ASSESSMENT OF THE QUALITY AND DISSOLUTION PROFILES OF BRANDED FIXED DOSE ARTEMETHER/LUMEFANTRINE TABLETS SOLD AT PHARMACIES IN KAMPALA - UGANDA

BY

KIDEGA FRANCIS KIMONG

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DECLARATION

I hereby declare that this research dissertation has not been submitted in full or part to any other institution for any purpose and that the views here are mine unless stated and where such has been the case, acknowledgement or reference has been quoted.

MR. KIDEGA FRANCIS KIMONG

RESEARHER: **SIGNATURE:** .....**DATE**..05../..05../..2012

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This research dissertation has been submitted to Kampala International University School of Pharmacy with my approval as the supervisor.

MR. SSENNYONJO F. HANNINGTON (MPS)

SUPERVISOR: **SIGNATURE:** .....**DATE**..07../..05../..2012

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DEDICATION

This work is dedicated to my mother, **Mrs. Hellen Oyoo** and my father, the late Dominic Oyoo (RIP). Your love for God and your children, and the sacrifices you made for our education have been a constant inspiration in good and bad times.

This work is dedicated with love and respect for both of you.

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LIST OF ABBREVIATIONS

ACT: Artemisinin based Combination Therapy

AGJ: Artificial Gastric Juice

AL: Artemether-Lumefantrine

BCS: Biopharmaceutical Classification System

BP: British Pharmacopoeia

DW: Distilled Water

FDA: Food and Drug Administration

HPLC: High Performance Liquid Chromatography

ICH: International Conference Harmonization

IP: International Pharmacopoeia

SALMOUS: Standards for Articles Legally Marketed Outside the United States

TLC: Thin Layer Chromatography

USP: United States Pharmacopoeia

WHO: World Health Organization

ABSTRACT

This study was done to evaluate the physicochemical properties, quality control parameters and the dissolution profiles of circulating samples of artemether/lumefantrine sold at Pharmacies in Kampala. The physicochemical parameters and assay of thirteen (13) brands of the products were assessed through the evaluation of uniformity of tablet weight, friability test, disintegration test and assay of active pharmaceutical ingredients according to established methods.

The dissolution rate was determined according to the USP SALMOUS Standard.

All brands complied with official requirements for uniformity of weight and friability.

The disintegration time had higher time in artificial gastric medium relative to distilled water.

Evaluation of content of active ingredients revealed that 38.5 percent of tested samples were failing test for assay.

With the exception of three brands, all the brands complied with the requirements for dissolution test.

Overall, 54 percent of the brands conformed to all the compendia specifications and 46 percent were substandard.

Future studies should test for a greater number of samples per batch, aim at comparing dissolution profile in various biological medium and investigate reasons for poor dissolution performance of some brands.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Tablets and capsules are prescribed widely and are very effective means of administering drugs to patients. A basic assumption is that when tablets are used by the patients, the drug from the tablet is released, dissolves, and is absorbed promptly and consistently. Drug product quality is needed for this to be a valid assumption.

In addition, many drugs are incompletely absorbed, due to factors relating to the drug, dosage form, and human physiology in the gastrointestinal tract. Optimal and consistent absorption of such drugs needs to be assured.

Bioavailability and bioequivalence become an important consideration in assuring optimal drug absorption. Changes in released characteristics of the drug from the dosage form can affect its bioavailability especially for modified released products.

Bioequivalence is an important consideration in several key situations involving batch to batch consistency, innovator to generic product therapeutic equivalence, and situations where formulation, manufacturing processes and dosage strength changes.

Bioequivalence testing is considered as the surrogate for the chemical evaluation of the therapeutic performance of drug products.

Pharmaceutically equivalent drug products are formulated to contain the same amount of active ingredient(s) in the same dosage form and meet all the existing physicochemical standards in official compendia or other applicable standards, but they may differ in characteristics such as shape, scorings, configuration, release mechanisms, packing, excipients, expiration time and within certain limits (FDA, 2003).

Pharmaceutical equivalence of drugs may be established by *in vitro* studies based on measurements intended to reflect the rate and extend to which the active pharmaceutical ingredient becomes available at the site of action. Based on the general consideration that *in vitro* drug dissolution test is predictive *in vivo* performance, *in vitro* drug dissolution test for

immediate release tablets and capsules are used among other things, to ensure conformity of drug products to official or set specifications.

Artemether and other artemisinin derivatives such as artesunate and dihydroartemisinin are potent drugs which have been widely used for the treatment of uncomplicated malaria. The use of any of arteminisin derivatives as monotherapy has however been discourage by the World Health Organization (WHO) to minimize development of resistance to these antimalarials. Instead WHO has since 2001 recommended the use of artemisinin based combination therapy (ACT) as first line malaria treatment in malaria endemic African countries.

In Uganda artemether/lumefantrine (AL) is one of the ACT of choice. This is because AL meets WHO prequalification criteria for efficacy, safety and quality. (Cousin M et al. 2008) Coartem® for example, a fixed dose combination of AL, has consistently achieved cure rates of more than 95 percent in clinical trials (Cousin M *et al.* 2008).

Hence ACT is the current standard of care for patients with uncomplicated falciparum malaria in Africa. Artemether is a semi synthetic polygenated amorphene containing a peroxide bridge that confers potent ether prodrug of dihydroartemisinin and a derivative of artemisinin (quiughaosu), the principal antimalarial constituent of Chinese herb Artemisia annua. Artemether is active against the erthrocytic stage of multi drug resistant strains of plasmodium *falciparum.* Lumefantrine (also called benflumetol, racemate of the dextrogyre and levogyre anantiomers) is a fluorine derivative (2, 3-benzindene) belongs to the amino alcohol class (Cousin M *et al.* 2008; WHO, 1990).

The compound was synthesized at the Academy of Military Medical Sciences, Beijing, China, and has undergone preliminary clinical trails in China. Lumefantrine, in combination with artemether (called coartemether), was registered for the oral treatment of malaria in China in 1987 (WHO, 1990). Lumefantrine inhibits metabolism of heam within the parasite acid food vacuole (WHO, 1990; Peter B, 2001).

The United States published a report from a study on the quality of anti-malarial drugs in Uganda and other African countries. The study was the first part of 10-country examination of anti-malarials in Africa by the United States and the World Health Organization. The report said that the most effective type of malaria fighting drugs sold in three African

countries including Uganda are often of poor quality therefore raising fears of increased drug resistance. Between 16 percent and 40 percent of artemisinin based drugs sold in Senegal, Madagascar and Uganda failed quality testing, for reasons including impurities or not containing enough active ingredients, the survey found. But Ugandan scientists have pointed out that drug resistance problem for malaria fighting drugs is of bad consumer behavior problem than the purity of drugs. The scientists urge that there are more pressing problems with malaria which kill at least 300 people per day mostly children under five years and pregnant women. National Drug Authority officials said they are dealing with counterfeits on the market but the laboratories are registering a downward trend in quality testing over the past decade. (Esther N, 2010)

1.2 STATEMENT OF THE PROBLEM

The proliferation of generics of fixed dose AL tablets in Uganda with variable prices is raising suspicion of difference in quality. In Uganda AL tablets is the commonest ACT product in the market and is available in various strengths from both local and foreign manufacturers. Drug quality is a source of great concern world wide, particularly in many developing countries. Use of the substandard and counterfeit drugs endangers lives and wastes scarce resources. It appears that poor quality of fixed dose AL tablets is linked to development of drug resistant strain of *Plasmodium falciparium* which causes malaria. Evidence abounds on the circulation of poor quality drugs in tropical areas of the world (WHO, 1990). Counterfeiting of drugs and circulation of unlicensed drugs may be a major concern in a land locked country like Uganda. The quality of these antimalarials if not properly safeguarded may lead to therapeutic failure in patients and the development of drugs resistance.

1.3 JUSTIFICATION OF THE STUDY

Studies done in Nigeria and Ghana have highlighted the quality and bioequivalence of some brands of AL tablets, artesunate tablets and other pharmaceuticals (Daniel A, 2010; Ogboma *et al*, 2011).

But there appear to be very little information on the quality of AL tablets published in Uganda. Lack of information on the quality of drugs may lead to serious health implications, waste scarce resources and contribute to drug resistance.

The benefit of having information on the quality and bioequivalent of drug products includes: generate data that will aid in drug policy making, promote consumer trust in the health system; help to strengthen the drug quality assurance system, strengthen law enforcement; enhance cooperation among stake holders; increase the availability of inexpensive, quality assured drugs and raise awareness of the problem of counterfeits or substandard drugs among health professionals and the consumers.

1.4 PURPOSE OF THE STUDY

The purpose of the study was to evaluate the quality control parameters and dissolution profiles of branded fixed dose AL tablets marketed in Kampala – Uganda.

1.5 SPECIFIC OBJECTIVES

i. To determine the physicochemical properties of branded AL 20/120mg tablets marketed in Kampala, Uganda.

ii. To assay branded AL 20/120mg tablets marketed in Kampala, Uganda.

iii. To determine the dissolution profile of branded AL 20/120mg tablets marketed in Kampala, Uganda.

1.6 SCOPE OF THE STUDY

This study focused on the quality of fixed dose AL 20/120 tablets available in community and wholesale pharmacies in Kampala, Uganda. The study also made insight into existence or otherwise substandard AL tablets in the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

International Conference Harmonization (ICH) Q8 (Lawrence X, 2008; FDA, 2006; Christine G, 2006) defines quality as "The suitability of either a drug substance or drug product for its intended use. This term includes such attributes as the identity, strength, and purity." ICH Q6A emphasizes the role of specifications stating that "Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities."

Woodcock (Lawrence X, 2008) defined a high quality drug product as a product free of contamination and reproducibly delivering the therapeutic benefit promised in the label to the consumer.

This definition of product quality focuses on the performance of the drug product while the ICH definition focuses on specifications. As Woodcock pointed out in her paper (Lawrence X, 2008) "this (ICH) definition can be considered correct to the extent that the quality attributes represent, and the quality system controls variability of, the parameters that are important for clinical performance."

Typical specifications for an immediate release oral solid dosage form, for example, include assay, uniformity, impurities, moisture, and dissolution.

2.1 Physicochemical properties of artemether/lumefantrine tablets.

In *vitro* testing or quality control of drugs is a set of studies or experiments undertaken during production and occasionally ought to be undertaken post-production by regulatory agencies and researchers.

Routine laboratory testing of drugs in the market is crucial to protect public health especially in developing countries where counterfeit and substandard drugs have become a major challenge to health care services (Ochekpe NA, et al 2006; N. C. Ngwuluka et al 2009).

Counterfeit and substandard medicines are a major cause of morbidity, mortality and loss of public confidence in drugs and health structures (Cockburn R *et al*, 2005).

For various products to pass a test carried out using the same test method and instruments but manufactured by different companies; it definitely has to go through the same manufacturing

processes. The basic processes of making of tablets consist mainly of three stages; granulation when the powder are converted to granules, compression when the granules are made into tablets, and some tablets are further coated with sugar coating, film coating or enteric coating (FDA, 2003; Opio S, 2008).

In tablet formulation development and during manufacture of tablets, a number of procedures are used to assess the quality of the tablets. Some test methods are described in pharmacopoeias and these are traditionally concerned with the content and the in *vitro* release of the active ingredient.

Test methods not described in pharmacopoeias are sometime referred to as non - compendial and concern with a variety of quality attributes that need to be evaluated, such as the porosity of tablets. Some of the tests used in the quality evaluation of tablets are;

2.1.1 Uniformity of content of active ingredient:

A fundamental quality attribute for all pharmaceutical preparation is the requirement for a constant dose of drug between individual tablets. Traditionally, uniformity of dose or dose variation between tablets is tested in two separate tests: uniformity of weight (mass) and uniformity of active ingredients.

In the case of potent drugs which are administered in low doses, the excipients form the greater part of the tablet weight and the correlation between tablet weight and amount of active ingredients can be poor. Thus the test for weight variation must be combined with a test for variation in drug dose, which makes the test useful as quality control procedure during tablets production (G. Alderborn, 2007).

2.1.2 Disintegration test:

The release process from tablets often includes a step at which the tablet disintegrates into smaller fragments. The test is carried out by agitating a given number of tablets in an aqueous medium at a defined temperature and the time to reach end point of test is recorded. Disintegration tests are useful for assessing the potential importance of formulation and process variables on the biopharmaceutical properties of the tablet and as a control procedure to evaluate the quality reproducibility of tablet during production (G. Alderborn, 2007; Edward M Rudric and Joseph B Schwartz, 2005).

2.1.3 Dissolution test:

Dissolution testing is the most important way to study, under *in vitro* conditions, the release of a drug from a solid dosage form and thus represents an important tool to assess factors that affect the bioavailability of a drug from a solid preparation.

During a dissolution test, the cumulative amount of drug that passes into solution is measured as a function of time. The test thus describes the overall rate of all the processes involved in the release of drug into a bioavailable form. Dissolution studies are carried out for several reasons:

- to evaluate the potential effect of formulation and process variables on the bioavailability of a drug,
- to ensure that preparations comply with product specifications, and
- to indicate the performance of the preparation under in vivo conditions (G. Alderborn, 2007; Edward M Rudric and Joseph B Schwartz, 2005).

2.1.4 Mechanical strength:

The mechanical strength is associated with the resistance of the solid specimen to fracturing and attrition. An acceptable tablet must remain intact during handling at all stages.

This test is carried out for the following reasons:

- to asses the importance of formulation and production variables for the resistance of a tablets to fracturing and attrition during formulation work,
- process design and scaling up; to control the quality of tablets during production, in-process and batch control; and
- to characterize the fundamental mechanism properties of materials used in tablets formulation.

The most commonly used methods for strength testing can be subcategorized into two main groups: attrition resistance methods (friability test) and fracture resistance methods (G. Alderborn, 2007; Edward M Rudric and Joseph B Schwartz, 2005).

A preliminary conclusion was reached by studies in Senegal, Madagascar and Uganda. It documented a sizeable proportion of samples of antimalarial medicines failing to meet quality tests. Forty four percent of samples in Senegal failed to meet specific standards. The corresponding failure rates in Madagascar and Uganda were thirty and twenty six percent respectively.

In Ghana, there was a report of fake Coartem tablets on the market. The fake drug found in Ghana did not contain the active pharmaceutical ingredients of the Novartis Coartem® product it was being sold as, posing a significant health threat to patients relying on the medication (Daniel A, 2010; Eurekalert, 2009).

Also, a study on the Quality of active ingredients in artemisinin-derivative antimalarials within Kenya and DR Congo gave the following findings; nine of the 24 drug samples analyzed did not comply with the pharmacopoeial requirements of 95–105 percent: seven samples were under dosed and two were slightly overdosed.
Dihydroartemisinin was the active ingredient in 57% of the under dosed samples. Arteether injections had the lowest drug content (77 percent). Two-thirds of the dry powder suspensions were either substandard or fake. Tablets were up to 23 percent out of range (Daniel A, 2010; Ogbonna *et al*, 2011

2.2 Assay on artemether and lumefantrine

As AL combination formulations have gained prominence in the treatment of malaria, one would expect that there would be several methods of assay for such formulations. However, there are very few documented methods for the assay of Artemether and Lumefantrine as individual products and also as combination formulations. In the tropical countries where malaria is endemic, it is important to ensure the quality of antimalarial drugs.
There are no monographs for both Artemether and Lumefantrine in both the B.P and the USP.
The international pharmacopoeia (IP) (IP, 2008) contains monographs of Artemether pure sample, the injection formulation as well as tablet and capsule formulations but no monographs on the combination formulation with Lumefantrine.
Monographs of Artemether tablets can be found in the USP SALMOUS standard. Monographs of Lumefantrine and its tablet formulations are available only in the USP

SALMOUS Standard and are now being drafted for inclusion in the IP. There are also a few published papers on the assay of Artemether and Lumefantrine.

According to USP SALMOUS standard, AL tablets should contain not less than 90 percent and not more than 110 percent of the labeled amount of artemether and of Lumefantrine (USP SALMOUS 2009).

2.3 In *Vitro* Bioavailability of AL tablets

To reduce the cost of medicines especially for low income group of developing countries, the WHO has continuously advocated the use of generic brands (WHO, 2004) but this approach has not provided sufficient evidence for the substitution of one brand for another. The difference in cost between brand and generic medicine may be as high as 90 percent.

To assist in substitution of branded with generics for affordability and at the same time achieve therapeutic efficacy, bioequivalence studies become paramount. Bioequivalence has been described as the absence of a significant difference in the rate and extend of a active ingredient or moiety in pharmaceutical equivalents or pharmaceutical alternatives become available at the site of action (that is, a significant difference in the bioavailability of the 2 drug products) when they are administered at the same molar dose under similar conditions in an appropriately designed study (FDA, 2003; Opio S, 2008; Cousin M *et al* 2008).

Two pharmaceutical products are considered to be equivalent when their bioavailability factors (from the same molar dose) are so similar that they are unlikely to produce clinically relevant differences in therapeutic and/or adverse effects (N. C. Ngwuluka *et al*, 2009).

Generic substitution could be considered when a generic copy of a reference drug contains identical amounts of the same active ingredient in the same dose formulation and route of administration as well as meet standards for strength, purity, quality and identity. However evidences over the years indicate that marketed products with the same amount of active ingredient exhibit marked differences in their therapeutic responses (N. C. Ngwuluka *et al*, 2009).

This may be due to the extent of absorption being dissimilar; perhaps due to different excipients employed. Bioequivalence studies focus on the release of drug from the formulation and subsequent absorption into the system's circulation. Bioequivalence studies may involve both *in vivo* and *in vitro* studies.

However, with the introduction of biopharmaceutical classification system (BCS), *in vivo* bioequivalence studies could be waived for immediate release solid oral dosage forms for classes I (high solubility and permeability) and III drugs (high solubility and low permeability) (N. C. Ngwuluka *et al*, 2009; FDA, 2000).

Hence only *in vitro* testing may be used to determine bioequivalence for highly soluble and highly permeable drugs (N. C. Ngwuluka *et al*, 2009).

Dissolution testing, a surrogate marker for bioequivalence test is indeed a practical and economic approach in developing countries where technology and resources are limited for *in vivo* studies. One of the values of dissolution test is that it can be used to identify bioavailability problems and assess the need for *in vivo* bio-availability (N. C. Ngwuluka *et al*, 2009).

The release of active pharmaceutical ingredient from drug product, the dissolution of the drug under physiological conditions and the permeability across the gastrointestinal tract determines the drug absorption. Based on this, *in vitro* dissolution may be vital in assessing *in vivo* performance. Dissolution testing also serves as a tool to distinguish between acceptable and unacceptable drug products (N. C. Ngwuluka *et al*, 2009).

However current dissolution acceptance limits are selected based on data from a small number of batches in the context of their ability to distinguish batches with limited regard to clinical relevance. Under the Quality by Design, the dissolution tests should be developed to reflect in vivo performance as much as possible.

For example, the acceptance criteria for BCS Class I and III IR tablets may be much wider than that from batch data because, for these BCS classes, dissolution is highly unlikely to be the rate limiting step in vivo. Similarly, dissolution tests for BCS Class II and IV drugs may need to be carefully examined to better reflect in vivo dissolution (Lawrence X, 2008).

The USP SALAMOUS Standard requires not less than 45 percent of labeled amount of artemether to dissolve in 1 hour and not less than 65 percent of the labeled amount of artemether to dissolve in 3 hours and not less than 60 percent of the labeled amount of lumefantrine to dissolve in 45 minutes (USP SALMOUS 2009).

2.4 AL tablets and it's Quality

The combination AL tablet is an ACT indicated for the treatment of acute uncomplicated *Plasmodium falciparum* malaria. Artemether is a derivative of artemisinin, and lumefantrine (or benflumetol) is an antimalarial drug. Coartem is an effective and well-tolerated malaria treatment, providing cure rates of up to 97%, even in areas of multi-drug resistance.

In 2001, Coartem became the first fixed dose artemisinin-based combination therapy to meet the World Health Organization's (WHO) pre-qualification criteria for efficacy, safety and quality. In 2002, AL tablets were added to the WHO's Essential Medicines list, an index of essential drugs which help guide the purchasing decisions of Member States and UN agencies (Essential Medicines, 2005; WHO, 2008).

There are several reports of sub-standard and counterfeit antimalarial drugs circulating in the markets of developing countries. A review of literature published recently on the quality of antimalarial drugs indicated that:

(i) most antimalarial products pass the basic tests for pharmaceutical dosage forms, such as the uniformity of weight for tablets,

(ii) most antimalarial drugs pass the content test and

(iii) in *vitro* product dissolution is the main problem area where most drugs fail to meet required pharmacopoeial specifications, especially with regard to sulfadoxine-pyrimethamine products (Amin *et al*, 2007; Daniel A, 2010).

A study was done to assess the intra batch quality control and bioavailability variability of branded artesunate tablets in Nigeria. It concluded that there were variations in the hardness, absolute drug content, dissolution and drug release profiles of the five selected batches. Thus, none of the batches fully met the BP requirement for tablet dosage form (Ogbonna O *et al*, 2011). The study expressed concern that this inadequate and variable bioavailability in bio-phase; could consequently lead to therapeutic failure and emergence of artesunate resistant strain of *P. falciparum*.

A similar study conducted in Kumasi, Ghana found out that there was sub-standard quality of artesunate tablets (K Oforie *et al*, 2008).

2.5 Malaria and the burdens.

About 14-17 million people die each year of infectious diseases and nearly all live in developing countries. Malaria, a vector borne infectious disease caused by the protozoan parasite *Plasmodium falciparum*, is wide spread in tropical and subtropical region; and has an incidence of about 515 million cases annually, killing 1-3 million people majority of whom are children and pregnant women in sub Saharan Africa. The control of malaria has been severely hampered by a persistent increase in the prevalence of drug resistance malaria parasites (Ogbonna O *et al*, 2011).

Antimalarial drug resistance has now become a serious global challenge and it is the principal reason for the decline in antimalarial drug efficacy. Several drugs are effective but the emergence of parasite resistance limits the choice in various parts of the world. Resistance to **mefloquine** and even to **quinine** has been reported in Southeast Asia (Daniel A, 2010).
Malaria endemic countries, which are mostly poor, need inexpensive and efficacious drugs. To counter the threat of resistance of *P. falciparum* to monotherapies, and to improve treatment outcome, combinations of antimalarials are now recommended by the WHO for the treatment of falciparum malaria. The most important of the combination of antimalarials are the ACTs which combine artemisinin based antimalarials with other antimalarials such as the aryl amino alcohol anitmalarials. Artemisinin-based combinations offer a new and potentially highly effective way to counter drug resistance (Daniel A, 2010; Karbwang *et al*, 1997). Currently, the WHO recommends the following ACTs in the treatment of uncomplicated malaria: Artemether + Lumefantrine; Artesunate + Amodiaquine; Artesunate + Mefloquine; Artesunate + Sulfadoxine–Pyrimethamine.

Recently, partial artemisinin-resistant *P. falciparum* malaria has emerged on the Cambodia–Thailand border. Exposure of the parasite population to artemisinin monotherapies in sub therapeutic doses for over 30 years, and the availability of substandard artemisinins, have probably been the main driving force in the selection of the resistant phenotype in the region (Daniel A, 2010).

2.6 Causes of poor quality drugs in the consumer market.

Recent reports in Asian countries indicate that the availability of substandard and counterfeit drugs has reached a disturbing proportion in resource-poor settings. The rising cost of drugs

generally may create a corresponding increase in incentive to produce counterfeit drugs because of bigger profit margins. According to available literature, some of the possible contributing factors to poor quality of drugs include (USP, 2004);

2.6.1 Weak national drug regulatory authority and weak enforcement of drug laws:

The regulatory authority may have limited capacity to function due to inadequate resources for drug regulation activities and training of personnel.

Although drug laws exist, they may be rarely or weakly enforced. Implementation of activities is difficult due to budget constraints and inadequate staff to monitor the problem.

Lack of budget often leads to corruption, which could affect enforcement of law by failure to arrest and punish counterfeiters.

2.6.2 Limited laboratory capacity in terms of qualified staff and equipment:

The capacity of some laboratories to conduct quality testing of drugs may be limited due to lack of qualified personnel and testing equipment.

2.6.3 Lack of competent drug inspectors:

Regular inspection may be rarely implemented due to lack of competent and adequately trained inspectors. Training on GMP and Pharmacy Practice including storage conditions is necessary.

Lack of inspectors means less control of pharmaceuticals at points of entry. In Cambodia, for example, counterfeit and substandard drugs are more of a cross-border problem than a local problem. In Vietnam, no inspection is done at customs warehouses.

2.6.4 Lack of inexpensive, quality-assured drugs:

Limited availability of low-cost genuine drugs may help promote counterfeiting.

2.7.0 Research questions

2.7.1 How can a patient know if buying a cheaper brand of AL tablets would be cost effective or not?

2.7.2 Can branded AL tablets drugs be used interchangeably?

2.7.3 Could it be that substandard AL tablets are a contributing factor to development of resistant traits of *Plasmodium falciparium*?

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Design

The study was a cross sectional experiment which involved pharmacopoeias and non-compendial tests. Quality attributes was described quantitatively.

3.2 Setting of the Study

The experiment was conducted at the National Drug Control Laboratory (NDCL).

3.3 Selection of Study Samples

AL tablets of different brands having strength ratio of 20:120 was selected for the study.

3.4.0 Sample Size

For convenience reasons, 240 tablets of AL tablets from every brand were included in the study. These tablets were purchased from drug outlets in Kampala, Uganda. This various brands of AL tablets were coded with letters (A, B, C, D and so on).

Artemether and Lumefantrine reference powder were provided by National Drug Quality Control Laboratory and used as they were. All the drugs were stored in their original pack under condition specified by the manufacturer prior to experimental manipulations.

3.4.1 Inclusion Criteria

Only AL tablets manufactured at least six months before the study were included in the study.

3.4.2 Exclusion Criteria

AL tablets labeled "not for sale" were not included in the study. Unlicensed brands of AL tablets were not included in the study.

3.5 Sampling technique

The AL tablets that were used for each experiment were selected randomly from the purchased AL tablets.

3.6 Data Collection Methods

3.6.1 Materials

Reagents were purchased from local suppliers in Kampala, Uganda and standardized in the laboratory.

Reagents:

a) Artemether reference standard

b) Lumefantrine reference standard

c) Hexane sulphonic acid sodium salt

d) Sodium dihydrogen phosphate monohydrate

e) Filtered distilled water (membrance filter 0.5 micrometer)

f) 85 % w/v ortophosphoric acid

g) Acetonitrile (HPLC GRADE)

h) Propanol (HPLC GRADE)

Apparatus:

a) Analytical balance

b) Ph meter

c) HPLC system

d) Magnetic stirrer

e) Centrifuge

f) Vacuum filtration system

g) Glassware (50ml, 25ml, & 100ml volumetric flaks, conical flaks funnels, watman filter paper no. 1)

h) Ultraviolet spectrophotometer systems

3.6.2 Preparation artificial gastric juice

Artificial gastric juice was prepared according to British Pharmacopoeia 2011 (British Pharmacopoeia 2011) **Refer to the procedures in Appendix Three.**

3.6.3 Visual Inspection

The shape, size and color of the different brands of tablets were examined visually. Search for the brand name, the manufacture, batch number and country of origin were done and the findings recorded.

3.6.4 Determination of Uniformity of weight

Twenty tablets from each of the brands were weight individuality with an analytical weighing balance. The average weight for each tablet from each brand as well as the percentage deviation from mean values was calculated using a scientific calculator. **Refer to the procedures in Appendix one.**

3.6.5 Friability test

Twenty tablets from each brand were weighted and subjected to abrasion using a tablets friability tester at 25 revolutions per minute. The tablets were then weighted and compared with their initial weight and percentage friability was calculated. **Refer to the procedures in Appendix two.**

3.6.6 Disintegration test

The disintegration time of randomly selected tablet of each brand was determined in distilled water and artificial gastric juice using a disintegration time test apparatus set at 50 rpm at 37°C. Artificial gastric juice was made according to the British Pharmacopoeia 2011 monogram. The time for the last tablet to break up into small aggregates was noted as the disintegration time.

3.6.7 Thickness and diameter test

Twenty tablets were selected from each brand and micrometer screw gauge was used to measure the thickness and diameter of each tablets. The mean values as well as standard deviations and coefficient of variation were evaluated for each of the brand.

3.6.8 Dissolution rate determination

The release of artemether and lumefantrine was determined in1000 milliliter of partially degassed water and 1000 milliliter of 0.1 M hydrochloric acid containing 1% of benzalkonium chloride. Dissolution test were performed according to USP SALMOUS 2009 standards. **Refer to the procedures in Appendix Four and Five.**

3.6.9 Content of active ingredient test

Tablets content were determined as described in the USP SALMOUS edition. **Refer to procedure in appendix six**

3.7 Ethical Consideration

An introductory letter was obtained from the Kampala International University School of Pharmacy to introduce the researcher to the relevant authorities; permission was also sought from the Director of National Drug Authority Control Laboratory (NDACL). Brands of AL tablets were coded with capital letters and there was maximum level of confidentially of data collected.

3.8 Limitation of the study

There was no assurance that tested brands were bioequivalent just because they pass the dissolution test. There was no assurance that the used apparatus complied with those adopted by the pharmacopoeias.

3.9 Statistical Analysis

The results of all the above experiment were expressed as mean ± standard deviation. Calculations were made using a scientific calculator.

3.10 Pretest

Data collection methods and tools were pretested at NDACL a week before the actual experiment. The purpose of the pretest was to test the effectiveness of the methods so that adjustment can be made

CHAPTER FOUR

4.0 RESULTS

4.1 Samples of AL Tablets

Table 1: Brands of AL tablets used (20 mg of artemether and 120 mg of lumefantrine).

Code	Brand name	Dosage form	Manufacturer	Country of origin	NDA registration number	Batch number	Price
A	LUMETHER	Tablet	Astra Lifecare (India) Pvt Ltd	India	7113/06/10	001	3000
B	Artefan	Tablet	Ajanta Pharma Limited	India	6408/06/08	P0311H	1100
C	Artrin®	Tablet	Medreich Limited	India	6516/06/09	600046	5000
D	Coartem®	Tablet	New York Novartis Pharmaceutical	U.S.A	4839/06/05	F2422	5000
E	LUMARTEM	Tablet	Cipla Ltd	India	5275/06/05	FD00751	900
F	Coartem®	Tablet	Beijing Novartis Pharmaceutical	China	4839/06/05	X1461	30000
G	Lonart	Tablet	BLISS GVS PHARMA LTD	India	5905/06/07	LN-438	5000
H	Cachet-ART	Tablet	Cachet Pharmaceutical PVT Ltd	India	6960/06/10	CHRT/0002E	1500
I	Fantem	Tablet	The Madras Pharmaceuticals	India	6830/06/10	M100393	1500
J	Laritem	Tablets	Ipca Laboratories	India	7371/06/11	BUQ004F	1000
K	CO-METHER	Tablet	Agog Pharma Ltd	India	6009/06/07	T0601	1000
L	Lumaren	Tablet	Rene Industries Limited	Uganda	7187/06/10	02510	1000
M	Amatem	Tablet	Micro Lab Limited	India	_____	AMMH0033	5000

4.2 PHYSIOCOCHEMICAL PROPERTIES OF BRANDED AL TABLETS

Table 2; Disintegration time, uniformity of weight and friability of the sampled tablets.

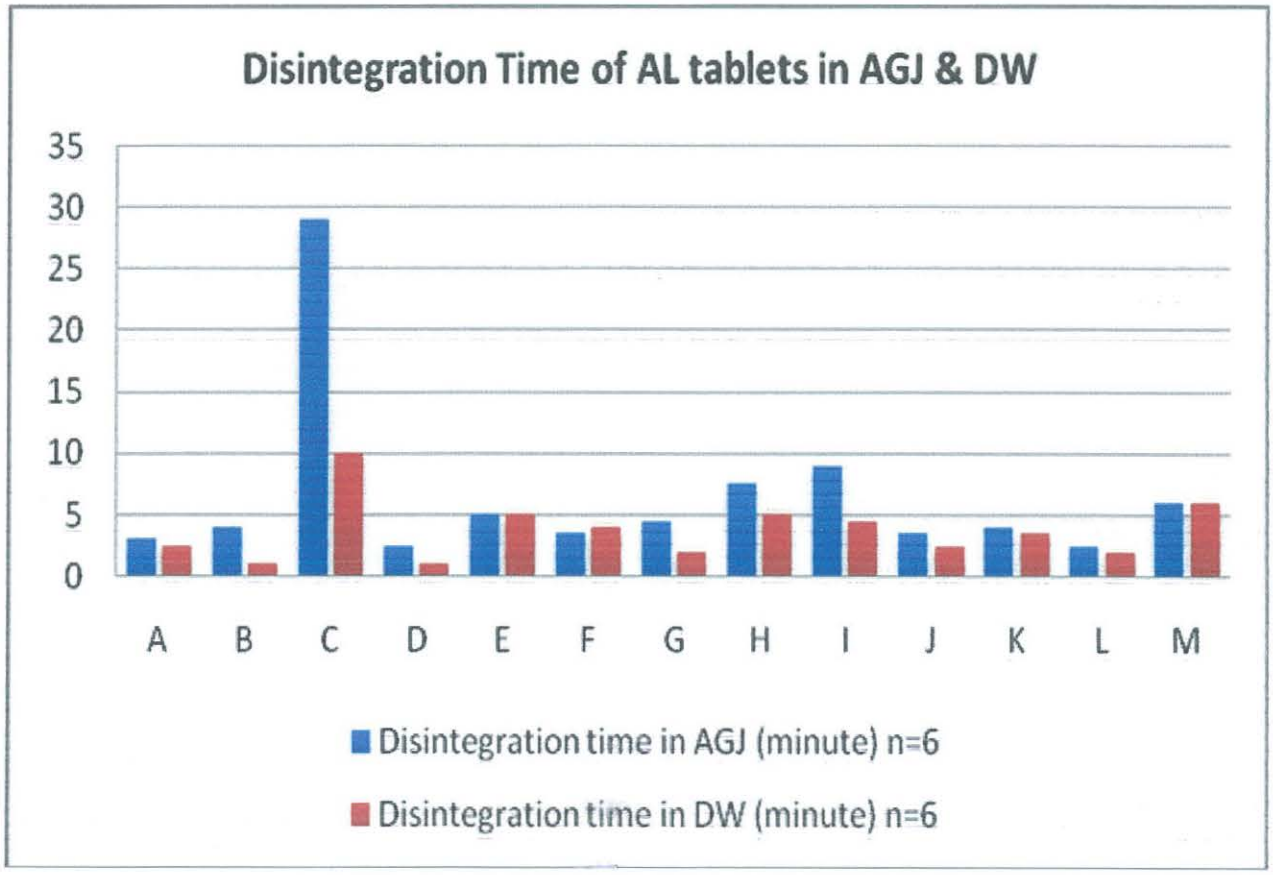
Brand	Tablet Thickness (mm), n=20	Tablet Diameter (mm), n=20	Uniformity of weight (gram), n=20	Friability (% w/w), n=20
A	3.78±0.048476	9.737575±0.034911	0.314172±0.006417	0.27127
B	3.225±0.073314	9.1357±0.027355	0.244113±0.00355	0.42297
C	3.24±0.0406202	9.1625±0.0311247	0.243275±0.00426	0.13098
D	3.0825±0.02385	9.0924±0.017817	0.241398±0.001863	0.41617
E	3.5225±0.029475	10.13±0.024495	0.350148±0.004688	0.02813
F	3.1175±0.0238485	9.0625±0.021651	0.242934±0.001094	0.05545
G	4.105±0.942324	10.335±0.035707	0.33918±0.006018	0.02630
H	4.878952±0.049282	9.54752±0.022066	0.334553±0.005323	0.32320
I	3.27415±0.0256696	9.61915±0.043057	0.283011±0.001997	0.26791
J	3.175±0.025	9.12±0.024495	0.246571±0.004771	0.21298
K	3.2225±0.024875	9.6225±0.024875	0.295866±0.001934	0.16340
L	3.425±0.07500	9.2375±0.086422	0.256006±0.005539	0.42915
M	3.705±0.015	9.6125±0.02165	0.330403±0.005154	0.27104

Table 3: Result of Disintegration test of AL tablets in Artificial Gastric Juice and Distilled water

Brand	A	B	C	D	E	F	G	H	I	J	K	L	M
Y (minute) n=6	3	4	29	2.5	5	3.5	4.5	7.5	9	3.5	4	2.5	6
Z (minute) n=6	2.5	1	10	1	5	4	2	5	4.5	2.5	3.5	2	6

Y represents average Disintegration time in Artificial Gastric Juice (AGJ) and Z represents average Disintegration time in Distilled Water (DW).

Figure 1: Graphical Representation of Tablet Disintegration test in Artificial Gastric Juice & Distilled water



4.3 ASSAY OF AL TABLETS

Table 4: Results showing percentage w/w of Artemether & Lumefantrine in a tablet

Brand	A	B	C	D	E	F	G	H	I	J	K	L	M
O (%w/w)	90	90	82	110	121	93	97	100	88	97	89	98	83
P (%w/w)	96	100	65	103	100	96	95	96	86	95	92	94	85

O represents results of artemether assay

P represents results of Lumefantrine assay.

4.4 RELEASE PROPERTIES OF AL TABLETS

Table 5: Result for Dissolution tests for lumefantrine and artemether

Brand	Average Percentage of Lumefantrine Released % (w/v), N=6	Average Percentage of Artemether released at 1st hour (% w/v), N=6	Average Percentage of Artemether released 3rd hour (% w/v)N=6
A	81.587±6.0911	81.623±3.7013	88.4513±7.2532
B	81.687±2.752	71.02±5.3362	84.662±1.54788
C	16.331±0.833	60.227±10.7351	78.4±8.0935
D	83.447±4.9117	86.1±7.52408	93.4±8.72518
E	90.66±4.3539	51.64±1.5244	74.13±3.254
F	92.75±12.91697	69.638±11.9178	88.236±7.81329
G	101.8867±4.7073	90.65±1.5021	92.92±2.641
H	64.075±2.2383	70.56±2.5687	88.7560±7.428
I	53.3683±2.5503	65.125±3.427	91.364±5.487
J	90.2683±6.1865	80.023±4.084	89.91±3.701
K	75.3567±3.509	72.009±6.7084	93.064±4.214
L	43.1656±3.9927	79.457±7.245	90.014±6.451
M	85.5667±1.4781	76.512±5.412	89.457±0.478

Figure 2: Graphical Representation of Artemether Dissolution in % (w/v)

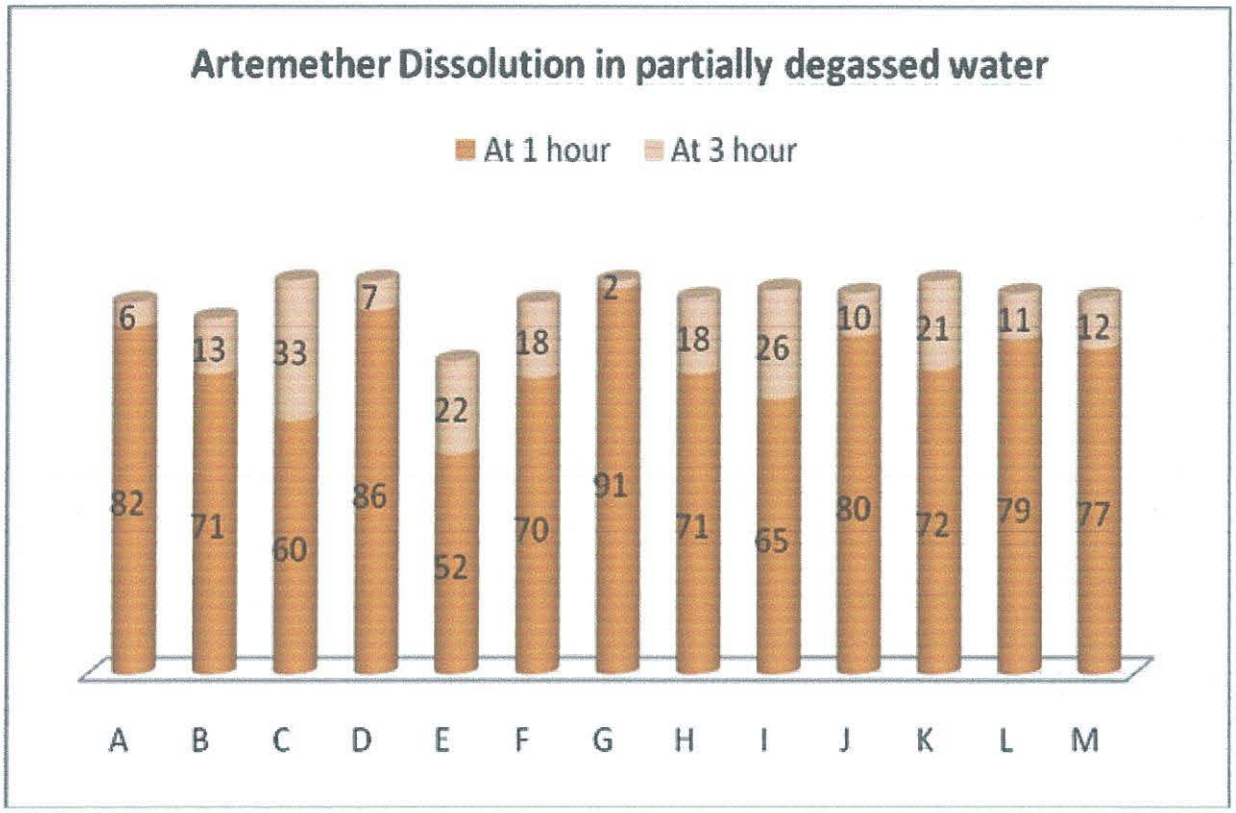
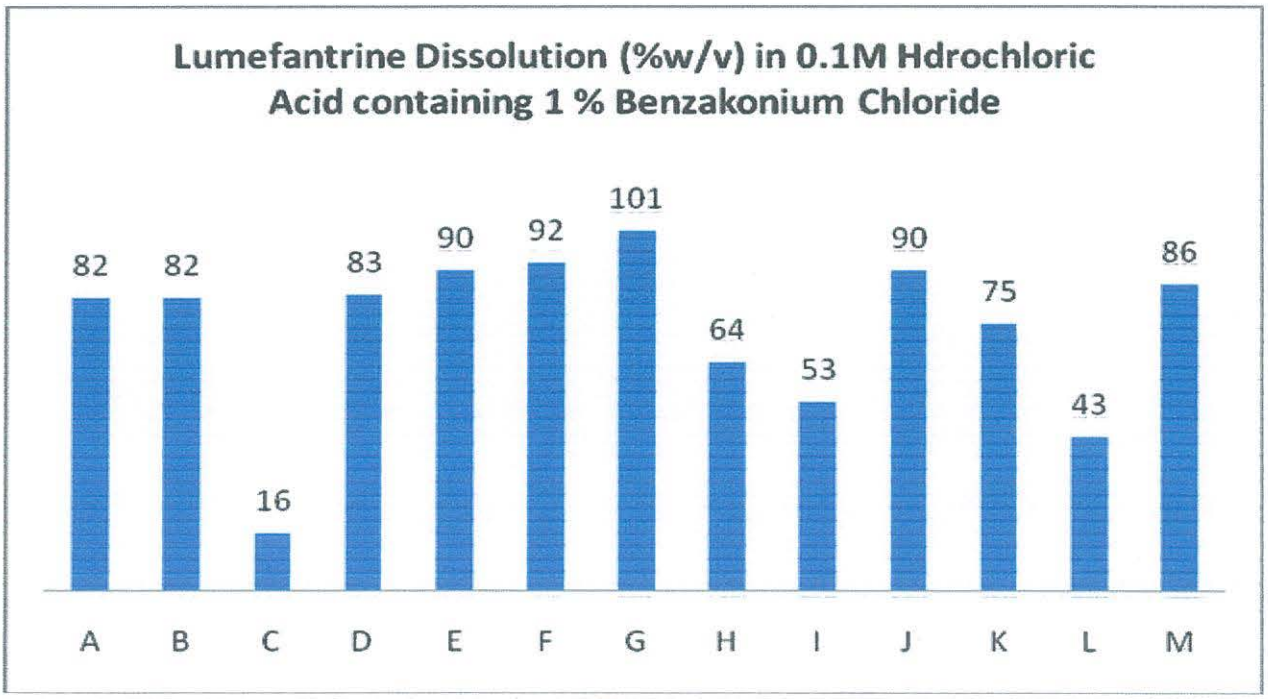


Figure 3: Graphical Representation of Lumefantrine Dissolution.



CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Physicochemical properties of brands AL 20/120 mg tablets

A summary of the results of tablets thickness, tablets diameter, uniformity of weight and friability are shown in table 2. Results of disintegration test in artificial gastric juice and distilled water are shown in table 3.

Most of the tablets passed appropriate mechanical test in terms of weight uniformity, friability and disintegration time in both artificial gastric juice and distilled water. The different brands of AL tablets showed acceptance weight uniformity with weight deviation of less than 10 percent w/w. The compendia require not more than 7.5 percent deviation for tablets weighing not less than 80 mg and not more than 250 mg. Uniformity of weight does serve as a pointer to good manufacturing practice as well as amount of the active pharmaceutical ingredients artemether and lumefantrine contained in the preparation.

The friability of all the AL brands was less than 1 percent w/w and the disintegration times were less than 15 minutes except for brand C which took 36 minutes to disintegrate in artificial gastric juice.

It could be inferred that all the AL brands studied could disintegrate readily in aqueous medium especially in the gastrointestinal tract except brand C.

The disintegration time had higher time in artificial gastric medium relative to distilled water.

Disintegration of tablet into primary particles is thus important, as it ensures that a large effective surface area of a drug is generated in order to facilitate dissolution and subsequent absorption.

However, simply because tablets disintegrate rapidly does not necessarily guarantee that the liberated primary drug particles in the gastrointestinal fluids and the extent of absorption are adequate. (M. Ashford 2007)

All the brands of AL tablet passed the friability test. It could thus be inferred that all the AL brands studied could withstand abrasion without loss of tablet integrity.

5.2 Assay of Artemether and Lumefantrine

According to USP SALAMOUS standard, AL tablets should contain not less than 90 percent and not more than 110 percent of the labeled amount of artemether and of Lumefantrine (USP SALMOUS 2009).

While brands **A, B. D, F, G, H, J** and **L** complied with the USP SALAMOUS specification for assay, brands **C, E, I, K, and M** did not meet USP SALAMOUS specification.

Therefore 38.5 percent of tested sample were failing test for assay.

Low quantity of artemether and Lumefantrine in a tablet may lead to therapeutic failure in patients and the development of drug resistance.

High quantity may contribute to increased drug toxicity.

A material may well fall within the assay limits stated in the individual monograph to a particular substance, and yet not be of suitable quality to conform to the complete specifications indicated for the compound, even though a substance meets the purity specifications of an official monograph, as established by a chemical or physical assay procedure, it is not of USP quality unless it conform to all of the specifications contained in the monograph for that material.

5.3 Dissolution profile of AL tablets

The USP SALAMOUS Standard requires not less than 45 percent of labeled amount of artemether to dissolve in 1 hour and not less than 65 percent of the labeled amount of artemether to dissolve in 3 hours and not less than 60 percent of the labeled amount of lumefantrine to dissolve in 45 minutes.

All the brands complied with these requirements except brand **C, I and L** as shown in **table 5**. Artemether / Lumefantrine have been classified as class 4/3 API according to the Biopharmaceutical Classification system. This implies that AL bioavailability may be dissolution rate limited.

The release rates and extent of absorption of active ingredients depend largely on the excipients and minute details of other physicochemical properties of both excipients and drug substance.

Factors that can influence the dissolution rate of drugs are particle size, the wettability, the solubility and the drug crystalline or amorphous form.

Different manufacturers are likely to adopt different methods during their tableting stages which will ultimately affect the dissolution characteristics of their product.

Multisource drugs may have varying performance due to the results of their dissolution behaviors. Good manufacturing practices therefore involves a critical analysis of the various key factors as to control the various key factors as to control the overall outcome of dissolution.

5.3 Conclusion

In this study, brand **A, B, D, F, G, J**, and **L** representing **54 percent** of studied brands conformed to all the compendia specifications evaluated.

On the other hand brand **C, L, E, I** and **K** representing **46 percent** of total brands studied were substandard because they did not conform to all the compendia specifications evaluated.

5.4 Recommendation

i. Future studies should test for a greater number of samples per batch if adherence to good pharmaceutical manufacture, distribution and trading practice is to be assured.

ii. The designed of dissolution studies should aim at comparing the similarity factor, difference factor and dissolution efficiencies so that possibility of interchangeably with other brands are established.

iii. Dissolution studies should be conducted in mediums which mimic in vivo condition conditions (for example in artificial gastric medium and simulated intestinal fluid).

iv. Reason(s) for poor dissolution and low or high content of artemether and lumefantrine should be investigated.

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APPENDIX 1

PROCEDURE FOR DETERMINATION OF UNIFORMITY OF MASS

a) Select 20 tablets randomly from the sample population of the brand.

b) Accurately weigh each tablet individually using a calibrated analytical balance.

c) Calculate the total values (Total mass) of the 20 tablets.

d) Calculate and record the mean weight using the formula: **Mean weight = Total weigh (mass) divide by 20.**

e) Calculate the different between the smallest mass and the largest mass.

f) Calculate and record the standard deviation.

APPENDIX 2

DETERMINATION OF FRIABILITY OF UNCOATED TABLETS:

Friability conditions;

Speed of rotation: **100 rpm**

Test time: **1 minute**

Number of tablet tested: **20 tablets**

Procedure:

a) Dedust 10 tablets,

b) Weigh using a calibrated analytical balance. Record the weight as A_1.

c) Clean the tablet friability apparatus.

d) Place in the drum.

e) Rotate the drum 100 times for 1 minute.

f) Remove the tablet

g) Remove any loose dust from the tablet.

h) Accurately weigh. Record the weight as A_2.

i) Calculate the percentage friability from the formula;

Percentage friability = $(A_1 - A_2) \times 100 / A_1$

Result specification: A maximum loss of mass obtained from a single test from the mean of three tests not greater than 1 percent is considered acceptable for most products.

APPENDIX 3

PREPARATION OF ARTIFICIAL GASTRIC JUICE

a) Dissolve 2 gram of sodium chloride and 3.2 gram of Pepsin powder in water.

b) Add 80 milliliter of 1 M Hydrochloric acid

c) Dilute to 1000 milliners with distilled water

APPENDIX 4

DETERMINATION OF DISSOLUTION OF ARTEMETHER:

Medium: water, partially degassed (6.5 to7 mg of O_2/L); 1000 milliliters

Apparatus 2: 100 rpm

Time: 1 and 3 hours.

Mobile Phase: Prepare a filtered and degassed mixture of acetonitrile, water, 1-propanol, and trifluoroacetic acid (500 : 400 : 100). Make adjustments if necessary.

Diluent: a mixture of water and acetonitrile (1 : 1).

Standard stock solution: Prepare a dilution in Diluent containing about 0.2 mg per ml USP Artemether RS.

Standard solution: Prepare a dilution of standard stock solution in Medium having a final concentration of about 0.02 mg per ml. Break down procedure is as below,

Weigh accurately 20mg of artemether reference standard into a 100ml volumetric flask.

a) Add about 70ml of dissolution medium, sonicate for about 30 minutes to dissolve, cool to 20 °C.

b) Top up to the mark, in dissolution medium.

c) Dilute 1ml to 10ml with dissolution medium.

Test solution: Pass a portion of test solution under test through a suitable 0.45 micrometer filter. Dilute quantitatively, and stepwise, if necessary with medium.

Chromatographic system: The liquid chromatography is equipped with a 210 nanometer detector and a 4 nanometer detector and a 4 micrometer x 12.5 centimeter column that contains a packing 5 micrometer L1. The flow rate is 1 milliliter per minute.

Chromatograph the standard solution, and record the peak response as directed for Procedure: the tailing factor is not more than 2, and the relative standard deviation for replicate injection is not more than 2.5%.

Procedure: Separately inject equal volumes (about 10 microliter) of the Standard solution and the test solution in to the chromatograph, record the chromatograms, and measure the peak response.

Calculate the percentage of artemether dissolved by the formula:

$(R_u \times C_s \times 100) / (R_s \times C_u)$ where,

R_u is the peak response obtained from the test solution,

R_s ist the peak response obtained fro the standard solution,

Cs is the concentration of standard solution, and

C_u is the concentration of the test solution

C_s = (actual weight of artemether RS x 1) / (100 x 10)

C_u = (Label claim for artemether / 1000 ml)

Tolerance: Not less than 45 % (Q) of the labeled amount of artemether is dissolved in 1 hour, and not less than 65% (Q) of the labeled amount of artemether is dissolved in 3 hours.

APPENDIX 5

DETERMINATION OF DISSOLUTION OF LUMEFANTRINE:

Medium: 0.1 M hydrochloric acid containing 1 % of benzakonium chloride; 1000 ml.

Apparatus 2: 100 rpm. **Time:** 45 minutes.

Standard solution: Dissolve an accurately weighed quantity of USP Lumefantrine RS in Medium, and dilute quantitatively, and stepwise, if necessary, with Medium to obtain a solution having a concentration of about 0.72 mg per ml. Break down procedure is as below,

a) Weigh accurately 12mg of Lumefantrine reference standard into a 100ml volumetric flask.

b) Add 70ml of dissolution media, sonicate for about 30 minutes to dissolve, cool to 20 °C.

c) Top up to the make, in dissolution media and filter through a Whatman filter paper No. 1.

d) Dilute 5ml to 25ml with dissolution media.

Test solution: Pass a portion of the solution under test through a suitable 0.5 micrometer filter. Quantitatively dilute with Medium. (Dilute 5ml of the test solution to 25ml with dissolution media).

Procedure: Determine the percentage of lumefantrine dissolved by employing UV absorption at the wavelength at about 342 nm, on solution in comparison with the standard solution, using 0.2 cm cells and Medium as the blank. Calculate the percentage of lumefantrine dissolved by the formula:

$(A_u \times C_s \times 100) / (A_s \times C_u)$ where,

A_u is the absorbance of test solution,

A_s is the absorbance of standard solution,

C_u is the concentration of test solution,

C_s is the concentration of standard solution, and

100 is conversion factor to percentage.

C_u = (Label claim for lumefantrine x 5) / (1000 x 25)

C_s = (actual weight of lumefantrin RS x 5) (100 x 25)

Tolerance: Not less than 60% (Q) of the labeled amount of lumefantrine is dissolved in 45 minutes

APPENDIX 6

PROCEEDURE FOR THE ASSAY OF ARTEMETHER AND LUMEFANTRINE:

Ion – pairing solution: Prepare a mixture of 5.65 g of sodium 1-hexanesulfonate and 2.75 g of monobasic sodium phosphate in 800 ml of water. Adjust the PH to 2.3 using phosphoric acids, dilute with water to 1000 mland filter.

Solvent: Prepare a mixture of 100 ml of ion-pairing solution, 100 ml of 1-propanol, and 30 ml of water, and dilute with acetonitrile to 500 ml.

Solution A: Prepare a mixture of Ion-pairing solution and acetonitrile (7:3).

Solution B: Prepare a mixture of acetonitrile and Ion-pairing solution (7:3).

Mobile phase: Use variable mixture of solution A and solution B as directed for Chromatographic system. Make adjustment if necessary.

Standard preparation: Dissolve an accurately weighed quantity of USP Artemether RS and USP Lumefantrine RS in solvent to obtain a solution having known concentration. The procedure for preparation of standard is as below,

a) Weigh approximately 25 mg of Artemether reference standard into a 25 ml volumetric flask, add about 15 ml of solvent mixture and sonicate for 15 minutes then cool to about 20°C and top up to the mark.

b) The concentration of this solution is about 1 mg per ml.

c) Weigh approximately 30 mg of lumefantrine reference standard in to a 50 ml volumetric flask, add 40 ml solvent mixture and sonicate for 15 minutes.

d) Cool to about 20°C and top up to the mark.

e) Pipette 1 ml of the stock solution of the standard Lumefantrine solution in to a 25 ml volumetric flask and top to the mark with the solvent mixture in to a 25 ml volumetric flask to make a concentration of about 0.024 mg per ml.

Chromatographic conditions:

Injection volume: 20 microliter.

Wave length: 210 nanometer for artemether & 265 nanometer for Lumefantrine.

Temperature: 30°C

Mobile Phase; 30:70 of Hexane sufonic acid Buffer (Ion-pairing solution): Acetonitrile HPLC GRADE.

Column: Symmetry C 18 5 micrometer; 4.6 x 250 nanometer.

Flow rate: 1.5 ml per minute.

System suitability Determination:

Using the above standard solution, inject 20 microliter of the same solution at least 5 times and record the chromatograms. The system is suitable if the relative standard deviation does not exceed 2.5 percent.

Preparation of sample solution:

The sample solutions are prepared separately for Artemether and Lumefantrine assays as follows:

Weigh 20 tablets individually and obtain the average weight and relative standard deviation.

Crush the 20 tablets in a mortar with a pestle and weight equivalent to the average weight of the 20 tablets (x).

For lumefrantrine Assay:

Take a weight of powder equivalent to 30 mg (x/4) in duplicate into 50 ml volumetric flask.

Add about 40 ml of the solvent mixture and sonicate for 15 minutes at about 20°C (Caution: maintain the specific temperature to prevent evaporation of 2-propanol).

Top up to the mark with the solvent mixture and shake to mix.

Filter the mixture through whatman filter no. 1.

Dilute 1 ml of the filtrate to 25 ml with the solvent mixture, sonicate for 2 minutes.

Inject 20 microliter of the dilute sample in to the HPLC system under chromatographic conditions given above.

For Artemether Assay:

Take a weight of powder equivalent to 25 mg (1.25x) in duplicate in to a 25 ml volumetric flask.

Add 20 ml of the solvent mixture.

Centrifuge for 10 minute at 4000 rpm and top up to volume.

Inject 20 microliter of the supernatant into HPLC system using chromatographic conditions specific previously.

Calculation:

Percentage assay = $(P_{au} \times C_s \times 100) / (P_{as} \times C_u)$ **where,**

P_{au} is the peak area of sample solution,

P_{as} is the peak area of standard solution,

C_s is the concentration of standard solution,

C_u is the concentration of sample solution, and

100 is the conversion factor to percentage.

C_s for artemether = weight of artemether RS / 25 ml

C_s for lumefantrine = (Weight of lumefantrine RS x 1 ml) / (50 ml x 25 ml)

Cu for artemether = (Actual weight of powder x 25 g) / (calculated weight of powder x 25 ml)

Cu for lumefantrine = (Actual weight of powder x 30 g) / (calculated weight of powder x 50 ml x 25 ml)

KAMPALA INTERNATIONAL UNIVERSITY
Western Campus

Office of the Associate & Acting Dean, School of Pharmacy
College of Health Sciences, Ishaka* P.O. BOX 71
Bushenyi, Uganda: Tel: +256 (0)782124067, (0)703 599 144.
E-mail: nduezeh@yahoo.com, nduezeh@gmail.com.

Our ref: SPHKIUS/NDA/11/0001
Your ref:
4th October 2011

The Executive Secretary
National Drug Authority
Kampala

Dear Sir/Madam,

LETTER OF INTRODUCTION

This is to introduce Mr Francis Kidega Kimong, a final year pharmacy student of the above named school who may wish to undertake his research work in your facility by accessing data from your laboratory in partial fulfillment of the requirements for the award of a Bachelor of Pharmacy degree. His research topic is: **Assessment of the Quality and *in vitro* Bioavailability of branded Fixed Dose Artemether/Lumefantrine Tablets in Kampala Uganda.**
Please, assist him as required.

Thank you for your anticipated co-operation.

Yours truly,

KAMPALA INTERNATIONAL UNIVERSITY
DATE 10/10/11
SIGN
WESTERN CAMPUS
SCHOOL OF PHARMACY

Ezeonwumelu, Joseph O.C
Associate & Acting Dean, School of Pharmacy.
For and on behalf of School of Pharmacy.

KAMPALA INTERNATIONAL UNIVERSITY
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Kampala International University

School of Pharmacy

P.O. Box 71,

Bushenyi – Uganda

Date: 04th October 2011

To:

The Head of Department

National Drug Authority Control Laboratory

Thru'

The Executive Secretary / Registrar

National Drug Authority

Thru'

The Director Academic Affairs

Kampala International University – Western Campus

Dear Sir / Madam,

Please kindly assist our student.

REF: REQUEST FOR PERMISION TO CONDUCT EXPERIMENTS IN YOUR LABORATORY

I am a final year student of Bachelor of Pharmacy at Kampala International University – Western Campus. As a partial fulfillment for the award of a degree, I am expected to carryout research and make a report.

It is on this ground that I have developed a research proposal to assess the quality control parameters and *in vitro* bioavailability of fixed dose artemether – lumefantrine tablets marketed in Kampala (Please kindly see a copy of proposal enclosed).

Therefore, I am kindly requesting your office to facilitate me with your laboratory facilities to help me conduct these experiments.

I will be very grateful if my request is taken in to kind consideration. Thank you in advance.

Yours Sincerely

Francis Kidega Kimong

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KIU Registration Number: BPHE/0003/81/DU

Mobile Phone Number: 0772911098 / 0701911098

Email: kidega.francis@gmail.com