A COMPARISON OF PHARMACOKINETICS OF TWO TABLET FORMULATIONS CONTAINING ARTEMETHER / LUMEFANTRINE – QUALITY CRITERIA FOR MALARIA TREATMENT ASSURANCE

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By

Ignace Alphonce

A Dissertation Submitted in Partial fulfillment of the requirement for the Degree of Master of Science in Clinical Pharmacology of the Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certifies that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation titled: **A Comparison of Pharmacokinetics of two Tablet Formulations containing Artemether/Lumefantrine –Quality Criteria for Malaria Treatment Assurance**, in partial fulfillment of the requirement for degree of Master of Science in Clinical Pharmacology of the Muhimbili University of Health and Allied Sciences.

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DECLARATION

I, **Ignace Alphonce**, declare that this dissertation is my original work and that it has not been presented and will not be presented to any other University for similar or any other degree award.

Signature…… Date……..

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Lastly but not least I thank SIDA-Sweden for their financial support though MUHAS Capacity Strengthening Subprogram.

DEDICATION

This dissertation is dedicated to my parents, Mr. and Mrs. Ignace Benedict Marealle. I will keep on remembering the hard work you did in making sure that I reach this level. I love you very much and God bless you.

EXECUTIVE SUMMARY

Background: Treatment of non-severe malaria remains a challenge to endemic areas including Tanzania. Since November 2006, Coartem® an artemisinin based combination therapy (ACT) containing artemether-lumefantrine (ALu), replaced sulphadoxine/pyrimethamine (SP) as first line drug for treatment of uncomplicated malaria in Tanzania because of emergence and spread of SP resistance to *Plasmodium falciparum*. Currently a number of generic artemether-lumefantrine drugs are available in resource limited settings such as Tanzania and yet few pharmacokinetics (PK) and bioequivalence (BE) data in these populations are available. Considering the liability to substandard manufacturing, there is a need to assess quality of generic ALu tablet formulations.

Objective: We assessed the quality of the most prevalent generic artemether-lumefantrine tablet formulation available in the Tanzanian market using clinical study for bioequivalence.

Methodology: Survey of available generics of artemether-lumefantrine tablet formulations was carried out in retail pharmacies in Dar es Salaam in which the most widely available generic was sampled (Artefan® from India) for quality assessment. The randomized, 2-treatment cross over study was conducted in 18 healthy Tanzanian male volunteers. Each volunteer received Artefan® (test) and Coartem® (reference) formulation under fed condition separated by 42 days of drug-free washout period. Serial blood samples were obtained over 168 hours after oral administration of each treatment. Quantitation of lumefantrine plasma levels was done using HPLC with UV detection. Formulation lumefantrine bioequivalence was assessed in accordance with the US Food and Drug Authority (FDA) bioequivalence criteria.

Results: All eighteen enrolled volunteers completed the study and both test and reference drug formulations were well tolerated. The mean ± SD for lumefantrine primary PK parameters for bioequivalence C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$ under fed condition for artefan®: coartem® were (4206.93±2942.48: 4438.81±2548.43), (6.11±2.70: 6.22±2.16), (123758.90±83527.51: 135430.70±86814.81) and (138189.70±94959.77: 149530.20±95109.42) respectively.

Ratios for geometric means of bioequivalence parameters for lumefantrine C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of artefan® to coartem® and 90% confidence interval limits were 84.01% (49.44% - 142.76%), 84.49% (52.70% - 136.81%) and 84.26% (52.46% - 135.35%) respectively. The geometric mean ratios (artefan® to coartem®) for lumefantrine C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were all within FDA recommended bioequivalence limits of 80% – 125%, but 90% confidence intervals were outside FDA recommended limits of 80% - 125%.

Conclusions: Although the ratios of AUCs and C_{max} were within the acceptable FDA range, bioequivalence between artefan® and coartem® tablet formulations was not demonstrated due to failure to comply with the FDA 90% confidence interval criteria. However based on the observed total drug exposure (AUCs) in comparison to other studies carried elsewhere, artefan® is likely to produce a similar therapeutic response as Coartem®.

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LIST OF ABBREVIATIONS

ACC	Analytical Clinical Concepts
ACT	Artemisinin based Combination Therapy
ALP	Alkaline Phosphatase
ALT	Alanine transaminase
ALu	Artemether-Lumefantrine
AST	Aspartate transaminase
AUC	Area Under the Curve
BA	Bioavailability
BE	Bioequivalence
BMI	Body Mass Index
CI	Confidence Interval
ECG	Electrocardiography
EO	Eosinophils
FBC	Full Blood Count
FDA	Food and Drug Authority
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HCT	Hematocrit
HGB	Hemoglobin
HPLC	High Performance Liquid Chromatography
IHI	Ifakara Health Institute
IS	Internal Standard

KCTF	Kingani Clinical Trial Facility
LLOQ	Lower Limit of Quantitation
LYMPH	Lymphocytes
MONO	Monocytes
MSD	Medical Stores Department
MUHAS	Muhimbili University of Health and Allied Sciences
NEUT	Neutrophils
PK	Pharmacokinetics
PLT	Platelets
QC	Quality control
QCH	Quality Control High
QCL	Quality Control Low
QCM	Quality Control Middle
RBC	Red Blood Cells
SD	Standard Deviation
SP	Sulphadoxine/Pyrimethamine
STD	Standards
TaSUBa	Institute for Arts and Culture Bagamoyo
TFDA	Tanzania Food and Drug Authority
WBC	White Blood Cells
WHO	World Health Organization

CHAPTER 1

1. Introduction

Malaria remains the most common public health problem in Tanzania and is the number one cause of morbidity and mortality especially in children below five years of age.[1] Since November 2006, Coartem® (Novartis Pharma, Basel Switzerland) an artemisinin combination therapy (ACT) containing artemether-lumefantrine (ALu) replaced sulphadoxine/pyrimethamine (SP) as first line drug for treatment of uncomplicated malaria in Tanzania due to development and spread of resistance of SP to *Plasmodium falciparum*.[2] Currently a number of generic artemether-lumefantrine drugs are available in resource limited settings including Tanzania and yet few pharmacokinetics (PK) and bioequivalence (BE) data in these populations are available.

The availability of some generic brands of ALu in the drug market today complicates the choice of a suitable brand or the possibility of switchability. In fact there are growing concerns that various ALu formulations available in the market may have different qualities. ALu is currently considered as the most highly effective and efficacious drug for treatment of uncomplicated malaria in Tanzania. [3-5]However, differences in the quality of some of its generics may lead to development of resistance of ALu to malaria parasites. Thus the quality of different brands of ALu available on the Tanzanian market needs to be evaluated and monitored from time to time as most drug manufacturers do not maintain the quality of drugs as initially submitted in dossiers for registration applications.

For diseases like malaria where progression from mild to severe disease is rapid, using drugs with little or no active ingredient has been said to be “tantamount to murder.” Using drugs with no active ingredient or with the wrong active ingredients means the patient will not be cured of malaria and there is a high chance such a patient will die. Giving patients anti-malarial drugs which can result into sub-therapeutic plasma levels means increased likeliness to development of drug resistance to malaria parasites. This in turn may lead to switching into newer and in most cases more expensive drugs. [6, 7]

While generic formulations are promoted to address accessibility and affordability of drugs, reports on the quality of medicines in African countries have generally portrayed generics as poor quality or fake products. [8, 9]

Our study assessed the quality of ALu tablets available on the Tanzanian market. The main focus being on comparative lumefantrine bioavailability between the most available generic drug in the market with the innovator's drug Coartem®.

1.1. Problem Statement

The introduction of ACTs in the National Guidelines for Diagnosis and Treatment of Malaria in 2005 led to change in malaria treatment approach in Tanzania. This necessitated the introduction of artemether-lumefantrine under the brand name of Coartem® (Novartis Pharma, Basel Switzerland) as a first line treatment for uncomplicated malaria. Thereafter, subsidized ALu was made available in all the public health facilities and now in the private sector. The cost of unsubsidized Coartem® available in the private sector is however significantly higher than the previously used first line antimalarial drugs such as chloroquine and sulfadoxine-pyrimethamine (SP). At current market prices, unsubsidized Coartem® costs 10 to 20 times more than chloroquine and SP. This has necessitated the need to introduce generic drugs from multiple sources in order to improve the availability, affordability and accessibility to this life serving drug. The government is currently supplying to the private sector subsidized ALu tablets to be sold at a price of 1000 Tanzanian shillings per dosage for both Coarten® and generics. The justification for generics might also be the concern of sustainability of the donor funded supplies. While generic formulations are introduced to address accessibility and affordability of drugs, reports on the quality of medicines in African countries have generally revealed generics as poor quality or fake products. [8, 10, 11]As such, the quality of generic ALu tablets in Tanzania has not been documented.

1.2. Study rationale

Poor quality of generic anti-malarial drugs has been associated with treatment failure, drug resistance, drug toxicity and health burden to both individual and state. To increase the availability, accessibility and affordability various generic drugs containing ALu are currently available on the Tanzanian market. Given the liability to substandard manufacturing, the issue of quality is of concern. Identifying poor quality drugs in terms of bioavailability could help improve the safety and effectiveness in the treatment of malaria through using ensured quality generic drugs.

1.3. Research Question

We wanted to find out whether generic brands of ALu tablets found on the Tanzanian market are of acceptable quality in terms of oral bioavailability.

1.4. Objectives

1.4.1. Primary objective

1.4.1.1. To determine the bioavailability and assess bioequivalence of the most available ALu generic tablet formulation in the Tanzanian market in relation to the innovator's drug Coartem®.

1.4.2. Secondary objectives

1.4.2.1. To determine the most prevalent generic ALu tablet formulation in the Tanzania market

1.4.2.2. To determine lumefantrine bioequivalence between the most prevalent ALu generic and Coartem®

1.4.2.3. To compare other lumefantrine pharmacokinetics parameters of the most prevalent ALu generic with those of Coartem®

CHAPTER 2

2. Literature review

Artemisinins are very rapidly acting blood schizonticides against all human malaria parasites with no effect on hepatic stages. Artemether, an artemisinin derivative is available as a single drug and as a fixed-dose combination with lumefantrine. Recrudescence is frequent when artemether is used as a monotherapy. [12]In the areas with multidrug resistance, many authorities advocate combination therapy with an artemisinin as the optimal treatment for falciparum malaria. Currently, WHO recommends the use of ACTs in all endemic areas and many countries including Tanzania have adopted this recommendation. [2]

Lumefantrine is an aryl alcohol related to halofantrine. Lumefantrine as with halofantrine oral absorption is highly variable and is improved when is taken with fatty foods.

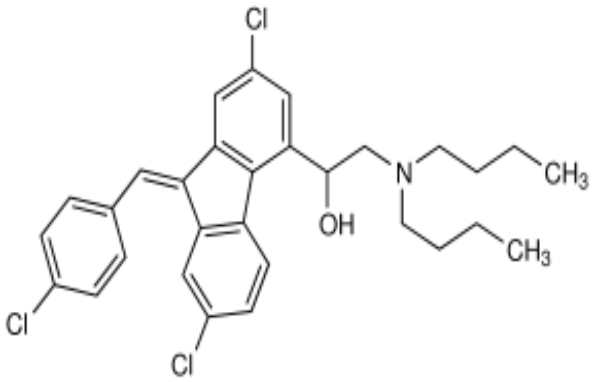

Figure 1.2: Chemical structure of lumefantrine

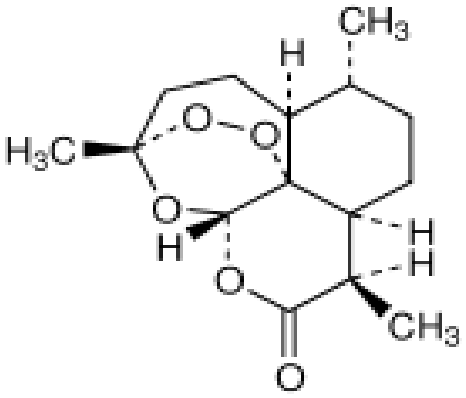

Fig 2.2: Chemical structure of artemether

The importance of lumefantrine in the combination is due to its longer elimination half-life of up to 10 days and thus it is associated with a low recrudescence rate. ALu therefore combines the benefits of the fast onset of action of artemether with the long duration of action and high cure rate of lumefantrine in a single oral formulation. It is highly efficacious even against multi drug resistant malaria parasites with clearance of the parasites from the blood within 2 days. In Tanzania, this drug is currently recommended as first line treatment of uncomplicated malaria. [2, 12]

Lumefantrine day 7 plasma concentration has been used to predict treatment outcome in malaria patients. Patients who had lumefantrine levels below 175ng/ml on day 7 are more likely to experience recrudescence by day 42 allowing prediction of treatment failure with 75% sensitivity and 84% specificity. [13]

Availability of substandard antimalarial products circulating on the market of developing countries poses a challenge on malaria control programs of many of these countries especially where analytical laboratories of the drug regulatory agencies are poorly developed. Substandard pharmaceutical products contribute to the emergence of resistance, as their use may result in low bioavailability, which may result in sub-therapeutic concentration. This, in turn, may promote the development and spread of drug resistance. In Tanzania for example, many studies have indicated the existence of poor quality antimalarial drugs. [6, 7, 9, 11, 14-17] The use of poor quality antimalarial drugs has been reported to be one of the key sources of treatment failure and eventually development of drug resistance.[3-5] The use of poor quality antimalarial drugs could be one of the major reasons which contributed to emergence of chloroquine and SP resistant *P. falciparum* strains. [18-21]

There are already reports of circulating substandard artesunate containing antimalarials in Kenya, DR Congo and south-east Asia.[22, 23] Substandard artesunate containing drugs will lead to emergence of ACT resistant strains as it has been already reported.[24-27] A number of studies carried out in Tanzania and elsewhere in the world to evaluate the pharmacokinetics and bioequivalence of different generic antimalarial drugs, like different brands of sulphadoxine-pyrimethamine; chloroquine and some dihydroartemisinin based tablets revealed an existence of low quality drugs in terms of one or more quality parameters such as dissolution, friability, disintegration and relative bioavailability. [3, 28-31]

This creates the need for continuous monitoring of the quality of antimalarial drugs marketed in Tanzania. Therefore this study assessed the quality of generic ALu tablets available in the Tanzanian market through comparison of lumefantrine oral bioavailability of the sampled most available generic in the market (artefan®) with Coartem®.

CHAPTER 3

3. Methodology

3.1. Determination of the most prevalent generic

A cross-sectional survey was carried out in which a list of all pharmacies in Dar es Salaam was obtained from TFDA. Thirty percent of the pharmacies were randomly sampled using lottery method whereby each name of the total 305 community pharmacies was written on a piece of paper and folded. These pieces of papers were picked randomly one at a time until the required sample of 90 pharmacies was reached. The selected retail pharmacies were visited by research assistants with a checklist and documented all of available generic ALu tablet formulations. The generic drug which was found in majority of the visited pharmacies was regarded as the most prevalent.

The survey revealed three generic ALu tablet formulations found in the Dar es Salaam market. These generics (% of pharmacies in which the product was found) were *artefan*® from Ajanta Pharma Ltd-India (72.7%), *lumaterm* from Cipla-India (31.8%) and Artemether-Lumefantrine (which did not have a branded name) from Ipca Laboratories Ltd-India (68.2%). All of them are registered in Tanzania.

Artefan® was found to be the most commonly dispensed generic and therefore for the purpose of this study it was selected for quality assessment.

3.2. Sampling of tablets

Artefan® tablet formulations were purchased from SALAMA pharmacy (wholesale pharmacy) through Alfo pharmacy (retail pharmacy) in Dar es Salaam. Two hundred and twenty four tablets of the same batch number equivalent to ten adult doses were purchased. Artefan® (test formulation) was a fixed-dose combination tablet consisting of 20 mg of artemether and 120 mg of lumefantrine (B.No./No.LOT.P0511H, TAN 09.085.PO1BAJA, expiration date July 2013; Ajanta Pharma limited, Made in India). The reference formulation; Coartem® (720 tablets of the same batch number) was donated by Dodoma Regional Hospital. Coartem® was a fixed-dose combination tablet consisting of 20 mg of artemether and 120 mg of lumefantrine (Batch No.F2491, expiration date August 2013; Novartis

Pharmaceuticals Corporation, Suffern, New York, USA for Novartis Pharma AG, Basle, Switzerland, under license from the PRC).

3.3. Clinical study

3.3.1. Study design

This was a randomized, single dose, open label, single center, two period, two sequence crossover BE trial. The study was performed in accordance with ICH-GCP guideline and laboratory analysis according to GLP. The study protocol was reviewed and approved by the MUHAS and IHI institutional review boards. To ensure confidentiality of study subjects, the names of the volunteers were not used, instead study ID code number were used.

3.3.2. Study area

The study was conducted at Kingani Clinical Trial Facility (KCTF) located in Bagamoyo district. KCTF is a newly established research facility which is part of Bagamoyo Research and Training Centre of Ifakara Health Institute (IHI) specially designed to conduct early phase studies where volunteers can be retained for the entire period of study. The facility has dedicated areas for essential study procedures including volunteer screening, blood sample collection, investigational product preparation, ICU for management of serious adverse events and research wards. Other facilities include a kitchen, washing area and lounge where volunteers can dine and relax. Determination of lumefantrine concentration in plasma samples was performed at MUHAS-Sida bioanalytical laboratory.

3.3.3. Study participants

These were healthy adult male college students from TaSUBa Bagamoyo in Coastal region.

3.3.3.1. Inclusion criteria

a) Healthy male > 18 years of age.
b) Availability during entire study period (two months).
c) Willingness to give written informed consent after being informed of the nature of study.
d) Body mass index (BMI) between 18 and 30 kg/m^2.

e) No history of antimalarial drug ingestion in the past one month.

f) Normal Laboratory range parameters from all performed laboratory tests at baseline (FBC, ALT, ASAT, ALP, serum createnine, total bilirubin and albumin)

g) Must be literate, can speak English and understand written English.

3.3.3.2. Exclusion criteria

a) Female

b) Hypersensitivity to artemether and/or lumefantrine or related compounds.

c) History of conditions that may alter absorption, metabolism, or passage of drugs out of the body (Sprue, Celiac disease, Crohn's, colitis, liver, kidney, or thyroid conditions).

d) History of mental illness, drug addiction, drug abuse.

e) A hematocrit value of $\leq 37.0\%$.

f) Receipt of an investigational drug within 4 weeks prior to study drug dosing.

g) Currently taking any prescription of systemically acting medications, within 7 days prior to study dosing or over-the-counter medication within 3 days of study dosing.

h) Smoking

i) Positive blood slide for malaria

j) Any abnormal biochemistry result

k) Any abnormal hematology result

l) Regular use of any drugs known to induce or inhibit hepatic drug metabolism (examples included barbiturates, carbamazepine, rifampicin, phenytoin, phenothiazines, cimetidine, omeprazole, macrolides, imidazoles, fluoroquinolones) within 30 days prior to study.

3.3.4. Participants Recruitment

Recruitment was done through sensitization meeting which was conducted at the college premises. The recruitment was carried out at KCTF in Bagamoyo. Permission to conduct sensitization meetings was sought from College administration and thereafter advertisement for the meetings was posted on the college notice board. Interested participants were asked to register their names and

were invited to attend sensitization meeting where detailed explanation and clarification about the study was given followed by screening of potential subjects in which the eligible participants were drawn from.

3.3.5. Screening for eligible participants

Screening was performed by IHI clinicians to all potential subjects at baseline. It was performed based on the inclusion and exclusion criteria to get eligible participants which were then enrolled. Baseline screening included taking medical history, performing physical medical examination and blood laboratory tests. Physical medical examination included taking vital signs and ECG. Laboratory tests included parasitology (blood slide test for malaria), haematology (FBC) and biochemistry (urea, createnine, total bilirubin, ALT, AST, ALP and albumin).

3.3.6. Enrollment

Eligible subjects were those who fulfilled the inclusion criteria and none of the exclusion criteria. Out of 31 screened subjects 18 were enrolled into the study. The enrolled subjects were randomized into treatment sequence and admitted at KCTF to undergo study related procedures described below.

3.3.7. Randomization and study procedures

Eighteen random treatment numbers were generated (Microsoft Visual FoxPro version 9.2). These numbers were randomly assigned to the two sequences of treatment i.e. RT (Period 1, reference drug and period 2, test drug) or TR (Period 1, test drug and Period 2, reference drug). This was done using block randomization in three blocks of six numbers (SAS version 9.2 for Windows Inc. NC, USA). Eligible volunteers were assigned any of the treatment numbers by the enrollment clinician. At drug administration a sealed envelope with the corresponding treatment number, which contained the drug allocation was opened by the pharmacist and appropriate drug was administered. The investigators were blinded on the drug the volunteer was taking. The randomization code was kept in the IHI Data Unit and was broken after all the PK analysis had been completed.

3.3.8. Drug administration

Each enrolled healthy volunteer received a single adult dose (80/480mg artemether and lumefantrine respectively) of either Coartem® or artefan® depending on his treatment randomization sequence and after a 42 day washout period the volunteers took the alternative drug (figure 1.3).

In order to facilitate absorption; tablets were swallowed with a glass of water (200ml) under supervision followed by standardized fatty food.

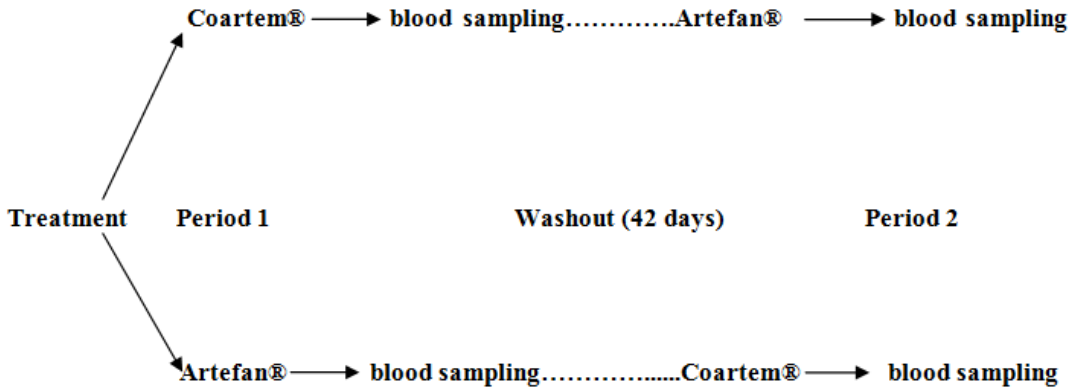

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Figure 1.3: Schematic presentation of the 2 period cross over trial

3.3.9. Blood sample collection and follow-up

On the day of enrolment participants were admitted and retained at the KCTF for three days (72 hours). Subjects were discharged and returned to KCTF at 168 hours (day 7) for a blood sample and allowed back home.

After a 42 days washout period, volunteers were recalled and retained at KCTF for another 3 days (72 hours). Subjects were discharged and returned to KCTF at 168 hours for one blood sample donation and allowed back home. On each period all subjects underwent clinical evaluations throughout the study to monitor for adverse drug reactions and assess medication tolerability at the following hour 0.5, 2, 4, 6, 8, 10, 12, 24, 48, 72 and 168 post each dose.

3.3.9.1 Blood sampling and sample processing

The enrolled volunteers arrived at the KCTF for stay after an overnight fasting. Subjects had a heparinized saline lock placed in an arm to obtain serial venous blood samples for plasma drug concentrations for the first 72 hours. Each subject had a predose blood sample drawn (time = 0) and took an observed dose of the test or reference ALu formulation with 200 mL of water followed by standardized fatty meal. Subsequent blood samples were drawn at 2, 4, 6, 8, 10, 12, 24, 48, 72 and 168 hours after the dose. After a 42-day washout period, the subjects took an observed dose of the alternative ALu formulation. Study procedures and blood sampling were repeated as described previously. Each subject had 22 blood samples drawn over the course of the study (3 ml each) for determination of lumefantrine plasma concentrations. The total amount of blood sample collected in the whole study period from each volunteer was 66 ml.

The blood samples were collected in heparinized vacutainers. Each vacutainers was appropriately labeled with Brady number, subject's identification number (ID), sampling time, sampling hour and date. Blood samples were kept in a cool box and transported within 45 minutes after sampling to the IHI laboratory about 2 km from KCTF for further processing including centrifugation at 1800xg at 4^0c to obtain plasma samples which were transferred into similarly labeled cryovials. The plasma samples were kept in IHI freezer at -80^{o}C until the day of transferring to MUHAS in which they were carried in a cool box packed with ice. At MUHAS plasma sample were kept at -80^0C until the day of analysis.

3.3.10. Participants Sample size

A total of 18 eligible volunteers were enrolled to participate in this study. FDA allows a minimum number of volunteers to be 12 for bioequivalence study.[32] The number of volunteers can be higher than 12 depending on the drug intra-subject variability so as to ensure at least 80% power. [32]Written informed consent was obtained from each volunteer before participation.

3.4. Bioanalytics

Blood samples were analyzed at MUHAS-SIDA Bioanalytical laboratory. The plasma analysis for Lumefantrine determination was done using an HPLC method with UV detection. The method used has been developed by ACC laboratory in German and validated in the MUHAS-SIDA Bio-analytical Laboratory. Details of the method have been published. [33]

3.4.1. Method validation

The method was validated in which inter-day method; linearity, precision and accuracy were assessed by processing one batch each day for three different days. Validation batches consisted of extracts of blank plasma spiked with internal standard, 8 calibration samples (50, 100, 200, 500, 1,000, 2,000, 5,000 and 10,000 ng/ml) and hexaplicates for each of the 4 QC samples (50, 100, 1,000 and 8,000 ng/ml).

3.4.2. Preparation of standard solutions, calibration and quality control samples

Lumefantrine stock solution was prepared by dissolving 10 mg of lumefantrine (double weighing) in a mixture of methanol: acetic acid (99.8:0.2, v/v) up to 20.0 ml. For standard solutions preparations, different volumes of the stock solution were diluted using 0.1% acetic acid solution in methanol: water (1:1, v/v) up to 20 ml. For preparation of the standard curves, 50.0 µl of the respective standard solution were added to 500.0 µl of blank plasma. The calibration curves prepared were in a concentration range of 0.05-10.0 µg/ml.

Lumefantrine quality control solutions were obtained by dilution of the stock solution to achieve 80.0 µg/ml, 10.0µg/ml, 1.0 µg/ml and 0.50 µg/ml as high, middle, low level quality control samples and lower limit of quantitation (LLOQ) respectively. Final QC samples were prepared by adding 50.0 µl of each QC solution to 500.0 µl of plasma. Halofantrine (internal standard) stock solution was prepared by dissolving 10.0 mg into 20.0 mL of methanol which was then diluted 4 times in methanol to obtain working internal standard solution.

3.4.3. Preparation of samples for HPLC analysis

Pooled blank plasma (500.0 µl) was mixed with 50.0 µl of lumefantrine standard solutions (for calibration/standard curve); 50.0 µl of the internal standard (halofantrine: 100.0 µg/ml); and 50.0 µl of hydrochloric acid (0.1 M). The mixture was vortexed for 5 s at 2000 rpm, then 2 ml of diethyl ether: ethyl acetate (2:1 v:v) was added and the mixture was vortexed for 20 sec at 2000 rpm and then centrifuged for 10 min at 2800 g. The organic layer (1200.0 µl) was transferred into a tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The nitrogen gas was purchased from Tanzania Oxygen Limited, Dar es salaam, Tanzania. The nitrogen gas evaporation system was designed by the MUHAS Bioanalytical laboratory team and assembled locally by a blacksmith. The residue was reconstituted in 300.0 µl of mobile phase and vortexed for 2 s at 2000 rpm. The solutions were transferred into auto sampler vials and 20.0 µl was injected into the chromatograph.

3.4.4. Chromatographic conditions

The mobile phase was prepared by dissolving 4.76 g of di-potassium hydrogen phosphate tri-hydrate in 350 ml distilled water. The obtained solution was mixed with 650 ml acetonitrile and the mixture was adjusted to a pH of 3.1 with ortho-phosphoric acid. The pre column (LiChrospher 100) RP 18, 5 µm; 5×4 mm and the column (LiChrospher 100) RP18, 5 µm; 125 × 4 mm was used. The flow rate was 1.2 ml/min, detection was achieved at 335 nm and the total run time was 20 min.

3.4.5. Analysis of test samples

Unknown volunteer samples were run in 8 different batches each with its own calibration curve and three QCs in triplicates.

A total of 394 test plasma samples were analyzed in the eight batches. Procedures for analysis of test samples were similar to those carried out in method validation except that for each test sample 50µl of methanol was added to the extraction mixture to make its volume similar to that of STD and QC. During run precision and accuracy of the method using quality control samples were determined for three different concentrations (n=3 each concentration) of the standard curve: High (QCH-

8000ng/ml); Medium (QCM-1000ng/ml); Low (QCL-100ng/ml). The mean accuracy and coefficients of variation (CV) for QCL, QCM and CQH on all the batch runs performed were determined.

3.4.6. Pharmacokinetic and Statistical Analysis of lumefantrine

This was achieved through comparison of PK parameters (student t-test) and through bioequivalence study which was designed as randomized, single center, open label, two period, two sequence, single dose crossover trial comparing the lumefantrine BA between artefan® and Coartem®. Analysis involved tablet formulations.

Non-compartmental PK analysis was employed to determine PK profile of lumefantrine using R Statistical software version 2:13. The parameters C_{max} and T_{max} were calculated directly from experimental observations of plasma concentrations of lumefantrine. Samples below LLOQ were assigned a 50ng/ml value during estimation of PK parameters. $AUC_{0\text{-}168}$: area under the plasma concentration–time curve from 0 hours to the last measurable plasma concentration ($C_{0\text{-}168}$) was calculated by a combination of linear and logarithmic trapezoidal methods. AUC extrapolated to infinity was calculated by the following equation: $AUC_{0\text{-}168} + C_{0\text{-}168}/\lambda z$, where λz is terminal elimination rate constant. The λz was estimated by performing log-linear regression on the concentration versus time data points that were determined to describe the terminal, linear elimination phase.

Bioavailability was determined using the pharmacokinetic parameters C_{max}, $AUC_{0\text{-}168}$, and $AUC_{0\text{-}\infty}$. Individual pharmacokinetic parameters were natural log-transformed according to FDA recommendations, and geometric means and standard deviations calculated. The ratio of the test to reference formulation for geometric mean C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ and the 90% confidence intervals around each mean ratio were determined. Average bioequivalence was met when the 90% confidence intervals around the C_{max}, $AUC_{0\text{-}168}$, and $AUC_{0\text{-}\infty}$ mean ratios for each drug all fall within the FDA's predefined limits of 0.80 to 1.25. [32]

CHAPTER 4

4. RESULTS

4.1. Volunteers and baseline characteristics

Healthy young male volunteers were enrolled in the study between February and April 2012 and completed the study (Table 1.4 – 3.4).

Table 1.4: Baseline Characteristics of the Study participants at Enrollment

N	*MA*	*RA*	*MW*	*RW*	*MH*	*RH*	*MBMI*	*RBMI*
18	27±7.3	20-43	67.3±12.6	44-99	172.1±10.0	155.5-189.3	22.7±3.0	18.4-29.8

N, number of enrolled participants; MA, mean age in years ±SD; RA, age range in years; MW, mean body weight in kg ± SD; RW, body weight range in kg; MH, mean height in cm ± SD; RH, mean height range in cm; MBMI, mean body mass index in kg/m^2 ± SD; RBMI, range body mass index in kg/m^2

Table 2.4: Baseline hematological indices in the Study participants

Test	*WBC ($X10^3$/ µL)*	*RBC ($X10^6$/µL)*	*HGB (g/Dl)*	*HCT (%)*	*PLT ($X10^3$/ µL)*	*NEUT%*	*LYMPH (%)*	*MONO (%)*	*EO (%)*
MEAN	5.98	5.082	14.61	42.506	230.89	46.09	40.46	13.38	4.372
MEDIAN	6.06	5.13	14.7	42.55	246	48.15	37.05	8.65	3.1

Table 3.4: Baseline Biochemical Characteristics of the Study participants

Test	*ALBUMIN (g/L)*	*ALP (U/L)*	*ALT (U/L)*	*AST (U/L)*	*CREATENINE (umol/L)*	*TOTAL BILIRUBIN (umol/L)*
MEAN	41.61	73.33	17.94	20.30	78.44	10.74
REFERENCE RANGE	37.00-53.00	35.00-117.00	2.0-60.00	15.00-55.00	20-97	0.1-25.7

4.1.1. Tolerability

The test and reference drug formulations were well tolerated. There were no adverse drug reactions, abnormal laboratory results or dropouts.

4.2. Bioanalytics

4.2. Lumefantrine method validation

4.2.2. Selectivity

The chromatograms showed no interference. Figure 1.4; demonstrates a chromatogram obtained from extracted blank plasma spiked with internal standard only. The peaks of halofantrine (IS) and lumefantrine eluted at 4.4 min and 8.6 minutes, respectively (Figure 2.4), and there were no interferences by endogenous plasma substances indicating good selectivity of method in analyzing biological samples. The retention times of the peaks obtained were always consistent. Individual chromatograms and assessment of the peak areas at respective retention time were documented.

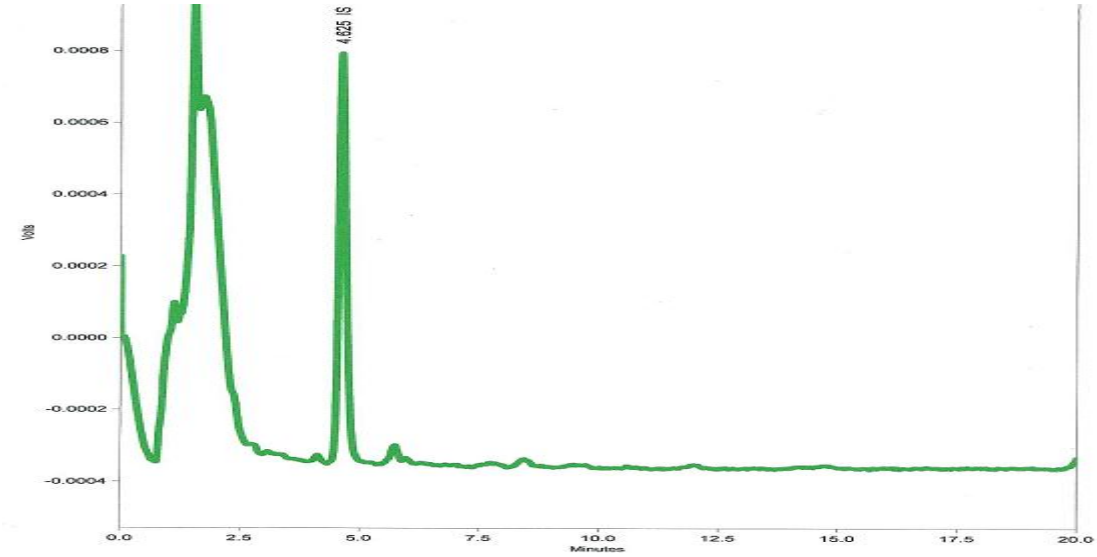

Figure 1.4: Chromatogram showing blank plasma spiked with halofantrine (IS) with no interfering peaks at the retention time of lumefantrine (8.6 min)

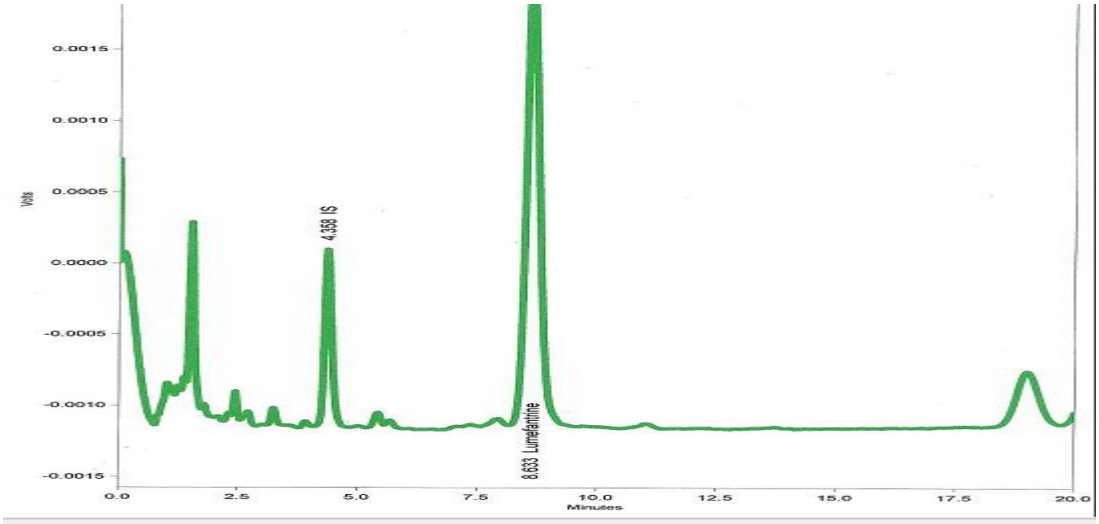

Figure 2.4: Chromatogram showing baseline separation between lumefantrine and halofantrine (IS) peaks with no interference from endogenous plasma substances

4.2.3. Linearity

At least 75% of the percentage relative deviations (RD %) of the calculated concentrations from the nominal values were within ± 15 %. The calibration curves were well repeatable for the three days. The plot of the mean peak areas against the analyte concentrations showed the calibration curve to be linear.

Tables with individual values and parameters of regression (slope, intercept, coefficient of correlation) and graphics of standard curves were documented.

4.2.4. Precision/Accuracy

The coefficients of variation (CV %) for QCL, QCM and QCH on all the three runs performed on different days were documented and all fulfilled the acceptance criteria of being within ±15%. The percentage relative deviations from the nominal value for QCL, QCM and CQH on all the runs were within ± 15 %. Tables with individual values and mean values (calculation of CV and relative percentage deviation from nominal values) are documented. The inter-day accuracy and precision are as shown in the table 4.4 below.

Table 4.4: Inter-day lumefantrine method validation accuracy and precision results

QCs	*Spiked plasma concentration (ng/ml)*	*Mean determined concentration (ng/ml)*	*Accuracy (%)*	*CV (%)*
QCL	100	103.3	3.3	5.4
QCM	1000	1055.6	5.6	3.4
QCH	8000	7894.9	-1.3	6.4

4.3. Test samples

A total of 394 test plasma samples were analyzed in eight batches. Each batch had its own calibration curve. Tables with individual values and parameters of regression (slope, intercept, correlation coefficient) and graphics of standard curves were documented. There was no interference from endogenous substances (figure 3.4).

The mean coefficients of variation (CV) for QCL, QCM and CQH on all the batch runs performed were documented and all fulfilled the acceptance criteria of being within ±15%. The percentage relative deviations from the nominal value for QCL, QCM and CQH on all the runs were within ± 15 %.

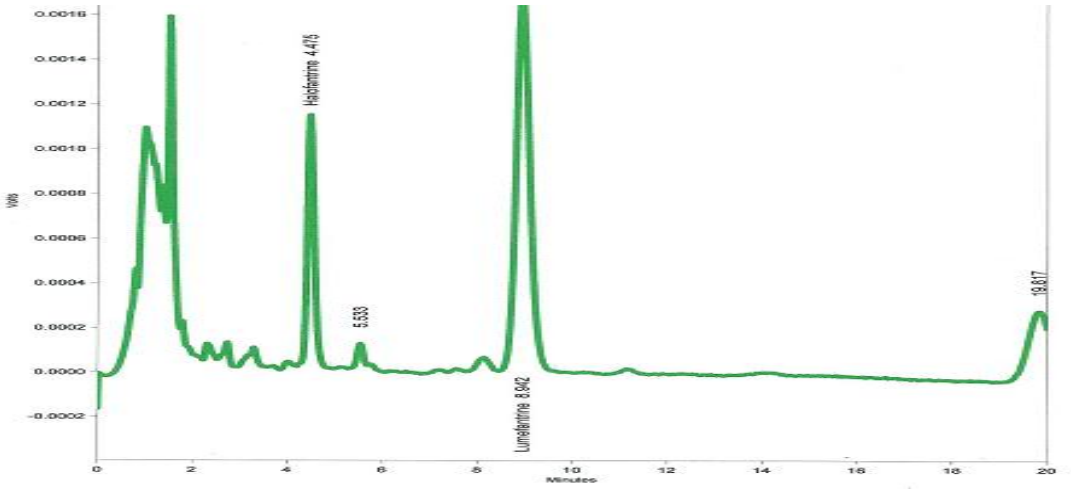

Figure 3.4: Chromatogram showing baseline separation between lumefantrine and halofantrine (IS) peaks with no interference from endogenous plasma substances in one of the unknown samples

4.4. Bioavailability and Average Bioequivalence

The mean (± SD) lumefantrine primary PK parameters C_{max}, T_{max}, AUC_{0-168} and $AUC_{0-\infty}$ under fed condition for artefan® and coartem® formulations were determined with the C_{max} of lumefantrine in plasma for both drugs achieved within 6.22 hours and the results were comparable to other studies carried some where else (Table 5.4). The percentage of AUC extrapolated to infinity was similar for both formulations with 10.44% for artefan® and 9.78% for coartem (p-value=0.95).

Plasma concentrations of lumefantrine attained by administration of a single dose of artefan® and coartem® formulations were comparable based on lumefantrine concentration-time profiles for artefan® and coartem® formulations (Figure 3.4). Day 7 lumefantrine plasma concentrations were comparable for both artefan® and coartem® (table 6.4).

Table 5.4: % Ratio of Untransformed Data (Artefan® to Coartem®) for Bioequivalence Assessment and Two More Studies for Comparison

Primary Parameter	*Artefan®* *Arithmetic mean ± SD* *(*)*	*Coartem®* *Arithmetic mean ± SD* *(*)*	*% Ratio of test to reference*	*Another study*** *Arithmetic mean ± SD*	*Norvatis Data on file (Riamet)* *Arithmetic mean*
Tmax (h)	6.11±2.698	6.22±2.157	-	8.5±2.15	6 – 8
Cmax (ng/ml)	4206.93±2942.484 (3144.017)	4438.81±2548.432 (3742.267)	84.01	4600±2280	5100-9800
AUC_{0-168} (ng.h/ml)	123758.90±83527.51 (93175.97)	135430.70±86814.81 (109729.0)	84.49	-	-
$AUC_{0-\infty}$ (ng.h/ml)	138189.7±94959.77 (102441.5)	149530.20±95109.42 (121576.3)	84.26	262500±129600	108000-243000

*Geometric mean **Study carried out in Pakistan in Healthy Volunteers[37]

Table 6.4: Volunteers Day 7 lumefantrine plasma concentration (ng/ml) for artefan® and coartem® (n=18)

Drug	Minimum	1st quartile	Median	Mean (SD)	3rdquartile	Maximum
Artefan®	50.00	65.35	102.10	163.00 (148.01)	218.15	610.90
Coartem®	50.00	99.83	146.95	171.82 (116.59)	196.10	489.95

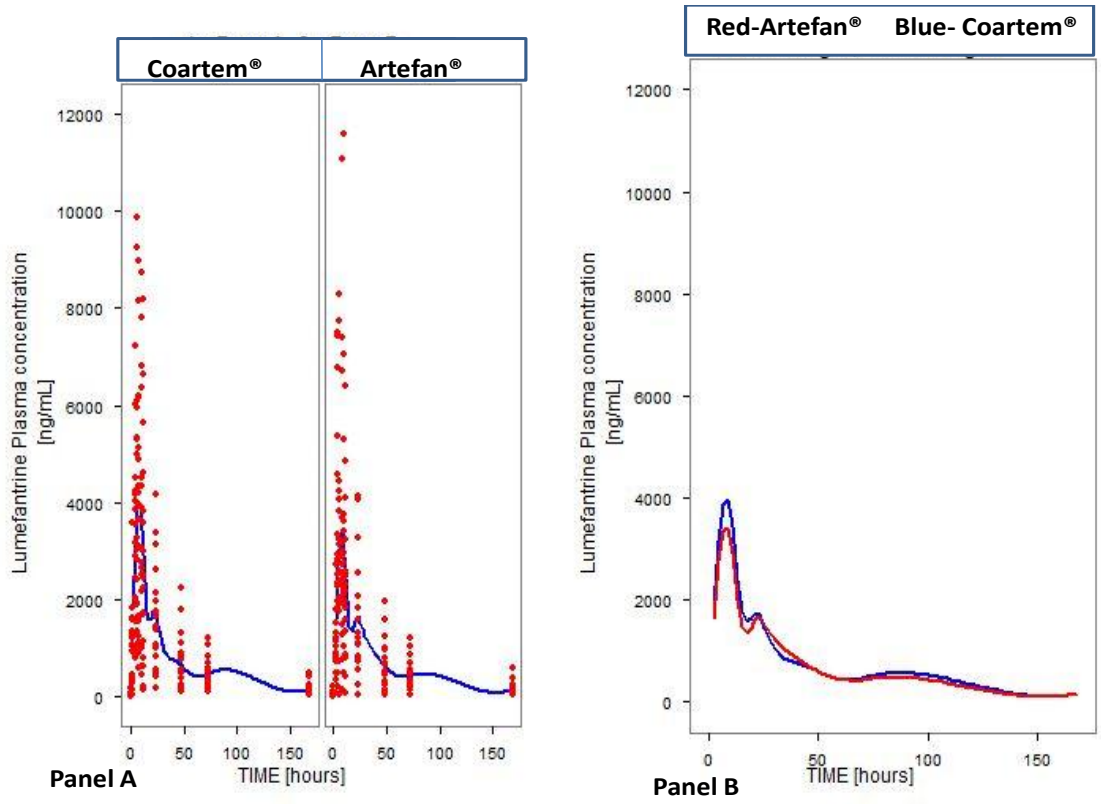

Figure 3.4: Panel A: Plasma concentration-time profiles (linear scale) of lumefantrine in healthy volunteers (n = 18) for artefan® and Coartem®. Panel B: Plasma concentration-time profiles (linear scale) of lumefantrine in healthy volunteers (n = 18) for artefan® and Coartem® on the same axes.

Mean ratios of artefan® to coartem® natural log-transformed C_{max}, AUC_{0-168}, and $AUC_{0-\infty}$ values and 90% confidence interval limits are summarized in Table 7.4. Other PK parameters were determined including elimination half life, volume of distribution and clearance (Table 8.4). One out of the 18 subjects had predose lumefantrine concentration above the lower limit of quantitation (50 ng/mL) in period 1 and 6 subjects had predose lumefantrine after the washout period. Lumefantrine predose concentrations ranged from 50.6 to 221.6ng/mL, and all were included in the lumefantrine pharmacokinetic analysis as concentrations at time zero. None of the subjects had a predose concentration greater than 5% of his lumefantrine C_{max} and thus all concentrations were acceptable for inclusion in average bioequivalence analysis in accordance with FDA recommendations. [34]

Artefan® lumefantrine C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ geometric means were all less by around 16% relative to Coartem®. However the geometric mean ratios for

lumefantrine of artefan® to coartem® C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were all within the acceptable FDA criteria for bioequivalence of 0.8 – 1.25. The 90% confidence intervals for lumefantrine (18 subjects) C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ mean ratios were not within 80% to 125% and therefore FDA criteria for average bioequivalence was not met. [34]

Table 7.4: 90 % Confidence Interval from Log Transformed Data for Bioequivalence Assessment

Pharmacokinetic Parameters	*Acceptance Criteria*	*% Ratio of artefan® to Coartem®*	*% Confidence Interval*	*P(normal)*
LnC_{max}	80 - 125%	84.01	49.44 - 142.76	0.559
$LnAUC_{0-168}$	80 - 125%	84.49	52.70 - 136.81	0.538
$LnAUC_{0-\infty}$	80 - 125%	84.26	52.46 - 135.35	0.575

Table 8.4: Other determined artefan® and coartem® mean lumefantrine pharmacokinetic parameters in the Study participants (n=18)

PK parameter mean ± SD	*Artefan®*	*Coartem®*	*p-value*
Elimination half life (hours)	61.372 ± 17.483	62.812 ± 13.971	0.80
Volume of distribution (L)	382.95 ± 262.73	405.05 ± 299.09	0.83
Clearance (L/hour)	7.81 ± 11.58	4.89 ± 3.23	0.31

4.5. Discussion

The mean percentage of residual AUC was similar for both formulations (10.44% for artefan® and 9.78% for coartem®) p value = 0.95. The estimated value of lumefantrine AUC_{0-168} for each drug artefan® and Coartem® being greater than 80% of the estimate value of $AUC_{0-\infty}$ implied that the sampling scheme was sufficiently long to ensure adequate description of the absorption phase and elimination phase of lumefantrine.

In comparison of lumefantrine bioavailability of artefan® with Coartem® and with other studies carried out elsewhere the mean lumefantrine pharmacokinetic parameters including day seven plasma concentrations were determined and compared. The mean PK parameters determined for artefan® and coartem were found to have no statistically significant difference under student t-test. Mean AUC values of lumefantrine for both artefan® and Coartem® were within reported range between 108,000 and 243,000ng.h/ml (Norvatis, data on file). This indicates that both artefan® and coartem have comparable quality in terms of achieving the expected plasma lumefantrine concentrations. The PK profile observed in Tanzanian volunteers was comparable to other populations. It was observed that lumefantrine elimination half life for both artefan® and Coartem® fell in the reported half life range of 2-3 days in healthy volunteers (Novartis, data on file). In a study of healthy Chinese volunteers the mean T_{max} was 6.1 hours which is comparable to 6.11 and 6.22 hours observed in our study for artefan® and Coartem® respectively, but the C_{max} was 2.034 mg/l, which is lower as compared to our study. [35]

Lumefantrine has a large volume of distribution (Vd) and in our volunteers there was no statistically significant difference between the observed mean Vd for artefan® and Coartem® (p-value 0.83). The observed mean Vd was 382.95 liters for artefan® and 405.05 liters for Coartem® compared to 276.5 liters and 159.2 liters in Pakistani and Malaysian volunteers respectively.[36, 37] Very large changes in AUC from dose to dose imply that changes in oral bioavailability rather than the Vd are the main contributor to inconsistency in plasma concentration profiles.[38]

Lumefantrine has an apparent low plasma clearance [39] and in our subjects, the mean total plasma clearance observed of lumefantrine was 4.89 L/hr for coartem and 7.81 L/hr for artefan® (p-value 0.31). A wide variation of this parameter is apparent from studies published by various researchers. A study conducted in Pakistani volunteers revealed a clearance of 2.44 L/hr.[37] Our results are comparable to reported values 6.6 L/hr and 7.2 L/hr in Chinese and Thai studies respectively. [40, 41]

The observed mean day 7 lumefantrine levels were similar for both artefan® and Coartem® (p-value=0.98). It has been reported that patients who had lumefantrine levels below 175 ng/ml on day 7 were more likely to experience recrudescence by day 42 allowing prediction of treatment failure with 75% sensitivity and 84% specificity.[13] With one sample t-test at a p-value of 0.05 to compare the observed means with the cutoff value for likeliness of recrudescence (175 ng/ml), both artefan® and Coartem® mean day 7 lumefantrine levels were found not to be statistically different from the cutoff value. This is indicating that both generic and innovator brands are probably therapeutically equivalent. The dose advised to the patients with uncomplicated falciparum malaria in the beginning of the therapy for both artefan® and coartem® are likely to produce adequate levels of the drug in plasma to exert antiplasmodial action, which may be maintained by administration of the subsequent doses.

In assessing the average bioequivalence of artefan® versus Coartem® it was observed that the geometric mean ratios for lumefantrine of artefan® to Coartem® C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were all within the acceptable FDA criteria for bioequivalence of 0.8 – 1.25. Artefan® lumefantrine C_{max}, AUC_{0-168} and $AUC_{0-\infty}$ geometric means were all less by around 16% relative to Coartem®. The ratios were all within the allowable FDA limit but very close to 0.8, the lower limit of the FDA acceptable range thus making it very difficult for drug with high intra-and inter-individual variability like lumefantrine to have the 90% confidence intervals within the accepted limits of 0.8 - 1.25.[34]

The 90% confidence intervals for lumefantrine (18 subjects) C_{max}, AUC_{0-168}, and $AUC_{0-\infty}$ mean ratios were not within 0.80 to 1.25 and did not meet the strict FDA criteria for average bioequivalence. [34] However one of the limitations of our study was high lumefantrine AUC and C_{max} intrasubject variability thus relative

bioequivalence may have been more appropriately assessed with a larger sample size or through a replicative study. [34] Based on reported intrasubject variability of 44% for lumefantrine AUC reported in a previous study with Coartem® (Novartis, data on file), a large sample size of at least 48 volunteers was required to ensure at least 80% power to obtain a 90% confidence intervals for lumefantrine AUC within a wider range than recommended by FDA. [42]

Lumefantrine high plasma concentration inter-individual variability observed can be explained on the basis of difference in metabolism of the drug by cytochrome 3A4 (CYP3A4). [43]Since cytochrome 3A4 (CYP3A4) is also expressed in the human small intestine, it contributes to the first pass effect. The wide variation in total CYP3A4 content seen among individuals has been attributed to both environmental and genetic factors which mean drugs cleared by this isoform by first pass metabolism are expected to have highly variable pharmacokinetics. [44] Due to observed high inter-individual variability in lumefantrine plasma concentrations, there is possibility of occurrence of treatment failure in some individuals when using either Coartem® or artefan® and this should not be confused with drug resistance. Genotyping to allow individualization of drug treatment could be proper but this is still a challenge in the developing countries like Tanzania.

4.6. Conclusions

The overall lumefantrine pharmacokinetic profile (pharmacokinetic parameters) for both artefan® and Coartem® in the healthy Tanzanian volunteers appears to be similar and comparable to other ethnic groups reported elsewhere. Although the ratios of AUCs and C_{max} were within the acceptable FDA range, bioequivalence between artefan® and coartem® tablet formulations was not demonstrated due to failure to comply with the FDA 90% confidence interval criteria. However, based on the observed total drug exposure (AUCs and day 7 plasma lumefantrine concentrations) in comparison to other studies carried somewhere else, artefan® is likely to produce a similar therapeutic response as Coartem®. The two formulations are therefore likely to produce the same cure rate.

4.6. Recommendations

Given the likelihood of finding low quality drugs in terms of bioavailability and the growing availability of low-cost generic drug products, PK and in particular bioequivalence studies are needed in specific populations in which these agents are used to account for unique characteristics that may influence drug disposition so that generics can be used exchangeably with the innovator's products. Drug regulatory authorities in countries in which generic medications are used should make an effort to test all drugs imported into their countries to ensure their quality. This can be done through providing infrastructural and technical assistance to bioanalytical laboratories and clinical trial facilities available in these countries so that they can serve as centers for training and running more bioequivalence studies in the region.

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6. APPENDICES

APPENDIX I

CHECK LIST FOR DETERMINATION OF THE MOST PREVALENT GENERIC DRUG

1. Number of checklist …… Date ……
2. Name of pharmacy ……
3. Municipal ……...
4. List all generic artemether-lumefantrine tablet formulations available in the pharmacy: Tick the available generic drug.

	Generic name	**Name of manufacturer**
i.	Artefan®	*Ajanta pharma limited, India*
ii.	Lumerax	*Ipca Laboratories Ltd, India*
iii.	Artemether 20mg + lumefantrine 120mg	*Ipca laboratories ltd, India*
iv.	Lumartem	*Cipla- India*

5. Any other generic available? Please mention:

	Generic name	**Name of manufacturer**
i.	……..	……
ii.	……..	……
iii.	……..	……

Appendix II

Consent information:

INFORMED CONSENT FORM

ID-NO | | | | | | | | | | | |

Consent to participate in the study entitled: ASSESSMENT OF QUALITY OF ARTEMITHER-LUMEFANTRINE TABLETS AVAILABLE ON THE TANZANIAN MARKET

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1. **Background:** Treatment of non-severe malaria remains a challenge to endemic areas including Tanzania. Since November 2006, Coartem® (Novartis Pharma, Basel Switzerland) an ACT containing Artemether-Lumefantrine replaced SP as first line drug for treatment of uncomplicated malaria in Tanzania. Considering the liability of antimalarial drugs to substandard manufacturing, a concern is raised on the quality of generic Artemether-Lumefantrine containing tablets being used in Tanzania. Identification of poor quality of Artemether-Lumefantrine tablets is therefore crucial in insuring efficiency of treatment of malaria infected patients. Therefore this study is aiming at assessing the quality of artemether-lumefantrine tablets available on the Tanzanian market.

2. **Purpose of the Study**

We are enrolling 18 health volunteers to participate in our study that aims at determining whether the generic drug is bioequivalent to innovator brand, Coartem®.

3. **What Participation Involves**

Your participation involves the following

1. To refrain from taking grape fruits, or juice and alcohol.
2. Not to take any other non prescription medication during the study period before consulting the investigator.
3. To be ready to take one antimalarial tablet containing artemether-lumefantrine and repeat another one of innovator brand after 42 days.

4. To allow blood sample to be taken from your fore arm at the following intervals after you have taken a single Generic anti-malarial tablet; 2, 4, 6, 8, 10,12, 24, 48, 72 and 168 hrs
5. To take diet that your study investigator have provided during the study
6. To report any adverse event that you observe during the study
7. To undergo laboratory and clinical investigations to determine your health status.

Explanation:

After you are found healthy you will take a single anti-malarial tablet containing artemether-lumefantrine followed by 3ml a serial blood sampling from your fore arm.

The blood sampling will be as follows; an indwelling cannula will be inserted in your fore arm vein for 72hrs and first blood sampling will be done before you take the tablet. Once you have taken the generic anti-malarial tablet further sampling will be done 2, 4, 6, 8, 10, 12, 24, 48 and 72hr. You will be discharged home and will be required to return in day 7(hr 168) for a single blood sampling at 08:00 in the morning. Blood sample collected in each volunteer during whole study period will be 66 ml. The total duration of blood sampling in each phase is 4 days.

4. Confidentiality

All information regarding testing and other evaluation will be confidential. We will not use names to store the information but the special study number which you will be given.

5. Risks

The expected risk in this study is drug sensitivity which some very few individual have been reported to experience. Also blood sample collection is a painful but tolerable procedure. Taking blood from you will not pose any risk as little blood will be taken at very safe intervals.

6. **Rights to withdrawal**

Taking part in this study is completely your choice. You are free to agree or refuse participating in this study.

7. Benefits

If you agree to take part in this study, you will benefit indirectly in providing information which can help policy makers in deciding the best brands of anti-malarial drugs suitable for use in the country. In addition you will benefit from medical check-up of your health status. We hope that the information we learn from this study will benefit others.

We will reimburse you the cost of public transport, time compensation and money for lunch on your appointment day. We do not expect that, there will be additional costs on your side resulting from participation in this study.

We will tell you about the new information from this or other studies that may affect your health, welfare, or willingness to stay in the study.

8. Handling of those who will be tested positive

Those who will be tested malaria positive will be counseled and channeled to the medical facilities according to the existing standard of care.

9. In Case of physical Injury or Sensitivity

We do not anticipate that any harm will occur to you as a result of participation in this study. However, if any physical injury or drug sensitivity resulting from participation in this research should occur, you should contact Dr Philip Sasi email address philipsasi@gmail.com and telephone number 0784836275, P.O. Box 65001 Dar es salaam.

10. Blood sample analysis and shipment

Plasma sample analysis will be done in Tanzania, there is a possibility to ship sample for analysis of artemether and this wil not inlcude DNA analysis.

11. Whom to Contact

If you have questions about this study, you should contact the Director of Research and Publications Prof M. Aboud. P.O. Box 65001, Dar es Salaam. Phone: 2150302-6. Secretary Ifakara research department 0686997582.

I ---------- confirm that I have read the contents of this form. My questions have been answered. I agree to participate in this study.

Signature of participant…… Date ……

Name of the participant…….…….

FOMU YA MAELEZO KUHUSU UTAFITI

ASSESSMENT OF QUALITY OF ARTEMITHER-LUMEFANTRINE TABLETS AVAILABLE ON THE TANZANIAN MARKET

ID-NO | | | | | | | | | | |

Kukubali kushiriki kwenye utafiti wa kujua ubora wa dawa za kutibu malaria

Jina langu ni ..... na tunafanya utafiti juu ya ubora wa dawa za mseto toka kwa watengenezaji wawili tofauti

1. Utangulizi.

Malaria imeendelea kuwa moja kati ya matatizo makubwa yanayoikumba sekta ya afya hapa Tanzania. Malaria inaongoza katika kusababisha vifo hasa kwa watoto walio chini ya umri wa miaka mitano. Tangu Novemba 2006, dawa ya mseto ya ALu ilianza kutumika badala ya SP kama dawa ya chaguo la kwanza kwa matibabu ya malaria isiyo kali. Tafiti mbalimbali zimeonesha kuwa dawa ya mseto ya ALu ni salama, na ina uwezo mkubwa wa kutibu malaria hata kwenye maeneo ambayo vimelea vya malaria vimekuwa sugu dhidi ya dawa ya SP. Kuzingatia namna sahihi ya utengenezaji dawa ni suala la muhimu sana katika kufanikisha matibabu na kadri mahitaji ya ALu yanavyoongezeka, kuna uwezekano mkubwa zaidi kwa viwanda vya dawa hizi kuzalisha dawa zisizo na ubora ukilinganisha na dawa za mgunduzi (innovator). Utumiaji wa dawa zenye ubora hafifu kunaweza kusababisha mgonjwa ashindwe kupona ipasavyo pamoja na kupelekea vimelea vya malaria kuwa sugu dhidi ya dawa za malaria.

2. Lengo la uchunguzi

Tunataka kuangalia ubora wa dawa mseto kwa kulinganisha kiwango cha dawa kinachoingia mwilini baada ya kumeza dozi moja.

3. Kinachotakiwa

1. Uwe tayari kukubali kuacha kula zabibu, kunywa juisi ya zabibu na kunywa pombe kwa kipindi utakachoambiwa na mtafiti (siku 10).
2. Usitumie dawa baridi yoyote kabla ya kuwasiliana na mtafiti muhusika kwa kipindi chote cha utafiti.
3. Uwe tayari kumeza vidonge vinne vya dawa mseto na baada ya siku 42 tutakupa aina nyingine vya vidonge vya dawa mseto.
4. Kila utakapoo meza vidonge hivi tutachukua damu toka kwako ml 3 kwa muda ufuatao tangu umeze dawa: 0, 2, 4 , 6,8, 12, 24, 48, 72 na 168.
5. Uwe tayari kula chakula kiatakachopendekezwa na mtafiti
6. Uwe tayari kusema madhara yoyote utakayoyapata katika kipindi chote cha utafiti
7. Uwe tayari kufanyiwa uchunguzi wa afya yako

UTARATIBU:

Tutakupima afya yako na kama utaonekana unatimiza vigezo tutakufahamisha kisha tutakuita kushiri. Kama utakuwa na tatizo lolote tutakueleza na kukushauri pa kwenda kupata matibabu. Baada ya kuafiki siku ya utafiti utapewa vidonge vinne umeze na glasi ya maji masafi na kisha utatolewa damu ml 3 kwa kipindi kilichoonyeshwa hapo juu na kurudia tena zoezi zima baada ya siku 42. Kiasi cha damu kitakachotolewa katika kipindi chote cha utafiti ni ml 66. Idadi ya siku utakazotolewa damu kwa kila awamu ni nne. Damu hiyo itatumika kuangalia kiasi cha dawa mwilini. Hapatakuwa na hatari za magonjwa ya kuambukizana wakati wa utoaji wa damu kwa kuwa tutatumia sindano na vifaa vingine vile vya mara moja na kutupa.Wakati tunachukua damu unaweza kusikia maumivu kidogo, lakini tunakuhakikishia kuwa tutakuwa na wataalamu wazuri ambao wataidhibiti hali hiyo.

Unao uhuru wa kujitoa kwenye utafiti huo bila maelezo kwa hiari yako maana zoezi zima ni la hiari.

4. **Usiri**:

Habari zote tutaziandika kwenye komputa na tutakupa namba ya ushiriki kwenye utafiti huu.

5. **Madhara:**

Hakuna madhara ya ziada tunayoyategemea kuwa utayapata maana zaidi ya kuchukua damu kidogo kwako hakuna liingine tutakalokufanyia. Dawa mseto si ngeni inatumika Tanzania.

6. **Haki ya kushiri au kukataa:**

Ushiriki kwenye utafiti huu ni hiari yako na unayo haki ya kukataa kushiriki bila kutoa sababu.

7. **Faida za kushiriki:**

Ukikubali kushiriki utakuwa umetoa mchango ambao utasaidia serikali kufanya maamuzi sahihi juu ya jinsi ya kuboresha uagizaji wa dawa aina hizi za kutibu malaria kwa nchi nzima na pia kupata uhakika wa ubora wa dawa zitumikazo kwa wagonjwa. Tutakupa majibu ya matokeo ya utafiti huu. Tutakulipa kiasi kidogo kama gharama ya kukucheleweshe na pia nauli ya kuja katika kitengo cha utafiti.

8. **Kama ukipata athari katika utafiti:**

Hatuoni chanzo chochote cha athari yoyote katika utafiti huu kwa kuwa dawa hizi zipo na zinatumika tayari kwa watanzania. Unaweza kuwasiliana na Dr Philip Sasi kwa barua pepe, philipsasi@gmail.com, simu ya mkononi 0784836275, au kupitia S. L. P 65001 Dar es salaam.

9. **Nani wa kumuona wakati ukiwa na shida ama dharura:**

Kama una maswali kuhusu haki zako za ushiriki unaweza kuulizia kwa Mkurugenzi wa tafiti, wa chuo, Prof. M. Aboud. P.O. Box 65001, Dar es Salaam. Simu: 2150302-6 na katibu wa kitengo cha utafiti cha Ifakara 0686997582

Mimi ---------- nimesoma yaliyomo kwenye fomu hii na kuyaelewa na maswali yangu yamejibiwa. Ninakubali kushiriki kwenye utafiti huu.

Sahihi ya mshiriki----------
Tarehe……

Jina la mshiriki----------

Sahihi ya mtafiti……Tarehe…….

Jina la mtafiti…….

APPENDEX III

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
DIRECTORATE OF POSTGRADUATE STUDIES

P.O. Box 65001
DAR-ES-SALAAM
TANZANIA
Telefax: 255-022-2150465
Telegrams: UNIVMED

E-MAIL dpgs@muhas.ac.tz
TEL: (255-022)-2150302-6 Ext. 207
Direct line: 2151378

Ref. No. MU/PGS/SAEC/Vol. VI/

13th October, 2011

Alphonce Ignace.
MSc. Clinical Pharmacology,
School of Medicine,
MUHAS.

Re: APPROVAL OF ETHICAL CLEARANCE FOR A STUDY TITLED "ASSESSMENT OF QUALITY OF ARTEMETHER-LUMEFANTRINE TABLET FORMULATIONS AVAILABLE ON THE TANZANIAN MARKET"

Reference is made to the above heading.

I am pleased to inform you that, the Chairman has on behalf of the Senate approved ethical clearance for the above-mentioned study.

Thus ethical clearance is granted and you may proceed with the planned study.

Prof. Z. Premji
DIRECTOR, POSTGRADUATE STUDIES

/emm

cc: Vice Chancellor, MUHAS
cc: Deputy Vice Chancellor-ARC
cc: Dean, School of Medicine

APPENDEX IV

IFAKARA HEALTH INSTITUTE
research | training | services

INSTITUTIONAL REVIEW BOARD
P O BOX 78373 DAR ES SALAAM, TANZANIA
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National Institute for Medical Research
P O Box 9653
Dar Es Salaam
Email, headquarters@nimr.or.tz

25th February, 2011

Ignas Alphonce
Ifakara Health Institute
P O Box 78373
Dar Es Salaam

IHIRB/No. 07 - 2012

INSTITUTIONAL CLEARANCE CERTIFICATE FOR CONDUCTING HEALTH RESEARCH

On 24th February 2012, the Ifakara Health Institute Review Board (IHI IRB) reviewed a project titled: *"Assessment of quality of artemether -lumefantrine tablet formulations available in the Tanzanian market"* submitted by the Principal Investigator Ignas Alphonce. The study was previously reviewed on the 30th January 2012

The following documents were reviewed.

1. Protocol
2. Budget
3. CVs

The study has been approved for implementation after IRB consensus. This certificate thus indicates that, the above- mentioned study has been granted an Institutional Ethics Clearance to conduct the above named study in Bagamoyo - Tanzania

The Principal Investigator of the study must ensure that the following conditions are fulfilled during or after the implementation of the study:

1. PI should submit a six month progress report and the final report at the end of the project
2. Any amendment, which will be done after the approval of the protocol, must be communicated as soon as possible to the IRB for another approval
3. All research must stop after the project expiration date, unless there is prior information and justification to the IRB.
4. There should be plans to give feedback to the community on the findings
5. Any publication needs to pass through the IRB

The IRB reserves the right to undertake field inspections to check on the protocol compliance

Chairperson
JOYCE K. IRINGURA

Secretary
BEVERLY MSAMBICHAKA

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