description of the quinine containing samples:

- Quinine injection containing 300mg of quinine dihydrochloride
- Quinine suspension containing 150mg of quinine sulphate
- Quinine mixture containing 50mg/5mL of quinine bisulphate BP

3.3.1.2.4.1 Colour and other reactions for quinine injection and other dosage forms [105]

a) *A quantity of quinine containing samples equivalent to 5mg was dissolved in 10mL of water. 1 drop of sulphuric acid (~100 g/l) TS was added to the solution.*

   Expected observation: Production of a strong blue fluorescence

b) *To another 10 ml of the solution prepared for test 1, was added 0.15 ml of bromine TS1 and 1 ml of ammonia (100 g/l) TS.*

   Expected observation: Production of an emerald-green colour.

3.3.1.2.4.2 Result for the basic tests

Table 11: Results for basic tests of quinine containing drug samples

Identity	Test a	Test b
1_1V_5	+	+

1_2V_5	+	+
1_3V_5	+	+
4_1Q_6	+	+
4_2Q_6	+	+
4_3Q_6	+	+
$4V_5$	+	+
4_1R_8	+	+
4_2R_4	+	+
4_3R_4	+	+
3_1Q_6	+	+
3_2Q_6	+	+
3_3Q_6	+	+

3.3.1.2.5 Sulphadoxine and Pyrimethamine drug samples

Description of the sulphadoxine and pyrimethamine drug samples:

- Fixed dose combination tablets of sulphadoxine / pyrimethamine: 500mg / 25mg.

Tests as described in sections **3.3.1.2.1.2** and **3.3.1.2.1.3**

3.3.1.2.5.1 Results for the basic tests

Table 12: Results for basic tests of sulphadoxine and pyrimethamine drug samples.

Identity	Sulphadoxine		Pyrimethamine	
	Test a	Test b	Test a	Test b
1_1P_{10}	+	+	+	+
1_1P_2	+	+	+	+
1_2P_2	+	+	+	+
1_4P_{10}	+	+	+	+
1_4P_2	+	+	+	+
1_6P_{10}	+	+	+	+
2_1P_{15}	+	+	+	+
2_1P_2	+	+	+	+
2_2P_2	+	+	+	+
2_3P_2	+	+	+	+
3_1P_{10}	+	+	+	+
3_1P_2	+	+	+	+
3_2P_{10}	+	+	+	+
3_2P_2	+	+	+	+
3_3P_{10}	+	+	+	+
3_5P_2	+	+	+	+
3_7P_{15}	+	+	+	+

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3_8P_{15}	+	+	+	+
4_1P_2	+	+	+	+
4_2P_2	+	+	+	+
4_4P_2	+	+	+	+
4_5P_2	+	+	+	+
4_8P_5	+	+	+	+
*2_4Y_{13}	+	+	+	+
*$4Y_{13}$	+	+	+	+
*3_4Y_{13}	+	+	+	+
*3_2Y_{13}	+	+	+	+

The results of the colorimetric tests demonstrated that all the samples contained the requisite APIs claimed by the manufacturers. The next phase of the work involved the SQ-TLC and HPLC assays to quantify the APIs in the samples and compare with pharmacopoeial specifications and manufacturers' claims.

3.3.2 Semi-Quantitative Thin Layer Chromatography (Sq-Tlc)

SQ-TLC is a tool that has been used to verify identity and quantity of the active pharmaceutical ingredients (APIs) in drugs. This is a semi-quantitative tool because it gives the estimated amounts of the active ingredients in the drugs. This technique has two designated solvent systems for each drug API in a formulation [92] and the Tables 13 and 14 below summarise the drug APIs, their respective solvent systems and spraying reagents.

Table 13: Solvent systems for artemisinin derived active pharmaceutical ingredients

API	**Solvent system 1 (S1)**	**Solvent system 2 (S2)**	**Spraying reagent**	**Colour**
Artesunate	Ethanol : Ammonia 100 : 0.5	Ethanol: Toluene: Ammonia 70 : 30 : 1.5	Anisaldehyde/ methanol	Purple
Artemether	Petrol: Ethyl acetate 70 : 30	Toluene: Ethyl acetate 70 : 30	Anisaldehyde/ methanol	Purple
Artenimol	Toluene: Ethyl acetate 60 : 40	Toluene: Ethyl acetate 70 : 30	Anisaldehyde/ methanol	Purple

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Table 14: Solvent systems for non-artemisinin derived active pharmaceutical ingredients

API	Solvent system 1 (S1)	Solvent system 2 (S2)	Spraying reagent	Colour
Chloroquine	Methanol: ammonia 100:1.5	Ethyl acetate: acetic acid: water 60:20:20	I_2-KI	Brown
Quinine	Methanol: ammonia 100:1.5	Ethyl acetate: acetic acid: water 60:20:20	I_2–KI	Brown
Amodiaquine	Ethanol: ammonia 100:1.5	Ethanol: Toluene: Ammonia 70:30:1.5	Cobaltous NO_3 saturated with NaCl	Green
Pyrimethamine	Ethyl acetate: methanol: ammonia 80:15:5	Ethyl acetate: acetic acid: water 60:20:20	I_2-KI	Brown
Sulphadoxine	Ethyl acetate: methanol: ammonia 80:15:5	Ethyl acetate: Methanol: acetic acid 75:25:1	I_2-KI	Brown
Lumefantrine	Ethyl acetate: acetic acid: toluene 4:2:18	Ethyl acetate: acetic acid 10:5	I_2-KI	Brown

3.3.2.1 Spotting, Development and Detection of Chromatogram

An origin line was marked at 1.5 cm from and parallel to the bottom end of the chromatoplate. Different amounts of a fixed 1mg/mL concentration of RS in µL (1.0-2.0) corresponding to the amounts in µg and a fixed amount of the drug sample (2µg) were spotted onto the plate. Solvent systems were placed in the TLC tanks lined with filter paper and allowed to stay for homogenous mixing and saturation of the solvents before the TLC plates were placed in them. The chromatoplates were left until the solvent front reached the upper limit line; then removed and left to dry. For alkaloidal drugs, drying was followed by spraying the appropriate reagent and then scanned onto a computer. For artemisinins, drying was followed by spraying with anisaldehyde and heated in an oven at 120 ^{o}C. The coloured (purple) spots (Figure 15) were scanned and saved on a computer.

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3.3.2.2 Data Capturing and Analysis

Using Microsoft Office Picture Manager, the brightness of the spots on the developed chromatograms was changed continuously and the rates at which the RS and test solution spots faded were compared simultaneously. The principle of the method is that the RS and the sample test solution spots on the chromatogram that had the same concentration would fade at the same time with the increase in the brightness of the computer screen. For example, if a spot of a sample test solution is presumed to contain 2 µg (µL), it must fade at the same time as 2 µg of the RS. Figure 14 below shows a sample of a developed TLC plate of an artemether containing antimalarial drug.

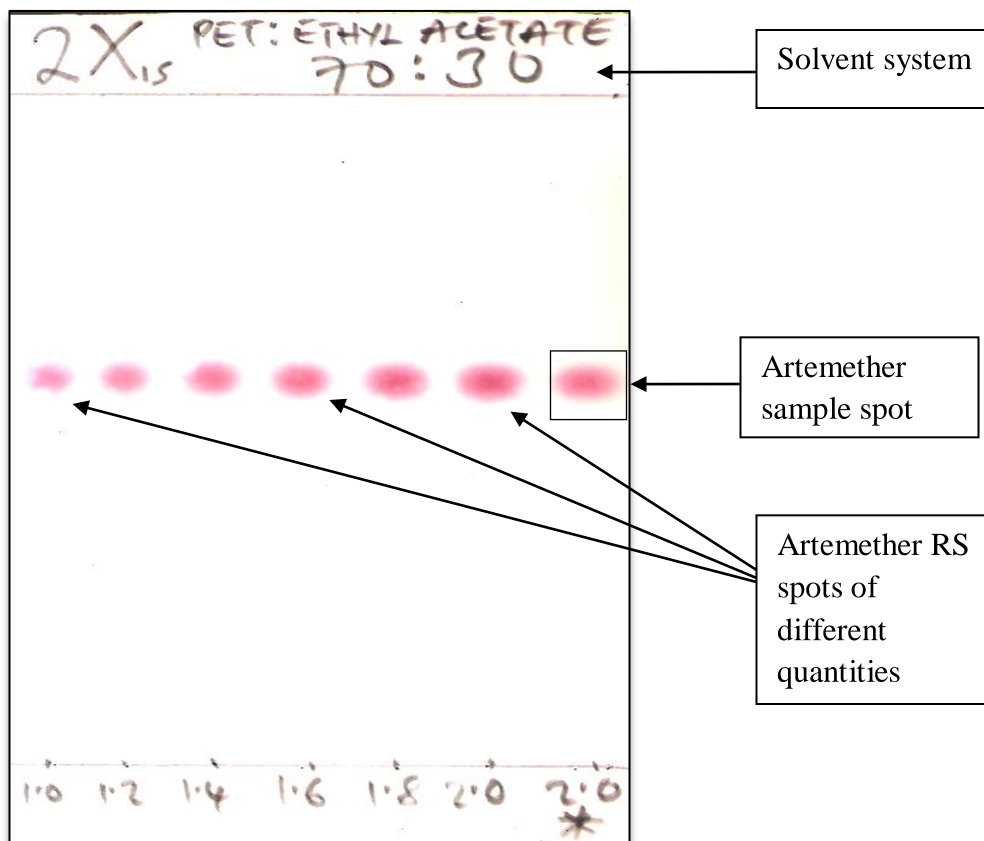

Figure 14: A sample of developed TLC plate of an artemether containing drug

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The concentration of a test solution is estimated by taking note of the RS spot that begins to fade simultaneously with the spot of the sample test solution and one that fades completely after the spot of the test solution. This gives the range in which the amount of the API test solution falls. For example, if the spot of the test solution (2µL) of a drug containing 200mg of an API expected to fade at the same time as the 2µL reference standard spot fades between 1.4µL and 1.6 µL spots of the RS, then;

1.4µL = lower limit

1.6µL = upper limit

of the API content of the test solution

In percentage equivalent:

Lower limit = 1.4/2.0 x 100 = 70.0%

Upper limit = 1.6/2.0 x 100 = 80.0%

Converting percentage to mass:

Lower limit = 1.4/2.0 x 200 mg = 140 mg

Upper limit = 1.6/2.0 x 200 mg = 160 mg

This implies that the API quantity is within 70 - 80% (140 – 160) mg, out of the 200 mg claimed by the manufacturer on the pack and it is also not compliant with the pharmacopoeial requirement that stipulates that each tablet must contain not less than 90% and not more than 110% of the manufacturers label claim. So this was done for all the 12 replicates, 6 from each of the two solvent systems. The average for the upper and lower limits was calculated for each drug sample. The data were transferred to Microsoft Windows Excel 2007 for further analysis.

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3.3.3 High Performance Liquid Chromatography (HPLC) Assays

This method was used as a validation tool for the results of the SQ-TLC method. There were different kinds of drug formulations whereby some drugs had 2 APIs while others had 3 APIs combined. A simultaneous assay was used where possible and single API analysis where the former was not feasible. The analysis was done with reference to pharmacopoeias and published validated assays subject to some adjustments in some cases where the available conditions and prescribed methods gave unrealistic results. The adjustments made have been outlined thoroughly in the experimental section. In all cases, an appropriate calibration curve using pure reference samples of the requisite API was plotted. These were used in calculating the API contents of the various dosage forms. Details are given in the experimental section.

3.3.3.1 Details of Various Drug Assays

3.3.3.1.1 HPLC Assay of Artesunate

The experimental conditions of the assay for the HPLC analysis of artesunate were formulated from the modification of a validated method by Ranher *et al.* [106]. Outlined below are the conditions of the adapted assay:

- Column measurements: Discovery C-18 bonded, 5µm, 25cm x 4mm.
- Mobile phase: 70: 30 v/v, 1% triethylamine (TEA) in methanol: buffer (10mM KH_2PO_4/ 85% H_3PO_4 , pH=2.5)
- retention time (average): 5.1 minutes
- detection wavelength: 216nm
- Flow rate: 1.2 mL/ min.
- volume of injection: 20 µL

The adapted assay was successfully implemented and produced well resolved chromatograms. Figure 15 below is a typical example of the chromatograms obtained.

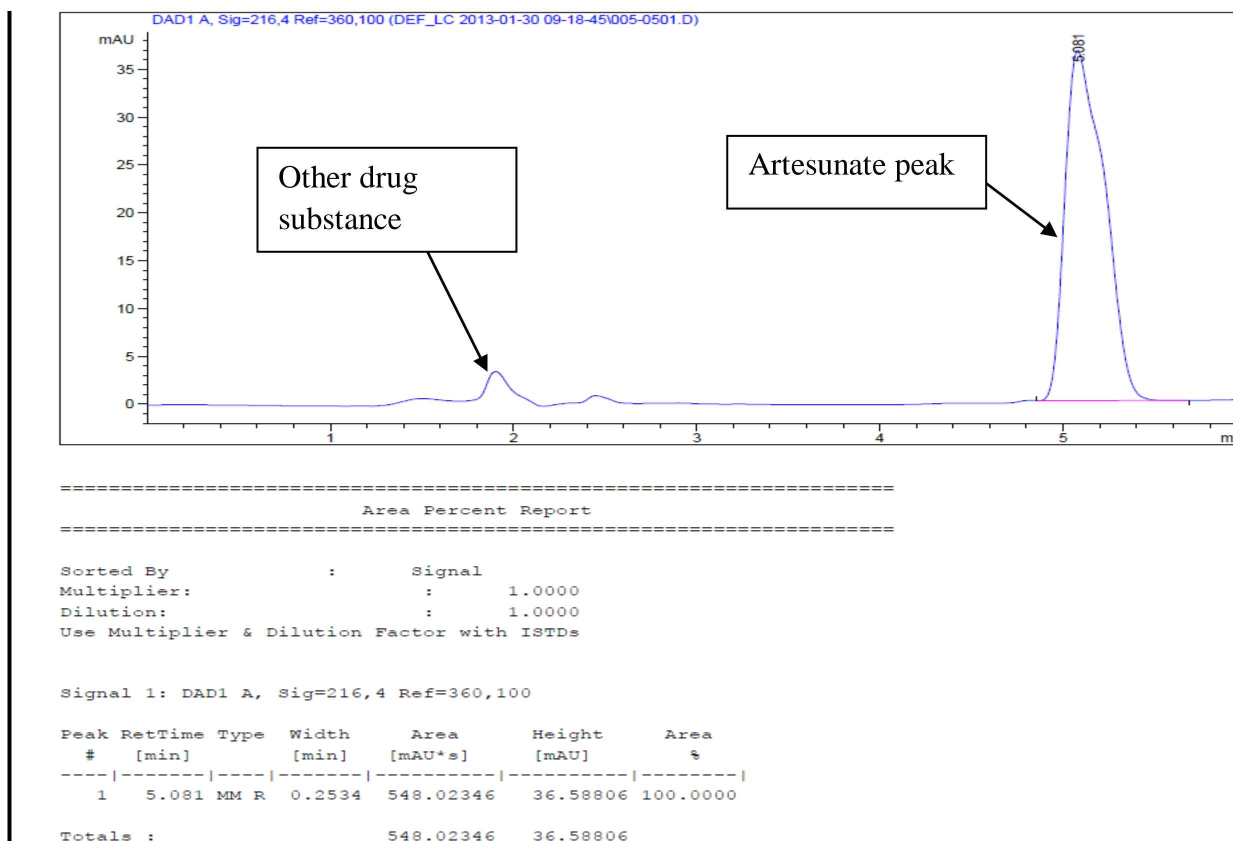


Figure 15: Chromatogram of a sample solution containing artesunate

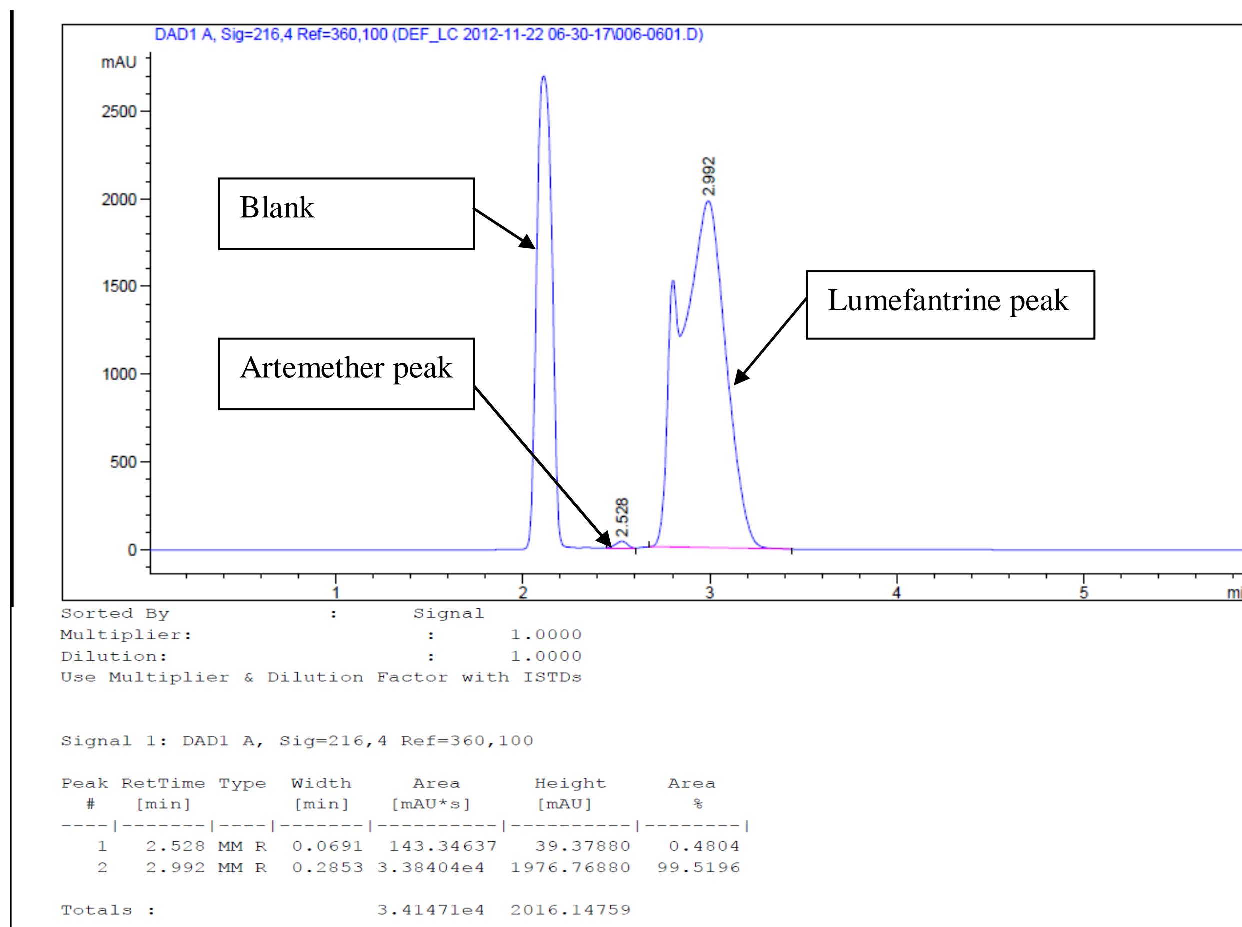
3.3.3.1.2 HPLC Assay of Artemether/Lumefantrine FDC Formulation

The assay of artemether/lumefantrine was carried out using a modified method of Arun and Smith (2011) [107] details of which are given below.

- Column measurements: Hyperprep PEP 300A C4, 8µm, 25cm x 4.6mm.
- Mobile phase: 70: 30 v/v, acetonitrile: 10mM buffer consisting of KH_2PO_4 mixed with 1 mL of triethylamine (TEA) per liter and pH changed to 2.5 using 85% H_3PO_4 mixture.
- Retention time (average): artemether appeared at 2.5 minutes and lumefantrine at 3 minutes.

- Detection wavelength: 216nm
- Flow rate: 1.5mL/min.
- Injection volume: 20 µL

A sample of the chromatograms produced from the modified experimental conditions is depicted in Figure 16 below.



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.528	MM R	0.0691	143.34637	39.37880	0.4804
2	2.992	MM R	0.2853	3.38404e4	1976.76880	99.5196
Totals :				3.41471e4	2016.14759	

Figure 16: Chromatogram of a sample solution containing artemether and lumefantrine

3.3.3.1.3 HPLC Assay of Dihydroartemisinin (Artenimol)

The assay of dihydroartemisinin was adopted from the Ph. Int. [105] with few modifications and the following experimental conditions were produced and used after the adaptations:

- Column measurements: Kramasil C8, 5 µm, 25cm x 4.6mm
- Mobile phase: 50: 50 v/v, water: acetonitrile
- Retention time (average): 5.2 minutes
- Flow rate: 1.5mL/min.
- Detection wavelength: 210nm
- Volume of injection: 10µL

Using these conditions, dihydroartemisinin was unequivocally analyzed and well resolved chromatograms were generated (Figure 17).

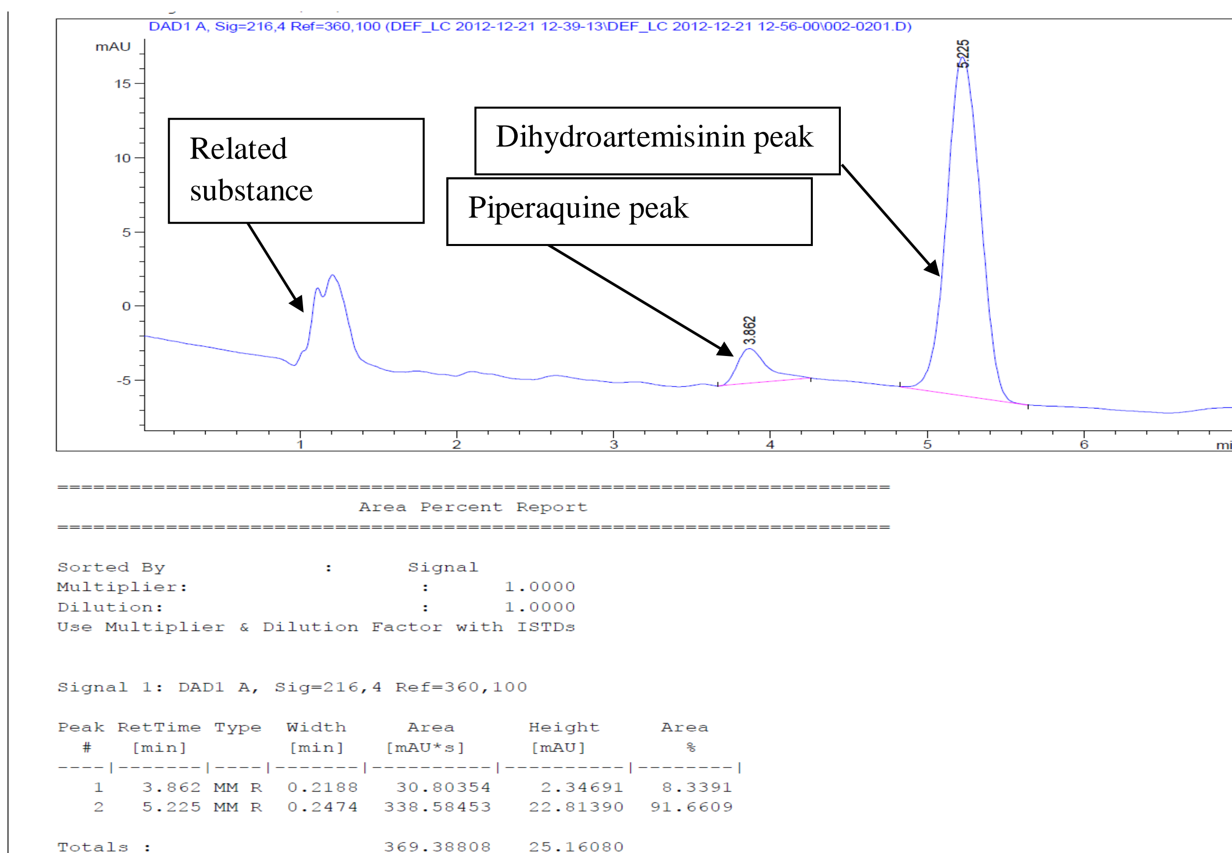


Figure 17: Chromatogram of a sample solution containing dihydroartemisinin

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3.3.3.1.4 HPLC Assay of Sulphadoxine/Pyrimethamine

WHO expert committee, 2011 adopted a method for the simultaneous assay of sulphadoxine and pyrimethamine due to the dearth of the assay in the previous editions of the Ph. Int., which was intended to be included in the subsequent edition (2010 edition) [108]. In this study, the method conditions were adapted as follows:

- Column measurements: Ascentis C-18 column, 5µm, 15cm x 4.60mm.
- Mobile phase: 65: 10: 25 v/v, 20mM buffer (KH_2PO_4/Na_2HPO_4 of pH 5.6: methanol: acetonitrile.
- retention time (average): 3.9 minutes for sulphadoxine and 8.7 minutes for pyrimethamine
- Flow rate: 1mL/min
- Detection wavelength: 240nm
- Volume of injection: 10µL

Figure 18 below is an illustration of a sample chromatogram that was produced from the application of the conditions of the modified assay.

3.3.3.1.5 HPLC Assay of Quinine

Quantitative determination of quinine described in the USP 24 [109] was modified and used in the HPLC assay of quinine preparations and the following conditions were generated after the changes:

- Column measurements: Discovery C-18 bonded, 5 µm, 25cm x 4mm
- Mobile phase: 80: 16: 2: 2 v/v, water: acetonitrile: methanesulfonic acid: TEA (pH 2.6)
- Average Retention time: 4.7 minutes.

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- Flow rate: 1.2mL/min.
- Wavelength for detection: 235nm
- Injection volume: 20 µL

Figure 19 below is a demonstration of one of the chromatograms produced from the assay conditions outlined above.

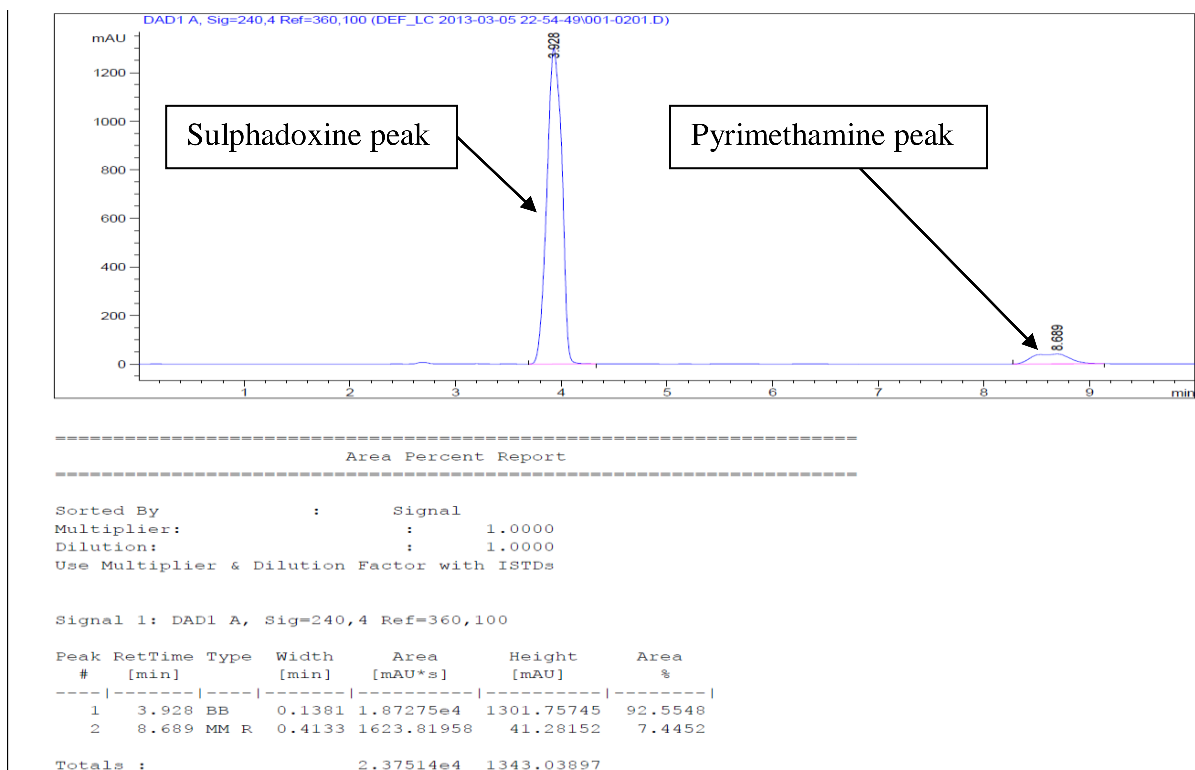

Figure 18: Chromatogram of a sample containing sulphadoxine and pyrimethamine

3.3.3.2 API Content from the HPLC Assay

The calculation of the API content in the HPLC analyzed samples is demonstrated below using sample, 3_1X_1: Label claim of API content per tablet = **ATM**/LUM: **20mg**/120mg (calculation of quantity of the artemether component only)

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Weight of 10 tablets = 2.9390g

Average weight of each tablet = 0.2939g = 293.9mg

Weight of tablets corresponding to 4 mg of artemether used for analysis is found as follows:

= 4mg/20mg x 293.9mg

= 58.78mg equivalent of artemether API

From the calibration curve;

AUC = 176.08 C + 74.287 and

C = (AUC – 74.287)/176.08; But AUC= 139.301

C = (139.301-74.287)/176.08

= 0.3692mg/mL

Sample solution prepared for the assay = 10mL

Quantity of artemether in 10mL = 0.3692mg/mL x 10mL

= 3.692mg

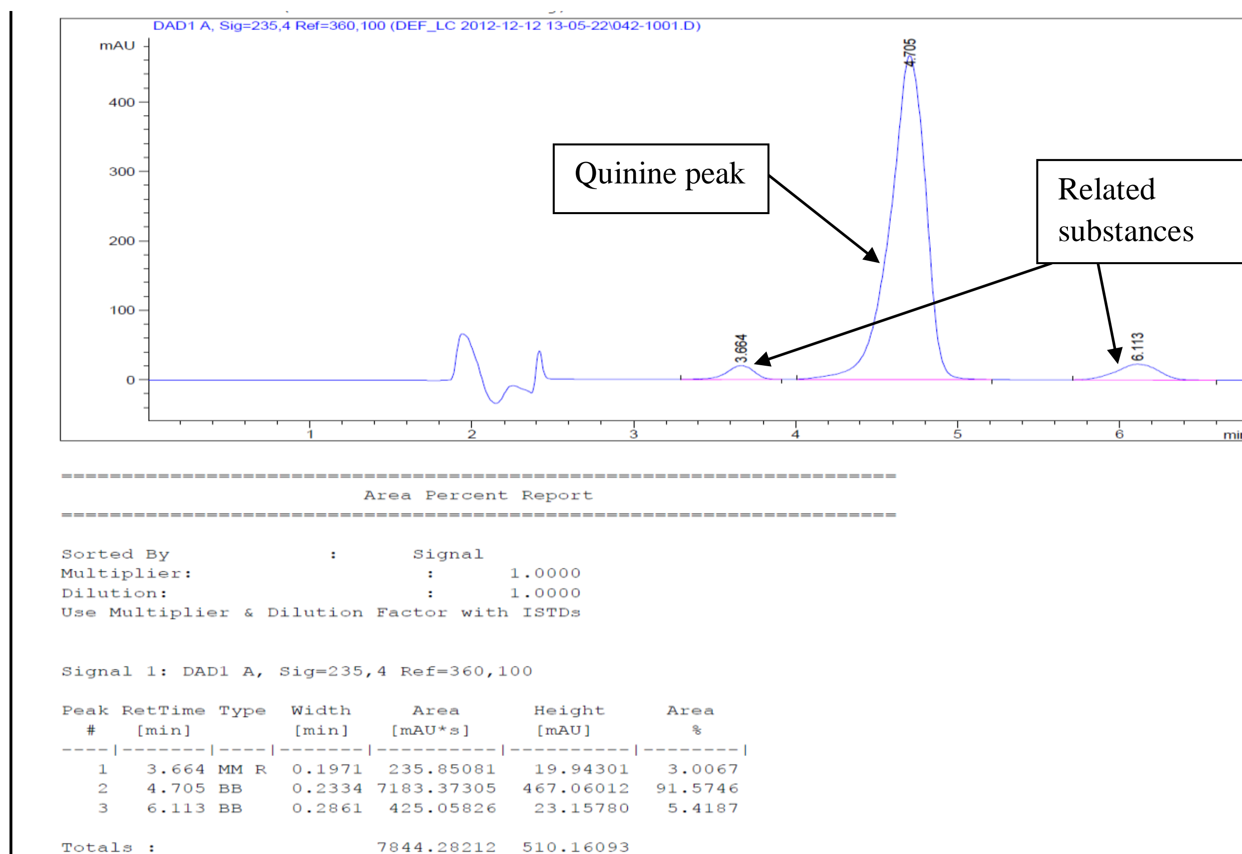
Percentage of the actual amount to the expected amount = 3.692mg/4mg x 100

= 92.3%

Therefore, the sample contains:

92.3/100 x 20mg = 18.46mg of artemether per tablet.

Since pharmacopoeial specifications require the drug to contain not less than 90% and not more than 110% of the manufacturer’s claim, sample 3_1X_1 will be deemed to have complied with pharmacopoeial specifications.



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=235,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.664	MM R	0.1971	235.85081	19.94301	3.0067
2	4.705	BB	0.2334	7183.37305	467.06012	91.5746
3	6.113	BB	0.2861	425.05826	23.15780	5.4187
Totals :				7844.28212	510.16093	

Figure 19: Chromatogram of a sample solution containing quinine

3.4 DISCUSSION

3.4.1 SQ-TLC and HPLC Results

There were three classes of results; identified as compliant (C), non-compliant (NC) and borderline compliant (BLC). A requisite API in a drug was classified as “compliant” if its quantity fell within acceptable limits of the Ph.Int.; “non-compliant” if the quantity was more (overdose) or less (under dose) than the acceptable limits and “borderline compliant” if the amount was marginally compliant with Ph. Int. limits by **±5% for SQ-TLC** and **±2% for HPLC**. In addition, a drug was graded as compliant if the entire component APIs in it were

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compliant; non-compliant if at least one of the API components was non-compliant and borderline compliant if all components are separately borderline compliant; one component or more is borderline amongst other compliant components of a drug.

3.4.1.1 SQ-TLC Method Results

3.4.1.1.1 Analysis of Drug API Components

Figure 20 below shows the quality status of the individual APIs when the SQ-TLC method was used to estimate their quantities.

The samples were analysed for artemether, artesunate, dihydroartemisinin, sulphadoxine, pyrimethamine, lumefantrine and quinine active pharmaceutical ingredients (APIs). For artesunate, there were 33.33% (3/9) of samples that were borderline compliant, whereas 44.44% (4/9) were compliant and 22.22% (2/9) non-compliant (under dose). 9.76% (4/41) of artemether samples were found to be borderline compliant, with 24.39% (10/41) and 65.85% (27/41) being compliant and non-compliant respectively, with the non-compliance arising due to 13 (48.15%) samples being overdose and 14 (51.85%) being under dose. The samples containing lumefantrine posted borderline qualification of 17.07% (7/41) and 31.71% (13/41) were compliant, with the remaining 51.22% (21/41) being non-compliant; whereby twelve (12) (57.14%) samples of the non-compliant samples (21) were overdose and 9 (42.86%) of them under dose.

For dihydroartemisinin, 7.69% (2/26) were found to be borderline compliant, 23.08% (6/26) compliant and 69.29% (18/26) non-compliant (under dose).

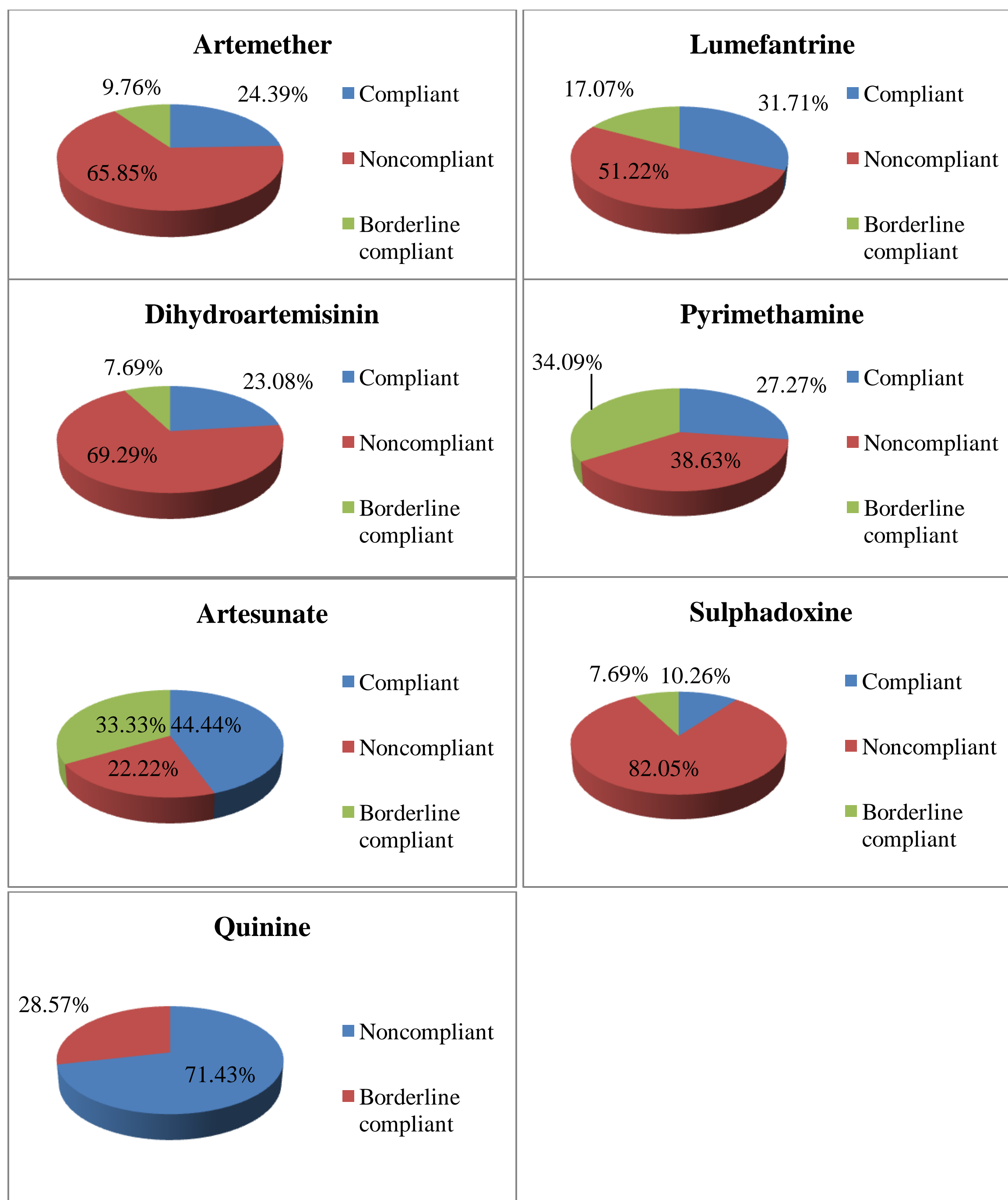

Figure 20: SQ-TLC results of individual APIs in the antimalarial drug samples

Out of the 39 samples with sulphadoxine component, 7.69% (3/39) were found to be borderline compliant, 10.26% (4/39) compliant with a further 82.05% (32/39) being non-compliant (under

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dose). The quantity of pyrimethamine samples found to be borderline compliant were 34.09% (15/44) with 27.27% (12/44) and 38.63% (17/44) being compliant and non-compliant respectively, with 5 out of the non-compliant samples (17) being overdosed. The analysis of the quinine samples showed that while 28.57% (2/7) were borderline compliant, 71.43% (5/7) were non-compliant, with all of the 5 (100%) non-compliant samples being overdose. Some quinine containing samples were not analyzed for the quinine API due to the insufficiency of the RS material. Therefore, the results demonstrated the widespread occurrence of substandard API quantities, with sulphadoxine being the most compromised.

3.4.1.1.2 Analysis of Drug Samples as a Whole Based on SQ-TLC Results

Classification of a drug as being compliant or otherwise in the case of fixed dose combination (FDC) drugs depends on compliance of all APIs present.

3.4.1.1.2.1 Artesunate/Sulphadoxine/Sulphamethoxypyridazine/Pyrimethamine Containing Samples

There were 9 samples of this API combination constituting 8.04% of the total samples (112). Out of the 9 samples, 5 samples had artesunate/sulphamethoxypyridazine/pyrimethamine while the rest (4) had artesunate/sulphadoxine/pyrimethamine API combination. Analysis of artesunate/sulphamethoxypyridazine/pyrimethamine samples showed that 40% (2/5) samples had been found to be non-compliant (under dose) for pyrimethamine component, whereas 20% (1/5) and 40% (2/5) samples were found to be compliant and borderline compliant respectively. In terms of artesunate component, 40% (2/5) were non-compliant while the other 40% (2/5) were compliant and the remaining 20% (1/5) of the samples was borderline compliant.

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Sulphamethoxypyridazine component was not successfully analyzed using SQ-TLC due to the procedural limitations of the analytical method, that is, although the spots of the sulphamethoxypyridazine on the TLC plate had the same retention time as the sulphadoxine spots, they could not be stained well with the spraying agent Iodine-Potassium iodide solution (I_2/KI). Therefore, these samples were analyzed as a whole using the other available components.

On the other hand, results of the components in the artesunate/sulphadoxine/pyrimethamine samples were as follows: samples with artesunate component being compliant were 2 (50%) and borderline compliant samples were also 2 (50%). For sulphadoxine component, all the samples were found to be non-compliant (under dose) and the pyrimethamine component registered 3 (75%) samples as being non-compliant all of which were also under dose and 1 (25%) sample being borderline compliant. Thus, on the whole, SQ-TLC tests showed that 22.22% (2/9) and 77.78% of these samples were borderline compliant (Ats/SmP) and non-compliant respectively.

3.4.1.1.2.2 Artemether / Lumefantrine Containing Samples

Out of the 41 artemether/lumefantrine samples, 27 artemether and 21 lumefantrine samples were non-compliant, with 13 (48.15%) and 12 (57.14%) of the non-compliant artemether and lumefantrine components respectively being overdose. Therefore, overall SQ-TLC results of the artemether/lumefantrine components showed that 2.44% (1/41) samples were borderline compliant, whereas 4.88% (2/41) were compliant and 92.68% (38/41) non-compliant.

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3.4.1.1.2.3 Dihydroartemisinin/Piperaquine Phosphate Containing Samples

These samples were analyzed for the dihydroartemisinin component only due to the lack of a piperaquine reference standard. Therefore, they were treated like a monotherapy drug in the analysis for dihydroartemisinin. Approximately 14.29% (2/14) of the samples were borderline compliant, with 42.86% (6/14) and 42.86% (6/14) being compliant and non-compliant correspondingly. All non-compliant components were under dose.

3.4.1.1.2.4 Dihydroartemisinin/Sulphadoxine/Pyrimethamine Containing Samples

For the samples of this combination, 83.33% (10/12) of the samples had sulphadoxine component non-compliance (under dose), with 8.30% (1/12) of samples being compliant and another 8.30% (1/12) being borderline compliant. For the pyrimethamine component, none of the samples was found non-compliant, with 50% (6/12) being found borderline compliant and the other 50% (6/12) found to be compliant. All the samples had 100% non-compliance (under dose) for the dihydroartemisinin component. Thus, out of the 12 samples that contained dihydroartemisinin/sulphadoxine/ pyrimethamine, none was found to be compliant, with the failure rate mainly due to the failure of the dihydroartemisinin and sulphadoxine components.

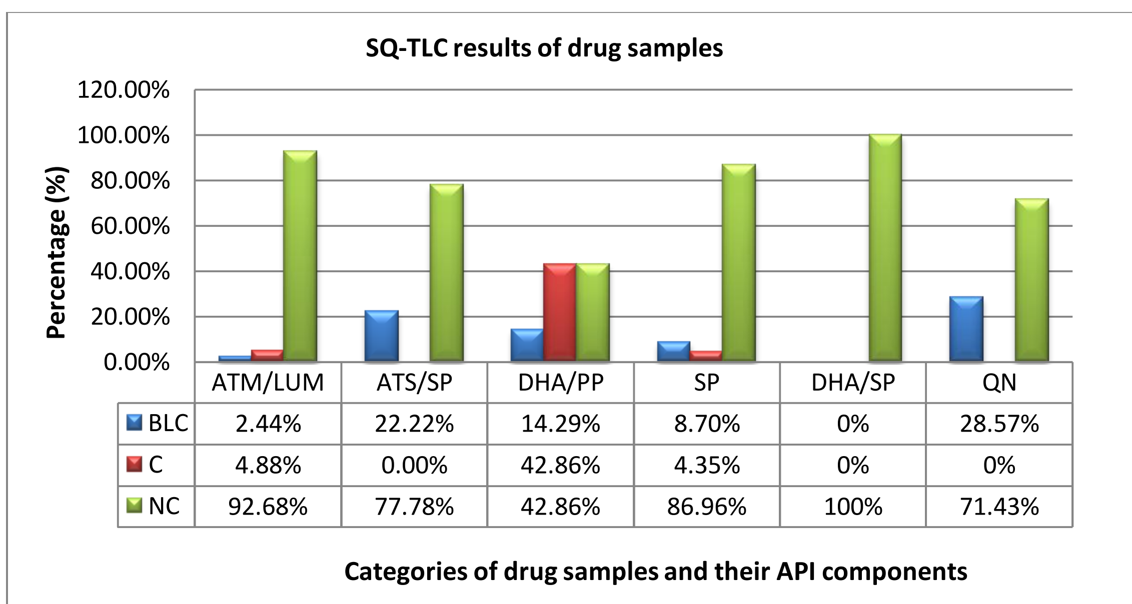
3.4.1.1.2.5 Sulphadoxine/Pyrimethamine Containing Samples

Out of the 23 samples, 78.26% (18/23) had sulphadoxine component being non-compliant, with 13.04% (3/23) being compliant and 8.70% (2/23) being borderline compliant. For the pyrimethamine component, 52.17% (12/23) samples were non-compliant (3 overdose, 9 under dose), with 21.74% (5/23) found to be compliant and 26.09% (6/23) being borderline compliant. Therefore, on the whole, of the 23 samples that contained the combined sulphadoxine and

pyrimethamine only, 8.70% (2/23) were found to be borderline compliant, 4.35% (1/23) being compliant and 86.96% (20/23) non-compliant.

3.4.1.1.2.6 Quinine Containing Samples

There were 13 samples consisting of quinine sulphate, quinine bisulphate and quinine hydrochloride and 7 samples were selected for SQ-TLC due to inadequate reference standard (RS). Out of this, 28.57% (2/7) of these samples were borderline compliant and 71.43% (5/7) being non-compliant, with 100% (5/5) of the non-compliance arising from overdosed API quantity. Figure 21 below summaries the statuses of the drugs as a whole based on SQ-TLC only.



	ATM/LUM	ATS/SP	DHA/PP	SP	DHA/SP	QN
BLC	2.44%	22.22%	14.29%	8.70%	0%	28.57%
C	4.88%	0.00%	42.86%	4.35%	0%	0%
NC	92.68%	77.78%	42.86%	86.96%	100%	71.43%

Figure 21: SQ-TLC results of drug samples when all components are considered

3.4.1.2 HPLC Method Results

3.4.1.2.1 Summary of Results of the HPLC Assay

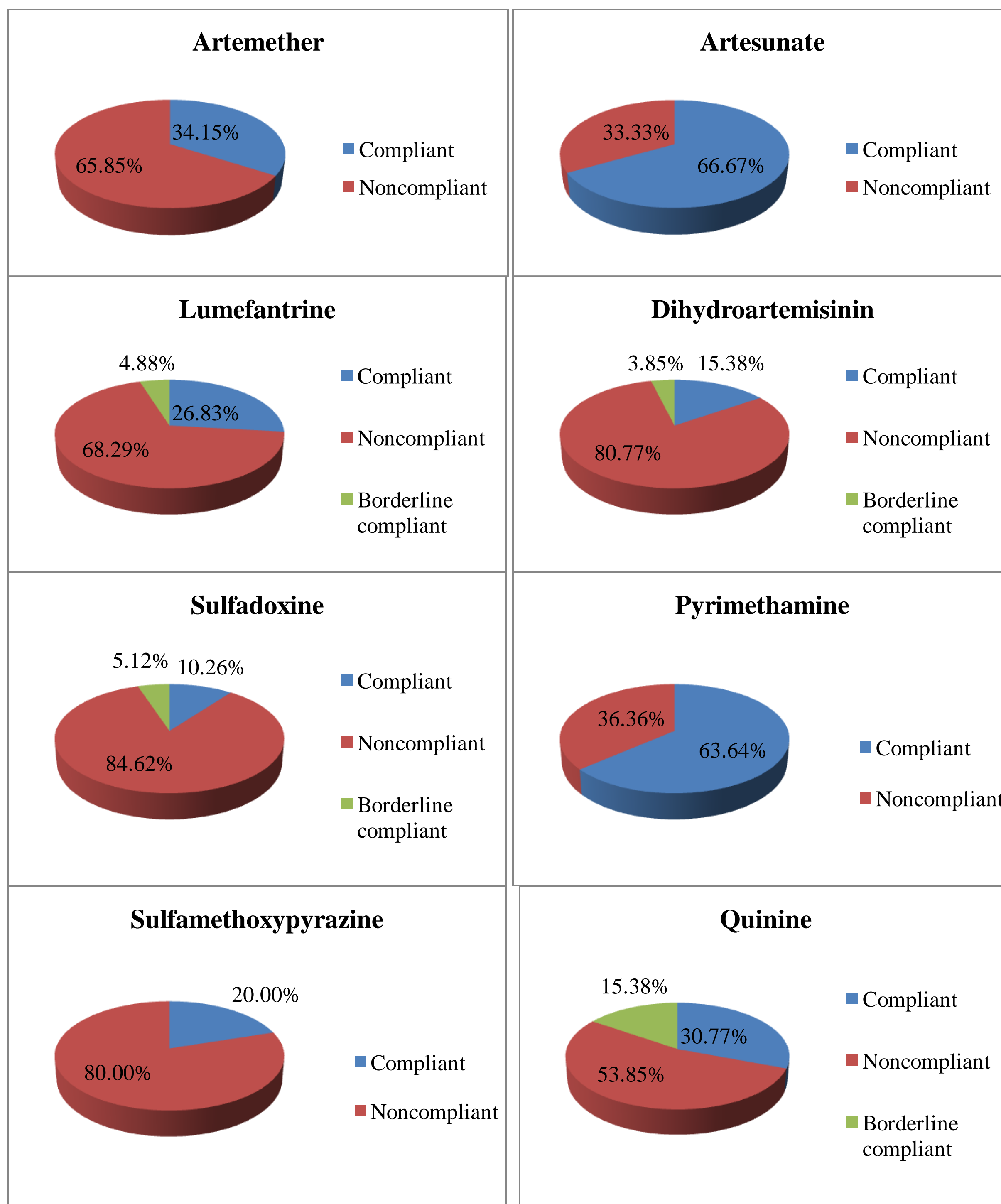


Figure 22: HPLC results of individual APIs of the antimalarial drug samples

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Figure 22 above demonstrates a summary of the results obtained from the HPLC analysis of the APIs. With the HPLC method, the samples were analysed for artemether, artesunate, dihydroartemisinin, sulphadoxine, sulphamethoxypyridazine, pyrimethamine, lumefantrine and quinine active pharmaceutical ingredients (APIs). For artesunate component, 66.67% (6/9) of the samples were compliant and the remaining 33.33% (3/9) were non-compliant (under dose). Artemether component of the samples posted 34.15% (14/41) and 65.85% (27/41) compliant and non-compliant results respectively, with 48.15% (13/27) of the non-compliant samples being overdose.

About 4.88% (2/41) of the lumefantrine component were found to be borderline compliant, whereas 26.83% (11/41) and 68.29% (28/41) were compliant and non-compliant respectively, and 50% (14/28) of the non-compliant samples were overdose. Of the samples that were analyzed for dihydroartemisinin, 3.85% (1/26) was found to be borderline compliant, with 15.38% (4/26) being found compliant and 80.77% (21/26) found non-compliant (under dose).

In the case of sulphadoxine and pyrimethamine components, the HPLC results showed a corresponding 5.12% (2/39) and 10.26% (4/39) for borderline compliant and compliant with respect to sulphadoxine component and the remaining 84.62% (33/39) were non-compliant (under dose). For pyrimethamine, 63.64% (28/44) and 36.36% (16/44) of the samples were compliant and non-compliant respectively, of which 6/16 (37.50%) of the non-compliant samples were overdose. Sulphamethoxypyridazine component had approximately 20% (1/5) of the samples being certified as compliant, with 80% (4/5) being declared non-compliant all of which were under dose. Finally, 15.38% (2/13) of the quinine samples were borderline

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compliant, while 30.77% (4/13) were endorsed as compliant and 53.85% (7/13) turned out to be non-compliant; 85.71% (6/7) of the non-compliant samples were found overdose (Figure 22).

These HPLC results were as a result of some SQ-TLC borderline samples in the numbers 3, 4, 1, 1, 13 and 1 for artesunate, artemether, lumefantrine, sulphadoxine, pyrimethamine and quinine respectively being found compliant using the HPLC method (indicated as *BLC in the table of results appendix II). Almost all the values for the latter method were also within the range of the TLC method estimation. In addition, borderline compliant samples for lumefantrine (2) and dihydroartemisinin (1) remained unchanged while the rest of the borderline samples were found non-compliant. Out of the total 139 non-compliant components in all the samples, 28.06% (39/139) were found to be overdose. Therefore, the HPLC results confirmed the widespread existence of substandard API component quantities in all the drug samples. Therefore, non-compliance was accounted for by both over dosage and under dosage of some of the components, both in significant proportions.

3.4.1.2.2 Analysis of APIs of the Drug Samples with Respect to their Country of Origin

The samples were also analysed for API quantity with respect to their country of origin (source) to find out a source that produced antimalarial drugs with good quality or better drug compliance rate. In essence, a recommendation would be made as to the private traders as to whom they should import from for quality drugs. Only the HPLC results were used based on individual APIs and the drug as a whole. All the samples in this study were purported to be manufactured and imported from India (68), Tanzania (13), Kenya (20), China (9) and the USA (2). Based on HPLC results, the samples were comparatively analysed for compliance/non-compliance.

Table 15 shows an outline of the sample APIs compliance status with respect to their sources and Table 16 shows the compliance status of the samples as a whole with respect to the same parameter. The results showed that failure rates for the individual APIs and the drug samples as a whole were similar, such that there were similar trends in all the samples imported from the different countries found to be of poor quality with very high failure rates recorded for all the samples and the sources. It was surprising to note that samples purported to be imported from both developing countries and well developed countries failed similarly.

3.4.1.2.3 Analysis of Drug Samples as a Whole (HPLC Results)

The samples were also analyzed in terms of a drug as a whole whereby a drug was qualified based on the collective qualification status of the individual APIs as well. Figure 23 below represents the overall drug's qualification status based on HPLC.

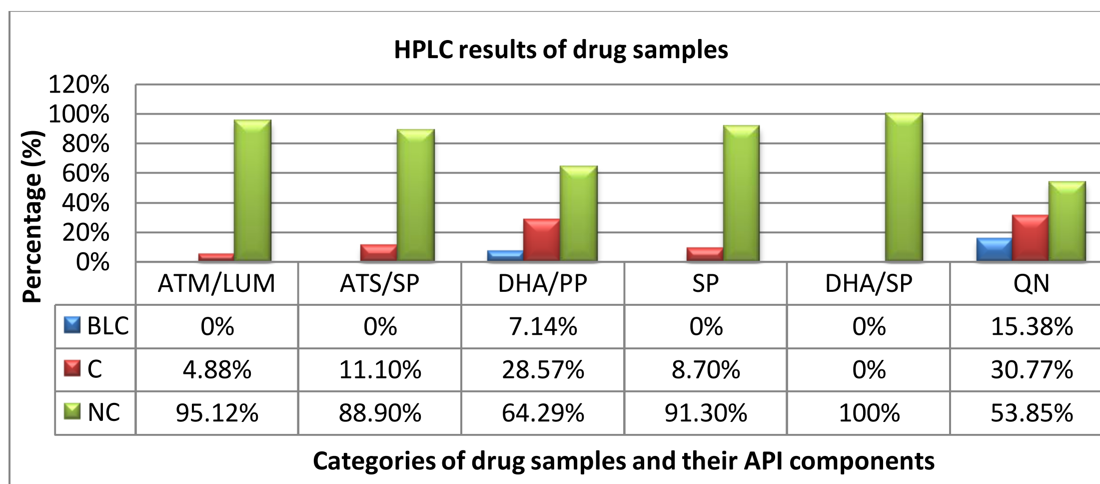

Figure 23: HPLC results of drug samples when all components are considered

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Table 15 : Comparative study of drug API compliance/non-compliance with regard to country of origin (Based on HPLC)

	India				Tanzania				Kenya				China				USA			
		NC				NC				NC				NC				NC		
APIs	**C**	**OD**	**UD**	**BLC**	**C**	**OD**	**UD**	**BLC**	**C**	**OD**	**UD**	**BLC**	**C**	**OD**	**UD**	**BLC**	**C**	**OD**	**UD**	**BLC**
Ats	4	-	-	-	-	-	-	-	2	-	3	-	-	-	-	-	-	-	-	-
Atm	11	11	12	-	-	-	-	-	1	1	3	-	-	-	-	-	1	1	-	-
Lum	9	10	13	2	-	-	-	-	2	2	1	-	-	-	-	-	-	2	-	-
Dha	3	-	13	1	-	-	-	-	-	-	-	-	1	-	8	-	-	-	-	-
S	1	-	20	1	2	-	10	1	1	-	3	-	-	-	-	-	-	-	-	-
Sm	-	-	-	-	-	-	-	-	1	-	4	-	-	-	-	-	-	-	-	-
P	18	-	4	-	6	3	4	-	4	3	2	-	-	-	-	-	-	-	-	-
Qn	3	2	1	1	-	-	-	-	1	4	-	1	-	-	-	-	-	-	-	-
Total	**49**	**23**	**63**	**5**	**8**	**3**	**14**	**1**	**12**	**10**	**16**	**1**	**1**	**-**	**8**	**-**	**1**	**3**	**-**	**-**

Table 16: Comparative study of compliance/non-compliance of drugs as a whole with regard to country of origin

APIs	**India**	**Tanzania**	**Kenya**	**China**	**USA**
	Non-compliance	**Non-compliant**	**Non-compliant**	**Non-compliant**	**Non-compliant**
Atm/Lum	91.18% (31/34)	-	100.00% (5/5)	100.00% (3/3)	100% (2/2)
Dha/Pp	20.00% (1/5)	-	-	88.89% (8/9)	-
Dha/SP	100.00% (12/12)	-	-	-	-
SP	100.00% (6/6)	92.31% (12/13)	50.00% (2/4)	-	-
Ats/S/SmP	75.00% (3/4)	-	100.00% (5/5)	-	-
Qn	57.14% (4/7)	-	80.00% (4/5)	-	-
Total	**83.82% (57/68)**	**92.31% (12/13)**	**84.21% (16/19)**	**91.67% (11/12)**	**100% (2/2)**

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3.4.1.2.3.1 Artesunate/Sulphadoxine/Sulphamethoxypyridazine/Pyrimethamine Containing Samples

With the HPLC, all the sample components were successfully analyzed. These samples consisted of 5 artesunate/sulphamethoxypyridazine/pyrimethamine (Ats/SmP) samples and 4 artesunate/sulphadoxine/pyrimethamine (Ats/SP) samples. In Ats/SP samples, 100% (4/4) of the artesunate components were compliant. For sulphadoxine component, 25% (1/4) was borderline compliant with the rest (3/4) being non-compliant due to under dosage and 50% (2/4) of the pyrimethamine component was compliant, while the remaining 2 were non-compliant (under dose). In Ats/SmP samples, 40% (2/5) of the artesunate component was compliant whereas the remaining 3 components were non-compliant and under dose. For sulphamethoxypyridazine component, 20% (1/5) was borderline compliant with the rest being non-compliant and under dose, whereas 40.00% (2/5) of pyrimethamine component were borderline compliant and 60% (3/5) were found to be non-compliant and overdose. Overall, the results showed that approximately 11.10% (1/9) and 88.90% (8/9) of the total sample were compliant and non-compliant respectively.

3.4.1.2.3.2 Artemether/Lumefantrine Containing Samples

Out of the 36.6% (41/112) samples of artemether/lumefantrine analyzed, the HPLC results showed that 4.88% (2/41) samples were compliant and 95.12% (34/41) non-compliant. The non-compliant samples here had the quantities within the range 35-89% for under dosed and 110-178% for over dosed (*see section 3.4.1.2.1 for details*). The HPLC tests found the SQ-TLC designated borderline compliant sample to be non-compliant in addition to those that were already non-compliant.

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3.4.1.2.3.3 Dihydroartemisinin/Piperaquine Phosphate Containing Samples

With the HPLC as well, these samples were analyzed for the dihydroartemisinin component only due to the lack of the piperaquine RS material. Therefore, it was treated like a monotherapy drug in the analysis for dihydroartemisinin. Approximately 28.57% (4/14) of the samples were found compliant, whereas 7.14% (1/14) turned out to be borderline compliant, with 64.29% (9/14) being non-compliant due to under dosage. Using the HPLC, one of the two SQ-TLC borderline compliant samples was found to be non-compliant and the other one remained the same.

3.4.1.2.3.4 Dihydroartemisinin/Sulphadoxine/Pyrimethamine Containing Samples

12 samples contained dihydroartemisinin/sulphadoxine/pyrimethamine APIs and all the samples were non-compliant due to the high failure rate in the dihydroartemisinin and sulphadoxine components. For example, while 100% (12/12) of the pyrimethamine component was compliant, 100% of the dihydroartemisinin was non-compliant and 8.33% (1/12) of the sulphadoxine was compliant; with the further 91.67% (11/12) being non-compliant. All the non-compliant samples in both dihydroartemisinin and sulphadoxine components failed because they were under dosed.

3.4.1.2.3.5 Sulphadoxine/Pyrimethamine Containing Samples

Out of the 23 samples that contained sulphadoxine and pyrimethamine only, 8.70% (2/23) were found to be compliant and the remaining 91.30% (21/23) were non-compliant. The failure rate was overwhelming due to the high non-compliant status of the sulphadoxine component unlike the pyrimethamine. As a matter of illustration, 13.04% (3/23) of the sulphadoxine alone was confirmed compliant, while 4.35% (1/23) was borderline compliant and 82.61% (19/23) non-compliant. On the other hand, the quantity of pyrimethamine that turned out to be compliant and

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non-compliant was 52.17% (12/23) and 47.83% (11/23) respectively. All the non-compliant components fell within the 47-84% range, except 27.27% (3/11) of the non-compliant pyrimethamine component that were overdosed. One (1) out of the 2 SQ-TLC borderline compliant samples turned out to be non-compliant whereas the other one was found to be compliant when the HPLC method was used.

3.4.1.2.3.6 Quinine Containing Samples

The quinine samples were in different forms of quinine sulphate, quinine bisulphate and quinine hydrochloride. All the samples were duly analysed using HPLC. Approximately 30.77% (4/13) of these samples were compliant, with 15.38% (2/13) and 53.85% (7/13) being borderline compliant and non-compliant respectively; 85.71% (6/7) of the non-compliant quinine samples were found overdosed. The HPLC tests of the SQ-TLC borderline compliant samples showed that 1 sample turned out to be compliant, whereas one sample became non-compliant and one SQ-TLC non-compliant sample turned out to be borderline compliant.

Therefore, majority of the samples have been found non-compliant with the Ph. Int. [96, 105] requirements. Some of the non-compliant samples had more than the requisite API amount, a situation which is abnormal for most substandard drugs. This might be attributed to either wrong calibration in a normal/licensed manufacturing company or counterfeiting, whereby production of the drugs takes place in substandard infrastructure using substandard materials and machinery by mostly unqualified personnel.

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The current study results are comparable to those found in recent studies conducted in Ghana by Osei-Safo *et al.* [92] and Konadu [68] as well as several other surveys in both Africa and Asia in which significant proportions of the samples were also found to be overdosed, with serious cases also reported in quinine samples as well.

The results therefore show that whereas a given FDC drug may have certain individual components complying with pharmacopoeial specifications, other components may render the drug as a whole non-compliant. If therefore a component complies and is in sufficient quantities to effect reduction of parasitaemia, the drug as a whole may produce some treatment success, giving a false impression of total efficacy. The danger, however is that the absence or insufficient quantities of the other component(s) of the fixed dose combination drug to do what it/ is/are supposed to do in the overall cure or reduction in parasitaemia, could lead to rapid development of drug resistance.

3.4.1.3 Comparison of SQ-TLC and HPLC Results

After analyzing the results with respect to SQ-TLC and HPLC methods separately, the results were compared as well to find out if they were comparable. Figure 24 below illustrates the comparison of the results derived from the two analytical methods.

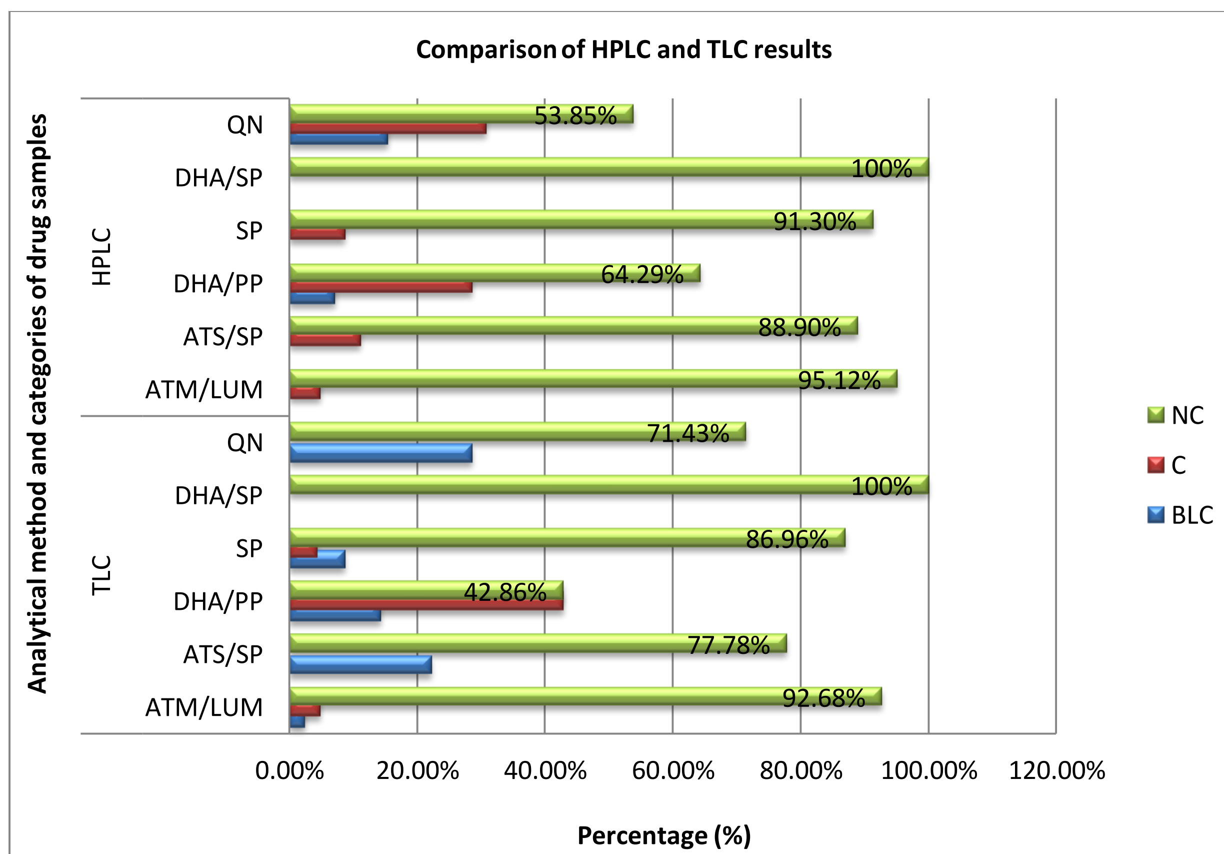

Figure 24: Comparison of HPLC and SQ-TLC results

The API quantity of the samples was analyzed using HPLC and SQ-TLC assays. The results for the two methods were analogous such that similar decisions were made from them in a significant majority of the results and they were comparable for the case where drugs were analyzed as a whole (inclusive of all APIs) except for DHA/PP and QN (Figure 24). For individual APIs, looking at the artemether, artesunate, dihydroartemisinin, sulphadoxine and pyrimethamine as well with non-compliant results as an example, the HPLC results of 65.85% (27), 33.33% (3), 80.77% (21), 84.62% (33) and 36.36% (16) non-compliance were very closely related and comparable to the SQ-TLC non-compliance results of 65.85% (27), 22.22% (2),

69.29% (18), 82.05% (32) and 38.63% (17) respectively. However, the HPLC and SQ-TLC results of lumefantrine and quinine APIs were slightly wider in difference. These slight and wide differences can be attributed to some of the borderline SQ-TLC compliant cases being found to be either fully compliant or non-compliant by HPLC. The HPLC assay was used to confirm and specify the estimated TLC results. Hence, the difference between the two methods was found to be insignificant because majority of the HPLC results fell within the TLC results ranges, whether compliant or non-compliant. For example, artesunate (3), artemether (4), lumefantrine (1), sulphadoxine (1), pyrimethamine (13) and quinine (1) samples changed from borderline compliant to compliant with HPLC assay (indicated as *BLC in the table of results, appendix II) and were still within the TLC range which made the TLC results statistics more closer to those of the HPLC results. Therefore, the HPLC and TLC results were comparable and equally important for the analysis (refer to appendix II). A correlation curve for each API in Figure 25 below further illustrates the comparability of the HPLC and SQ-TLC results; with a correlation coefficient of r = 0.949, showing a very strong similarity in the results.

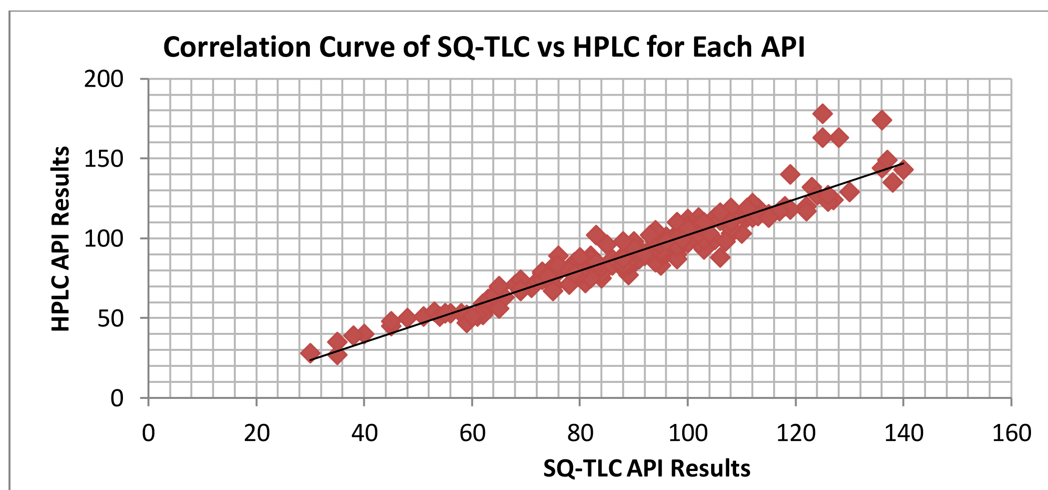

Figure 25: Correlation curve of HPLC and SQ-TLC results

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3.4.2 Combined HPLC and SQ-TLC Results

3.4.2.1 Artesunate/Sulphadoxine/Sulphamethoxypyrazine/Pyrimethamine Containing Samples

There were 9 samples constituting 8.04% of the total samples. 33.33% (3/9) of these samples had artesunate component failure, 77.78% (7/9) had sulphadoxine component failure and 55.56% (5/9) had a pyrimethamine component failure. The overall results for the drug samples with all the components analyzed showed that 1 out of 9 samples were fully compliant in all respects representing an overall 88.89% failure rate.

3.4.2.2 Artemether/Lumefantrine Containing Samples

41 samples containing artemether/lumefantrine were analyzed representing 36.6% of the total sample size. The results of the artemether component showed a 65.85% (27/41) failure rate and lumefantrine component was found to be 68.85% (28) non-compliant. On overall, 39 out of 41 samples were not compliant representing a 95.12% failure rate. Being the first line treatment, this is a devastating situation to the efforts against Malaria in a country burdened with acute, frequent and persistent drug shortages in public hospitals, thus making people resort to private hospitals and pharmacies for prompt and easily accessible treatment.

3.4.2.3 Dihydroartemisinin/Piperaquine Phosphate Containing Samples

These samples were analyzed for the dihydroartemisinin component only due to the lack of a piperaquine reference standard. Therefore, it was treated like a monotherapy drug in the analysis. 35.71% (5/14) of the samples were compliant. It is quite possible that a few more non-compliance cases would have been recorded if the piperaquine had also been analysed.

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3.4.2.4 Dihydroartemisinin/Sulphadoxine/Pyrimethamine Containing Samples

There were 12 samples containing this formulation. The components dihydroartemisinin, sulphadoxine and pyrimethamine recorded 100%, 91.7% and 0% failure rates respectively. Generally, all the samples were non-compliant with the Ph. Int. requirements, even though all the samples had 100% compliance for pyrimethamine. This situation where one component of a multi component formulation complies while the other does not, was found to be common in all the samples analysed. The implication is that the synergism effect that is aimed at preventing development of parasite resistance against the drug over a short period of its usage is compromised. Hence, the drug only reduces the malaria causing parasite, which eliminates the signs/symptoms and once the parasites re-group and one falls sick again, it is unlikely that s/he would get cured as the parasites might have developed resistance against the drug. In cases where the API is overdosed, one is likely to get cured with serious or unexpected side effects and/or organ damages even unintended self-poisoning that can cause death.

3.4.2.5 Sulphadoxine / Pyrimethamine Containing Samples

There were 23 samples that contained sulphadoxine and pyrimethamine only and 82.61% (19/23) of the sulphadoxine component were non-compliant, 4.35% (1/23) were borderline compliant and 13.04% (3/23) were compliant. In addition, 52.17% (12/23) of the pyrimethamine component were non-compliant and the rest were compliant. On overall, 91.30% (21/23) of the samples were non-compliant. This is also a worrisome development because sulphadoxine / pyrimethamine is still administered in Malawian hospitals especially to the vulnerable group of pregnant women, due to the lack of comprehensive studies on the possible adverse effects of ACTs on the developing foetus. Hence, administering of poor quality SP drugs could worsen the

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already fragile situation this group encounters and aggravate the parasite resistance the regimen has faced.

3.4.2.6 Quinine Containing Samples

There were 13 quinine containing samples consisting of quinine sulphate, quinine bisulphate and quinine hydrochloride. All the samples were analysed using HPLC, while some could not be analysed using SQ-TLC method because the extraction process was not efficient enough such that the results were not comparable to their HPLC counterparts like all other samples (see appendix II for details for such samples). The results showed that 53.85% of the samples were non-compliant.

The high failure rate of the drugs and their individual components is worrisome and poses a serious parasite resistance (for under dosed) and toxicity (for overdosed) threats to the use of quinine for the treatment of more complicated cases of malaria. In addition, most of the malaria cases in Malawi are diagnosed without microscopic determination. In most cases, most types of fever are presumed to be malaria first, and treated as such. If indeed, such an ad hoc diagnosis is also treated with substandard or poor quality antimalarials, this could lead to treatment failure and/or fast development of resistance. This inference comes with the background reported by Bate *et al.*[45] that resistance development of chloroquine and sulphadoxine in Africa in the 1990s and the devastating impact of malaria on the people was partly due to the use of substandard drugs.

3.4.3 Registration Status and Quality of the Samples

The results of the registered and unregistered samples were compared to determine whether the registration status necessarily had an influence on the quality of the drugs. Below is Figure 26 summarizing the relationship between drug registration and compliance.

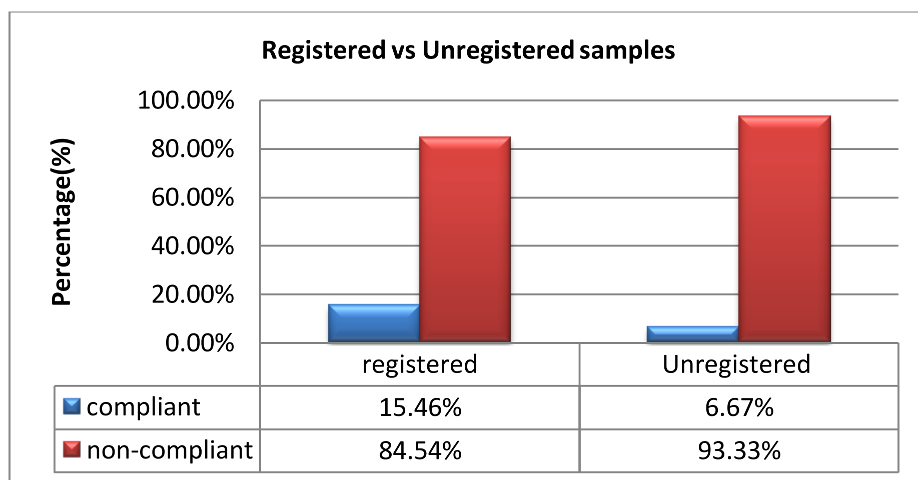

Figure 26: A comparison of failure rates for registered and unregistered samples

Out of the 97 registered samples, 15.46% (15) of the samples were compliant, while 6.67% (1) of the 15 unregistered samples were compliant. Although the registered samples had a better compliance compared to the unregistered ones, the overall results show that better registration status does not necessarily always guarantee the quality a drug. These results are similar to the observations made in the 2008/2009 Ghana study [67]. This implies that a registered drug does not mean good quality drug and the fact that a drug is registered with the national regulatory authority does not make it good. This might be caused by lack of adherence of a supplier to registration guidelines, whereby they register a particular batch number of drug products and duplicate lots of that batch number, which are not checked by authorities.

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In addition, this might also be caused by a supplier registering a particular product name or formulation and more products similar to it subsequently supplied without being checked. Furthermore, this problem might be attributed to lack of rigorous testing and assessment of the new products information required and presented for registration due to either lack of instruments for thorough testing or lack of qualified personnel. Additionally, this problem is also caused by supplier presenting a well manufactured drug product for the registration application process, which after passing the scrutiny, is diluted and subsequent products are prepared without regard to GMP. Finally, due to the lack of forensic testing of the drug products, some counterfeit products get their way into the market by mimicking the registered products, compromising the quality of the drug market as a whole. There is also the possibility of an importer simply not subjecting products for registration with all the tools involved, simply because the regulatory authorities are not vigilant enough in surveillance and application of appropriate sanctions.

3.4.4 Samples with the Same Batch Number

Such samples were expected to have the same quantities of APIs or negligible differences. On the contrary, majority of the samples had varying quantities within a pool of a batch number (see appendix III). This might be attributed to counterfeiting, lack of adherence to good manufacturing and laboratory practices, inconsistent quantities of excipients that are, among others, used to minimize the effect of adverse conditions (e.g. heat, humidity) on APIs and exposure to different environmental conditions that affect the stability of APIs significantly.

3.4.5 Overall Results

The detailed results on each of the samples analysed by both SQ-TLC and HPLC are as shown in appendix II. It has been shown that there was a 100 % compliance of the samples for the visual inspection of dosage form and packaging material as well as qualitative determination of active pharmaceutical ingredients (API). There was a good relationship between the former and the latter because the labelled contents on the packaging materials were correct. Therefore, there was an excellent correlation between the two results as expected. However, it has been reported earlier that with the advancement of counterfeiting today, visual inspection and basic tests could not qualify a drug as genuine despite chemical and physical similarities. Bate *et al.* state that determination of a drug as counterfeit or substandard requires a forensic examination of the trademarks, product designs and holograms [45].

Generally, using both HPLC and SQ-TLC results and the fact that if a component fails then the sample as a whole fails, there was an overall 85.71% (96) failure rate out of the 112 samples that were tested. The Table 17 and Figure 27 below illustrate the overall results.

Table 17: Overall results of the study

APIs	North			Central			South East			South West			Total		
	Total	**C**	**NC**	**Total**	**C**	**NC**	**Total**	**C**	**NC**	**Total**	**C**	**NC**	**Total**	**C**	**NC**
Atm/Lum	6	0	6	6	1	5	6	0	6	23	1	22	41	2	39
Dha/pp	4	0	4	3	1	2	2	2	0	5	2	3	14	5	9
Dha/sp	2	0	2	2	0	2	4	0	4	4	0	4	12	0	12
Sp	5	0	5	8	2	6	4	0	4	6	0	6	23	2	21
Ats/sp	5	1	4	3	0	3	1	0	1	0	0	0	9	1	8
Qn	7	3	4	3	2	1	0	0	0	3	1	2	13	6	7
Total	**29**	**4**	**25**	**25**	**6**	**19**	**17**	**2**	**14**	**41**	**4**	**37**	**112**	**16**	**96**

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The failure rates were more serious for dihydroartemisinin/sulphadoxine/pyrimethamine combination 100% (12/12), followed by artemether/lumefantrine 95.12% (39/41), sulphadoxine/pyrimethamine 91.3% (21/23), artesunate/sulphadoxine/pyrimethamine 88.9% (8/9), dihydroartemisinin 64.29% (9/14) and quinine 53.8% (7/13) as shown in Table 17 above. This means that in multi component FDC drugs, manufacturers have to rigorously apply good manufacturing practices (GMP) and ensure that every component meets the requisite pharmacopoeial specifications. Compliance in such circumstances is therefore more difficult to achieve.

The results have also shown that the failure rates were worst in the south west zone with 90.20% failure rate, followed by the north zone with 86.20%, then south east with 82.40% and the central with 76% as shown in the Figure 27 below.

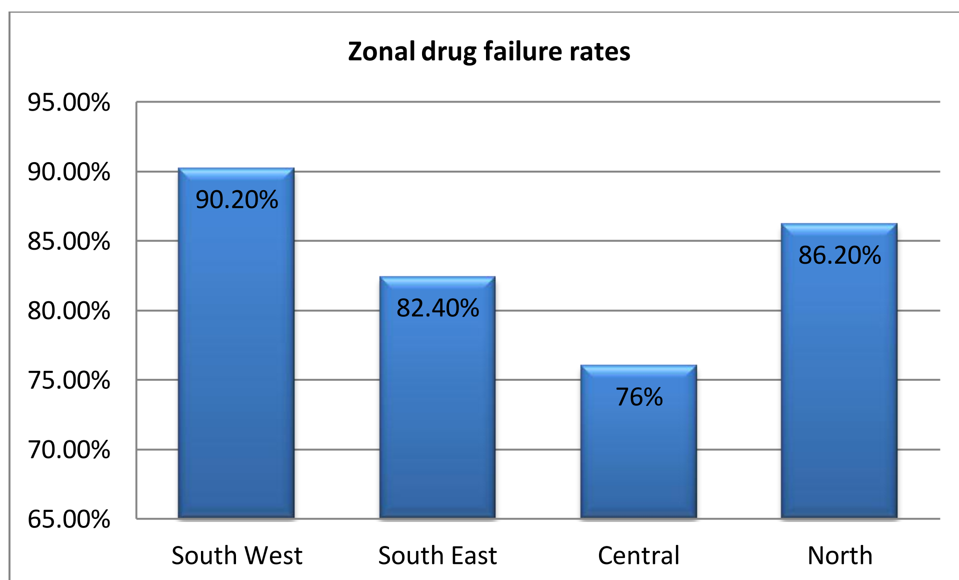

Figure 27: Failure rates of the drug samples by Zone

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The results suggest that there is a widespread circulation of poor quality antimalarial drugs with the south west zone being the worst affected. This has been greatly attributed to the presence of more economic activity places in south west region such as the Mulanje border, Mwanza border and Blantyre city that attract more imports and influence the import of poor quality drugs. In addition, this region has higher economic activities with higher likelihood of population increase, which provides a good market for goods. The north has two places of high economic activities such as Karonga border and Mzuzu and a large number of imports pass through this border from Tanzania and Kenya just like the Mwanza border in the south west zone. However, the other zones are equally centres of high economic activities because they also have cities and border posts like Zomba city and Mangochi district border for south east and Lilongwe city as well as Mchinji and Dedza border posts for the central zone, only that the activities are slightly lower than the former two zones with high failure rates. In addition, the borders are porous and despite the regulatory body carrying out some testing activities, some poor quality drugs are bound to be smuggled into the country; worse still the tests done at the border posts are not rigorous enough to detect a fake, substandard or degraded drug, other than just certifying that the sample contains the labelled APIs, which is not sufficient; that is, if at all they take place as per requirement because their occurrence is not well documented in the published literature.

The high circulation of poor quality drugs implies that many people are using antimalarial drugs with insufficient or too much amount of the requisite APIs, hence such drugs would either not be effective because they cannot eradicate all the parasites from the body and those that survive can easily develop and transmit resistance (for under dose) or cause some toxicity concerns (for overdose). These effects might lead to worsening of an acute to severe malaria case, high cost of

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treatment to get cured, loss of public trust in the treatment regimen and unreliable studies on medical drug efficacy resulting in high mortality rate. Despite the effort put in by the Malawi government and its partners to minimize the impact of Malaria over the years, Malaria remains the country's biggest health challenge and the most risky part is that almost the whole population is affected.

Several programmes have been rolled out and phased out, others are still in progress, but the problem seems to be getting worse and there have been cases of resistance against antimalarials as well. Nevertheless, the trend is similar to the areas where resistance and high treatment failures have been attributed to poor quality drugs. Some of these suggestions are supported by an article in which the Malawi government is quoted to have ordered the removal of some antimalarial drugs such as sulphadoxine/pyrimethamine from the shops' shelves. This particular antimalarial drug was suspected to have had its sensitivity to malaria parasites compromised, and has since been replaced with artemether/lumefantrine [110].

The post-marketing surveillance and pharmacovigilance system in Malawi has been under development since 2009 and therefore is still faced with severe limitations. This problem is also shared by other African countries like Tanzania and Kenya which are also sources of antimalarial drugs imported into Malawi. Unless this problem is solved with urgency, all efforts to kick out malaria will be in vain. Moreover, if there is development of resistance, this could be disastrous because currently there does not appear to be any new efficacious antimalarial drug in the pipeline to replace the current regimen.

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3.4.6 Comparison of Results with Recent Results from other African Countries

The results of the current Malawi study were compared with the results of other African countries from a recent survey reported in the WHO QAMSA report of 2011, where the participating countries Ethiopia, Kenya, Tanzania, Cameroon, Ghana and Nigeria had 0%, 5%, 11%, 37%, 39% and 64% failure rates respectively [55]. Another recent study report by Nayyar *et al*. (2012) was selected. In this survey, the authors reviewed several survey reports of poor quality antimalarial drugs from mostly Uganda, Cameroon, Kenya, Democratic Republic of Congo, Burkina Faso, Madagascar, Senegal, Ghana, Nigeria, Tanzania, Ethiopia, Gabon, Mali, Mozambique, Zimbabwe, Sudan, Burundi, Angola, Congo, Chad and Rwanda for the period between 1999 and 2011 [63].

The review report showed that the failure rates for all the surveys conducted in these countries within the stated period were in the range 12-69%, with the exception of one of the studies conducted in Ghana which reported an 82% failure rate in 2008 [63]. Therefore, the Malawian failure rate of 85.71% is very alarming. This problem might be attributed to lack of routine rigorous drug testing by international and local organizations as most international surveys have rarely included samples from Malawi and such activities are also rarely done internally if they take place at all because efforts to locate any such activity taking place at the required level have proved futile.

In addition, Caudron *et al.,* 2008 reported the results of a quality audit done by Medicin Sans Frontiers (MSF) pharmacists over a period of 4 years in 180 sites they visited, which showed that most regulated manufacturers bypassed their GMP compliance and set the standards of their drug

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products based on the recipient countries status with regard to the level of regulation capability and level of income as well as lack of prequalified standards by most developing countries to their suppliers [111]. So Malawi being one of the poorest countries apart from just being a developing country, with poor regulatory capability, without a pharmacovigilance system and lack of expertise in such areas, there is a possibility that these factors might be amongst the causes of the high failure rates, because most of the drugs circulating in these countries are manufactured by almost the same manufacturers.

3.4.7 Conclusions

The results have shown an alarming widespread use of poor quality drugs in Malawi, with the south west and the north zones appearing to be the hub of the circulation of substandard antimalarial drugs and the central zone having relatively high quality antimalarial drugs. In addition, the results have also shown that the failure rate is much higher compared to other African countries. The registration, visual and qualitative analysis of the drugs alone would not serve a purpose of certifying a drug as worth prescription and the fact that a drug is registered with the national regulatory authority does not guarantee its quality. As such, there is always a need to carry out thorough tests on the drugs if the purpose of such activities is to flush out the poor quality drugs. Some forms of drugs that are supposed to be phased out at the time of this study were found to be still in circulation. It has also been established that SQ-TLC method can be used if the HPLC method is beyond the capability of the community since the results of the two methods are comparable.

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Therefore, the results have shown that while the existence of a poor regulatory system is not a conclusion that can be drawn directly from the present study, it could be responsible for the high failure rate of medicines imported into the country. Hence, the regulatory system needs urgent attention by the authorities for improvement to be able to carry out thorough routine tests of drugs. This will result in better quality health care delivery through malaria and other diseases control, patients' safety, fight and eradication of parasite resistance.

3.4.8 Recommendations

Well-planned and implemented surveys need to be conducted to give a true reflection of the nature of the poor antimalarial drugs as to whether those drugs found to be non-compliant are degraded due to exposure to adverse conditions, counterfeited or substandard as a result of poor manufacturing practices. From the fact that some substandard drugs might not be necessarily counterfeit, it is recommended that analysis be carried out to determine the factors that contributed to the current state of being substandard. This is so because a substandard drug can result during production for a number of reasons like deliberate deviation from standards to maximise profits, non-compliance with standard operating procedures (SOPs) and good manufacturing practices (GMPs), equipment error or malfunctioning, degradation and/or mishandling at any part of medicine continuum. Knowledge generated from the study would help in choosing appropriate action to address the situation. The studies should also include dissolution tests, which is also another important parameter that defines the quality of a drug plus many other important parameters. Such studies should also be extended to public hospitals and pharmacies.

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Good procurement practices amongst the public and private institutions must be promoted and appropriate measures taken to prevent the illegal importing and smuggling of drugs into the country. The drug regulatory authority's capacity should be strengthened in terms of infrastructure and working personnel so that they will be able to conduct thorough initial quality and routine tests on new supplies, drug manufacturing and packaging companies and suppliers inspection to ensure strict adherence to the GMPs. Appropriate sensitisation of the public and drug handling personnel in charge of the supply chain is needed to let them know the appropriate quality control measures, proper drug storage and distribution.

A properly functioning and sustainable pharmacovigilance system should be developed. This could partly be achieved by incorporating the pharmacovigilance topics in the various training disciplines such as medicine, pharmacy, nursing colleges, public health schools or even secondary schools at an introductory level.

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CHAPTER FOUR

4 EXPERIMENTAL

4.1 THIN LAYER CHROMATOGRAPHY

4.1.1 Experimental Conditions

The TLC tests were carried out at room temperature under the following conditions:

a. Chromatograms were run in TLC tanks (22.5 cm x 10 cm) with filter papers lined on the inside walls, saturated with the mobile phase.

b. Silica gel 60 F_{254} (Merck) pre-coated on aluminium foil or Silica gel 60 (Fluka) pre- coated on aluminium foil were used as stationary phase.

c. Each drug API had two proposed solvent systems selected as mobile phase.

d. Test solutions: 1mg/mL solutions of the authentic Reference Standards (RS) of the active pharmaceutical ingredient (API) and the dosage forms were prepared daily and stored at 4 ^{0}C. The RS were obtained from the European Directorate for the Quality of Medicines and Healthcare (EDQM), France. These were prepared by dissolving 10 mg of RS in 10 mL of their respective solvents as shown in Table 18 below.

Table 18: Dissolution solvents of APIs for TLC tests

Active Pharmaceutical Ingredient (API)	Dissolution Solvent
Quinine	Methanol
Dihydroartemisinin	Methanol
Sulphadoxine	Methanol
Pyrimethamine	Methanol
artemether	Ethyl acetate
Lumefantrine	Ethyl acetate
Artesunate	80% methanol

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e. Application: different volumes of a 1mg/mL concentration reference standard (RS) in the range 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 and 2.4µL equivalent to the corresponding quantities in µg were applied to the plate at 1cm apart. The quantity of the dosage form was however kept constant; 2.0µL of a 1mg/mL solution equivalent to 2.0µg of each drug was applied.

f. Detection: each active ingredient had its specific detection agent as specified in the pharmacopoeia and published literature and these have been outlined (Tables 13 and 14).

4.1.2 Preparation of Dosage Form Solutions

An amount of the ground dosage form equivalent to 10mg of a given API was weighed into a clean dry beaker. 5 mL of a suitable dissolution solvent was added to the beaker, then the mixture shaken for at least 5 minutes and filtered. Another 3 mL of the solvent was added to the filtration residue, shaken gently, then allowed to settle for at least 5 minutes and filtered again. To check the effectiveness of the extraction, another 2 mL of the solvent was added to the residue and a TLC spot developed from residue solution alongside the RS. Absence of the spot from the residue meant complete API extraction. The filtrate was subsequently added to the other filtrates to make up 10 mL of the dosage form solution. So in effect, a 1mg/mL solution was prepared.

For each of the FDC formulations, each API component was assayed independent of the other API constituents. For example, if artemether/lumefantrine is assayed for artemether, a quantity that will give out 10mg of artemether is weighed, irrespective of what the amount of lumefantrine will be given. Likewise when lumefantrine is assayed, an amount that will give 10mg of lumefantrine is weighed irrespective of the amount of artemether. Therefore, in

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extracting an API of interest, a solvent is selected such that what will dissolve it will dissolve only the analysed API, and leave the others intact, so that there is a preferential extraction.

4.2 HPLC METHODS FOR THE ASSAY OF THE SELECTED ANTIMALARIALS

In addition to the SQ-TLC assay, the drug API contents were also assayed using HPLC procedures suitable for each particular API, adopted from the pharmacopoeias and the literature to authenticate the results of the SQ-TLC.

4.2.1 HPLC Assay of Artesunate

The assay for the HPLC analysis of artesunate was adopted from Ranher *et al.* validated HPLC method [106] with some few modifications to suit the current circumstances as shown in Table 19 below:

Table 19: Chromatographic and instrumental conditions for artesunate assay

Chromatographic parameters	Prescribed method	Adapted method
Column measurements	HiQ-Sil C8, 25cm x 4.6mm i.d.	Discovery C-18 bonded, 5µm, 25cm x 4mm.
Mobile phase	(70:30 v/v) acetonitrile: 1M sodium acetate buffer (pH 3 adjusted with *o*-phosphoric acid)	70: 30 1% triethylamine(TEA) methanol: 10mM KH_2PO_4/ 85% H_3PO_4 (pH=2.5)
Injection volume	20 µL	20 µL
Flow rate	1.0mL/min	1.2 mL/ min.
Retention time	4.883min.	5.1 minutes
Detection Wavelength(UV/Vis)	220nm	216nm
Internal standard	artemether	None

The prescribed conditions of the assay using an HiQ-Sil C8 column gives some tailing problems, hence modifications leading to the adapted conditions above which gave well resolved peaks.

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The modifying agent, 1% triethylamine (TEA) was added to methanol and the pH of the 0.01 M buffer potassium dihydrogen phosphate was adjusted to 2.5 with 10mM of phosphoric acid in the 70/30 v/v mobile phase.

4.2.1.1 Preparation and Assay of Artesunate RS Solution for Calibration Curve

Artesunate concentrations comprising 0.706 mg/mL, 0.807 mg/mL, 0.908 mg/mL, 1.009 mg/mL, 1.211mg/mL, 1.312mg/mL and 1.413mg/mL were prepared through dilution of pipette volumes of 0.7mL, 0.8mL, 0.9mL, 1.0mL, 1.2mL, 1.3mL and 1.4mL respectively, from a 10.1mg/mL stock solution of the artesunate reference material using 2mL of mobile phase (70:30 methanol: buffer) and 8mL of HPLC grade water to produce 10 mL of working solution. The area under the curve (AUC) for each concentration was determined with six replicates each and an average AUC resulting from them. This data was used to plot a graph of average AUC against concentration (C) using Microsoft Excel and the slope of the graph, intercept, correlation coefficient (r^2) as well as equation of the straight line, AUC = mC + b were deduced and calculated from it. The area under the curve (AUC) is the average area under a chromatographic peak, m = slope of the straight line and b = AUC intercept (Table 20 and Figure 28 below).

4.2.1.2 Preparation and Assay of Solutions of Artesunate Containing Tablets

A quantity of powdered dosage form equivalent to 10 mg of artesunate was weighed into a clean dry 10 mL volumetric flask. 5mL of the mobile phase (70:30 v/v, 1% triethylamine in methanol: buffer) was then added and the mixture shaken for 15 minutes on an ultrasonic sonicator. Then, more mobile phase was added to the mark and the solution filtered through a 0.45 µm filter.

Table 20: Concentrations of artesunate RS solutions and their corresponding AUCs

ID	Actual volume pipetted (ml)	Conc. (mg/ml)	Peak Area (AUC)						Average AUC	SD	RSD %
			1	2	3	4	5	6			
1	0.7	0.706	403.4	403.3	402.9	403.5	403.5	403.5	403.3897	0.22	0.055
2	0.8	0.807	455.9	455.8	454.9	455.9	455.0	455.9	455.6458	0.47	0.103
3	0.9	0.908	515.8	515.4	515.2	515.4	515.2	515.2	515.3963	0.21	0.041
4	1.0	1.009	570.2	569.9	570.2	570.3	570.3	569.9	570.1942	0.17	0.031
5	1.2	1.210	640.0	640.0	640.1	640.0	639.9	640.0	640.0629	0.05	0.008
6	1.3	1.311	714.2	714.2	714.1	714.2	714.2	713.9	714.1686	0.09	0.012
7	1.4	1.412	767.3	767.5	767.3	767.2	768.0	767.3	767.4849	0.26	0.034

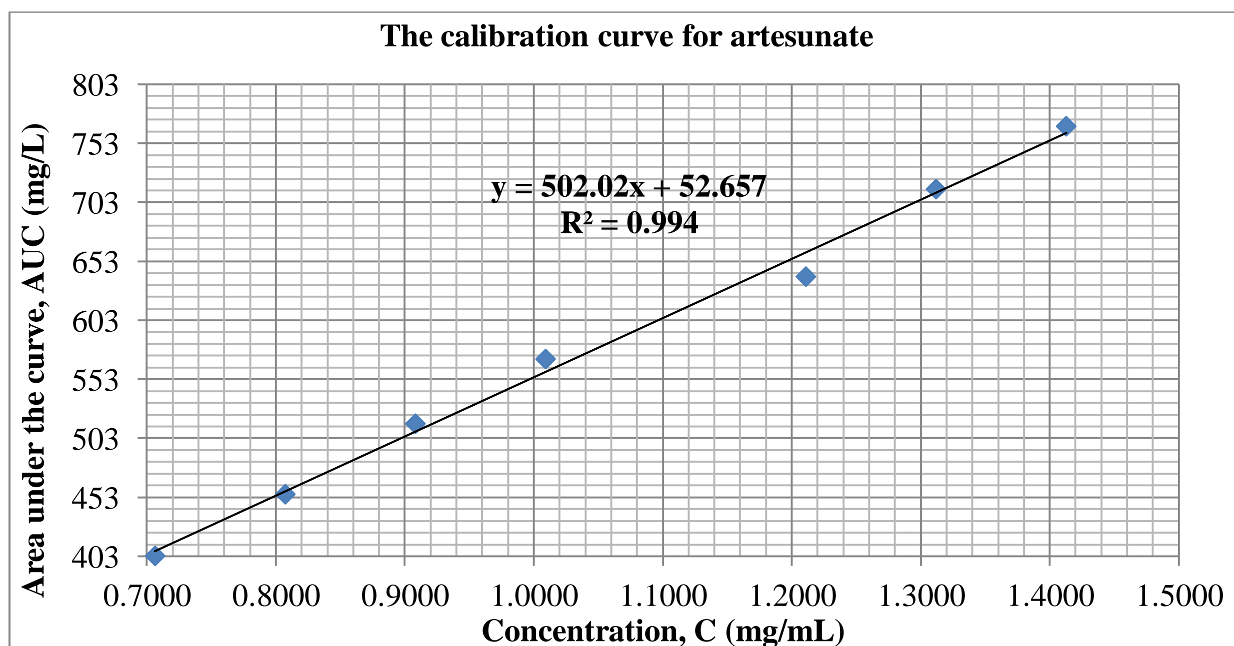

Figure 28: Calibration curve for artesunate RS Conc. as a function of AUC

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4.2.2 HPLC Assay of Artemether / Lumefantrine FDC Formulations

Artemether/lumefantrine drugs were analyzed using assay conditions derived from modifications of a method developed by Arun and Smith [107], to improve the excess retention times of the methods developed earlier by Sridhar *et al.* and Sunil *et. al.*; with retention times of 13.8 and 13.9 minutes respectively, which were deemed to be unfavourable for routine analysis. Table 21 below summarises the prescribed and adapted method conditions.

Table 21: Chromatographic and instrumental conditions of artemether/lumefantrine assay

Chromatographic parameters	Prescribed method	Adapted method
Column measurements	Hypersil (BDS) C18, 25cm x 4.6mm, 5µm.	Hyperprep PEP 300A C4, 25cm x 4.6mm, 8µm.
Mobile phase	(70: 30 v/v) acetonitrile: 0.01M Potassium dihydrogen orthophosphate buffer at pH 4.0.	(70: 30 v/v) acetonitrile: 10mM buffer consisting of KH_2PO_4 mixed with 1 mL of triethylamine per liter and pH changed to 2.5 using 85% H_3PO_4 mixture.
Injection volume	20 µL	20 µL
Flow rate	1mL/min.	1.5mL/min.
Retention time		Artemether = 2.5 minutes, lumefantrine = 3.0 minutes
Detection Wavelength(UV/Vis)	253nm	216nm

The counter-ion modifying agent triethylamine was added as well to get enhanced peak symmetry and minimized tailing. Additionally, due to the large difference in the ratio of artemether to lumefantrine of 1: 6, efforts were made to add an artemether amount detectable by using higher concentrations, whilst as well avoiding use of very high concentration of lumefantrine that would have been too much for the column or given peaks that shot over the height of the recording device.

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4.2.2.1 Preparation and Assay of Artemether RS Solution for Calibration Curve

Artemether concentrations comprising 0.2797mg/mL, 0.3168mg/mL, 0.3564mg/mL, 0.3960mg/mL, 0.4356mg/mL, 0.4752mg/mL and 0.5148mg/mL were prepared through dilution of 0.28mL, 0.32mL, 0.36mL, 0.40mL, 0.44mL, 0.48mL and 0.52mL pipette volumes of 9.9 mg/mL stock solution of the artemether reference material respectively and topped up to 10 mL using mobile phase (70:30 v/v acetonitrile: buffer). Area under the curve (AUC) for each concentration was determined with six replicates for each and an average AUC derived from them. The data was used to plot a graph of average AUC against concentration (C) using Microsoft Excel and the slope of the graph, intercept, correlation coefficient (r^2) as well as equation of the straight line, AUC = mC + b were deduced and calculated from it. The area under the curve (AUC) is the average area under a chromatographic peak, m = slope of the straight line and b = AUC intercept (Table 22 and Figure 29).

4.2.2.2 Preparation and Assay of Lumefantrine RS Solution for Calibration Curve

Similarly, lumefantrine concentrations comprising 1.6782mg/mL, 1.9008mg/mL, 2.1384mg/mL, 2.3760mg/mL, 2.6136mg/mL, 2.8512mg/mL and 3.0888mg/mL were prepared through dilution of 0.28mL, 0.32mL, 0.36mL, 0.4mL, 0.44mL, 0.48mL and 0.52mL pipette volumes of 9.9mg/mL stock solution of the lumefantrine reference material for each concentration and top up to 10mL using mobile phase (70:30 acetonitrile: buffer). Area under the curve (AUC) for each concentration was determined with six replicates each and an average AUC derived from them. The data was used to plot a graph of average AUC against concentration (C) using Microsoft Excel and the slope of the graph, intercept, correlation coefficient(r^2) as well as equation of the straight line, AUC = mC + b were deduced and calculated from it. The area under

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the curve (AUC) is the average area under a chromatographic peak, m = slope of the straight line and b = AUC intercept (Table 23 and Figure 30).

4.2.2.3 Preparation and Assay of Solutions of Artemether/ Lumefantrine Tablets

A quantity of powdered dosage form equivalent to 4 mg was accurately weighed into a clean dry beaker. 1mL of acetic acid was added, allowed to react for a few minutes after which 5 mL of the mobile phase (70:30 v/v acetonitrile: buffer) was added. The mixture was then shaken for 15 minutes on an ultrasonic sonicator. Then, the solution was filtered into a 10 mL volumetric flask through a 0.45 µm filter, then the beaker was washed with the mobile phase and the residue added to the mark through the filter and kept well in readiness for the analysis.

Table 22: Concentrations of artemether RS solutions and their AUCs

ID	Actual volume pipetted (ml)	Conc. (mg/ml)	Peak Area (AUC)						Average AUC	SD	RSD
			1	2	3	4	5	6			
1	0.28	0.2797	121.0	120.5	130.2	122.5	120.5	120.2	122.8	3.88	3.16
2	0.32	0.3168	134.4	133.8	135.3	130.5	129	130.8	131.9	2.56	1.94
3	0.36	0.3564	136.0	138.7	138	142.9	140.0	131	138.1	4.04	2.92
4	0.40	0.3960	145.2	143.8	146.1	144.3	145.8	144.3	144.9	0.93	0.64
5	0.44	0.4356	142.0	155.2	151.6	150.1	150.2	150.9	150	4.33	2.88
6	0.48	0.4752	158.4	158.6	159.1	157.4	158.5	158.3	158.4	0.55	0.34
7	0.52	0.5148	166.6	167.2	165.3	167.6	166.2	169.6	167.2	1.45	0.88

Table 23: Concentrations of lumefantrine RS solutions and their AUCs

ID	Actual volume pipetted (ml)	Conc. mg/ml	Peak Area (AUC)						Average AUC	SD	% SD
			1	2	3	4	5	6			
1	0.28	1.6782	28344	28337	28093	28315	28297	28090	28226	121	0.4
2	0.32	1.9008	30243	30245	30685	30544	30345	30262	30416	185	0.6
3	0.36	2.1384	32159	32131	30909	32160	32122	32091	31883	500	1.6
4	0.40	2.3760	33503	33500	32033	33493	33502	33541	33214	602	1.8
5	0.44	2.6136	35452	35404	33385	35462	35391	35460	35020	836	2.4
6	0.48	2.8512	36537	36533	34161	36512	36334	36537	36015	954	2.7
7	0.52	3.0888	37822	37848	36892	37805	37843	37867	37651	386	1.0

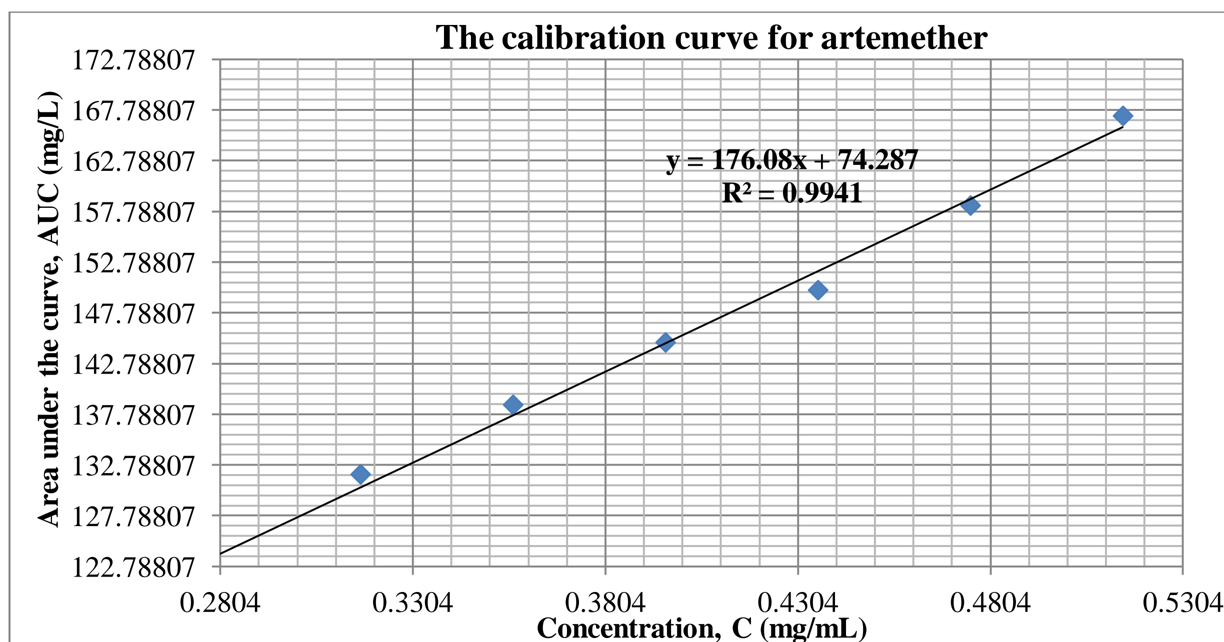

Figure 29: Calibration curve for artemether RS Conc. as a function of AUC

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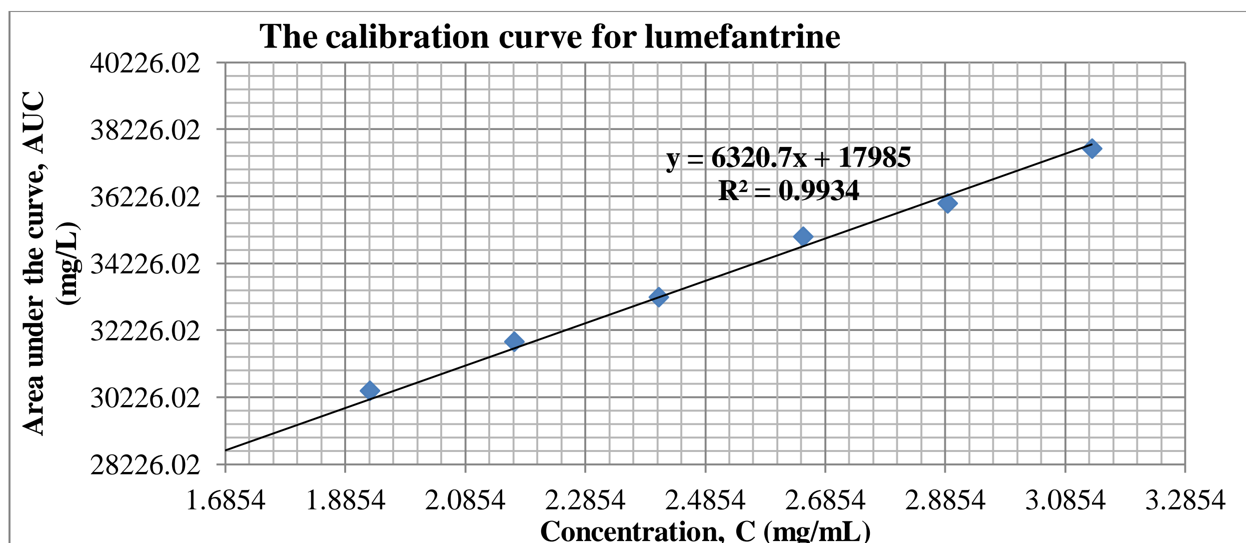

Figure 30: Calibration curve for lumefantrine RS Conc. as a function of AUC

4.2.3 HPLC Assay of Dihydroartemisinin

A method outlined in the Ph. Int. [105] was modified to suit the present study as shown in Table 24 below, with both the old method conditions and the new conditions outlined.

Table 24: Chromatographic and instrumental conditions of dihydroartemisinin assay

Chromatographic parameters	Prescribed technique	Adapted method
Column measurements	C-18 column, 10cm x 4.6mm, 3 µm	Kramasil C8, 25cm x 4.6mm, 5µm
Mobile phase	60:40 v/v, acetonitrile: water with gradient elution	50:50 v/v, water: acetonitrile
Injection volume	20 µL	10µL
Flow rate	0.6mL/min.	1.5mL/min.
Retention time	Total runtime of 30 min; no retention time given	5.2 minutes
Detection wavelength (UV/Vis)	216nm	210nm

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4.2.3.1 Preparation and Assay of Dihydroartemisinin RS Solution for Calibration Curve

Dihydroartemisinin concentrations comprising 0.7014mg/mL, 0.8016mg/mL, 0.9018mg/mL, 1.0020mg/mL, 1.1022mg/mL, 1.2024mg/mL and 1.3026mg/mL were prepared through dilution of pipetted volumes of 1.4mL, 1.6mL, 1.8mL, 2.0mL, 2.2mL, 2.4mL and 2.6mL respectively, from a stock solution of 12.5mg/mL dihydroartemisinin reference material and mobile phase (60:40 v/v acetonitrile: phosphate buffer) was added to make a 25 mL solution. Area under the curve (AUC) for each concentration was determined with six replicates each and an average AUC derived from them. The data was used to plot a graph of average AUC against concentration (C) using Microsoft Excel and the slope of the graph, intercept, correlation coefficient (r^2) as well as equation of the straight line, AUC = mC + b were deduced and calculated from it. The area under the curve (AUC) is the average area under a chromatographic peak, m = slope of the straight line and b = AUC intercept (Table 25 and Figure 31 below).

4.2.3.2 Preparation and Assay of Solutions of Dihydroartemisinin Containing Tablets

A quantity of powder equivalent to 10 mg of dosage form was accurately weighed into a clean dry beaker. 5 mL of the mobile phase (60:40 v/v acetonitrile: phosphate buffer) was added as a diluent and sonicated for 15 minutes on an ultrasonic sonicator. The solution was then filtered through a 0.45 μm filter into a 10 mL volumetric flask and more diluent was used to wash the beaker and the residue was added to the mark through the filter and the solution kept well in readiness for the analysis.

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Table 25: Concentrations of dihydroartemisinin RS solutions and the corresponding AUCs

ID	Actual Volume pipetted (mL)	Conc. (mg/mL)	Peak Area (AUC)						Average AUC	SD	RSD
			1	2	3	4	5	6			
1	1.4	0.7014	328.6	328.6	326.6	328.6	329.1	325.6	327.8271	1.40	0.428
2	1.6	0.8016	355.2	355.2	358.2	357.2	358.2	354.2	356.3465	1.72	0.484
3	1.8	0.9018	393.3	391.5	394.3	390.0	390.3	390.8	391.7017	1.74	0.445
4	2.0	1.0020	440.1	439.1	441.1	440.1	441.1	439.1	440.0619	0.89	0.201
5	2.2	1.1022	486.6	485.6	488.0	485.5	485.6	484.6	486.0071	1.17	0.240
6	2.4	1.2024	530.8	531.0	530.9	531.9	529.9	530.9	530.8868	0.63	0.119
7	2.6	1.3026	564.6	561	563.6	563.5	564.6	564.6	563.6343	1.39	0.246

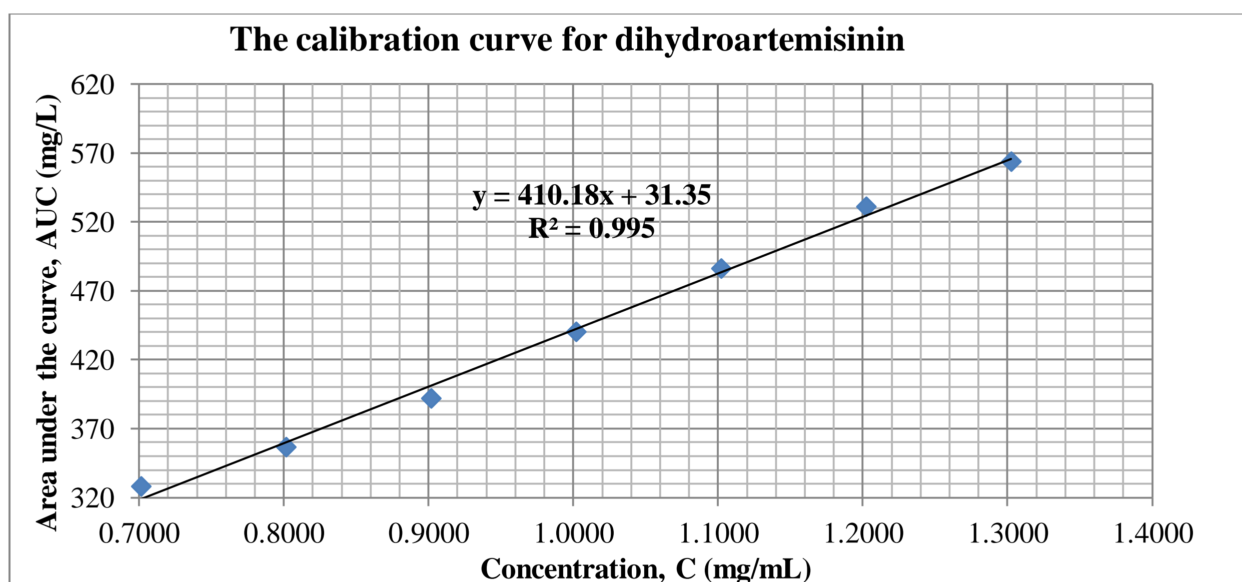

Figure 31: Calibration curve for dihydroartemisinin RS Conc. as a function of AUC

4.2.4 HPLC Assay of Quinine

An USP 24 HPLC assay [109] was modified and used in the analysis of quinine preparations and the changes brought out the analytical conditions shown under the adapted method in Table 26.

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Table 26: Chromatographic and instrumental conditions for quinine assay

Chromatographic parameters	Prescribed technique	Adapted method
Column measurements	C-18 Phenomenex luna, 15cm x 4.66mm, 5 µm	Discovery C-18 bonded , 25cm x 4.0 mm, 5 µm
Mobile phase	81:15:2:2 v/v, water: acetonitrile: methanesulfonic acid solution: triethylamine (TEA), pH 2.6	80:16:2:2 v/v, water: acetonitrile: methanesulfonic acid: TEA, pH 2.6
Injection volume	10 µL	20 µL
Flow rate	1.2mL/min.	1.2mL/min.
Retention time	3.6minutes	4.7 minutes
Detection Wavelength (UV/Vis)	235nm	235nm

4.2.4.1 Mobile Phase Preparation

Methanesulfonic acid solution:

- 3.5 mL of methanesulfonic acid
- 2.0 mL of glacial acetic acid
- Dilute with water to 50 mL of solution

Triethylamine solution:

- 10.0 mL of triethylamine
- Dilute with water to 100.0 mL of solution

Final composition:

- Water – acetonitrile – methanesulfonic acid solution – triethylamine solution
- (80:16:2:2 v/v) adjusted to pH 2.6

4.2.4.2 Preparation and Assay of Quinine RS Solution for Calibration Curve

50.6mg of Quinine reference standard was weighed into a 50mL volumetric flask to give a resultant solution of concentration 1.012mg/mL. Concentrations comprising 0.3542mg/mL,

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0.4048mg/mL, 0.4554mg/mL, 0.5060mg/mL, 0.5566mg/mL, 0.6072mg/mL and 0.6578mg/mL were prepared by diluting pipetted volumes of 3.5mL, 4.0mL, 4.5mL, 5.0mL, 5.5mL, 6.0mL and 6.5mL respectively, from a stock solution of the quinine reference material into a 10mL volumetric flask. Area under the curve (AUC) for each concentration was determined with six replicates each and an average AUC derived from them. The data was used to plot a graph of average AUC against concentration(C) using Microsoft Excel and the slope of the graph, intercept, correlation coefficient(r^2) as well as equation of the straight line, AUC = mC + b were deduced and calculated from it. Area under the curve is the average area under chromatographic peak, m = slope of the straight line and b = AUC intercept (Table 27 and Figure 32).

4.2.4.3 Preparation and Assay of Solutions of Quinine Containing Drug Samples

4.2.4.3.1 Quinine Suspensions / Mixtures /Injections

A certain amount of the quinine suspension was sonicated for about 15 minutes and a quantity equivalent to 5mg was pipetted into a 10 mL volumetric flask. 8 mL of methanol was then added to the content of the flask and made up to the mark with the mobile phase. Where necessary, the solution was also filtered.

For the injections, a 10mL solution was prepared in a volumetric flask after 5 ampoules of quinine injections were mixed together and 20 μL aliquot was measured from the stock quinine solution using a microlitre syringe. The prepared solution was diluted with 8mL of methanol and mobile phase was added to the 10mL mark.

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Table 27: Concentrations of quinine RS solutions and their corresponding AUCs

ID	Actual volume pipetted (mL)	Conc. mg/mL	Peak area (AUC)						Average AUC	SD	RSD
			1	2	3	4	5	6			
1	3.5	0.3542	7183	7178	7173	7173	7178	7178	7177	3.95	0.06
2	4	0.4048	8375	8416	8369	8445	8445	8374	8404	36.2	0.43
3	4.5	0.4554	9453	9433	9390	9445	9333	9463	9413	49.3	0.52
4	5	0.5060	10301	10471	10257	10309	10335	10470	10368	97.4	0.94
5	5.5	0.5566	11665	11450	11665	11664	11507	11445	11546	110.8	0.96
6	6	0.6072	12185	12348	12185	12329	12479	12312	12331	104.7	0.85
7	6.5	0.6578	13346	13364	13202	13439	13489	13398	13378	109.1	0.82

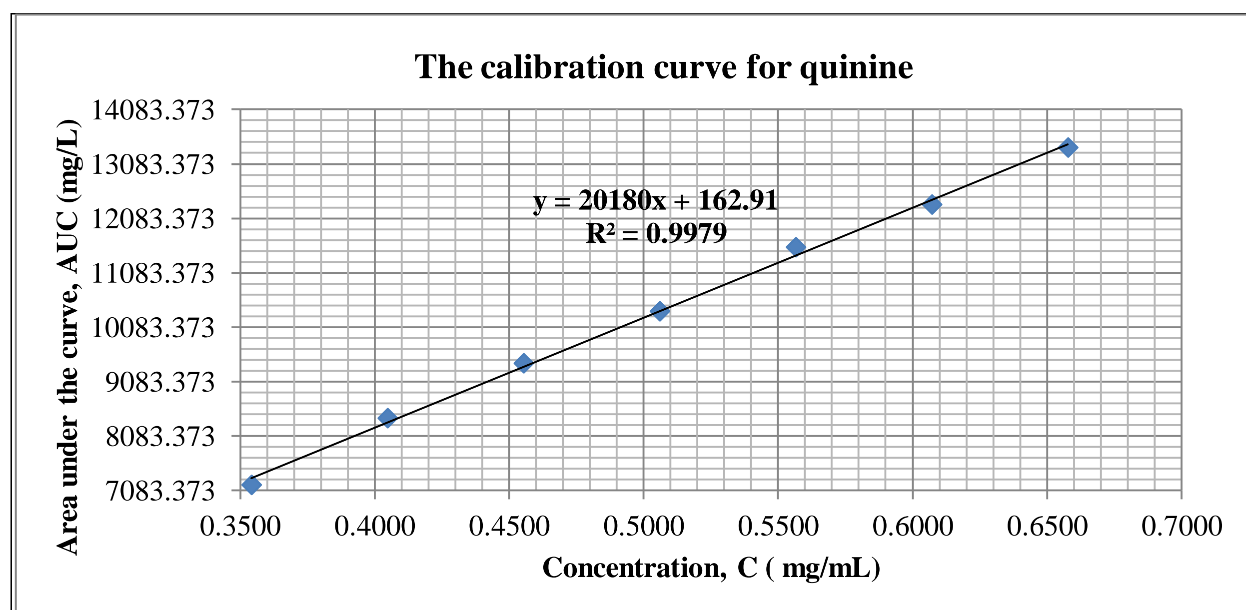

Figure 32: Calibration curve for quinine RS Conc. as a function of AUC

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4.2.5 HPLC Assay of Sulphadoxine/Sulphamethoxypyridazine/Pyrimethamine Formulations

Table 28 below shows experimental conditions that were used in the analysis of sulphadoxine / pyrimethamine containing drug samples, produced after modifying those of the WHO adopted monograph for inclusion into the Ph. Int. in 2010 [108].

Table 28: Chromatographic conditions of sulphadoxine / pyrimethamine assay

Chromatographic parameters	Prescribed technique	Adapted method
Column measurements	Phenomenex Luna®, 25 cm x 4.6 mm, 5 µm.	Ascentis C-18, 15 cm x 4.60 mm, 5 µm.
Mobile phase	80:20 v/v, 10 mL of acetic acid R and 0.5 mL of TEA R dissolved in 800 mL of H_20 R, diluted to 1000 mL and pH adjusted to 4.2 using NaOH (~400 g/L) TS: acetonitrile R.	65:10:25 v/v, 20 mM buffer (KH_2PO_4/Na_2HPO_4 of pH 5.6; methanol; acetonitrile.
Injection volume	20 µL	10 µL
Flow rate	2mL/min.	1 mL/min
Retention time	Run time, at least 25 minutes	sulphadoxine = 3.9 minutes, pyrimethamine = 8.7 minutes
Detection Wavelength(UV/Vis)	227 nm	240 nm

Like in artemether/lumefantrine, the ratio of sulphadoxine to pyrimethamine of 20: 1 made the detection of pyrimethamine difficult and this was overcome by using higher concentrations of pyrimethamine whilst still maintaining appropriate concentration of sulphadoxine for the column.

4.2.5.1 Preparation and Assay of Sulphadoxine RS Solution for Calibration Curve

Different concentrations of sulphadoxine RS were prepared using mobile phase (65:10:25 v/v

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buffer: methanol: acetonitrile) as shown in Table 29 and Figure 33 below. Area under the curve (AUC) for each concentration was determined with six replicates each and an average AUC derived from them. The data was used to plot a graph of average AUC against concentration (C) using Microsoft Excel and the slope of the graph, intercept, correlation coefficient (r^2) as well as equation of the straight line, AUC = mC + b were deduced and calculated from it. The area under the curve (AUC) is the average area under a chromatographic peak, m = slope of the straight line and b = AUC intercept (Table 29 and Figure 33).

4.2.5.2 Preparation and Assay of Pyrimethamine RS Solution for Calibration Curve

Similarly, different concentrations of pyrimethamine RS were prepared using mobile phase (65:10:25 v/v buffer: methanol: acetonitrile) as shown in the Table 30 and Figure 33 below. Area under the curve (AUC) for each concentration was determined with six replicates each and an average AUC derived from them. The data was used to plot a graph of average AUC against concentration (C) using Microsoft Excel and the slope of the graph, intercept, correlation coefficient (r^2) as well as equation of the straight line, AUC = mC + b were deduced and calculated from it. The area under the curve (AUC) is the average area under a chromatographic peak, m = slope of the straight line and b = AUC intercept (Table 30 and Figure 34).

4.2.5.3 Preparation and Assay of Solution of Sulphadoxine/Sulphamethoxypyridazine/ Pyrimethamine Containing Tablets

100mg of sulphadoxine/sulphamethoxypyridazine and 5mg of pyrimethamine equivalents of the dosage form were weighed simultaneously into a clean dry beaker and the APIs extracted three

times for completeness using acetonitrile and the mobile phase (65:10:25 v/v Buffer: methanol: acetonitrile) in a 50mL volumetric flask.

Table 29: Concentrations of sulphadoxine RS solutions and their corresponding AUCs

ID	Actual volume pipetted (mL)	Conc. (mg/mL)	Peak Area (AUC)						Average AUC	SD	SD %
			1	2	3	4	5	6			
1	1.6	0.7968	18728	18765	18756	18753	18785	18720	18751	24.2	0.13
2	1.8	0.8964	19558	19566	19530	19585	19569	19533	19557	21.6	0.11
3	2.2	1.0956	23670	23660	23657	23650	23653	23650	23656	7.71	0.03
4	3.8	1.8924	36670	36665	36666	36663	36664	36662	36665	2.9	0.01
5	4.2	2.0916	40433	40426	40430	40442	40449	40427	40434	9.3	0.02
6	5	2.4900	47100	47113	47112	47112	47110	47111	47111	2.6	0.01

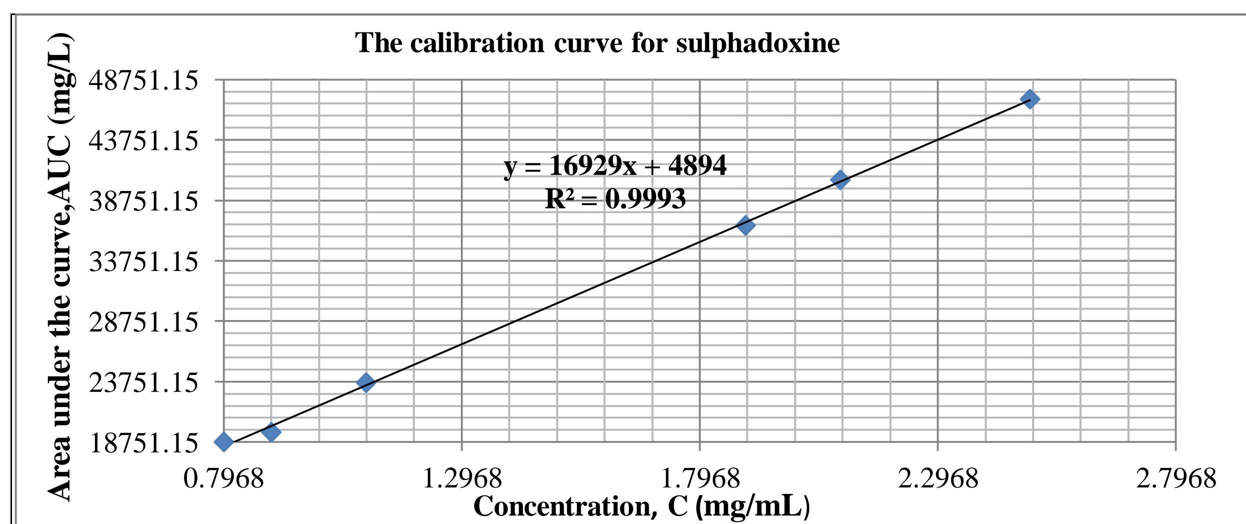

Figure 33: Calibration curve for sulphadoxine RS Conc. as a function of AUC

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Table 30: Concentrations of pyrimethamine RS solutions and their corresponding AUCs

ID	Actual volume pipetted (mL)	Conc. (mg/mL)	Peak Area (AUC)						Average AUC	SD	RSD
			1	2	3	4	5	6			
1	0.08	0.0402	1624	1624	1624	1624	1624	1624	1625	0.00	0.00
2	0.1	0.0503	1777	1777	1777	1777	1777	1727	1769	20.4	1.15
3	0.11	0.0553	1868	1868	1868	1868	1868	1868	1868	0.00	0.00
4	0.12	0.0604	1960	1960	1960	1960	1960	1960	1960	0.00	0.00
5	0.13	0.0654	2024	2024	2024	2024	2024	2024	2024	0.00	0.00
6	0.15	0.0743	2185	2185	2185	2185	2185	2185	2185	0.00	0.00

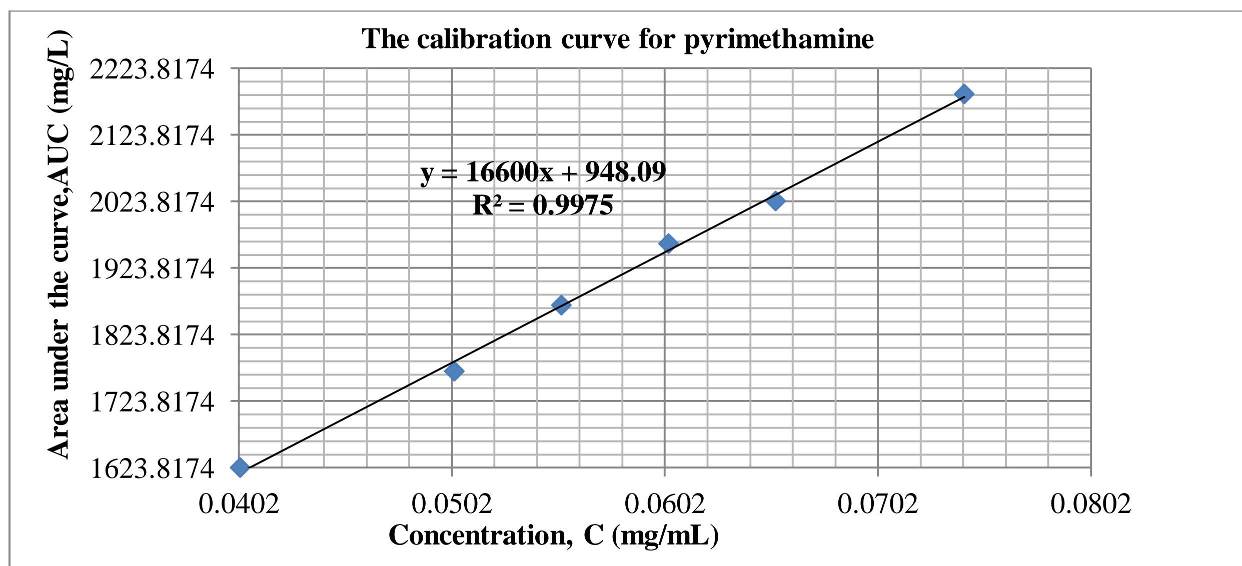

Figure 34: Calibration curve for pyrimethamine RS Conc. as a function of AUC

The list of antimalarial drugs purchased is as in appendix I. In addition, the detailed results of each of the samples analysed by both SQ-TLC and HPLC are given in appendix II. Samples of the SQ-TLC and HPLC chromatograms are given in appendix IV and V.

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APPENDICES

APPENDIX I

LIST AND DETAILS OF ANTIMALARIAL DRUGS PURCHASED

Table 31: List of artemisinin-based and non-artemisinin based antimalarial drugs purchased from various zones in Malawi

No.	Code	Name of drug	Dosage form	Active ingredient	Batch no.	Manufacturer	Man–Exp Date
ZONE 1: SOUTH WEST							
NON-ARTEMISININ-BASED DRUGS							
1	*1_4P_{10}	Malacure	Tablet	Sulphadoxine USP/ Pyrimethamine USP 500mg/ 25mg fixed dose	S-60	S Kant India	07/2010-06/2013
2	*1_6P_{10}	Malacure	Tablet	Sulphadoxine USP/ Pyrimethamine USP 500mg/ 25mg fixed dose	S-60	S Kant India	07/2010-06/2013
3	*1_1P_{10}	Malacure	Tablet	Sulphadoxine USP/ Pyrimethamine USP 500mg/ 25mg fixed dose	S-60	S Kant India	07/2010-06/2013
4	1_1P_2	Sulphadar	Tablet	Sulphadoxine USP/ Pyrimethamine USP 500mg/ 25mg fixed dose	10011	Shellys Tanzania	07/2010-06/2014

* Unregistered samples

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5	1_2P_2	Sulphadar	Tablet	Sulphadoxine USP/ Pyrimethamine USP 500mg/ 25mg fixed dose	10008	Shellys Tanzania	05/2010- 04/2014
6	1_4P_2	Sulphadar	Tablet	Sulphadoxine USP/ Pyrimethamine USP 500mg/ 25mg fixed dose	10011	Shellys Tanzania	07/2010- 06/2014
7	1_3V_5	Quinaquin 100ml	Mixture	Quinine Bisulphate BP 50mg/ml	OK 157	Elys Chemical Kenya	11/2010- 10/2012
8	1_2V_5	Quinaquin 100ml	Mixture	Quinine Bisulphate BP 50mg/ml	OK 159	Elys Chemical Kenya	11/2010- 10/2010
9	1_1V_5	Quinaquin 100ml	Mixture	Quinine Bisulphate BP 50mg/ml	OL 119	Elys Chemical	12/2010- 11/2012
ARTEMISININ-BASED DRUGS							
10	1_7X_1	Lonart-DS	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	LD-266	Bliss GVS India	09/2011- 08/2013
11	1_9X_1	Lonart-DS	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	LD-259	Bliss GVS India	08/2011- 07/2013

* Unregistered samples

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12	$1_{13}X_1$	Lonart-DS	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	LD-259	Bliss GVS India	08/2011-07/2013
13	$1_{10}X_1$	Lonart-DS	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	LD-259	Bliss GVS India	08/2011-07/2013
14	$1_{12}X_1$	Lonart-DS	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	LD-266	Bliss GVS India	09/2011-08/2013
15	1_5X_1	Lonart-DS	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	LD-266	Bliss GVS India	09/2011-08/2013
16	1_6X_{11}	Lonart	Suspension	Artemether/ Lumefantrine 180mg/1080mg fixed dose	LO-210	Bliss GVS India	08/2011-07/2013
17	1_4X_1	Lonart Forte	Tablet	Artemether/ Lumefantrine 40mg/240mg fixed dose	LF-249	Bliss GVS India	08/2011-07/2013
18	1_6X_1	Lonart Forte	Tablet	Artemether/ Lumefantrine 40mg/240mg fixed dose	LF-249	Bliss GVS India	08/2011-07/2013
19	1_3X_1	Lonart Forte	Tablet	Artemether/ Lumefantrine 40mg/240mg fixed dose	LF-239	Bliss GVS India	05/2011-04/2013
20	1_8X_1	Lonart	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	LN-450	Bliss GVS India	09/2011-08/2013
21	1_2X_1	Lonart-Dispersible	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	LS-39	Bliss GVS India	09/2011-08/2013
22	1_1X_1	Lonart	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	LN-449	Bliss GVS India	09/2011-08/2013

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23	1_2X_{11}	Artefan	Suspension	Artemether/ Lumefantrine 180mg/1080mg fixed dose	SB0100I	Ajanta India	09/2010-08/2012
24	1_1X_{11}	Artefan	Suspension	Artemether/ Lumefantrine 180mg/1080mg fixed dose	SB0100I	Ajanta India	09/2010-08/2012
25	1_4X_{11}	Artefan	Suspension	Artemether/ Lumefantrine 40mg/240mg fixed dose	C0490J	Ajanta India	10/2010-09/2012
26	1_3X_{11}	Artefan	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	C0520J	Ajanta India	10/2010-09/2012
27	1_5X_{11}	Artefan	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	C0480J	Ajanta India	10/2010-09/2012
28	1_1X_{20}	Coartem-Dispersible	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	F0493	Norvatis USA	08/2011-07/2013
29	1_1X_{17}	Fantem-Forte	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	M110371	Medinomics India	05/2011-04/2013
30	1_2X_{14}	Lum-Artem	Tablet	β-Artemether/ Lumefantrine 20mg/120mg fixed dose	1105062	Dawa Ltd Kenya	05/2011-04/2014
31	1_1X_{14}	Lum-Artem	Tablet	β-Artemether/ Lumefantrine 20mg/120mg fixed dose	1105062	Dawa Ltd Kenya	05/2011-04/2014
32	1_1X_{18}	LA-DS	Tablet	Artemether/ Lumefantrine 40mg/240mg fixed dose	1117	Global Pharma India	04/2010-03/2013
33	1_7Z_1	P-Alaxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40mg/320mg fixed dose	PX-161	Bliss GVS India	08/2011-07/2014

* Unregistered samples

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34	1_6Z_1	P-Alaxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40mg/320mg fixed dose	PX-161	Bliss GVS India	08/2011-07/2014
35	1_5Z_1	Alaxin	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60mg/500mg/25mg fixed dose	AP-18	Bliss GVS India	08/2011-07/2014
36	1_4Z_1	Alaxin	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60mg/500mg/25mg fixed dose	AP-17	Bliss GVS India	03/2011-02/2014
37	1_3Z_1	Alaxin	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60mg/500mg/25mg fixed dose	AP-18	Bliss GVS India	08/2011-02/2014
38	1_2Z_1	Alaxin	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60mg/500mg/25mg fixed dose	AP-18	Bliss GVS India	08/2011-02/2014
39	*1_4Z_3	Duo-Cotecxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40mg/320mg fixed dose	110123	Zhejiang Holley Nanhu China	01/2011-01/2013
40	*1_2Z_3	Duo-Cotecxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40mg/320mg fixed dose	110123	Zhejiang Holley Nanhu China	01/2011-01/2013
41	*1_1Z_3	Duo-Cotecxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40mg/320mg fixed dose	110123	Zhejiang Holley Nanhu China	01/2011-01/2013

* Unregistered samples

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ZONE 2: SOUTH EAST							
ARTEMISININ-BASED DRUGS							
42	2_3X_1	Lonart	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	LN-262	Bliss GVS India	02/2010-01/2012
43	2_1X_1	Lonart	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	LN-450	Bliss GVS India	09/2011-08/2013
44	2_2X_1	Lonart	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	LD-259	Bliss GVS India	08/2011-07/2013
45	2_4X_{14}	Lum-Artem	Tablet	β-Artemether/ Lumefantrine 20mg/120mg fixed dose	1105062	Dawa Ltd Kenya	05/2011-04/2014
46	$2X_{15}$	Co-Max	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	021367	Universal Kenya	08/2010-07/2012
47	2_5X_{11}	Artefan	Tablet	Artemether/ Lumefantrine 80mg/480mg fixed dose	C0601A	Ajanta India	01/2011-12/2012
48	*2_4Y_{13}	Spafil	Tablet	Artesunate/Sulphadoxine/Pyrimethamine 100/500/25mg co-packed on the same blister	T0458	Fourrts	02/2011-01/2014
49	2_5Z_1	Alaxin	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60mg/500mg/25mg fixed dose	AP-18	Bliss GVS India	08/2011-07/2014
50	2_4Z_1	Alaxin	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60mg/500mg/25mg fixed dose	AP-16	Bliss GVS India	02/2011-01/2014
* Unregistered samples							

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51	2_1Z_1	Alaxin	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60mg/500mg/25mg fixed dose	AP-18	Bliss GVS India	08/2011-07/2014
52	2_3Z_1	Alaxin	Tablet	Dihydroartemisinin/Suphfadoxine BP/Pyrimethamine BP 60mg/500mg/25mg fixed dose	AP-16	Bliss GVS India	02/2011-01/2014
53	2_6Z_1	P-Alaxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40/320mg	PX-161	Bliss GVS India	08/2011-07/2014
54	2_7Z_1	P-Alaxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40/320mg	PX-116	Bliss GVS India	02/2011-01/2014
NON-ARTEMISININ BASED DRUGS							
55	2_1P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/ 25mg fixed dose	10011	Shellys Tanzania	07/2010-06/2014
56	2_2P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/ 25mg fixed dose	10012	Shellys Tanzania	07/2010-06/2014
57	2_3P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/ 25mg fixed dose	10012	Shellys Tanzania	07/2010-06/2014
58	2_1P_{15}	Methomine S	Tablet	Sulphadoxine BP/Pyrimethamine BP 500mg/25mg fixed dose	021350	Universal Kenya	08/2010-07/2013

* Unregistered samples

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ZONE 3 : CENTRAL							
ARTEMISININ-BASED DRUGS							
59	3_1X_{11}	Artefan	Tablet	Artemether/Lumefantrine 80mg/480mg fixed dose	C0520J	Ajanta India	10/2010-09/2012
60	3_1X_1	Lonart-Dispersible	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	LS-39	Bliss GVS India.	09/2011-08/2013
61	3_2X_1	Lonart-Dispersible	Tablet	Artemether/ Lumefantrine 20mg/120mg fixed dose	LS-39	Bliss GVS India.	09/2011-08/2013
62	3_3X_1	Lonart Forte	Tablet	Artemether/Lumefantrine 40mg/240mg fixed dose	LF-249	Bliss GVS India	08/2011-07/2013
63	3_4X_1	Lonart-DS	Tablet	Artemether/Lumefantrine 800mg/480mg fixed dose	LD-227	Bliss GVS India	05/2011-04/2013
64	3_6X_1	Lonart-DS	Tablet	Artemether/Lumefantrine 80mg/480mg fixed dose	LD-227	Bliss GVS India	05/2011-04/2013
65	3_1Y_{12}	Co-Arinate FDC(Adult)	Tablet	Artesunate/Sulphamethoxypyridazine/Pyrimethamine 200/500/25mg fixed dose	081	Dafra Kenya	02/2011-02/2013
66	*3_2Y_{13}	Spafil(Adults)	Tablet	Artesunate/Sulphadoxine/Pyrimethamine 100/500/25mg co-packed on the same blister	TR0458	Fourrts India	02/2011-01/2014
67	*3_4Y_{13}	Spafil(Adults)	Tablet	Artesunate/Sulphadoxine/Pyrimethamine 100/500/25mg co-packed on the same blister	TR0458	Fourrts India	02/2011-01/2014

* Unregistered samples

68	*3_2Z_3	Duo-Cotecxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40/320mg fixed dose	210610	Zhejiang Holley Nanhu China	06/2010-06/2012
69	*3_3Z_3	Duo-Cotecxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40/320mg fixed dose	110123	Zhejiang Holley Nanhu	01/2011-01/2013
70	3_4Z_1	Alaxin +(plus)	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60/500/25mg fixed dose	AP-18	Bliss GVS India	08/2011-07/2014
71	3_5Z_1	Alaxin +(plus)	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60/500/25mg fixed dose	AP-16	Bliss GVS India	02/2011-01/2014
72	3_7Z_1	P-Alaxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40/320mg fixed dose	PX-146	Bliss GVS India	06/2011-05/2014
NON-ARTEMISININ BASED DRUGS							
73	*3_1P_{10}	Malacure	Tablet	Sulphadoxine/Pyrimethamine 500mg/25mg fixed dose	S-60	S Kant Mumbai	07/2010-06/2013
74	*3_2P_{10}	Malacure	Tablet	Sulphadoxine/Pyrimethamine 500mg/25mg fixed dose	S-60	S Kant Mumbai	07/2010-06/2013
75	*3_3P_{10}	Malacure	Tablet	Sulphadoxine/Pyrimethamine 500mg/25mg fixed dose	S-60	S Kant Mumbai	07/2010-06/2013
76	3_1P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/25mg fixed dose	10008	Shellys Tanzania	05/2010-04/2014

* Unregistered samples

77	3_2P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/25mg fixed dose	10012	Shellys Tanzania	07/2010-06/2014
78	3_5P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/25mg fixed dose	10011	Shellys Tanzania	07/2010-06/2014
79	3_7P_{15}	Methomine S	Tablet	Sulphadoxine BP/Pyrimethamine BP 500mg/25mg fixed dose	021350	Universal Kenya	08/2010-07/2013
80	3_8P_{15}	Methomine S	Tablet	Sulphadoxine BP/Pyrimethamine BP 500mg/25mg fixed dose	021350	Universal Kenya	08/2010-07/2013
81	3_1Q_6	Quinine Sulphate Suspension (QSM)	Suspension	Quinine Sulphate 150mg	L-491	Lebene Laboratories India	06/2011-05/2013
82	3_2Q_6	Quinine Sulphate Suspension (QSM)	Suspension	Quinine Sulphate 150mg	L-1180	Lebene Laboratories India	08/2010-07/2012
83	3_3Q_6	Quinine Sulphate Suspension (QSM)	Suspension	Quinine Sulphate 150mg	L-491	Lebene Laboratories India	06/2011-05/2013
ZONE 4 : NORTH							
NON-ARTEMISININ-BASED DRUGS							
84	$4V_5$	Quinaquin	Mixture	Quinine bisulphate BP 50mg/5ml	OK 159	Elys Chemical Kenya	11/2010-10/2012
85	4_1R_8	Kwinil	Injection	Quinine di-HCl	L01224	Intas Pharmaceuticals India	02/2010-01/2013

* Unregistered samples

86	4_1Q_6	Quinine Sulphate Suspension (QSM)	Suspension	Quinine sulphate 150mg/5ml	L-491	Lebene Laboratories India	06/2011-05/2013
87	4_2Q_6	Quinine Sulphate Suspension (QSM)	Suspension	Quinine sulphate 150mg/5ml	L-491	Lebene Laboratories India	06/2011-05/2013
88	4_3Q_6	Quinine Sulphate Suspension (QSM)	Suspension	Quinine sulphate 150mg/5ml	L-2100	Lebene Laboratories India	12/2010-11/2012
89	4_2R_4	Curaquin	Quinine syrup	Quinine HCl BP 100mg/5ml	110433	Regal Pharmaceuticals Kenya	04/2011-03/2014
90	4_3R_4	Curaquin	Quinine syrup	Quinine HCl BP 100mg/5ml	110433	Regal Pharmaceuticals Kenya	04/2011-03/2014
91	4_1P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/25mg fixed dose	10013	Shellys Tanzania	07/2010-06/2014
92	4_4P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/25mg fixed dose	10008	Shellys Tanzania	05/2010-04/2014
93	4_2P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/25mg fixed dose	10012	Shellys Tanzania	07/2010-06/2014
94	4_5P_2	Sulphadar	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/25mg fixed dose	10012	Shellys Tanzania	07/2010-06/2014
95	4_8P_5	Ekelfin	Tablet	Sulphadoxine USP/Pyrimethamine USP 500mg/25mg fixed dose	OA 92	Elys Chemical Kenya	01/2010-12/2013

* Unregistered samples

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ARTEMISININ-BASED DRUGS							
96	4_5X_{12}	Co-Artesiane	Suspension	Artemether/Lumefantrine 180mg:60ml/1080mg:60ml	24243	Dafra Pharma Kenya	06/2010-06/2012
97	$4X_{20}$	Coartem-Dispersible (Children)	Tablet	Artemether/Lumefantrine 20/120mg fixed dose	F0442	Novartis USA	05/2011-04/2013
98	4_4X_1	Lonart-DS	Tablet	Artemether/Lumefantrine 80/480mg fixed dose	LD- 259	Bliss Gvs India	08/2011-07/2013
99	4_3X_1	Lonart Forte	Tablet	Artemether + lumefantrine 80/480mg fixed dose	LF-249	Bliss Gvs India	08/2011-07/2013
100	4_2X_{11}	Artefan	Tablet	Artemether/Lumefantrine 20/120mg fixed dose	C0411G	Ajanta India	07/2011-06/2013
101	4_1X_{11}	Artefan	Tablet	Artemether/Lumefantrine 20/120mg fixed dose	P0761G	Ajanta India	07/2011-06/2013
102	$*4Y_{13}$	Spafil	Tablet	Artesunate/Sulphadoxine/Pyrimethamine 100/500/25mg co-packed on the same blister	TR0324	Fourrts India	05/2010-04/2013
103	4_3Y_{12}	Co-Arinate PDC	Tablet	Artesunate/Sulphamethoxypyridazine/Pyrimethamine 100/250/12.5mg fixed dose	079	Dafra Pharma Kenya	03/2011-03/2013
104	4_1Y_{12}	Co-Arinate PDC	Tablet	Artesunate/Sulphamethoxypyridazine/Pyrimethamine 200/500/25mg fixed dose	081	Dafra Pharma Kenya	02/2011-02/2013
105	4_2Y_{12}	Co-Arinate PDC	Tablet	Artesunate/Sulphamethoxypyridazine/Pyrimethamine 200/500/25mg	081	Dafra Pharma Kenya	02/2011-02/2013
106	4_4Y_{12}	C0-Arinate PDC	Tablet	Artesunate/Sulphamethoxypyridazine/Pyrimethamine 100/250/12.5mg	079	Dafra Pharma Kenya	03/2011-03/2013
107	$*4_4Z_3$	Duo-Cotecxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40/320mg fixed dose	110123	Zhejiang Holley Nanhu China	01/2011-01/2013

* Unregistered samples

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108	*4_3Z_3	Duo-Cotecxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40/320mg fixed dose	110123	Zhejiang Holley Nanhu China	01/2011-01/2013
109	*4_2Z_3	Duo-Cotecxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40/320mg fixed dose	110620	Zhejiang Holley Nanhu china	06/2011-06/2013
110	*4_1Z_3	Duo-Cotecxin	Tablet	Dihydroartemisinin/Piperaquine Phosphate 40/320mg fixed dose	110123	Zhejiang Holley Nanhu China	01/2011-01/2013
111	4_5Z_1	Alaxin	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60/500/25mg	AP-18	Bliss GVS India	08/2011-07/2014
112	4_6Z_1	Alaxin	Tablet	Dihydroartemisinin/Sulphadoxine BP/Pyrimethamine BP 60/500/25mg	AP-11	Bliss GVS India	01/2010-01/2013

* Unregistered samples

Total number of unregistered samples* = 15

APPENDIX II

HPLC AND TLC RESULT

Table 32: Percentage (%) and mass (mg) quantities of results of artesunate active pharmaceutical ingredient (API) by TLC and HPLC methods and their comparisons with the manufacturer's claim and pharmacopoeial requirements. Artesunate tablets must contain at least 90.0% and at most 110.0% of the labelled amount of artesunate on the pack.

Code	Manufacturer's Label Claim (mg)	Semi-quantitative TLC estimation of composition in % and mg quantities of dosage forms of artesunate compared to the manufacturer's label claim range of dosage forms (n = 6 for each solvent system, total n = 12)				Remarks based on TLC results	HPLC determination of composition of artesunate dosage forms in % and mg quantities (n = 6)		Remarks based on HPLC results
		Solvent system 1 Ethanol : Ammonia 100 : 0.5		Solvent system 2 Ethanol: Toluene: Ammonia 70 : 30 : 1.5					
		% range ± rsd	Quantity (mg)	% range ± rsd	Quantity (mg)		% ± rsd	Quantity (mg)	
*2_4Y_{13}	**ATS**/SDX/PYR:**100**/500/25	87-97±5	87-97	82-92 ± 4	82-92	***BLC**	97.57 ± 0.01	98	**C**
*$4Y_{13}$	**ATS**/SDX/PYR:**100**/500/25	90-100± 5	90-100	92-102 ± 4	92-102	**C**	94.8 ± 0.1	95	**C**
*3_4Y_{13}	**ATS**/SDX/PYR:**100**/500/25	100-110 ± 5	100-110	89-99 ± 5	89-99	**C**	97.65 ± 0.08	98	**C**
*3_2Y_{13}	**ATS**/SDX/PYR:**100**/500/25	90-100 ± 5	90-100	87-97 ± 5	87-97	***BLC**	98.11 ± 0.02	98	**C**
4_4Y_{12}	**ATS**/SM/PYR:**100**/250/12	90-100 ± 10	90-100	95-105 ± 10	95-105	**C**	90.4 ± 0.2	90	C
4_2Y_{12}	**ATS**/SM/PYR:**200**/500/25	87-97 ± 5	174-194	85-95 ± 5	170-190	***BLC**	92.77 ± 0.02	186	C
4_3Y_{12}	**ATS**/SM/PYR:**100**/250/12.5	65-75 ± 10	65-75	70-80 ± 10	70-80	**NC**	78.22 ± 0.04	78	**NC**

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4_1Y_{12}	**ATS**/SM/PYR:**200**/500/25	90-100 ± 5	180-200	89-99 ± 5	178-198	**C**	86.9 ± 0.2	174	**NC**
3_1Y_{12}	**ATS**/SM/PYR:**200**/500/25	65-75 ± 10	130-150	77-87 ± 5	154-174	**NC**	88.96 ± 0.05	178	**NC**

CODE: $ZONE_{Sample}API_{Manufacturer}$ = Fixed dose combination

$_*$ $ZONE_{Sample}API_{Manufacturer}$ = Single dose of ATS + single dose of SP on the same blister

Table 33: Percentage (%) and mass (mg) quantities of results of artemether active pharmaceutical ingredient (API) by TLC and HPLC methods and their comparisons with the manufacturer's claim and pharmacopoeial requirements. Artemether tablets must contain at least 90.0% and at most 110.0% of the labelled amount of artemether on the pack

Code	**Manufacturer's Label Claim (mg)**	**Semi-quantitative TLC estimation of composition in % and mg quantities of dosage forms of artemether compared to the manufacturer's label claim (n = 6 for each solvent system, total n = 12)**				**Remarks based on TLC results**	**HPLC determination of composition of artemether dosage forms in % and mg quantities (n = 6)**		**Remarks based on HPLC results**
		Solvent system 1 Petrol: Ethyl acetate 70 : 30		**Solvent system 2** Petrol: Ethyl acetate 60 : 40					
		% range ± rsd	Quantity (mg)	% range ± rsd	Quantity (mg)		% ± rsd	Quantity (mg)	
$2X_{15}$	**ATM**/LUM:**20**/120	127-137 ± 5	25-27	134-144 ± 5	27-29	**NC overdose**	144.1 ± 0.7	29	**NC overdose**
$4X_{20}$	**ATM**/LUM:**20**/120	95-105 ± 5	19-21	92-102 ± 4	18-20	**C**	100 ± 2	20	**C**
1_1X_1	**ATM**/LUM:**20**/120	59-69 ± 4	12-14	55-65 ± 5	11-13	**NC**	58.9 ± 0.6	12	**NC**
1_1X_{11}	**ATM**/LUM:**180**/1080	97-107 ± 5	175-193	99-109 ± 4	178-196	**C**	93 ± 2	167	**C**
1_1X_{14}	**ATM**/LUM:**20**/120	79-89 ± 4	16-18	78-88 ± 4	16-18	**NC**	75.3 ± 0.9	15	**NC**
1_1X_{17}	**ATM**/LUM:**80**/480	115-125 ± 15	92-100	125-135± 15	100-108	**NC overdose**	177.8 ± 0.3	142	**NC overdose**

1_1X_{18}	**ATM**/LUM:**40**/240	30-40 ± 5	12-16	29-39 ± 4	12-16	**NC**	26.5 ± 0.8	11	**NC**
1_1X_{20}	**ATM**/LUM:**20**/120	132-142 ± 4	26-28	134-144 ± 5	27-29	**NC overdose**	135 ± 4	27	**NC overdose**
1_2X_1	**ATM**/LUM:**20**/120	119-129 ± 4	24-26	115-125 ± 5	23-25	**NC overdose**	119.7 ± 0.8	24	**NC overdose**
1_2X_{11}	**ATM**/LUM:**180**/1080	105-115 ± 5	189-207	100-110 ± 5	180-198	***BLC**	103.3 ± 0.8	186	**C**
1_2X_{14}	**ATM**/LUM:**20**/120	30-40 ± 5	6-8	30-40 ± 5	6-8	**NC**	35 ± 2	7	**NC**
1_3X_1	**ATM**/LUM:**40**/240	92-102 ± 4	37-41	94-104 ± 5	38-42	**C**	96.8 ± 0.2	39	**C**
1_3X_{11}	**ATM**/LUM:**80**/480	35-45 ± 5	28-36	35-45 ± 5	28-36	**NC**	39.6 ± 0.5	32	**NC**
1_4X_1	**ATM**/LUM:**40**/240	64-74 ± 5	26-30	67-77 ± 5	27-31	**NC**	69 ± 2	28	**NC**
1_4X_{11}	**ATM**/LUM:**40**/240	75-85 ± 5	30-34	72-82 ± 4	29-33	**NC**	82.7 ± 0.3	33	**NC**
1_5X_1	**ATM**/LUM:**80**/480	65-75 ± 5	52-60	62-72 ± 4	50-58	**NC**	67.3 ± 0.3	54	**NC**
1_5X_{11}	**ATM**/LUM:**20**/120	40-50 ± 0	8-10	40-50 ± 0	8-10	**NC**	45 ± 1	9	**NC**
1_6X_1	**ATM**/LUM:**40**/240	125-135 ± 10	50-54	120-130 ± 5	48-52	**NC overdose**	163.2 ± 0.4	65	**NC overdose**
1_7X_1	**ATM**/LUM:**80**/480	84-94 ± 5	67-75	84-94 ± 5	67-75	**NC**	81.205±0	65	**NC**
1_8X_1	**ATM**/LUM:**20**/120	120-130 ± 5	24-26	120-130 ± 5	24-26	**NC overdose**	162.7 ± 0.3	33	**NC overdose**
1_9X_1	**ATM**/LUM:**80**/480	105-115 ± 5	84-92	105-115 ± 5	84-92	***BLC**	103.4 ± 0.4	83	**C**
2_1X_1	**ATM**/LUM:**20**/120	97-107± 5	19-21	97-107 ± 5	19-21	**C**	97 ± 1	19	**C**
2_2X_1	**ATM**/LUM:**80**/480	120-130 ± 5	96-104	115-125± 15	92-100	**NC overdose**	131.5 ± 0.2	105	**NC overdose**
2_3X_1	**ATM**/LUM:**20**/120	135-145 ± 5	27-29	129-139 ± 4	26-28	**NC overdose**	149 ± 1	30	**NC overdose**

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2_4X_{14}	**ATM**/LUM:**20**/120	52-62 ± 4	10-12	50-60 ± 0	10-12	**NC**	53.2 ± 0.3	11	**NC**
2_5X_{11}	**ATM**/LUM:**80**/480	105-115 ± 10	84-92	107-117 ± 5	86-94	**NC overdose**	113 ± 4	90	**NC overdose**
3_1X_1	**ATM**/LUM:**20**/120	85-95 ± 10	17-19	84-94 ± 5	17-19	***BLC**	92.4 ± 0.6	18	**C**
3_2X_1	**ATM**/LUM:**20**/120	92-102 ± 4	18-20	95-105 ± 5	19-21	**C**	97 ± 1	19	**C**
3_1X_{11}	**ATM**/LUM:**80**/480	32-42 ± 4	26-34	34-44 ± 5	27-35	**NC**	39 ± 1	31	**NC**
3_3X_1	**ATM**/LUM:**40**/240	74-84 ± 5	30-34	77-87 ± 5	31-35	**NC**	81.0 ± 0.3	32	**NC**
3_4X_1	**ATM**/LUM:**80**/480	95-105 ± 5	76-84	95-105 ± 5	76-84	**C**	102.1 ± 0.7	82	**C**
3_6X_1	**ATM**/LUM:**80**/480	99-109 ± 4	79-87	95-105 ± 5	76-84	**C**	104.8 ± 0.3	84	**C**
4_1X_{11}	**ATM**/LUM:**20**/120	105-115 ± 5	21-23	100-110± 10	20-22	***BLC**	106.2 ± 0.4	21	**C**
4_2X_{11}	**ATM**/LUM:**20**/120	24-34 ± 5	5-7	25-35 ± 5	5-7	**NC**	28 ± 1	6	**NC**
4_3X_1	**ATM**/LUM:**40**/240	132-142 ± 4	53-57	130-140 ± 0	52-56	**NC overdose**	173.8 ± 0.3	70	**NC overdose**
4_4X_1	**ATM**/LUM:**80**/480	135-145 ± 5	108-116	135-145± 10	108-116	**NC overdose**	142.6 ± 0.7	114	**NC overdose**
4_5X_{12}	**ATM**/LUM:**180**/1080	90-100 ± 5	162-180	90-100 ± 5	162-180	**C**	96.0 ± 0.9	173	**C**
$1_{10}X_1$	**ATM**/LUM:**80**/480	120-130 ± 5	96-104	124-134 ± 5	99-107	**NC overdose**	124.2 ± 0.5	99	**NC overdose**
1_6X_{11}	**ATM**/LUM:**180**/1080	100-110 ± 5	180-198	100-110 ± 5	180-198	**C**	99.3 ± 0.9	179	**C**
$1_{12}X_1$	**ATM**/LUM:**80**/480	92-102	74-82	90-100 ± 5	72-80	**C**	100.9 ± 0	81	**C**
$1_{13}X_1$	**ATM**/LUM:**80**/480	112-122 ± 4	90-98	115-125 ± 5	92-100	**NC overdose**	140.0 ± 0.5	112	**NC overdose**

CODE: $ZONE_{Sample}API_{Manufacturer}$ = Fixed dose combination

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Table 34: Percentage (%) and mass (mg) quantities of results of lumefantrine active pharmaceutical ingredient (API) by TLC and HPLC methods and their comparisons with the manufacturer's claim and pharmacopoeial requirements. Lumefantrine tablets must contain at least 90.0% and at most 110.0% of the labelled amount of Lumefantrine on the pack

Code	Manufacturer's Label Claim (mg)	Semi-quantitative TLC estimation of composition in % and mg quantities of dosage forms of lumefantrine compared to the manufacturer's label claim (n = 6 for each solvent system, total n = 12)				Remarks based on TLC results	HPLC determination of composition of lumefantrine dosage forms in % and mg quantities (n = 6)		Remarks based on HPLC results
		Solvent system 1 Ethyl acetate: acetic acid: toluene 4:2:18		Solvent system 2 Ethyl acetate: acetic acid 10:5					
		% range ± rsd	Quantity (mg)	% range ± rsd	Quantity (mg)		% ± rsd	Quantity (mg)	
$2X_{15}$	ATM/**LUM**:20/**120**	95-105 ± 5	114-126	95-105 ± 5	114-126	**C**	97 ± 2	116	**C**
$4X_{20}$	ATM/**LUM**:20/**120**	112-122± 4	134-146	113-123±5	136-148	**NC overdose**	119.5 ± 0.4	143	**NC overdose**
1_1X_1	ATM/**LUM**:20/**120**	104-114± 5	125-137	95-105 ± 5	114-126	**C**	112.5 ± 0.5	135	**NC overdose**
1_1X_{11}	ATM/**LUM**:180/**1080**	117-127± 5	1264-1372	120-130 ± 0	1296-1404	**NC overdose**	127 ± 1	1372	**NC overdose**
1_1X_{14}	ATM/**LUM**:20/**120**	60-70 ± 0	72-84	60-70 ± 0	72-84	**NC**	69.3 ± 0.3	83	**NC**
1_1X_{17}	ATM/**LUM**:80/**480**	94-104 ± 5	451-499	98-108 ± 5	470-518	**C**	101.0 ± 0.4	485	**C**
1_1X_{18}	ATM/**LUM**:40/**240**	95-105 ± 5	228-252	90-100 ± 0	216-240	**C**	92.9 ± 0.3	223	**C**
1_1X_{20}	ATM/**LUM**:20/**120**	109-119± 4	131-143	110-120 ± 0	132-144	**NC overdose**	114.5 ± 0.5	137	**NC overdose**
1_2X_1	ATM/**LUM**:20/**120**	100-110 ± 5	120-132	94-104 ± 5	113-125	**C**	113.0 ± 0.4	136	**NC**
1_2X_{11}	ATM/**LUM**:180/**1080**	122-132± 4	1318-1426	119-129 ± 4	1285-1393	**NC overdose**	123 ± 2	1328	**NC overdose**

1_2X_{14}	ATM/**LUM**:20/**120**	92-102 ± 4	110-122	90-100 ± 5	108-120	**C**	95.3 ± 0.2	114	**C**
1_3X_1	ATM/**LUM**:40/**240**	77-87 ± 5	185-209	74-84 ± 5	178-202	**NC**	81.1 ± 0.3	195	**NC**
1_3X_{11}	ATM/**LUM**:80/**480**	74-84 ± 5	355-403	75-85 ± 5	360-408	**NC**	85 ± 1	408	**NC**
1_4X_1	ATM/**LUM**:40/**240**	100-110 ± 5	240-264	109-119 ± 4	262-286	**BLC**	115.0 ± 0.9	276	**NC**
1_4X_{11}	ATM/**LUM**:40/**240**	100-110 ± 5	240-264	105-115 ± 5	252-276	**BLC**	114.0 ± 0.4	274	**NC**
1_5X_1	ATM/**LUM**:80/**480**	99-109 ± 4	475-523	103-113 ± 5	494-542	**BLC**	110.9 ± 0.2	532	**BLC**
1_5X_{11}	ATM/**LUM**:20/**120**	80-90 ± 10	96-108	90-100 ± 5	108-120	***BLC**	90.5 ± 0.3	109	**C**
1_6X_1	ATM/**LUM**:40/**240**	122-132± 4	293-317	120-130 ± 10	288-312	**NC overdose**	127 ± 1	305	**NC overdose**
1_7X_1	ATM/**LUM**:80/**480**	57-67 ± 5	274-322	59-69 ± 4	283-331	**NC**	59 ± 1	283	**NC**
1_8X_1	ATM/**LUM**:20/**120**	70-80 ± 0	84-96	72-82 ± 4	86-98	**NC**	79.19 ± 0.0	95	**NC**
1_9X_1	ATM/**LUM**:80/**480**	107-117± 5	514-562	109-119 ± 4	523-571	**NC overdose**	113.5 ± 0.4	545	**NC overdose**
2_1X_1	ATM/**LUM**:20/**120**	105-115 ± 5	126-138	109-119 ± 4	131-143	**NC overdose**	113 ± 4	136	**NC**
2_2X_1	ATM/**LUM**:80/**480**	57-67 ± 5	274-322	60-70 ± 0	288-336	**NC**	63 ± 2	302	**NC**
2_3X_1	ATM/**LUM**:20/**120**	97-107 ± 5	116-128	99-109 ± 4	119-131	**C**	103.3 ± 0.3	124	**C**
2_4X_{14}	ATM/**LUM**:20/**120**	112-122± 4	134-146	115-125 ± 5	138-150	**NC overdose**	117.5 ± 0.8	141	**NC overdose**
2_5X_{11}	ATM/**LUM**:80/**480**	90-100 ± 10	432-480	100-110 ± 5	480-528	**C**	112.2 ± 0.6	539	**NC**
3_1X_1	ATM/**LUM**:20/**120**	115-125 ± 5	138-150	119-129 ± 4	143-155	**NC overdose**	116.9 ± 0.5	140	**NC overdose**
3_2X_1	ATM/**LUM**:20/**120**	112-122± 4	134-146	112-122 ± 4	134-146	**NC overdose**	117 ± 2	140	**NC overdose**

3_1X_{11}	ATM/**LUM**:80/**480**	100-110 ± 10	480-528	105-115 ± 10	504-552	**BLC**	118.6 ± 0.6	569	**NC overdose**
3_3X_1	ATM/**LUM**:40/**240**	95-105 ± 5	228-252	95-105 ± 5	228-252	**C**	108.0 ± 0.2	259	**C**
3_4X_1	ATM/**LUM**:80/**480**	100-110 ± 10	480-528	102-112 ± 4	490-538	**BLC**	111.6 ± 0.3	536	**NC overdose**
3_6X_1	ATM/**LUM**:80/**480**	90-100 ± 5	432-480	95-105 ± 5	456-504	**C**	103.3 ± 0.1	496	**C**
4_1X_{11}	ATM/**LUM**:20/**120**	45-55 ± 5	54-66	40-50 ± 0	48-60	**NC**	50 ± 6	60	**NC**
4_2X_{11}	ATM/**LUM**:20/**120**	87-97 ± 5	104-116	90-100 ± 5	108-120	**BLC**	88 ± 2	106	**BLC**
4_3X_1	ATM/**LUM**:40/**240**	72-82 ± 4	173-197	75-85 ± 5	180-204	**NC**	81.8 ± 0.3	196	**NC**
4_4X_1	ATM/**LUM**:80/**480**	100-110 ± 5	480-528	95-105 ± 5	456-504	**C**	104.0 ± 0.2	499	**C**
4_5X_{12}	ATM/**LUM**:180/**1080**	125-135 ± 5	1350-1458	125-135 ± 5	1350-1458	**NC overdose**	129 ± 2	1393	**NC overdose**
$1_{10}X_1$	ATM/**LUM**:80/**480**	62-72 ± 4	298-346	60-70 ± 0	288-336	**NC**	63 ± 1	302	**NC**
1_6X_{11}	ATM/**LUM**:180/**1080**	107-117± 5	1156-1264	108-118 ± 5	1166-1274	**NC overdose**	119.3 ± 0.1	1288	**NC overdose**
$1_{12}X_1$	ATM/**LUM**:80/**480**	100-110 ± 5	480-528	94-104 ± 5	451-499	**C**	102.5 ± 0	492	**C**
$1_{13}X_1$	ATM/**LUM**:80/**480**	90-100 ± 5	432-480	95-105 ± 5	456-504	**C**	109.96±0.0	528	**C**

` CODE: $ZONE_{Sample}API_{Manufacturer}$ = Fixed dose combination

Table 35: Percentage (%) and mass (mg) quantities of results of dihydroartemisinin (Artenimol) active pharmaceutical ingredient (API) by TLC and HPLC methods and their comparisons with the manufacturer's claim and pharmacopoeial requirements. Dihydroartemisinin tablets must contain at least 90.0% and at most 110.0% of the labelled amount of Dihydroartemisinin on the pack

Code	Manufacturer's Label Claim (mg)	Semi-quantitative TLC estimation of composition in % and mg quantities of dosage forms of dihydroartemisinin compared to the manufacturer's label claim (n = 6 for each solvent system, total n = 12)				Remarks based on TLC results	HPLC determination of composition of dihydroartemisinin dosage forms in % and mg quantities (n = 6)		Remarks based on HPLC results
		Solvent system 1 Toluene: Ethyl acetate 60 : 40		Solvent system 2 Toluene: Ethyl acetate 70 : 30					
		% range ± rsd	Quantity (mg)	% range ± rsd	Quantity (mg)		% ± rsd	Quantity (mg)	
1_1Z_3	**DHA**/PPQ:**40**/320	100-110 ± 5	40-44	95-105 ± 5	38-42	**C**	98.7 ± 0.2	39	**C**
1_2Z_3	**DHA**/PPQ:**40**/320	84-94± 5	34-38	84-94± 5	34-38	**NC**	86 ± 2	34	**NC**
1_4Z_3	**DHA**/PPQ:**40**/320	70-80 ± 5	28-32	70-80 ± 5	28-32	**NC**	70 ± 1	28	**NC**
1_6Z_1	**DHA**/PPQ:**40**/320	90-100 ± 10	36-40	94-104± 5	38-42	**C**	97.5 ± 0.9	39	**C**
1_7Z_1	**DHA**/PPQ:**40**/320	85-95 ± 10	34-38	85-95 ± 10	34-38	**BLC**	85 ± 1	34	**NC**
2_6Z_1	**DHA**/PPQ:**40**/320	95-105 ± 10	38-42	95-105 ± 10	38-42	**C**	103 ± 1	41	**C**
2_7Z_1	**DHA**/PPQ:**40**/320	102-112± 4	41-45	100-110 ± 5	40-44	**BLC**	88 ± 5	35	**BLC**
3_2Z_3	**DHA**/PPQ:**40**/320	67-77 ± 5	27-31	66-76 ± 5	26-30	**NC**	73 ± 2	29	**NC**
3_3Z_3	**DHA**/PPQ:**40**/320	62-72 ± 4	25-29	64-74 ± 5	26-30	**NC**	71 ± 1	28	**NC**

3_7Z_1	**DHA**/PPQ:**40**/320	90-100 ± 5	36-40	95-105 ± 5	38-42		101 ± 6	40	**C**
4_1Z_3	**DHA**/PPQ:**40**/320	69-79 ± 5	28-32	67-77 ± 5	27-31	**NC**	74 ± 3	30	**NC**
4_2Z_3	**DHA**/PPQ:**40**/320	90-100 ± 10	36-40	95-105 ± 5	38-42	**C**	87 ± 1	35	**NC**
4_3Z_3	**DHA**/PPQ:**40**/320	70-80 ± 5	28-32	70-80 ± 10	28-32	**NC**	71 ± 3	28	**NC**
4_4Z_3	**DHA**/PPQ:**40**/320	90-100 ± 5	36-40	90-100 ± 5	36-40	**C**	88 ± 1	35	**NC**
1_5Z_1	**DHA**/SDX/PYR:**60**/500/25	47-57 ± 5	28-34	45-55 ± 5	27-33	**NC**	51.4 ± 0.2	31	**NC**
1_3Z_1	**DHA**/SDX/PYR:**60**/500/25	56-66 ± 4	34-40	60-70 ± 5	36-42	**NC**	56.8 ± 0.2	34	**NC**
1_2Z_1	**DHA**/SDX/PYR:**60**/500/25	57-67 ± 5	34-40	57-67 ± 5	34-40	**NC**	52.5 ± 0.1	32	**NC**
1_4Z_1	**DHA**/SDX/PYR:**60**/500/25	60-70 ± 5	36-42	55-65 ± 5	33-39	**NC**	61.6 ± 0.1	37	**NC**
2_1Z_1	**DHA**/SDX/PYR:**60**/500/25	60-70 ± 5	36-42	60-70 ± 5	36-42	**NC**	56.48±0.09	34	**NC**
2_3Z_1	**DHA**/SDX/PYR:**60**/500/25	45-55 ± 5	27-33	50-60 ± 5	30-36	**NC**	54.4 ± 0.2	33	**N C**
2_4Z_1	**DHA**/SDX/PYR:**60**/500/25	54-64 ± 5	32-38	52-62 ± 4	31-37	**NC**	52.5 ± 0.1	32	**NC**
2_5Z_1	**DHA**/SDX/PYR:**60**/500/25	55-65 ± 5	33-39	57-67 ± 5	34-40	**NC**	51.25±0.06	31	**NC**
3_4Z_1	**DHA**/SDX/PYR:**60**/500/25	49-59 ± 4	29-35	49-59 ± 4	29-35	**NC**	51.4 ± 0.1	31	**NC**
3_5Z_1	**DHA**/SDX/PYR:**60**/500/25	56-66 ± 5	34-40	52-62 ± 4	31-37	**NC**	51.8 ± 0.1	31	**NC**
4_5Z_1	**DHA**/SDX/PYR:**60**/500/25	57-67 ±5	34-40	57-67±5	34-40	**NC**	51.67±0.05	31	**NC**
4_6Z_1	**DHA**/SDX/PYR:**60**/500/25	57-67± 5	34-40	55-65 ± 10	33-39	**NC**	51.92±0.09	31	**NC**

CODE: $ZONE_{Sample}API_{Manufacturer}$ = Fixed dose combination

Table 36: Percentage (%) and Mass (mg) quantities of results of sulphadoxine active pharmaceutical ingredient (API) by TLC and HPLC methods and their comparisons with the manufacturer's claim and pharmacopoeial requirements. Sulphadoxine tablets must contain at least 90.0% and at most 110.0% of the labelled amount of Sulphadoxine on the pack

Code	Manufacturer's Label Claim (mg)	Semi-quantitative TLC estimation of composition in % and mg quantities of dosage forms of sulphadoxine compared to the manufacturer's label claim n = 6 for each solvent system, total n = 12)				Remarks based on TLC results	HPLC determination of composition of sulphadoxine dosage forms in % and mg quantities (n = 6)		Remarks based on HPLC results
		Solvent system 1 Ethyl acetate/methanol/ Ammonia 80:15:5		Solvent system 2 Ethyl acetate/acetic acid/ water 60:20:20					
		% range ± rsd	Quantity (mg)	% range ± rsd	Quantity (mg)		% ± rsd	Quantity (mg)	
1_1P_{10}	**SDX**/PYR:**500**/25	80-90 ± 5	400-450	77-87±5	385-435	**NC**	78 ± 2	390	**NC**
1_1P_2	**SDX**/PYR:**500**/25	82-92±4	410-460	80-90 ± 0	400-450	**NC**	87 ± 2	435	**NC**
1_2P_2	**SDX**/PYR:**500**/25	70-80 ± 5	350-400	70-80 ± 5	350-400	**NC**	67 ± 4	335	**NC**
1_4P_{10}	**SDX**/PYR:**500**/25	74-84± 5	370-420	77-87 ± 5	385-435	**NC**	85 ± 2	425	**NC**
1_4P_2	**SDX**/PYR:**500**/25	79-89± 4	395-445	78-88 ± 5	390-440	**NC**	82 ± 2	410	**NC**
1_6P_{10}	**SDX**/PYR:**500**/25	82-92± 4	410-460	80-90 ± 5	400-450	**NC**	83 ± 3	415	**NC**
2_1P_{15}	**SDX**/PYR:**500**/25	77-87± 5	385-435	78-88 ± 4	390-440	**NC**	82 ± 3	410	**NC**
2_1P_2	**SDX**/PYR:**500**/25	87-97± 5	435-485	80-90 ± 0	400-450	**NC**	77 ± 1	385	**NC**
2_2P_2	**SDX**/PYR:**500**/25	85-95 ± 5	425-475	83-93 ± 5	415-465	**NC**	83 ± 2	415	**NC**
2_3P_2	**SDX**/PYR:**500**/25	90-100± 5	450-500	90-100 ± 0	450-500	**C**	96 ± 2	480	**C**

3_1P_{10}	**SDX**/PYR:**500**/25	94-104±5	470-520	85-95 ± 5	425-475	**C**	83 ± 2	415	**NC**
3_1P_2	**SDX**/PYR:**500**/25	94-104± 5	470-520	90-100± 10	450-500	**C**	97 ± 3	485	**C**
3_2P_{10}	**SDX**/PYR:**500**/25	85-95 ± 5	425-475	80-90 ± 0	400-450	**NC**	81 ± 2	405	**NC**
3_2P_2	**SDX**/PYR:**500**/25	82-92 ± 4	410-460	82-92± 4	410-460	**NC**	85 ± 2	425	**NC**
3_3P_{10}	**SDX**/PYR:**500**/25	70-80 ± 5	350-400	69-79 ± 4	345-395	**NC**	82 ± 2	410	**NC**
3_5P_2	**SDX**/PYR:**500**/25	50-60 ± 0	250-300	50-60 ± 0	250-300	**NC**	53 ± 3	265	**NC**
3_7P_{15}	**SDX**/PYR:**500**/25	70-80 ± 5	350-400	75-85 ± 5	375-425	**NC**	71 ± 2	355	**NC**
3_8P_{15}	**SDX**/PYR:**500**/25	85-95± 10	425-475	85-95 ± 5	425-475	***BLC**	91.7 ± 0.2	459	**C**
4_1P_2	**SDX**/PYR:**500**/25	85-95 ± 5	425-475	80-90 ± 5	400-450	**NC**	89 ± 3	445	**BLC**
4_2P_2	**SDX**/PYR:**500**/25	55-65 ± 5	275-325	52-62 ± 4	260-310	**NC**	47 ± 2	235	**NC**
4_4P_2	**SDX**/PYR:**500**/25	72-82 ± 4	360-410	75-85 ± 10	375-425	**NC**	85.17 ± 0.06	426	**NC**
4_5P_2	**SDX**/PYR:**500**/25	82-92 ± 4	410-460	85-95 ± 5	425-475	**NC**	85.1 ± 0.2	426	**NC**
4_8P_5	**SDX**/PYR:**500**/25	87-97 ± 5	435-485	90-100± 10	450-500	**BLC**	85 ± 2	425	**NC**
*2_4Y_{13}	ATS/**SDX**/PYR:100/**500**/25	79-89 ± 4	395-445	73-83 ± 5	365-415	**NC**	72 ± 2	360	**NC**
*$4Y_{13}$	ATS/**SDX**/PYR:100/**500**/25	77-87 ± 5	385-435	77-87 ± 5	385-435	**NC**	89 ± 2	445	**BLC**
*3_4Y_{13}	ATS/**SDX**/PYR:100/**500**/25	65-75 ± 5	325-375	63-73 ± 5	315-365	**NC**	74 ± 4	370	**NC**
*3_2Y_{13}	ATS/**SDX**/PYR:100/**500**/25	64-74 ± 5	320-370	63-73 ± 5	315-365	**NC**	68 ± 5	340	**NC**
1_5Z_1	DHA/**SDX**/PYR:60/**500**/25	84-94 ± 5	420-470	88-98 ± 4	440-490	**BLC**	85.694 ± 0	428	**NC**

1_3Z_1	DHA/**SDX**/PYR:60/**500**/25	84-94± 5	420-470	82-92 ± 4	410-460	**NC**	86 ± 2	430	**NC**
1_2Z_1	DHA/**SDX**/PYR:60/**500**/25	72-82 ± 4	360-410	70-80 ± 10	350-400	**NC**	82.7 ± 0.9	414	**NC**
1_4Z_1	DHA/**SDX**/PYR:60/**500**/25	65-75± 10	325-375	70-80 ± 5	350-400	**NC**	79 ± 2	395	**NC**
2_1Z_1	DHA/**SDX**/PYR:60/**500**/25	72-82 ± 4	360-410	78-88 ± 5	390-440	**NC**	77 ± 2	385	**NC**
2_3Z_1	DHA/**SDX**/PYR:60/**500**/25	90-100± 5	450-500	90-100 ± 5	450-500	**C**	90 ± 2	450	**C**
2_4Z_1	DHA/**SDX**/PYR:60/**500**/25	70-80± 10	350-400	75-85 ± 10	375-425	**NC**	80 ± 2	400	**NC**
2_5Z_1	DHA/**SDX**/PYR:60/**500**/25	79-89± 4	395-445	75-85 ± 5	375-425	**NC**	86.7 ± 0.6	434	**NC**
3_4Z_1	DHA/**SDX**/PYR:60/**500**/25	80-90 ± 5	400-450	75-85 ± 5	375-425	**NC**	85.4 ± 0.2	427	**NC**
3_5Z_1	DHA/**SDX**/PYR:60/**500**/25	87-97 ± 5	435-485	80-90 ± 0	400-450	**NC**	84.9 ± 0.1	425	**NC**
4_5Z_1	DHA/**SDX**/PYR:60/**500**/25	74-84 ± 5	370-420	75-85 ± 5	375-425	**NC**	87.5 ± 0.8	438	**NC**
4_6Z_1	DHA/**SDX**/PYR:60/**500**/25	74-84 ± 5	370-420	80-90 ± 5	400-450	**NC**	75.2 ± 0.1	376	**NC**
4_4Y_{12}	ATS/**SM**/PYR:100/**250**/12.5	-	-	-	-		90 ± 3	225	**C**
4_2Y_{12}	ATS/**SM**/PYR:200/**500**/25	-	-	-	-		88.6 ± 0.2	443	**NC**
4_3Y_{12}	ATS/**SM**/PYR:100/**250**/12.5	-	-	-	-		87 ± 2	218	**NC**
4_1Y_{12}	ATS/**SM**/PYR:200/**500**/25	-	-	-	-		87.9 ± 0.5	440	**NC**
3_1Y_{12}	ATS/**SM**/PYR:100/**250**/12.5	-	-	-	-		86.6 ± 0.2	217	**NC**

Sm = sulphamethoxypyridazine. With TLC, it gave a spot with the same retention time as sulphadoxine, but it failed to stain well with I_2/KI for the SQ-TLC. However, HPLC analysis was successful.

CODE: $ZONE_{Sample}API_{Manufacturer}$ = Fixed dose combination

$_*$ $ZONE_{Sample}API_{Manufacturer}$ = Single dose of ATS + single dose of S/P on the same blister

Table 37: Percentage (%) and Mass (mg) quantities of results of pyrimethamine active pharmaceutical ingredient (API) by TLC and HPLC methods and their comparisons with the manufacturer's claim and pharmacopoeial requirements. Pyrimethamine tablets must contain at least 90.0% and at most 110.0% of the labelled amount of Pyrimethamine on the pack

Code	Manufacturer's Label Claim (mg)	Semi-quantitative TLC estimation of composition in % and mg quantities of dosage forms of pyrimethamine compared to the manufacturer's label claim n = 6 for each solvent system, total n = 12)				Remarks based on TLC results	HPLC determination of composition of pyrimethamine dosage forms in % and mg quantities (n = 6)		Remarks based on HPLC results
		Solvent system 1 Ethyl Acetate/methanol/ammonia 85:10:5		**Solvent system 2** Ethyl acetate/acetic acid/water 60:20:20					
		% range ± rsd	Quantity(mg)	% range ± rsd	Quantity (mg)		% ± rsd	Quantity (mg)	
1_1P_{10}	SDX/**PYR**:500/**25**	80-90 ± 0	20-23	90-100 ± 0	23-25	***BLC**	92±0	23	**C**
1_1P_2	SDX/**PYR**:500/**25**	105-115± 10	26-29	110-120 ± 5	28-30	**NC overdose**	114±0	29	**NC overdose**
1_2P_2	SDX/**PYR**:500/**25**	69-79 ± 4	17-20	69-79± 4	17-20	**NC**	76±0	19	**NC**
1_4P_{10}	SDX/**PYR**:500/**25**	92-102 ± 4	23-26	94-104 ± 5	24-26	**C**	110±0	28	**C**
1_4P_2	SDX/**PYR**:500/**25**	87-97 ± 5	22-24	89-99 ± 5	22-25	***BLC**	93±0	23	**C**
1_6P_{10}	SDX/**PYR**:500/**25**	87-97 ± 5	22-24	92-102 ± 4	23-26	**C**	92±0	23	**C**
2_1P_{15}	SDX/**PYR**:500/**25**	85-95 ± 5	21-24	85-95 ± 5	21-24	***BLC**	93±0	23	**C**
2_1P_2	SDX/**PYR**:500/**25**	87-97 ± 5	22-24	87-97 ± 5	22-24	**BLC**	89±0	22	**NC**
2_2P_2	SDX/**PYR**:500/**25**	60-70 ± 10	15-18	57-67 ± 5	14-17	**NC**	64±0	16	**NC**

2_3P_2	SDX/**PYR**:500/**25**	107-117± 5	27-29	107-117± 5	27-29	**NC overdose**	122±0	31	**NC overdose**
3_1P_{10}	SDX/**PYR**:500/**25**	80-90 ± 10	20-23	85-95 ± 5	21-24	**NC**	83±0	21	**NC**
3_1P_2	SDX/**PYR**:500/**25**	92-102 ± 4	23-26	94-104± 5	24-26	**C**	97±0	24	**C**
3_2P_{10}	SDX/**PYR**:500/**25**	85-95 ± 5	21-24	84-94 ± 4	21-24	***BLC**	94±0	24	**C**
3_2P_2	SDX/**PYR**:500/**25**	79-89 ± 4	20-22	80-90 ± 5	20-23	**NC**	96±0	24	**C**
3_3P_{10}	SDX/**PYR**:500/**25**	84-94 ± 5	21-24	82-92± 4	21-23	**NC**	87±0	22	**NC**
3_5P_2	SDX/**PYR**:500/**25**	40-50 ± 0	10-13	40-50 ± 0	10-13	**NC**	48±0	12	**NC**
3_7P_{15}	SDX/**PYR**:500/**25**	80-90 ± 5	20-23	70-80 ± 0	18-20	**NC**	78±0	20	**NC**
3_8P_{15}	SDX/**PYR**:500/**25**	99-109 ± 4	25-27	95-105 ± 5	24-26	**C**	103±0	26	**C**
4_1P_2	SDX/**PYR**:500/**25**	110-120 ± 5	28-30	110-120 ± 5	28-30	**NC overdose**	113±0	28	**NC overdose**
4_2P_2	SDX/**PYR**:500/**25**	60-70 ± 5	15-18	50-60 ± 0	13-15	**NC**	51±0	13	**NC**
4_4P_2	SDX/**PYR**:500/**25**	80-90 ± 15	20-23	75-85 ± 10	19-21	**NC**	102±0	26	**C**
4_5P_2	SDX/**PYR**:500/**25**	95-105 ± 5	24-26	99-109 ± 5	25-27	**C**	108±0	27	**C**
4_8P_5	SDX/**PYR**:500/**25**	104-114± 5	26-29	102-112± 4	26-28	***BLC**	110±0	28	**C**
*2_4Y_{13}	ATS/SDX/**PYR**:100/500/**25**	85-95 ± 5	21-24	90-100 ± 0	23-25	***BLC**	91±0	23	**C**
*$4Y_{13}$	ATS/SDX/**PYR**:100/500/**25**	80-90 ± 5	20-23	85-95 ± 5	21-24	**NC**	98±0	25	**C**
*3_4Y_{13}	ATS/SDX/**PYR**:100/500/**25**	60-70 ± 0	15-18	60-70 ± 0	15-18	**NC**	70±0	18	**NC**
*3_2Y_{13}	ATS/SDX/**PYR**:100/500/**25**	75-85 ± 5	19-21	72-82 ± 4	18-21	**NC**	78±0	20	**NC**

1_5Z_1	DHA/SDX/**PYR**:60/500/**25**	90-100 ± 0	23-25	87-97 ± 5	22-24	***BLC**	94±0	24	**C**
1_3Z_1	DHA/SDX/**PYR**:60/500/**25**	87-97 ± 5	22-24	88-98 ± 4	22-25	***BLC**	91±0	23	**C**
1_2Z_1	DHA/SDX/**PYR**:60/500/**25**	90-100 ± 5	23-25	88-98 ± 4	22-25	***BLC**	98±0	25	**C**
1_4Z_1	DHA/SDX/**PYR**:60/500/**25**	95-105 ± 5	24-26	95-105 ± 5	24-26	**C**	106±0	27	**C**
2_1Z_1	DHA/SDX/**PYR**:60/500/**25**	89-99 ± 4	22-25	88-98 ± 5	22-25	***BLC**	105±0	26	**C**
2_3Z_1	DHA/SDX/**PYR**:60/500/**25**	95-105 ± 5	24-26	90-100 ± 0	23-25	**C**	100±0	25	**C**
2_4Z_1	DHA/SDX/**PYR**:60/500/**25**	95-105 ± 5	24-26	90-100 ± 10	23-25	**C**	95±0	24	**C**
2_5Z_1	DHA/SDX/**PYR**:60/500/**25**	84-94 ± 5	21-24	90-100 ± 5	23-25	***BLC**	90±0	23	**C**
3_4Z_1	DHA/SDX/**PYR**:60/500/**25**	90-100 ± 0	23-25	92-102 ± 4	23-26	**C**	98±0	25	**C**
3_5Z_1	DHA/SDX/**PYR**:60/500/**25**	104-114± 5	26-29	100-110 ± 0	25-28	***BLC**	98±0	25	**C**
4_5Z_1	DHA/SDX/**PYR**:60/500/**25**	97-107 ± 5	24-27	100-110 ± 0	25-28	**C**	103±0.	26	**C**
4_6Z_1	DHA/SDX/**PYR**:60/500/**25**	94-104 ± 5	24-26	94-104 ± 5	24-26	**C**	99±0	25	**C**
4_4Y_{12}	ATS/SM/**PYR**:100/250/**12.5**	104-114± 5	13-14	107-117± 5	13-15	**NC overdose**	119±0	15	**NC overdose**
4_2Y_{12}	ATS/SM/**PYR**:200/500/**25**	95-105 ± 5	24-26	107-117± 5	27-29	**BLC**	116±0	29	**NC overdose**
4_3Y_{12}	ATS/SM/**PYR**:100/250/**12.5**	115-125 ± 5	14-16	105-115 ± 10	13-14	**NC overdose**	113±0	14	**NC overdose**
4_1Y_{12}	ATS/SM/**PYR**:200/500/**25**	90-100 ± 5	23-25	85-95 ± 5	21-24	***BLC**	101.73±0	25	**C**
3_1Y_{12}	ATS/SM/**PYR**:100/250/**12.5**	99-109 ± 4	12-14	97-107 ± 5	12-13	**C**	110±0	14	**C**

CODE: $ZONE_{Sample}API_{Manufacturer}$ = Fixed dose combination

* $ZONE_{Sample}API_{Manufacturer}$ = Single dose of Ats + single dose of SP on the same blister

*BLC= Samples that changed to C with HPLC analysis.

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Table 38: Percentage (%) and Mass (mg) quantities of results of quinine active pharmaceutical ingredient (API) by TLC and HPLC methods and their comparisons with the manufacturer's claim and pharmacopoeial requirements. Quinine tablets must contain at least 90.0% and at most 110.0% of the labelled amount of Quinine on the pack

Code	**Manufacturer's Label Claim**	**Semi-quantitative TLC estimation of composition in % and mg quantities of dosage forms of quinine compared to the manufacturer's label claim n = 6 for each solvent system, total n = 12)**				**Remarks based on TLC results**	**HPLC determination of composition of quinine dosage forms in % and mg quantities (n = 6)**		**Remarks based on HPLC results**
		Solvent system 1 Methanol: ammonia 100:1.5		**Solvent system 2** Ethyl acetate: acetic acid: water 60:20:20					
		% range ±rsd	Quantity (mg)	% range ±rsd	Quantity (mg)		% ±rsd	Quantity (mg)	
1_1V_5	**QUN:50mg/5ml**	102-112 ± 4	51-56	102-112 ± 4	51-56	***BLC**	109.7 ± 0.3	55	**C**
1_2V_5	**QUN:50mg/5ml**	110-120 ± 5	55-60	103-113 ± 10	52-57	**NC overdose**	120 ± 1	60	**NC overdose**
1_3V_5	**QUN:50mg/5ml**	104-114 ± 5	52-57	104-114 ± 5	52-57	**BLC**	112 ± 1	56	**NC overdose**
4_1Q_6	**QUN:50mg/5ml**	-	-	-	-	**-**	102 ± 4	51	**C**
4_2Q_6	**QUN:50mg/5ml**	-	-	-	-	**-**	56 ± 7	28	**NC**
4_3Q_6	**QUN:50mg/5ml**	-	-	-	-	**-**	97 ± 4	49	**C**
$4V_5$	**QUN:50mg/5ml**	107-117± 5	54-59	107-117± 5	54-59	**NC overdose**	112 ± 2	56	**BLC**
4_1R_8	**QUN:100mg/5ml**	130-140 ± 10	130-140	135-145 ± 5	135-145	**NC overdose**	291 ± 1	291	**NC overdose**
4_2R_4	**QUN:100mg/5ml**	125-135 ± 5	125-135	125-135 ± 5	125-135	**NC overdose**	151 ± 1	151	**NC overdose**
4_3R_4	**QUN:100mg/5ml**	117-127± 5	117-127	120-130 ± 0	120-130	**NC overdose**	156 ± 2	156	**NC overdose**

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3_1Q_6	**QUN:150mg**	-	-	-	-	**-**	110.4 ± 0.1	166	**C**
3_2Q_6	**QUN:150mg**	-	-	-	-	**-**	110.5 ± 0.1	166	**BLC**
3_3Q_6	**QUN:150mg**	-	-	-	-	**-**	122.1 ± 0.8	183	**NC overdose**

CODE: $ZONE_{Sample}API_{Manufacturer}$ = Fixed dose combination

$_*$ $ZONE_{Sample}API_{Manufacturer}$ = Single dose of Ats + single dose of SP on the same blister

*BLC= Samples that changed to C with HPLC analysis.

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APPENDIX III

Table 39: Samples with the same batch number and their respective masses (mg)

Batch No.	Samples with the same batch number and their masses						
S-60	1_4P_{10}	1_6P_{10}	1_1P_{10}	3_1P_{10}	3_2P_{10}	3_3P_{10}	
	85mg/110mg	83mg/92mg	78mg/92mg	83mg/83mg	81mg/94mg	82mg/87mg	
10011	1_1P_2	1_4P_2	2_1P_2	3_5P_2			
	87mg/114mg	82mg/93mg	77mg/89mg	53mg/48mg			
10008	3_1P_2	4_4P_2	1_2P_2				
	97mg/97mg	85mg/102mg	67mg/76mg				
OK 159	1_2V_5	$4V_5$					
	120mg	112mg					
LD-266	$1_{12}X_1$	1_5X_1	1_7X_1				
	101mg/103mg	67mg/111mg	81mg/59mg				
LD-259	1_9X_1	$1_{13}X_1$	$1_{10}X_1$	2_2X_1	4_4X_1		
	103mg/114mg	140mg/110mg	124mg/63mg	131mg/63mg	143mg/104mg		
LF-249	1_4X_1	1_6X_1	3_3X_1	4_3X_1			
	69mg/115mg	163mg/127mg	81mg/108mg	174mg/82mg			

LN-450	1_8X_1	2_1X_1					
	163mg/79mg	97mg/113mg					
LS-39	1_2X_1	3_1X_1	3_2X_1				
	120mg/113mg	92mg/117mg	97mg/117mg				
SB0100I	1_2X_{11}	1_1X_{11}					
	103mg/123mg	93mg/127mg					
C0520J	3_1X_{11}	1_3X_{11}					
	39mg/119mg	40mg/85mg					
1105062	1_2X_{14}	1_1X_{14}	2_4X_{14}				
	35mg/95mg	75mg/69mg	53mg/118mg				
PX-161	1_7Z_1	1_6Z_1	2_6Z_1				
	85mg/-	98mg/-	103mg/-				
AP-18	1_5Z_1	1_3Z_1	1_2Z_1	2_5Z_1	2_1Z_1	3_4Z_1	4_5Z_1
	51mg/86mg/94mg	57mg/86mg/91mg	53mg/83mg/98mg	51mg/87mg/90mg	56mg/77mg/105mg	51mg/85mg/98mg	(52/88/105)mg
110123	1_4Z_3	1_2Z_3	1_1Z_3	3_3Z_3	4_4Z_3	4_3Z_3	4_1Z_3
	70mg/-	86mg/-	99mg/-	71mg/-	88mg/-	71mg/-	74mg/-
AP-16	2_4Z_1	2_3Z_1	3_5Z_1				

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	53mg/80mg/95mg	54mg/90mg/100mg	52mg/85mg/98mg				
10012	2_2P_2	2_3P_2	3_2P_2	4_2P_2	4_5P_2		
	83mg/64mg	96mg/122mg	85mg/96mg	47mg/51mg	85mg/108mg		
021350	2_1P_{15}	3_7P_{15}	3_8P_{15}				
	82mg/93mg	71mg/78mg	91mg/103mg				
LD-227	3_4X_1	3_6X_1					
	102mg/112mg	105mg/103mg					
081	3_1Y_{12}	4_1Y_{12}	4_2Y_{12}				
	89mg/87mg/110mg	87mg/88mg/102mg	93mg/89mg/116mg				
TR0458	3_2Y_{13}	3_4Y_{13}					
	98mg/68mg/78mg	98mg/74mg/70mg					
L-491	3_1Q_6	3_3Q_6	4_1Q_6	4_2Q_6			
	166mg	183mg	102mg	56mg			
110433	4_2R_4	4_3R_4					
	151mg	156mg					
079	4_3Y_{12}	4_4Y_{12}					
	78mg/87mg/111mg	90mg/90mg/119mg					

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APPENDIX IV

SAMPLES OF TLC PLATES

ARTESUNATE SAMPLE

Ethanol: Toluene: Ammonia

(70:30:1.5)

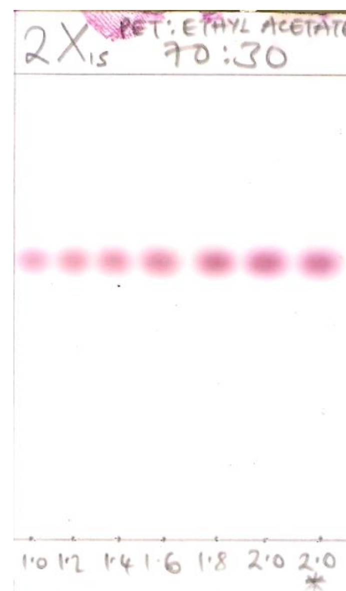
Ethanol: Ammonia

(100:0.5)

ARTEMETHER SAMPLE

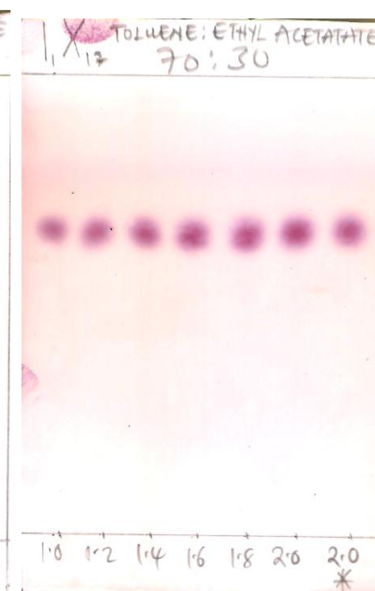
Pet. Ether: Ethyl Acetate

(70:30)



Toluene: Ethyl acetate

(70:30)



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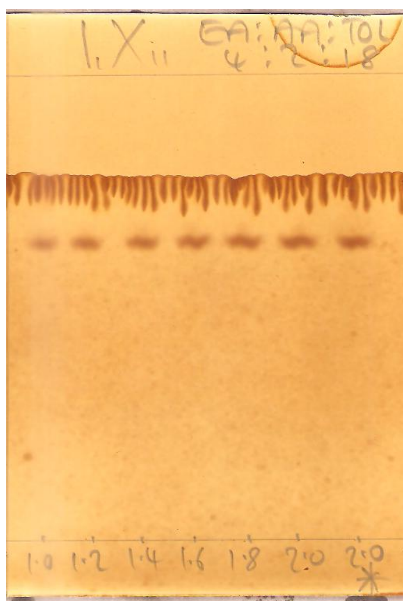
LUMEFANTRINE SAMPLE

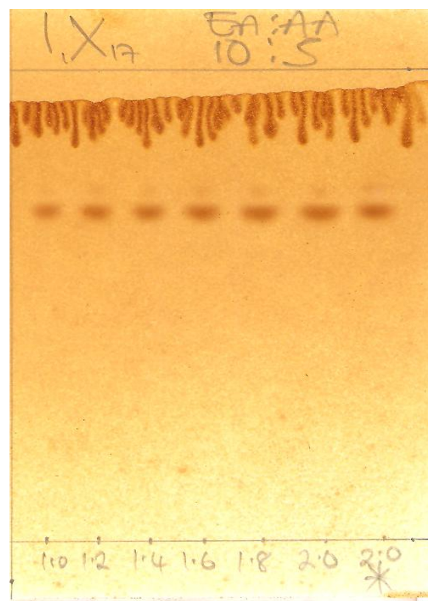
Ethyl Acetate: Acetic acid:Toluene

(4:2:18)



Ethyl acetate: Acetic acid

(10:5)



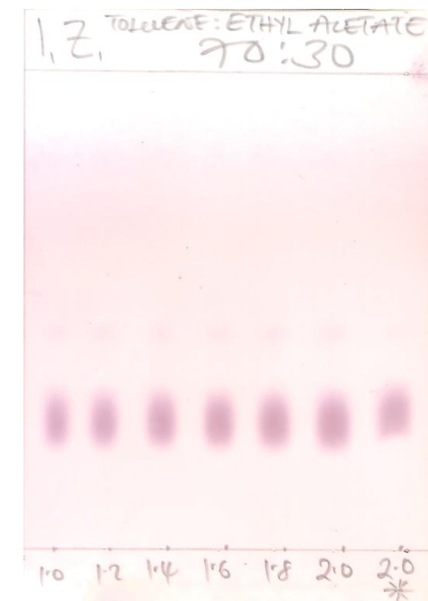
DIHYDROARTEMISININ SAMPLE

Toluene: Ethyl acetate

(60:40)

Toluene: Ethyl acetate

(70:30)



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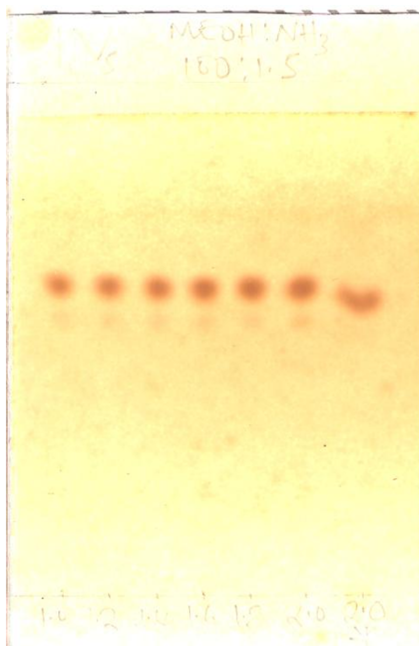
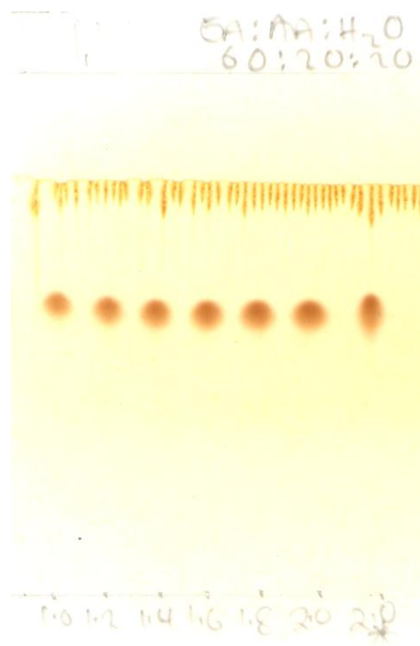
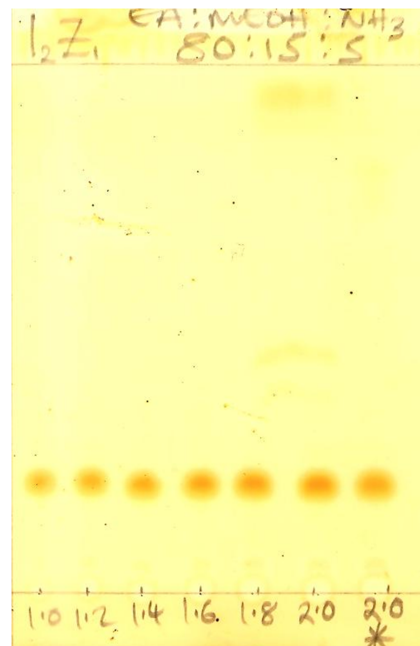
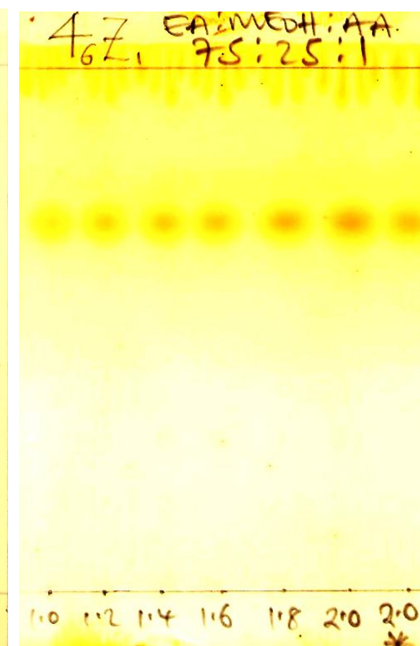
QUININE SAMPLE		**SULPHADOXINE SAMPLE**	
Methanol: Ammonia	Ethyl acetate: Acetic acid: Water	Ethyl acetate: Methanol: Ammonia	Ethyl acetate: Methanol: Acetic acid
(100:1.5)	(60:20:20)	(80:15:5)	(75:25:1)









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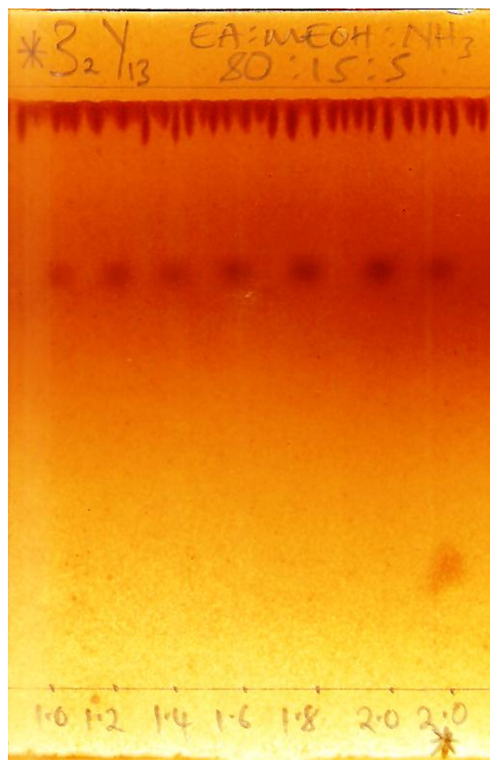
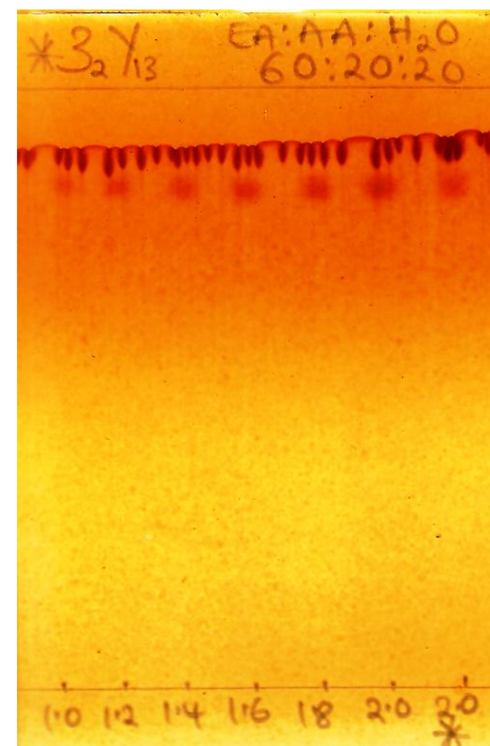
PYRIMETHAMINE

Ethyl acetate: Methanol: Ammonia (80:15:5)



Ethyl acetate: Acetic acid: Water (60:20:20)



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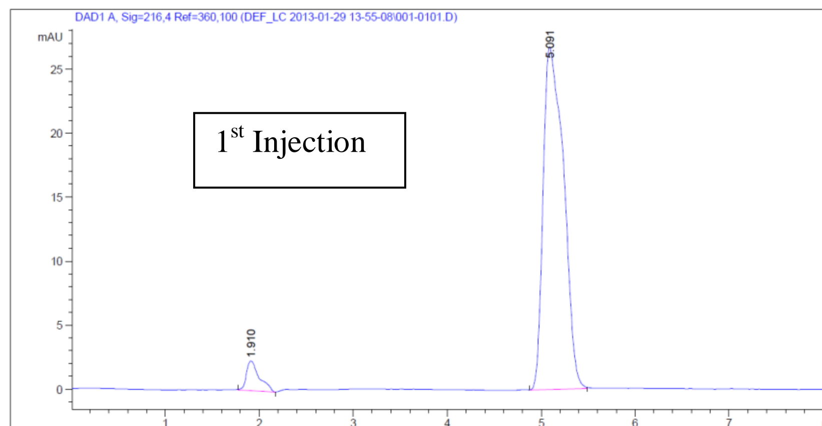
APPENDIX IV

SAMPLES OF API HPLC CHROMATOGRAMS

Samples of chromatograms of six replicate injections of a 0.7mg/mL test artesunate RS preparation.

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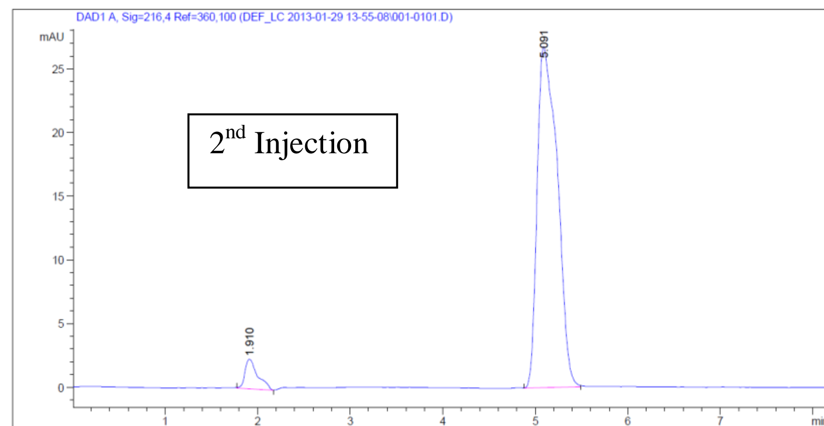
====================================================================
                       Area Percent Report
====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.910 BB    0.1418   22.23658    2.32120   5.2239
   2   5.091 BB    0.2100  403.42953   26.74580  94.7761

Totals :                   425.66611   29.06700
```

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====================================================================
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Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   1.910 BB    0.1418   22.36358    2.32120   5.2239
   2   5.091 BB    0.2100  403.36903   26.74580  94.7761

Totals :                   425.73261   29.06700
```

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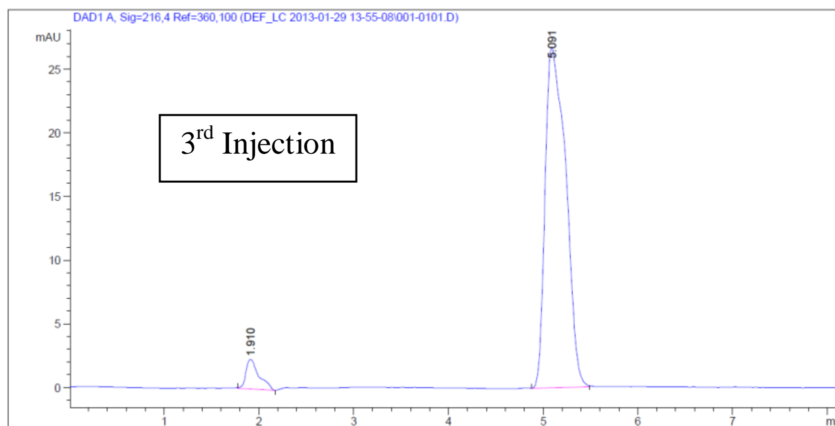

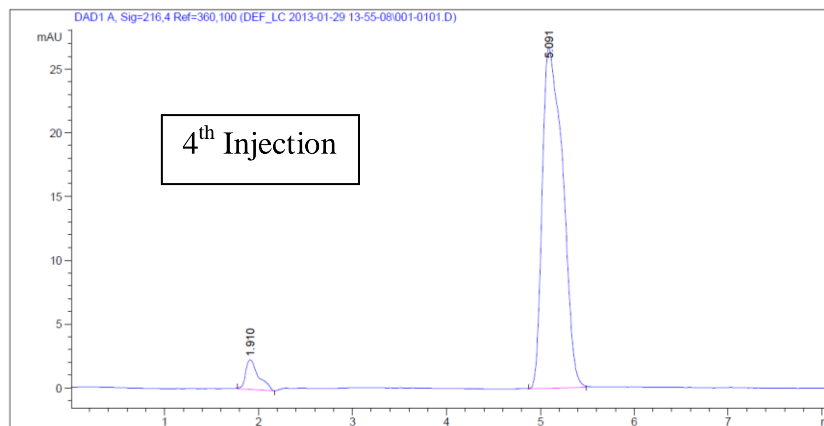


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Sorted By              :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   1.910 BB    0.1418   22.23658    2.32120   5.2239
   2   5.091 BB    0.2100  402.95253   26.74580  94.7761

Totals :                   424.18911   29.06700
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=====================================================================
                         Area Percent Report
=====================================================================

Sorted By              :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   1.910 BB    0.1418   22.35218    2.32120   5.2239
   2   5.091 BB    0.2100  403.53258   26.74580  94.7761

Totals :                   425.88476   29.06700
```

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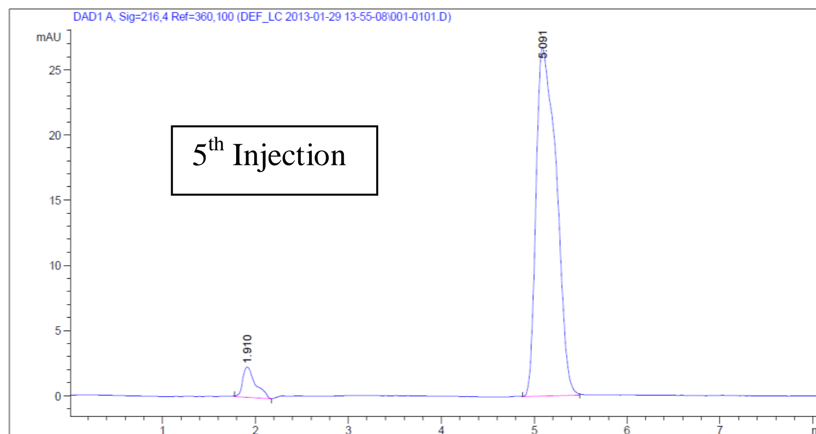


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=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   1.910 BB    0.1418   22.23658    2.32120   5.2239
   2   5.091 BB    0.2100  403.52453   26.74580  94.7761

Totals :                   425.76111   29.06700
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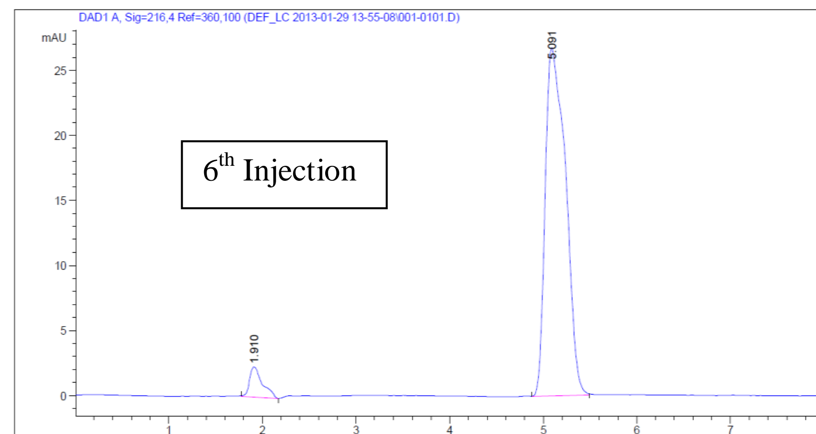


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                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   1.910 BB    0.1418   22.23658    2.32120   5.2239
   2   5.091 BB    0.2100  403.53023   26.74580  94.7761

Totals :                   425.76681   29.06700
```

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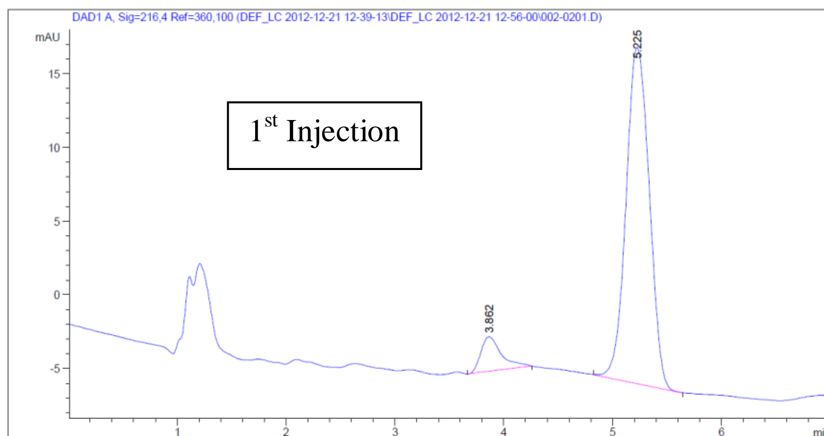
Samples of chromatograms of six replicate injections of a 0.7mg/mL test dihydroartemisinin RS preparation.

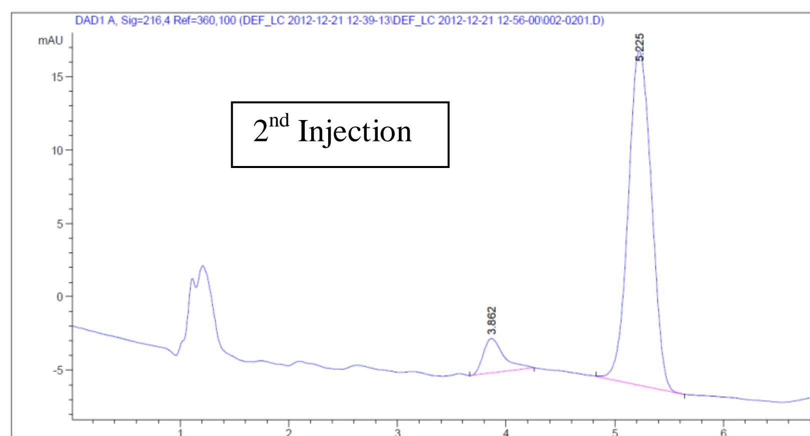


Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.862	MM R	0.2188	30.80354	2.34691	8.3391
2	5.225	MM R	0.2474	328.58453	22.81390	91.6609
Totals :				359.38808	25.16080	



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.862	MM R	0.2188	30.80354	2.34691	8.3391
2	5.225	MM R	0.2474	328.58020	22.81390	91.6609
Totals :				359.38374	25.16080	

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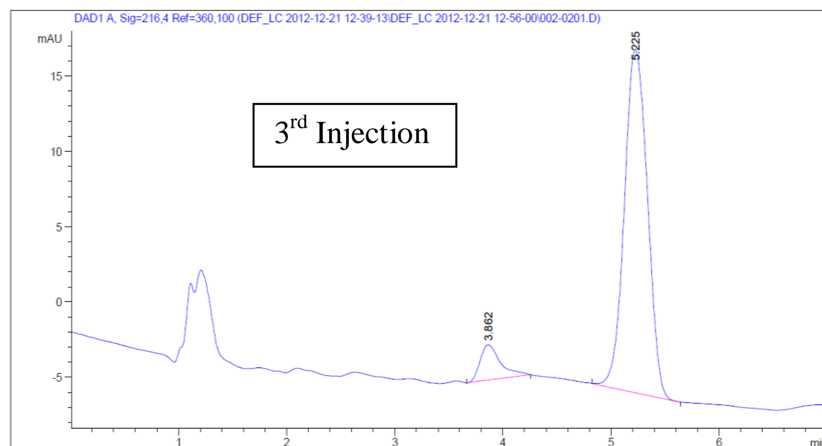


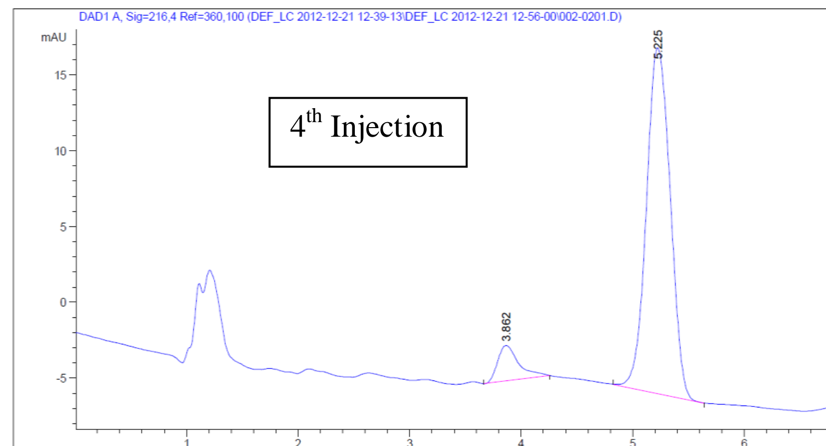
Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.862	MM R	0.2188	30.80354	2.34691	8.3391
2	5.225	MM R	0.2474	326.58111	22.81390	91.6609

Totals : 356.38465 25.16080



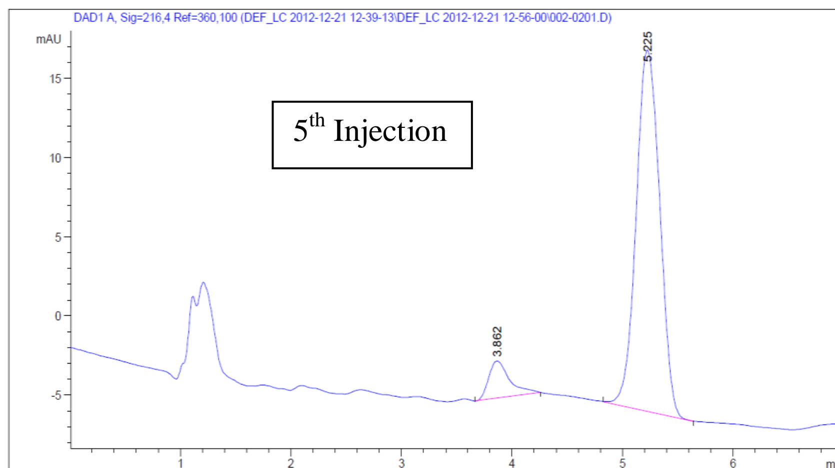
Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.862	MM R	0.2188	30.80354	2.34691	8.3391
2	5.225	MM R	0.2474	329.55553	22.81390	91.6609

Totals : 360.35908 25.16080

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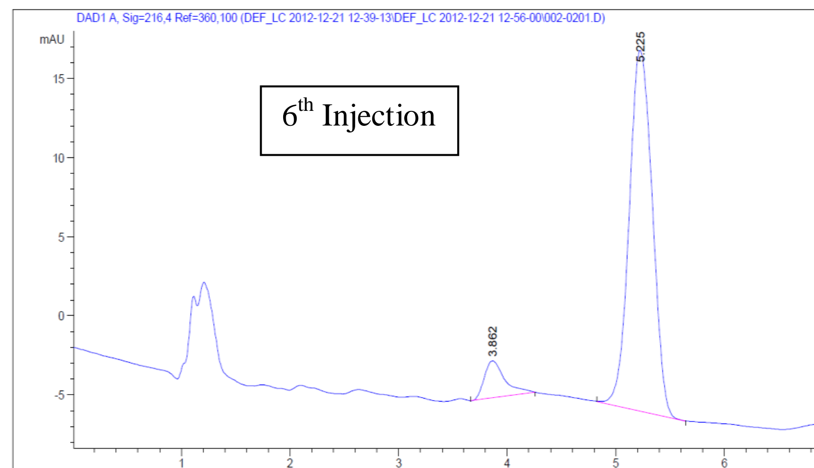
=====================================================================
                       Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   3.862 MM R  0.2188   30.80354    2.34691   8.3391
   2   5.225 MM R  0.2474  329.08003   22.81390  91.6609

Totals :                   359.88357   25.16080
```

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=====================================================================
                       Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   3.862 MM R  0.2188   30.80354    2.34691   8.3391
   2   5.225 MM R  0.2474  325.58123   22.81390  91.6609

Totals :                   356.38377   25.16080
```
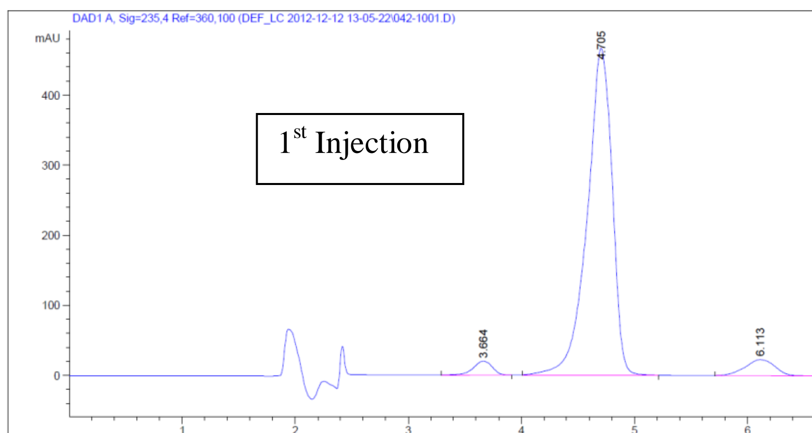
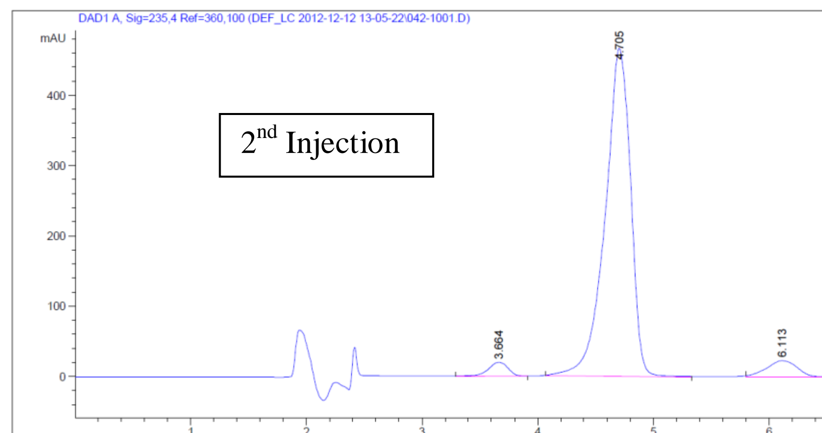

Samples of chromatograms of six replicate injections of a 0.4mg/mL test quinine RS preparation.




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=235,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.664 MM R  0.1971  235.85081   19.94301   3.0067
   2   4.705 BB    0.2334 7183.37305  467.06012  91.5746
   3   6.113 BB    0.2861  425.05826   23.15780   5.4187

Totals :                  7844.28212  510.16093
```




```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=235,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.664 MM R  0.1971  235.85081   19.94301   3.0071
   2   4.705 MM R  0.2561 7177.96924  467.15921  91.5190
   3   6.113 BB    0.2864  429.33032   23.36065   5.4740

Totals :                  7843.15038  510.46287
```

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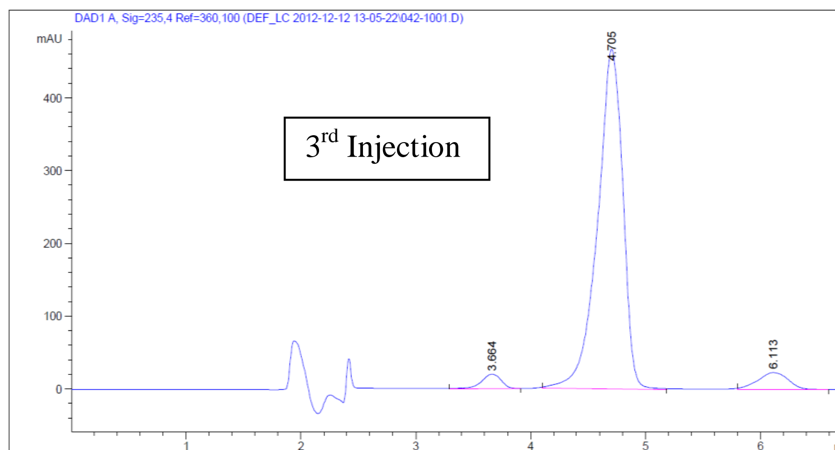
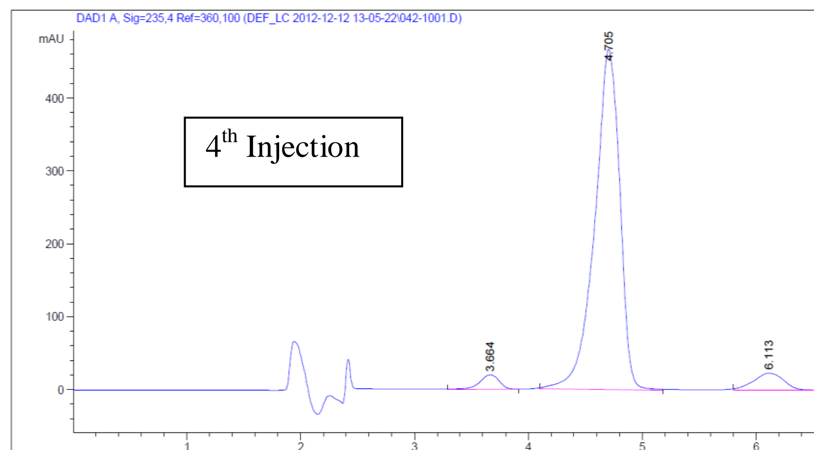
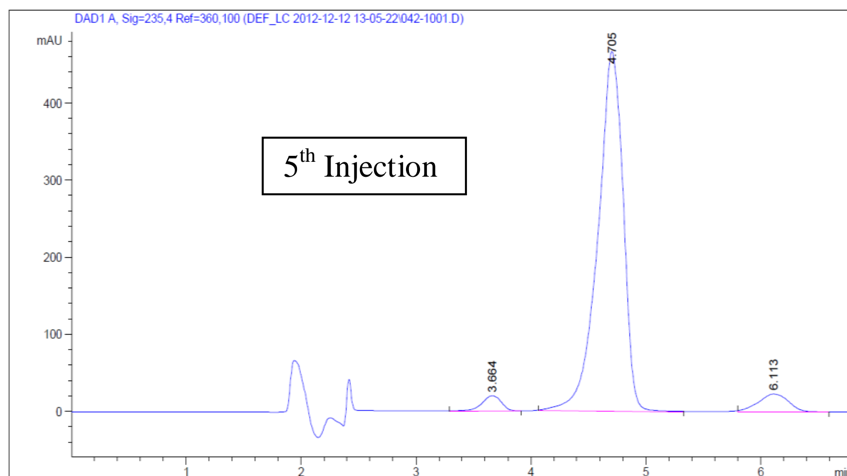


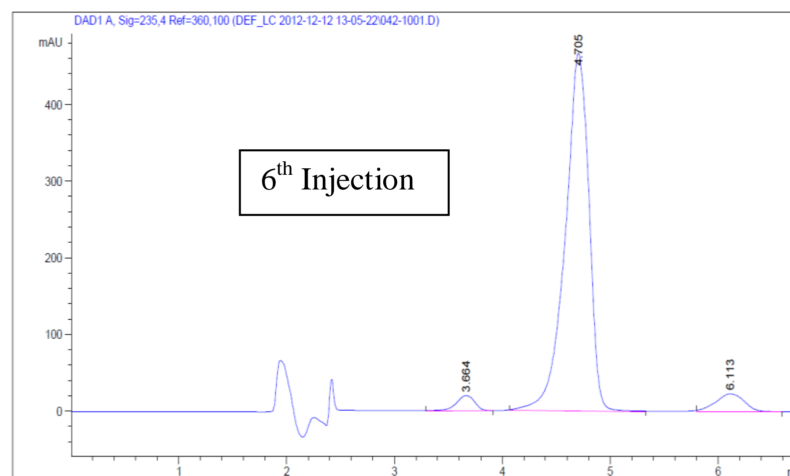

```
==================================================================
                       Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=235,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1   3.664 MM R  0.1971  235.85081   19.94301   3.0091
   2   4.705 MM R  0.2558 7172.83887  467.26971  91.5134
   3   6.113 BB    0.2864  429.33032   23.36065   5.4775

Totals :                  7838.02000  510.57338
```




```
==================================================================
                       Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=235,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1   3.664 MM R  0.1971  235.85081   19.94301   3.0091
   2   4.705 MM R  0.2558 7172.83887  467.26971  91.5134
   3   6.113 BB    0.2864  429.33032   23.36065   5.4775

Totals :                  7838.02000  510.57338
```

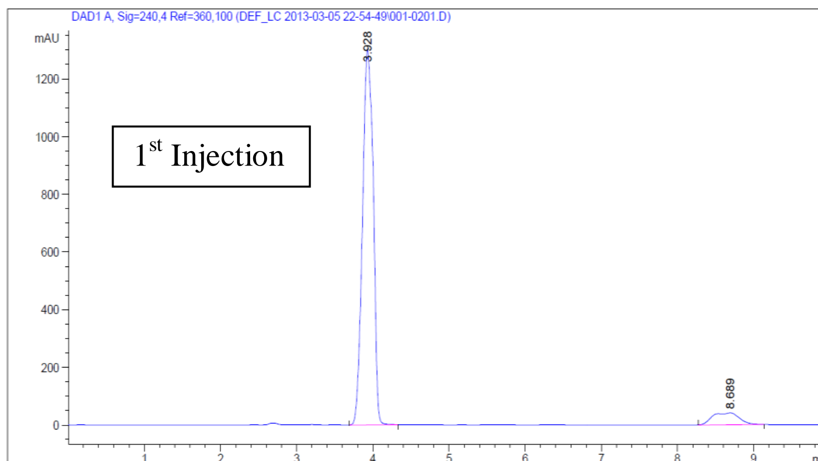



```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :       1.0000
Dilution:                     :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=235,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   3.664 MM R  0.1971  235.85081   19.94301   3.0071
   2   4.705 MM R  0.2561 7177.96924  467.15921  91.5190
   3   6.113 BB    0.2864  429.33032   23.36065   5.4740

Totals :                  7843.15038  510.46287
```

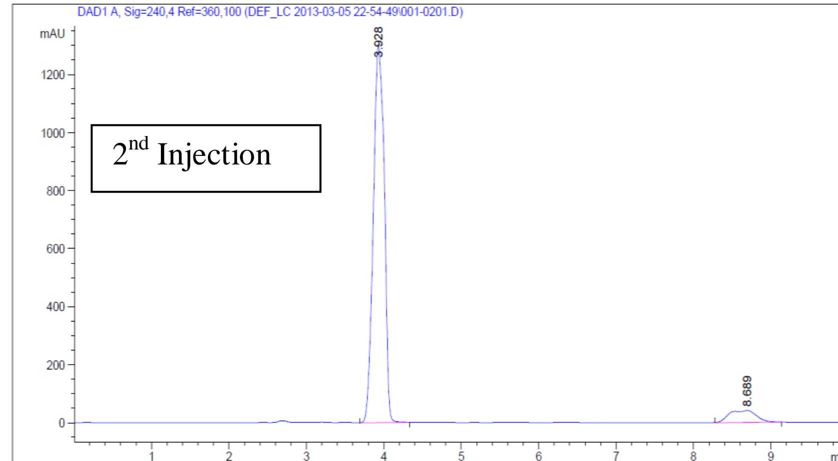



```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :       1.0000
Dilution:                     :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=235,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   3.664 MM R  0.1971  235.85081   19.94301   3.0071
   2   4.705 MM R  0.2561 7177.96924  467.15921  91.5190
   3   6.113 BB    0.2864  429.33032   23.36065   5.4740

Totals :                  7843.15038  510.46287
```

Samples of chromatograms of six replicate injections of a 0.8mg/mL and 0.04mg/mL test sulphadoxine and pyrimethamine RS solution respectively




```
======================================================================
                         Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=240,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   3.928 BB    0.1381 1.87275e4  1301.75745  92.5548
   2   8.689 MM R  0.4133 1623.81958   41.28152   7.4452

Totals :                  2.37514e4  1343.03897
```




```
======================================================================
                         Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=240,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   3.928 BB    0.1381 1.87654e4  1301.75745  92.5548
   2   8.689 MM R  0.4133 1623.81365  41.28152   7.4452

Totals :                  2.37892e4  1343.03897
```

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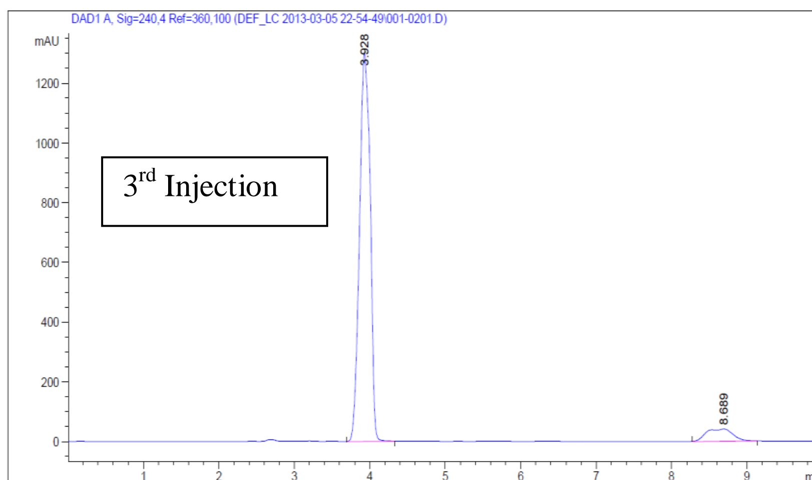
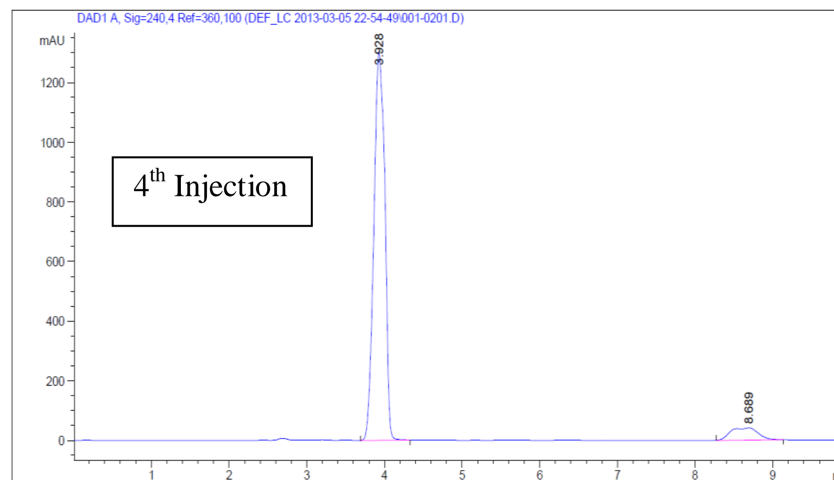




3rd Injection — Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=240,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.928	BB	0.1381	1.87564e4	1301.75745	92.5548
2	8.689	MM R	0.4133	1623.81875	41.28152	7.4452

Totals : 2.37802e4 1343.03897

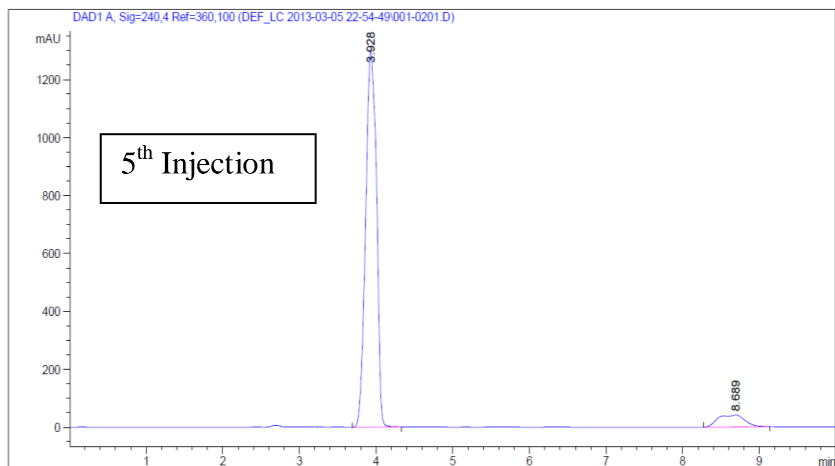
4th Injection — Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=240,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.928	BB	0.1381	1.87526e4	1301.75745	92.5548
2	8.689	MM R	0.4133	1623.81658	41.28152	7.4452

Totals : 2.37764e4 1343.03897



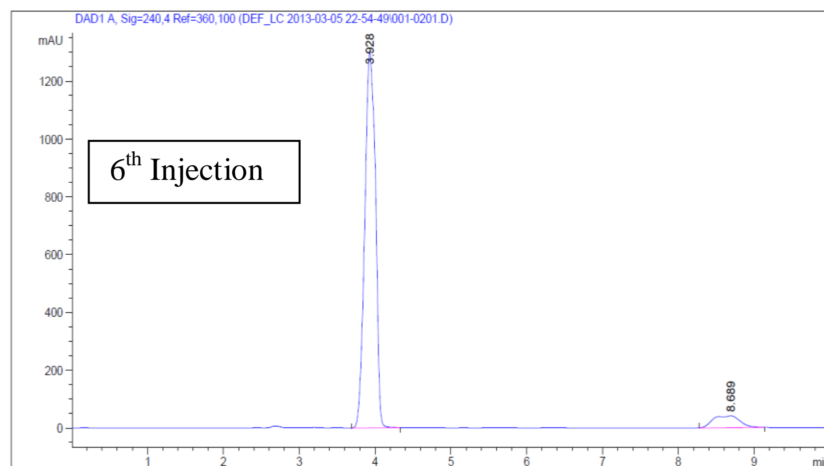
Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=240,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.928	BB	0.1381	1.87852e4	1301.75745	92.5548
2	8.689	MM R	0.4133	1623.81875	41.28152	7.4452

Totals : 2.38090e4 1343.03897



Area Percent Report

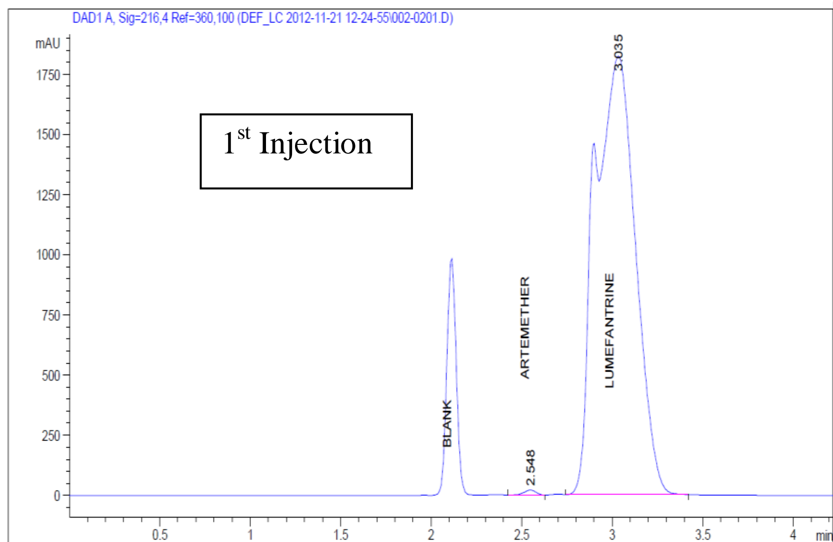
Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

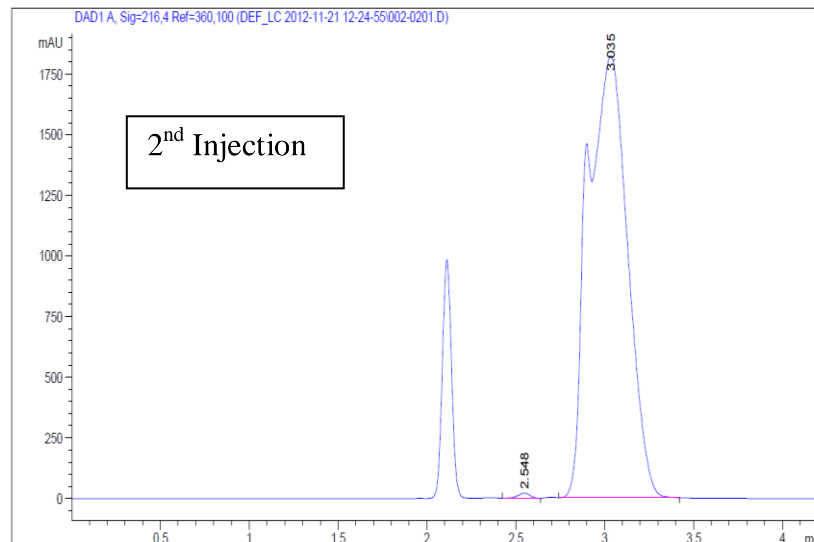
Signal 1: DAD1 A, Sig=240,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.928	BB	0.1381	1.87198e4	1301.75745	92.5548
2	8.689	MM R	0.4133	1623.81687	41.28152	7.4452

Totals : 2.37436e4 1343.03897

Samples of chromatograms of six replicate injections of a 0.3mg/mL and 1.7mg/mL test artemether and lumefantrine RS solution respectively.






```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                    :       1.0000
Dilution:                      :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   2.548 VV    0.0748  121.01455   22.20495   0.3797
   2   3.035 MM R  0.2591 2.83447e4  1823.05896  99.6203

Totals :                  2.84447e4  1845.26391
```

```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                    :       1.0000
Dilution:                      :       1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   2.548 MM R  0.0745  120.46783   21.14743   0.3323
   2   3.035 MM R  0.2591 2.83367e4  1823.05896  99.6677

Totals :                  2.84311e4  1844.20639
```

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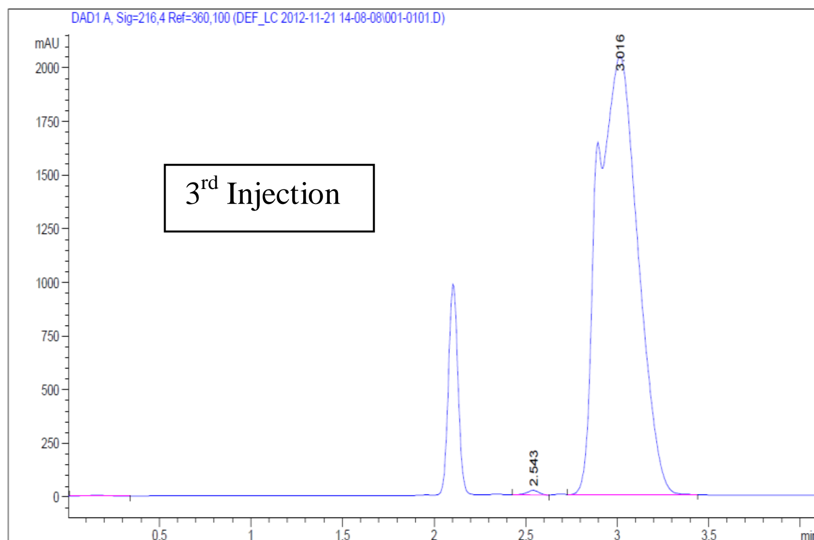


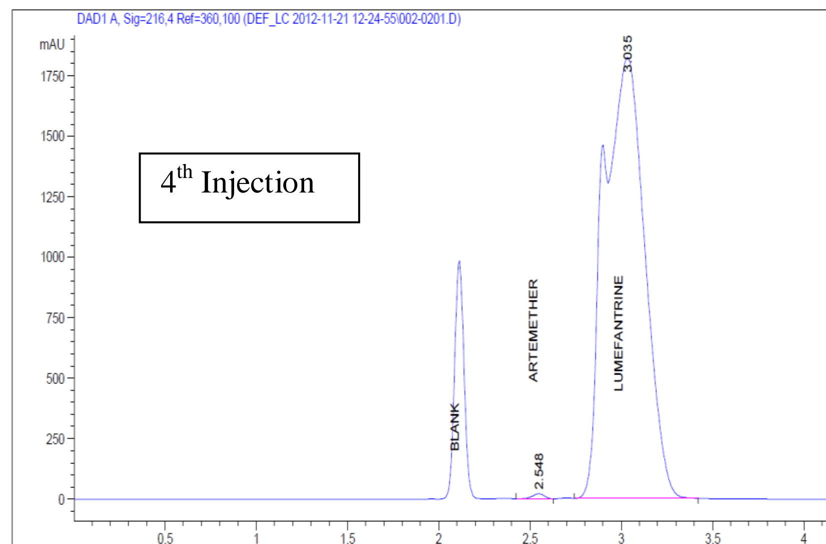

```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                    :      1.0000
Dilution:                      :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   2.543 MM R  0.0757   130.23293   21.42156   0.3115
   2   3.016 MM R  0.2536 2.80925e4  2043.54211  99.6187

Totals :                   2.81897e4  2068.38628
```




```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                    :      1.0000
Dilution:                      :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   2.548 VV    0.0748   122.54055   22.20495   0.3797
   2   3.035 MM R  0.2591 2.83147e4  1823.05896  99.6203

Totals :                   2.84232e4  1845.26391
```

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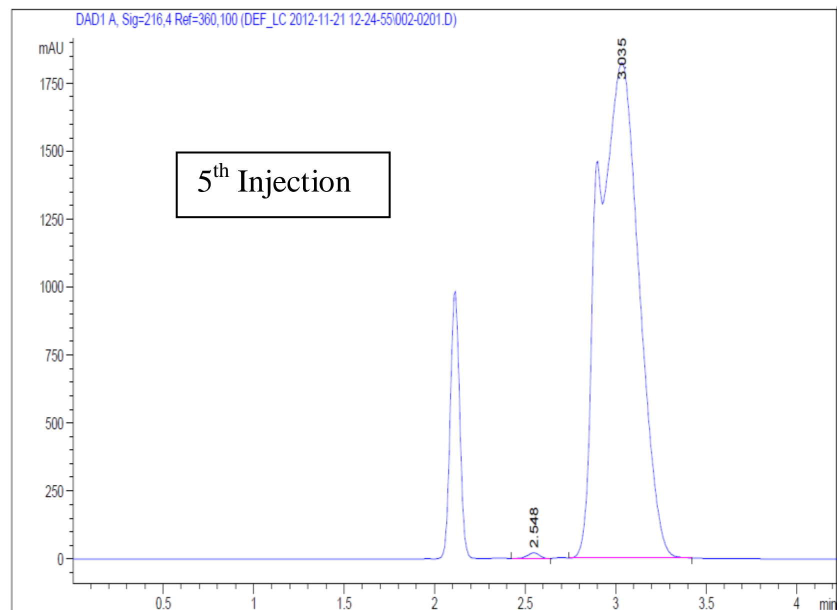


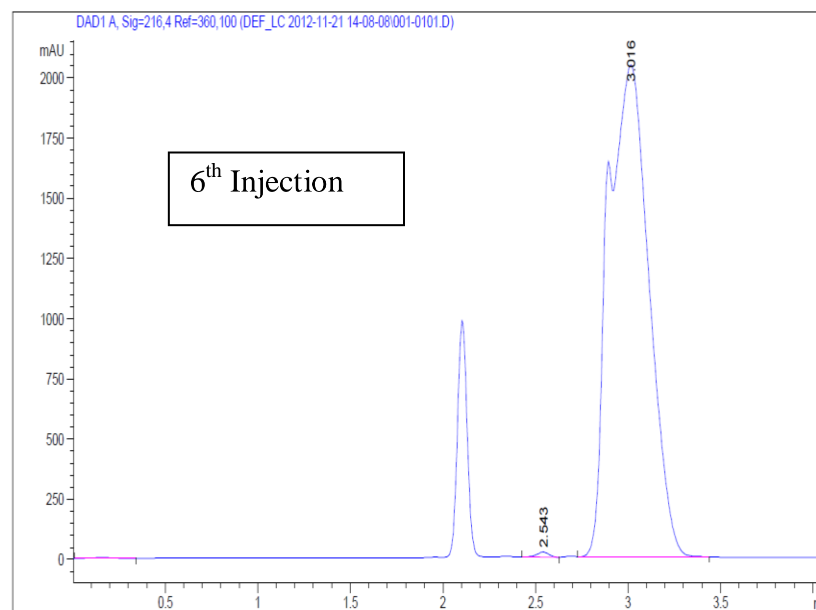

```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   2.548 MM R  0.0745   120.46701   21.14743   0.3323
   2   3.035 MM R  0.2591 2.82967e4  1823.05896  99.6677

Totals :                  2.83952e4  1844.20639
```




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=216,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   2.543 MM R  0.0757   120.23201   21.42156   0.3115
   2   3.016 MM R  0.2536 2.80895e4  2043.54211  99.6187

Totals :                  2.81877e4  2068.38628
```