ASSESSMENT OF PREVALENCE AND QUALITY OF ARTEMISININ BASED ANTIMALARIALS SOLD IN THE KUMASI METROPOLIS

By

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DECLARATION

I hereby declare that this submission is my own work towards the M.Phil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

The study was undertaken to assess the prevalence and quality of artemisinin based antimalarials sold in the Kumasi Metropolis of Ghana. The prevalence of antimalarials was assessed through a survey of 40 pharmacies (P) and 60 licenced chemical seller's shops (LCSS). One hundred (100) questionnaires consisting of 24 open ended and close ended questions were answered by respondents in P and LCSS in the Kumasi Metropolis through face-to-face interviews. The quality of various types of artemisinin based antimalarials, namely artesunate-amodiaquine tablets, artesunate (monotherapy) tablets, artemether-lumefantrine tablets and artemether injection purchased from various P and LCSS in the Kumasi Metropolis was evaluated. The uniformity of mass, disintegration time, hardness and percentage content of the tablets were determined using established methods. The authenticity of artesunate (monotherapy and combipack) was determined by colorimetric and thin layer chromatographic (TLC) methods while ultraviolet spectroscopy (UV) was employed in determining the percentage content of artesunate and amodiaquine tablets. The authenticity of artemether-lumefantrine tablets was determined by colorimetry while the content of artemether (including the injection) and lumefantrine was assessed by UV spectroscopy and non-aqueous titration, respectively.

Eighteen of the pharmacies surveyed were accredited by the NHIS as service providers while none of the LCSS was accredited. The Artemisinin based combination therapy (ACT) drugs were widely available in the Kumasi Metropolis with all the facilities visited (100 %) having in stock artemether-lumefantrine tablets while 93 % had artesunate-amodiaquine tablets. The bestselling ACTs were artemether-lumefantrine (24 % P; 36 % LCSS) and artesunate-amodiaquine (4 % P; 6 % LCSS). ACTs were generally more expensive than other antimalarials with artemether-lumefantrine being the most expensive ACT. Most pharmacists had knowledge of the WHO antimalarial policy while shop attendants and assistants had little or no knowledge of the policy. The cost of treatment of malaria ranged from GH¢5-GH¢7. Most of the respondents (94 %) had never suspected that antimalarials purchased for sale in their facilities could be counterfeit. Apart from artesunate tablet AT2 which failed the uniformity of mass test, all the artesunate and amodiaquine tablets passed both the uniformity of mass and disintegration tests. Artesunate tablets AT3 and AT4 and

amodiaquine tablets AM4 and AM6 failed the tablet hardness test while the rest passed the test. Colorimetric and TLC tests on the artesunate tablets showed all the samples contained artesunate. The artesunate and amodiaquine tablets were either overdose or underdose and therefore substandard. All artemether-lumefantrine tablets passed the uniformity of mass, disintegration and tablet hardness tests. Colorimetric tests showed the presence of artemether and lumefantrine in all the artemether-lumefantrine tablets and artemether in the artemether injection. Artemether-lumefantrine tablet AL2 passed the content uniformity test whilst 5 others failed the test (overdose or underdose). Artemether injection was underdose and hence of substandard quality.Whilst none of the samples analysed was counterfeit, all the antimalarial brands (except artemether-lumefantrine tablet AL2) did not contain the stipulated amount of the active pharmaceutical ingredient(s) and were therefore of substandard quality. There is therefore the need for a continuous evaluation of the quality of artemisinin based antimalarials on the Ghanaian market to safeguard the health of the population.

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INTRODUCTION

1.1 GENERAL INTRODUCTION

In 2008, there were an estimated 243 million cases of malaria worldwide. The vast majority of cases (85%) were in the African Region, followed by the South-East Asia (10%) and Eastern Mediterranean Regions (4%) (World Malaria Report, 2008). Malaria accounted for an estimated 863 000 deaths in 2008, of which 89% were in the African Region, followed by the Eastern Mediterranean (6%) and the South-East Asia Regions (5%) (World Malaria Report, 2008). Malaria continues to be the number one killer disease in third world countries especially in sub-Saharan Africa. Malaria is a major cause of morbidity and mortality in Ghana, directly contributing to poverty, low productivity and reduced school attendance. Malaria places a heavy economic burden on Ghana. According to the Ministry of Health, approximately 3.5 million cases of malaria are reported each year in public health facilities and over 900,000 of these cases are in children under five years. Malaria accounts for more than 60% of under-five hospital admissions, and 8% of under-five mortality and 9.2% of maternal deaths in Ghana (Malaria Case Management in Ghana: Training Manual for Pharmacists, 2010).

It is therefore important that antimalarial medications administered are genuine and of high quality. A national malaria policy is a set of recommendations and regulations concerning anti-malaria medicines and their utilization in the country. This policy is continuously evaluated, reviewed and updated whenever appropriate by the National Malaria Control Programme in consultation with experts. The most recent is the *Anti-Malaria Drug Policy for Ghana, 2nd Revised Version (MOH, 2009).*

According to the Pharmacy Council of Ghana, there are approximately 1600 pharmacies in Ghana. The pharmacy is the first point of call for most people in the community when seeking health services including treatment for malaria. In areas where pharmacies are absent, licensed chemical sellers shops serve the same purpose if available. In recent times, the emergence of resistant Plasmodium species to many of the cheap and readily available antimalarials has resulted in the continued use and dependence of artemisinins and its based

combination (Rober *et al*., 2002; Bhattacharya and Sharma, 1999). Management of uncomplicated malaria is achieved with the artemisinin based anti-malaria combination therapies (ACTs). The first line is Artesunate - Amodiaquine combination, with the second line combination therapies being; Artemether - Lumefantrine and Dihydroartemisinin Piperaquine. The second line is recommended for patients who cannot tolerate Artesunate - Amodiaquine. The increase in demand for ACTs, where the artemisinin derivative component is the expensive part, places these medications at the inevitable risk of being counterfeited or produced at lower cost using substandard technology. Formulations may contain insufficient active ingredients, no active ingredients, the wrong (possibly toxic) active ingredients, or fail to dissolve (Newton *et al.*, 2006).

The quality of formulations may also be affected by inappropriate storage and transportation. The problem of poor quality or counterfeit anti-malarials is well established in Africa and this might have contributed to the development of resistance by the cheap and hitherto effective chloroquine; when patients were exposed to sub therapeutic doses of a medication, resulting in low bioavailability, this will promote selection of resistance and treatment failure (Maponga and Ondari 2003; Petralanda1995). Poor quality of medications may also be due to inactive ingredients, incorrect excipients, contamination or degradation. Generally, there are two categories of poor quality medications; counterfeit and substandard. Counterfeit medications are deliberately and fraudulently mislabeled with respect to identity, source or both (*WHO, 1999).*

Substandard medications are genuine medicines that upon laboratory testing do not meet the quality specifications claimed by their manufacturer. This may reflect substandard manufacturing technology or inappropriate storage and transportation. Many developing countries do not have the technical, financial, or human resources required to inspect and police the medicines supply. The World Health Organisation has estimated that about 25% of the medicines consumed in developing countries are counterfeit. In some countries the figure is thought to be as high as 50*% (WHO, 2006).* In recent times, poor-quality medicines are conventionally classified into three main categories: counterfeit, substandard and degraded. Counterfeit and substandard medicines have already been defined above. Degraded medicines may result from exposure of good - quality medicines to light, heat

and humidity. It can be difficult to distinguish degraded medicines from those that left the factory as substandard, but the distinction is important as the causes and remedies are different (Keoluangkhot *et al*., 2008).

Poor quality of drugs may not only be as a result of low therapeutic doses but also inactive ingredients, incorrect excipients, contamination or degradation. Because of the declining efficacy of the quinolones and the antifolates, attention has shifted towards novel agents such as the artemisinin derivatives, to which resistance has not yet been reported. These compounds are characterized by a short half-life and rapid mode of action against gametocytes, the sexual stages of the parasite that infect mosquitoes (Ridely, 2002; Navaratman *et al*., 2000).

Following the WHO's adoption of the new malaria policy, advocating the use of artemisinin-combination therapy, many manufacturing companies have embarked on the production of artemisinin based combination regimens, a situation that has led to the proliferation of diverse brands on the market. It is required by Law that all pharmaceuticals used in Ghana have to be registered by the Ghana Food and Drugs Board to ascertain their quality, safety and efficacy. However unregistered pharmaceutical products can be found in medicine outlets in Ghana. These unregistered medicines are usually supplied by unlicensed drug "peddlers". These unlicensed suppliers usually source their medicines from neighbouring countries or are agents for pharmaceutical companies, mainly from Asia (India and China), whose products are not registered in Ghana. The quality of such medicines may be questionable and their use could be associated with health risks. This situation is not unique to Ghana; it is common in sub- Saharan Africa as a whole (Nyunt and Plowe, 2007; Barnes *et al*., 2007; Amin and Snow, 2005; Ahorlu *et al*., 1997).

In recent years increasing numbers of substandard and fake medications have been detected in the international markets but precise figures of the global situation are lacking. It is estimated that more than 10% of the globally traded medicines are counterfeits (WHO, 2006; Newton *et al*., 2002). Thus, simple, rapid and inexpensive assays are necessary to easily set up an on-site quality control unit before large scale quality evaluation can be performed by a reference laboratory (Basco, 2004; Green *et al*., 2001).

In this study, the quality of artemisinin-based antimalarials available in retail pharmacies and licensed chemical sellers shops were analyzed to determine their authenticity.

1.2 JUSTIFICATION AND SCOPE OF WORK

Counterfeit and substandard medications contribute to malaria deaths and may lead to an increase in the incidence of drug resistance (Amin *et al*., 2005). The presence of counterfeit and substandard medications on the market undermines public confidence in pharmaceutical products and may result in a reduced intake of potentially lifesaving medicines (Dondorp *et al*., 2004).

This study seeks to identify the anti-malarials available in retail pharmacies and licensed chemical sellers' shops in the Kumasi metropolis. With respect to the WHO anti-malaria policy, the study seeks to ascertain whether or not patients, pharmacists and licensed chemical sellers are aware that ACTs are currently to be used for treatment of uncomplicated malaria. The study is also to establish whether ACTs are being given to patients with Health Insurance by accredited retail pharmacies. Various brands and generic forms of both the first and second line ACTs are available in the Kumasi metroplolis and their quality will be investigated. This study involves the use of simple methods for qualitative analysis of some ACTs and the use of other methods for their quantitative analysis.

1.3 MAIN OBJECTIVE

To investigate the availability of artemisinin based antimalarials in the Kumasi metropolis and to evaluate the quality of those available.

1.4 SPECIFIC OBJECTIVES

- To ascertain through a survey;
    - whether ACTs are available in retail pharmacies and licensed chemical sellers shops in the Kumasi metropolis
    - the kinds of dosage forms of ACTs available

- the types of ACTs available
- the cost of available ACTs
- whether pharmacists, licensed chemical sellers, medical counter assistants etc. are aware of the WHO antimalarial policy and adhere to it in the management of cases of malaria
- Whether or not ACTs are given to patients who attend the facilities with National Health Insurance Scheme prescriptions.
- Whether pharmacists, licensed chemical sellers and medical counter assistants have ever encountered fake or substandard ACTs in the course of their work and the actions they take in such cases.

❖ To determine the quality of samples of ACTs available in the Kumasi metropolis by conducting;

- pharmacopoeia tests
- qualitative analysis
- quantitative analysis

Chapter 2
LITERATURE REVIEW

2.1 BACKGROUND

Because of widespread and unsupervised use of malaria drugs, chloroquine-resistant *P. falciparum* emerged in the early 1960s and rapidly spread around the world. Today there are reported cases of Plasmodium parasite resistance to most of the currently available antimalarial therapies, thus necessitating the development of new antimalarial treatments (Bloland, 2001). A new class of antimalarials—artemisinin derivatives—were first isolated and developed in China in the 1980s. The world is greatly indebted to Chinese scientists and traditional healers for their discovery and open sharing of the antimalarial properties of the plant *Artemisia annua*, or sweet wormwood, also named *quinghaosu* in Chinese (Klayman , 1985). Artemisinin extracted from the plant can be chemically converted into several active derivatives. Artemisinin derivatives such as artesunate, artemether, and dihydric-artemisinin (DHA) are extremely potent antimalarials that act rapidly against both the parasite's asexual and sexual stages, which could potentially help to reduce the rate of malaria transmission (Sutherland *et al*., 2005). In addition, artemisinin-derived drugs have been shown to be highly efficacious against parasites resistant to other antimalarial drugs (Olumese, 2006).

2.1.1 MALARIA SITUATION IN AFRICA

Malaria is a major endemic disease caused by parasites in the tropical regions of the world with more than two billion people exposed to it, 300-500 million of new clinical cases and 1-2 million deaths every year. More than 90% of these deaths occur in Africa, mainly among children. Since 2001, WHO suggests to use artemisinin combination therapies (ACT) to treat Plasmodium falciparum uncomplicated malaria (WHO, 2001). *Plasmodium falciparum* malaria continues to cause at least 1 million deaths per year, most of them in sub-Saharan Africa (WHO, 2000). Malaria accounted for an estimated 863 000 deaths in 2008, of which 89% were in the African Region, followed by the Eastern Mediterranean (6%) and the South-East Asia Regions (5%) (World Malaria Report, 2008).

Increasing drug resistance, particularly to chloroquine (CQ), and an expanding proportion of vulnerable young children in endemic regions will likely contribute to a further rise in malaria morbidity and mortality (Sachs and Malaney, 2002; Trape, 2001). As current malaria control in sub-Saharan Africa almost exclusively depends on treatment, safe, effective and therapeutic alternatives are urgently needed. Falciparum malaria is a mass killer that went out of control. The drug treatments for this potentially lethal infection that have been most widely recommended and provided over the past 50 years (i.e., chloroquine and sulfadoxine–pyrimethamine) no longer work in most tropical countries. Resistance to these drugs emerged in Asia and South America and spread to Africa. As resistance worsened, morbidity and mortality rose as a direct consequence. But this was not appreciated because surveillance was poor, and the clinical and epidemiologic methods used to measure morbidity, mortality, and drug resistance were insensitive. After much procrastination the seriousness of the situation was finally appreciated in the 1990s, although antimalarial policy did not change from ineffective to effective antimalarial drug treatment until the past 3 years in most countries. Replacing the failing chloroquine and sulfadoxine–pyrimethamine with effective drugs required increased donor support because most endemic countries could barely afford the failing medicines, let alone more expensive ones. This was not forthcoming initially.

Fortunately things are changing for the better. There is now considerably more funding available for malaria control in endemic countries, particularly from the Global Fund (GFATM). The treatments now recommended by the World Health Organization and supported by the GFATM for uncomplicated falciparum malaria are artemisinin-based combination treatments (ACT); these are combinations of an artemisinin derivative and another structurally unrelated and more slowly eliminated antimalarial. WHO has also "raised the bar" recently in recommending that cure rates should be at least 90% and preferably > 95% assessed at 28 days (WHO, 2006).

2.2 ANTIMALARIAL RESISTANCE

Antimalarial resistance of *Plasmodium falciparum* is constantly evolving. Failure to rapidly detect a decline in drug efficacy leads to greater morbidity and mortality (Zucker *et al.*, 2003; Trape, 2001), and greatly complicates malaria control. Resistance cases are at higher

risk of severe malaria (Olumese *et al.*, 2002) and anaemia (Bjorkman, 2002), are more infectious due to persistent parasitaemia and higher gametocyte carriage (Bousema *et al.*, 2003), and their diagnosis and management are more difficult and expensive. Antimalarial drug resistance presents a considerable challenge to malaria control, and is a major cause of malaria morbidity and mortality (Bremen *et al.*, 2001). Several trials have demonstrated the efficacy of artemisinin combination therapy for treating uncomplicated malaria and reducing gametocyte load in both resistant and nonresistant strains. A recent meta-analysis [International Artemisinin Study Group (IASG), 2004] examined 16 randomized trials and concluded that artemisinin combination therapy (ACT) has the potential to improve treatment outcomes and inhibit the transmission of malaria in areas where conventional treatment is failing. ACT has now been designated as the gold standard of malaria therapy by World Health Organization (WHO, 2001a, b). However, discussion continues on how to scale up the availability of ACT and how to reduce the cost to users (WHO, 2003a). Growing pressure is being put on international donors to provide funding for ACT in resource poor countries (Attaran *et al.*, 2004).

2.2.1 RESISTANCE TO SP AND CQ IN AFRICA

In Africa, Chloroquine resistance has resulted in increased malaria mortality (Trape, 2001) and morbidity e.g. transient clinical improvement and poor haematological recovery. Furthermore, poor efficacy results increase drug costs for patients and the health system (Bloland *et al.*, 1993). In Sub-Saharan Africa, Plasmodium falciparum chloroquine (CQ) resistance, first documented in eastern Africa in 1978, is now widespread and has required change of the first-line antimalarial treatment to sulphadoxine –pyrimethamine (SP) by several countries (Shretta *et al.*, 2000). In Rwanda, until recently, CQ and SP have been used as first and second-line drugs for the treatment of uncomplicated malaria. However, in vivo tests carried out in 1999-2000 in four sentinel sites showed clinical failure (early and late) to CQ ranging from 16.7% to 56.1%, three of them over 50% (http:www.eanmat.org). Similarly, in 2000 SP clinical failure in three sites ranged between 11.6% and 44.7%. The need to rapidly deploy an efficacious and cheap alternative to CQ prompted Rwanda to choose in 2001 amodiaquine (AQ) + Sulphadoxine-pyrimethamine (SP) as the first line treatment for uncomplicated malaria cases. A study in May-August 2001 (Rwagacondo *et al.*, 2003) showed that the AQ+SP was reasonably efficacious with a parasitological failure

around 10%, a significantly better result than the other combination tested during the same study, SP+ artesunate (AS).

2.2.2 RESISTANCE TO SP AND CQ IN ASIA

In South and Central Asia resistance to chloroquine (CQ) has reached unmanageable levels and resistance to sulfadoxine –pyrimethamine (SP) emerging. Amodiaquine (AQ) is widely used in the region, and elsewhere shows only partial resistance to CQ. In Afghanistan, one option for slowing spread of resistance and improving treatment outcomes is the use of artemisinin combination therapy (ACT) (Durrani *et al*., 2005).

2.2.3 RESISTANCE TO SP IN AFRICA

Sulfadoxine-pyrimethamine plays an important role in antimalarial chemotherapy in Africa. Due to the widespread occurrence of chloroquine-resistant *Plasmodium falciparum* infections since the 1980s, many African countries have resorted to sulfadoxine-pyrimethamine monotherapy for the first-line or second-line treatment of uncomplicated malaria (Sibley *et al*., 2001). In recent years, African countries have been adopting artemisinin-based combination therapy, but this novel strategy to enhance therapeutic efficacy and delay the emergence of drug-resistant parasites is not yet fully implemented in the field. During the transition period towards the generalized use of artemisinin-based combinations in Africa, sulfadoxine-pyrimethamine monotherapy continues to be useful in many African countries for the routine treatment of uncomplicated malaria in children and adults and for the intermittent preventive treatment in infants and pregnant women (Filler *et al*., 2006; Macete *et al*., 2006).

The massive use of antifolate drugs leads to a rapid development of drug resistance, as it occurred in Southeast Asia (Peters, 1987). Therefore, sulfadoxine-pyrimethamine monotherapy is not expected to remain highly effective in Africa in the future unless novel therapeutic strategies are applied in the field and target populations for the use of sulfadoxine-pyrimethamine are well defined. Treatment failures after sulfadoxine-pyrimethamine monotherapy have already been reported from many areas in Africa (WHO, 2005).

2.2.4 RESISTANCE TO SP IN GHANA

Ehrhardt *et al*. (2002; 2003)'s study is the first to assess the therapeutic efficacy of SP, SP+ AQ and SP+AS in West Africa in a placebo controlled trial. It is shown that in an area of intense CQ resistance in northern Ghana, SP achieves efficacy of <90% within two weeks of treatment and is associated with a considerable rate of parasitological and late treatment failure. In central Ghana, treatment with SP gave a cure rate of 99% within two weeks which dropped to 74% within two additional weeks (Driessen *et al*., 2002). The majority of late treatment failures was due to recrudescence and, thus, not a result of high transmission. Although not sufficient to provide a thorough picture of the status of SP resistance in Ghana, these data suggest that the drug is not appropriate for first- line treatment at the sites tested. Both SP+AQ and SP+AS were more effective than SP in treating uncomplicated falciparum malaria. SP +AS was quick- acting in parasite clearance and additionally carried the lowest risk of early treatment failure. The benefits of SP+AQ and SP+ AS over SP became even more obvious after 14 days of follow-up. This also underlines those trials applying the previous (WHO, 1996) protocol with a 14-day follow up period may have underestimated the true degree of drug resistance. PCR-genotyping supports this notion, because in the present study as well as in Rwanda (Rwagacondo *et al*., 2003); most failures after week 2 were due to recrudescence.

2.2.5 RESISTANCE TO ARTEMISININ MONOTHERAPIES

As monotherapies, artemisinin derivatives are effective treatments for uncomplicated malaria, but because of their very rapid clearance in plasma, complete cure requires a longer treatment (up to 7 days), which is often not completed. This has raised concerns of a higher potential for this class of drugs to induce drug resistance in *Plasmodium* parasites. To prevent the development of drug resistance against artemisinin derivatives, ACTs were developed; this method of drug combination is also used for the treatment of HIV and tuberculosis. The treatment consists of the simultaneous administration of two or three antimalarial drugs, each with a distinct mechanism of action against the parasite. The World Health Organization (WHO) first endorsed ACTs for the treatment of malaria in 2004 and recommended a switch to ACTs as the first-line malaria treatment in 2005 (WHO, 2005).
Unfortunately, because of their high cost, these therapies are not widely accessible to the people who most need them, many of whom resort to using inexpensive but failing drugs

such as chloroquine. Alternatively, some patients may use artemisinin monotherapies, which are generally cheaper than ACTs but could accelerate the development of parasite resistance, as has been recently suggested by the work of Jambou and others (Jambou *et al*., 2005). At present, there have been no documented cases of ACT treatment failure because of resistance in *Plasmodium* parasites, but the study of Jambou and others strongly supports the need to protect artemisinin derivatives from the development of parasite resistance. In 2006, the WHO requested the discontinuation of manufacturing and marketing of all artemisinin monotherapies, except for the treatment of severe malaria, in an effort to prevent the development of resistance. Many drug manufacturers should be commended for their responsible response to this call from the WHO.

Malaria is a disease that overwhelmingly affects areas of poverty, producing 300–500 million new infections and 1–3 million deaths each year, with most of the disease burden falling on African children younger than 5 years of age (Eline Korenromp *et al*., 2005). In the last few decades, *Plasmodium falciparum*—the parasite causing the most virulent form of malaria—has become increasingly resistant to first-line drug therapies. However, artemisinin-based combination therapies (ACTs) show nearly 100% effectiveness against these drug-resistant parasites. Unfortunately, because of their high cost, ACTs are still beyond the reach of the world's poorest people. This unique partnership is using innovative technology to reduce the cost of ACTs, thereby making these life-saving therapies more accessible to people in the developing world.

2.3 COMBATING MONOTHERAPY

The rapid expansion of artemisinin products marketed mostly as monotherapies is one of the most striking changes seen recently in the private sector of malaria endemic countries. Formerly, over 40 pharmaceutical companies manufactured artemisinin monotherapies as finished products and marketed more than 60 brands of these products in endemic countries, generally at prices lower than those of ACTs. With one exception (Sanofi-Aventis), they are all generic manufacturers from Africa (Cameroon, Ghana, Kenya, and Tanzania), Asia (China, Malaysia, India, and Vietnam), and Europe (Belgium, Cyprus, Denmark, Germany, Greece, Italy, Netherlands, and Switzerland). Most companies

manufacturing artemisinin products are based in India, China, and Vietnam (Bosman and Mendis, 2007).

Given the diverse and exploitative nature of the market, a major responsibility for maintaining the quality of medicines falls on regulatory authorities in countries. A total of nine countries with resistant *P. falciparum* malaria (Afghanistan, Brazil, Eritrea, Ethiopia, Iran, Malaysia, Philippines, Saudi Arabia, and South Africa) have never registered oral artemisinin monotherapies. Thailand registered oral artesunate in 1994, with a restriction on distribution regulated by the Ministry of Health, which resulted in very limited use of the product (i.e., as third-line treatment when quinine + tetracycline treatment failed). One country, Sudan, has withdrawn the marketing authorization for artemisinin monotherapies after it began implementing an ACT-based treatment policy.

In January 2006, WHO made a strong appeal to pharmaceutical companies, National Drug Regulatory Authorities, and international funding and procurement agencies to manufacture, procure, and promote ACTs as the best standard of care for malaria treatment. It called for an end to the deployment of artemisinin monotherapies for the treatment of uncomplicated malaria—a practice especially common in the private sector—to prevent the development of resistance to artemisinins. As a result, 17 of 40 pharmaceutical companies made public commitments to support the WHO position and to stop marketing artemisinin monotherapies within a short span of time and increase the production and marketing of ACTs in both public and private sectors. National health authorities of Benin, Comoros, and Gabon have taken formal steps to withdraw marketing authorizations for artemisinin monotherapies in their respective countries. Recently, India, Kenya, and 11 others in Southern Africa belonging to the Southern African Development Community (Angola, Botswana, Democratic Republic of Congo, Madagascar, Malawi, Mozambique, Namibia, Swaziland, United Republic of Tanzania, Zambia, and Zimbabwe) have made a formal commitment to withdraw marketing authorization for artemisinin monotherapies. In May 2006, with the launch of its new ACT-based treatment policy, the Ministry of Health of Cameroon withdrew marketing authorization for 42 antimalarial medicines. They included 25 different brands/ formulations of artemisinin monotherapies, 11 of amodiaquine, 3 of halofantrine, and 1 brand each of pyrimethamine, proguanil, and Peschiara Fuchsiaefolia

(Malarex). Forty-seven countries have yet to withdraw their marketing authorization for oral artemisinin monotherapies. Ghana has also withdrawn marketing authorization for oral artemisinin monotherapies. Injections of artemisinin monotherapy are used as first line treatment in complicated falciparum malaria.

2.4 ARTEMISININ- BASED COMBINATION TREATMENT

Artemisinin is isolated from the aerial parts of the herb *Artemisia annua* L. (quinghao, sweet wormwood). Because the effectiveness of artemisinin is hampered by its poor bioavailability, a number of semisynthetic derivatives have been developed. Artesunate (AS), a water-soluble hemisuccinate ester derivative of dihydroartemisinin (DHA), artemether (AM), and arteether two oil-soluble methyl and ethyl ether derivatives of DHA, respectively, as well as DHA, the active metabolite of all artemisinin-based compounds and which is also used as a drug itself, are the artemisinin derivatives most widely available in different pharmaceutical dosage forms, including tablets, injections, suppositories, and drug powders. The artemisinin derivatives cause a rapid and substantial decrease of parasite load. However, when used alone and for less than seven days, recrudescent infections are frequent because of the drug's short half-life (White and Olliaro, 1998). Combination with a longer- acting drug such as mefloquine or amodiaquine (AQ) solves the problem of recrudescence, allows a shorter course of treatment and protects against the emergence of resistant strains because parasites are less likely to be resistant to both drugs (Hastings and D'Alessandro, 2000).

Artemisinin-based combination treatments (ACT) are now advocated as the best therapy for the treatment of *Plasmodium falciparum* malaria (Nosten and White, 2007; White, 2006). WHO (2001) currently recommends long acting affordable antimalarial drugs such as piperaquine and amodiaquine to be administered with potent artemisinin derivatives for the treatment of uncomplicated falciparum malaria (Yeung *et al.*, 2004). The combination of a highly potent artemisinin compound, which rapidly reduces parasite numbers and an intrinsically less active partner drug that persists sufficiently long enough in the blood to prevent recurrences of malaria is logical and has proven to be effective. ACTs require a 3-day regimen to expose the parasite to the artemisinin derivative for two asexual life-cycles

to minimize the opportunity for resistance selection and provide maximum clinical response.

2.4.1 GLOBAL SUPPLY AND DEMAND FOR ACTs

The number of ACT treatment courses procured by national governments of endemic countries for use in their public sector increased from around half a million in 2001 to 31.3 million in 2005 (Figure 2.1) 25.5 million of these being for countries in Africa. More than 80% of these orders were placed through WHO. From January 2005 to July 2006, the total ACT procurements for the public sector made by WHO, UNICEF, and to a lesser extent, by Crown Agents has been 62.6 million treatment courses: 80.8% of these orders were for artemether/ lumefantrine, 12.5% were for artesunate plus amodiaquine, and 6.7% were for artesunate plus sulfadoxine/pyrimethamine

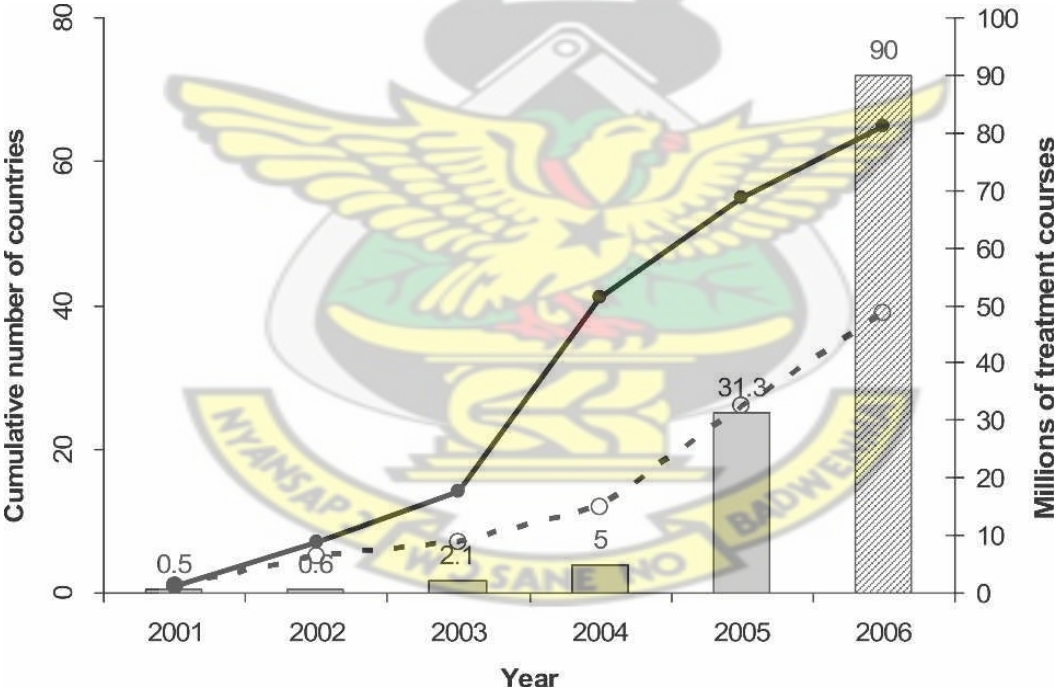


Figure 2.1 Cumulative number of countries adopting (continuous line) and deploying (interrupted line) ACTs as first-line treatment of malaria from January 2001 to July 2006. The annual ACT orders for the public sector in period 2001–2005 (solid bars) and the demand forecast (hatched bar) for 2006

(source; Bosman and Mendis, 2007).

2.4.2 AFFORDABILITY OF ACTs

In response to the increasing burden of malaria caused by parasite resistance to the conventional antimalarial medicines, WHO, in 2001, recommended the use of artemisinin based combination therapies (ACTs) in countries where *Plasmodium falciparum* malaria is resistant to the conventional antimalarial medicines: chloroquine, sulfadoxine-pyrimethamine, and amodiaquine (WHO, 2001). ACTs provide the highest cure rates (Adjuik *et al*., 2004) and could reduce the spread of drug resistance (White and Olliaro, 1996). At a current ex-manufacturer price of 1.35–2.40 US dollars (USD) per adult treatment course, ACTs are 20–40 times more expensive than conventional antimalarial medicines. The adoption by malaria-endemic countries of ACTs, which are 20-fold more expensive than conventional antimalarial medicines, has been made possible largely because of increased financial support provided by the Global Fund to fight AIDS, Tuberculosis, and Malaria (GFATM). Newer initiatives to support malaria control such as the US President's Malaria Initiative (PMI) and the World Bank Booster Program, which were launched in 2005, are additional sources of support for this change, but until now, their financial disbursements have been considerably less than those of the GFATM. These initiatives, will, collectively, contribute no more than one quarter of the global resource requirements for ACTs estimated at 500–600 million treatment courses per year (Schapira and Mendis, 2006). Nor are these external financial mechanisms likely constitute sustainable and predictable sources of financing in the long term, given that they themselves are subject to fluctuations in donor commitment. Lately, international attention is being focused on more innovative financing schemes. In particular, those that would generate more stable, predictable revenue with which to fund programs that require recurrent funding such as for the procurement of life-saving antimalarial medicines. A core group of countries is supporting a new initiative, the International Drug Purchasing Facility (IDPF/UNITAID), based on an "airline tax" to mobilize resources for the procurement of medicines and diagnostics for tuberculosis, HIV, and malaria. This initiative aims to influence market dynamics to lower drug prices and increase supply; it will also explore a global subsidy for ACTs (Laxminarayan *et al*., 2006) to reduce ex-manufacturer price in both the public and private sectors. An international subsidy to promote the universal

adoption of ACTs and discourage monotherapies will have both a humanitarian scope of saving lives and the economic rationale of protecting a public good, which artemisinin derivatives are (Committee on the Economics of Antimalarial Drugs, 2004).

2.4.3 CHALLENGES OF SUPPLYING AFFORDABLE ACTs

Despite notable efforts by pharmaceutical companies, governments, and nongovernmental organizations to make ACTs available to malaria patients, ACTs remain too expensive for the majority of people in endemic countries. Although there are many elements that influence the cost of these drugs, such as packaging and the non-artemisinin component of the combination drugs, the high cost of artemisinin itself (reported to be a range of $900–$1,600/kg in 2006; is currently a key cost driver for ACTs.

Artemisinin is a natural product, and like many other natural products in the current pharmacopoeia, they have presented a supply chain challenge for the pharmaceutical industry that—in the case of a few active pharmaceutical ingredients (APIs) derived from natural products—had to make substantial financial investments to manage the API manufacturing and supply chain. To manufacture APIs, pharmaceutical companies often prefer to use a consistent and reliable source of starting material—ideally a chemical that can be completely synthesized from common and inexpensive chemicals rather than extracted from the natural source. Artemisinin, the starting material for the derivatives used in ACTs, is currently extracted from dried leaves and influorescences from *A. annua*, an annual herb that is primarily cultivated throughout China and Southeast Asia. *A. annua* is a very labour-intensive crop with a lengthy growing cycle; the period from time of planting to artemisinin extraction is ~12–18 months (Grupper, 2005). In addition, the plant's artemisinin content is quite sensitive to genetic backgrounds, cultivation conditions, and harvesting periods. The commonly accepted artemisinin recovery yield is ~5 kg per 1,000 kg of dry leaves, produced from ~1 ha of *A. annua* plants (Grupper, 2005). Based on this yield, an estimated 17,000 ha are required to produce enough artemisinin to manufacture 100 million adult treatments per year. In 2004, there was only an estimated 4,700 ha of Artemisia grown in the world, mainly in China and Vietnam (Grupper, 2005). Recent efforts to scale-up the cultivation of *A. annua* in Asia and East Africa are forecasted to increase the total acreage to ~11,200 ha.

However, an additional source for artemisinin production is clearly needed to meet the projections of a global demand of 400 million ACT treatments per year (Grupper, 2005). In addition to the challenges inherent to the scale-up of *A. annua* cultivation, the artemisinin extraction and purification processes are difficult and costly. These processes currently rely on methods that use organic solvents such as hexane and petroleum ether, which are relatively inefficient, potentially unsafe, and environmentally damaging. The plants—and the many farmers who grow them—are currently the sole source of artemisinin worldwide and are responsible for saving the lives of countless numbers of people who are treated with ACTs each year. *A. annua* will continue to play an important role in the ACT manufacturing process in the future and provide the additional benefit of supporting the development of local economies in the malaria endemic countries where *A. annua* is cultivated. Nonetheless, it is also clear that there is a need for an additional source of artemisinin that is consistent, reliable, pure, and inexpensive. By reducing the cost of this important API intermediate, the cost of ACTs can in turn be reduced, and these life-saving drugs will be more accessible to the people who need them most.

2.4.3.1 AUTHENTICATION OF ARTEMISININ CONTAINING TABLETS IN SOUTH-EAST ASIA

Reports of the distribution of counterfeit artesunate tablets in South-east Asia are becoming more frequent. In 1999, an investigation into the sale of counterfeit artesunate revealed that 71% of the drug vendors and pharmacies sampled in Cambodia sold the counterfeit drug (Rozendaal, 2001). In response to this serious health threat, Green et al. (2000) developed and validated a colorimetric field test [ARTS-Fast red TR (FRTR)] to distinguish genuine artesunate from counterfeit tablets. In conjunction with organoleptic evaluation, the ARTS-FRTR test was used to survey the extent of the distribution of counterfeit artesunate tablets collected in South-east Asia. Initial testing has shown that 38% of artesunate tablets tested in South-east Asia contained no active ingredient (Newton *et al*., 2001). Distribution of counterfeit artemisinin derivatives, such as artemether and dihydroartemisinin has not been documented to date, but these drugs are similar in cost and appearance to artesunate and it is likely that they will become targets of counterfeiting. As artemether is not detected in the

ARTS-FRTR test and Green *et al.* (2000) modified this assay to identify the presence of artemether as well as dihydroartemisinin and artesunate.

2.5 MALARIA TREATMENT PRACTICES IN THE PRIVATE SECTOR

Studies on treatment seeking behavior of people have highlighted the importance of both the formal and informal private sectors as sources of malaria treatment (Laxminarayan *et al*., 2006; Ruebush *et al*., 1995; Deming *et al*., 1989). They also point to problems in the quality of health care (Kirigia *et al*., 1998, Ejezie *et al*., 1990; Deming *et al*, 1989) and those relating to the heterogeneous quality of medicines available in the market (WHO, 2003). A wide variety of antimalarial medicines is available in the retail sector of malaria-endemic countries, in sharp contrast to the limited product range deployed by the public sector. In 2002, a study in Kenya identified 218 different brands of antimalarials registered in the country, including 12 different brands of artemisinin monotherapies (Amin and Snow, 2005). Given the unruly nature of the market, it will be important to monitor and analyze on a continuous basis the quality and price of medicines available and to scrutinize the distribution systems of antimalarial medicines in both the formal and informal private sectors. The lucrative nature of the pharmaceutical market and the opportunities offered by the rapidly increasing demand for antimalarial medicines make them a high value commodity in the private sector—in particular, the artemisinin derivatives, because of the rapid clinical cure they produce, are prone to exploitation.

2.5.1 ARTESUNATE & AMODIAQUINE

Since 2001, WHO recommends the use of artemisinin combination therapies (ACT) to treat Plasmodium falciparum uncomplicated malaria (WHO, 2001). Many artesunate+amodiaquine combination therapies are formulated as 100mg of amodiaquine and 50mg of artesunate per tablet, which requires rather many tablets per day. To improve treatment compliance, Pfizer Laboratories developed two new formulations with 300/ 600 mg of amodiaquine per tablet and 100/200 mg of artesunate per tablet for malaria case management in children up to 7 years of age and in adults. This study confirmed the good efficacy and the tolerability of artesunate-amodiaquine combination treatment of Plasmodium falciparum uncomplicated malaria in African adults. It also demonstrates the

non-inferiority of AS+AQ dosed at 300 and 600 mg of amodiaquine per tablet in one dose per day versus Artemether - Lumefantrine in two doses per day. However, adverse events although are more frequent with this formulation. To achieve equally good results in normal circumstances, where the treatment is not supervised, good compliance on the part of patients is required. This new formulation with fewer tablets per dose, may lead to better compliance (Faye *et al.*, 2010).

2.5.2 ARTEMETHER - LUMEFANTRINE

Artemether is characterized by a rapid onset of schizontocidal action, but has a short elimination half-life (2–3hr), (Lefe`vre *et al.*, 2000; Lefe`vre and Thomsen, 1999; van Agtmael *et al.*, 1999) and recrudescence is frequent when artemether is used as monotherapy, (von Seidlein *et al.*, 1997) unless high dosages are given over several days (White, 1996; Karbwang *et al.*, 1994; Bunnag *et al.*, 1991). In contrast, lumefantrine has a longer elimination half-life of up to 10 days (Lefe`vre and Thomsen, 1999) and is associated with a low recrudescence rate, (Ezzet *et al.*, 1998; Skelton-Stroud and Mull, 1998) but has a slower onset of action. The rationale for the drug combination was to combine the benefits of the fast onset of action of artemether with the long duration of action and high cure rate of lumefantrine in a single oral formulation. Moreover, the short course of treatment with artemether-lumefantrine (over a two- or three day period) should lead to much better compliance, (Karbwang *et al.*, 1994) which remains a major problem with long treatment regimens (Looareesuwan *et al.*, 1996; Hien and White, 1993).

Each tablet consists of 20 mg artemether and 120 mg lumefantrine. The rationale for this combination is that artemether rapidly reduces parasite biomass, and the long-acting lumefantrine eliminates residual parasites. Accumulating data suggest that, for optimum efficacy, coartemether should be given as six doses and with food containing fat (Ashley and White, 2005). Although the four-dose regimen seemed sufficient to provide good cure rates in regions where patients were semi-immune and in areas without multidrug resistance, (Kshirsagar *et al.*, 2000) WHO expressed the desire to have a single global dosing regimen for coartemether to avoid confusion and to assure its long-term effectiveness. However, the safety and tolerability of the higher dose regimen is an obvious concern in using the six-dose regimen as the global standard. Clinical studies investigating

the six-dose regimen of coartemether showed high day 28 parasitological cure rates and good tolerability in adults and children from multi-drug–resistant areas of Southeast Asia and Africa (Falade *et al*., 2005; Piola *et al*., 2005; Lefevre *et al.*, 2001; Van Vugt *et al*., 2000). Moreover, a high adherence to the six-dose regimen was observed, with day 28 cure rates close to 100%, irrespective of whether given under supervision or under unsupervised conditions of routine clinic practice in Africa (Piola *et al*., 2005; Fogg *et al*., 2004). In a head-to-head study performed in Thailand, the six-dose administration schedule of coartemether was more efficacious and equally well tolerated than the four-dose regimen (Van Vugt *et al*., 1999). A recently performed pooled analysis of data from randomized clinical trials confirmed the superior efficacy of the six-dose over the four-dose regimen in adolescents and adults, without altering tolerability and safety (Mueller et al, 2006, 2005).

Clinical studies conducted in more than 2,000 adult and pediatric patients in China, The Gambia, Tanzania, Thailand, and India, and in travelers returning to Europe with acute falciparum malaria have demonstrated that artemether-lumefantrine is very well tolerated and highly efficacious, even against multidrug-resistant strains of the parasite (Bakshi *et al*., 2000; van Vugt *et al*., 1999; Hatz *et al*, 1998) . The therapeutic dosage regimen in adults consists of four doses of artemether-lumefantrine (80 mg of artemether plus 480 mg of lumefantrine per dose) given over a 48-hr period, starting at the time of onset of symptoms or diagnosis, and then at eight, 24, and 48 hours thereafter. This four-dose regimen was rapidly and highly effective with cure rates 95% (Kshirsagar *et al*., 2000; van Agtmael *et al*., 1999; Jiao *et al*., 1999) in areas with high malaria transmission (where patients have some immunity) and where *P. falciparum* is more drug sensitive. In areas such as Thailand, with low transmission but where the most drug-resistant *P. falciparum* strains occur, a six-dose regimen given over a three-day period is necessary to achieve similar efficacy (i.e., 95%) to that of the four-dose regimen in other regions (van Vugt *et al*., 1999). This higher six-dose regimen was chosen based on two dose-optimization trials (van Vugt *et al.*, 1999, 1998) which showed that cure rates were only suboptimal (approximately 80%) using the four-dose regimen in such areas with drug-resistant strains of the parasite. The six dose regimen is also recommended for stand-by emergency treatment (van Agtmael *et al*., 1999).

2.5.2.1 DIHYDROARTEMISININ-PIPERAQUINE

Dihydroartemisinin-piperaquine (DP) is an artemisinin-containing fixed-combination antimalarial treatment developed in China. It is being used increasingly in Southeast Asia. Piperaquine, an orally active bisquinoline discovered by Rhone-Poulenc in the early 1960s, was developed for clinical use in 1973. It is structurally related to chloroquine, with a similar mechanism of action that is, through the chemical inhibition of parasite haem detoxification. However, piperaquine is active against highly chloroquine-resistant Plasmodium falciparum (Raynes *et al.*, 1995; Vennerstrom *et al.*, 1992). The estimated terminal elimination half-life in adults is 17 days (Hung *et al.*, 2003). Piperaquine phosphate (PQP) replaced chloroquine as the recommended treatment for falciparum malaria in China in 1978 and was used extensively for mass prophylaxis and treatment. Reported adverse events are generally similar to those observed with chloroquine, although pruritus is uncommon (Tropical Medicine Institute, 2003). The results of 2 recent randomized clinical trials that assessed DP in Cambodia and Vietnam (Tran *et al.*, 2004; Denis *et al.*, 2002) indicate excellent tolerability and high rates of cure of multidrug-resistant falciparum malaria. This combination is now part of the nationally recommended antimalarial-treatment policy in Vietnam. Artemisinin derivatives have an excellent safety profile in humans, which has been documented in a large number of randomized, controlled trials and in more-extensive community use (Nosten, 1994). Dihydroartemisinin (DHA) is the active metabolite of the more widely used artesunate and artemether and is manufactured as an oral antimalarial drug in China. When taken orally, artesunate is almost entirely hydrolysed to DHA and, so, has equivalent therapeutic efficacy (Newton *et al.*, 2002). In the present study, the dose of DHA in an adult treatment course of DP was ~6.4 mg/kg given over the course of 48 h, which is ~4.7mg lower than that in current artesunate-containing combination anti-malarial regimens (10-12 mg/kg). This dose was chosen empirically and, so, raises the possibility that the DHA content may be insufficient for optimum antimalarial activity, especially in areas where P. falciparum is highly drug resistant.

2.6 SAFETY AND EFFICACY OF ACTs

Several studies have been conducted or are currently underway to evaluate artemisinin-based combination malaria therapy. Studies have shown that the combination therapies are

safe and effective. However, the WHO antimalarial policy currently permits the use of only three combinations, namely; artemether-lumefantrine, artesunate- amodiaquine and dihydroartemisinin –piperaquine.

2.7 COUNTERFEIT DRUGS

Public health is at increasing risk because of an apparent growing global epidemic of the manufacture and trade of counterfeit pharmaceuticals. For example, in Haiti, India, Nigeria, and Bangladesh some 500 children died of acute renal failure after ingesting counterfeit paracetamol (acetaminophen) and cough syrup made using diethylene glycol, a renal toxin (O'Brien *et al*., 1998; Hanif *et al*., 1995) Although there are few accurate estimates of the scale of the problem, only a few counterfeit drug incidents are reported to the appropriate enforcement agencies; thus, numbers of those affected by counterfeit drugs are likely to be grossly underestimated (Cockburn *et al*., 2005; Newton *et al*., 2002) . Since the late 1990s, counterfeit drugs manufactured to mimic antimalarial medicines have been detected in increasing numbers (Newton *et al*., 2006; Basco, 2004; Newton *et al*., 2001). Each year, 300–500 million people in Asia and Africa contract *Plasmodium falciparum* malaria and approximately 1.5 million die (Daviss, 2005). The control of malaria, which is dependent on effective antimalarial drugs, has been severely hampered by a widespread increase in the prevalence of drug-resistant malaria parasites (White, 2004).

Artesunate, an artemisinin derivative, is widely and increasingly used in the treatment of *P. falciparum* malaria in many Southeast Asian and African countries and is vital for the therapy of drug-resistant malaria (van Agtmael *et al*., 1999). In Asia, artesunate has become the target of an extremely sophisticated and prolific counterfeit drug trade that includes the counterfeiting of both the artesunate tablets and packaging, which look extremely similar to the authentic product (Newton *et al*., 2006; Aldhous, 2005; Dondorp *et al*., 2004; Newton *et al*., 2003). In response to this public health problem, Green and others developed a colorimetric Fast Red TR dye test (Green *et al*., 2001) that rapidly and inexpensively screens for the presence of artemisinin-derived compounds such as artemether, artesunate, and dihydroartemisinin in tablets. There are at least 10 different manufacturers of artesunate tablets in Asia. The tablets produced by them have a stated artesunate content of 50 mg. Surveys conducted in 1999–2000 and 2001–2002 in Cambodia, Lao People's Democratic

Republic(Laos), Burma (Myanmar), on the Thailand/Burma border, and Vietnam demonstrated that 38% and 53%, respectively, of artesunate tablets contained no active ingredient (Dondorp *et al*., 2004; Newton *et al*., 2001). To date, only artesunate labeled as made by Guilin Pharmaceutical Co., Ltd. (Guilin, Guangxi, China) has been found to be counterfeit. Visual inspection, including the examination of the holograms, bar codes, printing, crimping, colour, size, weight, and consistency of the putative artesunate tablets, was in good agreement with the colorimetric test results in the first survey, but not in the second survey. The counterfeiters appear to have responded to the increase in public awareness by producing more sophisticated counterfeit holograms and packaging, making it very difficult to distinguish the counterfeit and genuine drugs (Newton *et al*, 2006; 2003). Little is known about the chemical composition of these counterfeit medicines, the potential presence of toxic substances, or the fake artesunate production sources.

Therefore, a liquid chromatography-mass spectrometry (LC-MS) method to quantitatively evaluate the contents of artesunate tablets was developed to validate the Fast Red TR colorimetric test results, and to investigate the presence of other active ingredients. Raman spectroscopy was used to characterize the excipients present in the tablets and complement the LC-MS analysis. Multivariate pattern recognition methods were used to examine the chemical similarities and differences between the chemical fingerprints of different types of fake tablets, and to correlate these similarities with packaging characteristics and sample origin.

2.7.1 RECORDS OF COUNTERFEIT AND SUBSTANDARD DRUGS

Over the past two decades, there has been an increase in public awareness of the existence of counterfeit and substandard drugs (Reithinger, 2001; Afu, 1999; WHO, 1993). Increasing international and regional free trade, high demand for curative and preventive drugs and vaccines, proliferation of small pharmaceutical industries, and insufficient regulation of drug manufacture and trade are some of the factors that have contributed to the wide distribution of low-quality medicines, particularly in developing countries. Although practically all types of pharmaceutical products have been shown to be involved, the existing data suggest that anti-infectious agents, in particular antibiotics and anti-parasitic agents, are the most counterfeited products in developing countries

(Wondemagegnehu, 1999). The potential risk of counterfeit anti-infectious agents for individual and community health includes clinical aggravation leading to complications and even mortality from either the disease itself or possible toxic components in the product, increased health expenditures to attain cure, and selection of drug-resistant bacteria and parasites.

From the epidemiologic viewpoint, ineffective or partially effective counterfeits may lead to biased data on antimalarial drug efficacy, as well as to discordant results between clinical efficacy and molecular markers. To implement effective counter measures against counterfeit drugs, there is a need for more data to define the extent of the problem. Many of the previous reports on counterfeit drugs and vaccines have been based on case reports on failure to attain the expected therapeutic or prophylactic effect, chance discovery, or investigation on a small sample of products belonging to different classes of drugs (Wondemagegnehu, 1999; Petralanda, 1995; Roy, 1994).

2.7.2 FAKE ANTIMALARIALS IN SOUTHEAST ASIA

In much of the malaria-affected world, the majority of antimalarial drugs are purchased directly by the patient or carer from the private sector (shops, pharmacies, markets, itinerant drug sellers, etc.). In Southeast Asia, falciparum malaria has become resistant to most of the available antimalarials. The recommended treatment in most of the region has now changed to combinations containing the highly potent and active artemisinin derivatives combined with a second, slower acting, drug such as mefloquine. Resistance to the artemisinins has not developed yet. An important, but underappreciated, obstacle to malaria control in the region is the widespread dissemination of counterfeit antimalarial drugs, especially of the artemisinins. As fake antimalarials usually contain no active ingredient the unwitting patient is at substantial risk of developing severe malaria and dying. As fake, and thus ineffective drugs, cannot be easily distinguished from the genuine products, this undermines the confidence of the public and health care workers in the antimalarial. There have been several reports of artemisinin resistance in this region, which were ultimately attributed to fake drugs. In 1999–2000, Newton *et al.* (2001) conducted a multinational survey to document the prevalence of counterfeit artesunate in South East Asia, which demonstrated

that 38% of shop-bought 'artesunate' blisterpacks were counterfeit, containing no artesunate.

2.7.3 TACKLING COUNTERFEIT AND SUBSTANDARD MEDICINES

Significant resources have been devoted to tackle counterfeit medicines, but very little specific attention has been given to the far more serious and widespread problem of substandard medicines. This is partly a consequence of the poor differentiation made between these two distinct problems. However, reducing the problem of substandard medicines to a consequence of counterfeiting skews resources towards legal action alone, complicating efforts to define targeted strategies to specifically address the problem of the substandard medicines. The focus of attention should rather be on the detection and removal of poor quality medicines, whether they are counterfeit or not, while at the same time assisting legitimate manufacturers to improve the quality of their pharmaceutical production. The limited resources available for the development of efficient pharmacovigilance systems in developing countries compound the problem. Because the consequences of substandard medicines, both on individuals and on public health, often go unreported, there is no stimulus to intervene. The pre-qualification programme has recently been expanded but capacity remains limited, and the majority of essential drugs remains outside of the scope of the programme and are still purchased without a proper evaluation. Other recent initiatives by the WHO are important but remain financially fragile; moreover, these measures will be only successful if other actors involved in drug procurement assume their responsibilities.

Donors have an important role to play by strengthening quality clauses based on WHO standards in the tender mechanisms they impose on non-governmental organizations. Likewise, drug purchasers (NGOs, international organizations, charities, and national purchase centres in resource-limited countries) should assume their responsibility towards protecting patients' health and insist that producers and distributors supply drugs that meet WHO standards. Quality assurance is a mandatory preliminary to drug purchases in the West, and there is no rationale for this procedure to be any different when drugs are exported to poor populations. Governments could act now to reduce this problem by

granting export authorization only to pharmaceutical products that comply with the WHO standards for quality, efficacy and safety (Caudron *et al.*, 2008).

2.7.4 SUBSTANDARD DRUGS IN DEVELOPING COUNTRIES

In several developing countries drug quality is a source of concern. There is a general feeling that there is a high incidence of drug preparations which are not of acceptable quality. Instances which are quoted are often linked with terms such as counterfeiting and fake, which carry economic and perhaps political implications. Poor quality drug preparations may lead to adverse clinical results both in terms of low efficacy and encouraging drug resistance (ten Ham, 1992; Masland and Marshall, 1990; Silverman *et al.*, 1990).

In the industrialized world drug regulatory authorities have developed strict standards and controls to ensure drugs are effective and safe. However, in the less-developed world, lack of human and financial resources within the health sector as a whole limits the capacity of drug regulatory agencies, resulting in a sub-optimally regulated environment in which substandard drug production can persist without detection. Circulation of substandard drugs is further encouraged by the fact that drugs manufactured for export are often not regulated to the same standard as those manufactured for domestic use. An analysis done by the European Union and the French Ministry of Cooperation (Andriollo *et al.*, 1997) revealed many problems in the export legislation from European countries to developing countries, including imprecise controls regarding good manufacturing practices for exported products, lack of quality control of products that have not been marketed in Europe, and discordant information between drugs to be exported and drugs for European use (Andriollo *et al.*, 1997).

2.7.5 CAUSES OF SUBSTANDARD MEDICINES

Poor compliance with GMP standards can lead to substandard production. This may be accidental (such as human error) or the result of insufficient resources (expertise, appropriate manufacturing infrastructure, or human and financial resources). Other deliberate causes are often ignored or underestimated. Quality audits of manufacturing sites done by MSF pharmacists (180 sites visited over the last 4 years) have found that manufacturers that regularly pass the most stringent inspections adjust their standards to

that of the recipient country. In our observations, parallel productions can exist in the same 'GMP compliant' facilities: a high standard of production for the strictly regulated markets and for exacting clients such as UN organizations and international aid agencies; an intermediate standard of production for middle-income countries; and a much lower standard for poorly regulated countries (Caudron *et al*., 2008).

Developing country governments often purchase drugs without adequate reference to quality standards. While these are available through WHO publications and via the Internet (US Pharmacopeia 2008), local authorities in a number of countries have expressed their difficulty in accessing these documents and translating this information into clauses for tenders and contracts. Non-governmental organizations working in developing countries also issue drug tenders without applying minimum quality assurance procedures (European Commission Humanitarian Aid Department, 2006).

2.7.6 PROBLEMS ASSOCIATED WITH SUBSTANDARD PRODUCTION

Common problems associated with substandard medicines include under or over concentration, contamination, poor quality ingredients, poor stability and packaging problems. Contamination is a recurrent problem, and can have fatal consequences, particularly with intravenous products. In MSF's experience, microbial contamination of injections and infusions is often the result of poor sterilization management, obsolete equipment, inappropriate production environment or too short sterilization cycles (to cut costs). It can also be the result of poor quality packaging materials. Contamination of active pharmaceutical ingredient (API) with residues of solvents used in the synthesis or other toxic impurities is another frequent and important concern. The quality of the API is one of the major determinants of quality for all pharmaceuticals. However, it is also here that compromise can lead to the greatest cost saving as APIs can represent over 80% of the price of finished products (Pinheiro *et al*., 2006).

2.7.7 REGULATION OF SUBSTANDARD DRUGS

A World Health Assembly Resolution in 1988 requested WHO to initiate programmes for the prevention and detection of the export, import, and smuggling of substandard pharmaceutical preparations (WHO, 1988). More than a decade later the World Health

Assembly urged member states to 'establish and enforce regulations that ensure good uniform standards of quality assurance for all pharmaceutical materials and products manufactured in, imported to, exported from, or in transit through their countries (WHO, 1999a).

This was followed by a European Commission directive in 2003 stipulating that 'all medicinal products for human use, including medicinal products intended for export, are to be manufactured in accordance with the principles and guidelines of good manufacturing practices (European Commission, 2003).

However, the reality today is that the quality of drugs for export from developed to developing countries is still determined through a much less rigorous evaluation than for the domestic market (European Commission Humanitarian Aid Department, 2006). Efficacy and safety are often not evaluated at all. Drugs destined for international aid and development programmes are also often exempted from regulatory control (Andriollo *et al.*, 1997). The expectation is that the recipient country will evaluate the quality of the imported drug. While this may be an acceptable expectation between rich countries, placing this burden of responsibility on countries that do not have the resources to do it is impractical, even exploitative.

2.8 QUALITATIVE AND QUANTITATIVE ANALYTICAL METHODS FOR THE DETECTION OF COUNTERFEIT ACTs

A broad panel of techniques has been reported for the analysis of artemisinin derivatives, ranging from simple and cheap in-field ones (colorimetry and thin layer chromatography) to more advanced laboratory methods (mass spectrometry, nuclear magnetic resonance, and vibrational spectroscopies) through chromatographic methods, which remain the most widely used. Nowadays, the first step in detecting counterfeit drugs is to compare the physical appearance and text on packets, leaflet inserts, and blister packs (when present) of suspected samples with those of known genuine products. However, with increased counterfeiter sophistication, this careful visual inspection is not sufficient to distinguish between fake and authentic drugs. It must therefore be followed by chemical analysis, most often using high-performance liquid chromatography (HPLC), considered as the gold standard analytical method in drug analysis, but also with simple in-field assays [e.g.,

colourimetric test and thin-layer chromatography (TLC)] or more advanced laboratory techniques [e.g., mass spectrometry (MS), vibrational spectroscopies (Raman or IR), and nuclear magnetic resonance (NMR) spectroscopy]. Taken together, these analytical methods allow one to quantitatively determine the chemical composition of the drug [active pharmaceutical ingredients (APIs) as well as impurities and excipients] and hence to identify poor-quality medicines, which include not only counterfeit drugs but also substandard and degraded drugs.

2.8.1 COLORIMETRY

Several colorimetric tests have been developed to determine the authenticity of artemisinin derivatives. As artemisinin derivatives do not have particular chemical groups that easily react with certain reagents to yield colored products, they can be transformed by acid or base treatment to enolates/carboxylates or α,β-unsaturated decalones, which are more reactive compounds (Thomas *et al.*,1992). Thus, a diazonium salt, the commercial Fast Red TR (FRTR) salt, reacts with the alkali decomposition product of artesunate (AS), leading to the appearance of a distinctive yellow color at pH 4. The specificity of the test is pH-dependent. Indeed, at pH 6–8 not only AS but also other commonly used antimalarial drugs develop yellow (artemisinin, sulfadoxine) or orange (primaquine) colors (Green *et al.*, 2000). On the other hand, the FRTR salt added to the acid decomposition products of AM, DHA, or AS produces a yellow colour. A faint orange color appears in the same experimental conditions in the presence of paracetamol, whereas other common antimalarials (artemisinin, chloroquine, sulfadoxine, etc.) appear colorless (Green *et al.*, 2001). The method is thus specific to AM, AS, and DHA, but cannot distinguish selectively each of these drugs. The accurate quantitative analysis of the content of AS tablets after alkali decomposition and that of AM and AS tablets after acidic degradation can be accomplished, provided the yellow product is extracted into ethyl acetate and its absorbance measurement is performed at 420 nm with a spectrophotometer (Green *et al.*, 2001; 2000). The method is sensitive enough to require only 1% (0.5 mg) of AS tablets with the alkali decomposition process and 5% (2.5–5 mg) of AS, AM, and DHA tablets with the acidic decomposition process (Green *et al.*, 2001; 2000).

Table 2.1 The global pharma health fund minilab®

Manufacturer	Global Pharma Health Fund, Merck charitable organization
Presentation	Two suitcases (apparatus, chemical reagents, reference standards)
Worldwide diffusion	More than 350 units in use worldwide across 70 countries, mostly in Africa
Drugs analyzed	50 essential drugs from the WHO list
Steps	1. Visual inspection of the product, its packaging, and labeling versus the genuine product 2. Disintegration test 3.Quick specific color reaction versus a standard 4. Semi quantitative TLC versus a standard
Criteria of acceptance	≥80% of correct active ingredient with no upper-bound limit (estimated error ±10%)
Advantages	Simple, inexpensive, in-field, no trained personnel, detailed guides for the identification of each drug updated in 2008 and partly in 2010

2.8.2 CHROMATOGRAPHIC METHODS

2.8.2.1 THIN- LAYER CHROMATOGRAPHY

TLC is a simple, sensitive or moderately sensitive, rapid, and inexpensive technique that is employed for the analysis of artemisinin derivatives. Some publications deal with the specific quantitative determination of artemisinin derivatives in different matrices, such as artemisinin in *Artemisia annua* plant extracts (Marchand *et al.*, 2008), reconstituted mixtures of artemisinin derivatives (artemisinin, AM, AS, and DHA) (Gabriels and, Plaizier-Vercammen, 2004), or pharmaceutical formulations of AS (Agarwal *et al.*, 2007) or AM (Tayade and Nagarsenker, 2007) using normal- or reverse-phase TLC or high performance TLC. These assays can only be performed in specialized laboratories. Nevertheless, semi quantitative TLC determination using colored reaction tests is a potent technique to check the quality of AS and AM formulations in-field using the transportable Global Pharma Health Fund Minilab® (Global Pharma Health Fund, 2010; Jahnke, 2004; Basco, 2004).

Very recently, Ioset and Kaur (2009) developed two novel colour reaction assays utilizing TLC silica gel sheets and 2, 4-dinitrophenylhydrazine (DNP) or 4-benzoylamino-2, 5-dimethoxybenzenediazonium chloride hemi (zinc chloride) salt [Fast Blue RR salt (FBS)] as reagents, giving, respectively, a pink or a blue product in the presence of AS, AM, or DHA (but not artemisinin itself) clinically used in monoformulations as well as with ACTs. The identity of artemisinin derivatives can be determined unambiguously from their characteristic retention factor after elution on the TLC sheet. When the colour reaction obtained with both DNP and FBS reagents is combined with the separation of the pharmaceutical components by migration on TLC plates, the other antimalarial drugs tested as well as a range of commonly used excipients and other drugs readily accessible and frequently used in malaria-endemic countries such as anti (retro) virals, antibiotics, and analgesics are not detected. The DNP and FBS tests need 2–5 mg of material and can detect as little as 10% of nominal ART content in ACTs. As for any colorimetric method, assays should be conducted using a positive control (the declared ART drug) and a negative control (the solvent used for sample extraction). The method enables the detection of counterfeit as well as substandard medicines since a semi-quantitative measure of amounts of ARTs in the formulations analyzed is given by the depth of the color compared with that of the authentic suitable ART at various concentrations.

The method is specific, simple to use, rapid, robust, reproducible, inexpensive, and requires no trained staff. Developed in a kit format, it could quickly become a powerful, widely used in-field tool to ensure quality of artemisinin derivatives in ACTs. Compared with the FRTR test, the in-field method used nowadays (Green *et al*., 2001; 2000) these two novel reaction assays combined with TLC are not more sensitive but are much more specific. For example, AM, AS, and DHA cannot be discriminated in the FRTR test since they produce the same yellow colour after acidic decomposition (Green *et al*., 2001). Moreover, all the compounds colored yellow (as ACTs when co-formulated with other antimalarials such as amodiaquine, lumefantrine, and primaquine, etc) to orange before the reagent addition are not suitable for the FRTR test as they give false-positive results. When DNP and FBS reagents are used, only erythromycin among the 80 non-artemisinin based drugs tested reacts in a similar way as artemisinin derivatives, yielding a blue coloration with the FBS

reagent, whereas 24 compounds treated with the FRTR reagent as described by Green et al. (Green *et al*., 2001; 2000) yielded yellow to orange as well as red, brown, or pink colors, which can hinder the detection of artemisinin derivatives. It should be pointed out that the method of Ioset and Kaur (2009) is nevertheless specific as the second reagent (DNP) does not produce any colour with erythromycin.

2.8.2.2 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC is the most popular instrumental technique used for the analysis of pharmaceuticals. It is regarded as the reference or gold standard method for the quantitative determination of pharmaceutical formulation contents, especially artemisinin derivatives, and is used for validating alternative analytical methods. One of the advantages of HPLC is that many detectors can be coupled with it, such as electrochemical, evaporative light scattering (ELS), UV, photodiode array (PDA), and MS detectors, which provides more possibilities for detecting different types of constituents.

Nowadays, HPLC-PDA detection (or HPLC-UV detection), HPLC-ELS, and HPLC-MS are the preferred HPLC techniques for the quantitative analysis of artemisinin derivatives. Indeed, the maximum UV absorbance of artemisinin derivatives occurs at low wavelength (192 nm for artemisinin) and, even though it is weak, the absorption intensity is sufficiently high to allow the quantification of artemisinin, AS, and DHA at concentrations of 10, 400, and 340 µg/mL, respectively, using a PDA detector set at 192 nm (Ferreira and Gonzalez, 2009). Moreover, the quasi-universal, versatile, low-cost, and very sensitive ELS detector allows the quantification of ARTs with an improved sensitivity compared with UV detection at 220 nm (Ioset and Kaur, 2009) but close to that at 192 nm (Ferreira and Gonzalez, 2009). Hence, HPLC-PDA detection and HPLC-ELS have proved to be sensitive (even though sensitivity is not generally a crucial issue for the analysis of pharmaceutical formulation ingredients), accurate, precise, and reproducible methods for the quantification of ARTs in a time of less than 10 min (Gaudin *et al*., 2009; Ferreira and Gonzalez, 2009). HPLC coupled with the high sensitivity and selectivity of MS is the most powerful technique for the definitive structural identification of chemical entities present, even at low concentrations, in pharmaceutical formulations through detection of their major ions.

Quadrupole, ion trap, and time-of-flight (TOF) mass spectrometers using electrospray ionization (ESI) or atmospheric pressure chemical ionization with single ion monitoring or multiple reaction monitoring in tandem MS are currently employed for the determination of artemisinin derivatives.

Chapter 3

MATERIALS AND METHODS

3.1 MATERIALS

Artesunate, amodiaquine and artemether and lumefantrine powders were obtained as gifts from Kinapharma Ltd., Letap Pharmaceuticals Ltd., and Ipca industries Ltd., respectively. Six brands of artesunate and amodiaquine combi-pack tablets and two brands of artesunate monotherapy tablets were purchased from selected pharmacies and licensed chemical sellers' shops in the Kumasi metropolis. The combi-packs contained artesunate tablets and amodiaquine tablets packaged as co-blisters. Six brands of artemether-lumefantrine tablets and one brand of artemether injection were purchased from pharmacies and licensed chemical sellers' shops in the Kumasi metropolis. A dosage unit of artemether-lumefantrine tablets consists of a single tablet that contains both artemether (20 mg) and lumefantrine (120 mg); however, one sample consisted of artemether (40mg) and lumefantrine 240 mg. The packaging of the various brands of antimalarials was examined for any features of illegal prints. No further checks were done to distinguish the genuine ACTs from the fake ones. All analyses were performed before the expiry dates of the products.

The characteristics of the artemisinin based antimalarial samples are shown in Tables 3.1-Table 3.6.

Table 3.1 Artesunate and amodiaquine tablet samples used

SAMPLE CODE	**AT2/AM2**	**AT3/AM3**	**AT4/AM4**
BRAND NAME	CAMOSUNATE PLUS	_	_
GENERIC NAME	AMODIAQUINE/ ARTESUNATE	ARTESUNATE/ AMODIAQUINE	ARTESUNATE/ AMODIAQUINE
MANUFACTURER	DANADAMS	IPCA LABORATORIES LTD	PHYTO-RIKER PH'TICALS LTD
ORIGIN (COUNTRY)	GHANA	INDIA	GHANA
SHELF-LIFE	TWO YEARS	THREE YEARS	THREE YEARS
DATE OF MANUFACTURE	FEBRUARY, 2009	JUNE, 2008	SEPTEMBER 2009
EXPIRY DATE	FEBRUARY, 2011	MAY 2011	AUGUST 2012
STRENGTH OF ARTESUNATE	100 mg	50 mg	100 mg
STRENGTH OF AMODIAQUINE	300 mg	153.1 mg	300 mg
FDB REGISTRATION NUMBER	FDB/SD 05-5189	NA	NA
RETAIL PRICE(FULL COURSE)	GH¢ 3.00	GH¢ 3.00	GH¢ 2.00
DOSAGE FORM	TABLETS	TABLETS	TABLETS
PACKAGING	BLISTER PACK	BLISTER PACK	BLISTER PACK

NA=Not available on packaging

Table 3.2. Other artesunate and amodiaquine tablet brands used

SAMPLE CODE	**AT5/AM5**	**AT6/AM6**
BRAND NAME	GSUNATE 100 KIT	COMBICURE
GENERIC NAME	ARTESUNATE &AMODIAQUINE	ARTESUNATE+ AMODIAQUINE
MANUFACTURER	BLISS GVS PHARMA LTD	LETAP PH'TICALS LTD
ORIGIN (COUNTRY)	INDIA	GHANA
SHELF-LIFE	THREE YEARS	TWO YEARS
DATE OF MANUFACTURE	AUGUST, 2009	AUGUST, 2009
EXPIRY DATE	JULY, 2012	JANUARY, 2011
STRENGTH OF ARTESUNATE	100 mg	100 mg
STRENGTH OF AMODIAQUINE	300 mg	306 mg
FDB REGISTRATION NUMBER	NA	NA
RETAIL PRICE(FULL COURSE)	GH¢ 3.00	GH¢ 1.00----GH¢ 1.50
DOSAGE FORM	TABLETS	TABLETS
PACKAGING	BLISTER PACK	BLISTER PACK

NA=Not available on packaging

Table 3.3 Artesunate (monotherapy) tablet brands used

SAMPLE CODE	**AT 7**	**AT 8**
BRAND NAME	MALASATE 200	LEVER ARTESUNATE
GENERIC NAME	ARTESUNATE TABLETS	ARTESUNATE TABLETS
MANUFACTURER	ERNEST CHEMISTS LIMITED	ADAMS PHARMACEUTICALS LTD
ORIGIN (COUNTRY)	GHANA	CHINA
SHELF-LIFE	THREE YEARS	THREE YEARS
DATE OF MANUFACTURE	APRIL,2009	NOVEMBER, 2009
EXPIRY DATE	APRIL,2012	OCTOBER, 2012
STRENGTH OF ARTESUNATE	200 mg	50 mg
FDR REGISTRATION NUMBER	NA	NA
RETAIL PRICE(FULL COURSE)	GH¢ 4.50	GH¢ 4.00
DOSAGE FORM	TABLETS	TABLETS
PACKAGING	BLISTER PACK	BLISTER PACK

NA = Not available on packaging

Table 3.4. Artemether-lumefantrine tablet brands used

SAMPLE CODE	AL 2	AL 3	AL 4
BRAND NAME	COARTEM	LONART FORTE TABLETS	LUFART TABLETS
GENERIC NAME	ARTEMETHER LUMEFANTRINE	ARTEMETHER+ LUMEFANTRINE	ARTEMETHER+ LUMEFANTRINE
MANUFACTURER	NOVARTIS	BLISS GVS PHARMA LTD	MAXHEAL LABORATORIES PVT LTD
ORIGIN (COUNTRY)	SWITZERLAND	INDIA	INDIA
SHELF-LIFE	ONE YEAR	TWO YEARS	THREE YEARS
DATE OF MANUFACTURE	JANUARY, 2010	AUGUST, 2010	NOVEMBER, 2009
EXPIRY DATE	DECEMBER, 2011	JULY, 2012	OCTOBER, 2012
STRENGTH OF ARTEMETHER	20 mg	40mg	20 mg
STRENGTH OF LUMEFANTRINE	120 mg	240 mg	120 mg
FDB REGISTRATION NUMBER	NA	NA	NA
RETAIL PRICE(FULL COURSE)	GH¢10-GH¢ 12	GH¢ 5	GH¢ 5
DOSAGE FORM	TABLET	TABLET	TABLET
PACKAGING	BLISTER PACK	BLISTER PACK	BLISTER PACK

NA = Not available on packaging

Table 3.5 Other artemether-lumefantrine tablet brands used

SAMPLE CODE	AL 5	AL 6	AL 7
BRAND NAME	ARTRIFAN	IBATHER	ARTEFAN
GENERIC NAME	ARTEMETHER+ LUMEFANTRINE	ARTEMETHER+ LUMEFANTRINE	ARTEMETHER+ LUMEFANTRINE
MANUFACTURER	PHYTO-RIKER PH'TICALS LTD	LUPIN LIMITED	AJANTA PHARMA LIMITED
ORIGIN (COUNTRY)	GHANA	INDIA	INDIA
SHELF-LIFE	THREE YEARS	TWO YEARS	TWO YEARS
DATE OF MANUFACTURE	SEPTEMBER, 2010	MARCH, 2010	MAY,2010
EXPIRY DATE	AUGUST, 2013	FEBRUARY, 2012	APRIL, 2012
STRENGTH OF ARTEMETHER	20 mg	20mg	20 mg
STRENGTH OF LUMEFANTRINE	120 mg	120 mg	120 mg
FDB REGISTRATION NUMBER	FDB/SD.09.-12800	NA	NA
RETAIL PRICE(FULL COURSE)	GH¢ 4	GH¢ 5	GH¢ 5
DOSAGE FORM	TABLETS	TABLETS	TABLETS
PACKAGING	BLISTER PACK	BLISTER PACK	BLISTER PACK

NA = Not available on packaging

Table 3.6 Artemether injection brand used

SAMPLE CODE	**AL 8**
BRAND NAME	GVITHER FORTE INJECTION
GENERIC NAME	ARTEMETHER
MANUFACTURER	BLISS GVS PHARMA LIMITED
ORIGIN (COUNTRY)	INDIA
SHELF-LIFE	THREE YEARS
DATE OF MANUFACTURE	JULY, 2010
EXPIRY DATE	JUNE, 2013
STRENGTH OF ARTEMETHER	8Omg/ml
FDB REGISTRATION NUMBER	NA
RETAIL PRICE(FULL COURSE)	GH¢ 1.50
DOSAGE FORM	INJECTION
PACKAGING	AMPOULE

NA = Not available on packaging

3.1.1 REAGENTS

Fast Red TR salt (1, 5-Naphthalene-disulfonate salt, reagent grade) was obtained from Acros Organics (New Jersey, USA), dehydrated ethanol (absolute ethanol) was obtained from BDH Ltd. (UK). Sodium hydroxide, acetic acid, distilled water, methanol, Brady's reagent (2,4- dinitrophenylhydrazide), potassium iodide, ethyl acetate, hydrochloric acid, acetic anhydride, glacial acetic acid, perchloric acid, potassium hydrogen phthalate and oracet blue indicator were obtained from the Chemical Store of the Department of Pharmaceutics and the Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi. Dragendorff's reagent and vanillin/sulphuric acid reagent were prepared with reagents obtained from the Chemical Stores mentioned above.

3.1.2 EQUIPMENT AND APPARATUS

Scalpel blades, TLC sheet, capillary tubes, ultra sonic bath (Nickel-Electro Ltd., England), Cecil CE 2041 2000 series Ultraviolet spectrophotometer (Cecil Instruments, Cambridge, England), Erweka ZT4-4 disintegrating apparatus (Heusenstamm, Germany),tablet hardness tester (DBK Instruments, India) and digital analytical balance (PW Series, Adam Equipment Inc., U.S.A.).

3.2 SURVEY ON PREVALENCE AND COST OF ACTs IN THE KUMASI METROPOLIS

3.2.1 STUDY AREA

The survey was conducted in the Kumasi metropolis

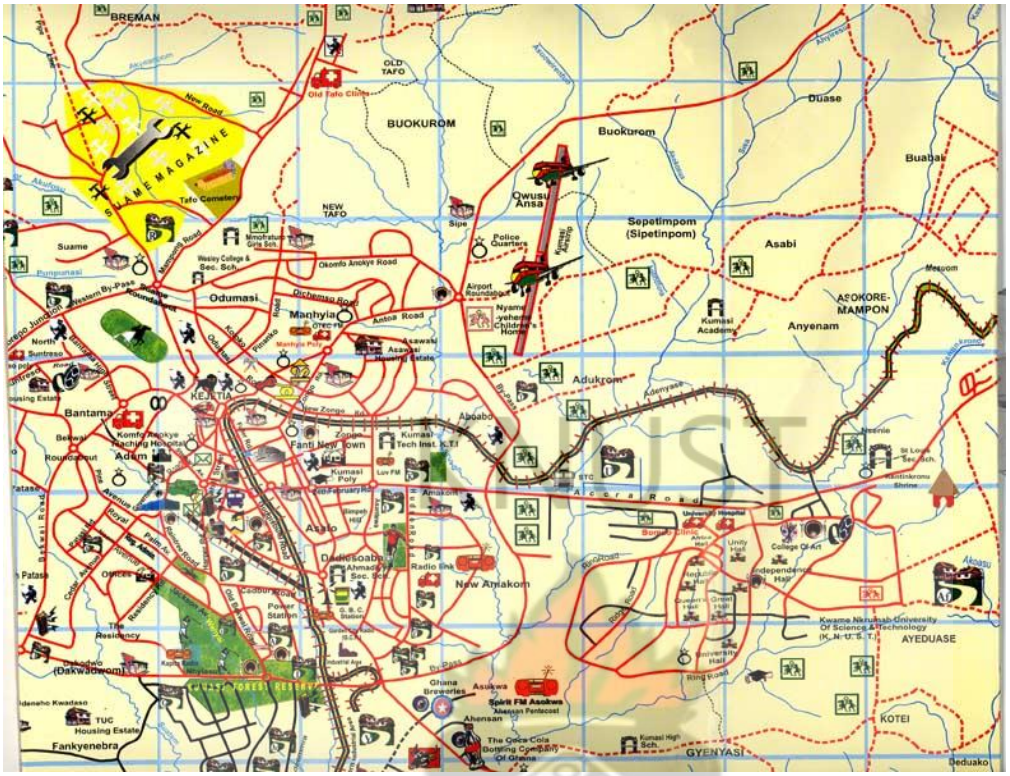


Figure 3.1 Map of Kumasi
(Source: Google images)

3.2.2 SELECTION OF FACILITIES

The selection of pharmacies and licensed chemical sellers' shops was done with reference to a list of all pharmacies and licensed chemical sellers' shops in the Kumasi metropolis obtained from the pharmacy council office in Kumasi. The distribution of selected facilities was across the length and breadth of the metropolis. To attain this, a map was used and the metropolis was divided into four quadrants. There was an even selection of facilities from each of the four quadrants. There are more licensed chemical sellers' shops than pharmacies in the Kumasi metropolis and thus, 60 licensed chemical sellers' shops and 40 pharmacies were randomly selected. Thus, in finding out the availability of ACTs in the Kumasi metropolis, a total of 100 questionnaires were answered by respondents in pharmacies and licensed chemical sellers' shops in the Kumasi metropolis through face-to-face interviews.

3.2.3 QUESTIONNAIRE DESIGN

The questionnaire used in the survey consisted of 24 questions and is presented in the Appendix I. The questionnaire consisted of both close ended and open ended questions. The close ended questions were multiple choices. The open ended questions were meant for interviewees to express themselves. Questionnaire testing through a pilot survey was carried out to assist in the design of the questionnaire.

3.2.4 DATA COLLECTION

The survey was carried out between 1st February, 2010 and 5th March, 2010. The data was obtained from a total number of forty (40) pharmacies and sixty (60) licensed chemical sellers' shops. This was done through face-to-face interviews using the questionnaires. The average interview time was about twenty minutes.

3.2.5 DATA ANALYSIS

The data obtained was transferred to the Statistical Package for Social Scientists (SPSS) version 16 data base. The SPSS version16 software was used in the analysis of the data collected. The results were presented as tables, bar graphs and pie charts in chapter 4

3.3 ASSESSMENT OF QUALITY OF ARTESUNATE AND AMODIAQUINE HYDROCHLORIDE TABLETS

3.3.1 UNIFORMITY OF WEIGHT OF ARTESUNATE AND AMODIAQUINE TABLETS

AT2, AT3, AT4, AT5, AT6, AT7 and AT 8 were the artesunate tablet brands used. The amodiaquine tablets used were AM2, AM3, AM4 and AM 5. Twenty tablets taken at random from each sample were weighed individually and then weighed together. The average mass of each sample was determined. The deviation of each of the individual tablets in each sample, from the average mass of the sample was determined. For a sample to pass the uniformity of weight test not more than 2 of the individual masses should deviate from the average mass by more than the percentage deviation shown in the table below and none should deviate by more than twice the percentage.

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

3.3.2 DISINTEGRATION OF ARTESUNATE AND AMODIAQUINE TABLETS

The samples used were; AT2, AT3, AT4, AT5, AT6, AT7 and AT8 (artesunate tablets), AM2, AM3, AM4 and AM 5(amodiaquine tablets). Six tablets from each sample were selected at random. One tablet was placed in each of the six tubes of the disintegration basket. The apparatus was operated using distilled water as the medium and immersion fluid and maintained at 37 ± 2 ^{o}C. The dosage units were observed during the process in order to record the time of disintegration. At the end of the specified time (15 minutes for uncoated tablets and 30 minutes for film coated tablets), the basket was lifted from the fluid and the dosage units observed. If 1 or 2 dosage units fail to disintegrate, the test is repeated on 12 additional tablets. The requirements of the test are met if not less than 16 of the 18 dosage units tested have disintegrated.

3.3.3 HARDNESS OF ARTESUNATE AND AMODIAQUINE TABLETS

The samples used were; AT2, AT3, AT4, AT5, AT6, AT7 and AT 8 (artesunate tablets), AM2, AM3, AM4 and AM5 (amodiaquine tablets). Ten tablets were selected at random from each of the samples. Each tablet was placed between the jaws of a hardness tester (DBK Instruments, India) taking into account where applicable, the shape, the break-mark and the inscription. For each test, the tablet was oriented in the same way with respect to the direction of application of the force. The test was carried out on 10 tablets from each sample and it was ensured that all fragments of tablets were removed before each determination. The force in Newtons required to break each tablet was recorded and the

mean and standard deviation determined. The results were expressed as the mean, minimum and maximum values of the forces measured, all expressed in newtons.

3.4 DETERMINATION OF AUTHENTICITY OF ARTESUNATE TABLETS

3.4.1 COLORIMETRY

The artesunate tablets in the artesunate - amodiaquine combipacks, were analyzed. These were; AT2, AT3, AT4, AT5 and AT6. Artesunate tablets that were packaged alone (monotherapy), namely: AT7 and AT 8 were also analyzed. The procedure of Green *et al*., (2000) was adopted in the analysis. Approximately 1% of the test artesunate tablet was scraped into a clean tube and labeled with the test number. An equivalent mass of artesunate powder was poured into a clean test tube and labeled as positive control. A clean test tube was left empty and labeled as negative control 0.5ml of 1M NaOH was added to all the test tubes and shaken gently and allowed to stand for 5 minutes. 1ml of 1.1M acetic acid was added to all test tubes and shaken gently 0.5ml of 5 mg/ ml Fast Red TR salt solution was added to all test tubes and shaken gently. The content of each tube was observed after 5 minutes for any colour change. Formation of yellow colouration indicates the presence of artesunate in the sample. In order to interpret the test of the artesunate tablet under test, the positive control must turn yellow and the negative control must remain colourless.

3.4.2 THIN LAYER CHROMATOGRAPHY OF ARTESUNATE TABLETS

The artesunate tablets in the artesunate - amodiaquine combipacks, were analyzed. The test samples were; AT2, AT3, AT4, AT5 and AT6. Artesunate tablets that were packaged alone were also analyzed; AT7 and AT 8. The method used by Ioset and Kaur, (2009) was employed in the thin layer chromatography of artesunate tablets. A quantity of pure artesunate powder was dissolved in 200ul (0.2ml) of methanol. The solution was mixed thoroughly by manual shaking and then placed in an ultrasonic bath for 30 seconds.

The test samples were each taken through the following procedure; the tablet was pulverized with a pestle in a mortar and 2ml of methanol was added. The mixture was placed in an ultrasonic bath for 30 seconds for solubilization to take place. The mixture was left on the bench for about 5-10 minutes to encourage sedimentation. The reference

standard and the pulverized tablet samples were each dissolved separately in 0.2ml and 2 ml of methanol respectively. The individual samples in methanol were each mixed thoroughly and a small quantity of each was applied in a graphite pencil drawn circle or a spot/ dot made by a graphite pencil on the TLC sheet. Brady's reagent was dropped on each spot. The reaction was allowed to proceed at room temperature. The observation of colour changes or otherwise was done.

3.5 DETERMINATION OF PERCENTAGE CONTENT OF ARTESUNATE TABLETS USING UV - VISIBLE SPECTROPHOTOMETRY

3.5.1 PREPARATION OF PURE ARTESUNATE SOLUTIONS FOR CALIBRATION CURVE

The procedure adopted was based on the method of Green *et al*. (2000). 0.025g, 0.05g, 0.075g, 0.1g, 0.2g and 0.3g of pure artesunate powder were weighed respectively and transferred into respective beakers. 35ml of 1M NaOH was added to each of the powders in the beakers. The mixtures were allowed to stand for 20 minutes to hydrolyze the artesunate. Each was transferred quantitatively into 100ml volumetric flasks and 40ml of 1.1M acetic acid was added into each volumetric flask. 10ml of 5mg/ml Fast Red TR solution was added into each volumetric flask. The samples were allowed to stand for 5 minutes to form a yellowish solution. The solutions were made up to 100ml each with 1.1M acetic acid. Assay was carried out using Ultraviolet (UV) spectrophotometer (Cecil CE 2041 2000 series, Cecil Instruments, Cambridge, England) at wavelength of 420nm. The test was repeated twice and the results presented are the mean of three determinations.

3.5.2 UV- VISIBLE ANALYSIS OF ARTESUNATE CONTENT

The samples used were AT2, AT3, AT4, AT5, AT6, AT7 and AT 8 and the procedure adopted was based on the method of Green *et al*., (2000). 2-3 tablets of artesunate were randomly selected from each sample and crushed. Powder equivalent to a single tablet were weighed respectively. Each of the samples prepared as above, were transferred into 100ml volumetric flasks respectively. 40ml of 1M NaOH were added to each sample. The mixtures were allowed to stand for 20 minutes to hydrolyze the drug. Each sample was filtered into 100ml volumetric flasks. 40ml of 1.1 M acetic acid was added to each sample, followed by the addition of 20ml of 5mg/ml FRTR solution. The resultant solutions were

allowed to stand for 5 minutes to form yellowish solutions. Assay was carried out using Ultraviolet (UV) spectrophotometer (Cecil CE 2041 2000 series, Cecil Instruments, Cambridge, England) at a wavelength of 420nm. The test was repeated twice and the results presented are the mean of three determinations.

3.6 ASSAY OF AMODIAQUINE TABLETS

The procedure described in the United States Pharmacopoeia monograph (2010) for amodiaquine tablets was used. The samples assayed were AM2, AM3, AM4 and AM5.
A mass of powdered amodiaquine tablet equivalent to 300mg of amodiaquine was weighed and transferred into a 200ml volumetric flask. Dilute hydrochloric acid (1 in 1000) was added to make up to volume. The resulting solution was well mixed. The absorbance of this solution and a solution of USP Amodiaquine Hydrochloride RS in the same medium with a known concentration of about 15ug per ml in 1cm cells at the wavelength of maximum absorbance at about 342nm. Hydrochloric acid (1 in 100) was used as the blank. The quantity in mg of amodiaquine hydrochloride in the proportion of amodiaquine hydrochloride taken was calculated by the formula;

20C (Au/As)

C= concentration in ug per ml of USP Amodiaquine Hydrochloride RS

Au= absorbance of the Amodiaquine HCl solution

As= absorbance of the standard Amodiaquine Hydrochloride solution

3.7 ASSESSMENT OF QUALITY OF ARTEMETHER-LUMEFANTRINE TABLETS

3.7.1 UNIFORMITY OF WEIGHT OF ARTEMETHER-LUMEFANTRINE TABLETS

The samples used were, AL2, AL3, AL4, AL5, AL6 and AL 7. The procedure described in **3.3.1** above was used in the assessment of the uniformity of weight.

3.7.2 DISINTEGRATION TEST FOR ARTEMETHER- LUMEFANTRINE TABLETS

The procedure described in 3.3.2 was used to determine the disintegrating time of AL2, AL3, AL4, AL5, AL6 and AL 7 samples respectively.

3.7.3 HARDNESS OF ARTEMETHER-LUMEFANTRINE TABLETS

AL2, AL3, AL4, AL5, AL6 and AL7 were the samples tested. The method described in 3.3.3 was employed.

3.7.4 AUTHENTICITY OF ARTEMETHER IN ARTEMETHER-LUMEFANTRINE TABLETS

The artemether component in the artemether-lumefantrine fixed dose tablets was analyzed. The samples analyzed were; AL2, AL3, AL4, AL5, AL6 and AL7. The procedure of Osei-Safo *et al*. (2010) was adopted.

a) To a quantity of the powdered tablets equivalent to about 80 mg of artemether was added 40 ml of dehydrated ethanol (absolute ethanol). The solution was shaken well to dissolve and filtered. Half of the filtrate was evaporated to about 1 ml and 100 mg of potassium iodide was added and heated on a water bath for about five minutes. A yellow colour is expected to be seen on the evaporating dish, signifying the presence of artemether.

b) The remaining filtrate from test (a) above was evaporated to about 5 ml. A few drops of this solution were placed on a white porcelain dish and 1 drop of vanillin/sulphuric acid TSI added. Development of a pink colour is expected if artemether is present.

3.7.5 AUTHENTICITY OF ARTEMETHER IN ARTEMETHER INJECTION

A sample of artemether injection (AL8) was also analyzed. The procedure of Osei-Safo *et al.* (2010) was adopted. To the contents of one ampoule equivalent to about 80 mg of artemether was added 6 ml of dehydrated ethanol and shaken well to dissolve. A few drops of this solution were put on a white porcelain dish and 1 drop of vanillin/sulphuric acid TSI added. Development of a pink colour is expected if artemether is present.

3.7.6 AUTHENTICITY OF LUMEFANTRINE IN ARTEMETHER - LUMEFANTRINE TABLETS

The procedure of Osei-Safo *et al*. (2010) was adopted with some modification.

a) To a quantity of the powdered tablets equivalent to 10 mg of lumefantrine in a test tube was added 5 ml of ethyl acetate. A few drops of 1M hydrochloric acid solution were added. The solution was stirred, warmed and filtered. To a portion of the test solution was added a few drops of Dragendorff's reagent. Formation of an orange precipitate implies that an alkaloid is present.

b) To a quantity of the powdered tablets equivalent to 10mg of lumefantrine was added 5ml of methanol and shaken well to dissolve the active ingredient. 20 mg of potassium permanganate was added to the solution and boiled for about a minute. The solution was filtered and a few drops of 2, 4-Dinitrophenylhydrazine solution (Brady's reagent) were added and shaken. A positive test for lumefantrine is observed if an orange precipitate is produced.

3.7.7 DETERMINATION OF PERCENTAGE CONTENT OF ARTEMETHER IN ARTEMETHER - LUMEFANTRINE TABLETS USING UV-VISIBLE SPECTROMETRY

The methods of Awofisayo *et al*. (2010) and Shrivastava *et al*. (2008) were used with some modifications as outlined below.

3.7.7.1 PREPARATION OF 1M METHANOLIC HCL

1M Methanolic HCl was prepared by diluting 85 ml of concentrated HCl with methanol up to one litre.

3.7.7.2 PREPARATION OF STANDARD SOLUTIONS OF PURE ARTEMETHER

Standard solutions of pure artemether were prepared in 10 ml volumetric flasks. This was achieved by weighing the required quantities, 0.01 g, 0.02 g, 0.03 g, 0.04 g, 0.05 g and 0.1 g

of pure artemether respectively. 5 ml of methanolic HCl was added to each and shaken for 5 seconds. The solutions were then heated in a water bath for 3 hours at 60^{o}C after which they were cooled at room temperature (25^{o}C). Each solution was made up to the required 10 ml volume with methanolic HCl. Absorbance was then taken with a Cecil 2000 series UV spectrophotometer at a wavelength of 254 nm.

The blank was prepared by heating methanolic HCl at the above-stated conditions and diluting up to the 10 ml mark.

3.7.7.3 ESTIMATION OF ARTEMETHER CONTENT IN ARTEMETHER – LUMEFANTRINE TABLET BRANDS

A quantity of powdered tablet equivalent to 20 mg of artemether was weighed for each brand except AL 3. For AL 3, the quantity weighed was equivalent to 40 mg of artemether. 5 ml of methanolic HCl was added to each sample and shaken for 10 minutes. Each solution was heated on a water bath for 3 hours at 60^{o}C. The solutions were allowed to cool at room temperature (25^{o}C), after which, 3 ml of methanolic HCl was added to the precipitate formed to dissolve. The samples were each filtered with Whatman filter paper. After filtration, each sample was made up to 10 ml and the absorbance taken as above at a wavelength of 254 nm.

3.7.7.4 ESTIMATION OF ARTEMETHER IN A BRAND OF ARTEMETHER INJECTION (80mg/ml)

1 ml of the solution inside the artemether injection ampoule (AL 8) was transferred to a 100ml volumetric flask and was shaken with 50 ml of methanoic HCl for 25 minutes after which the volume was made up to volume with the same solvent. 0.5 ml of this solution was transferred to a 10 ml volumetric flask and made up to volume with methanolic HCl. The estimation of the artemether content was done with a Cecil 2000 series UV spectrophotometer at a wavelength of 254 nm.

3.7.8 ESTIMATION OF THE PERCENTAGE CONTENT OF LUMEFANTRINE IN ARTEMETHER-LUMEFANTRINE TABLET BRANDS

3.7.8.1 PREPARATION OF 0.1M PERCHLORIC ACID SOLUTION IN GLACIAL ACETIC ACID

8.5 ml of 72% perchloric acid and 20ml of acetic anhydride were added to 400ml of glacial acetic acid in a 1000ml volumetric flask. The solution was shaken and diluted to full mark with glacial acetic acid. The perchloric acid was well diluted with acetic acid before adding the anhydride because of the danger of forming the explosive acetyl perchlorate. The solution was left for 24 hours in order for the acetic anhydride to mop up water in the glacial acetic acid and perchloric acid.

3.7.8.2 STANDARDIZATION OF 0.1 M PERCHLORIC ACID SOLUTION ($HCLO_4$)

0.5 g of potassium hydrogen phthalate ($C_8H_8KO_4$) was weighed and dissolved in 25ml of anhydrous acetic acid, and warmed gently for five minutes. The mixture was allowed to cool, protected from air and titrated with perchloric acid solution using Oracet Blue as indicator. Each milliliter of 0.1M perchloric acid is equivalent to 20.42mg of potassium hydrogen phthalate ($C_8H_8KO_4$). The expected colour change was from blue to pink.
The reaction equation is as follows $C_8H_8KO_4+HClO_4 \rightarrow C_8H_6O_4+KClO_4$

3.7.8.3 ASSAY OF LUMEFANTRINE BY NON AQUEOUS TITRATION

Twenty (20) whole tablets of each artemether-lumefantrine brand were weighed and the average weight recorded. The tablets were powdered with a porcelain mortar and pestle. A quantity of each powdered brand equivalent to 0.45 g of lumefantrine was first weighed and extracted with 60ml of hot acetone. The extract was allowed to cool and 50ml of glacial acetic acid was added. This was titrated with 0.1M acetous perchloric acid (already standardized). The end point was determined using Oracet Blue as indicator. The expected colour change was from green to yellow. A blank determination was carried out similarly but excluding the drug. The determinations were repeated twice and the average of the corresponding percentage content was calculated.

Chapter 4

RESULTS AND CALCULATIONS

4.1 SURVEY ON PREVALENCE AND COST OF ANTIMALARIALS IN THE KUMASI METROPOLIS

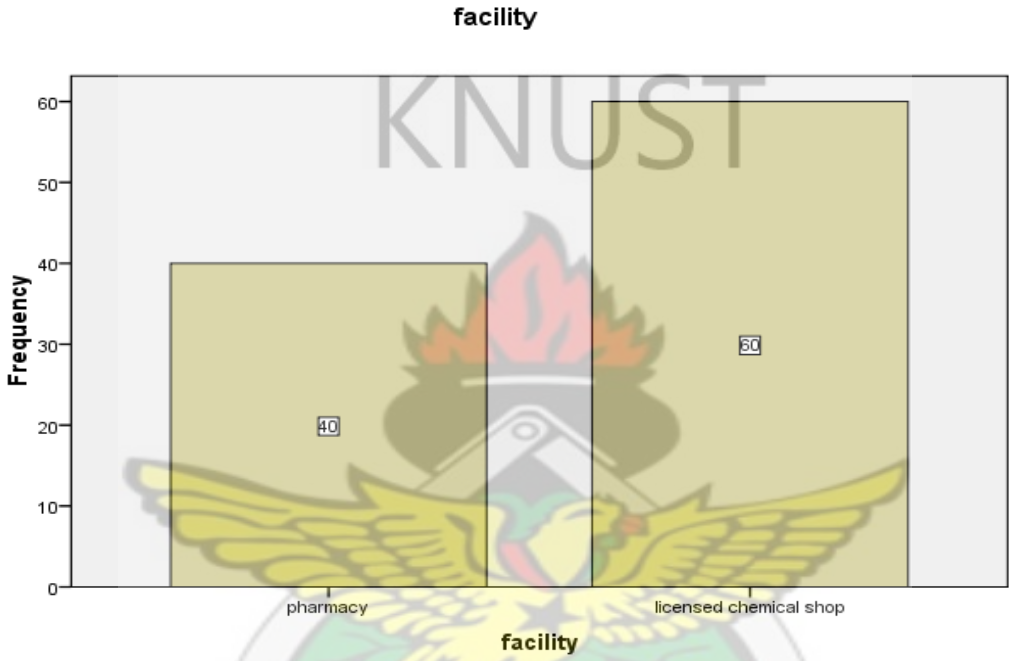


Figure 4.1 Type of facility visited

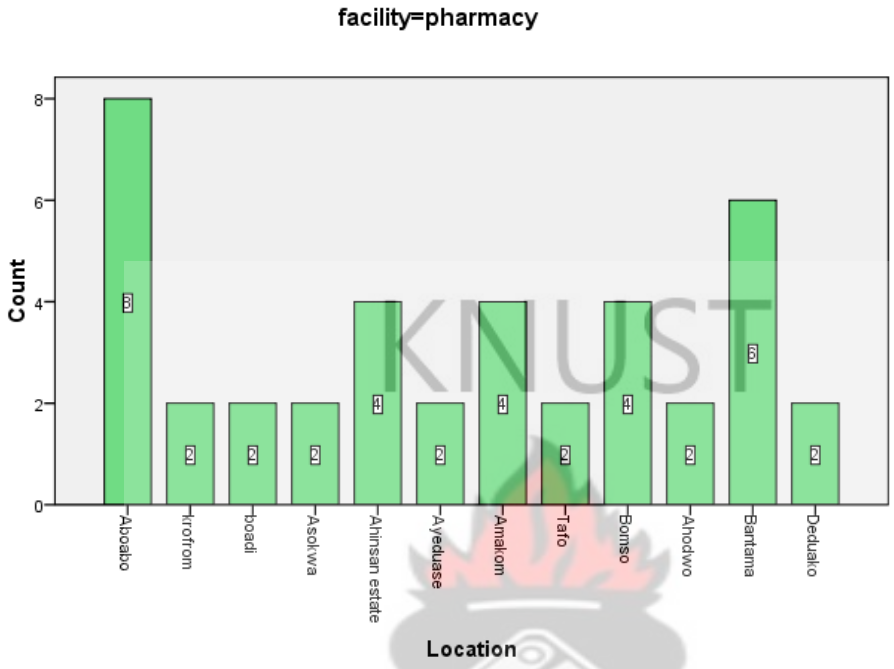


Figure 4.2 Locations of facilities visited

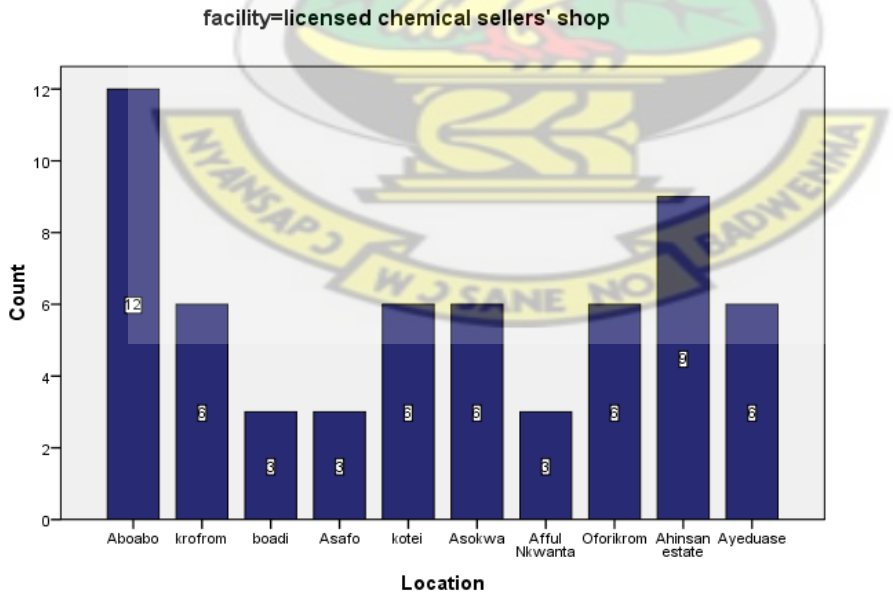


Figure 4.3 Locations of facilities visited

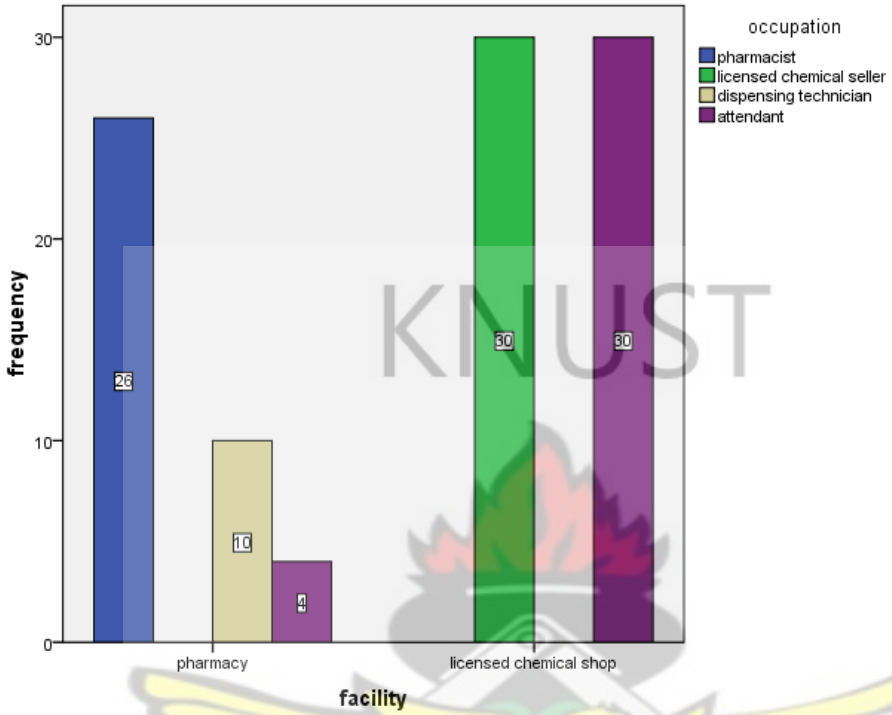


Figure 4.4Occupation of Respondents

Table 4.1 Sale of antimalarials by facilities

		Antimalarial	
		Yes	Total
facility	Pharmacy	40	40
	licensed chemical shop	60	60
Total		100	100

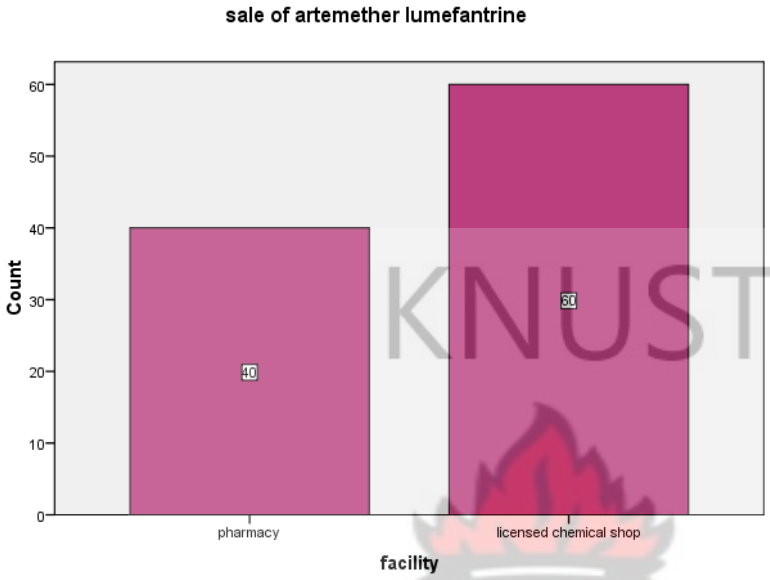


Figure 4.5 Sale of Artemether-Lumefantrine tablets

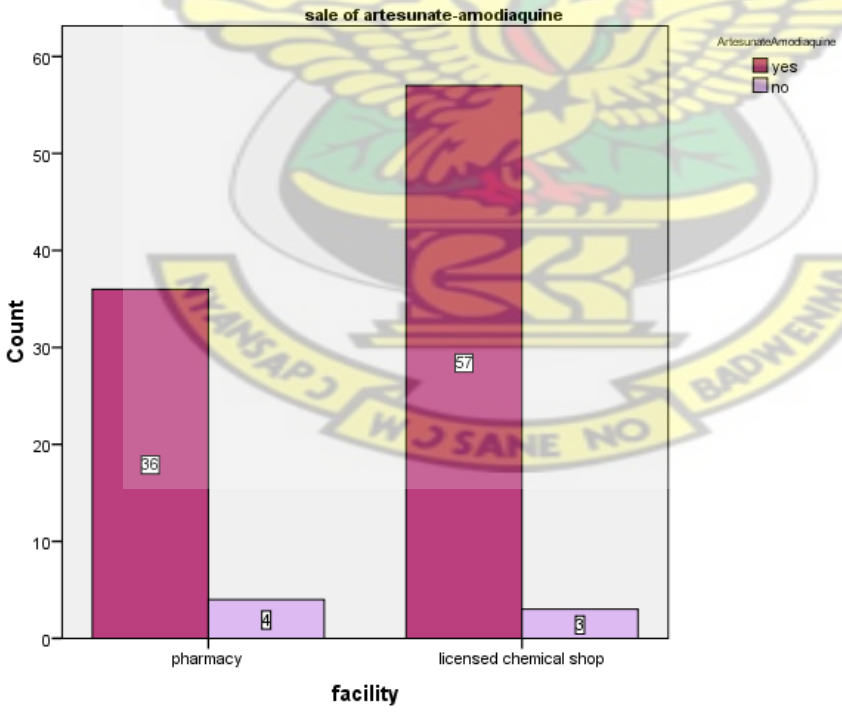


Figure 4.6 Sale of Artesunate-Amodiaquine tablets

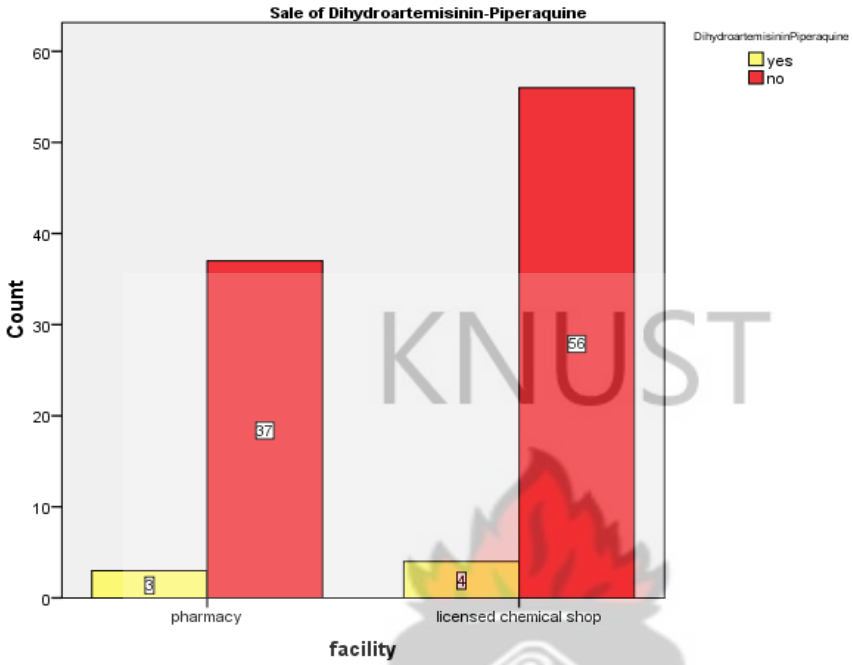


Figure 4.7 Sale of Dihydroartemisinin-Piperaquine tablets

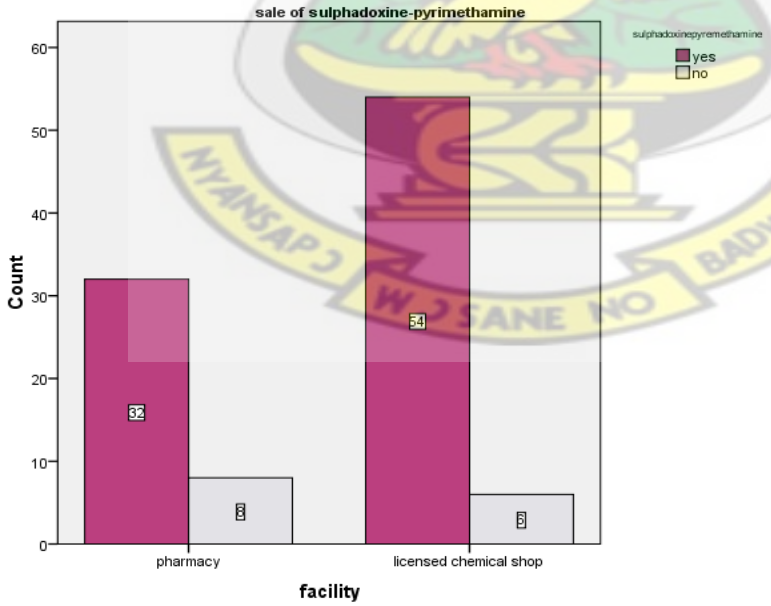


Figure 4.8 Sale of Sulphadoxine-Pyrimethamine tablets

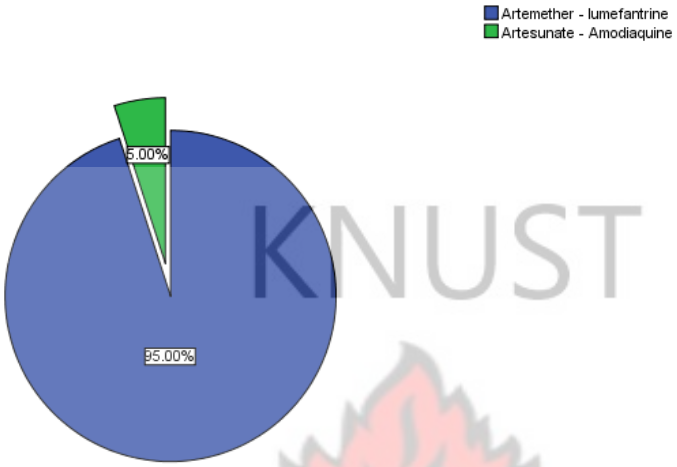


Figure 4.9 The most expensive antimalarial sold

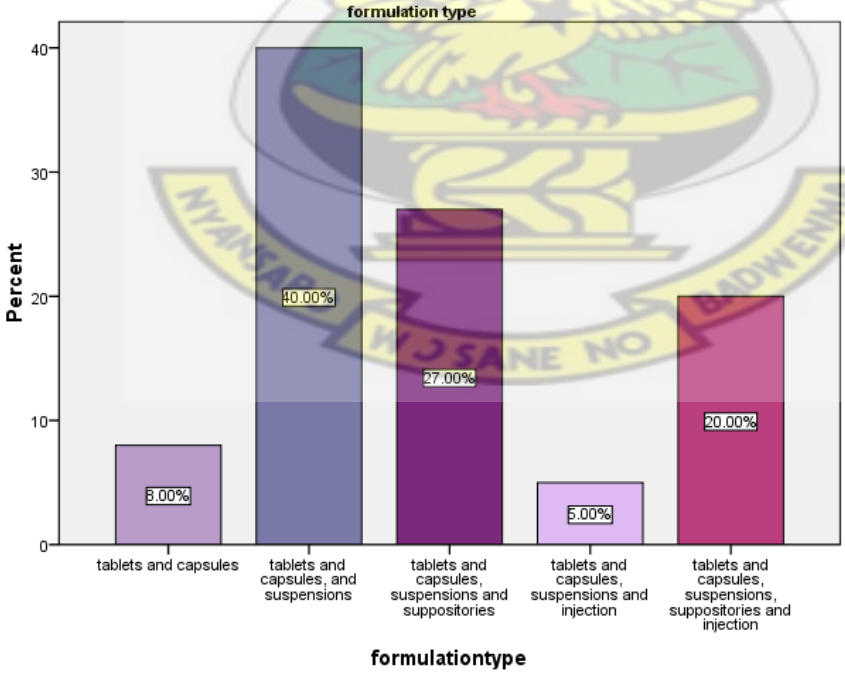


Figure 4.10 Various formulations of antimalarials available

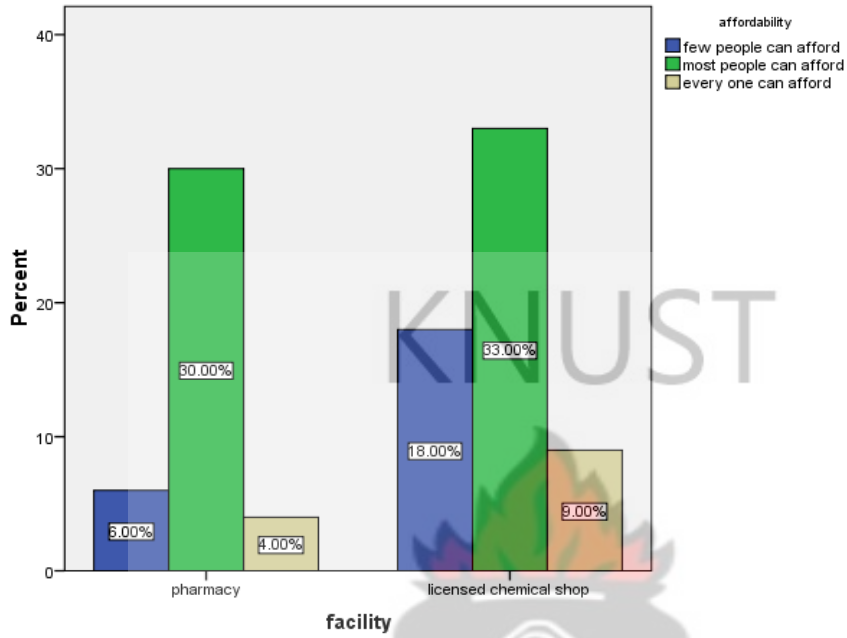


Figure 4.11 Affordability of antimalarials

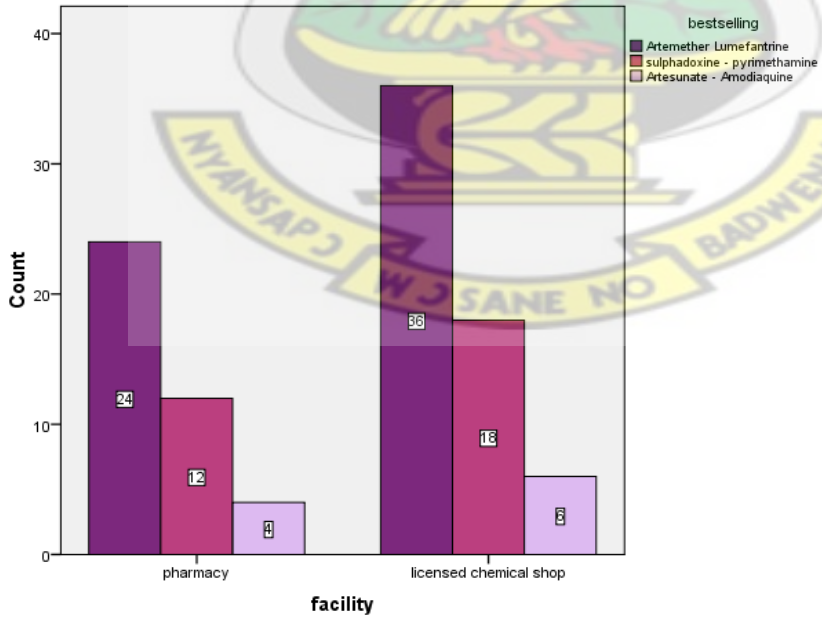


Figure 4.12 Bestselling antimalarial medications

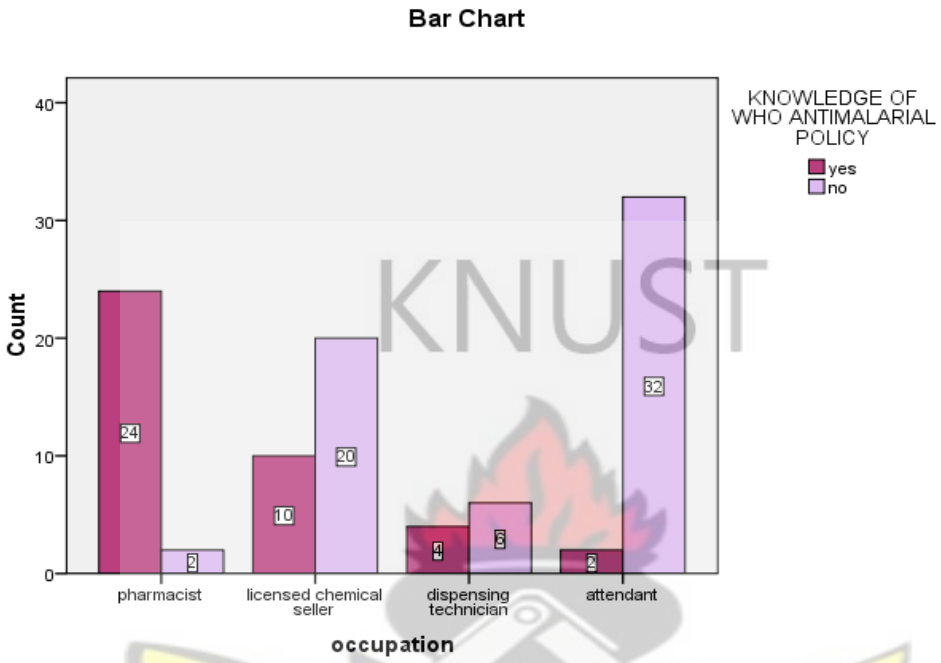


Figure 4.13 Knowledge of WHO antimalarial policy

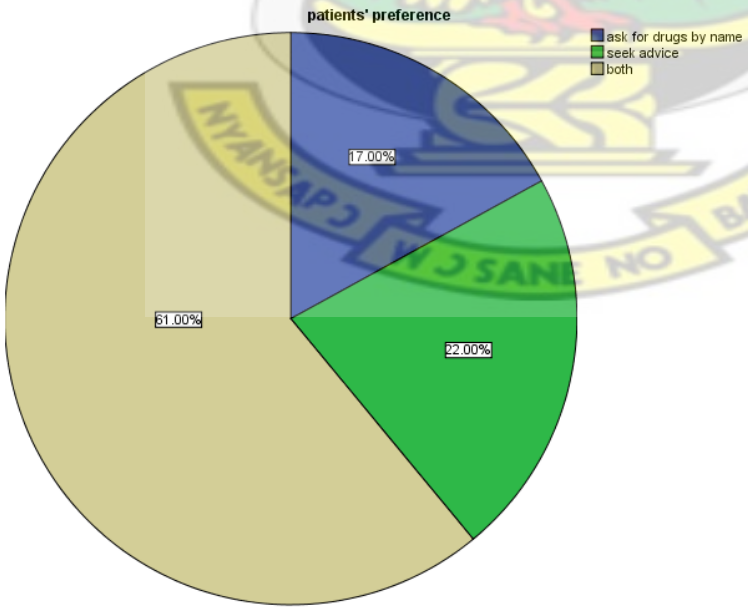


Figure 4.14 Patients' sources of information about antimalarials

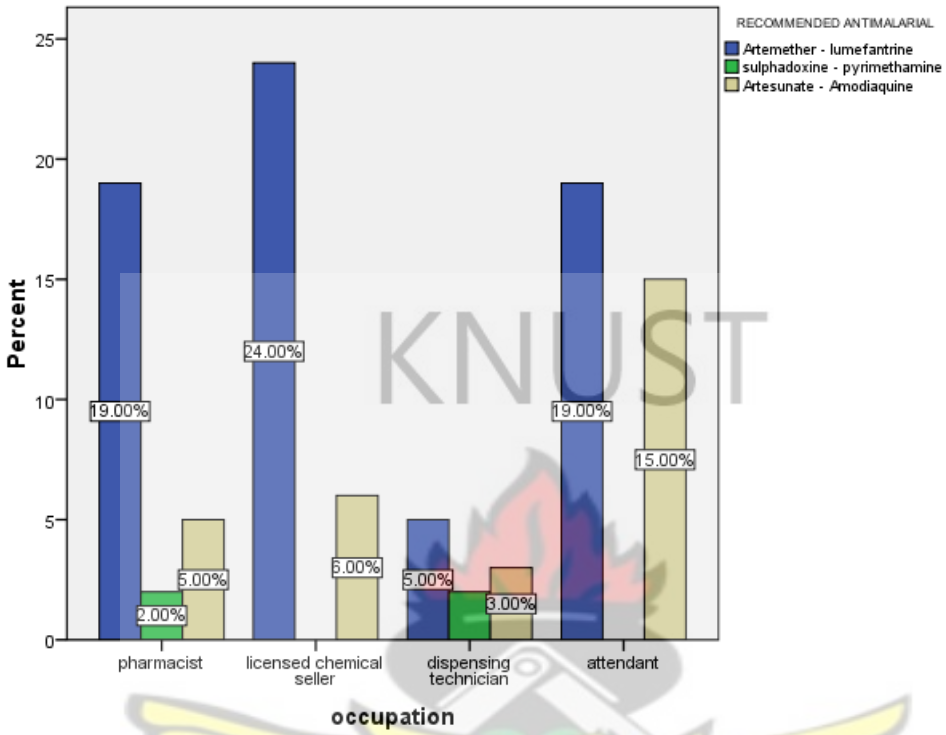


Figure 4.15 Antimalarials commonly recommended to patients

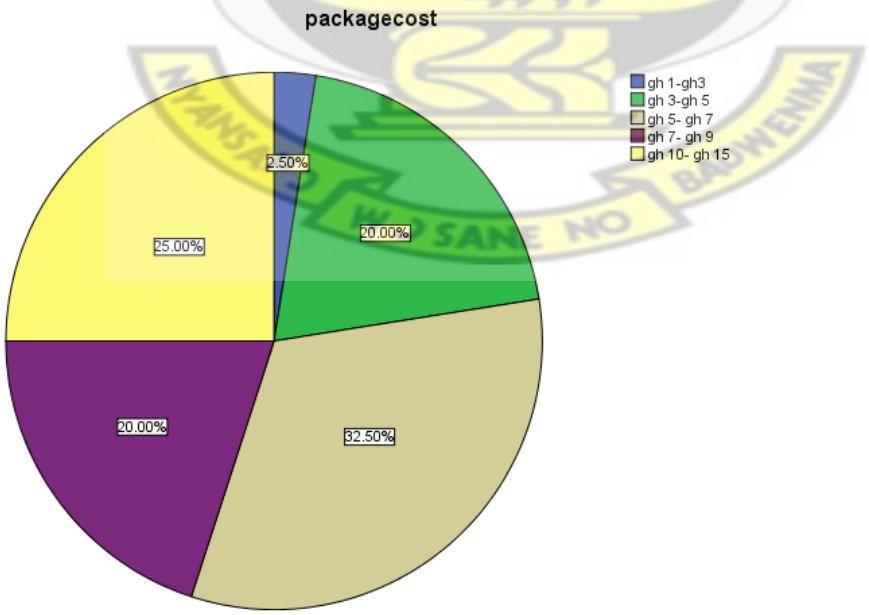


Figure 4.16 Cost of full course of treatment with antimalarials

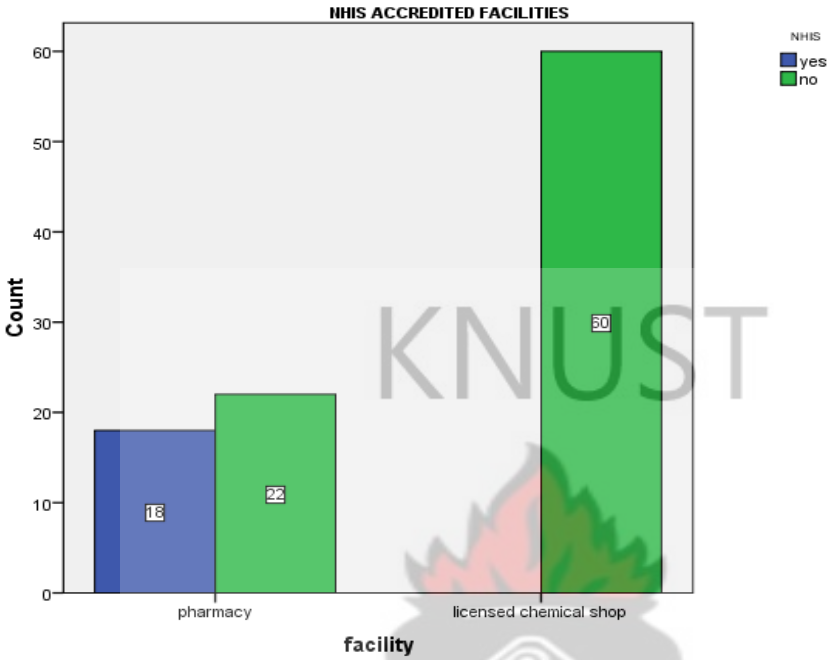


Figure 4.17 NHIS accredited facilities

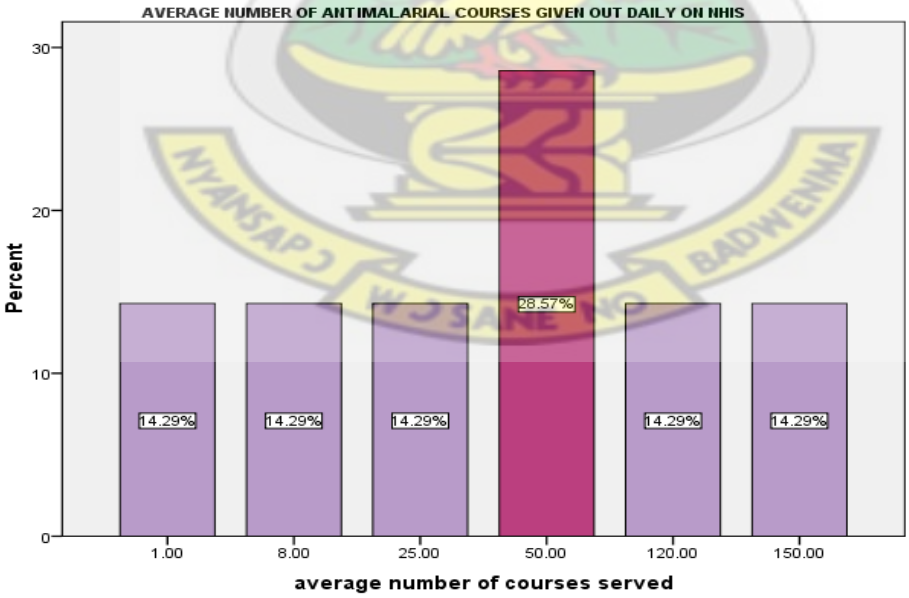


Figure 4.18 Average number of antimalarial courses served daily on NHIS by accredited pharmacies

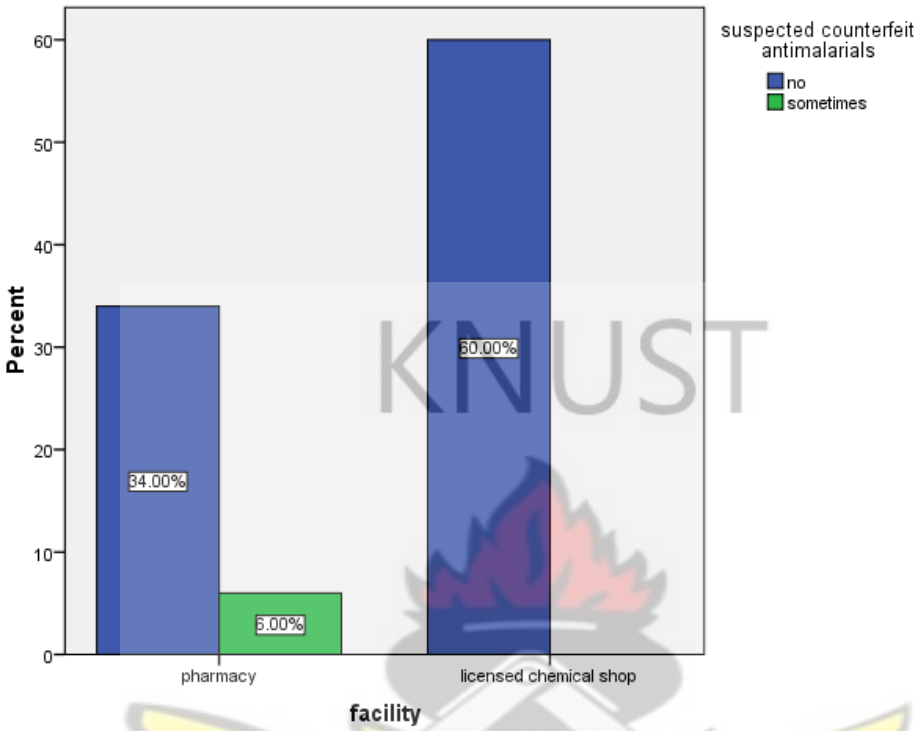


Figure 4.19 Purchase of suspected counterfeit antimalarial medication

Table 4.2 Uniformity of weight of artesunate and amodiaquine tablets

SAMPLE	MASS OF 20 TABLETS/G (X)	AVERAGE /G (X/20)	STANDARD DEVIATION (±)	DEVIATION BY >±5%	DEVIATION BY > TWICE ±5%	INFERENCE
AT 2	6.0428	0.3021	0.0168	5 TABLETS	1 TABLET	FAILED
AT 3	5.6298	0.2815	0.0040	0	0	PASSED
AT 4	6.9303	0.3465	0.0043	0	0	PASSED
AT 5	4.1478	0.2074	0.0030	0	0	PASSED
AT 6	7.0980	0.3549	0.0084	0	0	PASSED
AT 7	12.0620	0.6031	0.0008	0	0	PASSED
AT 8	5.9250	0.2963	0.0008	0	0	PASSED
AM 2	10.1544	0.5077	0.0201	2 TABLETS	0	PASSED
AM 3	6.1676	0.3084	0.0022	0	0	PASSED
AM 4	11.8509	0.5925	0.0067	0	0	PASSED
AM 5	10.3889	0.5194	0.1155	0	0	PASSED
AM 6	9.6333	0.4817	0.0061	0	0	PASSED

PASS- not more than 2 of the individual masses deviates from the average mass by more than 5% and none deviate by more than 10% (British Pharmacopoeia, 2007)

Table 4.3 Disintegration times of artesunate and amodiaquine tablets

SAMPLE CODE	TIME/MINUTES	OUTCOME
AT 2	1:47	PASSED
AT 3	2:35	PASSED
AT 4	3:22	PASSED
AT 5	9:50	PASSED
AT 6	2:43	PASSED
AT 7**	7:21	PASSED
AT 8	0:12	PASSED
AM 2	9:00	PASSED
AM 3	0:20	PASSED
AM 4	1:00	PASSED
AM 5	1:00	PASSED
AM 6	1:30	PASSED

PASS-for uncoated tablets, disintegration should be within 15 minutes, for coated tablets it should be within 30 minutes (British Pharmacopoeia, 2007)
**film coated tablet

Table 4.4 Hardness of artesunate and amodiaquine tablets

SAMPLE	FORCE FOR 10 TABLETS (NEWTONS)	MEAN (NEWTONS)	MINIMUM FORCE (NEWTONS)	MAXIMUM FORCE (NEWTONS)	INFERENCE
AT 2	475.30	47.53 **± 39.01**	2.00	100.90	PASSED
AT 3	265.60	26.56 **± 6.518**	14.70	35.30	FAILED
AT 4	230.20	23.02 **± 6.443**	12.70	35.30	FAILED
AT 5	398.00	39.80 **± 6.430**	25.50	51.00	PASSED
AT 6	923.30	92.33 **± 9.718**	75.50	105.80	PASSED
AT 7 **	1686.00	168.60 **± 0**	168.6	168.6	PASSED***
AT 8	493.00	49.30 **± 12.45**	30.40	64.70	PASSED
AM 2	1235.00	123.5 **± 34.88**	68.60	168.6	PASSED
AM 3	586.00	58.60 **± 17.56**	39.20	86.20	PASSED
AM 4	348.70	34.87 **± 6.164**	21.60	41.20	FAILED
AM 5	729.00	72.90 **± 11.11**	49.00	86.20	PASSED
AM 6	360.60	36.06 **± 19.60**	19.60	76.40	FAILED

**Film coated tablet

Table 4.5 Determination of authenticity of artesunate tablets by colorimetry

TEST	OBSERVATION	INFERENCE
Sample=pure artesunate powder (positive control)	Clear before addition of FRTR solution. Yellow colour developed 5minutes after addition of FRTR	Artesunate present
Sample=AT2	Clear before addition of FRTR solution. Yellow colour developed 5minutes after addition of FRTR solution.	Artesunate present
Sample =AT3	Clear before addition of FRTR solution. Yellow colour developed 5minutes after addition of FRTR solution.	Artesunate present
Sample =AT4	Cloudy before addition of FRTR. Yellow colour developed 5 minutes after addition of FRTR solution.	Artesunate present
Sample=AT5	Cloudy before addition of FRTR. Yellow colour developed 5 minutes after addition of FRTR solution.	Artesunate present
Sample =AT6	Clear before addition of FRTR solution. Yellow colour developed 5minutes after addition of FRTR solution.	Artesunate present
Sample =AT7	Clear before addition of FRTR solution. Yellow colour developed 5minutes after addition of FRTR solution.	Artesunate present
Sample =AT8	Cloudy before addition of FRTR. Yellow colour developed 5 minutes after addition of FRTR solution.	Artesunate present
NO sample (negative control)	Clear before and after the addition of FRTR solution.	Artesunate absent

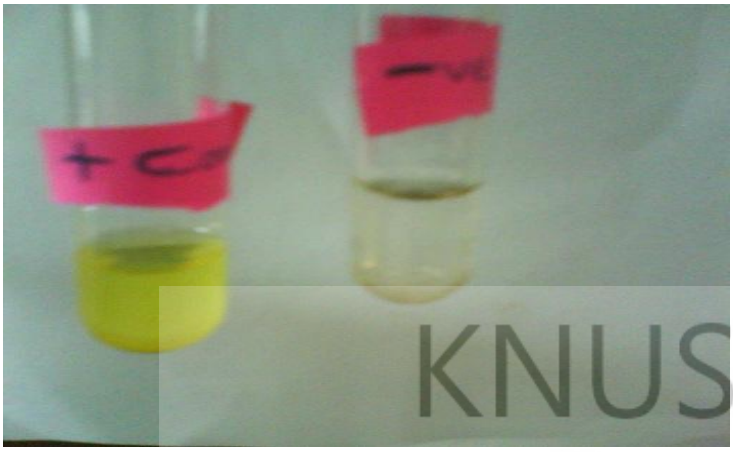


Figure 4.20 Positive and negative control for colorimetry results

+ = Positive control contains pure artesunate powder showing yellow colouration.
- = Negative control with sample showing no yellow colouration

Figure 4.21 TLC plate spotted with artesunate tablet samples

KEY
1=POSITIVE CONTROL
2-6 =AT2-AT 6
7=NEGATIVE CONTROL (showing no colour change from orange)

Table 4.6 Thin layer chromatography of artesunate tablets

TEST	OBSERVATION (COLOUR CHANGE)	INFERENCE
Sample =pure artesunate powder (positive control)	Orange to dark pink/ rust/ brown	Artesunate present
Sample =AT2	Orange to dark pink/ rust/ brown	Artesunate present
Sample =AT3	Orange to dark pink/ rust/ brown	Artesunate present
Sample =AT4	Orange to dark pink/ rust/ brown	Artesunate present
Sample =AT5	Orange to dark pink/ rust/ brown	Artesunate present
Sample =AT6	Orange to dark pink/ rust/ brown	Artesunate present
Sample=AT7	Orange to dark pink/ rust/ brown	Artesunate present
Sample = AT8	Orange to dark pink/ rust/ brown	Artesunate present
NO sample (negative control)	Orange colour maintained	Artesunate absent

4.2 DETERMINATION OF PERCENTAGE CONTENT OF ARTESUNATE TABLETS USING UV VISIBLE SPECTROPHOTOMETRY

TABLE 4.7 AMOUNT OF POWDERED ARTESUNATE TABLET BRANDS USED

SAMPLE CODE	TABLET WEIGHT/G	ACTUAL WEIGHT OF POWDERED TABLET/G
AT 2	0.2752	0.2781
AT 3	0.2907	0.2909
AT 4	0.3393	0.3406
AT 5	0.2647	0.2660
AT 6	0.3618	0.3628
AT 7	0.6042	0.6068
AT 8	0.2970	0.2983

Table 4.8 Absorbance of pure artesunate powder

CONCENTRATION %w/v	ABSORBANCE 1	ABSORBANCE 2	ABSORBANCE MEAN
0.025	**0.190**	**0.230**	**0.210** ± 0.0283
0.050	**0.277**	**0.345**	**0.311** ± 0.0481
0.075	**0.417**	**0.488**	**0.453** ± 0.0502
0.100	**0.513**	**0.550**	**0.532** ± 0.0262
0.200	**0.888**	**0.915**	**0.902** ± 0.0191

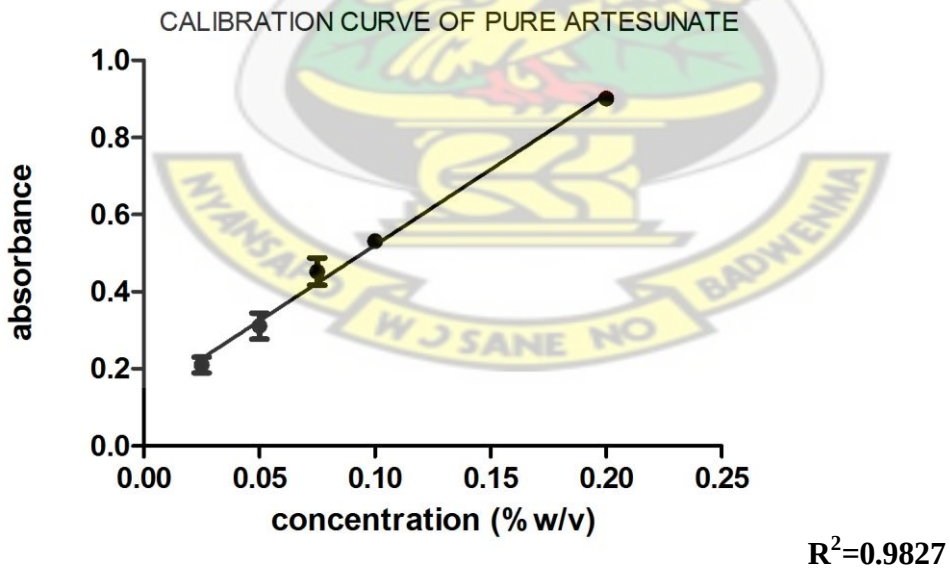


Figure 4.22 Calibration curve of pure Artesunate powder

Table 4.9 Absorbance of samples of artesunate tablets

SAMPLE CODE	ABSORBANCE 1	ABSORBANCE 2	MEAN ABSORBANCE
AT 2	**0.320**	**0.327**	**0.324**
AT 3	**0.230**	**0.257**	**0.243**
AT 4	**0.795**	**0.861**	**0.828**
AT 5	**0.472**	**0.459**	**0.466**
AT 6	**0.424**	**0.457**	**0.441**
AT 7	**0.577**	**0.636**	**0.607**
AT 8	**0.221**	**0.200**	**0.211**

4.2.1 Sample calculation of concentration of Artesunate in Artesunate tablets

From the calibration curve the equation of the graph of pure artesunate powder dissolved in 1M NaOH and 1.1M Acetic Acid;

$y = mx+c$

Where, y= absorbance x=concentration m =slope c= intercept

m=3.923 c=0.1282

Hence, x= y-0.1282/3.923

Thus for a tablet with an absorbance of 0.324,

Its concentration, x = (0.324-0.1282)/3.923

x=0.0499

Table 4.10 Percentage content of artesunate tablets

SAMPLE CODE	STATED STRENGHTH OF TABLET(mg)	CONC. OF PREPARATION %w/v	ACTUAL CONC. OF ARTESUNATE FOUND %w/v	ACTUAL CONTENT OF ACTIVE INGREDIENT(mg)	%CONTENT
AT 2	**100**	**0.1011**	**0.0499**	**49.36**	**49.36**
AT 3	**50**	**0.0500**	**0.0293**	**29.30**	**58.60**
AT 4	**100**	**0.1004**	**0.1784**	**177.69**	**177.69**
AT 5	**100**	**0.1005**	**0.0861**	**85.67**	**85.67**
AT 6	**100**	**0.1003**	**0.0797**	**79.46**	**79.46**
AT 7	**200**	**0.2009**	**0.1220**	**121.46**	**60.73**
AT 8	**50**	**0.0502**	**0.0211**	**21.02**	**42.03**

4.3 ASSAY OF AMODIAQUINE

Table 4.11 Amounts of powdered amodiaquine samples used

SAMPLE	MASS OF TABLET/G	MASS OF POWDERED TABLET USED
AM 2	0.4977	0.4980
AM 3	0.3091	0.3092
AM 4	0.5933	0.5938
AM 5	0.5146	0.5148
AM 6	0.4833	0.4836

Table 4.12 Absorbance of amodiaquine HCl

	Absorbance 1	Absorbance 2	Absorbance 3	Absorbance mean
Pure amodiaquine	0.518	0.523	0.522	**0.521** ± 0.0026
AM 2	0.593	0.604	0.600	**0.599** ± 0.0056
AM 3	0.403	0.395	0.391	**0.396** ± 0.0061
AM 4	0.750	0.739	0.743	**0.744** ± 0.0056
AM 5	0.591	0.590	0.588	**0.590** ± 0.0015
AM 6	0.748	0.755	0.759	**0.754**± 0.0056

4.3.1 SAMPLE CALCULATION OF PERCENTAGE CONTENT OF AMODIAQUINE HCL IN AMODIAQUINE HYDROCHLORIDE TABLETS

The quantity in mg of amodiaquine hydrochloride in the proportion of amodiaquine hydrochloride taken was calculated by the formula;

20C (Au/As)

C= concentration in ug per ml of USP Amodiaquine Hydrochloride RS

Au= absorbance of the Amodiaquine HCl solution

As= absorbance of the standard Amodiaquine Hydrochloride solution

As =0.521

If Au = 0.599 (AM 2)

C=15ug/ml

= (20 x 15ug/ml) x (0.599/0.521)

=344.9mg

Table 4.13 Percentage content of amodiaquine HCl in amodiaquine hydrochloride tablets

Sample	Stated amount of amodiaquine HCl(mg)	Actual amount of amodiaquine HCl (mg)	Percentage content of amodiaquine HCl
AM 2	300.0	344.9	115.0
AM 3	153.1	228.0	148.9
AM 4	300.0	428.4	142.8
AM 5	300.0	339.7	113.2
AM 6	306.0	434.2	141.9

Table 4.14 Quality of artesunate and amodiaquine tablets

BRAND	ARTESUNATE CONTENT(%)	AMODIAQUINE CONTENT(%)	QUALITY
AT2-AM2	49.36	115.00	SUBSTANDARD
AT3-AM3	58.60	148.90	SUBSTANDARD
AT4-AM4	177.69	142.80	SUBSTANDARD
AT5-AM5	85.67	113.20	SUBSTANDARD
AT6-AM6	79.46	141.90	SUBSTANDARD
AT7	60.73	-	SUBSTANDARD
AT8	42.03	-	SUBSTANDARD

Artesunate tablets 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.

Amodiaquine hydrochloride tablets contain an amount of amodiaquine hydrochloride ($C_{20}H_{22}ClN_3O.2H_2O$) equivalent to not less than 93.0% and not more than 107.0% of the labeled amount of amodiaquine ($C_{20}H_{22}ClN_3O$).

4.4 ASSESSMENT OF QUALITY OF ARTEMETHER-LUMEFANTRINE TABLET BRANDS

Table 4.15 Uniformity of weight of artemether-lumefantrine tablet brands

SAMPLE	MASS OF 20 TABLETS/G (X)	AVERAGE (X/20)	STANDARD DEVIATION (±)	DEVIATION BY >±5%	DEVIATION BY > TWICE ±5%	INFERENCE
AL 2	4.8820	0.2441	0.0017	0	0	PASSED
AL 3	11.5993	0.5800	0.0026	0	0	PASSED
AL 4	6.8227	0.3411	0.0060	0	0	PASSED
AL 5	5.3074	0.2654	0.0048	0	0	PASSED
AL 6	4.8533	0.2427	0.0011	0	0	PASSED
AL 7	4.9203	0.2460	0.0020	0	0	PASSED

PASS- not more than 2 of the individual masses deviates from the average mass by more than 5% and none deviate by more than 10% (British Pharmacopoeia, 2007).

Table 4.16 Disintegration times of artemether-lumefantrine tablet brands

SAMPLE CODE	TIME/MINUTES	OUTCOME
AL 2	**3:50**	**PASSED**
AL 3	**2:30**	**PASSED**
AL 4	**6:50**	**PASSED**
AL 5	**0:25**	**PASSED**
AL 6	**0:40**	**PASSED**
AL 7	**5:45**	**PASSED**

Table 4.17 Hardness of artemether-lumefantrine tablet brands

SAMPLE	FORCE FOR 10 TABLETS (NEWTONS)	MEAN (NEWTONS)	MINIMUM FORCE(NEWTONS)	MAXIMUM FORCE (NEWTONS)	INFERENCE
AL 2	1124.00	112.4 **± 39.99**	52.92	154.80	PASSED
AL 3	1096.00	109.6 **± 23.55**	47.00	129.40	PASSED
AL 4	817.30	81.73 **± 6.489**	72.50	92.10	PASSED
AL 5	466.50	46.65 **± 6.823**	39.20	56.80	PASSED
AL 6	482.10	48.21 **± 5.244**	39.20	54.90	PASSED
AL 7	719.30	71.93 **± 15.95**	47.00	92.10	PASSED

4.4.1 DETERMINATION OF AUTHENTICITY OF ARTEMETHER-LUMEFANTRINE TABLETS AND ARTEMETHER INJECTION

Table 4.18 Test for authenticity of artemether in artemether -lumefantrine tablets and in artemether injection

SAMPLE	OBSERVATION (TEST A)	OBSERVATION (TEST B)	CONCLUSION
Pure artemether powder (positive control)	Yellow colour produced	Pink colour produced	Artemether present
AL 2	Yellow colour produced	Pink colour produced	Artemether present
AL 3	Yellow colour produced	Pink colour produced	Artemether present
AL 4	Yellow colour produced	Pink colour produced	Artemether present
AL 5	Yellow colour produced	Pink colour produced	Artemether present
AL 6	Yellow colour produced	Pink colour produced	Artemether present
AL 7	Yellow colour produced	Pink colour produced	Artemether present
AL 8 (injection)	-	Pink colour produced	Artemether present
Negative control	No yellow colour produced	No pink colour produced	Artemether absent

Table 4.19 Test for authenticity of lumefantrine in artemether-lumefantrine tablets

SAMPLE	OBSERVATION (TEST A)	OBSERVATION (TEST B)	CONCLUSION
AL 2	Orange precipitate produced within 5 minutes	Orange precipitate produced	A=alkaloid present B=lumefantrine present
AL 3	Orange precipitate produced within 5 minutes	Orange precipitate produced	A=alkaloid present B=lumefantrine present
AL 4	Orange precipitate produced within 5 minutes	Orange precipitate produced	A=alkaloid present B=lumefantrine present
AL 5	Orange precipitate produced within 5 minutes	Orange precipitate produced	A=alkaloid present B=lumefantrine present
AL 6	Orange precipitate produced within 5 minutes	Orange precipitate produced	A=alkaloid present B=lumefantrine present
AL 7	Orange precipitate produced within 5 minutes	Orange precipitate produced	A=alkaloid present B=lumefantrine present
Negative control	No orange precipitate produced	No orange precipitate produced	A=alkaloid absent B=lumefantrine absent

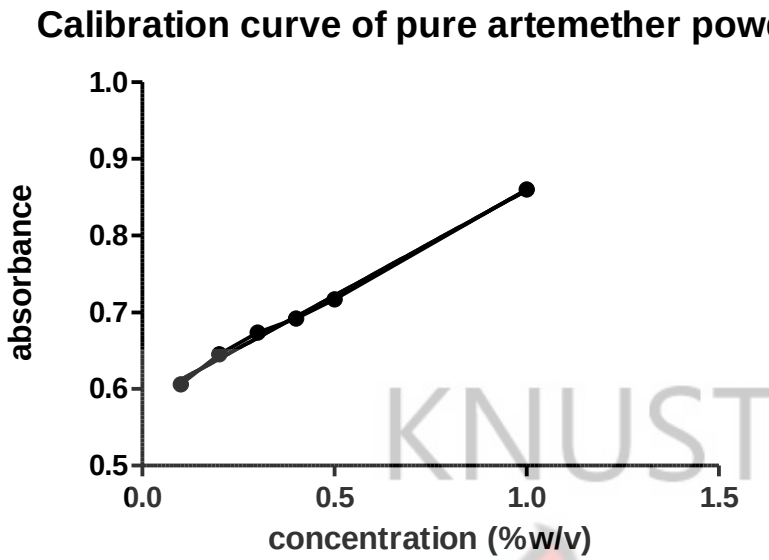
Table 4.20 Amount of Powdered Artemether-Lumefantrine Tablet Brands used

SAMPLE CODE	TABLET WEIGHT/G	ACTUAL WEIGHT OF POWDERED TABLET/G
AL 2	**0.2405**±0.0017	**0.2412**
AL 3	**0.5818**±0.0026	**0.5819**
AL 4	**0.3407**±0.0060	**0.3411**
AL 5	**0.2612**±0.0048	**0.2614**
AL 6	**0.2464**±0.0011	**0.2470**
AL 7	**0.2408**±0.0020	**0.2411**

AL 8 is an injection

Table 4.21 Absorbance of pure artemether powder

CONCENTRATION %w/v	ABSORBANCE 1	ABSORBANCE 2	ABSORBANCE 3	ABSORBANCE MEAN
0.1	**0.600**	**0.610**	**0.608**	**0.606** ±0.0053
0.2	**0.640**	**0.645**	**0.650**	**0.645** ±0.0050
0.3	**0.670**	**0.680**	**0.670**	**0.673** ±0.0058
0.4	**0.690**	**0.695**	**0.690**	**0.692** ±0.0029
0.5	**0.720**	**0.720**	**0.710**	**0.717** ±0.0058
1	**0.860**	**0.870**	**0.805**	**0. 845** ±0.0350



R^2 =0.9924

Figure 4.23 Calibration curve of pure artemether powder

Table 4.22 Absorbance of artemether in artemether-lumefantrine tablet brands

SAMPLE CODE	ABSORBANCE 1	ABSORBANCE 2	ABSORBANCE 3	ABSORBANCE MEAN
AL 2	**0.640**	**0.650**	**0.640**	**0.643**±0.0058
AL 3	**0.730**	**0.740**	**0.728**	**0.732**±0.0064
AL 4	**0.610**	**0.610**	**0.615**	**0.612**±0.0029
AL 5	**0.620**	**0.650**	**0.620**	**0.630**±0.0173
AL 6	**0.680**	**0.672**	**0.670**	**0.674**±0.0053
AL 7	**0.650**	**0.660**	**0.648**	**0.653**±0.0064
AL 8	**0.755**	**0.758**	**0.760**	**0.758**±0.0025

Table 4.23 Percentage content of artemether in artemether-lumefantrine tablet brands

SAMPLE CODE	LABEL CLAIM (mg)	CONC. OF PREPARATION %w/v	ACTUAL CONC. OF ARTEMETHER FOUND %w/v	ACTUAL CONTENT OF ACTIVE INGREDIENT (mg)	%CONTENT
AL 2	**20**	**0.2010**	**0.2137**	**21.26**	**106.32**
AL 3	**40**	**0.4001**	**0.5377**	**53.76**	**134.39**
AL 4	**20**	**0.2002**	**0.1008**	**10.07**	**50.35**
AL 5	**20**	**0.2002**	**0.1664**	**16.62**	**83.12**
AL 6	**20**	**0.2005**	**0.3265**	**32.57**	**162.84**
AL 7	**20**	**0.2002**	**0.2501**	**24.99**	**124.93**
AL 8	**80mg/ml**	**0.8000**	**0.6323**	**63.23**	**79.04**

Artemether - Lumefantrine tablets contain Artemether and Lumefantrine. They 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.

4.4.1.1 SAMPLE CALCULATION OF CONTENT OF ARTEMETHER IN ARTEMETHER-LUMEFANTRINE TABLETS

From the calibration curve the equation of the graph of pure artemether powder is ; y = mx+c

Where, y= absorbance x=concentration m =slope c= intercept

m=0.2747 c=0.5843

Hence, x= y-0.5843/0.2747

Thus for a tablet with an absorbance of, 0.643

Its concentration, x = (0.643-.5843)/0.2747

x= 0.2137

(0.2137/0.2010)x100=106.32%

(106.32/100) x 20 mg =21.26 mg.

4.4.2 ESTIMATION OF THE PERCENTAGE CONTENT OF LUMEFANTRINE IN ARTEMETHER-LUMEFANTRINE TABLET BRANDS

Table 4.24 Amount of powdered artemether-lumefantrine tablet brands used

BRAND	AVERAGE TABLET WEIGHT	WEIGHT OF POWDERED TABLET EQUIVALENT TO 0.45g OF LUMEFANTRINE/g
AL 2	**0.2405**±0.0017	0.9019
AL 3	**0.5818**±0.0026	1.0909
AL 4	**0.3407**±0.0060	1.2776
AL 5	**0.2612**±0.0048	0.9795
AL 6	**0.2464**±0.0011	0.9240
AL 7	**0.2408**±0.0020	0.9030

SAMPLE CALCULATION OF AMOUNTS USED

AL 2

If 0.2405g of Al 2 contains 0.12g of lumefantrine,

Then 0.45g of lumefantrine is contained in:

$\frac{0.45g \times 0.2405g}{0.12g}$

= 0.9019g of powdered AL 2

4.4.2.1 MILLIEQUIVALENT CALCULATIONS

STANDARDISATION OF 0.1M PERCHLORIC ACID

Molecular weight of potassium hydrogen phthalate ($C_8H_8KO_4$)=204.22g/mol

204.22g of ($C_8H_8KO_4$) in 1000ml =1M perchloric acid ($HClO_4$)

20.422g of ($C_8H_8KO_4$) in 100ml = 1M perchloric acid ($HClO_4$)

2.0422g of ($C_8H_8KO_4$) in 100ml =0.1M perchloric acid ($HClO_4$)

0.020422g of ($C_8H_8KO_4$) in 1ml = 0.1M perchloric acid ($HClO_4$)

Table 4.25 Standardisation of 0.1M Perchloric Acid

Burette Reading (ml)	1st Determination	2nd Determination	3rd Determination
Final reading (ml)	26.90	26.90	26.90
Initial reading (ml)	0.00	0.00	0.00
Titre volume (ml)	26.90	26.90	26.90

4.4.2.2 ASSAY OF LUMEFANTRINE

Molecular weight of lumefantrine = 528.94g/mol

528.94g of lumefantrine in 1000ml = 1M perchloric acid ($HClO_4$)

52.894g of lumefantrine in 100ml = 1M perchloric acid ($HClO_4$)

5.2894g of lumefantrine in 100ml = 0.1M perchloric acid ($HClO_4$)

0.052894g of lumefantrine in 1ml = 0.1M perchloric acid ($HClO_4$)

Table 4.26 Assay of Lumefantrine

Burette Reading (ml)	**Pure lumefantrine**	**AL 2**	**AL 3**	**AL 4**	**AL 5**	**AL 6**	**AL 7**
Final reading (ml)	**10.00** ±0.0	**10.80**±0.0	**13.10**±0.0	**12.20**±0.0	**13.20**±0.0	**11.50**±0.0	**11.10**±0.0
Initial reading (ml)	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Titre volume (ml)	**10.00**	**10.80**	**13.10**	**12.20**	**13.20**	**11.50**	**11.10**

Table 4.27 Blank Determination

Burette Reading (ml)	1st Determination	2nd Determination	3rd Determination
Final reading (ml)	0.40	0.40	0.40
Initial reading (ml)	0.00	0.00	0.00
Titre volume (ml)	0.40	0.40	0.40

4.4.2.3 FACTOR OF POTASSIUM HYDROGEN PHTHALATE ($C_8H_8KO_4$)

Actual weight of ($C_8H_8KO_4$) = (0.4901+0.5001+0.4992)g/3

Actual weight of ($C_8H_8KO_4$) = 0.4965g

Nominal weight = 0.5000g

Factor of =Actual weight / Nominal weight

= 0.4965g/0.5000g

=0.993

4.4.2.4 FACTOR OF PERCHLORIC ACID ($HCLO_4$)

Factor of ($HCLO_4$) x Volume of ($HCLO_4$) = Factor of ($C_8H_8KO_4$) x Volume of ($C_8H_8KO_4$)

Factor of ($HCLO_4$) = (0.993 x 25ml)/26.90ml

Factor of ($HCLO_4$) = 0.9229

4.4.2.5 SAMPLE CALCULATIONS OF PERCENTAGE CONTENT OF LUMEFANTRINE

PURE SAMPLE

10.00ml-0.40ml=9.60ml

9.60ml x 0.9229 = 8.86ml

From milliequivallence, 1ml of $HCLO_4$= 0.052894g of lumefantrine

Therefore 8.86ml of $HCLO_4$ = 0.4686g of lumefantrine

Actual mass of pure lumefantrine used =0.4561g

(0.4686/0.4561)g x 100

=102.74%

AL 2

10.80ml-0.40ml = 10.40ml

10.40ml x 0.9229 = 9.60ml

From milliequivallence, 1ml of $HCLO_4$ = 0.052894g of lumefantrine

Therefore 9.60ml of $HCLO_4$ = 0.5078g of lumefantrine

Actual mass of AL 2used =0.4590g

(0.5078/0.4590)g x 100

110.63%

Table 4.28 Percentage Content of Lumefantrine in A-L Tablet Brands

BRAND	AMOUNT IN (MG) OF LUMEFANTRINE (LABEL CLAIM)	ACTUAL AMOUNT OF LUMEFANTRINE PRESENT (MG)	PERCENTAGE CONTENT
AL 2	120	132.76	110.63
AL 3	240	328.8	137.76
AL 4	120	153.56	127.97
AL 5	120	166.66	138.88
AL 6	120	144.59	120.49
AL 7	120	139.36	116.13

Table 4.29 Quality of Artemether-Lumefantrine tablet brands analyzed

BRAND	ARTEMETHER CONTENT (%)	LUMEFANTRINE CONTENT (%)	QUALITY
AL 2	106.32	110.63	GOOD
AL 3	134.39	137.76	SUBSTANDARD
AL 4	50.35	127.97	SUBSTANDARD
AL 5	83.12	138.88	SUBSTANDARD
AL 6	162.84	120.49	SUBSTANDARD
AL 7	124.93	116.13	SUBSTANDARD
AL 8	79.04	-	SUBSTANDARD

Artemether -Lumefantrine tablets contain Artemether and Lumefantrine. They 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.

Chapter 5

DISCUSSION

5.1 SURVEY ON PREVALENCE OF ARTEMISININ BASED ANTIMALARIALS IN THE KUMASI METROPOLIS

The effective and rapid control of malaria requires not only proper diagnosis of the disease but the administration of good quality antimalarial medication. In line with the World Health Organization's antimalarial policy, artemisinin based combination therapies (ACTs) are first and second line treatment for uncomplicated falciparum malaria. In order to achieve effective case management of falciparum malaria, patients must have easy access to the ACTs and the quality of the ACTs must not be questionable. Poor quality of ACTs has resulted in treatment failure and may lead to drug resistant falciparum malaria in the future. It is therefore necessary to ensure that, ACTs available on the retail market that is, in pharmacies and licensed chemical sellers' shops are of good quality.

Retail pharmacies and licensed chemical sellers' shops (LCSS) are the first point of call for most people suffering from falciparum malaria who can afford to purchase medication and thus a good place to gather information about the availability of artemisinin based combination antimalarials. Others who are registered on the National Health Insurance Scheme (NHIS) do sometimes visit the above mentioned facilities when the antimalarials or other medication prescribed to them are not readily available at the hospitals or clinics they attend. Some pharmacies are accredited to serve prescriptions to patients under the NHIS Therefore assessment of the availability of artemisinin based antimalarials in selected Pharmacies or Licensed Chemical Sellers' shops in the Kumasi metropolis is a credible way of determining the availability and affordability of artemisinin based antimalarials in the Kumasi metropolis.

5.1.1 TYPE OF FACILITY VISITED AND LOCATION OF FACILITIES

With reference to Figure 4.1, forty (40) pharmacies and sixty (60) licensed chemical sellers' shops were visited in the Kumasi metropolis. According to the pharmacy council, licensed chemical sellers' shops are generally situated in areas where there are no retail pharmacies available to the communities. The locations of the facilities are indicated in Figures 4.2 and4.3.

5.1.2 OCCUPATION OF RESPONDENTS

Most of the respondents at the pharmacies were either pharmacists or dispensing technicians whereas the respondents in LCSS were the license holders themselves or shop attendants as seen in Figure 4.4.

5.1.3 AVAILABILITY OF ACTs AT FACILITIES

Rapid access to effective treatments at the first onset of symptoms has been clearly shown to prevent the progression of the disease to more life-threatening stages such as severe and cerebral malaria. All the facilities visited had on sale, antimalarials according to Table 4.1.From Figure 4.5, it is observed that all the facilities visited had on sale artemether-lumefantrine tablets whiles Figure 4.6 shows that 93 out of the 100 facilities visited had on sale artesunate-amodiaquine tablets This shows that ACTs are widely available in the Kumasi metropolis as the facilities visited covered the length and breadth of the metropolis as seen in Figures 4.2 and 4.3. Buabeng *et al.* (2008) reported that the availability of either the first or second-line policy-recommended medicines for uncomplicated malaria was higher in community pharmacies, hospitals and clinics in urban areas. This correlates with the findings made in this study whereby policy recommended ACTs are widely available in all retail pharmacies and licensed chemical sellers' shops visited and by extrapolation, it can be said that availability in the Kumasi metropolis is high. However at the time of the study, dihydroartemisinin-piperaquine, the other second line ACT used for uncomplicated falciparum malaria was not readily available in most facilities as seen from Figure 4.7. This was because it had been recently introduced and at the time of the study, only one brand was found in the facilities that had it in stock.

5.1.4 THE MOST EXPENSIVE ANTIMALARIAL

The cost of the ACTs is significantly higher than other antimalarials available in these facilities, with artemether- lumefantrine being the most expensive according to Figure 4.9.

5.1.5 VARIOUS FORMULATIONS OF ANTIMALARIALS AVAILABLE

From Figure 4.10, the survey also depicted that there were various formulations available namely, tablets, capsules, suspensions, suppositories and injections. Thus,

patients who could not take ACTs via a particular route of administration had other alternatives and this improves patient compliance and effective management of falciparum malaria.

5.1.6 BEST SELLING ANTIMALARIAL

The bestselling antimalarials in the Kumasi metropolis according to the survey included two ACTs; artemether- lumefantrine (24% P, 36% LCSS) and artesunate-amodiaquine (4% P, 6% LCSS). The relatively higher frequency of sale of artemether-lumefantrine tablets as compared to artesunate-amodiaquine may be associated with a public perception that artemether-lumefantrine is safer and better tolerated than artesunate-amodiaquine. Owing to earlier reported incidences of undesirable side effects of artesunate-amodiaquine, some patients no matter the education given to them by healthcare providers refuse to take artesunate-amodiaquine. SP was the second bestselling antimalarial in the metropolis according to Figure 4.12 (16% P, 18% LCSS). Although SP is restricted for use in Intermittent Preventive Therapy (IPT) in pregnant women and no longer recommended for treatment of uncomplicated falciparum malaria in Ghana respondents acknowledged that, due to the relatively low cost of SP, most patients who could not afford the more expensive ACTs opted for SP. The availability and sale of SP therapies defeats the purpose of the current policy, which seeks to promote ready access and appropriate use of effective antimalarials in households and in the community and health-care facilities (Buabeng *et al.*, 2008).

5.1.7 KNOWLEDGE OF W.H.O. ANTIMALARIAL POLICY

Most pharmacists had knowledge of the policy and shop attendants had little or no knowledge of the existence of the antimalarial policy according to Figure 4.13. A total of forty respondents representing 40% had knowledge of the policy. Out of this percentage, the majority was pharmacists and only 2 attendants out of 34 interviewed knew about the policy and the recommended ACTs. Community/retail pharmacies and licensed chemical sellers' shops have been identified as important sources for households and communities to access antimalarials for home-based management of malaria in Ghana (Agyepong and Kangeya-Kayonda, 2004; Ahorlu *et al*, 1997; Lang *et al*, 2006; Kazembe *et al*, 2007) and thus it is important for persons that dispense medications at these facilities to have extensive knowledge of the antimalarials they

dispense to patients in order to promote patient compliance and improve outcomes of the disease conditions. Regular training programmes must be organized for attendants at these facilities in order to improve their knowledge and skills in managing uncomplicated falciparum malaria in the communities.

5.1.8 PATIENTS' SOURCES OF INFORMATION ABOUT ANTIMALARIALS

It is important to establish whether the decision of patients to purchase particular antimalarials is influenced by the respondents, owing to the fact that a majority (60%) of them are not aware of the recommended first and second line therapy for uncomplicated falciparum malaria. According to Figure 4.14, most patients ask for specific antimalarials while others asked whoever was available at the facility to recommend a suitable antimalarial medication. The personnel at these community-based outlets should therefore be targeted to receive appropriate public health information so as to improve the quality of their services and to convince them of the need to dispense only medicines that have proven efficacy and that are recommended by the national policy for the management of malaria (Buabeng *et al.*, 2008).

5.1.9 ANTIMALARIALS COMMONLY RECOMMENDED TO PATIENTS

In situations when patients asked for the opinion of the person at the facility, artemether-lumefantrine was most recommended and SP was least recommended. For most attendants the recommendation of artemether-lumefantrine was not due to known efficacy but rather an attempt to improve daily sales at their respective facilities as artemether-lumefantrine is the most expensive antimalarial available in most facilities. However, irrespective of the reason for recommendation of artemether-lumefantrine, it is a good recommendation and in line with the WHO antimalarial policy. Artesunate-amodiaquine was also recommended according to Figure 4.15.

5.1.10 AFFORDABILITY OF ANTIMALARIALS

From Figure 4.11 30% of respondents from pharmacies and 33% from LCSS said most people could afford to buy antimalarials but they however went on to clarify that they were referring to sulphadoxine-pyrimethamine combinations (SPs). It is observed from Figure 4.8 that 86 out of the 100 facilities visited had on sale, SP tablets. Although SP is under the current policy for Ghana, reserved for Intermittent Preventive Therapy in

pregnant women, 86% of facilities in the Kumasi metropolis sold them to patients for the treatment of uncomplicated malaria.

5.1.11 COST OF FULL COURSE OF TREATMENT WITH ACTs

A full course of treatment of falciparum malaria in these facilities includes in addition to the antimalarial, haematinics, multivitamins and pain-killers. Such packages depending on what the components were ranged in cost from between GH¢1 to GH¢15. Most of such packages cost between GH¢5-GH¢7 with the least frequently dispensed package costing between GH¢1-GH¢3 as seen in Figure 4.16. According to Buabeng *et al.* (2008), a course of treatment of ACTs for malaria costs approximately $3-$9 (GH¢4.5-GH¢13.5), which is beyond affordability for many clients who patronize licensed chemical shops for antimalarial therapeutics. In contrast, the cost of unapproved alternatives such as chloroquine, amodiaquine only or S/P could be as low as 20-50 cents (50-70 pesewas) for the generic brands that are produced locally in Ghana.

5.1.12 NHIS ACCREDITED FACILITIES

None of the licensed chemical sellers' shops visited was accredited to serve prescriptions under the National Health Insurance Scheme (NHIS). Of the 40 pharmacies visited, eighteen were accredited by the NHIS as service providers as shown in Figure 4.17. Accredited service providers are expected to stock and supply policy recommended medicines to clients with health insurance. Thus, the number of clients or courses of ACTs served daily can give an indication as to the availability of policy recommended ACTs at the accredited facilities and by extrapolation, the Kumasi metropolis.

5.1.13 AVERAGE NUMBER OF ANTIMALARIAL COURSES SERVED DAILY ON NHIS

Artemisinin based combination therapy is prescribed under the NHIS and accredited pharmacies serve patients who attend their facilities with NHIS prescriptions for ACTs. Form Figure 4.18, it can be seen that 28.57% of these pharmacies serve up to 50 ACTs

daily and 14.29% serve as many as 150 ACTs per day. Thus, it can be inferred ACTs are widely available to patients in the Kumasi metropolis. Strict adherence to the policy by health providers is a measure of the success of its implementation. Therefore widening the net of facilities that are registered for NHIS services could be an efficient way to promote provider adherence to policy for malaria therapy in Ghana (Davis *et al*, 2005; Robertson and Hill, 2007; Kaona and Tuba, 2005; Afeyadu *et al*., 2005; Nsabagasani *et al*., 2007; Drager *et al.*, 2006).

5.1.14 PURCHASE OF SUSPECTED COUNTERFEIT ANTIMALARIAL MEDICATION

In 2008 substandard and counterfeit versions of thirteen key antimalarial medicines were uncovered in multiple locations across Ghana by the Medicines Quality Monitoring surveillance programme. Set up by the Ghana Food and Drugs Board (FDB) in collaboration with the U.S. Pharmacopeial Convention (USP) and the U.S. Agency for International Development (USAID), the programme sampled antimalarials across the public and private sectors (The Ghanaian Journal, 2010).

In 2009 the same programme uncovered a counterfeit version of Novartis' Coartem®, another widely used antimalarial. In that case an alert citizen notified the authorities after suspecting the drug he bought might be fake (The Ghanaian Journal, 2010). Culprits from both retail and whole sale pharmacies were made to pay huge fines. Thus, question of the existence of counterfeit or substandard medicines makes most owners of retail facilities uncomfortable. However the question was put in such a way to shift the blame to wholesalers. Thus to the question, whether respondents had ever suspected that antimalarials purchased for sale in their facilities irrespective of the source could be counterfeit, most respondents (94%), answered "no". The number who answered "sometimes" did so with a lot of explanations and was mostly, pharmacists as seen in Figure 4.19.

5.2 B. QUALITY ASSESSMENT OF ACTs

The ability to identify a poor quality formulation is the crucial component in a drug quality assurance system. Quality evaluation studies are primarily important to provide information on the drug content and second, to identify the (cause), if any, of the poor quality in the field. It is worth noting that at the time of the purchase of samples,

artesunate-amodiaquine existed as separate tablets packaged together, the combination tablets were not common as is the case currently. All samples collected were coded (for example, AT 2, AM 3, AL 5) and analysis was performed without the packaging and only codes identified the various samples and thus any possible bias was avoided. Tablet packaging, country of manufacture (origin), shelf-life and other relevant information on the packaging were recorded. Each of the samples purchased had at least 6 months left on the shelf-life and all analysis were carried out before the expiry dates were up.

5.2.1 UNIFORMITY OF WEIGHT OF ARTESUNATE AND AMODIAQUINE TABLETS

The amount of fill placed in the die of a tablet press will determine the weight of the resulting tablet. The volume of fill (granulation) or powder permitted to enter the dies adjusted with the first few tablets produced to yield tablets of the desired weight and content. The uniformity of weight test is one way of determining whether proper mixing or blending of ingredients occurred during manufacture. Also, even distribution of active ingredient is necessary in order to avoid overdosing or under dosing which can both be fatal to a patient. Out of the 7 samples of artesunate tablets tested, one sample failed the uniformity of weight test. Five tablets of the sample AT2, deviated by more than 5% and one tablet deviated by more than 10% of the average mass as shown in Table 4.2. All the five samples of amodiaquine tablets passed the test. The results shown in Table 4.2 , indicate that the five brands of amodiaquine hydrochloride tablets had uniform weights with little standard deviation and hence conformed to the British Pharmacopoeia (2007) specification of not more than 5% deviation for more than two of the individual masses and no deviation by more than 10% of the average mass of the tablets.

5.2.2 DISINTEGRATION TIMES OF ARTESUNATE AND AMODIAQUINE TABLET BRANDS

For a drug to be absorbed from a solid dosage form after oral administration, it must first be in solution, and the first important step toward this condition is usually the break-up of the tablet; a process known as disintegration. Looking at the results on Table 4.3, it can be seen that all the samples of both artesunate and amodiaquine tablets passed the disintegration test. The British Pharmacopoeia (2007) states that, for

uncoated tablets, disintegration should occur within 15 minutes and for coated tablets within 30 minutes. Sample AT7, upon physical examination was found to be coated. For the artesunate tablets, 3 brands disintegrated in less than five minutes and 1 in under a minute with 2 brands disintegrating at about 7 and 9 minutes respectively. For tablets that are bitter to taste or that possess unpleasant smell or taste, a very quick disintegration time is not the best since it can lead to the tablets disintegrating in the mouth of the patient. However this does not affect the therapeutic efficacy of the medication in any way.

All the amodiaquine tablet brands disintegrated in less than 15 minutes implying that their disintegration was satisfactory. As mentioned earlier, a very quick disintegration may produce certain unpleasant situations for the patient. One brand disintegrated in 20 seconds and if this brand is exposed to moisture before ingestion, the disintegration process may begin and this is undesirable. Two brands of amodiaquine tablets disintegrated in 1 minute and one brand in one and a half minutes. Only one brand disintegrated in 9 minutes. The disintegration time of all the brands of both artesunate and amodiaquine tablets fall within British Pharmacopoeia (2007) specification of disintegration time of less than or equal to 15 minutes. AT7 is coated but however passed the test.

5.2.3 HARDNESS OF ARTESUNATE AND AMODIAQUINE TABLETS

Hardness, which is now more appropriately called crushing strength determinations are made during tablet production and are used to determine the need for pressure adjustment on tablet machine. If the tablet is too hard, it may not disintegrate in the required period of time to meet the dissolution specifications; if it is too soft, it may not be able to withstand the handling during subsequent processing such as coating or packaging and shipping operations. The resistance of tablets to capping, abrasion or breakage under conditions of storage, transportation and handling before usage depends on its hardness. The instrument used, measures the force required to break the tablet.

The force required to break the tablet is measured in kilogrammes (but the result is expressed in Newton; IKG=9.8 NEWTONS) and a crushing strength of 4Kg (39.2 N) is usually considered to be the minimum for satisfactory tablets. According to table 4.4., AT3 and AT4 have a mean crushing strength of 26.56 and 23.02 Newton respectively. These values are lower than the minimum force required (39.2N) and thus AT3 and

AT4 can be described as soft tablets. If a tablet is described as soft, it may not be able to withstand the handling during subsequent processing such as coating or packaging and shipping operations. Easy breakage of tablet may also lead to loss of medicament which eventually leads to under dosing. AT7 is a coated tablet and thus hardness test is not a required test, however the regular force required to break all 10 tablet samples is an indication of the good quality of the tablet coating process. From table 4.4 the force required to break AT7 brand of artesunate tablets is 168.60N with no deviation from the mean. AT2, AT5, AT6 and AT8 were found to have passed the test with mean forces of 47.53 N, 39.8N, 92.33N and 49.3N respectively.

According to Table 4.4, two brands of amodiaquine tablets were found to be soft as they required a breaking strength of less than 4kg (39.2N). Although the other brands required a force of more than 4kg (39.2 N), tablets that are too hard are also undesirable. But since none of the tablet brands are chewable, the forces recorded were considered satisfactory.

5.2.4 DETERMINATION OF AUTHENTICITY OF ARTESUNATE IN ARTESUNATE TABLETS BY COLORIMETRY

The artemisinins do not have the particular chemical groups that easily react with certain reagents to yield coloured products, however, they can be transformed by acid or base treatment to more reactive compounds, i.e. enolate/carboxylates or a, b-unsaturated decalones (Zhao and Zeng, 1986; Thomas *et al*., 1992). These compounds react readily with diazonium salts (Zollinger, 1991). Green *et al*. (2000) demonstrated that the alkali-decomposition product of artesunate react with the diazonium salt, FRTR, to produce a yellow product correlating well with artesunate concentration. All the seven samples used gave the yellow colouration upon addition of the FRTR dye as seen in Table 4.5. Thus all the samples contained an artemisinin derivative (artesunate). The negative control yielded no yellow colouration thus; the yellow colouration can be attributed to the presence of an artemisinin derivative.

5.2.5 THIN LAYER CHROMATOGRAPHY OF ARTESUNATE TABLETS

All the spots with the exception of the negative control turned brown; hence all the samples contained an artemisinin derivative as seen in Figure 4.21 and Table 4.6. This method was validated by Ioset and Kaur (2009) by comparing the results of their assays

in parallel with the other available field test (Fast Red Test in the MiniLab®) and found that their assays specifically detected only the artemisinin component in the samples used. This assay is therefore robust, reproducible and sensitive. It has also been extensively validated using HPLC-PDA the "reference standard method" to compare the assay conducted by Ioset and Kaur (2009). The method of Ioset and Kaur (2009) provides two user friendly/field friendly methods to monitor the quality of ACT formulations for use in the developing countries. Ioset and Kaur (2009) further compared the results of their assays with the published colorimetric assay (Green et al., 2000; 2001) and established that their assays specifically detected the artemisinin derivatives clinically used as antimalarial drugs. According to Ioset and Kaur (2009), none of the other antimalarial drugs or a range of commonly used excipients, antiretroviral drugs or other frequently used drugs from the WHO essential drugs list such as analgesics or antibiotics are detected with their assays. It can therefore be said that all the artesunate tablet brands tested with results shown in Table 4.6 contain artesunate and thus are not fake.

5.2.6 PERCENTAGE CONTENT OF ARTESUNATE TABLETS BY UV VISIBLE SPECTROPHOTOMETRY

The International Pharmacopoeia (2003), states that Artesunate tablets 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. By these standards, all the artesunate brands tested do not contain the appropriate content of artesunate. Moreover the content of active ingredients found with reference to Table 4.10, do not correspond to the stated label claims. Six out of the seven samples were under dosed whereas one sample was overdosed. Out of the seven brands of artesunate tablets analyzed, six brands; AT 2, AT 3, AT 4, AT 6, AT 7 and AT 8 showed a very wide deviation of quantity of active ingredient as compared to the label claims as seen in table 4.10, this represents 85.7% of the samples analyzed. Only AT 5 with a percentage content of 85.67% came close to the lower level of acceptance which is 90%. According to a study by Esimone *et al.* (2008), five out of nine brands of artesunate tablets tested showed unacceptable quantity of active contents against the label claims which represented approximately 55.6% of total brands surveyed. However, Esimone et al conducted their survey and analysis in Nigeria. Ofori-Kwakye *et al.* (2008) reported that 64.7% of brands failed the WHO International Pharmacopoeia requirements for percentage content. Comparatively, the percentage

deviation from the stated label claims in this study gives rise to concern in Kumasi and Ghana as a whole. It is known that due to the presence of the unstable peroxide linkage in the chemical structure of artesunate, it tends to be unstable. Product stability is another parameter that has a direct influence on the quality and efficacy of the medicine. Pharmaceutical degradation is generally accelerated by heat and humidity and WHO recommends stability testing in tropical conditions (WHO, 2006c), but this is not always done (Omer, 1990; Arya, 1995).

Artesunate, an essential antimalarial drug, is extremely sensitive to heat and humidity. Stable formulations of Artesunate are difficult to produce (the quality of the packaging material is critical) (Fawaz and Millet, 2006) while co-formulations of Artesunate with other antimalarials are even more challenging as these latter molecules can increase the instability of Artesunate. It is difficult to obtain consistent stability data from producers, although this is crucial information for guaranteeing the efficacy of the product and avoid the emergence of resistance. When stability studies are performed they often do not adhere to WHO guidelines, especially for the testing in zone IV (tropical) conditions. It can therefore be inferred that owing to the unstable nature of the artesunate, the percentage content may have reduced during storage as the stability depends on heat and humidity. However there is no way of verifying that this was indeed the case and therefore, even though the artesunate tablet brands were found to contain artesunate the tablets were found to be substandard.

5.2.7 PERCENTAGE CONTENT OF AMODIAQUINE HCl IN AMODIAQUINE HYDROCHLORIDE TABLETS

Amodiaquine, like chloroquine, is a 4-aminoquinoline; it is effective against chloroquine-resistant strains of *P. falciparum*, although there is some cross-resistance. After oral administration amodiaquine is largely converted to desethylamodiaquine, which contributes the majority of the antimalarial activity. Amodiaquine is generally reasonably well tolerated and slightly more palatable than chloroquine, although in some areas it has not been a popular substitute. The serious adverse effects that have been associated with its prophylactic use (agranulocytosis and severe liver toxicity) are considered rare when amodiaquine is used in malaria treatment, although more data are needed to characterize the risks.

Amodiaquine hydrochloride tablets contain an amount of amodiaquine hydrochloride ($C_{20}H_{22}ClN_3O.2H_2O$) equivalent to not less than 93.0% and not more than 107.0% of the labeled amount of amodiaquine. ($C_{20}H_{22}ClN_3O$). From Table 4.13, it is observed that there is a great disparity between the amount of amodiaquine content stated on the product labels and the amount of amodiaquine actually present in the product. In all instances overdosing has occurred. Since the introduction of the new WHO antimalarial policy, manufacturers were obliged to produce amodiaquine tablets to be sold with artesunate monotherapy tablets that already existed on the market. It is rather unfortunate that content requirements for all the amodiaquine tablets are not satisfactory. From Table 4.14., all the samples contained more than 107 per cent of the labeled amount of amodiaquine. Over dosing is as harmful as under dosing. Owing to the undesirable side effects of amodiaquine, an over dose of the medicament is not a useful venture.

5.2.8 ASSESSMENT OF THE QUALITY OF ARTEMETHER-LUMEFANTRINE TABLETS

5.2.9 Uniformity of weight of artemether-lumefantrine tablet brands

As stated earlier, the importance of weight uniformity cannot be underestimated. However all the brands of artemether –lumefantrine tablets passed the test as shown on Table 4.15. Thus, it can be said that proper mixing, good flow of granules and accurate die filling of granules were achieved during manufacture.

5.2.10 Disintegration times of artemether-lumefantrine tablet brands

All the tablet brands disintegrated in less than 15 minutes and thus were satisfactory. It is worth noting that from Table 4.16, two tablet brands disintegrated in less than a minute. This implies that these tablets can disintegrate in the mouth before swallowing and this may reduce patient compliance. Moreover, exposure of these tablets to moisture, prior to administration may lead to disintegration and therefore storage conditions of these tablets must be satisfactory in order to maintain the integrity of the tablets.

5.2.11 Hardness of Artemether-Lumefantrine tablet brands

The hardness or crushing strength reflects the function and appropriateness of the type and quantity of binder and lubricant employed as well as the compression force used in preparing the tablets (Awofisayo *et al.*, 2010).

The force required to break the tablet is measured in kilogrammes (but the result is expressed in Newton; IKG=9.8 NEWTONS) and a crushing strength of 4Kg (39.2 N) is usually considered to be the minimum for satisfactory tablets. From Table 4.17, all the tablet brands are hard enough and can withstand handling and transportation without breaking since tablet breakage may lead to loss of active ingredient and eventually, under dosing.

5.2.12 Authenticity of artemether in artemether-lumefantrine tablet brands

Generally, the results seen in Table 4.18 indicate that each of the brands contained the stated active ingredient; artemether, since they all produced a pink colouration. The absence of a pink colour would have indicated the absence of artemether and thus a fake product. Osei –Safo *et al.* (2010) reported that all 49 drug samples tested with the same method produced a pink colouration which meant that all brands contained artemether. Thus, all the brands of artemether-lumefantrine sold in pharmacies and licensed chemical sellers' shops sampled in the current study contain artemether.

5.2.13 Authenticity of artemether in artemether injection

According to the National Antimalarial Drug Policy for Ghana, management of severe/ complicated malaria requires parenteral treatment. In order to provide adequate blood concentrations as quickly as possible, initially, parenteral treatment should start before oral treatment when the patient's condition permits. To this end, it is important that parenteral artemether is of the optimum quality in order to prevent fatalities as a result of severe or complicated malaria. Licensed chemical sellers' shops are by law not permitted to stock or offer for sale, parenteral medicines. The brand used was therefore purchased from pharmacies. From Table 4.18, the brand tested contains artemether since it produced the pink colouration. Thus the active ingredient present in the artemether injection brand is authentic.

5.2.14 Authenticity of lumefantrine in artemether-lumefantrine tablets

The fixed combination of artemether and lumefantrine achieves its antimalarial effect through the sequential large initial reduction in parasite biomass by artemether and the subsequent removal of all of the remaining viable parasites by the intrinsically less active but more slowly eliminated lumefantrine (White, 1997; 1998). From Table 4.19, test (A) is a general test for the presence of an alkaloidal antimalarial such as lumefantrine and piperaquine. It is therefore recommended that test (A) is used in combination with other tests and hence test (B) is also carried out and is based on the presence of an allyl alcohol in the structure of lumefantrine. Allylic hydroxyl groups are easily oxidized by MnO_2 to the corresponding carbonyl in high yields. Carbonyl compounds react with 2, 4-DNPH to give orange or deep yellow precipitates of the corresponding hydrazones (Osei-Safo *et al.*, 2010). All the brands produced an orange precipitate in both test (A) and test (B). The negative controls did not yield the orange precipitate since they did not contain any lumefantrine or alkaloidal antimalarial.
It must be noted however that other compounds containing an allylic alcohol functional group could also test positive with this reagent (Osei-Safo *et al.*, 2010).

5.2.15 Percentage content of artemether in artemether-lumefantrine tablet brands

The International Pharmacopoeia (2003) states that artemether-lumefantrine tablets contain artemether and lumefantrine and they must contain not less than 90.0% and not more than 110.0% of the amounts of artemether and lumefantrine stated on the label. From Table 4.29, five out of the six brands of artemether lumefantrine tablets were found not to conform to the International Pharmacopoeia standards. AL 3, AL 6 and AL 7 were found to be overdosed whereas AL 4 and AL 5 were underdosed. Only AL 2 was found to contain an appropriate amount of artemether. Awofisayo *et al.* (2010) reported that only two out of six brands purchased from different sources in Nigeria complied with the International Pharmacopoeia specifications. Over dosing is as harmful as under dosing due to the various adverse effects that a patient may encounter. Under dosing may lead to treatment failure and may eventually result in mortality.

5.2.16 Percentage content of artemether in artemether injection

Artemether injection is used as first line treatment for complicated *falciparum* malaria especially in cases where the patient is unable to take ACTs via the oral route. According to the International Pharmacopoeia (2003), artemether injection contains not less than 95.0% and not more than 105.0% of the amount of $C_{16}H_{26}O_5$ stated on the label. From Table 4.29, the artemether injection brand analysed (AL 8) was found to contain 79.04% of artemether. Thus AL 8 was found to be underdosed. Since artemether injection is used in the treatment of complicated *falciparum* malaria, underdosing can lead to life threatening situations.

5.2.17 Percentage content of lumefantrine in artemether-lumefantrine tablet brands

Lumefantrine is a blood schizonticide active against erythrocytic stages of *Plasmodium falciparum.* It is thought that administration of lumefantrine with artemether results in cooperate antimalarial clearing effects. Artemether has a rapid onset of action and is rapidly cleared from the body. It is thus thought to provide rapid symptomatic relief by reducing the number of malarial parasites. Lumefantrine has a much longer half- life and is believed to clear residual parasites. The International Pharmacopoeia (2003) states that artemether-lumefantrine tablets contain artemether and lumefantrine and they must contain not less than 90.0% and not more than 110.0% of the amounts of artemether and lumefantrine stated on the label. From Table 4.29, all the brands of artemether-lumefantrine tablets analysed were found to contain more than 110.0% of lumefantrine. However AL 2 contained 110.63% and thus can be said to be of better quality than the other brands analysed. An overdose of lumefantrine is undesirable and potentially dangerous owing to reports of possible serious adverse effects which includes QT prolongation which can lead to arrhythmias and other cardiac complications which are potentially life threatening.

5.3 CONCLUSION

From the study, it can be concluded that;

a) Artemisinin based antimalarials (ACTs) are widely available in retail pharmacies and licensed chemical sellers' shops (LCSS) in the Kumasi metropolis.

b) Various formulations of ACTs are available in the Kumasi metropolis with tablets, capsules and suspensions being most prevalent.

c) ACTs are more expensive than other antimalarials.

d) Pharmacists are aware of the WHO antimalarial policy but most pharmacy and LCSS attendants have no knowledge of the policy.

e) ACTs are available at NHIS accredited pharmacies for patients on the NHIS.

f) All the ACTs analyzed contained the appropriate active ingredients but most did not contain the right amounts.

g) Most of the ACTs analyzed were found to be substandard.

5.4 RECOMMENDATIONS

a) Further work should be carried out on new brands of ACTs emerging onto the market.

b) HPLC analysis should be carried out on more brands available on the market to validate the methods used in the current study.

c) Further work should be done to ascertain the causes of the substandard ACTs identified in the current study.

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APPENDIX

APPENDIX I - QUESTIONNAIRE

1. Location of facility……..
2. Type of facility a. Pharmacy b. Licensed Chemical seller's shop
3. Interviewee a. Pharmacist b. Licensed Chemical seller c. Dispensing technician d. other (specify)…….
4. Do you sell antimalarial medicines? a. Yes b. No
5. What types of formulations of antimalarial medicines are available at your facility?
   a. Tablets and capsules……
   b. Suspensions…….
   c. Suppositories ……
   d. Injections ……..
   e. Other (specify)……..
6. Do you sell any of these antimalarial medicines?
   a. Artemether-lumefantrine b. sulphadoxine-pyrimethamine c. Artesunate-Amodiaquine d. Quinine e. dihydroartemisinin-piperaquine f.proguanil g. other (specify)…….
7. Which of the antimalarial medicines is most expensive?
   a. Artemether-lumefantrine b. sulphadoxine-pyrimethamine c. Artesunate –Amodiaquine d. Quinine e. dihydroartemisinin-piperaquine f. proguanil g. other (specify)
8. How affordable are the different antimalarial medicines?
   a. Most people can afford b. Few people can afford c. Everyone can afford d. No one can afford e. other (specify)…….
9. What are the bestselling antimalarial medicines at your facility?

   …….

10. What factors do you consider in the selection of antimalarial medicines to be stocked and sold at your facility?.....

11. Do you know about the WHO antimalarial policy for Ghana? a. Yes b. No

12. Are you aware of the recommended first line antimalarial therapy? A. Yes b. No

13. If Yes, which one?.....

14. Do patients/ customers ask for antimalarial medicines by name or do they seek the advice of the pharmacist or attendant?.....

15. Which medicines do you usually recommend for patients with fever/malaria?

    ……
    ……

16. How much would a "package" of treatment cost?.....

17. Do some patients/ customers purchase incomplete courses of antimalarial medicines?

    a. Yes b. No

18. If yes, why?.....

19. Are there any literature on antimalarial medicines displayed in your facility?

    a. Yes b. No

20. What is the average number of patients who purchase antimalarial medicines each day?.....

21. Is your facility NHIS accredited? A. Yes b. No

22. If yes, which antimalarial medicines are usually prescribed by the NHIS?

……

23. Do you usually have those particular antimalarial medicines in stock?

    a. Yes b. No c. Sometimes

24. What is the average number of patients who are served with antimalarial medicines on NHIS at your facility per day?.....

APPENDIX II - PREPARATION OF TEST MATERIALS

1M NaOH

40g NaOH in 1000ml =1M NaOH

4g NaOH in 100ml = 1M NaOH

4g of NaOH is used to make a 100ml solution of 1 M NaOH with distilled water

1.1M $C_2H_4O_2$ (ACETIC ACID)

60g $C_2H_4O_2$ in 1000ml= 1M $C_2H_4O_2$

6g $C_2H_4O_2$ in 100ml = 1M $C_2H_4O_2$

6.6g $C_2H_4O_2$ in 100ml =1.1M $C_2H_4O_2$

Density=mass/volume

Density($C_2H_4O_2$)=1.048-1.051

Density ($C_2H_4O_2$)=(1.048+1.051)/2

Density ($C_2H_4O_2$)=1.0495

Volume=mass/density

Volume=6.6g/1.0495

Volume=6.28ml

5mg/ml Fast Red TR salt

100mg of Fast Red TR salt in 20ml of distilled water

1000ml of 1M NaOH

1000ml of 1.1M Acetic acid

250ml of 5mg/ml FRTR solution

1M NaOH

40g in 1000ml =1M

40g of NaOH was weighed and 1000ml of 1M solution was prepared with distilled water.

1.1M Acetic acid

60g in 1000ml =1M $C_2H_4O_2$

66g in 1000ml =1.1M $C_2H_4O_2$

Density=mass/volume

Density($C_2H_4O_2$)=1.048-1.051

Density ($C_2H_4O_2$)=(1.048+1.051)/2

Density ($C_2H_4O_2$)=1.0495

Volume=mass/density

Volume=66g/1.0495

Volume=62.887ml

Volume =63ml

5mg/ml FRTR

5mg-----1ml

1.25g in 250ml

1.25g of FRTR powder was weighed and a 250ml solution was prepared.

APPENDIX III - UNIFORMITY OF WEIGHT OF ARTESUNATE TABLET BRANDS

AT 2

WEIGHT OF 20 TABLETS=6.0428g AVERAGE WEIGHT =6.0428/20 =0.3021

TABLET NUMBER	TABLET WEIGHT/g	DEVIATION	% DEVIATION
1	0.3015	0.0006	0.1986
2	0.3518	-0.0497	-16.4515
3	0.2970	0.0051	1.6882
4	0.3208	-0.0187	6.1900
5	0.2762	0.0259	8.5733
6	0.3030	-0.0009	-0.2979
7	0.2946	-0.0075	-2.4826
8	0.3017	0.0004	0.1324
9	0.3000	0.0021	0.6951
10	0.3071	-0.0050	-1.6551
11	0.2929	0.0092	3.0453
12	0.3053	-0.0032	-1.0593
13	0.2760	0.0261	8.6395
14	0.2735	0.0286	9.4671
15	0.3102	-0.0081	-2.6812
16	0.3103	-0.0082	-2.7143
17	0.3037	-0.0016	-0.5296
18	0.3076	-0.0055	-1.8206
19	0.3016	-0.0005	-0.1655
20	0.2998	0.0023	0.7613

APPENDIX IV -HARDNESS OF AMODIAQUINE HYDROCHLORIDE TABLET BRANDS

AM 2

AVERAGE = 12.6 KG SD ± 3.558

TABLET NUMBER	FORCE/KILOGRAMMES	FORCE/NEWTONS
1	7.0	68.6
2	10.0	98.0
3	17.2	168.6
4	12.4	121.5
5	9.8	96.0
6	16.2	158.8
7	12.2	119.6
8	15.0	147.0
9	9.4	92.1
10	16.8	164.6