Formulation development of artemether 20 mg/ lumefantrine 120 mg fixed dose combination tablet

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Master of Pharmacy in Industrial Pharmacy Dissertation

Muhimbili University of Health and Allied Sciences

October, 2020

Muhimbili University of Health and Allied Sciences

Department of Pharmacy Practices and Pharmaceutics

"Formulation Development of Artemether 20 mg/ Lumefantrine 120 mg Fixed Dose Combination Tablet"

By

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A Dissertation submitted in (Partial) fulfillment of the requirements for the Degree of Masters of Pharmacy (Industrial Pharmacy)

Muhimbili University of Health and Allied Sciences

October, 2020

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a Dissertation entitled; ***"Formulation Development of Artemether 20 mg/Lumefantrine 120 mg Fixed Dose Combination Tablet"*** in partial fulfilment of the requirements for the degree of Masters of Pharmacy in Industrial Pharmacy of Muhimbili University of Health and Allied Sciences.

Prof. Mary Justin Temu

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Date

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DECLARATION AND COPYRIGHT

I, **Ibrahimu Kaswamila**, declare that this Dissertation entitled **"Formulation Development of Artemether 20 mg/Lumefantrine 120 mg Fixed Dose Combination Tablet"** is my own original work and it has not been presented and will not be presented in any other university for a similar or any other degree award.

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ACKNOWLEDGEMENT

I would like to give thanks to the Lord my God for giving me a healthy life and opportunity to pursue this study.

I convey my sincere gratitude to the Ministry of Health, Community Development, Gender, Elderly and Children, for sponsoring this study.

My heartfelt gratitude also goes to Zenufa Laboratories and Keko Pharmaceutical Industries for providing materials.

My special thanks are due to my Supervisors, Professor Mary J. Temu and Professor Eliangiringa Kaale, for their patience and guidance during the selection of the study topic, proposal development and data collection.

My special thanks are also extended to Dr. Betty Maganda, the Head of the Department of Phamacy Practices and Pharmaceutics, for her advice and assistance.

Last but not least, I wish to thank various people for their contribution; Mr. Prosper Tibalinda, Mr. Raphael Shedafa, Paul Malaba, Ms. Ruth, Mr.Edison and my fellow Mpharm students at MUHAS, for their valuable technical support.

DEDICATION

This work is dedicated to my parents, Mr. and Mrs. Kaswamila, also to my wife Zawadi Vyabadi, my children Ellen and Elvin. Thank you so much for your love, prayers and endless support.

ABSTRACT

Background: The World Health Organization (WHO) recommends artemisinin based combination therapies (ACTs) for the treatment of uncomplicated malaria caused by *P. falciparum* parasite. By combining two active ingredients with different mechanisms of action, ACTs are the most effective antimalarial medicines available today.

In Tanzania, access and affordability of antimalarials are limited, because there is only one Pharmaceutical Industry that manufactures Artemether Lumefantrine (ALU) tablets. Consequently, more than 90% of antimalarials are imported from other countries in abroad.

Aim of the study: The objective of this study is to develop a formulation of Artemether 20 mg/ Lumefantrine 120 mg Fixed Dose Combination Tablet.

Methods and Materials: This was an experimental study conducted at Muhimbili University of Health and Allied Sciences, specifically in the Pharmaceutical Analysis Laboratory and Research and Development (R and D) Laboratory. The Active Pharmaceutical Ingredients (APIs) used in pre-formulation and formulation development are Artemether and Lumefantrine, while the excipients are Hydroxypropyl Cellulose, Sodium Lauryl sulphate, Croscarmellose, stearate, Magnesium, Polysorbate 80 and Colloidal Silicon anhydrous.

Mortar and pestle were used to mix each excipient with Active Pharmaceutical Ingredient (API) in the ratio of 1:1; and the resultant mixtures were stored in Relative Humidity of 75±5% and Temperature of 40 ◦ C ±2 ◦C), room temperature 30◦C± 2◦C and in oven of 50◦C. Physical and chemical compatibility were assessed by using sense organs, HPTLC and NIR on day 0, after 14 and 90 days respectively.

D-Optimal design expert version 7 software was used to get eight trial formulations. The formulations were evaluated and results were used to obtain seven predicted formulations. From these seven formulations, only one formulation was selected for optimization.

Results: Pre-formulation showed that APIs and excipients were compatible within the studied period of 90 days. Formulation development was successfully performed by using a wet granulation method where by the optimized formulation which had Artemether 20 mg, Lumefantrine 120 mg, 2 milliliters of Polysorbate 80, Aerosil 4.75 mg, Hydroxypropyl cellulose 3.5 mg, Croscarmellose 4.5 mg and Microcrystalline cellulose 80 mg, gave good results of dissolution, tisintegration time, friability and assay that are comparable to the innovator drug.

Conclusion: A formulation of Artemether 20 mg and Lumefantrine 120 mg fixed dose combination tablet was successfully developed by wet granulation method. This indicates that, scale up by our local Pharmaceutical industries may be done by adopting this formula but they should adhere to official compendia. Adoption of this formula will lead to mass production of this medicine as a result it will improve its availability and affordability.

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ABBREVIATIONS AND ACRONYMS

ALU	Artemeter Lumefantrine
API	Active Pharmaceutical Ingredients
FDC	Fixed Dose Combination
HPTLC	High Performance Thin Layer Chromatography
ICH	International Council for Harmonization of Technical Requirements for Pharmaceutical Human use
MUHAS	Muhimbili University of Health and Allied Sciences
NIR	Near Infrared
R and D	Research and Development
SSA	Sub Saharan Africa
USP	United States Pharmacopoeia
WHO	World Health Organization
HCL	Hydrochloric Acid
RSD	Relative Standard Deviation
ACT	Artemether Combination Therapy

DEFINITION OF KEY TERMS

Excipients are pharmacologically inactive substances that serve as the vehicle or medium for a drug or other active substance.

Active Pharmaceutical Ingredient is a substance that is incorporated into a drug product to trigger pharmacological activity.

Formulation is a systematic combination of excipients and active pharmaceutical ingredients to produce a drug dosage form.

Disintegrants are substances included in the tablet formulations to aid the breakup of the compacted mass into primary particles to facilitate the dissolution or release of active ingredients in a fluid environment.

Binder is a substance that gives cohesiveness to the tableting mixture, facilitating the formation of a compact tablet.

Glidant is an excipient that allows granules to flow freely from the hopper to the die cavity, and hence facilitate uniform flow of the powder and ensure good uniformity in the tablet weight.

Assay is a qualitative or quantitative analytical procedure for detecting the presence of ingredient and estimating the concentration of a substance or ingredient.

Solubility is the ability of a given substance, the solute, to dissolve in a solvent. It is measured in terms of the maximum amount of solute dissolved in a solvent at equilibrium.

CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 Malaria

Malaria is a common and a life threatening disease which is caused by four different species of Plasmodium: *P.Falciparum*, *P. Malariae*, *P. Ovale* and *P. Vivax*. The malaria parasite is transmitted by female Anopheles mosquitos. Malaria has incubation period of 7 days or longer; and the most severe form is caused by *P.Falciparum*; and clinical features include headache, fever, muscular aching, chill and weakness, vomiting, cough, diarrhea and abdominal pains. The global effect of the disease threatens public health and impedes the progress of many countries towards prosperity (1).

Approximately 90% of the disease burden occurs in sub-Saharan Africa-SSA. Although SSA countries have dramatically reduced the total number of malaria cases and deaths since 2000, progress in recent years has stalled as in some countries, malaria is on the rise (2).

In 2017, there were estimated 219 million cases of malaria in 87 countries globally. The estimated number of malaria deaths globally was 435, 000 in 2017. In 2017, African regions constituted 92% of malaria cases and 93% of malaria deaths (2).

In Tanzania, Malaria has led to a high death toll, and inpatients malaria deaths in 2006 were reported at just over 5,000. According to Tanzania Demographic and Health Survey and Malaria Indicator Survey in 2015-16, Malaria cases raised from 9% in 2011 -12 to 14% in 2015-16, according to rapid diagnostic testing results. Estimated 6.5 million confirmed outpatient malaria cases were reported in 2016 (3).

1.1.2 Malaria and ALU formulation

Tablets are solid dosage forms which are prepared by mixing with suitable potential excipients. Depending on the intended use of the manufacturer, their characteristics which includes

disintegration, hardness, dissolution, weight, shape and size may vary. Oral drug delivery system is the most acceptable and comfortable route of drug administration. The solid dosage forms, specifically conventional tablets and capsules are among of the drugs which are administered orally and are found to be the most leading convenient and safe formulations due to their stability which is not easily compromised during storage or transportation (4). Despite the fact that solid dosage forms are not suitable for pediatrics and individuals who have difficult to swallow, still they are more safe and easy to administer when it comes to dose accuracy compared to liquid dosage forms.

Combination drugs of Artemisinin derivatives have been recommended in treatment of malaria so as to improve efficacy and prevent Plasmodium falciparum drug resistance.

The Artemether Lumefantrine (ALU) tablet is one of the essential drugs recommended by WHO for treatment of uncomplicated malaria caused by *Plasmodium falciparum* due to its efficacy, safety and quality. A fixed dose combination of ALU has consistently achieved cure rate of 95% in clinical trials (4). Solid and liquid formulations of this drug have been introduced for different targeted group of people.

In Tanzania, the recommended first line drug for treatment of uncomplicated malaria is ALU (5). Being one of the essential medicines, its availability should be ensured all the time and at affordable prices. However, due to inadequate number of formulation experts and poor technology, in Tanzania there is only one Pharmaceutical Industry that manufactures the ALU tablets, despite the fact that there are 13 registered local Pharmaceutical industries. It is, therefore, difficult for this single manufacturer to satisfy the market requirements in Tanzania; and this explains why most of these drugs are imported (6,7).

1.2 Literature review

1.2.1 Pre-formulation

Pre-formulation is the first step in the rational development of a dosage form of a drug substance alone or in combination with excipients. A pre-formulation study is a very vital stage in any

formulation development that ensures the drug does not cause harm to the user. Also, this study ensures careful selection of raw materials during formulation development is done based on the type and proposed quantity so as to ensure safety and efficiency of the drug all the time (8).

In 2004, Norvatis manufactured Coartem tablets that became the first fixed dose combination artemisinin drug for treatment of Malaria. The formulation of this drug involved Artemether Lumefantrine and various excipients whose functions and properties are obtained in various literatures (9). After ten years, generic Artemether Lumefantrine fixed dose combination tablets were allowed to be manufactured by other pharmaceutical industries in the world and most industries and scientific studies on this drug used almost the same excipients as used by Norvatis. Some of the excipients are discussed in the subsequent presentation.

Croscarmellose Sodium is one of the excipient which is regarded as essentially non-toxic and non-irritant material used in oral pharmaceutical formulations such as a tablet, capsule and granule disintegrant. In tablet formulations, Croscarmellose sodium may be used in direct compression and wet granulations, it should be added in both the wet and dry stages so that the wicking and swelling ability of the disintegrant is best utilized. Croscarmellose sodium may be used up to 5% w/w, although normally 2% w/w is used in tablets prepared by direct compression and 3% w/w in tablets prepared by a wet granulation process (10). Croscarmellose cellulose is insoluble in water and it rapidly swells up to 8 times its original volume.

In many different formulation studies, Hydroxypropyl cellulose is one of the excipients used as binders. The concentration of Hydroxypropyl cellulose of 2-6%w/w may be used in either wet or dry granulation and in direct compression tableting process. The concentration of 15-35 can also be used in manufacturing extended release drug (10).

Microcrystalline cellulose is widely used in pharmaceuticals, primarily as a diluent in oral tablets and capsule formulations by wet granulation and direct compression process. It is generally regarded as a relatively non-toxic and non-irritant material. It is not absorbed systematically following oral administration and thus has a little toxic potential (12,13). Uses of Microcrystalline are summarized in Table 1.

Table 1: Uses of Microcrystalline Cellulose

Use	Concentration
Adsorbent	20-90
Antiadherant	5-20
Binder/Diluent	20-90
Disintegrant	5-15

As seen in Table 1 above, the intended use of excipient depends on the quantity or concentration selected.

Polysorbate 80 is a series of partial fatty acid esters of sorbitol which is used as a solubilizing agent or surfactant in tablet formulation. This excipient has been used in various studies of ALU formulation development as a solubilizing agent. The WHO has set an estimated acceptable daily intake of up to 25mg/kg (13,14).

In some few studies for development of ALU drug, Ethanol 96% alcohol has been used as a solvent in binder preparation. This excipient is in class three of the International Council for Harmonization of Technical requirements for Pharmaceutical Human use on a guideline for residual solvent. The metabolites and unchanged Ethanol 96% alcohol are mainly excreted through urine. Toxic symptoms may be produced by 20milliliters (13).

Colloidal Silicon dioxide is a glidant that has the ability to improve powder property. Its functional categories are adsorbent, anticaking agent, emulsion stabilizers, glidant, and stabilizing agent (10).

In this study, it was used as a glidant because literature says when it is added at a typical level of 0.1% to 0.2%, it improves the flow characteristics of a compression mix (14). It is generally regarded as an essentially non-toxic and non-irritant excipient.

Magnesium Stearate is primarily used as a lubricant in tablets and capsules manufacture at the concentration of between 0.25% and 5.0% w/w. It is a widely used excipient and is generally regarded as being nontoxic upon oral administration (14).

1.2.2 Formulation development

Formulation development is a systematic combination of excipients and active pharmaceutical ingredient to produce a drug dosage form. There are two types of formulation development methods, namely direct compression and wet granulation method (15). In this study, the formulation development trial batches were done by wet granulation method based on a prior scientific knowledge of the materials and available technology.

Direct compression method does not involve any addition of fluid before the compression of tablets. According to literature (16), this method is recommended to be the first choice of any tablet formulation because of its uncomplicated manufacturability and cost effectiveness. Other advantages of this technique are production of drug product with less disintegration time and faster dissolution rate, and better stability of an API in a drug product due to less processing steps.

Literature says direct compression formulation can be developed with a minimal number of excipients (17). Typically, the minimum excipients needed are a diluent (filler-binder), a disintegrant and lubricant. Additional components may include a glidant, surfactant and stabilizing agents. Some of the commonly used excipients are Microcrystalline cellulose, Croscarmellose sodium, Crospovidone, Colloidal silicon dioxide, hydroxypropyl cellulose and Sodium Lauryl sulphate (18).

A similar and successful study was conducted in India by the use of wet granulation techniques. The materials used were Atemether, Lumefantrine, Microcrystalline cellulose, Aerosil, Croscarmellose sodium, Crosspovidone and Avicel. Other materials included Hydroxypropyl cellulose, Magnesium stearate, Polysorbate 80 and Isopropyl alcohol. Another study was conducted in January 2014 (12), using such materials as hydroxypropyl cellulose, Microcrystalline cellulose, Croscarmellose, Magnesium stearate, Aerosil, Polysorbate 80, and Isopropyl alcohol. The mentioned materials gave the best results on formulation development.

Based on the above literature and prior knowledge, the selected materials and methods were suitable for this study. Therefore, the batch trials were developed by wet granulation method and the evaluation of the produced batches was conducted and gave good results.

1.2.3 Optimization

Optimization in formulation development of a drug is generally the process of making it as perfect as possible within a given set of restrictions or constraints. The physical, chemical and biological properties must all be taken into consideration in the selection of components and processing steps for that dosage form or product. Accordingly, there must be a better method than trial and error to determine the best formulation process. In formulation development, we generally experiment by a series of logical steps, carefully controlling the variables and changing one variable at a time until a satisfactory system is produced (19).

The development of solid, semisolid or liquid formulations usually involves a number of variables. Mathematically, they can be divided into two groups, namely independent and dependent variables. On the one hand, independent variables are directly under the control of the formulator; these might include the level of a given ingredient or the mixing time for a given process step. On the other hand, the dependent variables are the responses or the characteristics of the resulting product, and these are a direct result of any change made in the formulation or process. In any formulation study, we must be able to distinguish between the two variables (20).

D-optimal design expert version 7 software is one of the tools used in optimization and it has no limitations to the area of applicability. The steps involved in this type of optimization procedure are as follows; (a) selection of independent and dependent variable (b) performance of set of statistically designed experiments (c) measurement of properties of interest, that is dependent variable and then make judgement based on how best the best results obtained were (22).

1.2.4 Malaria and Antimalarial

Malaria is a common and a life threatening disease in many tropical and subtropical areas. Human malaria is caused by four different species of Plasmodium: *P.Falciparum, P. Malariae, P. Ovale and P. Vivax* (23). The malaria parasite is transmitted by female Anopheles mosquitos. Malaria is an acute febrile illness with incubation period of 7 days or longer. The most severe

form is caused by *P.Falciparum*; clinical features include headache, fever, muscular aching, chill and weakness, vomiting, cough and diarrhea (24).

The key interventions to Malaria include effective treatment with Artemisinin-based combination therapies. Artemisinin and its delivatives are very potent and effective anti-malarial drugs. For patients who are *P. Falciparum* malaria-resistant to the common antimalarial drugs, the use of artemisinin and its derivatives is essential (9).

Artemether

Arthemether is chemically named as [3R-(3R,5aS,6S,8aS,9R,10R,12S,12aR)] decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin. The Artemether molecular formula is C16H26O and it has the molecular weight of 298.4 g/mol (9)**.**

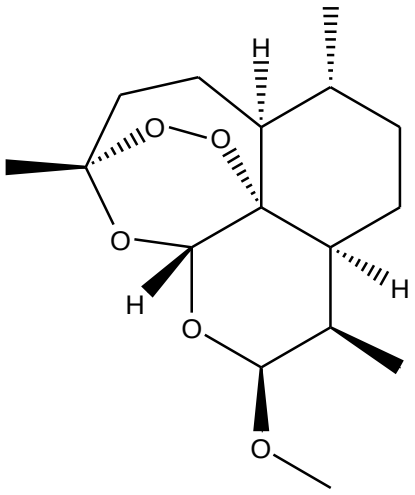

Figure 1: Artemether Chemical Structure

Description: It is a white crystalline powder.

Solubility: Artemether is practically insoluble in water and soluble in oil, freely soluble in Ethyl Acetate and Dehydrated Ethanol, and very soluble in Dichloromethane and Acetone. It is a Biopharmaceutic class two, meaning it has low intestinal solubility and high intestinal permeability. Its solubility classification is based on a United States Pharmacopoeia and its permeability is based on a comparison to intravenous injection (25).

Melting point: Range is 86 – 90°C as stated in International Pharmacopoeia.

Specific Optical Rotation: +166° to +173°.

Pka value: 4.6.

Indication: Artemether alone has anti-malarial property with rapid onset of action but due to its short half-life (2 to 3 hours), it is used in combination with Lumefantrine to improve its efficacy for the treatment of acute uncomplicated malaria caused by *Plasmodium Falciparum*. It is indicated for use in adult and children above 5kg (5).

Pharmacodynamic: In the body, Artemether is metabolised into the active metabolite dihydroartemisinin. The drug works against the erythropoietic stages of *P. falciparum* by inhibiting nucleic acid and protein synthesis. Artemether is administered in combination with Lumefantrine for improved efficacy. It has a rapid onset of action and rapidly clears the parasites with a shorter duration of action (28).

Mechanism of action: It involves an interaction with ferriprotorphyrin IX (heme), or ferrous ions, in the acidic parasite food vacuole, which results into generation of cytotoxic radical species.

Absorption: Food increases its absorption, but grape juice may increase toxicity of atemether by inhibiting its metabolism.

Protein binding: Artemether, 95.4%; Dihydroartemisinin, 47-76%.

Metabolism: Rapidly metabolizes into its active metabolite, Dihydroartemisinin.

Lumefantrine

Lumefantrine is a dichlorobenylidine derivative for the treatment of various types of malaria. Its chemical name is Lumefantrine and chemically formulated as 2-Dibutylamino-1-[2,7-dichloro-9-(4- chlorobenzylidene)-9Hfluoren-4-yl]-ethanol with molecular formula C30H32Cl3NO. Its average weight is 528.94 and the brand names of available drugs are Coartem and Riamet with the ingredients of Artemether + Lumefantrine (24).

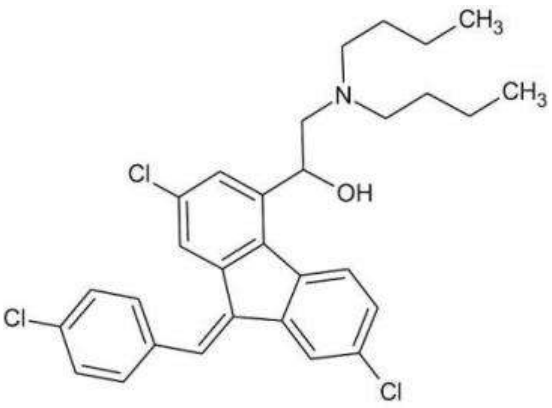

Figure 2: Lumefantrine Chemical Structure

Description: It is a yellow crystalline powder

Solubility: It is insoluble in water, soluble in oil.

Melting point: Range is 128 – 132℃ as stated in the USP SALMOUS Standard (26).

Indication: Lumefantrine alone has antimalarial effect with a long elimination half-life (approximately 4.5 days) but slow onset time of action, it has been used in combination with Artemether so as to improve its efficacy for the treatment of acute uncomplicated malaria caused by Plasmodium Falciparum. It is indicated for use in adult and children above 5kg (5).

Pharmacodynamics: Lumefantrine is a blood schizonticide active against erythropoietic stage. Lumefantrine has a much longer half-life and is believed to clear residual parasites left by Artemether.

Mechanism of action: Available data says it inhibits the formation of Beta hematin by forming complex with hemin and inhibits nucleic acid and protein synthesis of Plasmodium falciparum.

Absorption: Food increases its absorption.

Artemether and Lumefantrine combination.

Artemether and Lumefantrine fixed dose combination has been found to have a good efficacy with a cure rate of 95% in clinical trials (4). The rationale of this combination is that, Artemether has the ability to clear faster the Malaria parasites and provide a symptomatic relief but it has no ability to clear all the parasites due to its short half-life (2 to 3 hours). Due to longer half-life

(approximately 4.5 days) of Lumefantrine, it is combined with Artemether so as to clear the Malaria parasites left by Artemether and also helps to reduce the gametocytes carriage (9).

1.3 Statement of the Problem

Although the Artemether Lumefantrine (ALU) tablet is one of the essential medicines, its availability in Tanzania is still inadequate and inconsistent because more than 90% of this medicine is imported from abroad. In TFDA reports of 2016, ALU tablets were found to be the leading generic formulation among the top 20 drugs imported in Tanzania (6).

In the year 2019, one of the objectives of the Tanzania National Malaria Control Program (NMCP) was to improve access of ACT in public and private facilities so as its availability is improved up to the minimum of 90% by the year 2025 (27).

1.4 Rationale of the Study

Despite the fact that this study gives experience to the researcher on the formulation development and optimization of conventional tablets by using wet granulation method, the rationale of this study was to develop a formulation of Artemether 20mg/ Lumefatrine 120mg fixed dose combination tablet which will give the following benefits to the society; firstly, the results of this study will call for adoption by local manufacturing industries that are not manufacturing this drug to start its production that will lead to its increase and ensure consistent availability within the country because for now, more than 80% of this drug is imported from abroad (6). Secondly, the study supports and contributes to our national agenda of industrialization which is in the five years of development plan II (28). Last but not least, is to improve its affordability because this study has succeeded to come up with the formulation that uses cheap and few materials.

1.5 Research Questions

1. Are Active Pharmaceutical Ingredients and Excipients compatible?
2. How is Artemether Lumefantrine (ALU) fixed dose tablet developed?
3. How is optimization conducted during development of Artemether Lumefantrine (ALU) fixed dose tablet?

4. How is a developed formulation of Artemether Lumefantrine evaluated?

1.6 General Objective

To develop a formulation of Artemether Lumefantrine (ALU) fixed dose combination tablet.

1.6.1 Specific Objectives

Specifically, this study sought to:

1. Conduct a pre formulation study of Artemether Lumefantrine using potential excipients.
2. Develop a formulation of Artemether Lumefantrine tablet.
3. Optimize the formulation of Artemether Lumefantrine tablet.
4. Evaluate a developed formulation of Artemether Lumefantrine tablet.

1.7 Ethical Clearance

Ethical Clearance was granted by the Institution Review Board (IRB) of Muhimbili University of Health and Allied Science.

CHAPTER TWO

MATERIALS AND METHODS

This was an experimental study conducted at the Muhimbili University of Health and Allied Sciences (MUHAS) in the Research and Development (R and D) laboratory.

2.1 Materials and Instruments

2.1.1 Materials

Active Pharmaceutical Ingredients (APIs) that were used in the pre-formulation and formulation steps are Artemether and Lumefantrine batch number CASNo 71963-77 and CASNo 82186-77-4 both made by Hennan Senyuan Biological Tecnology, in Zhengzhou-China, donated by Keko Pharmaceuticals Ltd. Excipients that were used are Hydroxypropyl Cellulose and Sodium Lauryl sulphate both manufactured by Henan Chuange Industry Co., Ltd, Croscarmellose,stearate, manufactured by Anhui Sunhere Pharmaceutical excipients C., Ltd-China, Magnesium Stearate, manufactured by Hangsun Plastic Additives Co., Ltd (both donated by Keko Pharaceuticals Ltd), Polysorbate 80 and Colloidal anhydrous both manufactured by Ahhui Sunhere Pharmaceutical excipients Co., Ltd (both donated by Zenufa Laboratory Ltd).

During the pre-formulation and formulation stages, the following reagents were used; Ethyl acetate, n-hexane, Methanol, Acetone and Glacial acetic acid both made from Techno Pharmchem Bahandurggarh, Haryana, India. Moreover, distilled water was prepared by the Institute of Traditional Medicine at MUHAS, while Concentrated Sulphuric Acid manufactured by Techno Pharmchem Bahandurggarh, Haryana, India was donated by Keko Pharmaceutical Ltd.

2.1.2 Instruments

The instruments that were used included analytical balance (made by MS Mettler Toledo Germany), Near Infrared Spectrometer (made by Lab spec 5000 ASD Inc. USA), and HPTLC machine (Linomat 5 applicator and CAMAG TLC Scanner 4 both made in Germany). Other instruments were Single Trough Development chamber, Tubular mixer (Analytical Technology, Bangalore, India), Tablet press, EKOI 2 made in Germany, and Kenwood planetary mixer made

in the United Kingdom. Also a Dissolution tester (USP apparatus II), made in Germany, and Kotternmann 2712 Oven, made in Germany.

D-Optimal design version 8 was used to get the trial batches. HPTLC glass plates (20 X 10) pre coated with Silica gel 60F 254 were also used; these were made by Merck, Darmstadt in Germany.

2.2 Methods

2.2.1 Pre-formulation Studies

The characterization of chemical and physical properties of API was conducted by evaluation of their description by physical and chemical observations. Five grams of each API powder were placed in a petri dish; and their colour, form and taste were observed by using sense organs. The observed organoleptic properties were compared with the physical properties stated in API certificate of analysis which indicated that Artemether was a white crystalline powder, while Lumefantrine was a yellow powder.

A compatibility study was conducted through preparation of binary mixture of drug-excipient study; and each excipient was mixed with Active Pharmaceutical Ingredient (API) in a ratio of 1:1 by using Mortar and Pestle. The prepared sample materials were stored in three different environmental conditions; climatic condition of Relative Humidity of 75±5% Temperature 40 ◦C ±2 ◦C), room temperature 32◦C± 2◦C and at oven chamber of 50◦C. Through chemical analytical tests, and also by using eyes and other sense organs, the prepared samples were physically observed for caking, liquefaction, discoloration, odour formation and API content in the mixture at the interval of 0, 14 and 90 days. The binary mixture of samples prepared at day zero were scanned by Near Infrared (NIR) spectroscopy so as to have a calibration file that was later used to compare with the same binary mixture that were kept in the three mentioned environmental conditions for 90 days.

2.2.2 Formulation Development

Formulation development was conducted by starting with the determination of particle size distribution of API by using sieve analysis method. Also the mixture of API with potential excipients was done and the flow property of the powder mixture was evaluated through the

calculations of bulk density, tapped density, hausner's ratio and compressibility index. The results for particle size distribution of APIs and powder flow evaluation revealed that the formulation process has to undergo a wet granulation method because the distribution of particles of Lumefantrine was poor. Also the flow property of the powder mixture could not fall within the acceptable limits. The formulation development involved three major steps, namely dry mixing, wet mixing and compression (19).

2.2.2.1 Dry Mixing

Ten minutes were used to mix the required amount of Artemether, Lumefantrine, Microcrystalline Cellulose and half of the required amount of Aerosil. The preparation of binder was done by mixing the required amount of Hydroxyl propyl cellulose with a polysorbate 80 and Ethanol 96% so as to solubilize the hydroxyl propyl cellulose. Then a small amount of water was added to make the binder ready for use (29).

2.2.2.2 Wet Mixing

This stage involved addition of a binder solution to the mixture of a dry powder. The mixing was done in a mixture granulator machine for 15 to 20 minutes. Water was added during the mixing until granule formation was attained by a snow ball test. The granules were dried in an oven at a temperature of 50◦C for 16 hours. The powder granules were evaluated through the calculation of Bulk Density, Tapped Density, Carr's Index and Hausner's ratio so as to ensure good flow ability of powder granules (30).

2.2.2.3 Compression

Production of tablets was done on a single punch compression machine where by the upper and lower punches had the size of 10 mm. Eight formulation trial batches were produced with the aid of D-Optimal Design expert version 7 software which ensured the use of appropriate amount of materials to give the acceptable results of friability and disintegration time. The formulated 8 batches were evaluated for their friability, tablet weight uniformity, disintegration time, dissolution and then it was followed by analyzing the tablet API content by using a validated in house HPTLC method (31).

Tablet content uniformity

A sample of 10 tablets was taken and subjected to a validated HPTLC analytical test where by each tablet was analyzed for API content individually (21).

Tablet weight uniformity

A sample of 20 tablets was picked randomly, weighed and their average weight was calculated. Then, each tablet was weighed individually and compared to the average weight of 20 tablets.

Disintegration test

A sample of 6 tablets was placed in the test tubes which were immersed in a water bath with a maintained temperature of 37◦C. The machine containing the test tubes was switched on and allowed to start. The machine enabled up and down movement of tubes 28 – 32 times per minute (21). The disintegration time of the tablets was recorded.

Friability test

A friability tester was used to test ten tablets but the tablets were dedusted first and weighed before the testing began. The tester was allowed to start and undergo 100 rotations in 4 minutes. The tablets were dedusted and weighed again, and then the weight loss was recorded (21).

Dissolution tests

Three dissolution tests were conducted separately by using three different dissolution media (900mls of Hydrochloric acid pH 1.2 buffer solution, acetate and phosphate buffer solutions of pH 4.5 and 6.8 respectively set at 37 ◦C). A sample of 6 tablets were taken randomly and placed into the machine beakers filled with a respective medium. The machine was switched on and started then stopped after 180 minutes. The volume of withdrawn liquid was replaced with an equal amount of buffer. The amount of API in the sample was determined using a HPTLC method (31). All the compendial tests were conducted as per European Pharmacopoeia and Monographs (32).

2.2.3 Optimization

Results (disintegration time and friability) for 8 formulated batches were filled in the D-optimal design expert software. The software was able to give another seven formulations among which the best formulation could be obtained. The suggested formulations were produced and one among the seven formulations was taken for optimization by producing three batches of this formulation. These three batches were further evaluated for dissolution, tablet content uniformity, assay, disintegration time, friability and tablet weight uniformity by using the procedures explained above. The dissolution test was conducted in both three media (0.1M HCL buffer of pH 1.2, Phosphate buffer pH 6.8 and Acetate buffer pH 4.5) as stated in Pharmacopeia (33).

CHAPTER THREE

RESULTS

3.1 Pre-formulation Study

The five grams of Artemether and five grams of Lumefantrine separately placed in a petri dish for description and identification showed the following results in Table 2 below.

Table 2: Description of Artemether and Lumefantrine

Organoleptic properties	**Lumefantrine**	**Artemether**
Form	Powder	Crystalline Powder
Colour	Yellow	White
Odour	Odourless	Odourless

For the binary mixture made and kept in three different environmental conditions, namely uncontrolled room temperature 30 ◦C ± 2 ◦C, climatic condition with temperature 40 °C ± 2 °C and relative humidity (RH) of 75±5% and oven with temperature of 50 °C these sample mixtures did not show any significant change in colour, content and form after 90 days of observation (Table 3).

Table 3: Appearance of Samples in Day 0,14 and 90

Name of excipient	Ratio	Initial (0 day)	Observations at room temperature 30°C ± 2°C		Observations at climatic condition 40°C±2°C/ 75±5 % RH		Observations at Oven (50°C)	
			After 14 days	After 90 days	After 14 days	After 90 days	After 14 days	After 90 days
Art	-	White	White	White	White	White	White	White
Lu	-	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Art, Lu and Mcc	1:1	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Art, Lu and MgS	1:1	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Art, Lu and SodL	1:1	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Art, Lu and Hpc	1:1	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Art, Lu and Crsc	1:1	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Art, Lu and Iso	1:1	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Art, Lu and PolyS	1:1	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Art, Lu and ColH	1:1	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Art, Lu and AllExc	1:1	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow

KEY:

Art-Artemether

Lu-Lumefantrine

Mcc-Microcrystalline cellulose

PolyS-Polysorbate 80

ColH-Colloidal anhydrous

AllExc-All excipients

Hpc-Hydroxy propyl cellulose

Crsc-Croscamellose sodium

MgS-Magnesium stearate

SodL-Sodium Lauryl Sulphate

Scanning of the samples APIs and all Excipients were conducted by using Near Infrared; the results are shown in Figures 3, 4 and 5 below.

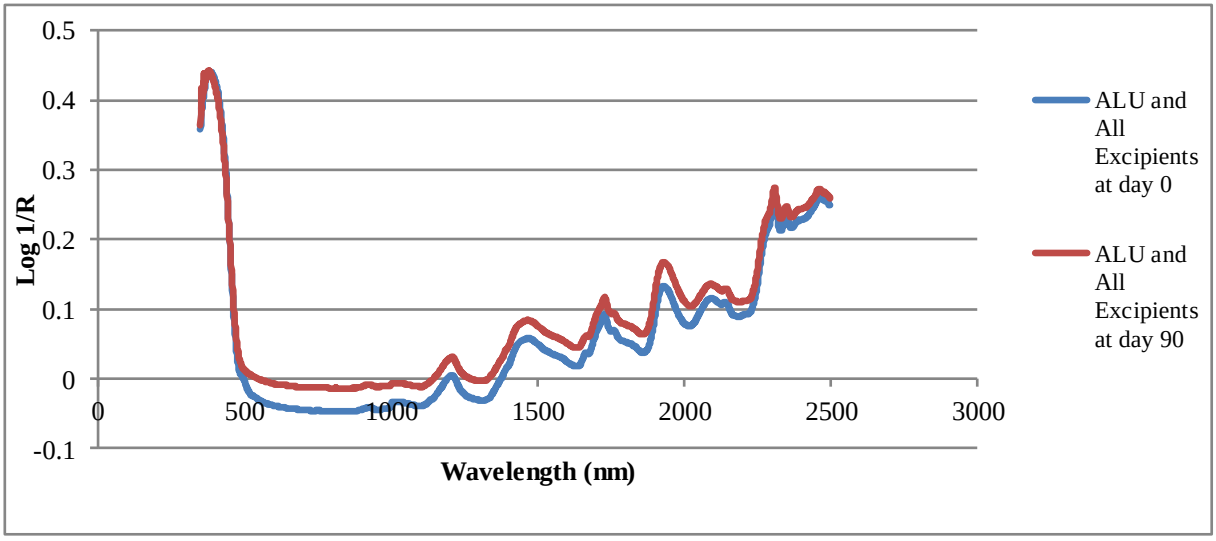

Figure 3: NIR Results for Artemether, Lumefantrine and all Excipients Kept in Climatic Condition (RH 75/ Temperature 40 °C ± 2 ◦Cat Day zero and Day 90

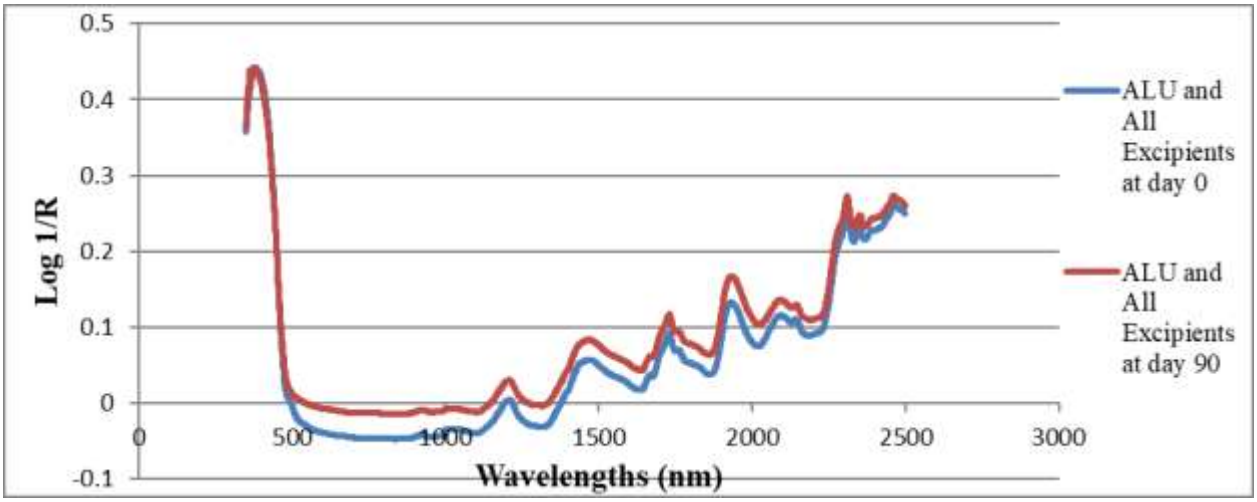

Figure 4: NIR Results for Artemether, Lumefantrine and all Excipients Kept in Oven (temperature 50 °C ± 2 ◦C) at Day Zero and Day 90

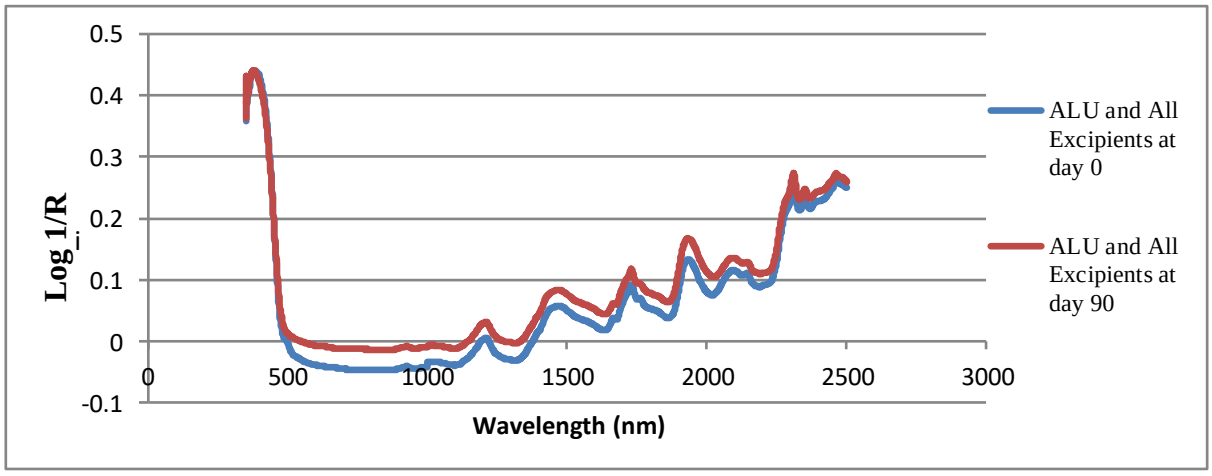

Figure 5: NIR Results for Artemether , Lumefantrine and all Excipients Kept in Uncontrolled Room Temperature (32 °C ± 2 °C) at Day Zero and Day 90

Based on the above results, although the samples were kept in three different environments, the results in figures 3, 4 and 5 shows no any change for the mixture of Artemether/Lumefantrine and all Excipients after 90 days. This signify that the mixture of APIs with all potential excipients were compatible because the results for observations on day 0 are the same as the results after 90 days.

Experimental observations of drug content for samples kept in three different environmental conditions was conducted in day 0, 14 and 90 by using a validated HPTLC method (31). The results are as seen in Tables 4 and 5 below.

Table 4: Percentage of Artemether Content of Samples Studied at Day 0, 14 and 90

s/n	Mixture	Day 0	Day 14 at climatic condition (RH 75 temp 40 °C ± 2°C)	Room temp (30 °C ± 2 °C) at day 14	Samples in oven (50 °C) at day 14	Samples in room temperature (30c +-2c) at day 90	Samples in oven (50 °C) at day 90	Samples in climatic condition (RH 75 temp 40 °C ± 2°C) day 90
1	Art	100	100	100	100	100	100	100
2	Art and Lu	98.5	97.4	98.4	98.2	95.7	96.85	96.97
3	Art, Lu and Mcc	108.9	99.5	103.2	98.2	99.4	97.96	95.86
4	Art, Lu and Magn	107.3	99.4	107.1	105.1	103.4	103.39	98.89
5	Art, Lu and SodLa	106.5	106.4	106.2	104.4	100.9	101	96.75
6	Art, Lu and Hpc	108.3	107.7	101.9	98.5	101.1	98.72	101.48
7	Art, Lu and Crosc	101.2	99.3	97.1	101.6	97.1	100.99	96.87
8	Art, Lu and CoHd	96.2	96.5	96.2	96.5	96.8	96.12	95.61
9	Art, Lu and allexc	98.2	97.4	96.3	96.5	96.1	96.46	97.23

Table 5: Lumefantrine Content of Samples Studied at Day 0, 14 and 90

s/n	Mixture	Day 0 **Assay in %**	Day 14 at climatic condition (RH 75 temp 40 °C ± 2°C) **Assay in %**	Room temp (30 °C ± 2 °C) at day 14 **Assay in %**	Samples in oven (50 °C) at day 14 **Assay in %**	Samples in room temperature (30c +-2c) at day 90 **Assay in %**	Samples in oven (50 °C) at day 90 **Assay in %**	Samples in climatic condition (RH 75 temp 40 °C ± 2°C) day 90 **Assay in %**
1	Lu	96.51	96.28	96.18	96.2	95.73	95.87	95.54
2	Art, Lu and Mcc	101.37	98.44	100.84	100.3	99.85	99.55	95.01
3	Art, Lu and Magn	105.33	102.49	100.48	102.49	100.4	102.32	96.97
4	Art, Lu and SodLa	107.14	104.07	107.3	97.8	97.45	97.99	95.78
5	Art, Lu and Hpc	99.9	99.14	99.97	98.35	99.19	97.29	99.41
6	Art, Lu and Crosc	103.54	103.46	97.38	101.64	97.24	101.4	102.11
7	Art, Lu and CoHd	105.11	104.4	105.74	104.83	104.58	98.94	103.27
8	Art, Lu and allexc	97.98	97.03	97.35	96.17	95.9	96.27	96.14

The results presented in tables 4 and 5 above, shows that the drug content of each sample mixture was not affected after 90 days. For all samples kept in three different environmental conditions, the drug content was in the acceptable range, that is 95% to 105 ± 5% (25).

3.2 Formulation Development

3.2.1 Analysis of Individual Powder Particle Distribution and Flow Property of the Powder Mixture

Sieve analysis for both Artemether and Lumefantrine was performed separately by arranging sieves in descending order (1400µm, 1000µm, 710µm, 500µm, 355µm, 250µm, 180µm, 180µm, 125µm, 90µm and 45µm. The percentage of amount retained in each sieve was calculated for each API and the graph was plotted to show the particle size distribution of powders (34). Results are as seen in table 6,

Table 6: Particle Size Distribution of Artemether Powder

S/n	Sieve size in µm	Weight of sieve alone (X)	Weight of sieve and retained material (X_2)in gm		Amount of material retained in gm (X_2-X)		% of amount retained in gm $\frac{(X_2-X)100}{100}$	
		Arte	Arte	Lume	Arte	Lume	Arte	Lume
1	45	292.9	292.9	292.9	0	0	0	0
2	90	296.5	297	296.6	0.5	0.1	0.5	0.1
3	125	299.1	304	299.4	4.9	0.3	4.9	0.3
4	180	307.4	337.5	308.8	30.1	1.4	30.1	1.4
5	250	307.9	362.3	326.2	54.4	18.3	54.4	18.3
6	355	320.3	324.1	321.5	3.8	1.2	3.8	1.2
7	500	330.2	332.6	344.6	2.4	14.4	2.4	14.4
8	700	362.9	365.3	375.1	2.4	12.2	2.4	12.2
9	1000	345.1	346	395.2	0.9	50.1	0.9	50.1
10	1400	368.9	369.1	370.8	0.2	1.9	0.2	1.9

The above results in table 6, shows that most of Artemether powder particles were retained in sieve number 5 and 4 which are sieve size of 250µm and 180µm. This means that the proper sieves to be used in this method of wet granulation are the ones that have the size above 250 µm because the granules will be having larger size than the powder before granulation (34).

For the Lumefantrine powder, there was unevenly distribution of powder particles indicating that it has a poor flow to the extent that a direct compression method was impossible. The unevenly distribution of this powder is shown in figure 7.

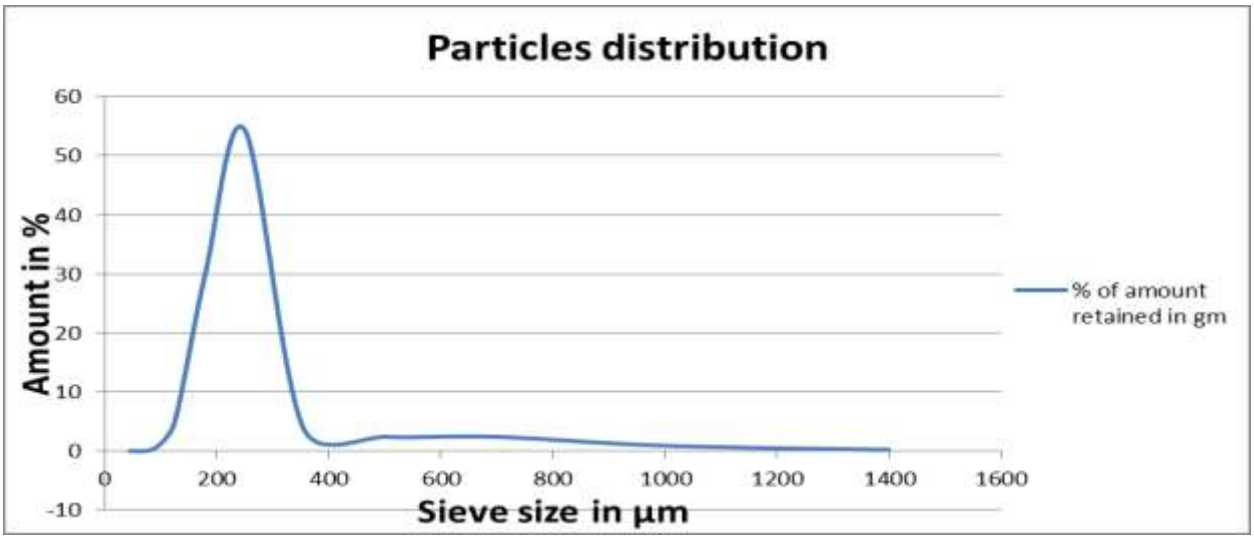

Figure 6: Amount Retained versus Sieve Size Showing Particle Size Distribution of Artemether Powder

The figure presented above shows that most powder particles were retained in sieve that had the size below 250 µm. This means that the appropriate sieve is the one with the size above 250 µm.

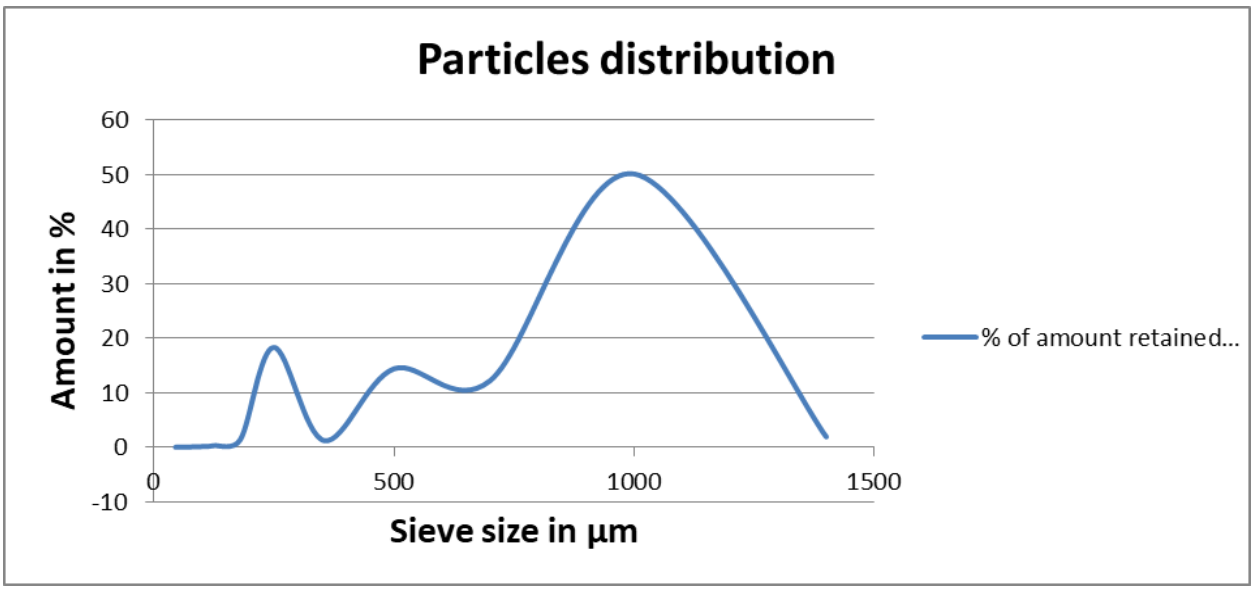

Figure 7: Amount Retained versus Sieve Size Showing Particle Size Distribution of Lumefantrine Powder

Table 6 and figure **(7)** above shows that there was unevenly distribution of powder particles for Lumefantrine powder. The figure **(7)** did not give a sigmoid curve meaning there was unevenly distribution. These results conclude that the flow property of this powder is poor, therefore it is not easy to use direct compression method in formulation development for tablet of this drug (35).

3.2.2 Determination of Flow Property for the Powder Mixture before Granulation

Despite the fact that sieve analysis was conducted to study the distribution of powder particles, the results for sieve analysis are still not strong enough to decide the method to be used during formulation development (8). Evaluation of the flow ability for the prepared powder mixture was conducted by calculating Bulk density, Tapped density then Hausner's ratio and Compressibility Index. The results are summarized in Table 7.

Table 7: Parameters for Determination of Powder Flow Property

S/n	Parameter	Value	Acceptable range
1	Bulk density (g/milliliters)	0.385	
2	Tapped density (g/milliliters)	0.625	
3	Hausner's ratio	1.623	1.12 – 1.25
4	% Compressibility Index	38.5	11 - 25
5	Moisture content (%)	1.4	

The above values for Compressibility Index and Hausner's ratio were compared with the standard limit values for good flow ability.

Based on the results obtained, the flow of the powder mixture was poor because the value for Compressibility index obtained was 38 which is above the acceptable value (Compressibility Index is supposed to be below 25). Therefore, it was not possible to use direct a compression method in this study (8).

3.2.3 Production of Trial Formulations by Wet Granulation Method

D-Optimal Design Expert was used to get the mixture presented in table 8 below.

Table 8: Amount of Materials for Trial Formulations

	Ingredient	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Constants	Artemether (%)	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3
	Lumefantrine (%)	50	50	50	50	50	50	50	50
	Magnesium stearate (%)	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
	Polysorbate 80 (milliliters)	4	4	4	4	4	4	4	4
	Colloidal anhydrous (%)	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Variable components	Sodium Lauryl sulphate (%)	1.75	2.5	-	-	-	-	-	-
	Ethanol 96% (milliliters)	35	35	16	10	10	10	10	10
	Water (milliliters)	-	-	20	30	30	30	30	30
	Hydroxypropyl cellulose (%)	0.21	0.42	0.58	0.5	2.0	3.0	3.0	2.4
	Croscamellose (%)	2.5	2.5	3.0	3.0	4.5	5.5	3.0	6.0
	Microcrystalline Cellulose(%)	33.3	32.5	34.4	36.7	30.4	28.3	30.4	28.3

Table 8 above shows the amount of ingredients that were used in trial formulations. In variable components, the amount kept on changing while the ingredients for constant variables were not changed.

Table 9: Results for Powder Granules Flow Property

S/n	Parameter	F1	F 2	F3	F4	F5	F6	F7	F8
1	Bulk density (g/milliliters)	0.47	0.49	0.475	0.483	0.47	0.44	0.40	0.42
2	Tapped density (g/milliliters)	0.54	0.59	0.572	0.584	0.59	0.53	0.51	0.56
3	Hausner's ratio	1.15	1.22	1.21	1.209	1.25	1.20	1.27	1.33
4	% Compressibility Index	16.9	17.67	16.98	17.3	16.9	15.96	17.2	17.01
5	Moisture content (%)	0.64	1.0	1.9	1.83	1.2	1.0	1.3	1.91

The results in Table 9 above were compared with the standard Compressibility Index specifications; and the powder was found to have good flow property that produces good tablets with minimum weight variations. Compression of powder granules of different batches was conducted by using upper and lower punches and dies which had the size of 10 mm.

3.2.4 Evaluation of Tablets

For trial formulations, tablets of each batch were evaluated for their disintegration time, friability and relative standard deviations of tablets weight. The results are as seen in Table 10.

Table 10: Summary Results for Tablets Evaluation of Disintegration, Friability and Relative Standard Deviation (RSD) of Tablet

Trial formulation	Maximum disintegration time (Min:Sec)	% Friability	Average weight of tablet	RSD of tablet weight
F1	2:29	0.5	240.5	1.6
F2	6:35	0.2	236.8	1.73
F3	3:02	0.3	242.3	2.07
F4	5:12	0.23	246.2	1.59
F5	10:17	0.09	242.55	0.66
F6	10:34	0.1	242.65	0.98
F7	5.17	0.3	139.6	1.53
F8	5.21	0.35	243.2	1.02

The above results in table 10 were the desired outcome for dependent variables in this study. Although there were variations of the results between formulations, but still all the results are within the acceptable ranges.

3.2.5 Optimization

The results obtained in trial formulations were inserted in Design expert version 7 software, and the software gave seven predicted formulations to be performed. The ingredients amount of these formulations are as shown in Table 11 below.

Table 11: Amount of Materials for Predicted Formulations

	Ingredient	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
Constants	Artemether (%)	8.3	8.3	8.3	8.3	8.3	8.3	8.3
	Lumefantrine (%)	50	50	50	50	50	50	50
	Magnesium stearate (%)	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	Polysorbate 80 (milliliters)	2	2	2	2	2	2	2
	Colloidal anhydrous (Aerosil) in %	1.98	1.98	1.98	1.98	1.98	1.98	1.98
Variable components	Sodium Lauryl sulphate (%)	2.1	2.9	-	-	-	-	-
	Ethanol 96% (milliliters)	35	35	10	10	10	10	10
	Water (milliliters)	-	-	30	30	30	30	30
	Hydroxypropyl cellulose (%)	0.35	0.63	0.21	0.15	1.6	1.04	1.0
	Croscamellose (%)	0.42	0.58	2.4	0.63	1.9	1.46	1.25
	Microcrystalline cellulose (%)	34.17	31.25	35.42	37.5	33.3	32.71	35

The predicted amount of these formulations had a difference from the ones that were used in trial formulations. Predicted formulations are always obtained based on the results obtained in trial formulations, the best formula for formulation development will possibly be obtained in these formulations after optimization process is done.

Evaluation of flow property for powder granules of the above formulations was done; and the results are as seen in Table 12 below.

Table 12: Parameters for Determination of Powder Granules Flow Property

S/n	Parameter	F1	F 2	F3	F4	F5	F6	F7
1	Bulk density (g/milliliters)	0.64	0.59	0.49	0.51	0.41	0.52	0.55
2	Tapped density (g/milliliters)	0.68	0.659	0.50	0.54	0.43	0.58	0.524
3	Hausner's ratio	0.94	0.90	1.02	1.05	1.049	1.20	1.11
4	% Compressibility Index	5.9	10.5	12.4	9.3	7.3	11.4	8.5
5	Moisture content (%)	2.1	3.3	2.4	3.2	1.2	3.0	1.3

The presented results in table 12 above shows that the powder granules had a good flow property because all the results were within the acceptable range.

Tablets for the above predicted formulations were produced and evaluated. The results in Table 13 below are evidence showing that all formulations gave the desired outcome.

Table 13: Results for Tablets Evaluation of Predicted Formulations

Trial Formulation	**Maximum Disintegration Time (Min:Sec)**	**% Friability**	**Average Weight of Tablet**	**RSD of Tablet Weight**
F1	5:41	0.58	241.8	1.965
F2	5:44	0.4	239.6	1.02
F3	3:21	0.305	247.55	1.168
F4	1:15	0.15	242.04	1.09
F5	2:29	0.45	240.7	1.368
F6	3:21	0.35	241.2	1.301
F7	2:58	0.49	240.4	1.82

Based on the above results, the predicted formulation 5 had the best results compared to other predicted formulations. By using the formulation 5, three batches were produced, followed by evaluation of disintegration time, friability, tablet variation, tablet content uniformity, assay and dissolution (21). The average results of these three batches were calculated and compared to the dissolution and assay of the innovator drug (Coartem).

3.2.6 Dissolution

Six tablets were taken randomly from each three batches of the optimized formulations, and the dissolution test was conducted in three different dissolution buffer media (HCL buffer of pH 1.2, Acetate buffer of p H 4.5, and Phosphate buffer). The results are as seen in Tables 14 to 16 and in Figures 9 to 14.

Table 14: Dissolution Profile of Artemether and Lumefantrine in 0.1 HCL p H 1.2 Buffer Media

Time in minutes	**Lumefantrine in HCLpH 1.2**		**Rt – Tt**	**(Rt – Tt)²**	**Artemether in HCL p H 1.2**		**Rt – Tt**	**(Rt – Tt)²**
	Coartem (Rt)	**optimised (Tt)**			**Coartem (Rt)**	**optimised (Tt)**		
15	47.27	36.74	10.52	110.775	51.9	43	8.9	79.21
30	61.71	53.90	7.80	60.846	66	60.6	5.4	29.16
45	87.43	75.23	12.20	148.894	84.2	79	5.2	27.04
90	94.27	88.40	5.87	34.40628	96.9	90.7	6.2	38.44
120	98.92	99.42	-0.49	0.241724	99.5	96.7	2.8	7.84
180	100.00	100.95	-0.96	0.915846	100.2	97.47	2.73	7.4529
Total	489.59		34.94	356.0804	498.7		31.23	189.142
SIMILARITY FACTOR (F2) (Acceptance criteria = 50-100				**85.03**	**89.72**			
DIFFERENCE FACTOR (F1) (Acceptance criteria = 0 – 15)				**7.13**	**6.26**			

The above results presented in table 14 shows that within 45 minutes, more than 75% of the drug was already released for both Coartem and optimized formulation. Also, these results show that the reference drug and the drug of optimized formulation were comparable because the similarity (F2) and difference (F1) are within the acceptable range.

The above presented results are supported by the figures 9 and 10 below which shows the dissolution profiles of the innovator drug and the drug of optimized formulation.

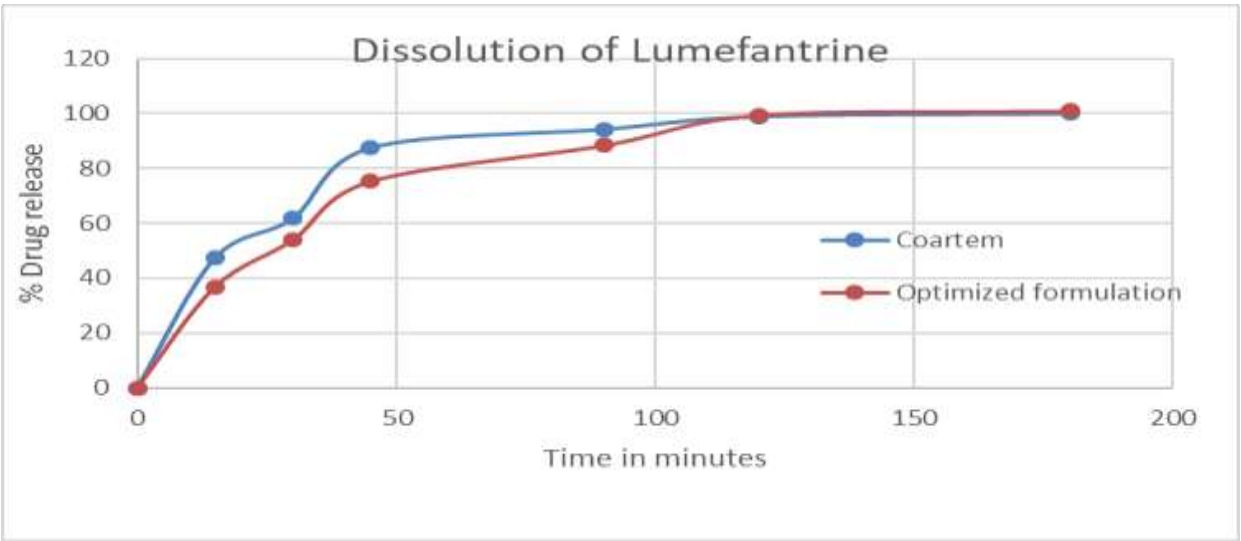

Figure 8: Percentage of Dissolution of Lumefantrine in Coartem and Optimized Formulation in HCL Buffer with a pH 1.2

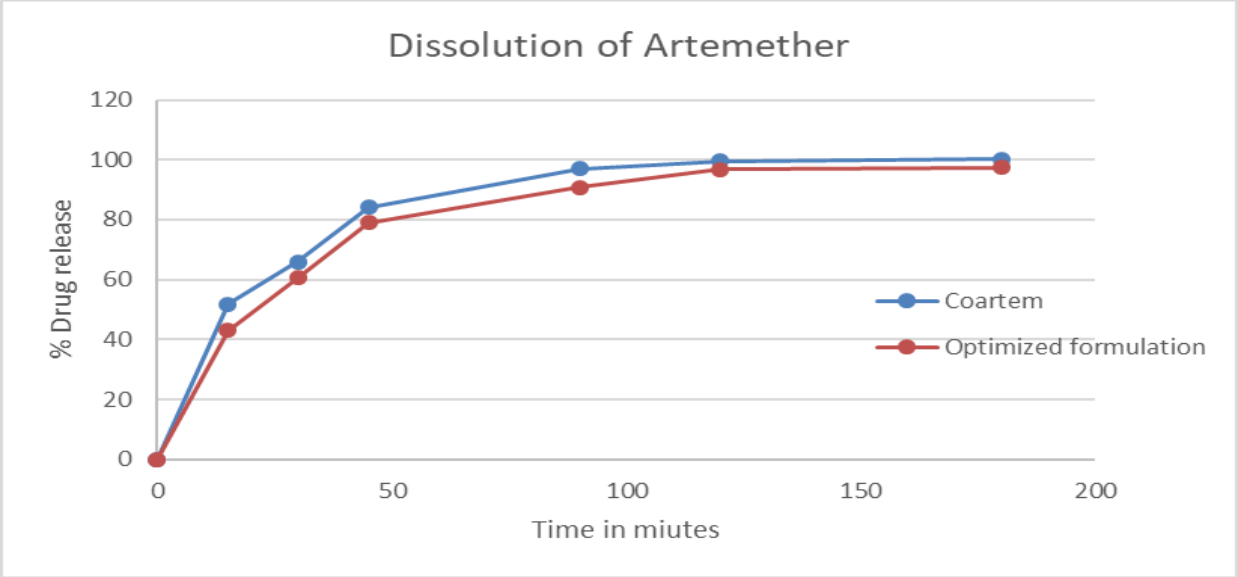

Figure 9: Percentage of Dissolution of Artemether in Coartem and Optimized Formulation in HCL pH 1.2

The reults presented in figure 9 and figure 10 are the graphical presentation of drug release shown in table 14 above. The graphs shows that there were no difference between the release of both Artemether and Lumefantrine when they were compared with that of innovator drug (Coartem).

Table 15: Dissolution Profiles of Artemether and Lumefantrine in Acetate Buffer pH 4.5

Time in minutes	**Lumefantrine in Acetate buffer pH 4.5**		**Rt – Tt**	**(Rt – Tt)²**	**Artemether in HCL p H 4.5**		**Rt – Tt**	**(Rt – Tt)²**
	Coarte m (Rt)	**F5 (Tt)**			**Coarte m(Rt)**	**F5 (Tt)**		
15	59.6	65.70	-6.081	36.985	48.08	36.19	11.89	141.48
30	68.6	76.06	-7.441	55.369	66.15	52.24	13.92	193.64
45	87.7	89.67	-1.911	3.655	76.38	81.25	-4.87	23.67
90	94.2	97.14	-2.897	8.397	90.17	96.29	-6.11	37.39
120	100.6	100.24	0.390	0.152	97.38	98.27	-0.89	0.79
180	100.9	100.98	-0.049	0.002	100.18	98.67	1.51	2.28
Total	511.7		-17.991	104.562	478.3461		15.45	399.25
SIMILARITY FACTOR (F2)				**84.09**	**93.19**			
DIFFERENCE FACTOR (F1)				**3.515**	**3.2**			

The above results presented in table 15 above shows that, within 45 minutes both formulations released more than 75% of the drug. The results above prove that the dissolution of optimized formulation was comparable with the dissolution of the innovator drug because the similarity factor (F2) and difference factor (F1) were within the acceptable ranges (35). These results were also supported by the graphs shown in figures 11 and 12 below.

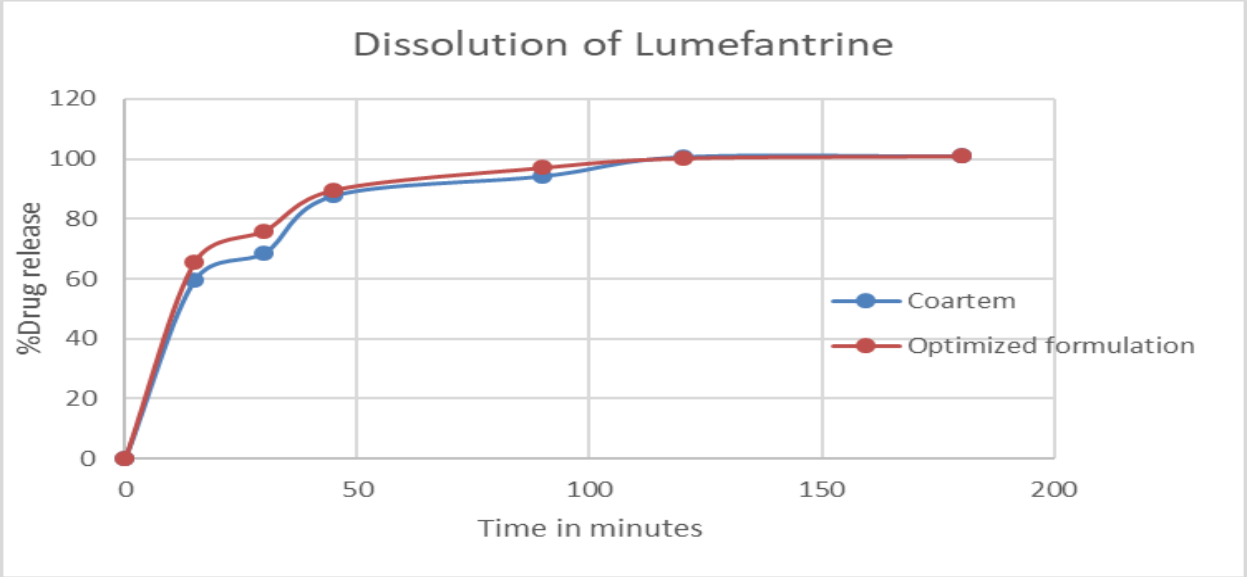

Figure 10: Dissolution Profile of Lumefantrine (120mg) for Coartem and Optimized Formulation (Medium: Acetate Buffer pH 4.5)

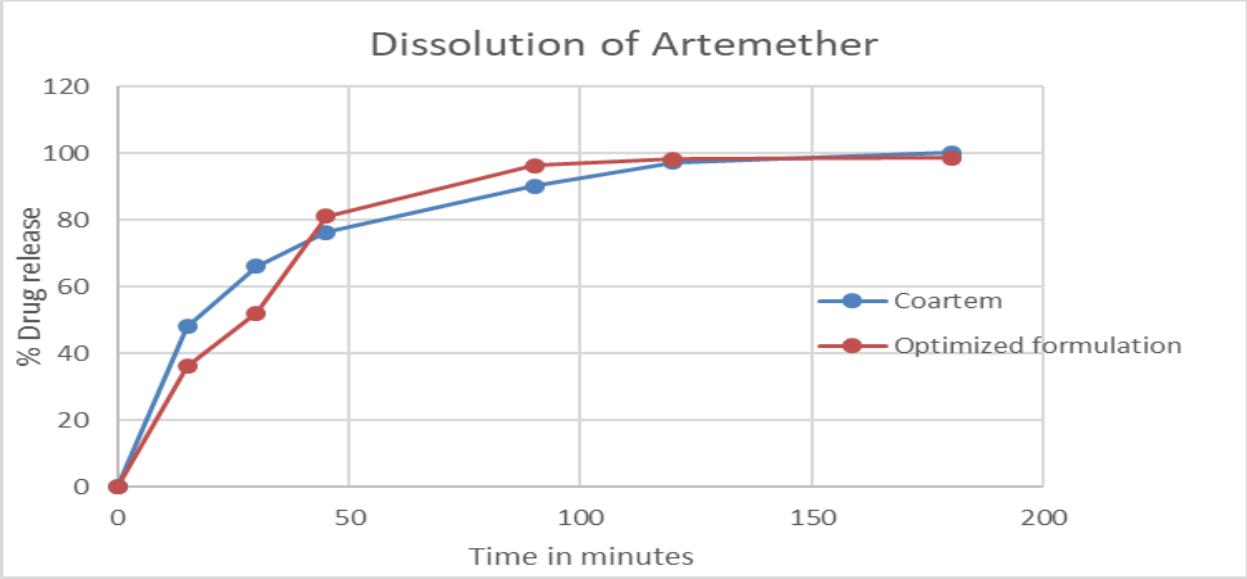

Figure 11: Dissolution Profile of Artemether (20mg) for Coartem and Optimized Formulation (Medium: Acetate Buffer pH 4.5)

The results presented in figures 11 and 12 are the graphical presentation of drug release as shown in table 15 above, the above figures support the results shown in table 15 that the drug of optimized formulation was comparable with the innovator drug because the similarities and differences were both within the acceptable ranges.

Table 16: Dissolution of Artemether and Lumefantrine for Coartem and Optimized Formulation in Phosphate Buffer p H 6.8

Time in minutes	Lumefantrine in Phosphate pH 6.8		Rt – Tt	(Rt – Tt)²	Artemether in Phosphate p H 6.8		Rt – Tt	(Rt – Tt)²
	Coartem (Rt)	F1 (Tt)			Coartem(Rt)	F1 (Tt)		
15	36	28	8	64	44.6	41	3.6	12.96
30	54	44.4	9.6	92.16	63.1	59.6	3.5	12.25
45	83.8	76.03	7.77	60.3729	84	78.9	5.1	26.01
90	86.6	83	3.6	12.96	92	87.3	4.7	22.09
120	93.3	89.4	3.9	15.21	94.3	91.6	2.7	7.29
180	97.9	95.1	2.8	7.84	98.3	96.2	2.1	4.41
Total	451.6		35.67	252.5429	476.3		21.7	85.01
SIMILARITY FACTOR (F2) (Acceptance criteria = 50-100				87.7	94.18			
DIFFERENCE FACTOR (F1) (Acceptance criteria = 0 – 15)				7.89	4.55			

The results presented in table 16 above shows that within 45 minutes, the dissolution of the drug was more than 75%. Also, the result show that the optimized formulation had the comparable dissolution profile with the innovator drug because the similarities and differences were within the acceptable limit. These results are supported by the figures 13 and 14 below

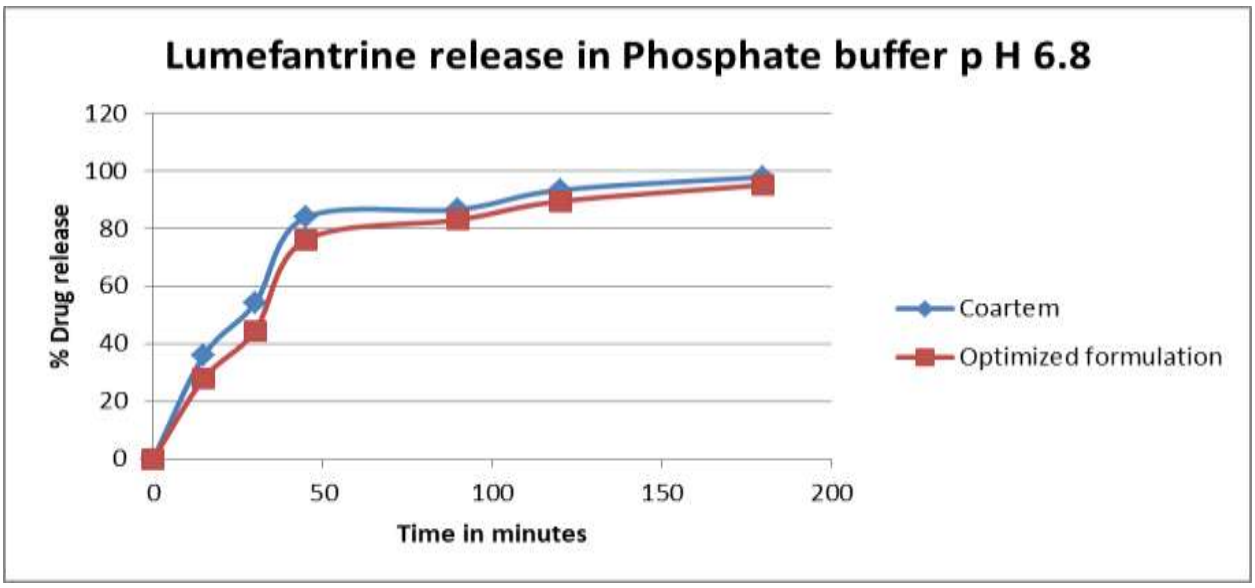

Figure 12: Dissolution Profile of Lumefantrine (120mg) for Coartem and Optimised Formulation (Medium: Phosphate Buffer pH 6.8)

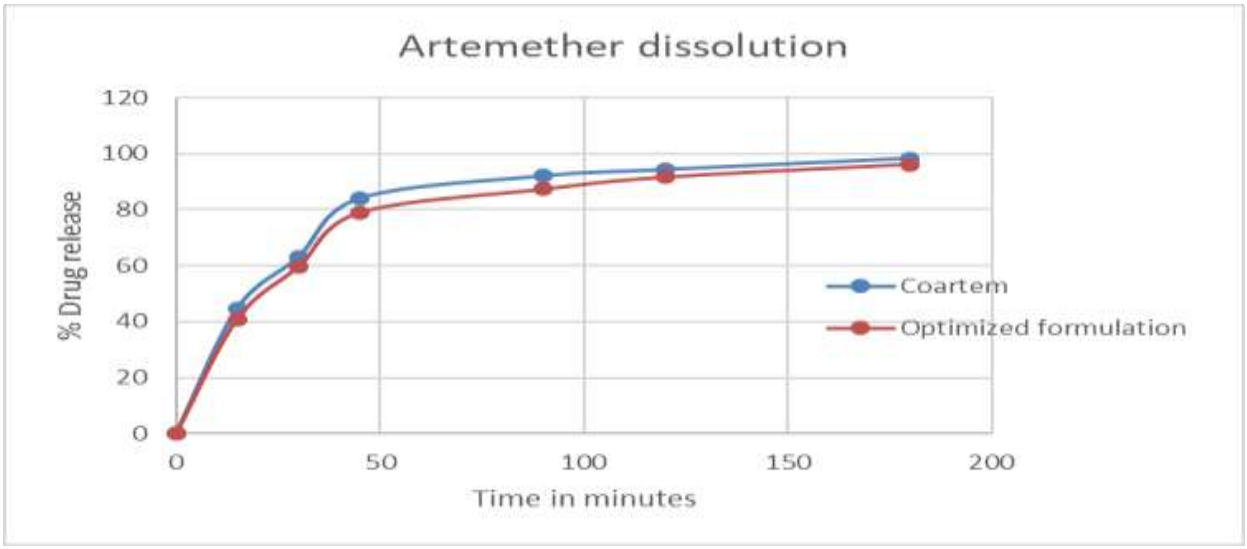

Figure 13: Dissolution Profile of Artemether (20mg) for Coartem and Optimized Formulation (Medium: Phosphate buffer pH 6.8)

Results presented in figures 13 and 14 support the results shown in table 16 which show that the dissolution of the innovator drug and the drug of optimized formulation are comparable.

After the dissolution tests, further evaluations for optimized batches were conducted as follows;

Tablet content uniformity

Ten tablets for each batch of optimized formulations were randomly taken and the assay was conducted to determine the content of API in each tablet separately. The average results are as seen in Table 17 below.

Table 17: The average Content Uniformity for optimized formulation

Tablet	BATCH OF THE OPTIMIZED FORMULATION	
	Artemether	**Lumefantrine**
1	97.4	95.2
2	100.7	105.1
3	98.2	99.5
4	95.3	105.6
5	95.1	103
6	97.7	103.3
7	98.1	102.2
8	98.8	99.3
9	97.2	101.7
10	102.5	101.2

The results presented in table 17 above shows that all examined tablets had the acceptable composition of drug content as specified in European pharmacopoeia, which is 95% to 105 ± 5%. (32).

Assay

For each concentration obtained, the assay was conducted by a validated HPTLC method; and the results are as seen in Table 18 below.

Table 18: Coartem and Optimized Formulation

Formulation	**Average weight (mg)**	**API content (%)**		**Content uniformity**		**Friability**	**Disintegration time (min:sec)**	**Relative standard deviation**
		Arte	**Lu**	**Arte**	**Lu**			
Coartem	-	100.4	101.3	-	-	-	-	-
Optimized formulation	241.2	98.1	99.2	98.04	101.6	0.65	2:29	1.368

The above results of table 18 prove that the optimized formulation has the drug content that is acceptable as stated in the International Pharmacopoeia (25).

CHAPTER FOUR

DISCUSSION

Formulation development involves stages that ensure the final product has a desired efficacy and safety to the consumer. The compatibility of API and potential Excipients is more important to be studied before the formulation process is initiated. During formulation, the evaluation of a formulated product should be conducted so as to have a final product that meets user requirement because the quality is always built in the process. This chapter explains how the study was conducted and what does the results obtained signify.

4.1 Pre-formulation Study

For the small amount of API that was taken for organoleptic property test, the results showed that Artemether was a white crystalline powder that is odourless and slightly bitter in test as specified in the certificate of analysis of this API. Lumefantrine was found to be a yellow powder which was odourless and bitter just as mentioned in the issued certificate of analysis. These results prove that the APIs used were the correct ingredients specified in the certificate of analysis and required in this study (8).

The binary mixture of APIs and excipients powder samples prepared and kept in three different environmental conditions (uncontrolled room temperature 30 ◦C ± 2 ◦C, climatic condition with temperature 40◦C ± 2◦C and relative humidity (RH) of 75±5%, oven with temperature of 50◦C), the observation of physical and chemical tests (assay) were made after every 14 and 90 days. The results showed no any change in colour or decrease or increase in the amount of APIs. Similarly, there was no any change in form or formation of new compound as it was observed when the NIR scanning was done at day 0 and day 90. These results conclude that the selected potential excipients were compatible with the APIs and trhe formulatin development can be done successifully as it was written in literatures (8).

4.2 Formulation Development and Optimization

In this study, wet granulation method was used based on the prior scientific knowledge; and the results obtained after conducting a sieve analysis showed poor distribution of particle size for

Lumefantrine. Furthermore, the powder mixture was found to have poor flow property with a compressibility index of 38 which is within the acceptable Pharmacopoeia range (17). Poor flow property of powder can result in unequal amount of powder that will be flowing in the die.

As a result, there will be variations in tablet weight and poor tablet content uniformity (21). Therefore, for this study to have a better formulation with good quality tablets, a wet granulation method was the best option.

Wet granulation method involved three main stages, namely dry mixing, binder preparation and wet mixing. During granulation, the use of Ethanol 96% was omitted in trial formulations number 4, 5, 6 and 7 by replacing it with distilled water. Since the use of alcohol (Ethanol) is limited in GMP because it is a class three solvent in ICH Q3. (13), it is suggested that its use should be minimized or avoided. Unfortunately, the solubility of the binder used (hydroxypropyl cellulose) depended on alcohol and not on water and, therefore, a small amount of alcohol had to be used to dissolve the binder. Similarly, in trial formulations 5, 6, and 7, the Sodium Lauryl sulphate ingredient was not used. Despite the fact that the mentioned materials were reduced and discarded, the flow properties of the powder granules and tablets quality had no significant difference with the ones that had all the ingredients incorporated in formulations number 1, 2 and 3.

Compression was done on a single station tablet machine (Korsh EK 02) by using a 10mm punch. In each batch, a specified number of tablets were taken randomly for evaluation of their friability, disintegration, dissolution, weight variation and tablet content (30).

4.3 Disintegration

All trial batches had good disintegration time within the acceptable range, which is less than 15 minutes for uncoated tablet (28). Although formulations F5, F6, F7 and F8 had the maximum amount of binder, their disintegration time was low compared to formulation F1, F2, F3 and F4, which had lower amount of binder. This is because, increase in the amount of binder was associated with the increase in the amount of disintegrant (30). These results signify that, increasing the amount of binder should be associated with the increase in the amount of disintegrant. Since the production of drugs should focus on the use of few and low amount of

excipients, formulations F1, F2, F3 and F4 should be considered as better than F5, F6, F7 and F8.

4.4 Friability

According to the findings, for all trial batches formulated, the percentage of friability was within the limits (less than 1% for uncoated tablets). Friability depends on the amount of binder used and the compression force. Specifically, the higher the amount of binder, the lower the friability and the vice versa is true. Also, the higher the compression force, the lower the friability. Despite the fact that all the trial formulations had good friability, formulations 5 and 6 (F5 and F6) showed a bit high time of disintegration, and therefore, it would be advisable to reduce the compression force and amount of binder (30).

4.5 Tablet Weight Variation

All batches in trial and predicted formulations underwent the tablet weight variation tests. Relative standard deviations of tablet weight were calculated and the results revealed that all the tablets tested in each batch had the acceptable weight variation range (not more than 7.5%). There were acceptable deviations in the tablet weight because all the powder granules prepared showed a good flow property and there was no any sticking of powder granules to the punch. In most cases, tablet weight variation is mainly caused by poor powder flow that results in variations in the volume of powder filled in the die, other factor that may lead to tablet variation is sticking of powder granules to the punch (30).

4.6 Tablet Content Uniformity

For 10 tablets of each batch of optimized formulations that were evaluated, the results revealed that they all had the acceptable range of API content (95% to 105%). These results proved that the homogeneity of the mixed ingredients and excipients were achieved. The drug particle size was controlled so as to avoid agglomeration. Furthermore, a proper selection of filler, binder and mixing scheme were taken into consideration because these are the main reasons for content uniformity failure (30).

4.7 Assay

Based on the results obtained, the average results of the optimized formulations had the API content that is within the acceptable range. The acceptable range for assay is 95% to 105% (30). These results prove that the mixing process of excipients and APIs was perfect, therefore the formulated tablets are of the required quality as stated in the International Pharmacopoeia.

4.8 Dissolution

The independent approach model that uses similarity (f2) and difference (f1) factor were used to compare the dissolution profiles of optimized formulation with that of reference drug (Coartem). Drugs of formulation 5 and that of innovator had percentage drug release of more than 75% after 45 minutes of dissolution (33). Also, the percentage difference and similarity for drugs of formulation 5 and innovator drugs were calculated and the results proved that both optimized formulation and innovator drug are comparable because the similarity (F2) and difference (F1) factors had the acceptable values for both two dissolution media (HCL buffer 1.2 pH Acetate buffer 4.5 pH and Phosphate buffer of 6.8 pH). Since comparative dissolution the dissolution test is one of the critical parameter, good results obtained in this test proves that the drugs obtained by this optimized formulation (F5) are comparable to the innovator drug.

CHAPTER FIVE

LIMITATION, CONCLUSION AND RECOMMENDATIONS

5.1 Limitation

All experiments were conducted successfully despite of the following;

i. Time was a limiting factor. Due to the presence of COVID 19, lab work was not conducted for 60 days that made a pre-formulation study to be conducted in the interval of day 0, 14 and 90 only, instead of day 0, 7, 14, 30, 60 and 90.

Because the results of day 90 revealed that the mixture was still stable (no any change of physical, chemical and the amount of API), this proves that even at day 30 and 60 the mixture was also stable.

5.2 Conclusion

A formulation of Artemether 20mg/Lumefantrine 120mg fixed dose combination tablet for treatment of Malaria has been developed and optimized by using a wet granulation method. The optimized formulation used 10 milliliters of Ethanol 10% together with Artemether 20mg, Lumefantrine 120 mg, 2 milliliters of Polysorbate 80, Aerosil 4.75mg, Hydroxypropyl cellulose 3.5 mg, Croscarmellose 4.5 mg and Microcrystalline cellulose 80 mg. The use of Magnesium stearate as lubricant and the presence good proportion of binder and disintegrant, gave good results on powder flow and dissolution profile of the drug. The of use Sodium Lauryl Sulfate which is mainly used in the innovator drug tablets as solubilizing agent and lubricant was not utilized in the optimized formulation. All evaluations were done according to the US Pharmacopoeia of 2014 and British Pharmacopoeia, fourth edition of 2017. The tablets of this formulation were compared to a reference drug product (innovator drug) that was also evaluated the same way. The results showed that the drug product of this formulation is comparable to that of innovator drug (Coartem).

5.3 Recommendations

Scale up by our pharmaceutical industry may be done by adopting this formula but should adhere to the official compendia.

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