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[Comparación de las propiedades fisicoquímicas y la biodisponibilidad *in vivo* de comprimidos genéricos e innovadores arteméter-lumefantrina en Kumasi, Ghana]

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Abstract

Context: Malarial remains a leading course of death in developing countries. Current treatment protocol involves the use of artemisinin-based combination therapy. In endemic areas, cost of treatment is a concern hence generic prescription is on the high. It is therefore necessary to investigate how equivalent or otherwise the generics are to the innovator brand Coatem®.

Aims: To compare the physicochemical properties and *in vivo* bioavailability of a locally manufactured generic artemether-lumefantrine tablet formulation and that of the innovator brand sold on the Kumasi market, Ghana.

Methods: The most used locally manufactured generic and the innovator brands were sampled from retail pharmacies. The samples were confirmed by colorimetry. Pharmaceutical equivalence of the brands was determined using compendial tests. *In vivo* bioavailability study on the two brands was done using a two-period, single dose, cross-over design involving 20 healthy rabbits. Pharmacokinetic parameters (AUC_{0-72}, $AUC_{0-\infty}$, and C_{max}) for both brands derived from the study were analysed statistically.

Results: Both the generic and innovator brands passed the physicochemical tests. The artemether component of both brands complied with the pharmacopoeia specification for dissolution testing while the lumefantrine did not. Average bioequivalence was demonstrated per the FDA criterion with the geometric mean ratios and corresponding 90% confidence intervals falling within the acceptable limits of 0.80 – 1.25.

Conclusions: Based on the similarity demonstrated between the two brands, evidence have been shown to support substitutability of the often-expensive innovator brand with the affordable locally produced brand.

Keywords: artemether-lumefantrine; bioequivalence generic-substitution; pharmacokinetics.

Resumen

Contexto: La malaria sigue siendo una causa principal de muerte en los países en desarrollo. El protocolo de tratamiento actual implica el uso de terapia de combinación basada en la artemisinina. En áreas endémicas, el costo del tratamiento es una preocupación y la prescripción genérica está auge. Por lo tanto, es necesario investigar qué tan equivalentes o genéricos son los medicamentos para la marca innovadora Coatem®.

Objetivos: Comparar las propiedades fisicoquímicas y la biodisponibilidad *in vivo* de una formulación de tableta de artemeter-lumefantrina genérica, fabricada localmente, y la de la marca innovadora vendida en el mercado de Kumasi, Ghana.

Métodos: Se tomaron muestras de las marcas genéricas e innovadoras más utilizadas en las farmacias minoristas. Las muestras fueron confirmadas por colorimetría. La equivalencia farmacéutica de las marcas se determinó mediante pruebas compendiales. El estudio de biodisponibilidad *in vivo* en las dos marcas se realizó utilizando un diseño cruzado de dosis única de dos períodos en el se utilizaron 20 conejos sanos. Los parámetros farmacocinéticos (AUC_{0-72}, $AUC_{0-\infty}$ y C_{max}), para ambas marcas derivadas del estudio, se compararon estadísticamente.

Resultados: Tanto las marcas genéricas como innovadoras pasaron las pruebas fisicoquímicas. El componente de arteméter de ambas marcas cumplió con la especificación de la farmacopea para las pruebas de disolución, mientras que la lumefantrina no lo hizo. La bioequivalencia promedio se demostró según el criterio de la FDA con las razones de medias geométricas y los correspondientes intervalos de confianza del 90% dentro de los límites aceptables de 0,80 a 1,25.

Conclusiones: En base a la similitud demostrada entre las dos marcas, se ha demostrado que las pruebas respaldan la sustitución de la marca innovadora, a menudo cara, con la marca de producción local asequible.

Palabras Clave: arteméter-lumefantrina; farmacocinética; sustitución genérica de bioequivalencia.

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INTRODUCTION

Malaria is a parasitic disease endemic in parts of the world where moisture and warmth permit the disease vector, mosquitoes of the genus Anopheles, to exist and multiply. Malaria poses as a serious life– threatening disease with almost half of the world's population at risk of malaria. As at December 2015, there were 214 million cases with 438,000 deaths (World Health Organisation, 2015b). A disproportionately high number of malaria cases and deaths occur in the Sub – Saharan region of Africa. As of 2015, this region reported 88% of cases and 90% of the malaria deaths. From 2000 to 2015, even though there has been a steady decline in the malaria incidence (about 53%) globally, that of sub – Saharan Africa has lagged behind (32%). Malaria's impact on endemic countries is the imposition of a growth penalty of over 1.2% of the gross domestic production (Sachs and Malaney, 2002; World Health Organization, 2014). Even though some of the success story in the control of malaria has been through the preventive measures, early diagnosis and treatment has been the mainstay of reducing transmission and preventing deaths. Case management of malaria involves basically initiating antimalarial therapy to eliminate the infection within 48 to 72 h and to prevent complications.

The World Health Organization in 2001 recommended the use of combination therapy (based on the additive potential of two or more drugs) for malaria treatment not only to improve therapeutic efficacy but also delay the development of resistance. Artemisinin-based combination therapy, using artesunate-amodiaquine (AS/AQ), or artemether-lumefantrine (AL) is currently considered as the first-choice treatment for *Plasmodium falciparum* malaria in endemic areas (World Health Organisation, 2015b). Coartem® (artemether 20 mg and lumefantrine 120 mg), a fixed-dose combination of the two antimalarial agents is the first fixed dose artemisinin-based combination therapy to satisfy the World Health Organization's (WHO) pre-qualification criteria for efficacy, safety and quality (Makanga and Krudsood, 2009). Thus, it is considered as the innovator brand for the fixed-dose combination therapy for malaria. Nonetheless, over the past decade, a lot of pharmaceutical manufacturing companies have ventured into the manufacturing of generics of this fixed – dose combination. The flux of substandard locally manufactured generic antimalarials with low bioavailability has been a major setback in the rapid clearance of parasites from blood and the fight against drug resistance (Dondorp et al., 2004).

Some reviews have shown that a lot of marketed drug products having dissimilar amount (or even sometimes similar amount) of drug displayed marked difference in their therapeutic effects. This difference in response has been well attributed to dissimilar plasma levels mainly due to impaired absorption (Rani and Pargal, 2004). Furthermore, a study on the quality and authenticity of 14 brands of artemisinin – based antimalarials in some licensed retail pharmaceutical outlets in Ghana revealed that about 90% of samples contained either less or higher amount of the specified drug making them to be of substandard quality (El-Dua and Ofori-Kwakye, 2012). Thus, prescribers and pharmacists are placed in a quandary as to which generic to substitute with the innovator brand when patients cannot afford the innovator

This study sought to compare the *in vivo* bioavailabilities of one locally manufactured generic tablet formulation of artemether-lumefantrine (fixed-dose combination) with the innovator product Coartem® from Novartis, Basel-Switzerland.

MATERIAL AND METHODS

Chemicals and samples

Pure samples of lumefantrine, and artemether were obtained from the Department of Pharmaceutics chemical store. Pure artesunate was also obtained as a gift from Guillin Pharma, China. HPLC grade Methanol, glacial acetic acid, trifluoroacetic acid TFA (98%) acetonitrile (BDH, PROLABO), Sodium Acetate (Fisons Laboratory), Hydrochloric acid fuming GR (Merck, Germany), Potassium Iodide (BDH, Poole England) were used. All other reagents used were of analytical grade and obtained from the Department of Pharmaceutical Chemistry chemical store, Kwame Nkrumah University of Science and Technology (KNUST), Ghana.

Table 1. Details of the two tablet brands used in the study.

Sample	Code	Batch No.	Ingredients	Expiry Date
Innovator brand	INN	K0902	Artemether (20 mg), lumefantrine (120 mg) (API), polysorbate 80, hypromellose, microcrystalline cellulose, colloidal anhydrous silica, croscarmellose sodium and magnesium stearate.	May 2016
Local brand	LMG	1005P	Artemether (20 mg), lumefantrine (120 mg) (API), dicalcium phosphate, maize starch, pregelatinized starch, Prosolv 50, croscarmellose sodium and magnesium stearate	May 2016

The names of the manufacturers have been omitted for ethical reasons. API: active pharmaceutical ingredient; INN: innovator brand; LMG: locally manufactured brand.

Determination of most dispensed locally manufactured generic formulation

A pilot survey to identify the most available locally manufactured formulation containing a fixed combination of artemether-lumefantrine (20 mg/120 mg) was carried out. An open and close ended questionnaire was designed, validated and taken to 25 pharmaceutical retail outlets in and around KNUST campus, Kumasi. The questionnaire asked questions relating to facility identity and demographics, stocking preferences of facilities, dispensing preferences and pricing. The most available locally manufactured generic (coded LMG) from the study was selected, purchased and used for the quality control and bioequivalence study with the innovator brand Coartem® (which was coded INN) as the reference. Both LMG and INN had manufacturing and expiry date of June 2014 and May 2016 respectively. LMG had a batch number 1005P.

Details of the formulations are given in Table 1.

Identification tests

Colorimetric test for artemether in reference artemether sample and artemether-lumefantrine tablets

Both active ingredients (artemether-lumefantrine), which are being studied fall under the Biopharmaceutics Classification System (BCS) Class IV (have low permeability and low solubility). Six tablets were crushed and a quantity equivalent to 20 mg of lumefantrine was used for the test.

A quantity of 50 mg artemether powder was weighed and dissolved in 2 mL of dehydrated ethanol. About 0.2 g of KI was added to the mixture and heated on a water bath for about 2 minutes. A similar procedure was employed in the test for artemether in the powered artemether-lumefantrine tablets. A yellow coloration indicated the presence of artemether

Colorimetric test for lumefantrine in reference lumefantrine sample and artemether-lumefantrine tablets

Six tablets were crushed and a quantity of the powder equivalent to 20 mg of lumefantrine was used for the test. Methanol (10 mL) and 20 mg of $KMnO_4$ were added to the powdered drug and boiled for about a minute. The mixture was filtered and a few drops of Brady's reagent (2, 4-dinitrophenylhydrazine solution) was added and shaken and observed for a precipitate formation. A similar procedure was carried out on the pure lumefantrine powder (World Health Organisation, 2015a).

Identification on pure sample by melting point determination

The open capillary method using Stuart melting point apparatus (Bibby Scientific Ltd., UK) was utilized to determine the melting point of the artemether and lumefantrine reference samples. Small amount of the samples was packed into a sample capillary tube. The sample tube was inserted into the melting point apparatus and the temperature range over, which the crystals melt in the tube was

recorded as the melting point (World Health Organisation, 2015a).

Uniformity of weight test

Twenty tablets were selected randomly from each brand and collectively weighed to ascertain the average weight. The individual weights were then taken. The percentage deviation of each tablet from the mean was then determined. This procedure was repeated for the other brand. The percentage deviations of the tablets from the average weight were calculated (British Pharmacopoeia, 2013).

Friability test

In this test, 10 tablets randomly selected from a particular brand, were dusted and weighed together. They were placed in an Erweka Friabilator (TA20, Germany), and operated at 25 rpm for 4 minutes. After the revolutions, the tablets were removed and re-weighed after de-dusting (British Pharmacopoeia, 2013). The percentage weight loss was then calculated for each brand and represented as the percentage friability. The procedure was performed in triplicates for both brands.

Disintegration test

Six tablets from both brands were randomly selected and used for the test. They were placed separately into each of the six cylindrical tubes of the basket rack of an Erweka Disintegrating Test Apparatus (ZT 3/1-GmbH Heusenstamm, Germany Nr 68318). The test was carried out at 37 ± 0.5°C and the bottom of the basket rack was positioned such that it was at least 15 mm below the surface of the distilled water. The time it took for complete disintegration, i.e. no granule of any tablet was left on the mesh, was recorded as the disintegration time. The procedure was performed in triplicates for both brands.

Determination of percentage content of artemether-lumefantrine by HPLC

The assay of the sampled artemether-lumefantrine tablets was carried out using methods described by earlier works (da Costa et al., 2008; Sunil et al., 2010; Arun and Smith, 2011; Gupta et al., 2013) with minor modifications. The response factor approach of determining the concentration of unknown sample was used to estimate the percentage content of the artemether and lumefantrine. Artesunate was utilised as the internal standard. The method was validated based on the International Conference on Harmonisation guidelines on linearity, precision and accuracy (ICH, 2005).

The HPLC system consisted of a model-Spectra P100Series Isocratic pump (Analytical Scientific Instruments, California, USA), rheodyne injector with a 20 µL loop, UV-Visible detector, eDAQ powerchrom series 280 integrator. Data acquisition and reporting was by eDAQ powerchrom software version 2.6.4 (eDAQ Pty, Deniston East, Sydney, Australia).

A Shandon Hypersil ODS 5 µm 4.6 x 250 mm column (Thermo Fischer Scientific Inc, Massachusetts, USA) was used to achieve the separation of the analytes; artemether (ART) and lumefantrine (LUM) and the internal standard, artesunate (AST). All HPLC measurements were done at 230 nm. An isocratic elution approach of 33% sodium acetate buffer and 67% acetonitrile was settled on after it produced distinct peaks with good resolution. The analysis was performed at a flow rate of 1.3 mL/min for all samples with an injection volume of 20 µL.

An aliquot of powdered artemether-lumefantrine tablets equivalent to the dose of drug in one tablet (20 mg artemether /120 mg lumefantrine) was weighed accurately and dissolved in a 100 mL volumetric flask containing 25 mL of acetonitrile. It was made up to volume and sonicated for 20 min to achieve enough dissolution. The solution was filtered through Whatman filter paper (No. 5) into a 100 mL volumetric flask. An aliquot of 1 mL of the filtrate was transferred into a 10 mL volumetric flask and an aliquot of the internal standard (artesunate) was added and made up to volume with the mobile phase to yield a concentration of 250 µg/mL for artemether and 60 µg/mL for lumefantrine. Twenty µL of the final solution was analysed using the validated HPLC method. The injection was done in triplicates and the peak response of the analytes and internal standard recorded. The response factor and peak response were used to calculate the amount of drug present (Gupta et al., 2013). The procedure was repeated for the other brand.

In vitro dissolution study and data comparison

Six vessels of the USP Dissolution Apparatus 2 (DT6-GmbH Heusenstamm, Germany) were filled with 900 mL of 0.1 M HCl containing 1% tween 80 and equilibrated to a temperature of 37 ± 0.5°C (Umapathi et al., 2011). The paddle was set at 50 rpm. One tablet was placed in each vessel and sampling was done at 5, 15, 30, 45, 60, 90 and 120 minutes. At each sampling period, 5 mL samples were withdrawn from a zone midway between the top of the paddle blade and the surface of the medium (pH 3). Five mL of fresh medium was withdrawn from the reservoir vessel and added to the vessel from which the volume was withdrawn. The samples were filtered and diluted 50 folds and analysed using the validated HPLC method in duplicates. The procedure was repeated for the other brand. The concentrations of artemether-lumefantrine in the samples were calculated and the percentage cumulative release values were then calculated. Mean percentage cumulative drug dissolved and their respective time points were plotted on a graph to obtain the release profiles of each formulation. The similarity factor of the release profiles of the two formulations was determined. The dissolution efficiency (DE) was calculated for each brand according to the equation (1):

$$(DE)= \{({}_0\int^t Y.dt) / 100. (t_2 - t_1)\} \times 100 \quad (1)$$

Where:

$({}_0\int^t Y.dt)$ = area under the dissolution curve (AUC)

Y=the percentage dissolved at t_2

t_2=time for all active ingredient to dissolve

t_1=time at which first sample was withdrawn

Bioequivalence study using animal models

Animal experimentation was approved by the Department of Pharmacology, KNUST Ethics Committee in 2016. Animals were handled humanly throughout the experiment in line with animal welfare regulations (Public Law 99-198, Food Security Act of 1985, Subtitle F - Animal Welfare) and the Public Health Services Policy on Humane care and Use of Laboratory Animals. Animals were allowed 24 h access to water and food except the last 24 h prior to drug administration during which they were starved. All the animals were euthanized at the end of the experiment by CO_2 exposure.

The study was a randomized, single dose, open label, two - period, two sequence crossover study and was conducted in line with previous studies (Nishimura et al., 1992; Eikelboom et al., 2005). Twenty healthy rabbits were procured for the study (Food and Drug Authority, 2013). They were weighed and randomized into two groups and starved for 24 h before the day of drug administration.

Each subject in the first group was administered the innovator product while those in the second group were administered the locally produced product at a dose of 4 mg/kg of artemether and 24 mg/kg of lumefantrine. After a 60–day wash–out period, the first group subjects were administered the locally produced product while the second group animals were given the innovator product in a cross-over fashion.

Blood sampling and plasma analysis of artemether-lumefantrine

Xylene was applied to the ears of rabbits of both groups to make their marginal ear veins more superficial. One mL of blood sample was then collected from each rabbit's marginal ear vein to serve as the baseline readings. The blood samples were collected into EDTA tubes, appropriately labelled with subject identification number and sampling times. Blood samples were kept in a cool box and transported to the laboratory for centrifugation to obtain plasma samples. The plasma samples obtained after centrifugation at 9000 rpm were transferred into Eppendorf tubes, shaken with 1 mL of mobile phase to extract the drug. Supernatant (500 µL) was withdrawn and a volume of the internal standard (artesunate), was added to the sample to an effective concentration of 1 mg/mL. The samples were analysed using the developed HPLC method described earlier. The procedure was repeated for blood samples taken at 0.25, 0.5, 2, 6, 8, 12, 24, 72 h after drug administration.

The concentrations of the samples were calculated using the response factor together with peak area of the analyte and internal standard.

Pharmacokinetic assessment and statistical analysis

A plasma drug concentration - time curve was plotted using Microsoft Excel and GraphPad Prism version 6. The pharmacokinetic parameters AUC_{0-72} and $AUC_{0-\infty}$ of the reference and test drug were calculated using Non–compartmental Pharmacokinetic Data Analysis with PK Solutions 2.0 (Summit Research Services). The C_{max} and T_{max} were determined directly from the plasma concentration–time curve and the AUCs were calculated using the linear trapezoidal rule. $AUC_{0-\infty}$ was calculated by the summation of AUC_{0-72} and residual AUC.

The pharmacokinetics parameters were log–transferred and the geometric mean with standard deviation calculated according to the FDA guidelines.

Statistical analysis

Standard deviations, the geometric mean ratio of the test to the reference formulation and the 90% confidence interval around each mean ratio was determined non-parametrically using GraphPad Prisms version 6 (GraphPad Software, California, USA). The intervals were compared to the FDA predefined limits of 0.80 to 1.25 on a forest plot.

RESULTS AND DISCUSSION

The survey conducted showed a 100% availability of at least one brand of artemether-lumefantrine fixed combination tablet (generic or innovator).

Nine of the brands that were observed in the survey were imported mainly from India whiles only two brands were locally manufactured. This high influx of foreign brands although making drugs available to patients, does not encourage the growth of the local manufacturing industry. The high cost of production in terms of energy, inputs and capital can be said to be the main reason for most pharmaceutical companies opting to import artemether-lumefatrine tablets and repackage them rather than manufacture them locally. These challenges are not peculiar only to Ghana but most African countries. In his review, Sarkar pointed out that India accounted for 17.7% (up from 8.5% in 2002) of African pharmaceutical imports in 2011. In addition to the reasons mentioned earlier, domestic manufacturers most often struggle to implement good manufacturing practices (GMP) and ensure quality production (Sarkar, 2014).

Identification tests

The samples were subjected to colorimetric tests and melting point determination (for pure reference sample only). The samples gave positive identification results for the colorimetric test. The melting point obtained for the pure samples were similar to and complied with the melting point stated in the International Pharmacopoeia (IP) (World Health Organisation, 2015a). It can thus be inferred that the pure samples are of appropriate standards to be used as reference samples. The two sampled brands of artemether-lumefantrine combination tablets also showed positive results for the presence of artemether and lumefantrine.

The pure artemether and lumefantrine powder used in the study had melting point of 86 - 89°C and 128 - 130°C respectively. The melting point range of the compounds confirmed their identity and relative purity.

Quality control tests

The physicochemical properties of the brands of artemether-lumefantrine after the quality control tests are shown in Table 2. The brands possessed satisfactory physicochemical properties. For a batch of solid oral dosage forms, a fundamental quality feature is a constant dose of the active ingredients among the individual dosage forms. Practically, there exist variations in the individual weight; nonetheless specifications in the pharmacopoeias establish acceptable limits. These established limits tend to ensure that the variations with respect to weight and eventually the dose is reduced to a minimum as well as maintaining a consistency of dosage units during compression.

The brands of artemether-lumefantrine weighed less than 250 mg and thus to pass the uniformity of weight test, not more than two of the individual weights should deviate from the average weight by more than a percentage deviation of ± 7.5%. Furthermore, none should deviate by more than twice of the permissible range (British Pharmacopoeia, 2013). From the results obtained, the sampled brands passed the uniformity of weight test. This can be attributed to factors such as good amount of fill

placed in the die, good flow properties of granules and uniform compression force employed during the tableting process. Since the variation of each tablet weight is a valid indication of the corresponding drug content variation, it can be deduced that the sampled brands are likely to have a low variation in their respective drug content (Rawlins, 1979).

Disintegration test as applied to solid dosage forms evaluates the disintegration capability of such tablets, which is very crucial when it comes to the immediate release of the drug from the tablets. Disintegration is said to be complete when any residue of the dosage unit except fragments of insoluble coating or capsule shell remaining on the screen of the test apparatus is a soft mass with no palpable firm core (British Pharmacopoeia, 2013). The specification for disintegration test stipulates that for uncoated tablets the disintegration time should be less than 15 min and within 30 min for film coated tablets. Results of the disintegration test showed that all the brands passed the test.

The friability test, which tends to assess a tablet's ability to withstand the tendency to break, crumble or fail to maintain its integrity after compression specifies a weight loss of not more than 1% of the weight of tablet as satisfactory. All the sampled brands (both generic and innovator) passed the friability test with the percentage weight loss of less than 1%. Optimal force of compression, content of disintegrants and binder can be said to be the reasons the brands passed the disintegration and friability test.

A study by Awofisayo et al. (2010) showed that only two brands (out of six) of artemether-lumefantrine tablets on the Nigeria market complied with the specifications according to the IP. Another study in 2012 showed that only one brand of artemether-lumefantrine tablet on the Ghanaian pharmaceutical retail market actually contained the correct amount of the drugs (El-Duah and Ofori-Kwakye, 2012). These studies together with others give rise to concern with respect to how cheap substandard antimalarial flood the African market (Esimone et al., 2008; Ofori-Kwakye et al., 2008; Tipke et al., 2008; Osei-Sarfo et al., 2010).

The IP specifies that artemether-lumefantrine co-formulation tablet should contain not less than 90.0% and not more than 110.0% of the labelled amount of artemether and lumefantrine. From the assay all the brands are within the monograph specification for both active ingredients. This shows that the sampled brands are of the right quality in terms of drug content. Strong regulations and strong compliance of the pharmaceutical industry with the WHO good manufacturing practices might have contributed to this success.

HPLC method development and summary of validation parameters

The selected mobile phase system composed of 33% acetate buffer (pH=2.5) and 67% acetonitrile. This mobile phase system produced peaks with good resolution, symmetry distinct retention times and stable baseline. A flow rate of 1.3 mL/min was selected after different flow rates show peak tailing and prolong retention times. A Shandon Hypersil ODS 5 µm 4.6 x 250 mm column was used to achieve the separation of the analytes. All HPLC measurements were done at 230 nm.

Table 2. Physicochemical properties of sampled artemether-lumefantrine tablet brands.

Sample code	Uniformity of weight		Disintegration time (min) ± SD	Friability (%)	Assay (%)	
	Mean Weight (g) ± SD	NTD by ± 7.5%			Artemether	Lumefantrine
LGM	0.2429 ± 0.004	none	2.33 ± 0.08	0.886	90.47 ± 10.85	94.94 ± 4.97
INN	0.2400 ± 0.002	none	3.325 ± 0.08	0.499	107.37 ± 17.8	92.98 ± 4.78

Twenty tablets were used for the uniformity of weight determination and six tablets each for the disintegration test and assay. INN: innovator brand; LMG: locally manufactured brand; NTD: number of tablets deviating.

Using the average peak areas of the internal standard and the two analytes, artemether and lumefantrine, the mean response ratios were calculated. A linear regression analysis was carried out on the concentrations of each analyte and its corresponding mean response ratio (Fig. 1A-B). The good correlation between the mean response ratio and concentration is an indication of a responsive method towards concentration variations. The validation parameters (Table 3) proved the method to be precise, accurate repeatable and robust.

In vitro dissolution studies

The IP monograph for artemether-lumefantrine tablet does not include specifications for dissolution test. However, the USP Salmous Standards (2009) contain separate specifications for each drug. Due to different solubility characteristics of the two substances, getting a common dissolution medium is a huge challenge. A study in 2011 developed and validated a method to be used as a single dissolution test for both artemether and lumefantrine (Umapathi et al., 2011). This method was adopted for the study of the release characteristics of artemether and lumefantrine in the sampled brands with slight modification (1% tween 80 replacing 2% myrj 52).

The USP Salmous Standards (2009) specifies that for artemether, not less than 45% of the labelled amount is dissolved in 60 min while for lumefantrine not less than 60% of the labelled amount is dissolved in 45 min. From the results obtained in the dissolution study, the release of artemether from both brands passed the tolerance standard (Fig. 2).

Conversely, when the specifications for lumefantrine was juxtaposed with the results obtained (Fig. 3), at 45 min the percentage release was less than the 60% threshold. Thus, both the innovator and generic brands failed the dissolution test with respect to the release of lumefantrine. The slight modification in the dissolution medium could have accounted for the slight deviation from the acceptance criterion as dissolution medium significantly affect dissolution rate of drug.

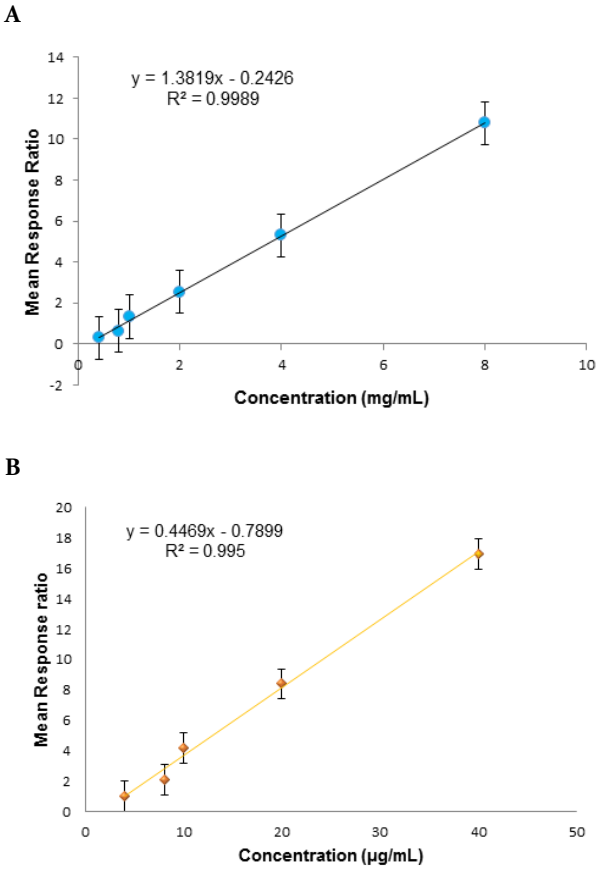

Figure 1. Linearity curve of mean response ratios against concentration for artemether **(A)** and lumefantrine **(B)**.

Table 3. Summary of validation parameters.

Parameter	Artemether	Lumefantrine
Mean % recovery	98.85 ± 0.59	99.35 ± 1.07
Precision (%RSD)		
Inter-day (n=3)	0.603	0.784
Intra-day (n=6)	1.005	0.944
Robustness	Robust	Robust
Retention time (min)	8.06	10.29

The validation results indicate the method was both accurate and robust.

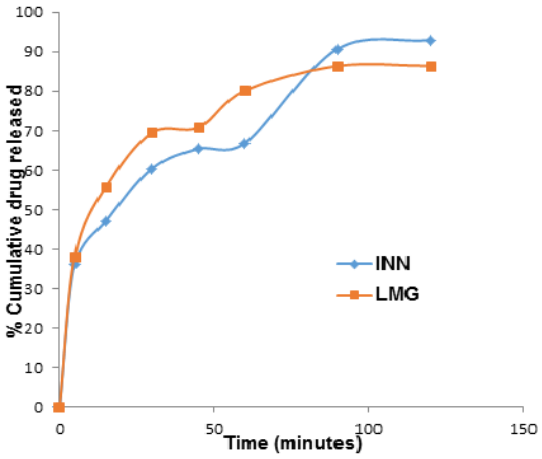

Figure 2. Dissolution profile of artemether in 0.1 M HCl containing 1% w/v tween 80 from the two formulations. INN: innovator brand; LMG: locally manufactured brand.

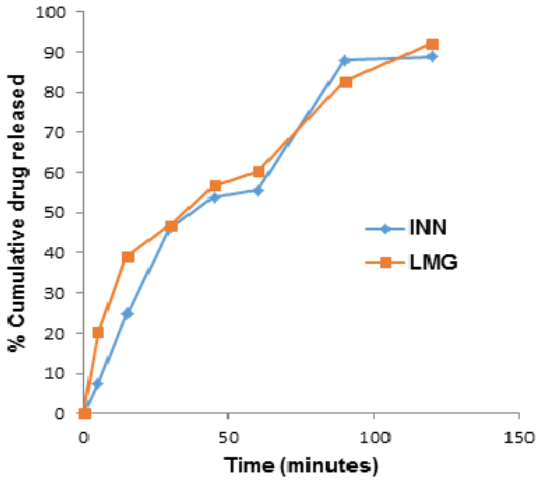

Figure 3. Dissolution profile of lumefantrine in 0.1 M HCl containing 1% w/v tween 80 from the two formulations. INN: innovator brand; LMG: locally manufactured brand.

Dissolution profile comparison

In the absence of *in vivo* bioequivalence testing, comparison of dissolution profiles using f2, a model-independent mathematical method developed by Moore and Flanner (1996) can help in assuring similarities between products. The f2 which is the similarity factor measures the closeness of the two dissolution profiles. For comparison of dissolution profile of different products, the regulatory bodies tend to focus more on the f2 comparison as it is tilted more towards knowing how similar the profiles are and to know a measure, which is more sensitive to large difference at each time point (Costa, 2001). Normally, f2 from 50 - 100 indicates similarity between two profiles. From the results, comparing the dissolution profiles of artemether, the similarity factor was 91 and that for lumefantrine was 99. It can be inferred that when it comes to the release of artemether and lumefantrine, the dissolution profiles are similar.

The dissolution efficiency (DE) as suggested by Khan and Rhodes (1972) is the area under the dissolution curve up to a certain time t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. It is determined so as to ascertain the extent to which a brand or batch will be effective in releasing the active ingredient. Generally, the higher the DE the more efficient a brand is in releasing the active ingredient. LMG was more efficient (DE 88.35%) in the release of artemether than the innovator brand (DE 78.32%). The DE (69.41 and 69.94 for LMG and INN, respectively) of both brands in the case of lumefantrine was low indicating the release of that drug was not efficient. This relatively low DE value can be attributed to formulation factors such as amount and type of excipients e.g. disintegrants, granule size and its distribution. The dissolution medium could have also contributed to the low DE observed (Ghayas et al., 2013).

In vivo bioavailability studies using animal models

In the *in vivo* bioavailability study, lumefantrine was the only analyte to be adequately detected and quantified by the method. A study in 2013, which also compared the bioavailabilities of different artemether-lumefantrine formulations in Tanzania also reported that lumefantrine was the only analyte quantified *in vivo*. It was subsequently used as the drug to assess the bioequivalence between the formulations (Minzi et al., 2013). Two reasons might have contributed to this observation. The quantity of artemether per dose in the combination formulation is lower relative to lumefantrine (ratio of 1:6). Thus, a low amount coupled with first pass effect and lack of absorbing chromophore might have contributed to the non-detection of the artemether

from the extracted plasma by the Ultra-Violet detector.

Comparable plasma concentration – time curves (Fig. 4) of lumefantrine was obtained after the administration of a single dose of the two samples. The mean pharmacokinetic parameters obtained from the concentration–time profiles showed no statistical difference ($p = 0.05$) between both brands except Tmax (Table 4). This implies the AUCs and Cmax, which measure the extent and rate of absorption of drug from both brands were similar even though the Tmax is dissimilar. This is because most regulatory bodies rely on Cmax rather than Tmax to estimate the rate of absorption in bioavailability studies (Chen et al., 2001).

The pharmacokinetic parameters obtained were all within the range of values by Novartis Pharma (manufacturers of the innovator brand), thus giving a strong indication of both brands achieving comparable plasma levels of lumefantrine (Minzi et al., 2013). In the bioequivalence assessment of the two brands, the geometric mean ratios of all the various pharmacokinetics parameters (AUC_{0-72}, $AUC_{0-\infty}$, and C_{max}) were within the FDA criterion of bioequivalence of 0.80 – 1.25. In comparing the 90% confidence interval obtained for the pharmacokinetic parameters to the criterion range, the ratio of INN to LMG with respect to $lnAUC_{0-72}$, $lnAUC_{0-\infty}$, and lnC_{max} were 100.36, 94.42 and 96.76, respectively. Their respective 90% confidence intervals of the geometric mean were 93.2 - 108.2, 83.9 - 106.3 and 86.8 - 103.4 and were within the standard range of 80 - 125 ($0.1 \leq p \geq 0.93$).

The confidence intervals of the ratios of AUC_{0-72}, $AUC_{0-\infty}$, and C_{max} on a forest plot (Fig. 5) showed the intervals to be within the boundary of the FDA criterion range. Accordingly, it can be inferred that based on this study the two tablet formulations meet the FDA bioequivalence criteria. Thus, the innovator can adequately be interchanged with the generic brand. In recent times, a number of studies in the post marketing setting have highlighted some lack of equivalence between generic and innovator brands (Elkoshi et al., 2002; Del Tacca et al., 2009; Minzi et al., 2013). Nonetheless the similarity that has been exhibited in this study can be taken as evidence in support of therapeutic effectiveness and safety of the generic product LMG.

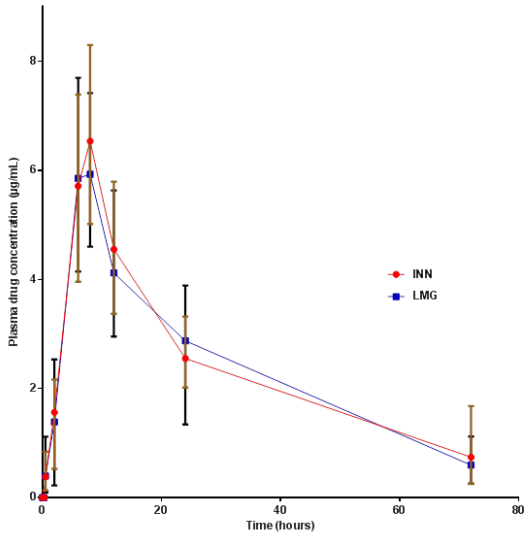

Figure 4. Mean plasma drug concentration – time profiles of lumefantrine in subjects after oral administration of both innovator brand (INN) and locally manufactured brand (LMG).

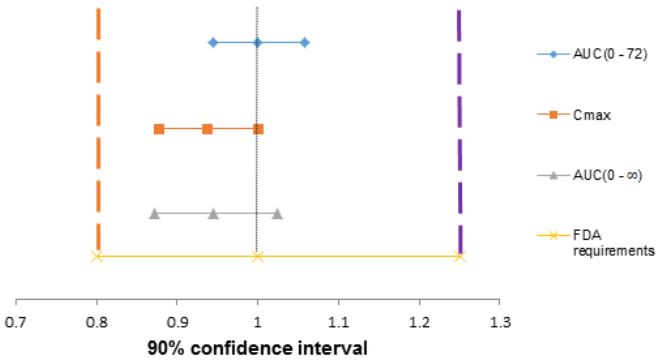

Figure 5. A forest plot showing the 90% confidence interval of the pharmacokinetic parameters plotted over the FDA requirement boundaries (dotted lines on the right and left represent the upper and lower limits respectively).

Table 4. Ratio of average untransformed data and data obtained by Norvatis Pharma (n=20).

Pharmacokinetic parameters	LMG	INN	P value	% Ratio of test to reference	Novartis data
(AUC_{0-72}) (µg·hr/mL)	173.150 ± 18.12	172.336 ± 16.14	0.91	100.47	--
($AUC_{0-\infty}$) (µg·hr/mL)	193.209 ± 19.56	207.493 ± 47.57	0.31	93.12	108.0- 243.0
(C_{max}) (µg/mL)	6.173 ± 0.63	6.531 ± 0.84	0.21	94.52	5.1 - 9.8
T_{max} (h)	6.6 ± 0.97	8 ± 0.00	0.0013	82.50	6 – 8

Significantly different (p = 0.10). Since each subject received both the locally manufactured brand and the innovator brand, each subject served as its own control and the n-value is the number of experimental subjects (20). INN: innovator brand; LMG: locally manufactured brand.

CONCLUSIONS

Even though artemether-lumefantrine coformulation tablets are widely distributed in the pharmaceutical retail outlets, most of them are imported brands. The brands of artemether-lumefantrine sampled for this study satisfied the specification for identification, disintegration test, uniformity of weight test, friability test and percentage content. The sampled brands satisfied the specification for dissolution testing with respect to artemether but failed that of lumefantrine. Statistical analysis showed similar dissolution profiles for artemether and lumefantrine. Average bioequivalence between LMG and INN was demonstrated due to compliance with the FDA 90% confidence intervals. This makes them bioequivalent and thus LMG can be interchanged with INN as it is likely to produce a similar therapeutic response in the treatment of uncomplicated *P. falciparum* malaria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Kuntworbe N	Acquah FA	Johnson R	Ofori-Kwakye K
Concepts or ideas	X	X		
Design	X	X		
Definition of intellectual content	X	X		
Literature search		X		
Experimental studies		X		
Data acquisition		X		
Data analysis		X		
Statistical analysis		X		
Manuscript preparation		X		
Manuscript editing	X		X	X
Manuscript review	X		X	X

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