COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY AND PHARMACOGNOSY

Quality Assessment of Common Antimalarials Marketed in Gambella National Regional State, South Western-Ethiopia.

A Thesis Submitted to the Department of Pharmaceutical Chemistry and Pharmacognosy in Partial Fulfillment of the Masters of Science (M.Sc.) Degree Requirements in Pharmaceutical Analysis and Quality Assurance.

By: Feruza Ahmed (B. Pharm)

June, 2023
Addis Ababa, Ethiopia

Addis Ababa University

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This is to certify that the thesis prepared by Feruza Ahmed, entitled "Quality Assessment of Common Antimalarials Marketed in Gambella National Regional State, South Western-Ethiopia." submitted to the Department of Pharmaceutical Chemistry and Pharmacognosy Presented in Partial Fulfillment of the Masters of Science (M.Sc.) Degree Requirements in Pharmaceutical Analysis and Quality Assurance complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

By: Feruza Ahmed (B. Pharm)

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Abstract

Background: Over the past years, there has been a growing alarm that a considerable amount of antimalarial supply in the underdeveloped world particularly in the private sector, is of poor quality. According to a WHO research, poor quality antimalarials are common in malaria-endemic locations, with 1 in 10 drugs allegedly being either substandard or falsified and one-third of antimalarial drugs from these regions failing chemical content examination.

Objective: This study intends to assess the quality of the most widely used antimalarial medications [artemether-lumefantrine tablets, chloroquine tablets, primaquine tablets, artesunate, and artemether injections] in Gambella, South-West, Ethiopia.

Methods: A total of 52 samples were collected on July, 2022 from Gambella national regional state. Six among the twelve districts (woredas) located in the four zones of the region were selected by simple random technique. All drug retail outlets available in the selected woredas were included in the study. All products were subjected to visual inspection with a tool adopted from the WHO/FIP and USP checklists. The pharmacopeial tests for identification, uniformity of dosage forms, assay, thickness, diameter, hardness, friability and disintegration and dissolution tests were carried out according to the USP 44-NF 39, 2023 and International pharmacopoeia, 2020 protocols.

Result and Discussion: Only 25% of the samples were registered on the EFDA's electronic registration system. This results the likelihood of exposing the local population to potentially dangerous medical items of varying efficacy leading to an increased incidence of adverse drug reactions. 88.8% of artemether injection products were presented in clear glass ampoules, which might expose the products to photochemical degradation and result in loss of anti-plasmodial activity. 50% of the artemether brand products were bioequivalent with the comparator product.

Conclusion: The study findings reveal a high prevalence (58.3%) of substandard antimalarial drugs in the region.

Key words: malaria**,** antimalarials, poor quality, quality assessment, Gambella

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Table of Contents

List of Figures Page

List of Tables Page

Abbreviation and Acronyms

ACT	Artemisinin based Combination Treatments
AL	Artemether Lumefantrine
API	Active Pharmaceutical Ingredient
AV	Acceptable Value
ARTM	Artemether
BP	British Pharmacopoeia
CQ	Chloroquine
EPHARM	Ethiopian Pharmaceuticals Manufacturin Share Company
GMP	Good Manufacturing Practice
GPHF	The Global Pharma Health Fund
HPLC	High Performance Liquid Chromatography
IP	International Pharmacopoeia
L	Label Claim
LC	Liquid Chromatography
LUM	Lumefantrine
MQCL	Medicine Quality Control Laboratory
N	Newton
NLT	Not Less Than
NMT	Not More Than
PAD	Paper Analytical Device
Ph. Eur	European Pharmacopoeia
PQA	Poor Quality Antimalarials
RS	Reference Standard
RSD	Relative Standard Deviation
SD	Standard Deviation
SF	Substandard and/or Falsified
UHC	Universal Health Coverage
USP	United States Pharmacopoeia
WHO	World Health Organization

1. Introduction

1.1. Background

Malaria remains a major public health problem worldwide (Dembele *et al.*, 2022). It is caused by *plasmodium* parasites, which are transmitted to people through the bites of female *Anopheles mosquitoes* as a vector. Although there are multiple *Plasmodium* species, *P. falciparum* predominates in Sub-Saharan Africa (WHO, 2020a).

The WHO claims malaria is endemic in 84 countries. These nations' health care systems frequently lack adequate treatments, and poverty is widespread (WHO, 2022a). The lack of availability of antimalarial medication therapy and the escalating resistance of mosquitoes and malaria parasites to insecticides and artemisinin remains to pause significant concerns in malaria control and eradication (Walker *et al.*, 2018).

The access to safe, effective, high-quality, and affordable medicines is vital for the success of equitable health outcomes (Walker *et al.*, 2018). On the contrary, drugs that are substandard or falsified have a negative impact on consumers by causing treatment failure, prolonged or severe illness or even death (Al-Worafi, 2020). In addition, pharmaceutical industries, drug regulatory agencies, and economies worldwide will be affected by poor quality antimalarials (PQAs) (Al-Worafi, 2020). This puts further strain on already scarce resources at the provider level and erodes trust in healthcare professionals (Lalani *et al.*, 2015). According to the WHO research, PQAs are common in malaria-endemic locations, with 1 in 10 drugs allegedly being either substandard or falsified and one-third of antimalarial drugs from these regions failing chemical content examination (WHO, 2017a).

Lethal outcomes and severe illness can be avoided by receiving an early diagnosis and effective antimalarial treatment (Vugt *et al.*, 2011). Therefore, it is essential that the antimalarial medications supplied are of good quality (Newton *et al.*, 2011).

Different methods of analysis have been used to assess the quality of antimalarials involving the official and non-official methods of analysis. Visual & physical inspection (Mufusama *et al.*, 2018: Kaur *et al.*, 2010), UV (Abdo-Rabbo *et al.*, 2005), HPLC (Kaur *et al.*, 2010: Yabré *et al.*, 2020), TLC (Mahano *et al.*, 2021), and GPHF minilab (Bate *et al.*, 2008: Visser *et al.*, 2015) are some of the techniques commonly employed to check the quality of antimalarial drugs.

1.2. Antimalarial Drugs

Drug therapy is one approach of combating malaria (Jhon, 2011). Antimalarial drugs are employed in the management and prevention of malaria infection. Most antimalarial drugs target the disease's erythrocytic stage, which is the stage of infection that causes symptoms of illness (Travassos and Laufer, 2023). The Ethiopian malaria diagnosis and treatment guideline includes quinine, chloroquine, primaquine, artesunate, artemether and lumefantrine as a treatment of choice depending on the parasitological confirmed diagnosis, severity, and additional issues with the patient, such as pregnancy (FMOH, 2017).

1.3. Classification of Antimalarials

The stage of the parasitic life cycle that an antimalarial medicine affect can help classify them into gametocides, tissue schizontocides, blood schizonticides, and sporontocides. Additionally, antimalarial medications can be grouped according to the chemical family they belong to, which also in turn establishes which stage of the life cycle they influence. According to pharmacological category and activity, the primary antimalarial medications examined for this study are given in Table 1 (O'Rourke, 2010).

Table 1. Classification of principal antimalarial drugs analyzed in this study

Antimalarial groups	Principal drugs	Activity
4-Aminoquinolines	Chloroquine	Rapid-acting blood schizonticides. Some gametocytocidal activity.
8-Aminoquinolines	Primaquine	Tissue schizonticide. Also, gametocytocidal activity and some activity at other stages of the parasite's life-cycle.
Artemisinin and its derivatives (Sesquiterpene lactones)	Artemether Artesunate	Blood schizonticide.
Dichlorobenzylidines	Lumefantrine	Blood schizonticide.

1.3.1. 4-Aminoquinolines

4-Aminoquinolines derivatives were the first class of compounds used for the successful malaria therapy and are also drugs of choice at the present time (Meshnick , 2001).The 4-aminoquinolines are the closest of the antimalarials that are based on the quinine structure. This group is replaced at the same position 4 as quinine and have an asymmetric carbon equivalent to quinine's C-9 position. The racemic combinations of 4-aminoquinoline are employed, and both isomers are active, just like quinine (Jhon, 2011).

1.3.1.1. Chloroquine

Chloroquine was the first aminoquinoline derivative medicine produced on a significant scale for the treatment and prevention of malaria infection (Derek, 2020). It is still the treatment of choice for non–*P. falciparum* and *P. falciparum* malaria in few locations where resistance has not been observed. Additionally, it is generally fast acting and well tolerated for infections with *P. vivax* and *P. ovale* (Kamya, 2020). This weak base accumulates in the parasite's digestive vacuole, a lysosomal compartment where hemoglobin from the host cell cytosol is broken down into its component peptides and heme by an endocytotic feeding process. It disrupts the process in the vacuole that turns potentially hazardous heme monomers into inert crystal hemozoin, leading monomeric heme with toxic ferriprotoporphyrin IX (FeII-PPIX) moiety to build up to amounts that are lethal to the parasite (Foley and Leann, 1998; Martin *et al.*, 2009).

P. falciparum, the cause of the deadliest variety of malaria is now chloroquine resistant (CQR) in nearly all malarious regions of the globe. The *P. falciparum* chloroquine resistance transporter (pfcrt-K76T) which is located in the food vacuole (FV) of CQR parasites mutation is likely to be the reason for an excessive amount of CQ being exported from its site of action in the parasite FV. Thus, in their digesting vacuole, CQ-resistant parasites have a significantly lower concentration (or CQ efflux occurs) (Martin *et al.*, 2009). However, as opposed to a drug export mechanism, other research linked this resistance to a reduced amount of accumulation. A changed vacuolar pH may be the cause of this reduced accumulation. Alternatively, the loss of an intracellular receptor can be the cause of the decreasing CQ concentration (Kouznetsov and Gómez-Barrio, 2009).

Chemistry of Chloroquine: Chloroquine, chemically referred to as a 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline is a dibasic compound (pKa 8.1 and 10.2) that enters malaria parasites by diffusion (Bray *et al.*, 2006 ; USP, 2023a). It has a molecular weight of 319.87 ***amu***

with a chemical formula of $C_{18}H_{26}ClN_3$. Figure 1 shows the chemical structure of chloroquine phosphate. It is a white, odorless, crystalline powder, which slowly discolors on exposure to light. It is freely soluble in water; practically insoluble in alcohol, in chloroform, and in ether and its solutions have a pH of about 4.5 (O'Rourke, 2010). It is classified as highly soluble and highly permeable (biopharmaceutical classification system (BCS) class (I) (Lindenberg *et al.*, 2004). It is quickly absorbed when taken orally, and the liver only partially metabolizes it before it is excreted in the urine. Since it is kept and trapped in lysosomes, the maximal effect is observed between one and two hours after consumption, and its terminal elimination half-life is between one and two months (Derek, 2020).

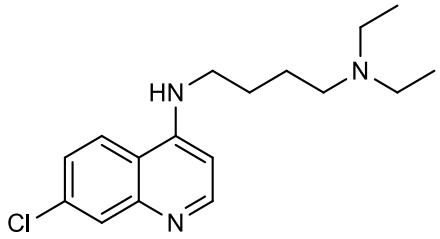


Figure 1.Structure of Chloroquine Phosphate

1.3.2. 8-Aminoquinolines

The first synthetic antimalarial medications to be introduced into medicine were 8-aminoquinolines (Aronson and Meyler, 2016).

1.3.2.1. Primaquine

It eradicates tissue (exoerythrocytic) infection. In that way, it prevents the growth of the blood (erythrocytic) phase of the parasite which are the reason for relapses in *vivax* malaria. The *P. falciparum* gametocytes are also responsive to primaquine phosphate. It marks a fundamental cure and averts relapse of *vivax* and *ovale* malaria. Primaquine is quickly absorbed from the digestive system. It is classified in the pharmacopeial standards as "soluble" in water (USP, 2023b). According to Mihaly *et al.*, (1985) the absolute bioavailability was well over 90 %, showing that the active pharmaceutical ingredient (API) was "very permeable." With a stated elimination half-life of 3 to 6 hours, peak plasma concentrations are reported to reach 1 to 2 hours after a dose is administered and then rapidly decline. It is dispersed broadly throughout body tissues. Primaquine is quickly metabolized in the liver into its main metabolite, carboxyprimaquine, and is hardly ever

eliminated unchanged in the urine. On repeated doses, carboxyprimaquine accumulates in the plasma (O'Rourke, 2010).

Chemistry of Primaquine Phosphate: Primaquine Phosphate having a chemical formula of $C_{15}H_{21}N_3O \cdot 2H_3PO_4$ (Figure 2) is also referred to as 8- [(4-Amino-1-methyl butyl) amino]-6-methoxyquinoline phosphate, a synthetic compound with potent antimalarial activity. It has a molecular mass of 455.3 ***amu*** (O'Rourke, 2010). It is an orange-red, odorless, and crystalline powder. It is soluble 1 in 15 parts of water; insoluble in chloroform and in ether (USP, 2023b). The pH of a 10 mg/mL solution revealed to be acidic with a pH range of 2.5–3.5 (Ph. Int., 2020 ; USP, 2023b).

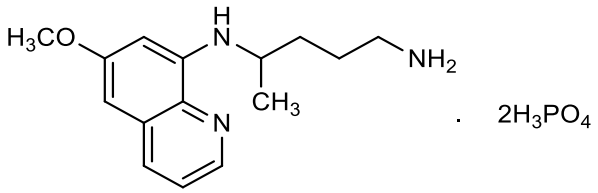


Figure 2. Chemical Structure of primaquine phosphate

1.3.3. Artemisinin and its derivatives (Sesquiterpene lactones)

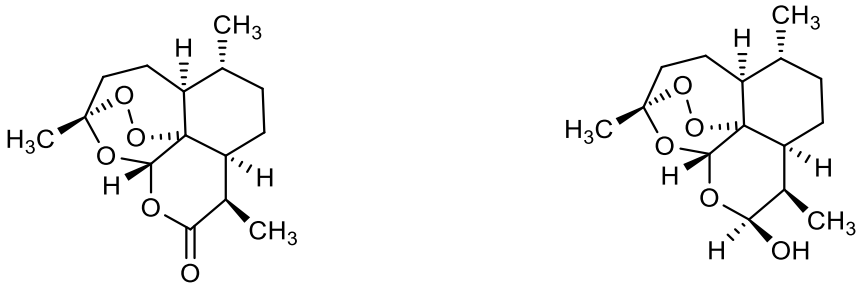
Artemisia annua (also known as sweet wormwood or ***qinghao***), which was originally used to cure hemorrhoids, belongs to a class of chemicals that have been utilized for at least 2000 years by practitioners of Chinese herbal medicine to treat malaria. The plant's active component was refined and given the name ***qinghaosu***, or essence of *qinghao*, in 1972 (Fidock *et al.*, 2000). *Artemisia annua L* has been applied for centuries in the traditional Chinese medicine and in Africa for treatment of fever and malaria (Berdelle *et al.*, 2011; Septembre-Malaterre *et al.*, 2020).

1.3.3.1 Artemisinin

A sesquiterpene containing artemisinin (***2, qinghaosu***) has a peroxide bridge link, which appears to be necessary for the anti-malarial effects. For intramuscular injections, pills, and suppositories, artemisinin was created in China in both oil-soluble and water-soluble formulations. Chinese researchers were inspired to create more soluble derivatives, leading to the creation of dihydroartemisinin and its esters or ethers, artesunate and artemether (for the chemical structures see Figure 4). In comparison to all other antimalarial drugs currently in development, these

derivatives appear to act quickly and have more potent antimalarial activity than the original molecule. (Lin, Klayman *et al.*, 1987; Lucumi *et al.*, 2010).

(+)-Artemisinin (Figure 3a) has been the subject of many total syntheses and structure-activity relationship (SAR) studies. 4-Simple derivatives obtained by stepwise modification to the lactone carbonyl such as artemether (2, R)CH_3 or sodium artesunate (2, R) $COCH_2CH_2$- COONa) have provided clinically useful drugs that have been marketed in Southeast Asia (Avery *et al.*, 1996).



a **b**

Figure 3.Chemical Structure of Artemisinin (a) and Dihydroartemisnin (b)

1.3.3.2. Artemether

It is used in conjunction with lumefantrine as an antimalarial to treat strains of falciparum that are resistant to multiple drugs (PubChem, 2021). Artemether is well absorbed from the gut and rapidly hydrolyzed to an active metabolite that has a short half-life of 2–3 hours. Despite having a high resurgence rate when used as a monotherapy, artemether has quite a few proposed mechanisms of action, including interference with plasmodial transport proteins, interference with mitochondrial electron transport, and the production of free radicals to reduce blood antioxidants and glutathione (Stover *et al.*, 2012).

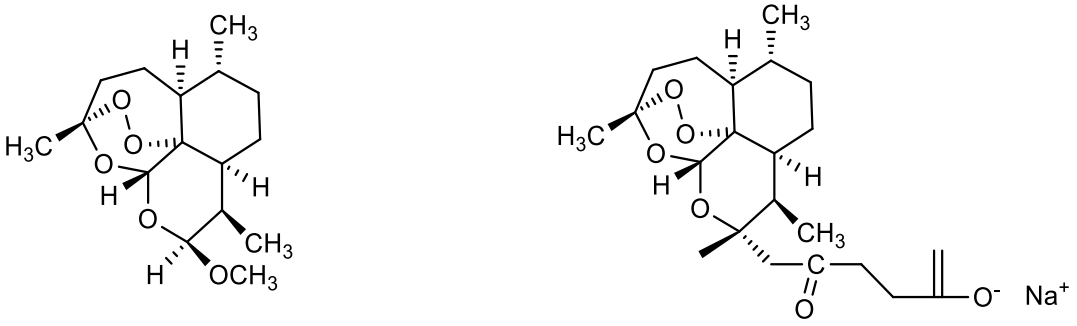
Chemistry of Artemether. It is a dihydroartemisinic methyl ether with a chemical formula of $C_{16}H_{26}O_5$. It's a sesquiterpenoid, cyclic acetal, organic peroxide, artemisinin derivative, and semisynthetic derivative. Artemether is also known by its chemical name [3R-(3*α*, 5a*β*, 6*β*, 8a*β*, 9*α*, 10*α*, 12*β*-12aR)]-Decahydro-10-methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano [4,3-j]-1,2-benzo-dioxepine. It has a 298.38 ***amu*** molecular weight. It is nearly insoluble in water, readily soluble in acetone, soluble in methanol, and soluble in ethanol (Webster and Lehnert, 1994).

1.3.3.3 Artesunate

It is a semi-synthetic derivative of the natural substance artemisinin (Berdelle *et al.,* 2011; Septembre-Malaterre *et al.,* 2020). Artesunate is active against chloroquine and mefloquine-resistant variants of the parasite. Compared to the other qinghao derivatives (arteether, artemisinin, and artemether), artesunate is the most successful *in vitro* (Barradell and Fitton, 1995).

Increased oxidative stress in intra-erythrocytic plasmodia is how artesunate works. The active endoperoxide bridge moiety of artesunate releases heme from parasite-infected red blood cells, resulting in reactive oxygen species and carbon-centered radicals that have been shown to kill the malaria parasite (Li *et al.*, 2008 ; Gopalakrishnan and Kumar, 2014) .

Chemistry of Artesunate: Artesunate is a fine, white crystalline powder with a chemical formula of $C_{19}H_{28}O_8$ and molecular weight of 384.4 ***amu***. It has a chemical name of (3R,5aS,6R,8aS,9R,10S, - 12R,12aR)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano- [4,3-j]-1,2-benzodioxepin-10-ol hydrogen succinate. It is very slightly soluble in water; very soluble in dichloromethane R; freely soluble in ethanol (~750 g/l) TS and acetone R (Ph.Int. 2020).



a **b**

Figure 4.Chemical Structure of Artemether (a) and Artesunate (b)

1.3.4. Artemisinin-based Combination Treatments (ACT's)

In the early 2000s, the WHO has suggested that all antimalarials should contain a combination of an artemisinin derivative with a co-drug, such as lumefantrine, amodiaquine, piperaquine or mefloquine (WHO, 2001). Ever since, ACTs are employed for use as first-line treatment against malaria (Kaur *et al.*, 2010). During that time the situation in Southeast Asia was miserable;

chloroquine, sulfadoxine-pyrimethamine (SP) and mefloquine had been deployed sequentially and fallen to resistance (Ashley and Phyo, 2018).

ACTs are now largely acknowledged as a viable option for slowing the formation and progression of malaria resistance while also extending the usable therapeutic life (UTL) of antimalarial medicines (Kokwaro *et al.,* 2007). The use of artemisinin derivatives as a monotherapy necessitates a multiple dose regimen lasting seven days due to their extremely short half-life. Three important potential advantages exist when one of these medications is used with an antimalarial medication with a longer half-life. It enables a shorter course of artemisinin treatment, increases the antimalarial efficacy through an additive or, ideally, synergistic impact. The third, and arguably most crucial, benefit of combination therapy is that it reduces the likelihood of resistance development to the partner drug (Banet and Brasier, 2014).

ACTs are effective against multi-drug resistant parasites, they cause a sharp decline in a parasite biomass in addition to a rapid reduction in fever, and they are gametocidal and could potentially reduce malaria transmission. They are also quickly eliminated and thus possess a lower likelihood of favoring resistant organisms (Kokwaro *et al.,* 2007). Globally, ACTs are very successful and efficacious, and they are still the first-line treatment for uncomplicated malaria (Kaur *et al.,* 2010; Adebayo *et al.,* 2020).

1.3.4.1 Artemether and Lumefantrine (Coartem)

Combining a fast-acting artemisinin derivative with a slow-acting antimalarial medication from a different class of antimalarials is usually the idea behind ACT's. Coartem contain not less than 90.0% and not more than 110.0% of the amounts of artemether ($C_{16}H_{26}O_5$) and lumefantrine ($C_{30}H_{32}Cl_3NO$) stated on the label (Ph.Int, 2020). It is the only fixed-dose ACT that is currently approved and pre-qualified by the WHO (Kokwaro *et al.,* 2007). Lumefantrine is thought to inhibit β-hematin formation, a vital detoxification pathway for the parasite (Stover *et al.,* 2012). Compared to artemether, lumefantrine acts more slowly, assisting in the removal of any remaining parasites and reducing recrudescence (Djimdé and Lefèvre, 2009).

1.3.4.2. Lumefantrine

It exists as yellow crystalline powder. It is freely soluble in *N, N*-dimethylformamide, in chloroform, and in ethyl acetate; soluble in dichloromethane; slightly soluble in ethanol and in

methanol; substantially water insoluble. It has a chemical formula of $C_{30}H_{32}Cl_3NO$ (Figure 5) and have a molecular weight of 528.94 ***amu*** (USP, 2023c). It is not active at gametocytes, hypnozoites, or pre-erythrocytic stages, lumefantrine is widely known for its potency against both *P. falciparum* and *P. vivax* erythrocytic stages. It is assumed that lumefantrine and other drugs fall under this category interact with heme in the acidic feeding vacuole of the parasite to create their antimalarial effect, despite the fact that the exact mechanism of action is unknown (Bennett *et al.*, 2020)

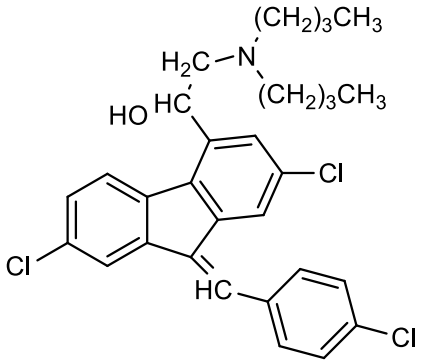


Figure 5.Chemical Structure of Lumefantrine

2. Statement of the problem

Treatment for numerous ailments and diseases is made difficult by the circulation of low-quality medications, namely those that are falsified (i.e., intentionally manufactured fraudulently) or substandard (i.e., damaged due to unintentional production faults or poor storage/handling practices). Forecasts of the burden of substandard and falsified medications indicate that the burden may account for up to 10% of all medications in low- and middle-income countries (LMICs) at a cost of between US$10 billion and US$200 billion (WHO, 2017a). Anti-infectives, especially anti-malarias, are among the class of medications most frequently linked to quality issues and vulnerable targets in low-income countries (Newton *et al.*, 2011; WHO, 2011).

Due to their simultaneous effects on people at different levels of the population affecting both individuals and communities, these medications constitute a serious risk to public health in all endemic countries. Reports of low-quality ACTs in Africa have increased alarmingly (Bate *et al.*, 2008; Newton *et al.*, 2011). A study conducted in south-west of Ethiopia Jimma indicated out-of-specification results for the chloroquine tablets' weight variation, hardness, and visual inspection tests indicating substandard and counterfeit acts that could jeopardize the quality of these products (Abuye *et al.*, 2020). There was a quinine tablet case in Gambella without API found by USP—PQM and reported to WHO alert system. The investigation found out that it was circulated from Kenya to South Sudan then Gambella (Unpublished data, annual internal report of USP-PQM and EFDA Post Marketing Surveillance Study, 2009).

Moreover, it was estimated that PQAs were responsible for 12,300 deaths and costs $892 million annually in Nigeria (Beargie *et al.*, 2019). In 2013 alone, in a sample of 39 sub-Saharan countries, it was estimated that 122,350 deaths in children younger than five years old (representing 3.75% of all deaths for that age group) were caused by PQAs (Renschler *et al.*, 2015; Walker *et al.*, 2018). Additionally, PQAs without APIs may promote resistance (Hall *et al.*, 2006; Newton *et al.*, 2011; Phillips *et al.*, 2017; Shibeshi *et al.*, 2020).

The PQAs in the markets of endemic countries is majorly attributed to weak law enforcements, corruption, inaccessibility, high price of quality ACTs, limited regulatory oversight, lack of penalties, self-prescribing practices, poor knowledge about product authenticity, and a large unregulated private sector for purchasing pharmaceuticals. This pause threat to the treatment and eradication of malaria (Walker *et al.*, 2018).

The national regulatory authority's capacity as a developing nation can't cover all aspects of quality and couldn't possibly collect larger sample sizes from a single regional state. Due to inadequate surveillance methods, instances involving the distribution and use of substandard medications frequently go undetected and are concealed from the public record by governments and pharmaceutical companies. Besides, the median duration between the collection of drug samples and publication in studies conducted in Southeast Asia and sub-Saharan Africa was 3 years (with a range of 0 to 6) (Nayyar *et al.*, 2012). Most nations where malaria is endemic lack sufficient reports on the efficacy of antimalarial medications (Tabernero *et al.,* 2014). A review done by the Worldwide Antimalarial Resistance Network (WWARN) antimalarial quality database found no reports of antimalarial quality for 17 of the 44 malaria-endemic sub-Saharan African countries (Renschler *et al.*, 2015). Ethiopia have always been underreported in this matter. This might be due to the lack of thorough tests conducted concerning PQAs in the nation. And that in turn reflects back to lack of resources, well organized lab, and lack of well-trained personnels in the quality control field.

According to the Ethiopian malaria eco-epidemiological strata map, Gambella region was listed on a stable, year-round transmission area in Ethiopia (President's Malaria Initiative, 2013). The high temperature and annual humidity which is characteristic of the Gambella region (World weather online, 2022), favors the degradation and change of the biopharmaceutical properties of the drugs. This could lead to sub-therapeutic dosage and formation of degradation products toxic to humans. Thus, the parasites are highly to be expected to develop resistance to antimalarials and, therefore, the treatment goals may not be achieved (Castro Souza *et al.,* 2019). Additionally, Gambella is a border town where refugees come and exit the region.

Generally, the use of high-quality antimalarial medicines has the potential to save hundreds of thousands of lives annually. ACTs have been critical to recent worldwide malaria control successes, and maintaining their efficacy for malaria management is a public health priority. It comes as no surprise that continuing research on the assurance of the antimalarial drug quality is a primary concern (Nkumama *et al.,* 2017; WHO, 2017b). Thus, PQAs are encouraged to be counteracted as a critical yet frequently disregarded public health issue (WHO, 2015a).

In order to counteract low-quality medications, a variety of intervention strategies are utilized. Strong legislative and regulatory frameworks that provide a reliable quality assurance system that

ensures medication quality across the pharmaceutical supply chain are the main ways of intervention (Hajjou *et al.,* 2015; Nayyar *et al.,* 2015).

Quality control of medicines in the distribution system according to the proper specifications is an important prerequisite in ensuring optimal treatment outcomes. This study aims to evaluate the quality of frequently employed antimalarial drugs in Gambella region. Quality surveys of marketed products such as this study would also act as a preventive strategy against the exposure of substandard medicines by manufacturers and importers.

3. Significance of the Study

Despite the fact that the Ethiopian Pharmaceuticals Supply Agency (EPSA) offers antimalarials to the regional states of Ethiopia for free under donor supported programs, there are many private sectors that profit from selling antimalarials during periods of high malaria prevalence when there is typically a shortage of antimalarial supplies. Therefore, quality evaluation studies such as this one are essential instruments to guarantee the distribution of high-quality, secure, and efficient artemether-lumefantrine tablets, chloroquine tablets, primaquine tablets, artesunate, and artemether injections having predictable and consistent therapeutic APIs.

This study provides a hint as to the therapeutic success or failure of malaria management since they can provide information on the quality of these items consumed and distributed throughout the distribution chain.

It also produces initial data that drug regulatory authorities could use to support and enforce their regulatory actions or take preventive or remedial action. Additionally, the condition of the samples that were taken from this sampling area was not well documented, and the findings of this inquiry will assist establish a baseline for future extensive, well-planned, and statistically sound drug quality studies. It also assesses whether the origin, collection site, and manufacturers have an impact on the tested products' quality. Therefore, this study is conducted with the goal of determining whether the artemether-lumefantrine tablets, chloroquine tablets, primaquine tablets, artesunate, and artemether injection currently being sold in Gambella Southwest Ethiopia comply with pharmacopeial requirements.

Overall, the study's findings will contribute to the national effort to eradicate malaria by ensuring that the general public has access to high-quality antimalarials.

4. Objectives

4.1. General Objective

To evaluate the quality of the frequently employed antimalarial drugs [artemether-lumefantrine tablets, chloroquine tablets, primaquine tablets, artesunate, and artemether injections] in Gambella, South-West, Ethiopia.

4.2 Specific Objectives

- To perform visual inspection assessment of the commonly marketed antimalarials in the Gambella National Regional State.
- To assess the physical properties such as, uniformity of dosage forms, hardness test, friability, dissolution, and disintegration time.
- To compare the dissolution profiles of the tablets of different manufacturers.

5. Literature review

5.1. Malaria

Plasmodium parasites, which individuals' contract through the bites of infected female *Anopheles* mosquitoes, are the source of the acute fever sickness referred as malaria. *Anopheles arabiensis* is the primary malaria vector in Ethiopia, with *An. funestus*, *An. pharoensis*, and *An. nili* as secondary vectors (President's Malaria Initiative, 2020).The two most dangerous parasite species, *P. falciparum* (70 %) and *P. vivax* (30%), are among the five that cause malaria in humans. The most common and deadly malaria parasite on the African continent is *P. falciparum* (WHO, 2022b). It is estimated to be the cause of 500 million cases and over 1 million deaths per year, mostly in women and children under the age of 5 years (Kaur *et al.*, 2010).

5.2. Malaria burden and Epidemiology

The most prevalent endemic disease in Africa and other Asian nations with the greatest number of cases is malaria. The fatality rate from malaria ranges between 0.3-2.2% globally, and in situations of severe forms in tropical climates it increases from 11-30% (Talapko *et al.*, 2019). Globally, there were 228 million cases in 2018, with the majority occurring in the WHO African Area (Ashley and Phyo, 2018). 95% of cases and 96% of deaths from malaria occurred in the area in 2021 (WHO, 2022b). It was estimated that 85% of the worldwide influence was carried by 19 sub-Saharan African nations and India (WHO, 2019a). According to the World Malaria Report (WHO, 2021) there were 627,000 malaria-related fatalities and 241 million malaria infections globally in 2020. This characterizes about 12 million additional cases in comparison to 2019, and 69,000 more deaths (WHO, 2022b).

In Ethiopia, malaria transmission is mostly limited to altitudes below 2000 meters, while endemic areas over 2000 meters have been documented (Figure 6 shows the malaria strata in Ethiopia). With the exception of the southern international border low land area, where transmission occurs year-round, the levels of malaria risk and transmission intensity show significant seasonal, inter-annual, and spatial variations (Taffese *et al.*, 2018; President's Malaria Intiative, 2020).

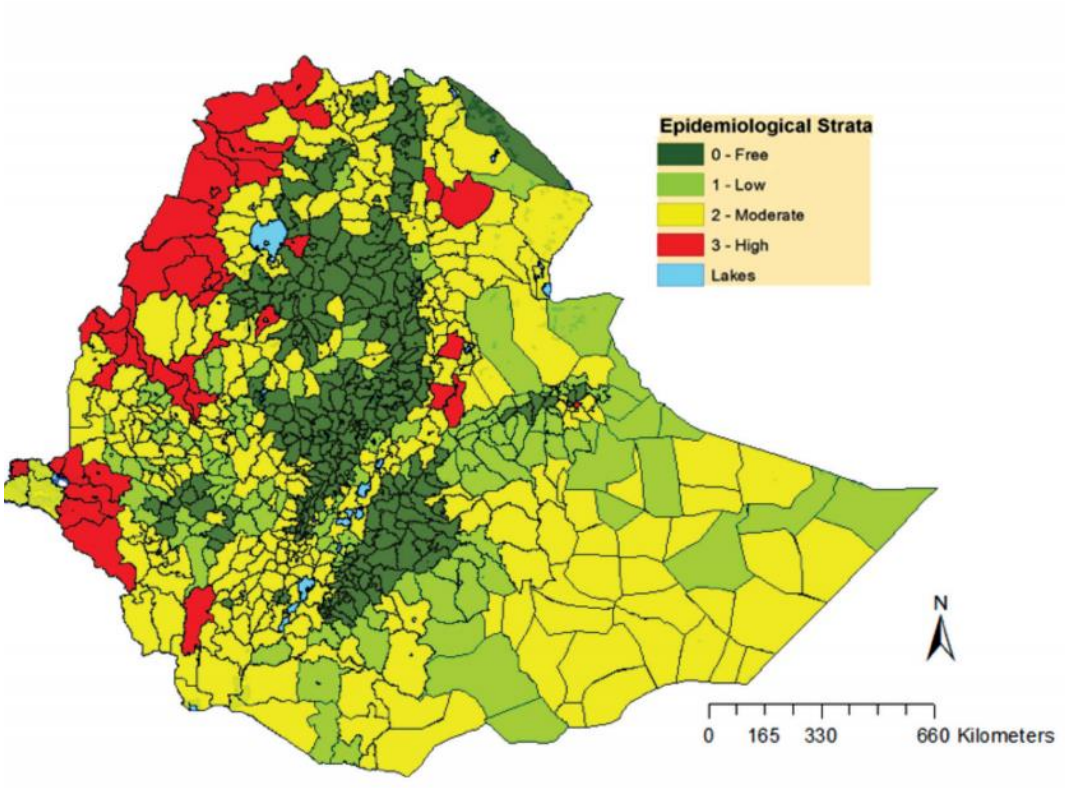


Figure 6. Malaria Strata in Ethiopia

It is thought to be prevalent in more than 75 % of Ethiopia, placing more than 60 million people at risk (or 60% of the country's total population). In past years, it was estimated that there were roughly 4-5 million cases of malaria and 70,000 deaths that resulted from it each year (Girum *et al.*, 2019). In addition, it is the most frequent cause of out-patient (10-40%) and in-patient admission (13-26%) nationwide with corresponding mortality rates of 13–35%. Moreover, it ranks in the top 10 major causes of morbidity and mortality in both adults and children under the age of five (Deribew *et al.*, 2017; Haileselassie *et al.*, 2022). In spite of the unceasing struggle to abolish malaria nationwide, it represented 30% of the total disability-adjusted life lost, which makes it a significant hindrance to the social and economic course of events (Girum *et al.*, 2019).

5.3. Quality of medicines

The pharmaceutical industry has suggested fitness for use, which denotes that the drug satisfies any predetermined regulatory requirements or quality criteria for the definition of "Quality". Additionally, it has been claimed that pharmaceuticals produced in accordance with cGMPs are of an exceptional good quality (Woodcock, 2004).

A global vision to assure equity, quality, and financial security in access to healthcare by 2030 has been established by the Universal Health Coverage (UHC) development target, which was announced in 2015 as one of the 17 Sustainable Development Goals (SDGs) of the United Nations (WHO, 2020b). However, numerous studies conducted over the past ten years have shown that all classes of medicines suffer from poor quality, and no country is exempt (Srivastava, 2021; Waffo Tchounga *et al.,* 2021; Karungamye, 2022).

A poor-quality medicine, as defined by the WHO, is one that is either substandard or falsified (SF). A substandard medication does not adhere to the necessary requirements, which typically include the active ingredient (s) content, impurity limitations, microbiological activity, and dissolving profile. On the other hand, medical items that purposefully or fraudulently misrepresent their identity, content, or source are considered counterfeit medicines (CDs) (WHO, 2017c).

Poor quality medications could spread quickly throughout the world due to pharmaceutical industry globalization before effective detection and intervention are available (Newton, Green and Fernández, 2010). About 1 million people can lose their lives around the globe every year, due to CDs and SFs related cases. SF and CDs are causing indescribable suffering to the general public particularly in certain nations of Africa where it represents an alarming proportion from the total drugs in circulation. Hence, it is one of the major global public health risk (Renschler *et al.*, 2015).

5.3.1. Quality of Antimalarials

Patients having access to medications with good quality, is a gravely underappreciated part of malaria control. Any plan for effectively reducing malaria-related mortality and morbidity depend on the effectiveness and safety of antimalarial medications, as determined by their quality. Hence, assurance of the medicine's quality is crucial for the perception of superior medical care and effective treatment among patients and medical professionals also for preventing the development of antimalarial drug resistance (WHO, 2011).

5.3.1.1. Poor quality Antimalarials

Over the past years, there is a growing concern that a significant percentage of the antimalarial supply, particularly in the private sector, in the developing countries is of poor quality. This issue is not new; falsified antimalarials were a severe problem in the 17th century when replicas of the

primary potent antimalarial drug, cinchona bark (the source of quinine), were widely marketed in Europe (Newton *et al.,* 2016). According to a study by Hajjou *et al.*, (2015), antimalarial medications made up to 92.6 % of the total detected fake medications and 52.5 % of the entire substandard medications in Africa, Asia, and South America.

A WHO study that attempted to assess the effectiveness of particular antimalarial drugs in six sub-Saharan African nations (Cameroon, Ethiopia, Ghana, Kenya, Nigeria, and United Republic of Tanzania) in 2008 found out that 28.7% of the 267 fully tested samples collected between April and June 2008 failed to meet predetermined, internationally recognized quality standards. From these, deviations from specifications which are anticipated to be related with health implications were 11.6% (WHO, 2011; Hamed and Stricker, 2016).

False Coartem® samples had been discovered in Ghana by Batson *et al., (*2016), in Benin by Yemoa *et al.,* (2017). Another Four batches, mostly discovered in the black market of Nigeria, were reportedly implicated in this suspected falsification (Kaur *et al.*,2015). Similar results were indicated in Angola and Cameroon (Newton *et al.,* 2011; Newton *et al.,* 2014) From these African nations, Nigeria was reported to have the greatest incidence of drug-related falsifying offenses in 2016 (USAID-Funded Health Communication Capacity Collaborative (HC3), 2016).

A study conducted in 2017 showed inactive or adulterated malaria medications upon sampling of ACTs in Benin, the Democratic Republic of the Congo (DRC), Kenya, Madagascar, Nigeria, Tanzania, Uganda, and Zambia. It amounts to 20 % of Kenya's private-sector market and in 42 % in the DRC (Brower, 2017).

Most of the reported data's are frequently of poor quality (Newton *et al.*, 2009) but some stand out as important hotspots of poor quality, such as the discovery of 1.4 million packets of ACTs that were faked in Angola (Newton *et al.,* 2014) and the finding that 88% of oral artesunate monotherapy in the private sector of Laos in 2003 were faked (Sengaloundeth *et al.,* 2009). All major surveys have discovered substandard medications, frequently possessing a lower percentage of APIs (ACT Consortium Drug Quality Project and IMPACT Study Team., 2015; Tabernero *et al*., 2015).

A WHO quality survey on antimalarials found a considerable portion of collected samples (41%) that were not registered, which raises concerns about the market's susceptibility to infiltration by

products with questionable qualities (WHO, 2011). Similar results were found by the Food, Medicines, Health Care Administration and Control Authority (FMHACA) of Ethiopia determining 34 % unregistered antimalarials. Additionally, hubs for the smuggling of illegal drugs were also found. The difficulties are reportedly made worse by the country's lack of enforcement of pharmaceutical regulations (President' Malaria Initiative, 2013).

The sale of fake medications has recently surpassed the trade in illegal drugs and prostitution as the biggest market for criminal traffickers worldwide. This dire circumstance may have long-lasting negative effects on one's health (Bottoni and Caroli, 2019). The widespread availability of falsified and substandard pharmacological products poses one of the biggest obstacles to the treatment of malaria and other infectious diseases in less developed nations, where these diseases are extremely important to public health (Ten Ham, 2003). Furthermore, PQAs can increase the risk of therapeutic failure and raise medical costs by causing the growth of parasites that are immune to the antimalarials (Tahar and Basco, 2007).

Stock-outs of malaria medications which usually open the market for the fake ones are frequent as a result of unmet financing requirements, inadequate training in medicine procurement, poor distribution, storage, and transportation procedures. Many patients are forced to seek therapy in unofficial settings due to frequent stock-outs; they frequently purchase unregistered antimalarials from drug hawkers, unlicensed pharmacies, or open drug markets or even licensed pharmacies and drug vendors (Bate *et al.,* 2010).

5.4. Methods to detect poor quality antimalarials

Distinguishing poor-quality medicines is an essential step in the drug quality assurance system. The peculiarity of locally produced, imported, or donated formulations used in a nation’s healthcare system can be confirmed by an affordable and well-established medicine quality control laboratory (MQCL). The WHO member states are encouraged to maintain MQCLs, but miserably this is not possible in many malaria-endemic African countries due to lack of economic resources. There are only two WHO Prequalified Quality Control Laboratories in malarious Africa as of 2009 (Kaur *et al.*, 2010). The quality of antimalarial medications is evaluated using a number of different techniques. Rapid testing techniques and pharmacopeial approaches are the two ways that are commonly employed methods for assessing the quality of medications (WHO, 2011).

5.4.1. Rapid testing techniques

Numerous easy-to-use analytical techniques have been developed on account of efforts to verify the quality of pharmaceuticals travelling in international trade. These techniques were created for the high-throughput identification, content, and physical attribute modifications of pharmaceuticals. Typically, they provide illustrative or estimated findings. It's crucial to remember that a product's quality is in question if it fails a rapid testing method (WHO, 2011). This approach, which employs various screening techniques, may be helpful in regions where gold standard techniques like HPLC (Kovacs *et al*., 2014), which are pricy, time-consuming, and require specialist knowledge, are not easily accessible. Additionally, this method is capable of quickly identify any CD's and/or SF anti-malarial medicine goods on the market, protecting the public health and enhancing quality of life (Opuni *et al.*, 2019).

The Global Pharma Health Fund (GPHF) Minilab kit (Bate *et al*., 2009; Petersen *et al.*, 2017), the Counterfeit Drug Indicator (CoDI) (Green *et al*., 2015), colorimetry (Green *et al.*, 2001; Koesdjojo *et al*., 2014), CD3+ (Batson et al., 2016) and the TruScan handheld Raman spectrometer (Bate *et al*., 2009) and reflectance infrared spectroscopy (Lawson *et al.*, 2018), Paper Analytical Devices (PADs) (Weaver and Lieberman, 2015) were some of the methods used to address a variety of analytical issues in low-resource situations. But, the sensitivity and specificity of screening tools and methodologies might occasionally provide false positive results (Roth *et al*., 2018).

5.4.2. Pharmacopeial methods

According to published monographs, pharmaceutical analyses adhere to tried-and-true procedures. Pharmacopeial procedures evolve throughout time as new technology become available. The International Pharmacopoeia (Ph. Int.), European Pharmacopoeia (Ph. Eur.), United States Pharmacopoeia (USP), British Pharmacopoeia (BP), and Japanese Pharmacopoeia (JP) are only a few of the pharmacopoeias that exist. Pharmacopeial requirements for product quality include identification requirements, API content requirements, related substance requirements, and testing of dosage forms such as uniformity of mass/content, friability requirements, hardness requirements, disintegration requirements and dissolution test. They also cover the standard specifications for the labeling and packaging of the sold formulations (WHO, 2011).

6. Materials and methods

6.1 Study Area and Study Period

The Ethiopian malaria eco-epidemiological stratum map was used to guide the choice of sample collection location (Dillu *et al.,* 2017). Accordingly, Gambella region was selected. Figure 7 shows the map of sampling area.

Gambella National Regional State borders South Sudan to the west, Oromia region to the north and east, and the Southern Nations, Nationalities and Peoples' Regional State (SNNPRS) to the south. It is situated in the southwest of Ethiopia, 777 kilometers west of Addis Ababa. The capital, Gambella is mostly flat, and has a hot and humid climate. The minimum and maximum temperatures are typically 21.1^0C and 35.9^0C, respectively, with an annual rainfall average of 600 mm. The area is primarily lowland with a few midlands (Ethiopian Demography and Health, 2014). According to the 2017 Ethiopian population projection, the total population of the region was approximately 436,000 (Central Statistical Agency, 2018). The region hosts the largest refugee population in Ethiopia; 337,421 refugees from South Sudan, a population almost equal to its own (UNHCR, 2021). While having a relatively small territory, the region has a sizable ethnic diversity, with one-fifth of its residents living in urban areas. There are four administrative zones and 12 woredas in Gambella Regional State. Agnuak zone (Gambella woreda, Abobo woreda, Gog Woreda, Jor woreda, Dimma woreda), Nuwer zone (Lare woreda, Jikawo Woreda, Wantawa Woreda, Akobo Woreda), Mezhenger-Zone (Godare woreda, Mengesh woreda) and Itang Special Zone (Ithang woreda) (Central Statistical Agency, 2018). There are 64 private drug retail outlets throughout the region. From these 2 are rural drug shops, 60 are drug stores and 2 are Pharmacies as communicated by the Gambella health bureau. Samples were collected during July, 2022.

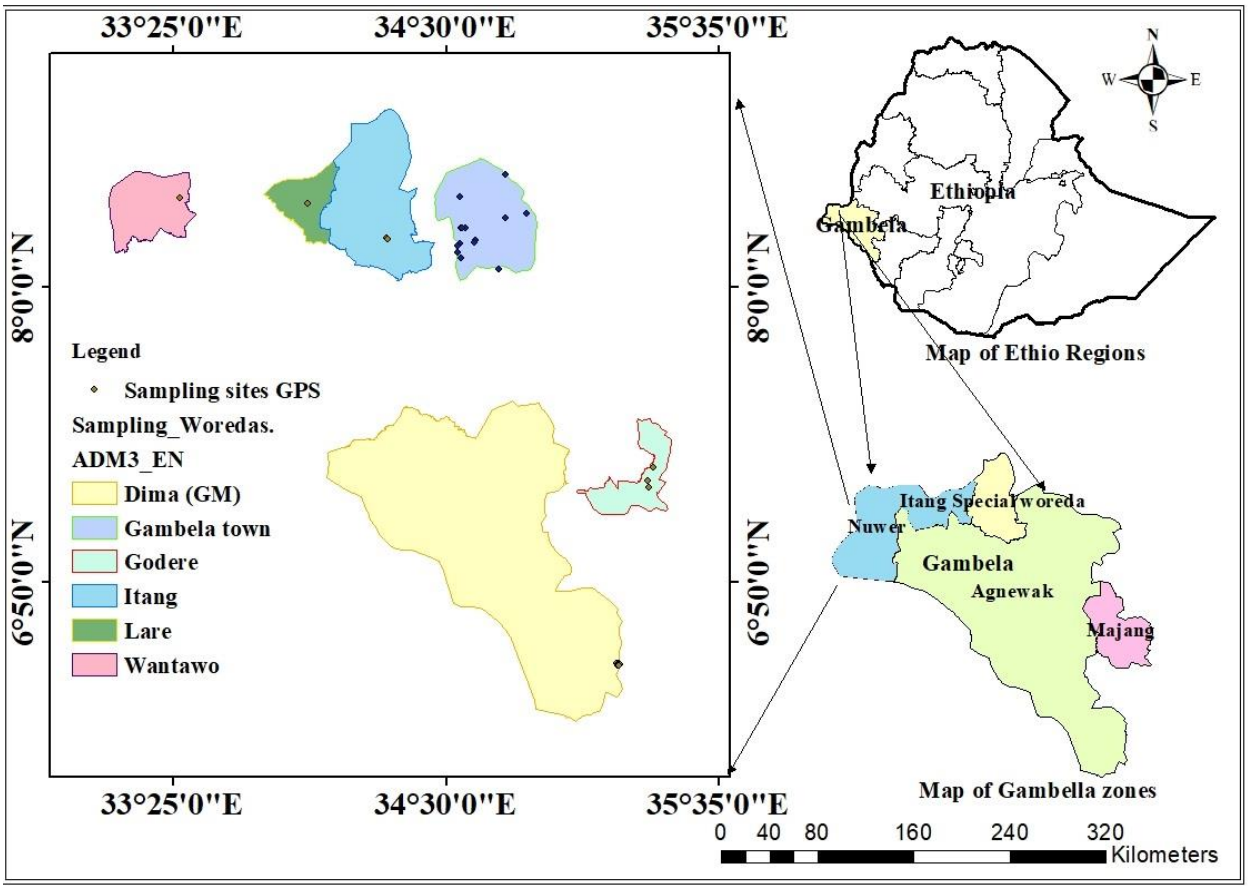


Figure 7.Map of sampling area

6.2. Sampling technique and sample size

Half of the (six) woredas located in the four zones of the region were randomly chosen. There are 59 private drug retail outlets in the 6 woredas. In the second stage of sampling, half of (29) the private drug retail outlets were randomly selected using simple random sampling technique. All available artemether lumefantrine tablets, chloroquine tablets, primaquine tablets, artesunate and artemether injections samples were collected.

6.3. Sample Collection

Mystery shopping method was applied to buy the samples from the private drug outlets (drug stores, pharmacies, and rural drug shop). The sample collectors appeared as a random walking customer anonymously by local nationals. The mystery shopper mimics a “normal shopper” for the neighborhood wherein the outlet is located and dressed, spoke and behaved appropriately for

the community. After leaving the surveyed place, the mystery shopper recorded details of the purchase including price and name of the provider/outlet. Collected medicines were properly identified and stored (WHO, 2015a).

Samples were gathered in accordance with their availability in the drug outlets and existing brands/batches of the antimalarials were obtained from the drug outlets of the selected Woredas in the region. The samples were then preserved in the original packages supplied by the manufacturers. The packaging was examined for any features of illegal prints. From each sample, the origin, labeled dose, registration status, and shelf-life were noted. All tests were completed before the product's expiration dates and different batches of a single product were purchased. The laboratory test was conducted at the Ethiopian Food and Drug Authority's Medicine quality control laboratory (EFDA) and Addis Ababa University, College of Health Sciences, School of pharmacy, pharmaceutical analysis lab.

Annexes 2 and 3, show the details of all 52 samples regarding collection site, origin (country of manufacturer), name of manufacturer, brand names of the drug and coding of the samples that were covered by the study.

6.4. Instruments

HPLC (Shimadzu, Japan), Analytical Balance (Mettler Toledo, Greifensee, Switzerland), Dissolution tester (ERWEKA, Heusentamm, Germany), PH meter (HANNA instruments), UV–Vis Spectrophotometer (Shimadzu, Germany), Vaccum Pump (China), Vortex, Drying Oven, Ultra Sonicator (Mumbai,India), Flask Shaker (Stuart), Disintegration tester Hardness Tester (Pharma Test, Hainburg, Germany), Friability Tester (Pharma Test), Water Purification System (Thermo Scientific, Model-7143, Waltham, MA, USA), and Pipett fillers were used in this study. The following glasswares were also used in the experiment: beakers, volumetric flasks, conical flasks, measuring cylinders, pipettes, and funnels were used for the study. HPLC columns: ACE (150*3.9mm), Waters: C18-102 (4.6*150mm, 5µm), Waters: C18-194 (3.9*150 mm, 5µm), Waters: C8 (15cm*4.6mm, 5µm), Waters: ODS2: (4.0*250mm, 5µm), Waters: C18-090 (4.6*50mm, 5µm), Phenomenex Kinetex-C18 column,

6.5. Chemicals and Reagents

Acetonitrile HPLC grade (Lot: I1161030-127,merck, Germany : Lot: 2189056,Sisco research laboratories, India), 1-propanol (Lot:1851527, Fisher Scientific, U.K), benzalkonium chloride AR (Lot: 44790, Blulux, India), Trifluoracetic acid AR (Lot: #STBH1190, Sigma-Aldrich, Germany), methanol HPLC grade (Lot: I116211-132, and Lot: I116211-132, Fisher Scientific Germany, Lot: 4549892, India), monobasic sodium phosphate (Lot: 62840, Sigma-Aldrich, Germany), Phosphoric acid (Lot: STBH5477, Sigma-Aldrich, Switzerland), orthophosphoric acid (Lot: 22182/1C,Surechem products, U.K), hydrochloric acid (Lot: 1003271452, 37 %, Sigma-Aldrich, USA), methane sulphonic acid (Lot: TB62586V, Sigma-Aldrich, U.K), potassium dihydrogen orthophosphate (Lot: 9680/109, Park scientific limited, U.K), glacial acetic acid (Lot: 60062, Sigma-Aldrich, Germany), sodium hexane sulfonate (Lot: 1201934, Fischer scientific, U.K), tetrahydrofuran (Lot:K3310, Honeywell, Germany), sodium hydroxide pellets (Lot:336155, Oxford Lab Fine Chem LLP, India), analytical grade reagent ethanol (Lot: 221084034, VWR chemicals, France), potassium hydrogen phthalate (Lot:43169, Blulux laboratories, India), 1-Pentane Sulfonic acid sodium salt (CAS No.22767-49-3, Fischer scientific U.K), perchloric acid (Lot: Scharlau Chemie S.A, Spain), Vanilin (Lot:A0371481,ACROS Organics,) and ultra-pure water, distilled water, primary reference standards of artemether USP RS 100mg (Lot: R12050, China) and lumefantrine USP RS 100 mg (Lot: R04X10, India), chloroquine phosphate USP RS 500 mg (Lot: R07550, India), amodiaquine hydrochloride 500mg USP RS (Lot: R0T8LO, India), artesunate USP RS, phenolphthalein powder (Lot: 43644 India), ,primaquine phosphate 200 mg USP RS (Lot: R1U0RO, China) Primaquine phosphate related compound A 100mg USP RS (Lot: R06080,China) and Artesunate USP RS (Lot:R07680,India) , were employed during the study.

6.6. Inclusion and exclusion criteria

6.6.1. Inclusion criteria

All tablet/injectable dosage forms of artemether lumefantrine tablets, artemether injection, artesunate powder for injection, primaquine phosphate tablets and chloroquine phosphate tablets available at the collection site during data collection.

6.6.2. Exclusion criteria

Identical antimalarials from the same sample collection site with similar batch numbers were excluded.

7. Methods

7.1. Physical Evaluation

Visual examination of the dosage form's physical properties, the container, and the labeling data was carried out. The WHO/FIP/USP joint check list was used (Annex 1). Each sample was visually examined for the brand name, active ingredient name, manufacturer's name, and complete address. The labeled medication's potency, dosage type, number of units per container, dosage statement, batch/lot number, manufacturing date, and expiration date were also evaluated. In a similar manner, storage details, the existence of leaflets or package inserts, color and size uniformity, breaks, cracks, and splits, embedded surface stains, and visual contaminations were looked into (WHO, 2015b).

Following a moderate shake for 30 seconds, the artemether injections were checked for the occurrence of particulate matter by looking into each ampoule in natural light (Ph. Int., 2020). The results of physical characteristics, packaging and labelling information were illustrated in Annex 2. All the samples obtained were within their shelf lives throughout the time of the experiment.

7.2. Identification Tests

High-performance Liquid chromatography (HPLC) was used for artemether-lumefantrine tablets, artemether injections, chloroquine and primaquine tablets. Comparing chromatographic peak retention times as a technique of identification is suggested in their monograph. To confirm their identity, retention times of the tested products' peaks were compared to those of standard references.

7.2.1. Identification test for artemether lumefantrine Tablets

Identification test for artemether-lumefantrine tablets was performed according to the International Pharmacopoeia (IP) using the test described under identification (Ph. Int., 2020) .

7.2.2. Identification test for artemether Injection

Identification test for artemether injection was performed according to the IP using the test described under identification (Ph. Int., 2020).

7.2.3. Identification of artesunate powder for Injection

Identification test for artesunate powder for injection was performed according to the described method on the IP using the test described under identification (Ph. Int.,2020).

7.2.4. Identification test for chloroquine Phosphate Tablets

Identification test for chloroquine phosphate tablets was performed according to the USP using the test described under identification (USP, 2023a).

7.2.5. Identification test for primaquine Phosphate Coated Tablets

Identification test for primaquine phosphate coated tablets was performed according to the described methods on the USP using the test described under identification (USP, 2023b).

7.3. Uniformity of Weight

The degree of consistency for the drug component contained in dosage units is known as "uniformity of dosage unit" (USP, 2022a). Either content uniformity or weight variation can be used to illustrate the homogeneity of dose units. When the API of the drug product is ≥ 25 mg, or when the API from the drug substance ratio is larger than 25%, the USP 44-NF 39 suggests employing weight variation. For formulations containing 25 mg API or 25% of the ratio of the API from the drug component, content uniformity is advised in all other cases (USP, 2022a). The (Ph. Eur., 2008: Ph. Int., 2020) on the other hand, uniformity of mass for single-dose preparations to be carried out for uncoated tablets and film-coated tablets formulated to contain 5% or more of the active ingredient should comply with the deviation of individual masses of minimum of 18 and maximum of 2 tablets should not exceed by (±7.5% and ±15% from average mass, respectively) (Ph. Eur., 2014; WHO, 2019b). Accordingly, the two techniques were used for the determination of uniformity of mass for the samples.

7.3.1. Artemether Lumefantrine fixed dose Combination

From each artemether-lumefantrine samples, twenty tablets were randomly selected. Using a calibrated analytical balance, these samples were weighed individually (Wx) and their average weight (Wav) was determined. From this the Standard Deviation (SD) was computed and assessed with the IP (WHO, 2019b). The deviation of individual masses of minimum of 18 and maximum of 2 tablets should not exceed by ±7.5% and ±15% from average mass, respectively (Table 2).

Table 2. Acceptable % deviation for uniformity of mass for single dose preparation

Average mass of tablet	Deviation %	Number of tablets
less than 80 mg	±10.0	minimum 18
	±20.0	maximum 2
80 mg to 250 mg	±7.5	minimum 18
	±15.0	maximum 2
more than 250 mg	±5.0	minimum 18
	±10.0	maximum 2

7.3.2. Extractable volume of parenteral preparation of artemether injection

The IP (Ph. Int., 2020) suggests for the determination of an extractable volume of parenteral preparation for a single dose parenteral preparation container (Ph. Int, 2020). Hence, artemether injection extractable volume was performed accordingly.

7.3.3. Artesunate Powder for Injection

The IP (WHO, 2019b) recommends a uniformity of mass for a single-dose preparations to be conducted for powder for injections with a content greater than 40 mg. Thus, this was done according to the method described in the IP. Individual net mass deviation from average net mass shouldn't go above the limits listed in Table 3.

Table 3. Acceptable % deviation for uniformity of mass for single dose powder for injection

Deviation %	Number of containers
± 10	minimum 18
± 20	maximum 2

7.3.4. Chloroquine Phosphate Tablets

For uniformity of dosage units of chloroquine phosphate tablets, a weight variation test was done using a method specified in USP (2022a) whereby the criteria will be met if the acceptance value of the first 10 dosage units is less than or equal to 15. Then, assay (content) of individual bolus (% xi), was obtained by the formula:

$$AV=|M-X|+Ks \quad \text{Equation (1)}$$

Where:

M – Reference value

X – Average content percentage of 10 tablets

K – Acceptability constant, which is 2.4, for number of tablets = 10

s – Standard deviation

7.3.4. Primaquine Phosphate tablets

The IP states that the uniformity of mass for single-dose preparations to be carried out for uncoated tablets and film-coated tablets formulated to contain 5% or more of the active ingredient should comply with the deviation of individual masses of minimum of 18 and maximum of 2 tablets should not exceed by (±7.5% and ±15% from average mass, respectively) (Ph. Eur., 2014; WHO, 2019b).

7.4. Quantitative Assay Test

The primary goal of an assay test is to validate that the active component is present in the requisite quantity and meets the compendial specifications. Examining the chemical assay of each drug utilized in the study was done with the aid of official methodologies that are outlined in the pertinent monographs. The techniques for each antimalarial drug were selected taking into account the accessibility of tools, chemicals, and reagents. The assay test for all the collected samples was done according to their respective USP and IP monographs. Then each result was evaluated whether or not passed the preset acceptance criteria.

7.4.1. Artemether Lumefantrine tablets

The contents of artemether and lumefantrine were determined using HPLC according to IP (Ph. Int., 2020).

7.4.2. Artemether Injection

The assay of artemether injection was also performed according to the method described under the IP. The areas of the peak responses obtained in the chromatograms from the standard and sample solutions were measured and the percentage content of $C_{16}H_{26}O_5$ was calculated (Ph. Int., 2020).

7.4.3. Artesunate Powder for injection

A titrimetric method was used to determine artesunate in artesunate powder for injection according to the IP (Ph. Int., 2020). Each sample was analyzed in triplicate. The content of artesunate per

sealed container was calculated (Affum *et al.*, 2013). Positive control (prepared from Artesunate USP RS) and negative controls were titrated as the samples.

7.4.4. Chloroquine Phosphate Tablet

The assay of chloroquine phosphate was performed according to the USP. The areas of the peak responses obtained in the chromatograms from the standard and sample solutions were measured and the percentage content of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ was calculated using equation 2 (USP, 2023a).

$$(\frac{r_u}{r_s}) \times (\frac{C_s}{C_u}) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{Equation (2)}$$

Where r_u peak response from the sample solution

r_s peak response from the standard solution

C_S Concentration of USP Chloroquine phosphate RS in the standard solution (mg/ml)

C_u nominal concentration of chloroquine phosphate in sample solution (mg/ml) (USP, 2023a).

7.4.5. Primaquine Phosphate Tablets

The assay of primaquine phosphate was performed according to the USP. The areas of the peak responses obtained in the chromatograms from the standard and sample solutions were measured and the percentage content of $C_{15}H_{21}N_3O \cdot 2H_3PO_4$ was calculated as per equation 3 USP (2023b).

$$(\frac{r_u}{r_s}) \times (\frac{C_s}{C_u}) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{Equation (3)}$$

Where r_u peak response from the sample solution

r_s peak response from the standard solution

C_S Concentration of USP primaquine phosphate RS in the standard solution (mg/ml)

C_u nominal concentration of primaquine phosphate in sample solution (mg/ml)

7.5. Thickness, Diameter, Hardness, Friability and Disintegration

The only dimensional factor involved in the compression of a tablet is its thickness. Typically, a micrometer is used to measure it. In addition to controlling for patient approval and simplifying tablet packing, the thickness should be within 5% of a defined value. The USFDA advises that the tablet's diameter should be 8 mm or less and 22 mm at the most. Tablets need to be strong enough

to resist the severe handling and transit they endure in the manufacturing facility, across the medication distribution chain, and outside, by patients and other end users. The force applied during production processes such as coating, packaging, and printing must not damage the tablets. Due to these factors, a range of tests are available to assess the compact's durability and how it will react when in touch with liquids like water. Disintegration, dissolution, tablet friability, and tablet breaking force are some of the tests included in this group (Pharmaeducation, 2021).

7.5.1. Measurement of diameter and thickness

Using a Mitutoyo® Absolute Micrometer Gauge, the diameter and thickness measurements of twenty randomly selected tablets were determined. The average diameter and thickness of the tablets was determined. For tablets with a diameter of 12.5 mm or more, the deviation from the average diameter shouldn't be more than 3%, and it shouldn't be more than 5% for tablets with a diameter of less than 12.5 mm. Each tablet's thickness shouldn't be more than 5% larger than the average thickness of 20 tablets (Salako *et al.*, 2020).

7.5.2. Hardness

The tablet samples to be tested were held between a fixed and moving jaw. Tablet orientation in diametral compression of round tablets without any scoring is unequivocal. Ten tablets from each sample were tested. The tablet is placed between the platens so that compression occurs across a diameter. The force applied to the edge of the tablet was gradually increased as the tester will be set to automatically measure the hardness by the movement of the indenter forward until the tablet breaks. Resistance of tablets to the applied force was used to measure the minimum and maximum force (newton) to crush each of 10 tablets Ph. Eur, (2008). The force at which each tablet crushed/damaged was recorded. The mean hardness was then determined by dividing the total hardness by the number of tablets USP (2022b). The hardness limits for tablets should lie in the 50–100 Newton (N) range (BP, 2000).

7.5.3. Friability

As per USP (2023d) recommendation, for tablets with a unit mass equal to or less than 650 mg, take a sample of whole tablets corresponding to 6.5 g. For tablets with a unit mass of more than 650 mg, take a sample of 10 whole tablets. The tablets were accurately weighed and placed in the drum of the friability tester and rotated at 100 revolutions for 4 mins. Clean the tablets of any loose

dust, then precisely weigh them. A single run of the test is typical. After being tumbled, the tablet sample fails the test if there are any plainly cracked, cleaved, or broken tablets. The test should be done again, and the mean of the three tests should be calculated, if the results are ambiguous or the weight loss exceeds the desired amount. For the majority of items, a maximum mean weight loss from the three samples of no more than 1.0% is regarded as acceptable (USP, 2023d). According to the USP, friability was evaluated as the percentage of weight loss as follows:

$$\% \, Friability = \frac{W1-W2}{W1} \times 100\% \quad \text{.....Equation (4)}$$

7.5.4. Disintegration Test

From the samples of each tablet brand, six tablets were randomly selected and placed into the six tubes of the basket rack assembly in a disintegration apparatus with distilled water kept at 37.25 °C. The disintegration duration of 15 minutes for uncoated tablets and 30 minutes for film-coated tablets and hard gelatin capsules, with no particle remaining on the basket after the allotted time, is a requirement for acceptance (USP, 2023e). The disintegration time was measured as the time it has taken for all six dose units to completely dissolve through the sieve, leaving only a soft mass in the basket having no pulpable firm core.

7.6. Sterility test

The test is applied to substances or preparations which, according to the pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating microorganism has been found in the sample examined in the conditions of the test. *Fluid thioglycolate* and *Soya-bean casein digest* culture media have been found to be suitable for the test of sterility. *Fluid thioglycolate medium* is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. *Soya-bean casein digest medium* is suitable for the culture of both fungi and aerobic bacteria. Preparation of the media was conducted according to the IP for both artemether injection samples and artesunate powder for injection samples. Sterility test was conducted for candida albicans and E.coli and direct inoculation method was employed. Sterility can be confirmed by incubation portions of the media for 14 days and the detection of no growth of microorganisms (Ph.Int., 2020).

7.7. Dissolution Test

Dissolution was performed at a single withdrawal point for every batch of tablet sample according to the specifications set by USP and IP. The dissolution was carried out using ERWEKA dissolution tester. Accordingly, six tablets of each brand/batch/sample of the investigated drugs were randomly taken. Dissolution profile was performed for generic brands available on each antimalarial tablet sample. The dissolution data were analyzed using the DDSolver® software.

Dissolution profile comparison

Model dependent methods, which involve fitting a particular dissolution curve to the data of each sample and comparing the parameters of the two fitted curves, or model independent methods, which compare the two profiles only at the observed time points, can be used to compare dissolution profiles (Freitag, 2001). To compare the dissolution profiles of the innovator and the generic brands available, a difference factor (f1) and a similarity factor (f2) were established. The difference factor (f1) measures the relative inaccuracy between the two curves by calculating the percent (%) difference between the two curves at each time point.

$$f1 = \{[\Sigma^{n}_{t=1} / R_t - T_t /] / \Sigma^{n}_{t=1} R_t]\} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation (5)}$$

Where (n) is the number of time interval points, (Rt) is the dissolution value of the innovator at time (t) and (Tt) is the dissolution value of the generic product under test at time (t).

The similarity factor (f2) is a logarithmic reciprocal square root transformation of the sum of squared error, it is a measurement or the similarity in the percent (%) dissolution amongst the two curves (Freitag, 2001).

$$f2 = 50 * Log \{[1 + (1/n) \Sigma^{n}_{t=1} (R_t - T_t)^2]^{-0.5} * 100 \ldots\ldots\ldots\ldots\ldots\ldots \text{Equation (6)}$$

Curves can be determined similar when (f1) values are close to 0, and (f2) close to 100. (f1) values from (0-15) and (f2) values from (50-100) certify similarity or bioequivalence of the two curves and the performance of the product under test with innovator product (Alzomor *et al.,* 2022).

7.7.1. Dissolution profile of Artemether

Calibration curve for Artemether dissolution test method. The seven concentration levels 0.004, 0.008, 0.012, 0.016, 0.02, 0.024, 0.028 mg/ml were then prepared from the stock by diluting 1, 2, 3, 4, 5, 6, and 7 mL of the stock solution to 50 mL with medium (distilled). Their peak area was assessed using HPLC. Besides, concentrations of artemether against peak area were plotted to obtain the calibration curves.

The dissolutions of all brands of artemether were evaluated using a dissolution apparatus type II (paddle) following the USP Salmous standards guideline (The USP Convention, 2009). The temperature and the paddle rotation speed were set to 37 ± 0.5°C and 100 rpm for 3hours and 30minutes, respectively. Artemether lumefantrine tablets from each brand were randomly assigned to the six dissolution vessels. Five (5.0) mL samples were withdrawn at predetermined time points (15, 30, 45, 60, 90,120, 150, 180,195 min). And replaced with an equal volume of fresh dissolution medium at the same temperature. The quantity in mg of artemether dissolved was calculated by the formula

$$\frac{ru \times Cs \times 1000 \times 100}{rs \times L} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{Equation (7)}$$

Where r_u and r_s are the peak responses obtained from the test solution and the standard solution, respectively; Cs is the concentration, in mg per ml, of artemether in the standard solution; 1000 is the volume, in mL, of medium; 100 is the conversion factor to percentage; L is the Tablet label claim for Artemether, in mg.

Tolerances- Not less than 45%(Q) of the labeled amount of artemether is dissolved in 1 hour, and not less than 65% (Q) of the labeled amount of artemether is dissolved 3hours. (Q) Is the amount of dissolved active ingredient specified in the individual monograph, expressed as a percentage of the content stated on the label (WHO, 2011).

7.7.2. Dissolution profile of Lumefantrine

Calibration curve for Lumefantrine dissolution profile. The five concentration levels 0.0128, 0.0144, 0.016, 0.0176, 0.0196 mg/mL were then prepared from the stock by diluting 1.56, 1.76, 1.95, 2.15, and 2.38 mL of the stock solution to 25 mL with medium (distilled). Their absorption was assessed using UV-Visible spectroscopy at wavelength of 342 nm. Besides, concentrations of Lumefantrine against absorption were plotted to obtain the calibration curves.

The dissolutions of lumefantrine tablets were evaluated using a dissolution apparatus type II (paddle) following the (The USP Convention, 2009). The temperature and the paddle rotation speed were set to 37 ± 0.5°C and 100 rpm for 1 hour and 10 minutes, respectively. Artemether lumefantrine tablets from each batch were randomly assigned to the six dissolution vessels and five (5.0) mL samples were withdrawn at predetermined time points (5, 15, 30, 45, 60 and 65 min) and replaced with an equal volume of fresh dissolution medium at the same temperature. And the samples were analyzed at wavelength of 342 nm spectrophotometrically (The USP Convention, 2009).

Test solution. Pass a portion of the solution under test through a suitable 0.45µm syringe filter. Considering complete dissolution of the tablet label claim. The solution was diluted quantitatively, and stepwise, if necessary, with medium to obtain a solution having a concentration of about 0.12 mg per mL of concentration for single point dissolution and 0.016 mg/mL for dissolution profile. The percentage of lumefantrine dissolved was calculated by the formula:

$$\frac{A_u \times C_S \times 1000 \times 100}{A_S \times L} \quad \text{.....Equation (8)}$$

Where:

Au is Absorbance of the sample solution

Cs refers to Concentration of the reference standard solution (mg/mL)

As refers to absorbance of the reference standard

L refers to label claim (mg/tablet)

Tolerances: Not less than 60% of the labeled amount of $C_{30}H_{32}Cl_3NO$ dissolved in 45 minutes (The USP convention, 2009).

7.7.3. Dissolution profile of Primaquine Phosphate Tablets

Calibration curve for Primaquine Phosphate dissolution test method. A series of six concentration levels 0.0016, 0.0032, 0.0048, 0.0064, 0.008 and 0.0096 mg/mL were then prepared from the stock by diluting 1, 2, 3, 4, 5, 6, and 7 mL of the stock solution to 50 mL with medium (distilled). Their peak area was assessed using HPLC. Besides, concentrations of Primaquine Phosphate against peak area were plotted to obtain the calibration curves.

The dissolutions of all brands of primaquine Phosphate tablets were evaluated using a dissolution apparatus type II (paddle) following the United States Pharmacopoeia protocol (USP, 2023b). Tablets from each brand were randomly placed in to six dissolution vessels. Five (5.0) mL samples were withdrawn at predetermined time points (5, 10, 15, 20, 30, 45, 60, 65 and 70 min).). And replaced with an equal volume of fresh dissolution medium at the same temperature.

The peak area of each sample was determined at 254 nm using a UV detector. A 0.01 N HCL was used as the blank solution. The percentage quantity in mg of primaquine phosphate ($C_{15}H_{21}N_3O{\cdot}2H_3PO_4$) dissolved was calculated by the formula:

$$\frac{r_u}{r_s} \times \frac{C_s}{L} \times V \times D \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{Equation (9)}$$

Whereby:

r_u is peak response of primaquine from the sample solution

Rs is peak response of primaquine from the standard solution

Cs concentration of USP primaquine phosphate RS in the standard solution (mg/mL)

L is label claim (mg/tablet)

V is volume of the medium

D is dilution factor for the sample solution, if applicable

Tolerances: NLT 80% (*Q*) of the labeled amount of primaquine phosphate ($C_{15}H_{21}N_3O{\cdot}2H_3PO_4$) is dissolved at 60 minutes.

7.7.4. Dissolution profile of Chloroquine Phosphate Tablets

Calibration curve for dissolution test of chloroquine phosphate method. In order to create a stock solution, 28 mg of USP chloroquine phosphate RS was dissolved in 100 mL of medium (water). The stock solution was then diluted by 2, 4.5, 2.5, 5.5, and 3 mL to 50 mL with medium to produce concentrations of 0.0112, 0.0126, 0.014, 0.0154, and 0.0168 mg/mL. Spectrophotometric analysis was used to determine their absorbances. Additionally, the calibration curves were obtained by plotting chloroquine phosphate concentrations against absorbance.

Following the recommendations of the USP, the dissolutions of chloroquine phosphate tablets were assessed using a dissolution apparatus type II (paddle) (USP, 2023a). Each batch of chloroquine phosphate tablets had six dissolving vessels randomly assigned to them. At specified intervals (5, 15, 30, 45, 60, and 65 min), five (5.0) mL samples were taken out and replaced with an equivalent volume of fresh dissolution medium at the same temperature. With a 0.45 µm syringe filter, the samples (5.0 mL) were immediately filtered, and the filtrate was employed in the analysis of the dissolution profile of chloroquine phosphate tablets. Then the absorbance of these samples was assessed using UV spectrophotometer on 343 nm wavelength. The quantity in mg of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$) dissolved was calculated by the formula:

$$\frac{A_u \times C_S \times 1000 \times 100}{A_S \times L} \quad \text{.....Equation (10)}$$

Whereby:

Au is Absorbance of the sample solution

Cs refers to Concentration of the reference standard solution (mg/mL)

As refers to absorbance of the reference standard

L refers to label claim (mg/tablet)

Tolerance: NLT 75% (*Q*) of the labeled amount of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$) is dissolved at 45 minutes.

7.8. Data quality control

The consistency and completeness of the data were examined. System suitability tests, method linearity (calibration curve), and careful adherence to the steps outlined in the specified monographs of the pharmacopeia were all performed to ensure the quality of the experimental results. Laboratory experiments were performed in an ISO 17025 accredited laboratory following their standard operating procedures in each experiment.

7.9. Data management and analysis

The coded data were entered into a Microsoft excel 2016 worksheet. Then it was analyzed by using excel, DD Solver and Origin software. Descriptive statistics was used to summarize the data. The mean, standard deviation, and relative standard deviation (RSD) were used in the quality determination of artemether lumefantrine tablets, artemether injection, artesunate powder for injection, chloroquine and primaquine phosphate tablets.

8. Ethical approval

Ethical clearance was obtained from the Ethical Review Board of Addis Ababa University, College of Health Sciences. Ref.No (ERB/SOP/471/14/2022)

9. Results and Discussion

9.1. Sample information

A total of 52 samples were collected during July, 2022 from retail outlets comprising of one pharmacy, 27 drug stores and one rural drug shop located in six woredas (Figure 8). Of the 52 collected samples, 46.15 % (24/52) were ACT's (artemether + lumefantrine), 19.23% (10/52) artesunate powder for injection, 17.3 % (9/52) artemether injection, 9.61 % (5/52) chloroquine phosphate Tablets and 7.6 % (4/52) primaquine phosphate. The drug samples packaging was noted, along with the place of origin, shelf life, and other pertinent information.

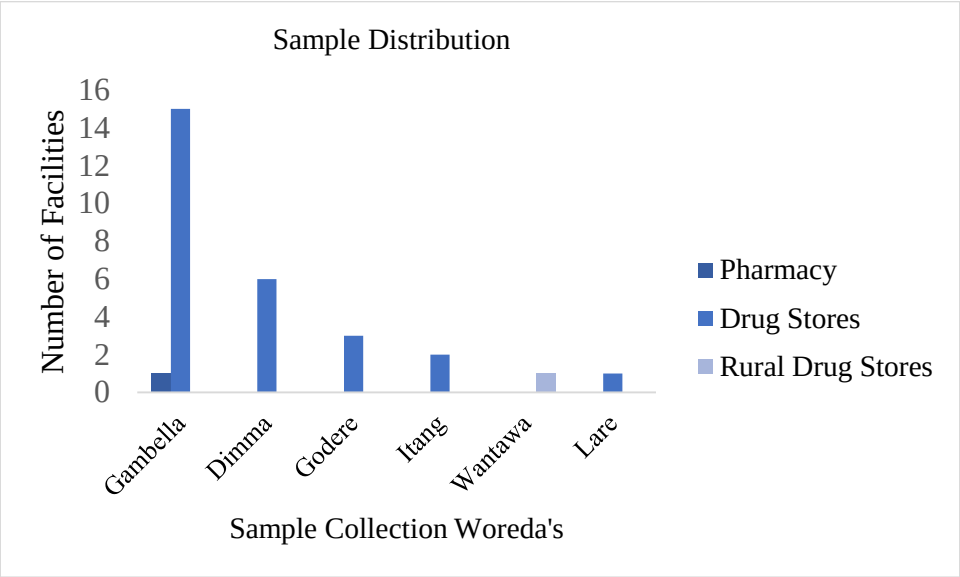


Figure 8. Distribution of sample collection sites

The highest number of samples were originated from India and the least from Germany. Figure 9 shows the percentage distribution for the country of origin of the collected samples in the form of pie chart. The World Bank found out during an audit that the malaria medications it had purchased in India from a local manufacturer were clinically substandard, according to an internal report that was released by the Wall Street Journal (Bate *et al.*, 2008).

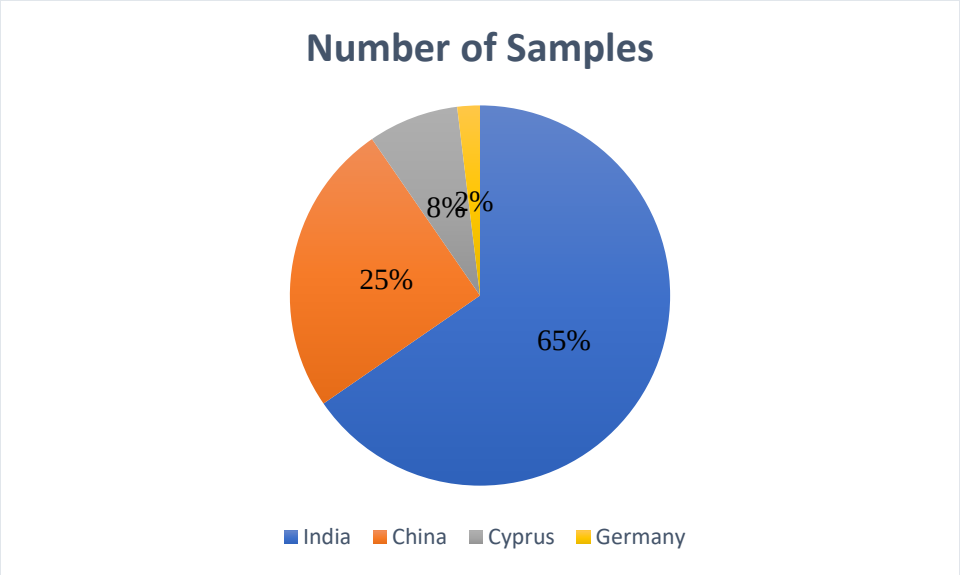


Figure 9. Country of origin for the collected samples

9.2. Physical characteristics, packaging and labelling information

Using a checklist developed by the WHO/ FIP/USP, all the samples were visually examined for tablet physical characteristics (such as shapes, colors, breaks, cracks, and splits), packaging, and labeling information (such as the name of the API, the country of origin, the manufacturer, expiration date, manufacturing date, batch/lot number, number of units per strip/package, and the labelled dose/strength of the active ingredient). The results of visual inspection of packaging and labelling information on the tested brands of artemether lumefantrine, artesunate injection, chloroquine phosphate tablets and primaquine phosphate tablets did not show any signs of counterfeit products as defined by the joint WHO/ FIP/USP checklist of visual inspection tool (Annex 1). The packaging of the medicines under investigations corresponded with the WHO recommendations for pharmaceutical product packaging (WHO, 2002). Opposing to this result, studies showed damp packages and blue-stained chloroquine and primaquine samples (Nogueira *et al.*, 2011) and lack of chloroquine leaflets (Abuye *et al.*, 2020). About 2% of artemether–lumefantrine samples failing the visual assessment because they had different shelf life than other samples of the same brand name (Mufusama *et al.*, 2018).

In this study, 8 artemether injection products (88.8%) were presented in clear glass ampoules, with only one product (11.1%) in an amber colored ampoule. Moreover, two-third (6/9) of artemether injection samples did not declare the vehicle used for the injectable formulation (Annex 2).

Similar to our study, a post-marketing surveillance done on artemether injections marketed in Southwest Nigeria indicated that the majority of the samples examined were packaged in plain ampoules, and about 81.8% of the samples lacked information about the formulation's oil basis (Hassan *et al.*, 2020).

In order to protect pharmaceutical items from environmental and transportation stress, which are risk factors for product quality issues, appropriate packaging is crucial. As a result, there may not be a risk of stability issues brought on by packaging for the products under investigation (Angeli and Trezza, 2009).

Artemisinin derivatives' endoperoxide bridge is a potential site for photochemical reaction (Hassan *et al.*, 2020). After exposure to UV radiation, a pilot investigation on the artesunate, another artemisinin derivative, found photochemical degradation with loss of anti-plasmodial action (Batty *et al.*, 1996). In order for an artemether injection to be accepted as being of high quality, it must meet both the general requirements for parenteral preparations and additional, product-specific quality standards. First, an ampoule that is amber in color or another container that provides enough light protection should be used to package artemether injection (Ph.Int., 2020). Additionally, each product's label should include information about the oil base utilized as a vehicle in the formulation. The information on the oil base is crucial since, if Arachis oil is included in the formulation, the recommended HPLC method to screen for the presence of related chemicals may not be appropriate (Ph.Int.,2020).

Only 25% of the products in this study were registered on EFDA electronic registration information system (ERIS). This result is in line with a surveillance done by EFDA's MQCL on the quality of antimalarials showing 34 % unregistered medications (The President's Malaria Initiative, 2013). A systematic review indicated 47 researches on the incidence of unlicensed/unregistered drugs, however none of them included details on why a drug was not approved but was nevertheless utilized (McManus and Naughton, 2020). Unregistered/unlicensed medical items that have not been evaluated and/or approved by the National or Regional Regulatory Authority for the market in which they are distributed, used, or sold, according to the

restrictions permitted by national or regional law and legislation are included in the 2017 WHO definition of poor-quality medicines (WHO, 2017c). Despite advancements in medicine regulation and advice from professional groups, there is evidence suggesting that low levels of implementation of specified standards for unregistered medications. This results in the raise of the possibility of exposing the local population to potentially dangerous medical items of varying efficacy leading to an increased incidence of adverse drug reactions (Sutherland and Waldek, 2015).

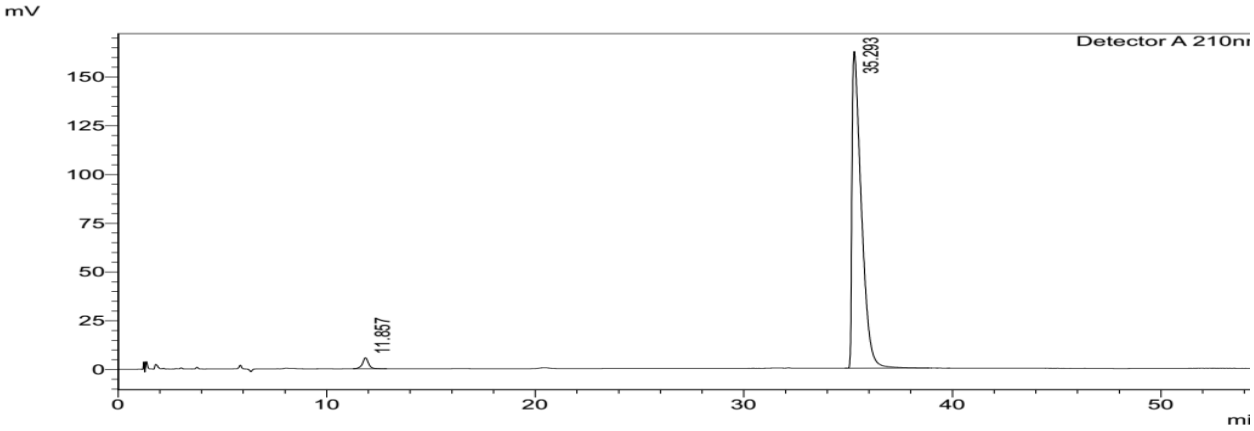
The prevalence and impact of unregistered medicines are greater in low- and middle-income countries due to less developed regulatory mechanisms, financial constraints, and human resource shortages (Ndomondo-Sigonda *et al.,* 2017).

Generally, visual inspection is a useful screening strategy for identifying low-quality medications, particularly those that have been tampered with to appear genuine or be packaged differently. One of the restrictions is that in order to compare sample and reference products, the manufacturer's original packaging is necessary (Dégardin and Roggo, 2015). Visual inspection of container and labeling is crucial when evaluating whether a certain type of medicine is substandard, damaged, or fake. On the other hand, some products come in phony packaging despite having real active pharmacological components (Newton *et al.,* 2006).

9.3. Identification

9.3.1. Artemether Lumefantrine Tablets

All artemether lumefantrine tablet samples analyzed displayed retention times corresponding with that of the respective standards (Figures 10 and 11), indicating the tested artemether and lumefantrine products contain the correct APIs.

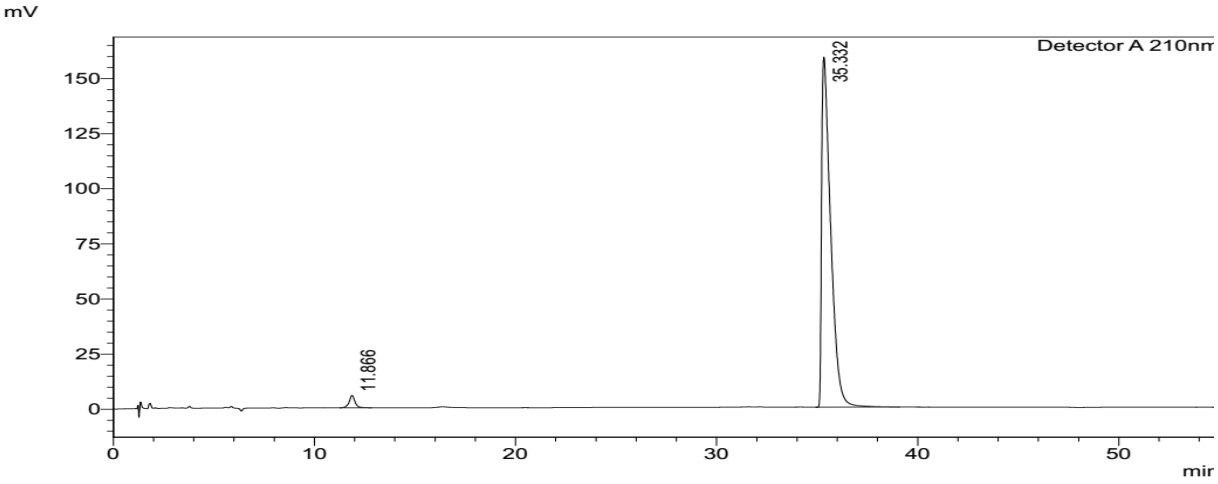


<Peak Table>

Detector A 210nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	11.9	113258	1.02	9394	--
2	35.3	5071046	2.81	29936	35.911
Total		5184304			

Figure 10. HPLC chromatogram of artemether lumefantrine USP RS



<Peak Table>

Detector A 210nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	11.9	115103	1.03	9146	--
2	35.3	4973587	2.72	30014	35.770
Total		5088690			

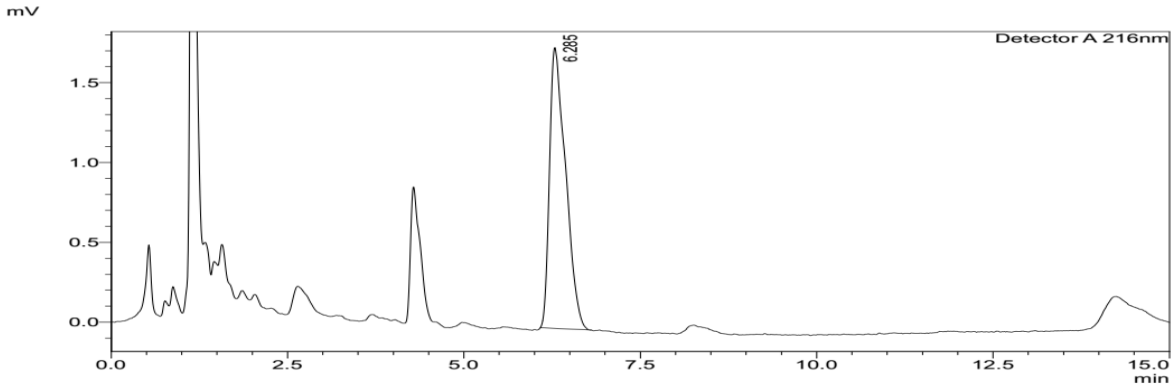
Figure 11. HPLC chromatogram of artemether lumefantrine tablets (Artem001)

From the above two figures it can be seen that the USP RS of artemether and lumefantrine peaks showed a retention time of 11.9 minutes and 35.3 minutes for artemether and lumefantrine respectively. All samples had the desired APIs, as demonstrated by the positive identification test findings. The findings of the identity test showed that none of the samples had erroneous APIs.

This result is in line with the study conducted in Jimma by Belew *et al., (*2019) on quality of fixed dose artemether/lumefantrine products. And another study on the impact of substandard and falsified antimalarials in Zambia indicated a similar results Jackson *et al.*, (2020). However, a Gabonese study done by Visser *et al.* (2015), API was absent in the sample of Coartem ® batch no. F2261, according to the analysis no peak on the spectra was detected.

9.3.2. Artemether Injection

All of the samples contained the API specified on the label, as shown by a comparison of the retention time (6.3 minutes) from the HPLC chromatogram (Figure 12) for the RS of artemether with that of the examined products. (e.g., Figure 13 for Artem-Inj-004).

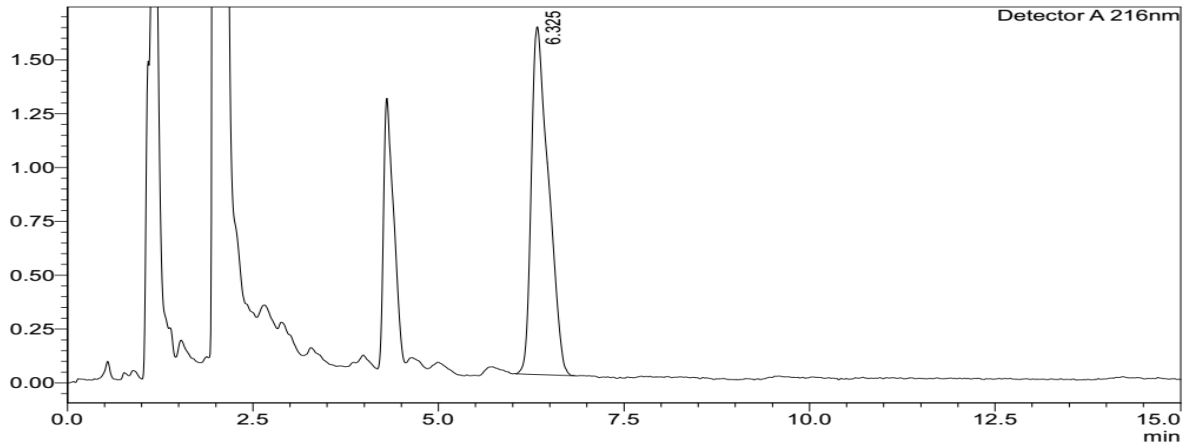


<Peak Table>

Detector A 216nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	6.3	27782	1.68	3086	--
Total		27782			

Figure 12.Chromatogram of Artemether Reference standard



<Peak Table>

Detector A 216nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	6.3	25927	1.57	3312	--
Total		25927			

Figure 13.Chromatogram of Artemether Injection (Artem-Inj-004)

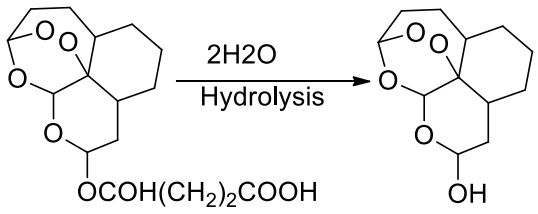
A Nigerian study also indicated that the identity tests performed on artemether injections using color test and HPLC retention times of the sample and reference standard established that the investigated products did contain the correct API (Hassan *et al.,* 2020). Quantitative testing on all the different brands of artemether injections used to prove the presence of artemether in the artemether injections indicated the presence of artemether in the collected samples of artemether injections sold in the Cape Coast Metropolis (Prah *et al.*, 2016).

9.3.3 Artesunate Injection

The color (Figure 14) chemical reaction between an artesunate powder for injection and vanillin/sulphuric reagent produced a bright red color distinct to artesunate (Ph. Int.,2020), confirming that the products had API.

Figure 14. Color identification of Artesunate powder for Injection

The primary mechanism underlying the reaction between artesunate and vanillin/sulphuric reagent is shown on Figure 15. And that is the hydrolysis of artesunate using concentrated sulphuric acid to produce dihydroartemisinin (Lactol). The alcohol dihydroartemisinin then reacts with the carbonyl component vanillin to produce a condensation product called a hemiacetal compound. Concentrated sulfuric acid is used to catalyze the process. The most plausible mechanism for this reaction is a nucleophilic addition reaction catalyzed by an acid. The creation of a positive charge in a carbonyl molecule begins with a proton attaching to the carbonyl oxygen. The positively charged carbon center is then joined by a DHA (DihydroArtemisinin) alcohol molecule to create a hemiacetal group (Attih *et al.*, 2015).

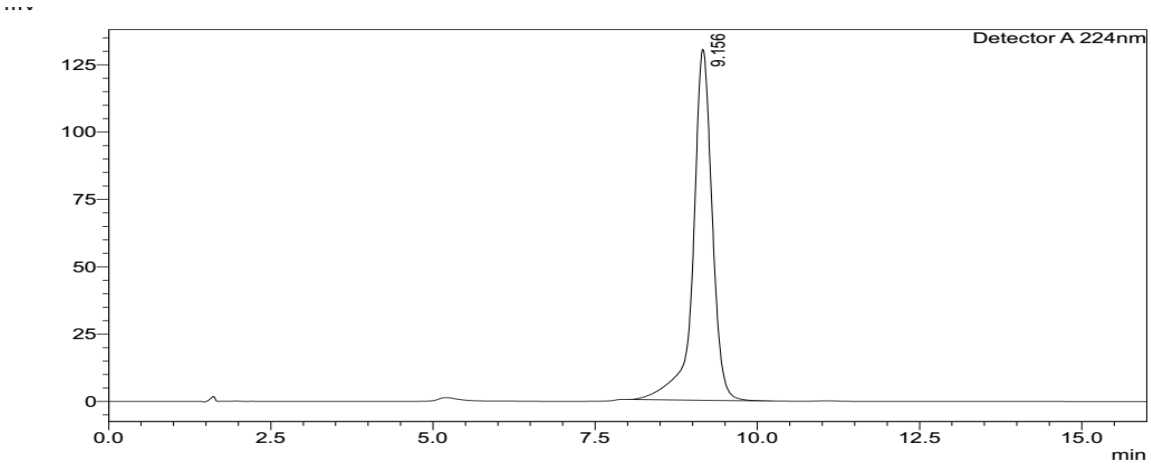


Artesunate **Dihydroartemisinin**

Figure 15. Artesunate conversion to Dihydroartemisinin

9.3.4. Chloroquine Phosphate tablets

A retention time of 9.2 minutes for both the reference standard and the sample solution on the chromatogram shown on Figure 16 and Figure 17 shows a positive result for the identification test. All of the chloroquine phosphate samples that were examined showed retention times that were consistent with the matching standards, revealing the presence of API.

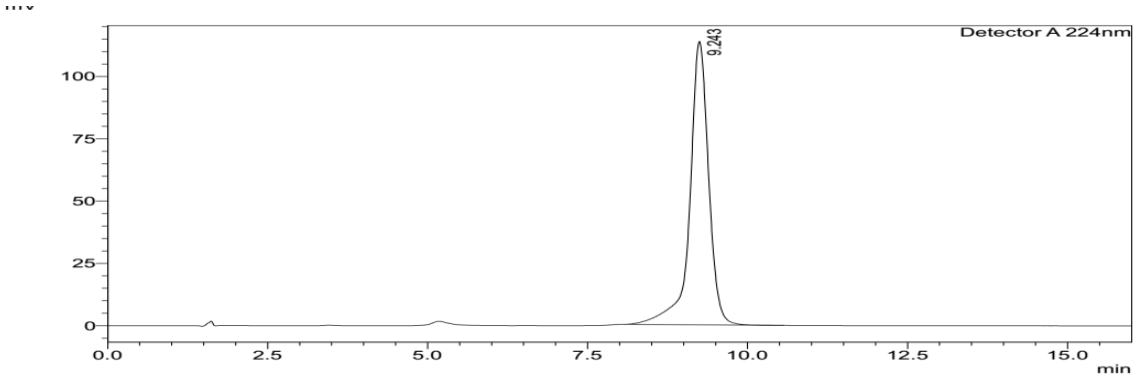


<Peak Table>

Detector A 224nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	9.2	2661068	0.84	5503	--
Total		2661068			

Figure 16.HPLC chromatogram of Chloroquine reference standard



<Peak Table>

Detector A 224nm

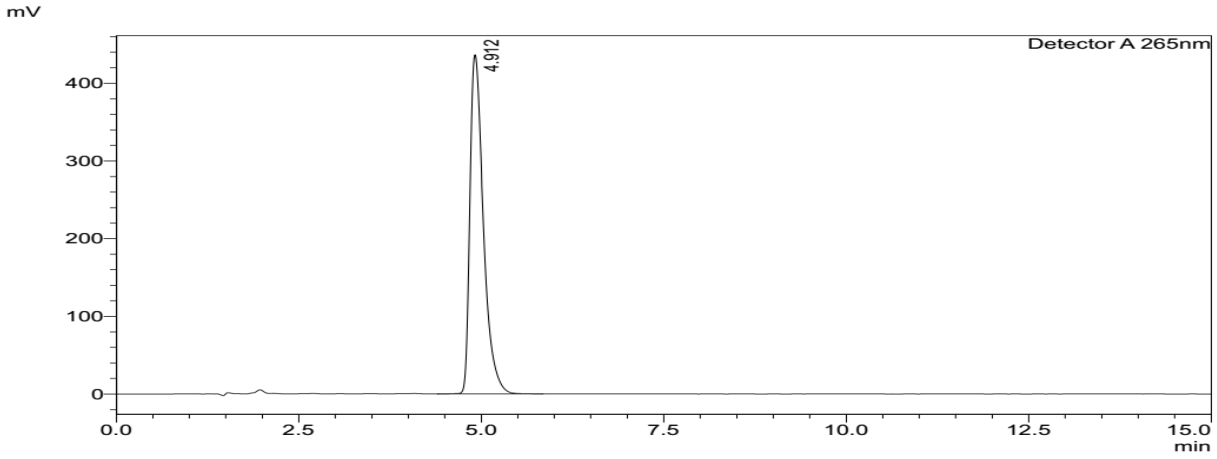
Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	9.2	2337507	0.84	5561	--
Total		2337507			

Figure 17. HPLC Chromatogram of Chloroquine phosphate tablets (Chlor001)

Similar to our study, Abuye *et al.*, (2020) reported that all investigated quinine sulfate and chloroquine phosphate samples were positive for identification tests.

9.3.5. Primaquine Phosphate Tablets

The retention time (4.9 minutes) of the major peak of the sample solution (Figure 19) corresponds to that of the standard solution (Figure 18).

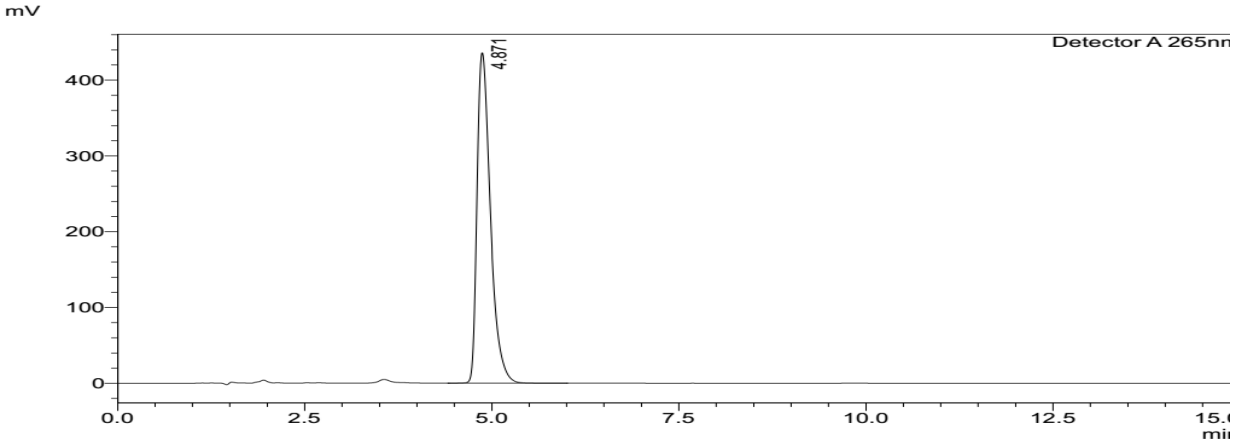


<Peak Table>

Detector A 265nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	4.9	5638113	1.65	3466	--
Total		5638113			

Figure 18.HPLC chromatogram of Primaquine USP RS



<Peak Table>

Detector A 265nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	4.9	5446249	1.59	3608	--
Total		5446249			

Figure 19.HPLC chromatogram of Primaquine phosphate tablets (Pri-Gam-001)

Even though this test revealed no issues related to identification test, results from other studies revealed antimalarials without the labeled API. Identification of quinine sulfate samples showed that four batches did not contain quinine sulfate; and one batch had an active ingredient other than quinine sulfate (Mahano *et al.*, 2021). Another case study also exposed an artesunate with no API which resulted in a treatment failure (Chaccour *et al.*, 2012).

9.4. Uniformity of Weight

9.4.1. Artemether Lumefantrine Tablets

Table 4 indicates that the uniformity of weight of the artemether lumefantrine tablets ranging from 238.73 ± 2.67 to 354.57 ± 3.64 ($\bar{x}$ (gm) ±SD).

Table 4.Uniformity of mass of Artemether Lumefantrine Tablets

Sample ID	**Brand name**	**Country of manufacture**	**$\bar{x}$(gm)**	**SD**	**Minimum**	**Maximum**	**Remarks**
Artem001	Artem/ Lum	India	248.22	2.04	244.7	251.7	Passed
Artem002	Artem/ Lum	India	250.46	3.37	243.4	257.6	Passed
Artem003	Artem/ Lum	India	248.45	2.07	244.3	252.2	Passed
Artem004	Artem/ Lum	India	248.17	2.68	242.2	251.7	Passed
Artem005	Artem/ Lum	India	245.04	3.06	238.3	253.1	Passed
Artem006	Artem/ Lum	India	252.89	3.47	242.7	261.6	Passed
Artem007	Artem/ Lum	India	250.68	3.01	242.6	256.1	Passed
Artem008	Artem/ Lum	India	251.51	2.08	247.3	254.9	Passed
Artem009	Artem/ Lum	India	248.9	1.33	246.1	250.8	Passed
Artem010	Artefan	India	238.73	2.67	234.1	242.9	Passed
Artem011	Artefan	India	241.52	2.56	236.9	245.1	Passed
Artem012	Artefan	India	240.63	2.17	237.1	243.7	Passed
Artem013	Artefan	India	240.72	1.22	238.9	246.4	Passed
Artem014	Artefan	India	238.85	1.06	237.3	240.5	Passed
Artem015	Artefan	India	241.44	1.57	239.2	244.4	Passed
Artem016	Lumiter	India	253.97	2.93	245	259.3	Passed
Artem017	Lumiter	India	253.33	1.94	248.8	258.5	Passed
Artem018	Artefan Dispersible	India	351.18	2.72	347.8	356.5	Passed
Artem019	Artefan Dispersible	India	352.87	3.25	320	397.7	Passed
Artem020	Artefan Dispersible	India	354.57	3.64	349.2	359	Passed
Artem021	Artefan Dispersible	India	351.74	2.21	347.8	358.1	Passed

Artem022	Artefan Dispersible	India	354.31	2.74	349.5	358.5	Passed
Artem023	Comether	China	244.54	1.95	241.9	251.1	Passed
Artem024	Lonart	India	247.19	2.48	242.4	251.7	Passed

The results of the % mass deviation distributed among the brands of artemether-lumefantrine products are shown in Table 5. The mean weight of the twenty tablets sampled was lowest for Artem010 (±2.67) and highest for Artem020 (±3.64), while the standard deviation, was lowest for Artem014 and highest for Artem006 (±3.47). Artem006 had the least uniform brand and the biggest dispersion/clustering of sample weight around the mean weight, whereas Artem014 had the best uniformity of weight variation. However, 90% of the evaluated items were more consistently uniform, and the percent deviation of all samples was lower than the upper acceptability limits.

Table 5.Results of % mass deviation distributed among brand products of artemether lumefantrine tablets.

	% Mass Deviation		
Brand Name	**Minimum**	**Maximum**	**Mean**
Artemether/Lumefantrine	1.33	3.47	2.56
Artefan	1.06	2.67	1.87
Lumiter	1.94	2.93	2.43
Artefan Dispersible	2.21	3.64	2.91
Comether	1.95	1.95	1.95
Lonart	2.48	2.48	2.48

The uniformity of weight test's goal is to ensure that each batch is uniform, which ultimately represents the consistency of the drug content in all batches of formulation. The findings of this study on the uniformity of mass of artemether-lumefantrine is in line with a study conducted in Jimma by Belew *et al.*,(2019). A study done by Prah *et al.*, (2016) also indicated that the percentage weight deviation of the various brands of artemether-lumefantrine tablets from their respective mean weights was less than 10%.

9.4.2. Artemether Injection

8.4.2.1. Extractable Volume of Artemether Injection

The extractable volume analysis's specifics are displayed in Table 6. Compared to the anticipated extracted volume of 3 mL from 3 ampoules, the extractable volumes of five items (55.5%) were much lower.

Table 6. Extractable volume of Artemether injection

Sample ID	**Volume of extractable Injection**				
	1st	**2nd**	**3rd**	**Total Volume**	**Remarks**
Artem-Inj 001	0.89	1.1	1.1	3.09	Passed
Artem-Inj 001	1	1.1	0.9	3	Passed
Artem-Inj 002	1	1	1	3	Passed
Artem-Inj 003	0.9	0.9	0.9	2.7	Failed
Artem-Inj 004	1.1	0.9	1.0	3	Passed
Artem-Inj 005	0.9	0.9	0.8	2.6	Failed
Artem-Inj 006	0.9	0.8	0.8	2.5	Failed
Artem-Inj 007	1.1	1	1	3.1	Passed
Artem-Inj 008	0.8	0.9	0.9	2.6	Failed
Artem-Inj 009	0.9	1.1	0.7	2.7	Failed

Each container of an injectable product must be filled with a volume that slightly exceeds the labeled content, according to the USP general chapter on injections. The extra volumes are intended to be enough to enable withdrawal and administration of the volumes specified on the label. According to FDA regulations at 21 CFR 201.51(g), the declaration of net quantity of contents on the label is considered to express the minimum quantity of contents for drugs in ampules or vials that are intended for injection, and it further requires that variations above the stated measure must comply with the excess volumes set forth in USP. The term "allowable extra volume" (sometimes known as "overfill") should not be confused with the term "overage," which is covered in a different set of guidelines. A single dose injection is anticipated to have more volume than the labeled amount in order to facilitate the drawing of precise amounts during administration (USFDA, 2015).

The results of this study showed a higher percentage of failure than the study carried out in southwest Nigeria which showed a 27.3% of shortfall in the extractable volume of an artemether injection from each ampoule of the goods under investigation. This result raises the likelihood that GMPs manufacturing protocols weren't followed. When using the assessed artemether injection products, accurate dosage may not be possible due to nonconformity with the need for extractable volume analysis (Hassan *et al.*, 2020). A reduced extractable volume may cause an inconsistent dose of artemether to be delivered, which may ultimately entail a poor therapeutic outcome. Given the advent of resistance to the artemisinin-based regimen in some areas of the world, the subtherapeutic dose is quite concerning (Ouji *et al.*, 2018).

9.4.3. Artesunate Powder for injection

The uniformity of mass for artesunate powder for injection was conducted according to the (Ph. Int., 2020). Table 7 shows that all the artesunate samples complied with the criteria set for uniformity of mass for single dose preparation with a SD range 1.03 to 14.34.

Table 7. Uniformity of dosage forms of artesunate powder for injection

Sample ID	Brand Name	Country of manufacturer	x̄(gm)	SD	min	max	Remarks
Artesun001	Artesunate injection	India	58.99	1.65	54.78	61.32	Passed
Artesun002	Artesun	China	59.09	3.65	45.74	64	Passed
Artesun003	Artesun	China	59.58	2.39	54.78	64.8	Passed
Artesun004	Artesun	China	58.91	3.34	47.89	64.1	Passed
Artesun005	Artesun	China	60.14	2.87	51.39	64.78	Passed
Artesun006	Artesun	China	60.39	1.59	56.94	64.78	Passed
Artesun007	Artesun	China	61.15	2.78	51.47	64.89	Passed
Artesun008	SCOSUNATE-60	India	61.69	1.77	59.97	64.89	Passed
Artesun009	Artemark	India	61.29	1.03	59.14	63.14	Passed
Artesun010	GSUNATE 60	India	56.51	14.34	14.67	63.12	Passed

9.4.4. Chloroquine Phosphate Tablets

The acceptance value (AV) found on the result ranges from 2.89 to 8.48 and all the chloroquine phosphate tablet samples met the uniformity of dosage criteria set by the USP. Table 8 shows the AV for the five chloroquine phosphate tablet samples. The SDs for randomly selected twenty tablets weighed per batch for each generic product varied from 2.47 % to 10.63 %.

Table 8. Uniformity of mass for chloroquine phosphate tablets

Sample ID	Brand name	Country of manufacturer	x̄(gm)	SD	Min	Max	AV (NMT 15)	Remark
Chlor001	Chloroquine	India	309.76	5.25	300.2	318.6	4.07	Passed
Chlor002	Chloroquine	India	315.47	2.47	300.2	318.9	2.89	Passed
Chlor003	Chloroquine	India	310.8	4.67	304.1	320.5	4.26	Passed
Chlor004	Chloroquine	India	312.13	6.55	302	318.7	5.07	Passed
Chlor005	Chloroquine	India	302.57	10.63	283	315	8.48	Passed

A similar study on the quality assessment of chloroquine phosphate tablet samples conducted in Ethiopia by Abuye *et al.*, (2020) showed a different result indicting a 6.8% of chloroquine phosphate tablet samples failing to meet the USP acceptance criteria for weight uniformity. The RDS for randomly selected twenty tablets weighed per batch for each generic product varied from 1.25% to 4.15%. As per the USP-2015, the weight variation limit for the tablet which is weighting 134 and 300 mg is 7.5% (Abuye *et al.*, 2020). In contrast, similar results were noticed from an Indian study demonstrating that none of the chloroquine brands assessed on the study exhibited a percent deviation in weight greater than 5%, (SD with a range of 2.5 to 11.5) as required by the British Pharmacopoeia, concluding all brands shown sufficient uniformity of weight (Patel *et al.*, 2005).

9.4.5. Primaquine Phosphate Tablet

The uniformity of weight of the primaquine phosphate tablets range from 83.22± 1.02 to 84.84 ± 0.90 (x̄ (gm) ±SD). Table 9 shows the mass in gram and SD of the four primaquine phosphate tablet samples.

Table 9. Uniformity of mass of Primaquine Phosphate Tablets.

Sample ID	Brand	Country of manufacturer	x̄(gm)	Minimum	Maximum	SD of wt	Remarks
Pri-Gam-001	Primaquine Phosphate	Cyprus	83.82	82	85.7	0.96	Passed
Pri-Gam-002	Primaquine Phosphate	Cyprus	83.22	82.2	85.1	1.02	Passed
Pri-Gam-003	Primaquine Phosphate	Cyprus	83.68	82.8	85	0.79	Passed
Pri-Gam-04	Primaquine Phosphate	Cyprus	84.84	83.8	86.7	0.90	Passed

The primaquine phosphate tablets that were subjected to analysis were generic, meaning that the items were manufactured using the same active components, excipients, and/or manufacturing

processes by several manufacturers. The mean weights of the four primaquine samples are quite similar. Hence all the four primaquine Phosphate samples comply with regard to uniformity of dosage units (Ph.Int.,2020).

However, changes in weight may be caused by the use of different proportions of the same excipient type.

It might also be the result of flawed formulation and production procedures, such as incorrect API and excipient weighing, insufficient component mixing, and adjustments to the force used to compress tablets. Additionally, variations can occur as a result of formulation procedures that do not adhere to good manufacturing practices (GMP) (Llusa *et al.,* 2013; Abuye *et al.*, 2020). These variations can affect the content of the tablet. A change in the weight of the tablets may indicate that the API content of the drug products has changed. When taking such formulations, patients are exposed to pharmacodynamic and pharmacokinetic variations (Abraham *et al.,* 2021).

Generally, the study's findings showed that the tested products complied with the mass uniformity requirements set forth in the USP and IP for uncoated and film-coated tablets.

9.5. Assay Results

9.5.1. Artemether Lumefantrine 20mg/120mg tablets

9.5.1.1. System Suitability

The results for system suitability are presented in Table 10 which shows a % RSD of 0.508 and 0.485 for the five replicate injections of artemether USP RS and lumefantrine USP RS respectively.

Table 10. System suitability parameters for Artemether Lumefantrine tablets

System Suitability Parameters for Artemether				
Standard Injections	**Peak Area**	**Tailing Factor**	**TPN(N)**	**Retention Time**
Reference Standard 1st Inj.	141834	0.98	2217.034	13.02
Reference Standard 2nd Inj.	142748	0.99	2214.237	12.995
Reference Standard 3rd Inj.	142303	1.00	2144.82	12.97
Reference Standard 4th Inj.	141575	1.00	2107.32	12.954
Reference Standard 5th Inj.	143385	0.99	2084.921	12.938
Mean Value	142369	0.99	2153.6664	12.9754
RSD	0.508563139	0.784691538	2.80872436	0.251485683
Limit	**NMT 2.0%**	**NLT 1.5**	**NA**	**NA**
System Suitability Parameters for Lumefantrine				
Standard Injections	**Peak Area**	**Tailing Factor**	**TPN(N)**	**Retention Time**
Reference Standard 1st Inj.	6989452	1.98	59646.47	33.209
Reference Standard 2nd Inj.	7085862	1.95	59853.354	33.2
Reference Standard 3rd Inj.	7038095	1.98	59930.544	33.181
Reference Standard 4th Inj.	7038209	1.97	60268.313	33.183
Reference Standard 5th Inj.	7042490	1.98	60660.544	33.183
Mean Value	7038821.6	1.694	60071.845	33.1912
RSD	0.485147042	0.598219822	0.6626437	0.037894858
Limit	**NMT 2.0%**	**NA**	**NA**	**NA**
Resolution: Limit NLT 3.5	**Result: -8.6**			

The results of amount of artemether and lumefantrine in the fixed dose combination artemether-lumefantrine tablet samples revealed that all samples complied with the pharmacopeial acceptance specification limit (i.e., 90.0 – 110.0% (% label claim). Table 11 shows that the amount of artemether API in samples analyzed ranges from 95.4 ± 2.3 to 106.3 ± 0 ($\bar{X}$:100.85%, RSD:7.75%) while that of lumefantrine ranges from 91.3 ± 0.8 to 103.7± 0.9 ($\bar{X}$: 97.5, RSD: 8.7 %).

Table 11.Assay results of Artemether Lumefantrine tablets

Sample ID	Brand Name	Collection site	Country of Origin	%mean Artem±RSD	%mean Lum±RSD	Remark
Artem001	Artem/ Lum	Gambella	India	103.7±1.4	98.8±0.8	Passed
Artem002	Artem/ Lum	Gambella	India	105.3±0.4	102.9±3.0	passed
Artem003	Artem/ Lum	Ithang	India	98.9±0.5	100.0±1.5	passed
Artem004	Artem/ Lum	Gambella	India	105.8±1.1	100.0±0.9	passed
Artem005	Artem/ Lum	Gambella	India	101.4±2.9	100.9±1.1	passed
Artem006	Artem/ Lum	Lare	India	95.4±2.3	97.3±1.2	passed
Artem007	Artem/ Lum	Dimma	India	97.86±1.0	95.9±0.8	passed
Artem008	Artem/ Lum	Dimma	India	100.7±0.2	98.7±1.0	passed
Artem009	Artem/ Lum	Gambella	India	98.1±0.9	95.1±0.3	passed
Artem010	Artefan	Godere	India	99.6±0.6	93.6±0.6	passed
Artem011	Artefan	Ithang	India	98.8±0.6	98.2±2.2	passed
Artem012	Artefan	Ithang	India	98.2±1.0	91.7±1.7	passed
Artem013	Artefan	Lare	India	100.6±0.2	99.2±1.2	passed
Artem014	Artefan	Lare	India	103.3±1.8	98.5±3.0	passed
Artem015	Artefan	Gambella	India	95.7±0.8	91.3±0.8	passed
Artem016	Lumiter	Wantwa	India	105.3±0.3	99.8±2.9	passed
Artem017	Lumiter	Gambella	India	99.3±2.7	103.7±0.9	passed
Artem018	Artefan Dispersible	Gambella	India	101.9±1.1	97.1±0.8	passed
Artem019	Artefan Dispersible	Dimma	India	98.5±2.9	96.1±2.4	passed
Artem020	Artefan Dispersible	Ithang	India	97.1±0.3	95.1±0.5	passed
Artem021	Artefan Dispersible	Ithang	India	103.4±2.8	98.6±1.8	passed
Artem022	Artefan Dispersible	Ithang	India	100.8±1.2	95.7±1.3	passed
Artem023	Comether	Lare	China	97.8±2.7	96.7±1.6	passed
Artem024	Lonart	Ithang	India	106.3±0.0	96.0±0.4	passed
Specifications				**Content**	**90.0% - 110.0%**	
				RSD	**NMT 3.0%**	

Percent label claim distributed among different brands of artemether/lumefantrine tablets was also assessed. It was found that the brand Comether contains the least artemether API (97.8 ± 2.7, mean ± SD) than the rest of the 6 brand products. In contrast the brand Lonart possessed the highest amount (106.3 ± 0.0, mean ±SD) of artemether API. Table 12 shows that the percent label claim for lumefantrine in among the six brands ranges between 95.4(Artefan) to 101.8 (Lumiter).

Table 12. Percent label Claim of ARTM and LUM APIs distributed among generic products

Product Name	API	Minimum	Maximum	Mean	SD
1.Artemether Lumefantrine	ARTM	95.4	105.8	100.8	3.58
	LUM	95.1	102.9	98.8	2.45
2. Artefan	ARTM	95.7	103.3	99.4	2.53
	LUM	91.3	99.2	95.4	3.59
3. Lumiter	ARTM	99.3	105.3	102.3	4.27
	LUM	99.8	103.7	101.8	2.76
4. Artefan Dispersible	ARTM	97.1	103.4	100.3	2.55
	LUM	95.1	98.6	96.5	1.35
5. Comether	ARTM	97.8	NA	97.8	2.7
	LUM	96.7	NA	96.7	1.6
6 Lonart	ARTM	106.3	NA	106.3	0.0
	LUM	96.0	NA	96.0	0.4

Similar study conducted in Jimma, Ethiopia depicts that except for one generic product (IPCA Laboratories Ltd., India), which failed to comply having a 111.9% label claim for lumefantrine API, the results of the amount of artemether and lumefantrine in the fixed dose combination (FDC) artemether-lumefantrine tablet samples showed that all samples complied with the pharmacopeial acceptance specification limit (Belew *et al.,* 2019). A Cape Coast study indicated the percentage of artemether in the samples ranging from 98.04 to 102.82 in the artemether lumefantrine tablet and the percentage of lumefantrine ranging from 98.70 to 111.87 suspension samples indicating the artemether having a full compliance with the IP criteria (Prah *et al.*, 2016). In contrast a Gabonese study claimed that the questionable Maloxine ® sample contained APIs, however the amount was only roughly half the dose (Visser *et al.*, 2015). A study done in the DRC discovered that 69% of the tested samples failed in which 11.3% samples had artemether concentrations above 110 percent and that 30.7% samples had artemether contents below the label's specified 90%. 5.3% samples exhibited lumefantrine values above 110 percent, whereas 21.7% samples had lumefantrine contents below 90 percent using HPLC assays (Mufusama *et al.*, 2018).

9.5.2. Artemether Injection

9.5.2.1. System suitability

System suitability of the artemether injection assay is shown in Table 13 where by the RSD was found to be 0.5670 and the tailing factor 1.476.

Table 13. System suitability parameters of Artemether Injection

System suitability parameters of Peaks				
Standard Injections	**Peak Area**	**Tailing Factor**	**TPN (N)**	**Retention Time**
Reference Standard 1st inj.	28153	1.28	3805	6.6
Reference Standard 2nd inj.	28116	1.27	3831	6.6
Reference Standard 3rd inj.	28217	1.35	3744	6.4
Reference Standard 4th inj.	27804	1.78	2661	6.2
Reference Standard 5th inj.	28067	1.7	2964	6.3
Mean Value	28071.4	1.476	3401	6.42
RSD	0.5670		2.786377542	
Limit	**NMT 2%**			

Results for the assay of artemether injection indicated that all the artemether injection samples fulfilled the specification set by Ph. Int., (2020) which states that artemether injection contains not less than 90.0% and not more than 110.0% of the amount of $C_{16}H_{26}O_5$ stated on the label. Table 14 shows that the % mean content ranges from 95.1 to 101.5. Table 14 depicts the results of assay results for artemether injection samples.

Table 14. Results of assay for artemether injections.

Sample ID	Brand Name	Collection site	Country of origin	%mean content	±RSD	Remark
Artem-Inj 001	Artem	Gambella	China	98.1	3.0	Passed
Artem-Inj 002	Artum	Lare	India	95.1	0.8	Passed
Artem-Inj 003	Artemether Injectable	Dimma	China	96.1	0.0	Passed
Artem-Inj 004	Artemether Injection	Dimma	Germany	96.5	2.7	Passed
Artem-Inj 005	Artemether Injectable	Gambella	China	98.3	2.2	Passed
Artem-Inj 006	Artemether Injection	Dimma	China	101.0	2.1	Passed
Artem-Inj 007	Artum	Godere	China	97.3	0.1	Passed
Artem-Inj 008	Artemether Injection	Dimma	China	101.5	2.6	Passed
Artem-Inj 009	Artum	Dimma	China	99.6	2.9	Passed
Specifications			**Content**	**90.0% - 110.0 %**		
			RSD	**NMT 3.0%**		

The results of this study indicated that all the artemether injections passed the assay test. Another study also met the criteria for the assay test of artemether injection set by the IP having an artemether content on the collected artemether injections 99.92% and 98.17% (Prah *et al.*, 2016). However, according to Hassan *et al.*, (2020), a study done on the 'Post-marketing surveillance of quality of artemether injection marketed in Southwest Nigeria' more than half (59.1%) of the examined samples failed the requirements for the content assay stated in Ph.Int. (2019).

9.5.3. Artesunate Powder for Injection

A titrimetric method was used to determine artesunate in powder for injection preparation as the IP suggested. Table 15 shows that the artesunate content ranges from 90.59 to 109.04%. All the artesunate samples passed the criteria set by the Ph.Int. (2020) which states that an artesunate powder for injection should contain not less than 90.0% and not more than 110.0% of the amount of artesunate ($C_{19}H_{28}O_8$) stated on the label.

Table 15. Assay of artesunate powder for injection

Sample ID	Brand Name	Collection site	Country of manufacturer	%Mean Content	±RSD	Remark
Artesun001	Artesunate injection	Gambella	India	93.28	0.3	Passed
Artesun002	Artesun	Lare	China	90.59	1.1	Passed
Artesun003	Artesun	Godere	China	100.58	5.8	Passed
Artesun004	Artesun	Gambella	China	92.19	1.6	Passed
Artesun005	Artesun	Gambella	China	109.04	1.2	Passed
Artesun006	Artesun	Gambella	China	102.51	0.4	Passed
Artesun007	Artesun	Gambella	China	92.32	2.8	Passed
Artesun008	SCOSUNATE-60	Dimma	India	97.45	1.6	Passed
Artesun009	Artemark	Wantwa	India	91.74	1.0	Passed
Artesun010	GSUNATE 60	Wantwa	India	91.17	0.2	Passed
Specifications				**Content**	**90.0% - 110.0 %**	
				RSD	**NMT 3.0 %**	

A Nigerian study indicated that all of the artesunate and amodiaquine antimalarial combination drugs examined had the necessary number of active components and complied with quality specifications (Uzondu and Okafo, 2016). A pilot investigation on the quality of artesunate and amodiaquine tablets used in the Ghanaian fishing village of Tema, artesunate samples passed the quality test with regard to assay (Affum *et al.,* 2013).

9.5.4. Chloroquine Phosphate tablet

Chloroquine Phosphate Tablets should contain NLT 93.0% and NMT 107.0% of the labeled amount of chloroquine phosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$) as stated in the USP. The assay of chloroquine phosphate tablets was done according to the specifications set on USP (2023a).

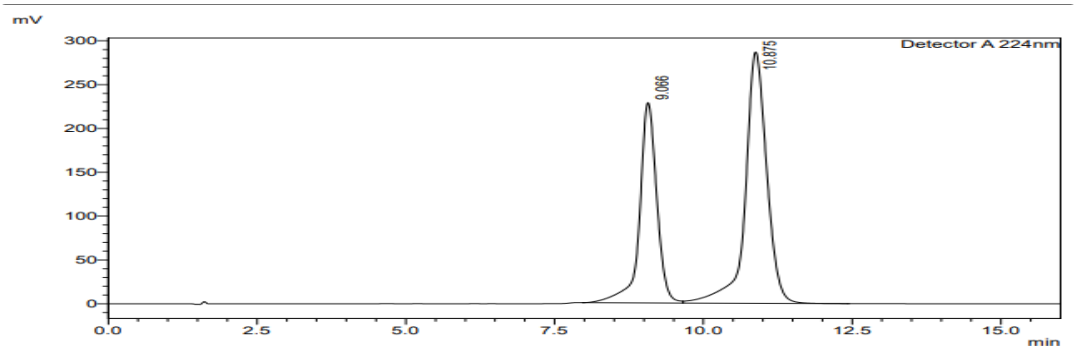
9.5.4.1. System Suitability

The system suitability requirements satisfy the criteria set on the USP having a mean resolution of 3.3002, a tailing factor of 0.872 and an RSD of 0.138 as shown on Table 16. The resolution between chloroquine phosphate USP RS and amodiaquine hydrochloride is shown on Figure 20.

Table 16.System Suitability Parameters of chloroquine phosphate tablets

System Suitability Parameters of peaks

Standard Injections	Peak Area	Tailing Factor	TPN (N)	Retention Time	Resolution
Reference Standard 1st inj.	4629714	0.88	5467	9.2	3.323
Reference Standard 2nd inj.	4614504	0.87	5451	9.1	3.306
Reference Standard 3rd inj.	4619520	0.87	5455	9.1	3.300
Reference Standard 4th inj.	4619427	0.87	5456	9.1	3.288
Reference Standard 5th inj.	4613627	0.87	5446	9.1	3.284
Mean Value	4619358.4	0.872	5455	9.12	3.3002
RSD	0.138			0.5	
Limit	**NMT 2%**	**NLT 1.5**			**NLT 1.5**



<Peak Table>

Detector A 224nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	9.1	4613627	0.87	5446	--
2	10.9	7215723	0.88	5075	3.284
Total		11829350			

Figure 20. Resolution between amodiaquine hydrochloride and chloroquine phosphate

Tables 17 shows the results of the drug assay of all chloroquine phosphate tablet samples indicating that the amount of APIs available in all these drug substances was within their acceptance limit. The APIs contained in samples Chlor002 and Chlor003 were in the upper limit.

Table 17.Assay results of Chloroquine phosphate tablets

Sample Id	Brand Name		Collection site	Country of Origin	%mean content	±RSD	Remark
Chlor001	Chloroquine tablets	Phosphate	Gambella	India	99.98	2.14	Passed
Chlor002	Chloroquine tablets	Phosphate	Gambella	India	102.46	0.55	Passed
Chlor003	Chloroquine tablets	Phosphate	Gambella	India	102.08	1.30	Passed
Chlor004	Chloroquine tablets	Phosphate	Dimma	India	100.79	1.02	Passed
Chlor005	Chloroquine tablets	Phosphate	Dimma	India	100.61	0.98	Passed
Specifications					**Content**	**93.0% -107.0%**	
					RSD	**NMT 3.0%**	

In line with this study, *in-vitro* evaluation of the quality of essential drugs on the Tanzanian market showed that the assayed amount of chloroquine phosphate for all the assessed samples was within the USP 24 acceptance range of 93–107% (Risha *et al.*, 2002). With the exception of one brand, the assay results from an Indian investigation on the *in-vitro* evaluation of commercially available antimalarial chloroquine phosphate tablets ranged from 92.5 to 107.5% of the amount listed on the label for chloroquine phosphate. Low content in brand may be caused by inadequate formulation and production processes (Patel *et al.*, 2005).

9.5.5. Primaquine Phosphate Tablet

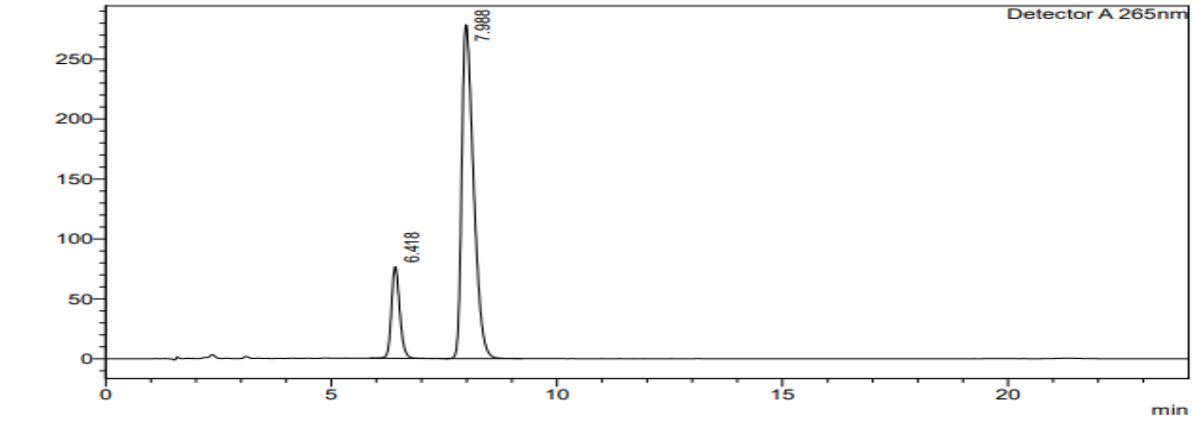
9.5.5.1. System Suitability

The resolution between primaquine phosphate and primaquine related compound A, system suitability solution was 3.8265 which is NLT 2.5. Table 18 illustrates the RSD of primaquine phosphate standard solution, which was 0.0464. The resolution between primaquine related compound and primaquine phosphate USP RS is shown on Figure 21. The chromatogram of primaquine related compound "**A**" having a 6.3min retention time is shown on Figure 22.

Table 18. System Suitability Parameters of Primaquine Phosphate assay

System Suitability Parameters of peaks

Standard Injections	Peak Area	Tailing Factor	TPN (N)	Retention Time	Resolution
Reference Standard 1st inj.	5614005	1.47	3733	4.743	3.835
Reference Standard 2nd inj.	5615619	1.474	3749	4.746	3.818
Reference Standard 3rd inj.	5609995	1.483	3799	4.74	
Reference Standard 4th inj.	5614685	1.471	3739	4.771	
Reference Standard 5th inj.	5616897	1.492	3750	4.746	
Mean Value	5614240.2	1.478	3754	4.7492	3.8265
RSD	0.0464			0.2618	
Limit	**NMT 1%**				**NLT 2.5**

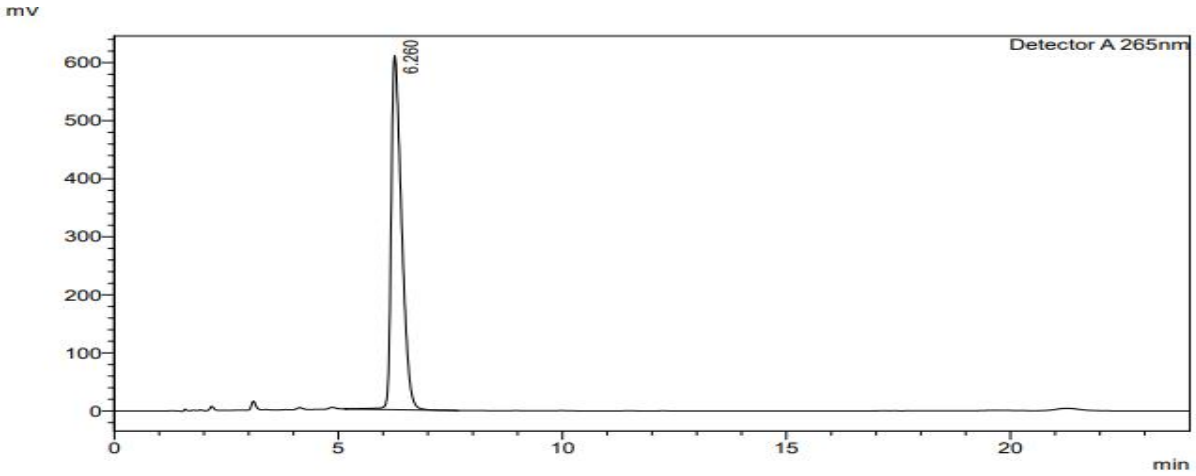


<Peak Table>

Detector A 265nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	6.4	974697	1.19	5856	--
2	8.0	5069094	1.54	4302	3.818
Total		6043791			

Figure 21. Resolution solution between primaquine related compound **A** and primaquine phosphate USP RS



<Peak Table>

Detector A 265nm

Peak#	Ret. Time	Area	Tailing Factor	Number of Theoretical Plate(USP)	Resolution(USP)
1	6.3	9839608	1.65	3349	--
Total		9839608			

Figure 22.Chromatogram of Primaquine related compound A

Figure 21 and 22 demonstrates that the primaquine related compound and primaquine phosphate USP RS showing a different retention time. The assay results for primaquine phosphate indicated that one batch of primaquine phosphate sample failed to meet the requirements set by the USP as shown in Table 19.

Table 19.Assay results for primaquine phosphate

Sample ID	**Brand name**	**Collection site**	**Country of Origin**	**%mean content**	**±RSD**	**Remark**
Pri-Gam-001	Primaquine Phosphate	Gambella	Cyprus	94.1	2.5	Pass
Pri-Gam-002	Primaquine Phosphate	Wantwa	Cyprus	93.7	1.0	Pass
Pri-Gam-003	Primaquine Phosphate	Gambella	Cyprus	95.9	0.5	Pass
Pri-Gam-004	Primaquine Phosphate	Gambella	Cyprus	80.0	5.4	Fail
Specifications				**Content**	**93.0% - 107.0%**	
				RSD	**NMT 3.0%**	

According to the quantity of API results of this study, there is little chance of treatment failure (Newton *et al.,* 2006; Chaccour *et al.,* 2012), a longer parasite clearance time and larger

recrudescence (Keoluangkhot *et al.,* 2008), or the establishment of resistance (White *et al.,* 2009) in the dose units of the majority of the products under investigation. Although a number of factors affect a drug's efficacy, including food intake, age, nutritional status, pharmacokinetics, and a parasite's IC50 (Mu *et al.,* 2010; Jamsen *et al.,* 2012), the presence of enough APIs, which are present in all of the investigated products, is crucial for increasing the recommended parasitological and clinical cure rate (90%) (WHO, 2010).

According to Philippe Guérin, director of the WWARN (World Wide Antimalarial Resistance Network), there are currently cases of artemisinin resistance that have been verified in Cambodia, Laos, Thailand, and Myanmar. The mechanisms underlying resistance are intricate and still poorly understood. Drug resistance is likely to be fueled by a variety of variables, such as sub-therapeutic stated APIs levels in ACT formulations (Kaur *et al.,* 2016). One of the key techniques for assessing the quality of drugs is assay. As a result, cGMP implementation, monitoring, and adjustment of the nation's medication distribution system must be done with considerable care.

9.6. Thickness, Diameter, Hardness, Friability and Disintegration

9.6.1. Artemether Lumefantrine

The study conducted showed that all the artemether lumefantrine tablet samples examined had passed the specification criteria set by the USP except two samples with a percent friability of 2.615% and 1. 299%. The percent friability values range from 0.008% to 2.615%. Table 20 depicts thickness, diameter, hardness and disintegration of artemether- lumefantrine tablets. All examined artemether-lumefantrine samples, except two, gave a hardness value >50 N which is the acceptable criteria set by BP (2000). Two of the samples that failed the hardness test had 41.4N and 48.8N hardness values. The average hardness of the products is different from each other, i.e., it is observed that tablet hardness ranged from 52.2 N to 84.4N.

Table 20. Summary of thickness, diameter, hardness and disintegration of artemether lumefantrine tablets.

Sample ID	Batch Number	Average Thickness± SD	Average Diameter (mm) ± SD	Average Hardness(N) ± SD	% Friability	DT (Sec)	time
Artem001	HWE101315	3.1 ±0.03	9.0±0.02	81.5±4.8	0.075	4	
Artem002	HWE111316	3.1±0.04	8.9±0.02	79.8±11.4	0.119	330	
Artem003	HWE110595	3.1±0.02	8.9±0.12	84.4±9.3	0.284	260	
Artem004	HWE110599	3.1±0.03	8.9±0.05	81.5±9.3	0.404	356	
Artem005	HWE1120560	3.1±0.02	9.0±0.02	80.5±7.4	2.615*	318	
Artem006	HWE1120561	3.1±0.04	9.0±0.04	83.6±15.4	1.299*	341	
Artem007	HWE110602	3.1±0.03	9.0±0.03	74.8±11.6	0.600	247	
Artem008	HWE110615	3.2±0.04	9.0±0.03	71.8±11.8	0.313	280	
Artem009	HWE110618	3.1±0.01	9.0±0.01	70.3±11.8	0.074	180	
Artem010	PA01830	3.2±0.03	9.0±0.02	68.5±6.9	0.266	11	
Artem011	PA0540B	3.2±0.02	9.0±0.02	67.2±8.9	0.501	10	
Artem012	PA18101	3.1±0.03	9.0±0.01	55.1±7.7	0.021	20	
Artem013	PA18121	3.2±0.04	9.1±0.16	41.4**±**12.5	0.164	10.3	
Artem014	PA18381	4.3±0.01	9.6±0.01	72.2±6.1	0.269	40	
Artem015	DJ03481	3.3±0.02	9.0±0.01	52.2±4.3	0.060	11	
Artem016	NAA21240A	3.3±0.02	8.8±0.01	48.8*****±4.3	0.326	361	
Artem017	NAA006A	3.2±0.02	8.8±0.01	52.2±5.1	0.144	352	
Artem018	PA07851	4.3±0.01	9.6±0.01	76.4±4.6	0.013	50	
Artem019	DJ1460L	4.4±0.03	9.6±0.01	67.7±10.1	0.316	43	
Artem020	PA14160	4.4±0.01	9.6±0.02	71.7±6.5	0.250	68	
Artem021	PA18271	4.3±0.05	9.6±0.02	73.7±6.0	0.973	48	
Artem022	PA18281	4.3±0.12	9.6±0.02	75.1±5.4	0.055	49	
Artem023	21KA	3.8±0.01	9.0±0.01	58.9±6.8	0.008	119	
Artem024	A3AAD013	3.1±0.01	8.9±0.01	75.0±14.4	0.102	257	

NB! * Failed

The reason for variability of between artemether-lumefantrine brands may have been related to pharmaceutical manufacturer's formulation conditions such as alteration in machine speed, granulation methods, and amount of lubricants added during manufacturing processes . A study conducted in Cape Coast, Ghana by Prah *et al.*, (2016) indicated that all the artemether-

lumefantrine tablets disintegrated in aqueous medium in less than 15 minutes (900 seconds) (range: 554 ± 2.2 to 866 ± 5.2). The study also indicated that the breaking strength (hardness) of the tablets ranged from 33.34 N to 51.97 N. Unlike this study, the cape coast study revealed that the percentage friability for all the artemether-lumefantrine tablets tested was lower than 1% (range: 0.01 to 0.23%). The disintegration time in this study ranges from 4- 356 seconds (5.93 minutes) which is much lower than the study done in Ghana. The bioavailability is ultimately impacted by the disintegration time, which increases if the hardness goes above a specific threshold (Nabila Morshed, 2015).

9.6.2. Chloroquine Phosphate Tablets

Among the five chloroquine phosphate tablets assessed for % friability test one failed to meet the criteria set by the USP having a % friability of 2.45% which was found to be more than 1%. The disintegration time of five brands of chloroquine phosphate is shown in Table 21. The disintegration test performed on the chloroquine phosphate samples showed a minimum of 123 seconds (2.05miutes) and a maximum of 198 seconds (3.3 minutes). The results showed that all the brands passed the disintegration test.

Table 21.Summary of thickness, diameter, hardness, friability and disintegration test of chloroquine phosphate tablet

Sample ID	**Batch Number**	**Average Thickness ± SD**	**Average Diameter ± SD**	**Hardness (N) ±SD**	**% Friability**	**DT (sec)**
Chlor001	L8080020	4.1±0.0	9.6±0.1	87.9±1.2	0.168	154
Chlor002	L8080021	4.2±0.1	9.6±0.0	90.9±1.2	0.160	145
Chlor003	L8080022	4.1±0.0	9.4±0.0	74.9±2.3	0.126	195
Chlor004	L8080044	2.6±0.0	5.9±0.0	30.7*±0.8	2.457*	123
Chlor005	9ME47	3.1±0.0	8.9±0.0	67.1±3.5	0.829	198

NB: * Failed

A Nigerian study showed that the results of assessment of the physicochemical properties of the chloroquine phosphate tablet preparations showed that the disintegration of chloroquine tablets ranged from 2.33 minutes to 32.75 minutes, with an average of 6.27minutes (locally produced) and 15.03 minutes ("imported" products) (Ofonaike *et al.,* 2008).

9.6.3. Primaquine Phosphate Tablets

The friability test done for the primaquine phosphate tablets showed that all the samples passed the criteria set by the USP, (2023d) as shown in Table 22. This might be because of the coating on the tablet that prevents abrasion (Gwaziwa *et al.,* 2017). The results of disintegration range between 154 to 233 seconds.

Table 22. Summary of thickness, diameter, hardness, friability and disintegration of Primaquine Phosphate tablets.

Sample ID	**Batch Number**	**Average Thickness± SD**	**Average Diameter± SD**	**Hardness (N) ± SD**	**%Friability**	**DT (sec)**
Pri-Gam-001	89175	2.6 ± 0.0	5.9 ± 0.0	27.5*± 8.0	0.029	185
Pri-Gam-002	89176	2.6 ± 0.0	6.0 ± 0.3	27.4*± 9.3	0.059	233
Pri-Gam-003	92736	2.6 ± 0.02	5.9 ± 0.0	30.7*± 7.7	0.011	154
Pri-Gam-004	21667	2.6 ± 0.02	5.6 ± 1.1	31.6* ± 7.2	0	221

The results of primaquine phosphate were found to be lower than the minimum required value, 50N which is in line with the results found by (Oishi *et al.*, 2011).

Tablets' mechanical strength is essential for quality control and product development, and its size and shape affect esophageal transit and administration methods (USP, 2022b; Center for Drug Evaluation and Research, 2019). Breaking force determines tablet's ability to tolerate mechanical shocks (Khar *et al.,* 2015). Simple tablet fracture may result in medication loss, which ultimately results in underdosing. Too hard tablets are also undesirable since they might not dissolve quickly and might leave the body without dispensing the required medication (Akinleye *et al.,* 2012). Hence, tablets must be able to tolerate mechanical shocks during handling, packaging, and shipping (Girma *et al.*, 2022).

Disintegration is the breakdown process of a tablet into smaller particles and is the first step towards dissolution. To be compliance with USP standards, the tablets should disintegrate, and the particles must pass through the 3-inch-long glass tubes and held against a 10-mesh screen within the time given (Banker and Anderson, 2009).

The disintegration test, which is a necessary condition for dissolution measures the time required for tablets to disintegrate into particles. It is a rate-determining step in the process of drug

absorption. The onset of action of a dosage form of a drug depends on the time to be taken by the tablets to unharness the active ingredients into the gastric juice. The tablets must be disintegrated within the acceptable time or the prescribed course will be affected and the drug may not exert its effect properly (Patel *et al.,* 2005).

It is possible to draw the conclusion that the various manufacturing methods are responsible for the comparatively long disintegration times seen on this study. Excipients like binders and the type of coating employed have a significant impact on how quickly a tablet disintegrates into smaller particles and how the API is released. More time is needed before the API is released for absorption if the binders have an affinity for the API (Gwaziwa *et al.,* 2017).

9.7. Test for sterility

Sterility testing is done in an aseptic environment. The test environment must be adjusted to the manner in which the sterility test is conducted in order to attain these conditions. Any microorganisms that are to be revealed in the test are unaffected by the efforts taken to prevent contamination. By taking an acceptable sample of the work area and implementing an appropriate set of controls, the working circumstances under which the tests are conducted are routinely checked. Both Soya-bean casein digest medium, suitable for the culture of both fungal and aerobic bacteria, and fluid thioglycollate medium, principally designed for the culture of anaerobic bacteria but also able to detect aerobic bacteria, were shown to be suitable for the test for sterility. Portions of the media were incubated for 14 days, and neither artemether nor artesunate injection resulted in any microbial development. Figure 23 shows the process of incubation during sterility test for artemether injection and artesunate powder for injection. The pictures show that there is no turbidity or fogginess which indicates that there is no microbial growth during the 14-day incubation period. Likewise, no turbidity was seen in the culture mediums after the third day for bacteria and the fifth day for fungi, showing that there was no microbial growth on the injectable antibiotics' samples in Benin (Dohou *et al.,* 2022).

Figure 23. Sterility test for Artemether and Artesunate powder for injection

The discovery of microbial contamination in the water for injection (WFI) batches in a study conducted in Dar Es Salaam, Tanzania suggests that the WFI products were in reality of poor quality (Mwambete *et al.,* 2009). Post-market quality surveillance project done on maternal healthcare products (oxytocin and ergometrine) on the Ghanaian market showed 87.5% of the Oxytocin injections and 69.2% of the Ergometrine injections in the random sample that underwent sterility testing failed (Eric, 2013).

Sterility testing, however, does not by itself guarantee the sterility of a batch; rather, it is an additional check, and continuing adherence to the test does provide assurance regarding the effectiveness of a sterilization or aseptic procedure. Despite its flaws, failing to perform a sterility test could have serious legal and moral repercussions (Mwambete *et al.,* 2009).

9.8. Dissolution

Dissolution test was performed to obtain information about the possible differences in the bioavailability of the antimalarial samples. It was conducted for the tablet drug samples based on USP individual monographs.

9.8.1. Artemether

9.8.1.1. System Suitability

Prior to the dissolution profile test, system suitability parameters (theoretical plate numbers, tailing factor, and repeatability of peak areas) were assessed using the analysis of USP primary standard solutions to determine whether the aforementioned HPLC method was appropriate for the intended purpose. The system suitability was performed according to the USP Salmous standards guideline specification (The USP convention, 2009). The tailing factor was 0.99 as shown in Table 23. The guideline also states that the RSD for replicate injections should not be more than 2.5% which the result in this work showed an RSD value of 0.9%.

Table 23.System suitability parameters of dissolution of Artemether by HPLC.

Standard Injections	**Peak Area**	**Tailing Factor**	**TPN (N)**	**Retention Time**
Reference Standard 1st inj.	27473	1	4597	6.21
Reference Standard 2nd inj.	27281	0.89	5026	6.21
Reference Standard 3rd inj.	27701	0.94	4317	6.22
Reference Standard 4th inj.	27845	1.2	4134.46	6.21
Reference Standard 5th inj.	27291	0.92	4802	6.23
Mean Value	27518.2	0.99	4575.292	6.216
RSD	0.908745449			0.143891118
Limit	**NMT 2.5 %**	**NMT 2**	**NA**	**NA**

9.8.1.2. Calibration Curve

Calibration curve was constructed in order to assess the linearity of concentration. The measured peak areas were plotted against the respective concentration of the standard solutions. A linear regression equation was Y = 315.0714X + 1223504, where Y is the peak area and X is the concentration in mg/mL, as shown in the calibration curve (Figure 24). The percentage release values of samples taken at intervals of 15, 30, 45, 60, 90, 120, and 150, 180 and 195 minutes were computed using the equation derived from the calibration curve. The concentration of the tested substances and the peak area values were correlated on this curve over the concentration of 0.028, 0.024, 0.02, 0.016, 0.012, 0.008, 0.004 mg/mL (r^2 = 0.974998).

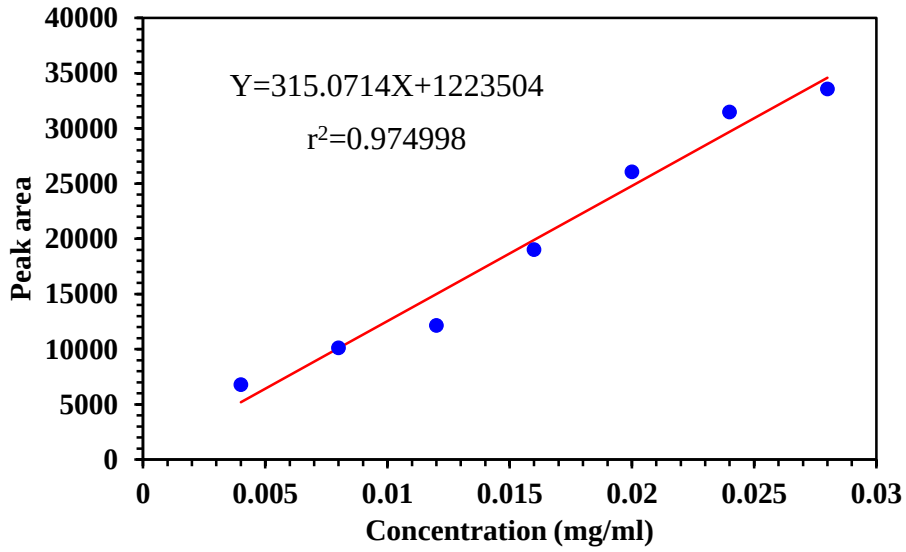


Figure 24.Calibration Curve of dissolution profile of Artemether Tablets

9.8.1.3. Dissolution profile of Artemether

The dissolution profile of artemether tablets is depicted in Figure 25. From the six brands of artemether lumefantrine tablets analyzed, Artem01 and Artem06 have failed to release their contents in the tolerance limits specified as a dissolution requirement. Aretm01 released 47.95% and 68.21% at 60 and 180 minutes respectively. Artem06 released 44.68 % of its content at the 60th minute and 65.28% at the 180th. These results are below the tolerance limits at both time references. Artem03 had a higher release percentage than the other generic brands shown under Table 24 and Figure 25.

Table 24. Dissolution Profile of Artemether

	%API± RSD released						
Time (Minute) of sampling	**ArtemC****	**Artem01**	**Artem02**	**Artem03**	**Artem04**	**Artem05**	**Artem06**
15	37.48±13.28	24.30±18.37	28.12±15.17	39.69±16.01	26.71±9.27	24.50±12.90	24.43±8.60
30	45.32±16.48	33.61±16.37	42.23±5.60	48.71±10.63	34.51±4.67	37.86±8.65	28.01±9.06
45	53.48±14.48	39.71±10.62	50.47±12.72	55.74±7.15	41.26±9.53	43.67±9.64	36.09±9.01
60	55.81±16.02	47.95±7.52*	56.70±10.79	65.03±9.89	51.89±5.92	51.20±8.01	44.68±4.80*
90	63.70±5.25	50.60±13.90	64.48±12.77	71.06±11.30	61.57±13.11	60.59±9.95	49.10±15.41
120	69.36±7.83	56.37±10.72	72.49±6.20	76.42±6.15	72.32±12.33	63.52±11.96	59.32±15.25
150	68.51±5.98	60.99±5.13	76.16±2.92	78.24±6.78	84.17±3.58	68.57±7.66	64.15±8.33
180	83.69±16.05	68.21±15.49*	74.87±4.97	91.75±13.11	89.10±6.76	70.94±9.00	65.28±5.80*
195	93.58±18.17	71.02±14.87	73.40±21.07	94.08±10.26	91.66±5.32	64.20±8.07	69.73±6.00

NB! ** Comparator product

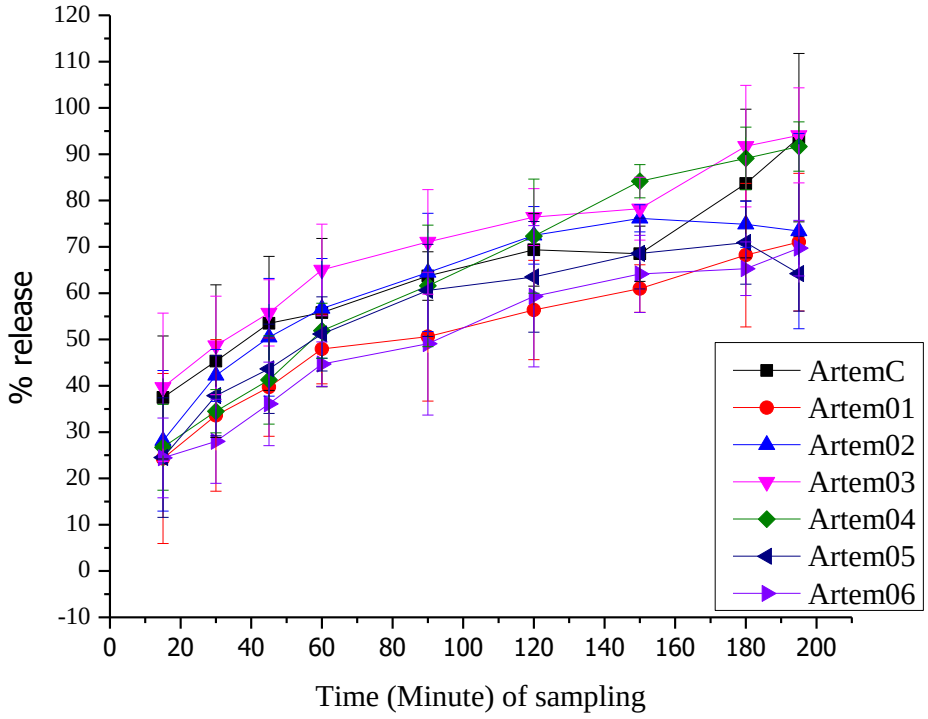


Figure 25. Dissolution profile of different brands of Artemether

9.8.1.4. Dissolution profile Comparison

Since the value (f1) factor is within the limit (0-15) and the (f2) factor is greater than 50, three of the generic products are measured to be similar and bioequivalent with the innovator product as shown in Table 25. The similarity of the product with respect to dissolution means that the test (generic) product has a dissolution performance no different than the reference (comparator) product.

Table 25. The difference factor (f1) and similarity (f2) for all generic artemether lumefantrine tablets brand with respect to Innovator (ArtemC).

	f1	f2
ArtemC01	20.7	43.0
ArtemC02	10.0	53.2
ArtemC03	8.7	59.4
ArtemC04	11.5	52.8
ArtemC05	15.1	45.0
ArtemC06	22.8	40.6

Similar research was conducted in Nigeria, where it was determined that three (50%) of the six types of ciprofloxacin tablets examined were not pharmaceutically identical to the original Cipro brand (Ngwuluka *et al.,* 2009).

9.8.1.5. Single point Dissolution of Artemether and Lumefantrine

Single point dissolution test was performed following similar steps for the dissolution profile except sample was withdrawn at only two points (60 minutes and 180 minutes) according to the USP. Table 26 showed that 10 samples out of 24 (41.6%) failed to meet the percent (%) release criteria of artemether. Artem024 did not meet the criteria for the both 60 and 180 minutes. On the other hand, Artem011 had a percent (%) release of 67.65% by the 180 minute which is lower than the set standard 65% (Q) while fulfilling the 60 minutes release tolerance by 52.28. Three lumefantrine samples out of 24 (12.5%) failed to meet the percent (%) release criteria of lumefantrine in artemether-lumefantrine tablet as shown in Table 26.

A study done on the effect of different excipients on formulation of immediate release artemether/lumefantrine tablets showed that the dissolution of lumefantrine from virtually all formulations was more than 80%, which is considered to be extremely acceptable, with the exception of two samples with a dissolution value of less than 10%. And it was indicted that the dissolution of nine artemether samples was greater than 55% after one hour, while the dissolution of aforementioned formulae was more than 78% after three hours. Two samples had a lower dissolution even after 1 hour having a percent release of 42.3% and 35.53%. The dissolution was more than 85% after 3 hours, though (Alburyhi *et al.,* 2013).

Table 26.Percent of Artemether released from the dosage forms at the specified sampling time (minutes)

Sample ID	**%API released**					
	Artemether				**Lumefantrine**	
	60min	RSD	180min	RSD	45min	RSD
Artem001	48.63*	9.71	73.33	4.13	78.17	0.38
Artem002	49.60*	6.25	70.10	6.51	96.98	2.33
Artem003	48.48*	4.35	72.41	7.44	89.47	5.28
Artem004	51.89	5.92	89.10	6.76	85.59	0.99
Artem005	48.83*	5.30	83.00	5.84	66.07	10.46
Artem006	45.65*	3.64	74.61	8.00	87.06	5.67
Artem007	43.61*	13.21	75.20	5.46	76.19	7.52
Artem008	47.31*	11.55	76.33	2.35	88.57	18.86
Artem009	49.23*	6.77	76.94	2.50	105.63	2.87
Artem010	56.70	10.79	74.87	4.97	62.05*	8.73
Artem011	52.28	15.16	67.65*	4.46	63.51*	19.50
Artem012	68.13	10.79	111.47	6.87	89.2	2.01
Artem013	65.66	5.35	91.43	10.71	92.96	2.45
Artem014	61.61	5.83	73.67	1.13	66.85	2.34
Artem015	59.86	1.47	77.09	9.05	74.79	4.81
Artem016	51.20	8.01	70.94	9.00	90.82	1.54
Artem017	67.83	15.42	84.64	7.29	40.62*	16.96
Artem018	80.88	11.57	94.03	9.36	90.15	8.28
Artem019	65.03	9.89	91.75	13.11	93.24	3.08
Artem020	55.74	7.15	91.75	77.27	105.05	2.29
Artem021	67.80	22.10	81.03	14.02	72.71	5.23
Artem022	77.97	8.41	90.86	23.10	84.18	6.33
Artem023	55.81	16.02	83.69	16.05	71.57	2.24
Artem024	44.68*	4.80	65.28*	5.80	84.55	6.82

NB* Failed

9.8.2. Lumefantrine

Prior to the dissolution profile test, calibration curve was done. This was constructed in order to assess the linearity of concentration.

9.8.2.1. Calibration Curve

The measured absorbance was plotted against the respective concentration of the standard solutions. The linear regression equation is Y = -0.0415X + 20.1554 where Y is the absorbance and X is the concentration in mg/mL, as shown on the calibration curve in Figure 26. The percentage release values of samples taken at intervals of 5, 15, 30, 45, 60 and 65 minutes were computed using the equation derived from the calibration curve. The concentration of the tested substances and the absorbance values were correlated on this curve over the concentration of 0.0128, 0.0144, 0.016, 0.0176, 0.019 mg/mL.

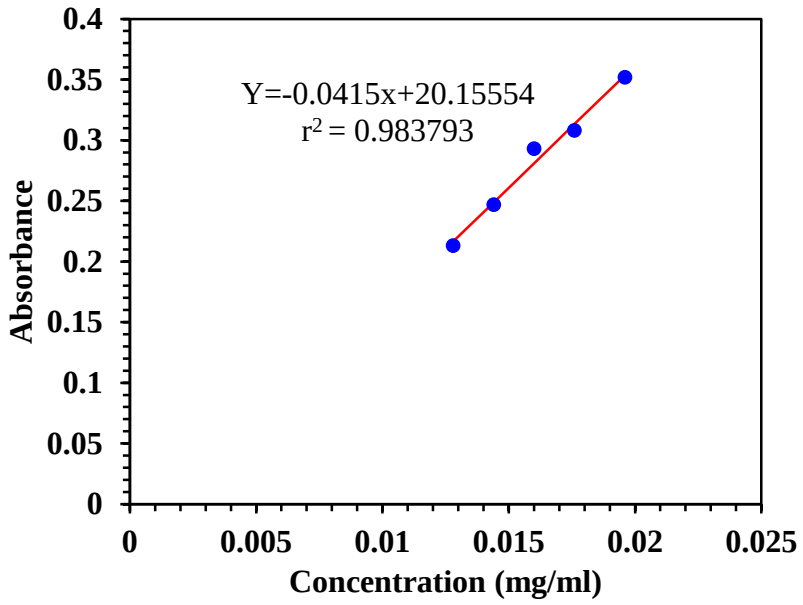


Figure 26. Calibration Curve of dissolution profile of Lumefantrine Tablets

9.8.2.2. Dissolution profile of Lumefantrine

The amount of lumefantrine released from the respective artemether-lumefantrine tablet products put in dissolution media were determined. The readings taken for each sample were quantified for lumefantrine using calibration curve equation. The dissolution profile of lumefantrine tablets is depicted in Figure 27. Table 27 demonstrates that all the generic brands passed the above USP requirement.

Table 27.Dissolution profile of Lumefantrine

	%API±RSD released						
Time (Minute) of sampling	**LumC****	**Lum01**	**Lum02**	**Lum03**	**Lum04**	**Lum05**	**Lum06**
5	59.62±2.91	38.82±2.17	36.90±4.19	33.86±3.60	37.41±3.73	44.06 ±4.53	42.47±1.50
15	81.12±1.72	66.07±4.92	68.02±2.17	48.83±3.19	64.22±2.98	65.16±0.65	62.04±2.04
30	86.22±1.15	82.93±2.14	84.35±1.16	56.27±1.21	81.11±0.90	68.81 ±2.10	74.97±1.99
45	93.78±1.33	86.39±0.34	86.36±1.78	67.66±1.32	88.39±2.41	80.87 ±1.56	79.36±0.91
60	97.25±1.29	90.68±3.54	73.43±1.62	74.39±2.88	92.84±2.96	80.84 ±2.04	85.18±1.51
65	96.06±0.37	94.57±2.06	66.23±4.67	73.17±1.10	90.84±2.25	78.96 ±0.91	84.55±1.74

NB! ** Comparator product

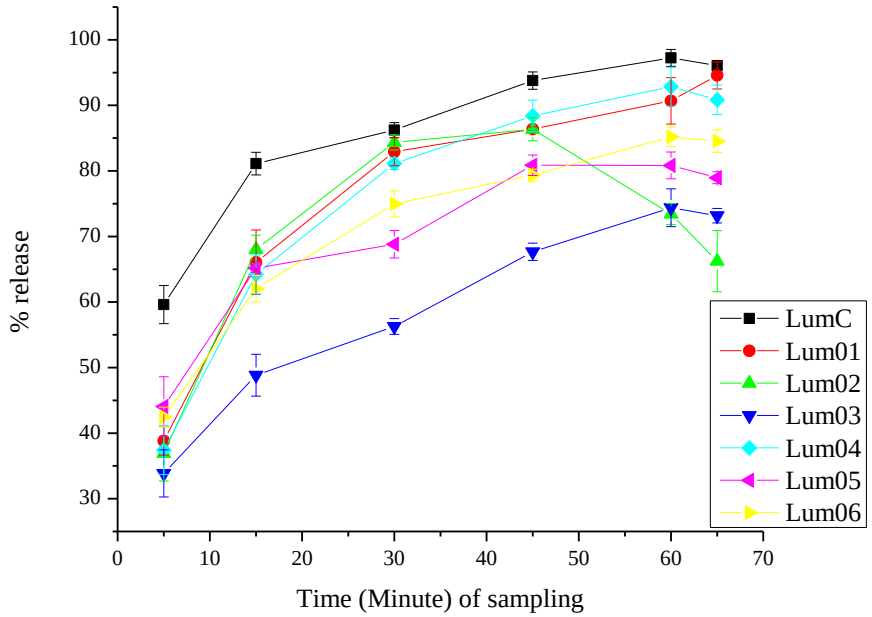


Figure 27.Dissolution profile of Lumefantrine

9.8.2.3. Dissolution profile Comparison

None of the generic products were found similar with the innovator product regarding the dissolution profile as shown on Table 28.

Table 28.The difference factor (f1) and similarity (f2) for all generic artemether lumefantrine tablets brand in respect to Innovator (LumC).

	f1	f2
Lum01	10.62	47.21
Lum02	19.21	35.85
Lum03	31.10	28.52
Lum04	11.53	45.76
Lum05	18.55	39.81
Lum06	16.63	41.80

9.8.3. Chloroquine Phosphate

Dissolution profile and single point dissolution test for chloroquine phosphate tablet was done according to (USP, 2023a).

9.8.3.1. Calibration Curve

The measured absorbances were plotted against the respective concentration of the standard solutions which gives a straight line. A linear regression equation is Y = 0.019056 X - 0.0044 and a correlation coefficient of 0.995879, where Y axis absorbance and X is the concentration in mg/mL, as shown on the calibration curve on Figure 28. The concentration of the tested substances and the absorbance values were correlated on this curve over the concentration of 0.0112, 0.0126, 0.014, 0.0154 and 0.0168 mg/mL.

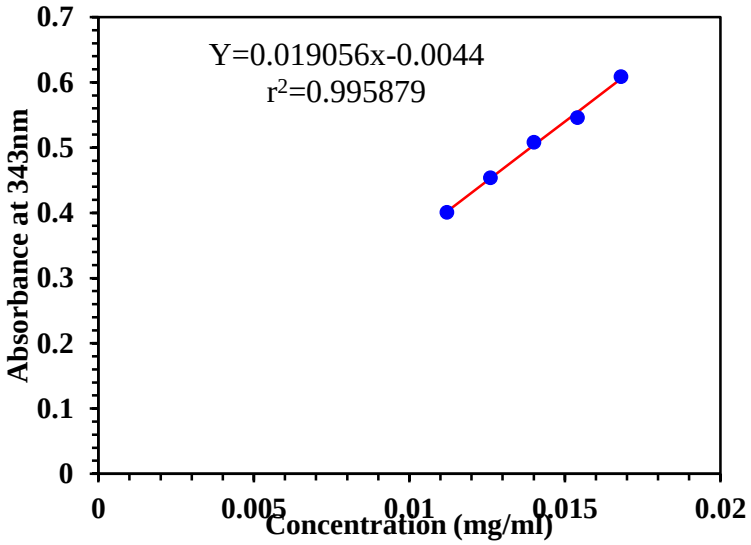


Figure 28. Calibration curve for Chloroquine Phosphate Dissolution

9.8.3.2. Dissolution profile of Chloroquine Phosphate

The dissolution profile of chloroquine phosphate was done according to the spectrophotometric method recommended in (USP, 2023a). The percentage release values of samples taken at intervals of 5, 15, 30, 45, 60 and 65 minutes were computed using the equation derived from the calibration curve (Figure 28). Table 29 shows that the generic brand Chlor001 released 97.13 ± 0.37% while Chlor002 released 97.35 ± 1.18%. The release profiles show nearly the same percentage as shown in Figure 29. In the two tested batches, 85% of the active ingredient dissolves within 15 minutes, therefore the dissolving profiles are assumed to be identical (Committee for Proprietary Medicinal Products, 1998; Australian Government department of health and aged care 2019).

Table 29.Dissolution profile of chloroquine phosphate tablet

	%API± RSD released	
Time	Chlor01±RSD (%)	Chlor02±RSD (%)
5	89.01±1.73	56.20± 10.65
15	94.83 ±0.92	94.61±1.76
30	96.04±0.52	95.90±1.75
45	97.13±0.37	97.35±1.18
60	99.29±0.90	95.53±1.04
65	96.78±1.22	96.02±0.36

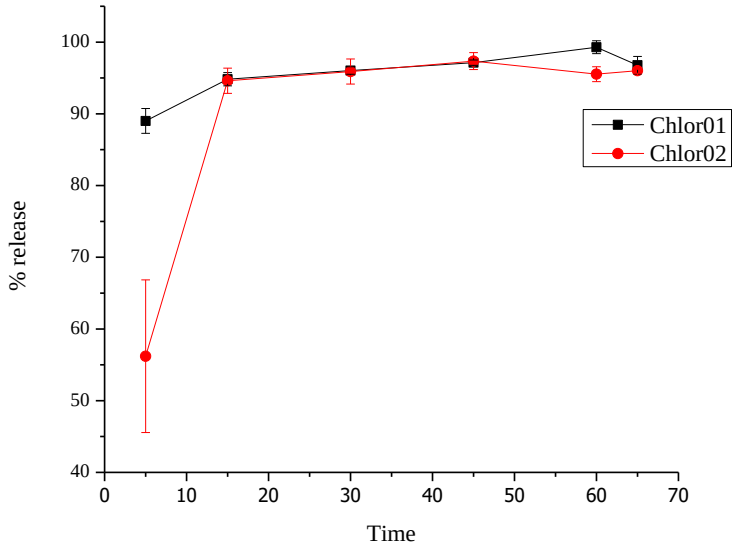


Figure 29. Dissolution profile of Chloroquine Phosphate tablets

9.8.3.3. Single Point dissolution of Chloroquine Phosphate tablet

The result for a single point dissolution test showed that all of the 5 samples passed the dissolution test. The results depicted on Table 30 show that chloroquine phosphate tablets released from 94.09 % to 98.72 % of the API within the specified minute set by USP, (2023a). This result is found to be comparable with Patel *et al.*, (2005) which showed a dissolution of 90.68% to 98.47%. Risha *et al.*, (2002) also indicated a chloroquine tablet drug release remaining well above 80% of labelled potency.

Table 30.Percent chloroquine released from the dosage forms at the specified sampling time

Sample ID	**Mean**	**RSD**
Chlor001	97.13	0.37
Chlor002	97.35	1.18
Chlor003	98.32	2.45
Chlor004	98.72	1.18
Chlor005	94.09	2.03

9.8.4. Primaquine Phosphate tablets

9.8.4.1. System Suitability

The analysis of USP primary standard primaquine solution was used to evaluate the system suitability parameters (theoretical plate numbers, tailing factor, and repeatability of peak areas) prior to the dissolution profile test to see if the aforementioned HPLC method was suitable for the intended purpose. According to the USP, (2023b) the RSD for replicate injections should not be greater than 3%; however, the results of this study revealed an RSD value of 0.16 % which satisfies the requirement mentioned as stated on Table 31.

Table 31. System suitability parameters of dissolution of Primaquine by HPLC.

Standard Injections	**Peak Area**	**Tailing Factor**	**TPN (N)**	**Retention Time**
Reference Standard 1st inj.	262757	0.9	3114	4.7
Reference Standard 2nd inj.	262697	0.9	3116	4.6
Reference Standard 3rd inj.	262758	0.9	3120	4.6
Reference Standard 4th inj.	262407	0.9	3133	4.6
Reference Standard 5th inj.	263542	0.9	3139	4.6
Mean Value	262832.2	0.9	3124.4	4.62
RSD	0.160751853			0.967994795
Limit	**NMT 3 %**	**NA**	**NA**	**NA**

9.8.4.2. Calibration Curve

The measured peak areas were plotted against the respective concentration of the standard solutions which gives a straight line. The linear regression equation is Y = 1671.333X + 31224129, where Y is the peak area and X is the concentration in mg/mL, as shown on the calibration curve on Figure 30. The concentration of the tested substances and the peak area values were correlated on this curve over the concentration of 0.0016, 0.0032, 0.0048, 0.0064, 0.008 and 0.0096mg/mL (r^2 = 0.994857).

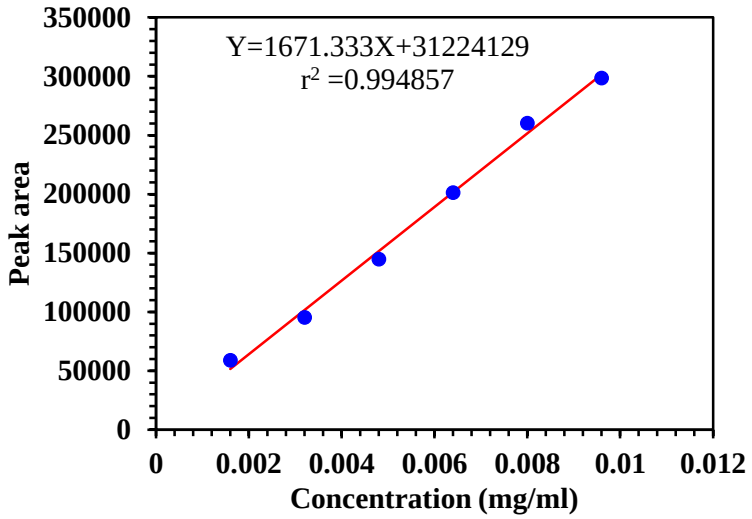


Figure 30.Calibration curve of Primaquine Phosphate dissolution profile

9.8.4.3. Dissolution profile of Primaquine Phosphate Tablet

The percentage release values of samples taken at intervals of 5, 10, 15, 20, 30, 40, 50, 60 and 70 minutes were computed using the equation derived from the calibration curve. Results were computed and there was only one generic brand of primaquine phosphate which is the product of Remedica, Cyprus as shown on Table 32. Hence profile was done for that specific generic brand selected randomly from the rest of 4 primaquine samples. And the sample where profile was done for had a low release profile and didn't meet the dissolution criteria. The dissolution profile of primaquine phosphate tablet is shown on Figure 31.

Table 32.Dissolution profile of primaquine phosphate tablets

Time	%API± RSD released
5	50.75 ± 4.00
10	72.09 ±4.60
15	73.27± 1.48
20	73.14 ±2.36
30	73.39 ± 1.12
40	72.91 ± 1.49
50	71.19 ± 3.02
60	71.17 ± 1.79
70	71.20 ±2.67

In contrast to our study, Nair *et al.*, (2012) found that the two commercially available tablet formulations examined for dissolution revealed that they were "very rapidly dissolving" products. The dissolving properties of these compounds were comparable to those of pure API.

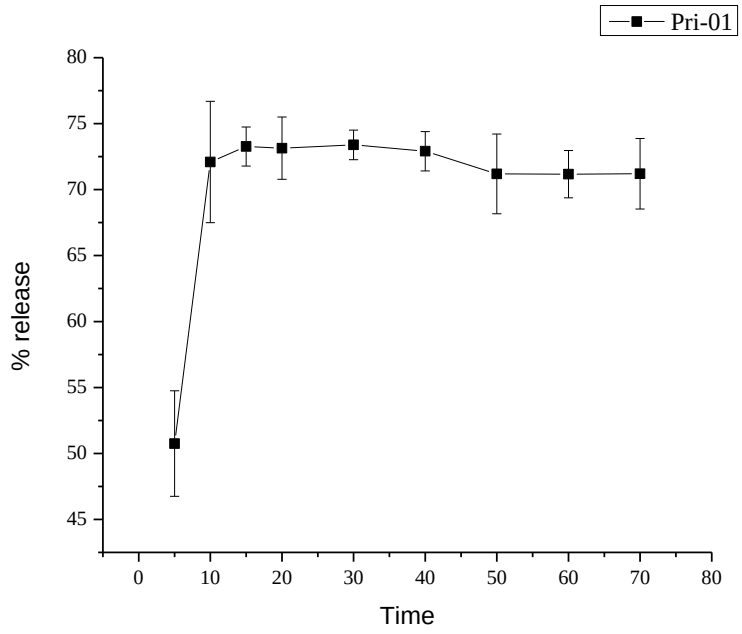


Figure 31.Dissolution profile of Primaquine Phosphate (Remedica, cyprus)

9.8.4.4. Single Point dissolution of Primaquine Phosphate

Two samples passed the single point dissolution test from the total primaquine phosphate samples collected. Table 33 shows the mean percentage of primaquine released from the dosage forms at 60minute.

Table 33. Percent primaquine released from the dosage forms at the specified sampling time (minutes)

Sample ID	**Mean**	**RSD**
Pri-Gam-001	67.33*	1.61
Pri-Gam-002	66.71*	2.21
Pri-Gam-003	95.67	2.77
Pri-Gam-004	94.59	1.88

NB! * Failed

Bioequivalence studies are essential for generic drugs to ensure that therapeutic components are available at the site of pharmacological action (Shargel, Wu-Pong and Yu, 2012). Dissolution is a crucial quality control test that evaluates a drug's *in-vitro* availability from the formulation and, consequently, its absorption potential, particularly if it includes drugs that aren't very soluble (Shah, 2001; Risha *et al.*, 2002).

The dissolution tests for immediate release solid oral dosage forms, like tablets, are used to evaluate the consistency of a drug's quality from batch to batch. It also direct the development of new formulations, and guarantee that the product's quality and performance remain after specific changes, like those to the formulation and the manufacturing process (Menegola *et al.*, 2007). Additionally, the *in-vitro* solubility of a medicinal product is important for predicting how well it will work *in-vivo* (Freitag, 2001).

Chemically identical drug products that are also bio pharmaceutically equivalent must share the same standards for rates of dissolution (Hassali *et al.*, 2012). Failure of a drug formulation to comply with pharmacopoeia dissolution requirements can may be a sign that there is a possible bioavailability issue (Shah, 2001; Risha *et al.*, 2002).

Due to the lack of information regarding the formulations' precise composition, it was challenging to determine the reason why some of the samples in this study failed to meet their respective dissolution criteria. Disintegrants like maize starch, which can lose its ability to expand with age or exposure to high humidity or temperature, may have been present in the formulations (Risha *et al.*, 2002). It is known that the drug may go through polymorphism or crystal modifications in high temperature and humidity settings, which could reduce its natural solubility. Additionally, high temperature and humidity storage conditions may cause excipient-excipient and/or excipient-drug interactions, which may slow the dissolution of a formulation containing a chemically stable medication (Saville, 2001). The amount of medication released in the paracetamol and chloroquine formulations that failed the stability test was reduced by more than 40% following 3 and 6 months of stability testing (Risha *et al.*, 2002).

Dissolution test isn't frequently used in the quality control of samples from markets in developing nations. The findings presented here reveal that even samples with the proper amount of the active ingredient may not always exhibit the same therapeutic qualities in terms of bioavailability. When bioequivalence studies are not conducted, it is not necessarily accurate to assume that a medicine

with the appropriate amount of active ingredient is "in standard," especially when the active ingredient is poorly soluble (Gaudiano *et al.*, 2007).

10. Conclusion

In this study, physicochemical quality assessment of the commonly marketed antimalarials in the Gambella National Regional State was conducted.

The study findings reveal a high prevalence (58.3%) of substandard antimalarial drugs in the region. The availability of unregistered antimalarials in the region indicates the possibility of exposing the local population to unregulated medicines. High demand in the region, combined with the accessibility of obtaining anti-malarial agents over the counter in the private sector may have caused the distribution of substandard agents. Additionally, the porous borders in the region may have contributed to the entry and distribution of low-quality antimalarials.

11. Limitations and Recommendation

The microbiological quality assessment of product samples was not included within the current study's purview. The samples collected in this study contained a limited number of brands. And a dissolution profile with comparator products and different pH media may generate a different perspective of the results. The EFDA should conduct a more thorough post marketing surveillance tests on antimalarials with a larger sample size from malarious regions. This will contribute highly to the malaria eradication program. Moreover, the regulatory authorities must take a follow-up measure for the proper application of GMPs. Investing in strengthening the national drug regulator's competence to regulate the distribution of high-quality antimalarials in the country is critical for malaria eradication.

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Annexes

Annex 1: Visual inspection tool for packaging and labeling of pharmaceutical formulations; modified from WHO/FIP/USP checklist.

sample code:_____Product Name:_____Date of Inspection:_____/_____/_____

1. Packaging			
Any drug should be packaged in a container, which can be anything from a glass bottle to a blister pack, to a tube of glass, plastic or metal. A folding carton bearing the label very often protects the container. Check the type of packaging and compare it to known containers for thesame drug from the same manufacturer. The packaging and the labeling of pharmaceutical products is a very complex and expensive business. Thus, the process and the quality of packaging material are very difficult to counterfeit. This is why a thorough visual inspection could be an important screening step for drug quality control. However, producers of counterfeit drugs are quick to copy special labeling and holograms.			
	Yes	No	Other Observations
1.1 Container and Closure			
Do the container and closure protect the drug from the outside environment e.g. properly sealed?			
Do they assure that the drug will meet the proper specifications throughout its shelf life?			
Are the container and the closure appropriate for the drug inside?			
Is the container safely sealed?			
1.2 Label			
The information written on the label is very important. The information can be printed on a label adhered to the container, or printed directly onto the container itself, but all information must be legible and indelible.			
If there is a carton protecting the container, does the label on the carton match the label on the container?			

Is all information on the label legible and indelible?			
1.2.1 The trade name:			
Is the trade name spelled correctly?			
Is the drug (trade name) registered in the country by the DRA (drug regulatory authority)?			
Is the drug legally sold in the country?			
Does the symbol ® follow the trade name?			
1.2.2 The active ingredient name (scientific name):			
Is the active ingredient name spelled correctly?			
Do the trade name and the active ingredient name correspond to the registered drug?			
1.2.3 The manufacturer's name and logo:			
Are the manufacturer's name and logo legible and correct?			
Does the logo or hologram (if applicable) look authentic?			
Does it change colour when viewed from different angles?			
1.2.4 The manufacturer's full address:			
All manufacturers are required by international law to print their complete address on the label. Many companies making substandard or counterfeit drugs do not have a traceable address on the label.			
Is the manufacturer's full address legible and correct?			
Has the company or its agent registered the drug in the country?			
1.2.5 The drug strength (mg/unit):			
Is the strength - the amount of active ingredient per unit - clearly stated on the label?			
1.2.6 The dosage form (e.g., tablet/capsule):			

Is the dosage clearly indicated?			
Is the indicated drug under this dosage form is registered and authorized for sale in the country?			
1.2.7 The number of units per container:			
Does the number of tablets listed on the labelmatch the number of tablets stated on the container?			
1.2.8 The batch (or lot) number:			
Drugs under the same batch/lot number are expected to be equivalent. In a continuous process, a batch corresponds to a defined portion of the production, based on time or quantity. Drugs from the same batch should have the same history of manufacturing, processing, packing, and coding. All drug quality control testing should be based on batch/lot numbers.			
Does the numbering system on the package correspond to that of the producing company?			
1.2.9 The date of manufacture and the expiry date:			
An expired drug should not be sold under any circumstances.			
Are the manufacture and expiry dates clearlyindicated on the label?			
1.2.10 Storage information:			
Are the storage conditions indicated on the label?			
Has the drug been properly stored?			
1.3 Leaflet or package insert:			
All drug packages should contain a leaflet explaining dosage, the drug content, the adverse effects, the drug actions, and how the drug should be taken. The only exceptions are where the packaging includes all the information that would otherwise be in the leaflet.			
Is the package insert printed on the same coloredor same quality paper as the original?			
Is the ink on the package insert or packagingsmudge-proof?			

Physical Characteristics of Tablets/Capsules

All types of medicines can be and have been counterfeited from cough syrups to injections. As mentioned above, it is important to check the packaging of these drugs. Additionally, medicines inthe form of tablets or capsules can be checked for signs of moisture, dirty marks, abrasion erosion, cracks, or any other adulteration.

	Yes	No	Other Observations
2.1 Uniformity of Shape:			
Are the tablets/capsules uniform in shape?			
2.2 Uniformity of Size:			
Are the tablets/capsules uniform in size?			
2.3 Uniformity of Color:			
Are the tablets/capsules uniform in color?			
2.4 Uniformity of Texture:			
Tablets can be film-coated, sugar-coated or enteric-coated.			
Do the tablets have a uniform coating?			
Is the base of the tablets fully covered?			
Are the tablets uniformly polished, free of powder, and non-sticking?			
2.5 Markings (scoring, letters, etc.):			
Are markings uniform and identical?			
2.6 Breaks, Cracks and Splits:			
Are the tablets/capsules free of breaks, cracks, splits or pinholes?			
2.7 Embedded surface spots or contamination:			
Are the tablets/capsules free of embedded surface spots and foreign particle contamination?			
2.8 Presence of empty capsules in the case of a sample of capsules:			

Is the sample examined free of empty capsules?			
2.9 Smell			
Does the medicine smell the same as the original?			

Name of inspector:_____ Signature:_____Date: _/____/____

Name of inspector:_____Signature:_____Date:____/_____/

_____

Annex 2. Details of identification and packaging of Artemether Injection

Sample ID	Batch Number	Date of Manufacture	Expiry date	Place of manufacture	Ampoule color	Oil-based	Pack size
Artem-Inj 001	20200803	08/20	07/24	China	Transparent	Arachis oil	80mg 6*1mL
Artem-Inj 002	PCP-2684	12/21	11/24	India	Amber	Not stated	80mg 6*1mL
Artem-Inj 003	210586	05/21	05/24	China	Transparent	Not Stated	80mg 6*1mL
Artem-Inj 004	200189	01/20	01/23	Germany	Transparent	Not Stated	80mg 6*1mL
Artem-Inj 005	20181202	12/18	11/22	China	Transparent	Arachis oil	80mg 6*1mL
Artem-Inj 006	20200801	08/20	07/24	China	Transparent	Arachis Oil	40mg 6*0.5mL
Artem-Inj 007	211099	10/21	10/24	China	Transparent	Not Stated	80mg 6*1mL
Artem-Inj 008	210842	08/21	08/24	China	Transparent	Not Stated	80mg 6*1mL
Artem-Inj 009	211045	9/24	9/24	China	Transparent	Not Stated	80mg 6*1mL

Annex 3: Detailed information of samples collected for the study

Sample ID	Brand Name	Dosage form	Strength	Batch Number	Manufacturer	Manufacring Date	Expire y Date	Country of Origin	Sampling Area	Registration Status
Artem001	Artemether Lumefantrine	Tablet	20/120mg	HWE101315	Ipca Laboratories Ltd.	Jul-20	Jul-25	India	Gambella	N
Artem002	Artemether Lumefantrine	Tablet	20/120mg	HWE111316	Ipca Laboratories Ltd.	Nov-21	May-24	India	Gambella	N
Artem003	Artemether Lumefantrine	Tablet	20/120mg	HWE110595	Ipca Laboratories Ltd	20-Nov	Oct-23	India	Ithang	Y
Artem004	Artemether Lumefantrine	Tablet	20/120mg	HWE110599	Ipca Laboratories Ltd	Oct-20	Sep-23	India	Gambella	Y
Artem005	Artemether Lumefantrine	Tablet	20/120mg	HWE1120560	Ipca Laboratories Ltd.	Oct-21	Mar-24	India	Gambella	Y
Artem006	Artemether Lumefantrine	Tablet	20/120mg	HWE1120561	Ipca Laboratories Ltd.	Jun-20	Sep-23	India	Lare	Y
Artem007	Artemether Lumefantrine	Tablet	20/120mg	HWE110602	Ipca Laboratories Ltd.	Nov-20	Nov-23	India	Dimma	Y
Artem008	Artemether Lumefantrine	Tablet	20/120mg	HWE110615	Ipca Laboratories Ltd.	Dec-20	Nov-23	India	Dimma	Y
Artem009	Artemether Lumefantrine	Tablet	20/120mg	HWE110618	Ipca Laboratories Ltd.	Dec-20	Nov-23	India	Gambella	Y
Artem010	Artefan	Tablet	20/120mg	PA01830	Ajanta Pharma Ltd	20-May	23-Apr	India	Godere	Y
Artem011	Artefan	Tablet	20/120mg	PA0540B	Ajanta Pharma Ltd	Feb-20	Jan-23	India	Ithang	Y
Artem012	Artefan	Tablet	20/120mg	PA18101	Ajanta Pharma Ltd	Oct-21	Sep-24	India	Ithang	Y
Artem013	Artefan	Tablet	20/120mg	PA18102(PA18381)	Ajanta Pharma Ltd	Oct-21	Sep-24	India	Lare	Y
Artem014	Artefan	Tablet	20/120mg	PA18121	Ajanta pharma Ltd	21-Oct	24-Sep	India	Lare	Y
Artem015	Artefan	Tablet	20/120mg	DJ03481	Ajanta pharma Ltd	21-Apr	24-Mar	India	Gambella	Y

Artem016	Lumiter	Tablet	20/120mg	NAA012A	Ajanta pharma Ltd	Sep-22	Dec-23	India	Wantwa	N
Artem017	Lumiter	Tablet	20/120mg	NAA006A	Ajanta pharma Ltd	Jul-22	Dec-23	India	Gambella	N
Artem018	Artefan Dispersible	Tablet	20/120mg	PA07851	Ajanta Pharma Ltd	May-21	Apr-24	India	Gambella	N
Artem019	Artefan Dispersible	Tablet	20/120mg	DJ1460L	Ajanta pharma Ltd	Sep-21	Aug-24	India	Dimma	N
Artem020	Artefan Dispersible	Tablet	20/120mg	PA14160	Ajanta pharma Ltd	Oct-21	Sep-23	India	Ithang	N
Artem021	Artefan Dispersible	Tablet	20/120mg	PA18271	Ajanta pharma Ltd	21-Oct	24-Sep	India	Ithang	N
Artem022	Artefan Dispersible	Tablet	20/120mg	PA18281	Ajanta pharma Ltd	21-Oct	24-Sep	India	Ithang	N
Artem023	Comether	Tablet	20/120mg	21KA	Tabuk Pharmaceutical mfg. Co.,	21-Nov	24-Oct	China	Lare	N
Artem024	Lonart	Tablet	20/120mg	A3AAD013	Bliss GVS pharma Ltd	21-Jun	23-May	India	Ithang	N
Artem-Inj001	ARTEM	Tablet	80mg/mL	20200803	KPC Pharmaceuticals,Inc	20-Aug	24-Jul	China	Gambella	N
Artem-Inj002	Artum 80	Tablet	80mg/mL	PCP-2684	Park-N-Chem Pharmaceuticals	21-Dec	24-Nov	India	Lare	N
Artem-Inj003	Artemether Injection	Injection	80mg/mL	210586	Jiangsu Ruinian Qianjin Pharmaceutical Co.,Ltd.	21-May	24-May	China	Dimma	N
Artem-Inj004	Artemether Injection	Injection	80mg/mL	200189	ZMC Hamburg GMBH Germany	20-Jan	23-Jan	Germany	Dimma	N
Artem-Inj005	ARTEM	Injection	80mg/mL	20181202	KPC Pharmaceuticals,Inc	18-Dec	22-Nov	China	Gambella	N
Artem-Inj006	ARTEM 0.5Ml:40mg	Injection	40mg/0.5mL	20200801	Kunming Pharmaceutical CORP.	20-Aug	24-Jul	China	Dimma	N
Artem-Inj007	Artemether	Injection	80mg/mL	211099	JIANGXI XIERKANGTAI Pharmaceutical CO., LTD	21-Oct	24-Oct	China	Godere	N

Artem-Inj008	Artemether Injection	Injection	80mg/mL	210842	Shanghai Trifecta Pharma co LTD.	21-Aug	24-Aug	China	Dimma	N
Artem-Inj009	Artum80	Injection	80mg/mL	PCP-2563	Park-N-chem Pharmaceuticals Pvt.Ltd	Dec-21	Nov-24	India	Dimma	N
Artesun001	Artesunate injection 60mg	Powder for Injection	60mg	BNI0721066	BRAWN laboratories limited	21-Jul	23-Jun	India	Gambella	N
Artesun002	Artesun 60mg Injection	Powder for Injection	60mg	ZA1200908	Guilin Pharmaceutical Co.,Ltd.;	20-Aug	23-Aug	China	Lare	N
Artesun003	Artesun 60mg Injection	Powder for Injection	60mg	ZA1210708	Guilin Pharmaceutical Co.,Ltd.;	21-Jun	24-Jun	China	Godere	N
Artesun004	Artesun 60mg Injection	Powder for Injection	60mg	ZA1200905	FOSUN Pharma	20-Aug	23-Aug	China	Gambella	N
Artesun005	Artesun 60mg Injection	Powder for Injection	60mg	ZA1210128	Guilin Pharmaceutical Co.,Ltd.;	21-Jan	24-Jan	China	Gambella	N
Artesun006	Artesun 60mg Injection	Powder for Injection	60mg	ZA1210712	Guilin Pharmaceutical Co.,Ltd.;	21-Jul	24-Jul	China	Gambella	N
Artesun007	Artesun 60mg Injection	Powder for Injection	60mg	ZA1200907	Guilin Pharmaceutical Co.,Ltd.;	20-Aug	23-Aug	China	Gambella	N
Artesun008	SCOSUNATE-60	Powder for Injection	60mg	XD1J003	Scott-Edil Pharmacia Ltd.	21-Oct	24-Sep	India	Dimma	N
Artesun009	Artemark	Powder for Injection	60mg	Aneb-004A	Marksans Pharma Ltd.	20-Aug	23-Jul	India	Wantwa	N
Artesun010	GSUNATE 60	Powder for Injection	60mg	ARI-032204	BLISS GVS PHARM LTD.	22-Mar	25-Feb	India	Wantwa	N
Chlor001	Chloroquine	Tablet	250mg	L8080020	IPCA Laboratories Ltd.	20/Apr	23/March	India	Gambella	N
Chlor002	Chloroquine	Tablet	250mg	L8080021	IPCA Laboratories Ltd.	May-20	Apr-23	India	Gambella	N
Chlor003	Chloroquine	Tablet	250mg	L8080022	IPCA Laboratories Ltd.	May-20	Apr-23	India	Gambella	N
Chlor004	Chloroquine	Tablet	250mg	L8080044	IPCA Laboratories Ltd.	22-May	25-Apr	India	Dimma	N
Chlor005	Chloroquine	Tablet	250mg	9ME49	Medopharm	20/Apr	04/23	India	Dimma	N

Pri-Gam-001	Primaquine Phosphate	Tablet	7.5mg	89175	Remedica Ltd	21-Aug	23-Aug	Cyprus	Gambella	N
Pri-Gam-002	Primaquine Phosphate	Tablet	7.5mg	89176	Remedica Ltd	21-Nov	23-Nov	Cyprus	Wantwa	N
Pri-Gam-003	Primaquine Phosphate	Tablet	7.5mg	92736	Remedica Ltd	21-Feb	25-Feb	Cyprus	Gambella	N
Pri-Gam-004	Primaquine Phosphate	Tablet	7.5mg	21667	Remedica Ltd	21-Jan	25-Jan	Cyprus	Gambella	N

Y: Registered; N: Not Registered; Y/E: Registered but expired

Annex 4: Sample collection form

1. Name of site where sample was taken_____Sample code:_____
2. Names of individuals who collected the sample_____
3. Product name of the sample (brand name)_____
4. Generic name of the sample with strength_____
5. Dosage form_____
6. Number of tablets per blister_____
7. Number of blisters per box_____
8. Quantity of boluses collected per sample_____
9. Batch/lot number:_____
10. Date of manufacture:_____Expiry date_____
11. Name and address of the manufacturer:_____

Date:_____

Signature of person/s taking samples

1._____

Annex 5. Ethical Clearance

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Addis Ababa University

School of Pharmacy
Ethical Review Committee

ቀን Date: July 11, 2022

ቁጥር Ref. No: ERB/SOP/471/14/2022

To: **Feruza Ahmed**
School of Pharmacy

Re: Ethical Clearance

It is to be recalled that you submitted a research proposal entitled **"Quality Assessment of Antimalarial Tablets Marketed in Gambella Region, Ethiopia".** The committee thoroughly reviewed the proposal based on its operational guideline and found that, it fulfills all the ethical requirements stipulated in the guideline. This is, therefore, to inform you that the proposal is ethically approved for implementation.

With best regards,

Shiemsu Umer (PhD)
Chairperson, ERC
School of Pharmacy
College of Health Sciences
Addis Ababa University

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