https://dx.doi.org/10.4314/jpb.v15i2.13
Vol. 15 no. 2, pp. 188-196 (September 2018)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Effect of fixed-dose sizes on *in vitro* properties of artemether-lumefantrine tablets

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Received 19th July 2018; Accepted 20th August 2018

Abstract
The World Health Organization recommends artemisinin combination therapy (ACT) for the treatment of uncomplicated malaria. Artemether-lumefantrine (A-L) is a fixed-dose combination (FDC) of two active antimalarial ingredients. Pharmaceutical equivalence (PE) of three FDC: Coartem® (20/120 mg); B (40/240 mg) and C (80/480 mg) were investigated. Assay was performed for A-L separately with a validated HPLC method. Assay for artemether in Coartem®, B and C were 93.34%, 98.27% and 100.78% while for lumefantrine 96.44 %, 97.34 % and 93.52 % respectively were obtained. From the dissolution test, the mean percent of artemether released after 1 h for Coartem®, B, and C were 38.67%, 32.86% and 50.74% respectively. Lumefantrine after 45mins gave 93.89%, 64.39% and 64.76% respectively. The data obtained were compared using ANOVA which was not statistically significant (P>0.05). The f2 values for B & C was greater than 50 suggesting their similarity with Coartem®. The focus of this study is to evaluate their *in vitro* properties and their overall performance.

Keywords: Artemether-lumefantrine tablets; Fixed-dose combination; Pharmaceutical equivalence; Antimalarial

INTRODUCTION

Nearly all human malaria is caused by four species of obligate intracellular protozoa of the genius Plasmodium. The malaria caused by *Plasmodium falciparum* is the world's most devastating human parasitic infections [1]. The world Health Organization recommends artemisinin combination therapy (ACT) for the treatment of uncomplicated malaria [2]. A combination of an artemisinin derivative and another structurally unrelated and slowly eliminated antimalarial [3]. Artemether-lumefantrine (A-L) is a fixed-dose combination of two antimalarial active ingredients, which is indicated for the treatment of acute, uncomplicated malarial infection due to *Plasmodium falciparum* in patients. The chemical name of artemether is (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin; CAS Reg. No. 71963-77-4. It is a white crystals or a white, crystalline powder with a molecular formula of $C_{16}H_{26}O_5$ and weight of 298.4 [4]. The chemical name of lumefantrine is (1*RS*)-2-(dibutylamino)-1-{(9*Z*)-2,7-dichloro-9-[(4-chlorophenyl) methylidene]-9*H*-fluoren-4-yl}ethanol; CAS Reg. No. 82186-77-4 also known as benflumetol. It is a yellow crystalline powder with a molecular formula

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ISSN 0189-8442

of $C_{30}H_{32}Cl_3NO$ and weight of 528.9 [4] (Figure 1). Artemether-lumefantrine has been shown to be effective against uncomplicated malaria [1]. A three-day treatment schedule of artemether-lumefantrine with 80 mg of artemether and 480 mg of lumefantrine per dose is normally administered to adult patients with a total of six doses recommended. In children, the dosage is according to individual body weight.

A fixed-dose combination (FDC) can be defined as a combination of two or more actives in a fixed ratio of doses. They are particularly useful in the management of infectious diseases that pose as serious threats in the world today [5] diseases such as human immunodeficiency virus/ acquired immuno-deficiency syndrome (HIV/AIDS), malaria and tuberculosis. Fixed dose combinations have advantages in that they are safe and effective, which help to improve patient compliance through reduction in pill burden, improved tolerability, reduce prescription cost, ease of storage, portability, ease of administration, and accuracy in dosing. However, the *in vitro* release of these dose sizes may be different due to formulation properties and this may affect drug absorption [5]. For example, artemether-lumefantrine tablets are presented in different fixed dose combinations of ratio 1:6 (20mg/120mg, 40mg/240mg, 60mg/360mg and 80mg/480mg). For the 20mg/120mg dose size of artemether-lumefantrine tablets, four tablets are taken together as single adult dose, while for the 80 mg/480 mg dose size, only one tablet is taken per dose by adult patients.

The generic formulations of fixed-dose size of A-L are expected to be pharmaceutically and therapeutically equivalent to the reference listed drug/innovator, Coartem®, even when there are differences in their physical characteristics in terms of sizes, shape and excipient used in their formulations. Some generic drug products have shown differences in *in vitro* properties when compared to innovator brands and in some cases; these generics may be of poor quality [6-8]. The rate and extent to which the amount of drug substance dissolved over a period of time is called dissolution. Dissolution testing is the primary pharmaceutical test that is designed to probe the performance of dosage forms. The dissolution method developed is compared with the innovator's reference product to evaluate the release pattern and establish the method comparison for estimating the drug released [9].

However, some studies on in vitro assessments and pharmaceutical equivalence of anti-malarial revealed that most of the antimalarial drugs assessed were of good quality [6-8]. Artemether lumefantrine tablets are widely manufactured in different fixed dose size combination ratios (20/120mg, 40/240mg, 60mg/360mg and 80/480mg). It is a known fact that smaller tablets generally have significantly faster transit times. Products are pharmaceutical equivalents if they contain the same amount of the same actives in the same dosage form, if they meet comparable standards, and if they are intended to be administered by the same route. Pharmaceutical equivalence does not necessarily imply therapeutic equivalence, as differences in the excipients and/or manufacturing process and some other variables can lead to differences in product performance.

The focus of this study is to investigate the effect of the differences in the fixed-dose combination ratios of two selected brands of A-L manufactured locally here in Nigeria in comparison with Coartem®, the innovator brand, on the *in vitro* properties of these tablets and its implication on product quality and performance before further bioequivalence studies will be conducted.

EXPERIMENTAL

Chemicals and reagents. Three (3) brands of A-L tablets with different fixed- dose size presentations namely; 20 mg/120 mg (which is innovator Coartem® Novartis, Basel, Switzerland); 40/240mg (B) and 80/480mg (C), were purchased from retail Pharmacy outlets in the FCT Abuja, Nigeria. Artemether and lumefantrine pure reference standards were obtained from USP, Rockville USA. Glacial acetic acid HPLC grade acetonitrile purchased from Sigma-Aldrich. Tetrahydrofuran, benzalkonium, cyclohexane, ethyl acetate were all analytic grade. Freshly distilled water was also used.

Instrumentation and analytical conditions. High Performance Liquid Chromatography (HPLC) was carried out on Agilent 1200 series (Santa Clara, California) composed of a binary pump with a ChemStation Software for data acquisition and analysis. The separation was performed on Ultrasphere ODS Column C_{18} (5µ particle size, 4.6 x 250mm) and detected on Diode array Detector DAD at 216 nm wavelength for artemether with the mobile phase composition of acetonitrile: water (70:30) at a flow rate of 2.0 ml/min. and injection volume of 20µl. The retention time was 6 min. For lumefantrine, column used was Zorbax eclipse C8 (5µ particle size, 4.6 x 150mm) at a detector wavelength of 265 nm. The mobile phase consisted of acetonitrile: 25mM KH_2PO_4 (70:30) and pumped at a flow rate of 1.0 ml/min. The retention time of lumefantrine was 4.5 min. The dissolution was performed on INTECH® USP dissolution Test Apparatus (USP Apparatus 2 with Eight-vessel Paddle). Tablets were weighed individually using Ohaus analytical plus balance (Model number AP50 capacity 210g-0.01mg serial number 1127070902). Disintegration test was carried out using Erweka station GmbH Germany (Model number 2tu-4, serial number 9416930). The Hardness (Erweka) test was done using a Hardness tester (Model number HT, serial number 65770). Roche friabilator single Erweka (Model number TAR, serial number 66939) was used to carry out the friability test on the tablets. Other items include: porcelain mortar and pestle for crushing the tablets; vortex mixer; Sonicator (Decon®) to degas the solvents used; pressure pump model xx5522050 for filtration of mobile phase through a 0.45µm membrane filter.

Quality assessment and physicochemical properties

Visual inspection. The packaging of the artemether-lumefantrine tablets were checked for correctness labelling and were checked for the expiration date, manufacturer's information, and batch number. The appearance of the tablets was also examined for discolouration.

Uniformity of weight test. For each of the dose size, twenty (20) tablets were weighed individually using Ohaus analytical plus balance. The average weight were calculated as well as their percentage deviation from the average weight.

Disintegration test, crushing force test and friability test. The test was carried out using Erweka station. Six tablets were randomly selected for each dose size and their disintegration times were determined using distilled water as the medium. The times for the tablets to disintegrate were observed. Six tablets in each dose size was selected and tested for hardness. Ten tablets were weighed and was allowed to tumble at 25 rpm in a Roche friabilator. After a time interval of 5 min., it was de-dusted and re-weighed and the percentage loss in weight was calculated.

Dissolution test for artemether. The dissolution tests was performed using standard USP apparatus 2 (paddle) at 100 rpm and a temperature range of 36.5-37.5°C. The dissolution medium for was 0.1M of HCL. Four tablets from each dose size were tested for artemether 5 ml aliquots was withdrawn

from the dissolution medium at (5, 15, 30, 45, 60, 75, 90, 120 minutes) and replaced with 5 ml of the dissolution medium at each time point in order to maintain a constant volume (900 ml) of the dissolution medium in the vessel. The samples were filtered through a 0.45 μm membrane filter and the first few ml discarded. The assay of the drug was performed with HPLC and the amount of the drug released at each point released was calculated [10-14].

Dissolution test for lumefantrine. The dissolution tests was performed using standard USP apparatus 2 (paddle) at 100 rpm and a temperature range of 36.5-37.5°C [15]. The dissolution medium for lumefantrine was 1% benzalkonium in 0.1M HCl and four tablets of each dose size were used and procedure for artemether was repeated.

Preparation of 25mM buffer. Accurately weighed sample (3.4001 g) of potassium dihydrogen orthophosphate (KH_2PO_4) was carefully transferred into a 1000 mL volumetric flask about 250ml of distilled water was added and thoroughly mixed by shaking mechanically to dissolve the salt. The volume was made up to the mark with distilled water .and the pH was adjusted to 3.5 with orthophosphoric acid.

Preparation of artemether and lumefantrine stock solutions. Stock solution of artemether was prepared by accurately weighing 10 mg of the reference standard. It was transferred into a 10 mL volumetric flask and about 5 ml of acetonitrile was added into the flask. It was whirl mixed for 5 minutes and sonicated for 10 minutes before adding acetonitrile to make up to the 10 mL mark giving a concentration of 1 mg/ml. This was labelled as stock solution for artemether. On the other hand, 10 mg of lumefantrine reference standard was accurately weighed and carefully transferred into a 10 mL volumetric flask. About 2.5 ml of tetrahydrofuran was added to dissolve the powder and about 2.5 ml of acetonitrile was added. It was whirl mixed for 5minutes and sonicated for 10 minutes. The volume was made up to the 10 mL mark with 25% tetrahydrofuran in acetonitrile as diluent. This gave a concentration of 1 mg/ml and was labelled as stock solution for lumefantrine.

Preparation of calibration curves. The working solutions containing artemether and lumefantrine were prepared using serial dilutions of the stock solutions and a diluent of acetonitrile: water (70:30) for artemether and acetonitrile: buffer for lumefantrine. Concentration of 100 μg/ml for artemether and lumefantrine was obtained**.** A calibration curve of 10-100 μg/ml was prepared for artemether and 60-600 μg/ml was prepared for the lumefantrine.

Assay for artemether. Twenty tablets from each dose size were weighed and powdered using a porcelain pestle and mortar. An accurately weighed portion of the powder, equivalent to 10 mg of artemether, was carefully weighed and transferred to a 10 ml volumetric flask. To this, 4.0 ml of 60% acetic acid and 3.0 ml acetonitrile were added. The resulting solution was sonicated for 10 min and the volume was made up to the 10 ml mark with acetonitrile. It was labelled as stock solution. The solution was filtered using 0.45 μm membrane filter discarding the first few ml. The solution was vortex mixed for 5 min. Three concentrations 20 μg/ml, 40 μg/ml and 80 μg/ml were prepared using acetonitrile: water (70:30) as diluent and analyzed at 216 nm.

Assay for lumefantrine sample. Twenty tablets from each dose size were weighed and powdered. A weight of powder equivalent to 10 mg of lumefantrine was carefully weighed and transferred into a 10 ml-volumetric flask. To this, 2.5 ml of tetrahydrofuran and 3.0 ml acetonitrile were added. The resulting solution was sonicated for 10 min, the volume made up to 10 ml with acetonitrile and labelled as stock solution. The solution was

filtered using 0.45 μm filter paper and the first few ml was discarded. The filtrate was vortex mixed for 5 minutes, a concentration of 100 μg/ml solution was prepared and labelled as first dilution and further dilutions were made to prepare three concentrations; 60 μg/ml, 240 μg/ml and 480 μg/ml using acetonitrile: buffer (70:30) as diluent and analyzed at 265 nm.

Statistical analysis and similarity factor. Two-way ANOVA was used for the statistical analysis. $P < 0.05$ was taken as the significant level. The similarity factor f2 and dissimilarity factor f1 was calculated for the Dissolution in the three fixed dose combination to explore the level of similarity and interchangeability. The dissolution values were compared using similarity factor (f2) and difference factor (f1) which were calculated using equations 1 and 2.

$$f1 = \{[\_t = 1\ n\ (Rt - Tt)] / [\_t = 1\ n\ Rt]\} \times 100 \quad \ldots\ldots (1)$$

$$f2 = 50x \log \{[1 + (1/n)\_t = 1\ n\ (Rt - Tt)2] - 0.5 \times 100\} \quad \ldots\ldots\ldots (2)$$

where Rt and Tt are the cumulative percentage dissolved at each of the selected 'n' time points of the reference and test product respectively.

RESULTS AND DISCUSSION

The visual inspection carried out on the three brands all the packaging of the tablets were found to be correctly labelled and there was no discoloration observed in any of the brands samples. There was also no excessive chipping observed indicating proper packaging, storage and handling. The results of the *in vitro* assessment carried out on the three dose sizes are summarized in the Tables, 1 and 2 while the plots of percentage of drug released versus time are represented in figures 1 and 2.

The weight uniformity test carried out on the three fixed dose combination tablets fell within the allowed percentage deviation in BP [5] specification of not more than two tablets deviating from the average weight by more than 5% and no tablet deviating by more than 10% from the average weight. The crushing force from the hardness test for the tablets ranged from 4.67to 5.30 kg/cm^2 while the percent friability for all the tablets from the three dose sizes tested ranged from 0.002 to 0.186 %. All the tablet samples conformed to percentage friability of less than 1%. This result showed that losses from tablets during handling are within limits.

The disintegration times for the three dose sizes of artemether-lumefantrine ranged from 2:23 minutes – 2:43 minutes. Disintegration is a critical step in the process of drug absorption in to the systemic circulation for therapeutic effect. From the results obtained passed the required standard as stated in the BP [5] that disintegration time for uncoated tablets should not be more than 15 min.

For the assay, percentage content for artemether in Coartem®, B and C were 93.34%, 98.27% and 100.78% while for lumefantrine it was 96.44 %, 97.34 % and 93.52 % respectively. These values conform with the requirement stated in the United States Pharmacopoeia [15] which stated that artemether-lumefantrine should contain not less than 90.0 % and not more than 110.0 % of the label claim. The variation in contents in the three different dose sizes were not statistically significant with $P > 0.05$.

According to the USP 2009 Salmous [15] specification for dissolution test carried out on artemether and lumefantrine, tolerance limits of dissolution of active pharmaceutical ingredient (API) of not less than 45% (Q%) of the labelled amount of artemether should be dissolved in 1 hour and not less than 65% (Q%) of labelled artemether should be dissolved in 3 hours. For lumefantrine, not less than 60% (Q %) of the labelled amount should be dissolved in 45 minutes. From the results of dissolution for artemether in these brands, the percent released in Coartem®, brands B and C tablets were 38.67%, 32.86% and 50.74% respectively while for lumefantrine, percent released after 45

minutes were 93.8970%, 64.3912% and 64.7628%.

The difference or dissimilarity values of f1 for artemether in samples B and C gave 21.68 and 60.44 respectively while for lumefantrine 41.52 and 40.91 (Table 3). Similarity factor (f2) for artemether, in brand B and C was 158.88, 180.19 while for lumefantrine 180.19 and 180.34 respectively. Ideally f1 values should be between 0-15 and f2 greater than 50-100 this ensures sameness and equivalence of the two curves and, thus, of the performance of the test (brand) and reference/innovator (brand) products. However the f1 (dissimilar values) and f2 (Similarity values), the dissimilar values is less than the similarity values obtained hence they are similar [16-19].

Dissolution method for FDC drug products may be challenging due to differences in physicochemical properties of the active ingredients (e.g., form, pH-solubility profile and pH dependent stability profile) which could prevent the selection of a common dissolution medium. In addition, large dose disparity (e.g. a low dose component such as in the case of artemether 20 mg in combination with a high dose of 120 mg lumefantrine a ratio of 1:6), differing release mechanisms (e.g. an immediate release in combination with an extended release or a modified release) could also present challenges for development of a single dissolution method to assess *in vitro* release of multiple components in a combination drug [5]. Dissolution medium for the lumefantrine was 0.1% HCl and 1% benzakonium chloride because of its poor solubility [15].

Conclusion. From the results obtained the assay of artemether-lumefantrine with the different fixed dose combination ratios were within required limits according to the USP, which states that artemether and lumefantrine should contain not less than 90.0 % and not more than 110.0 % of the label claim. The variation in contents in the three different dose sizes were not statistically significant with P >0.05.

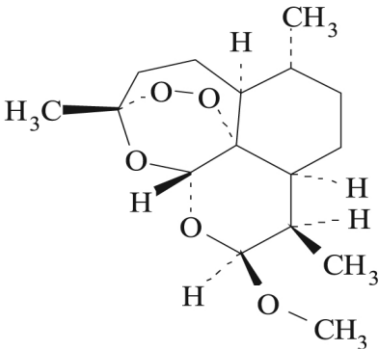

Figure 1a: Artemether $C_{16}H_{26}O_5$298.4 g mol^{-1} (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin;

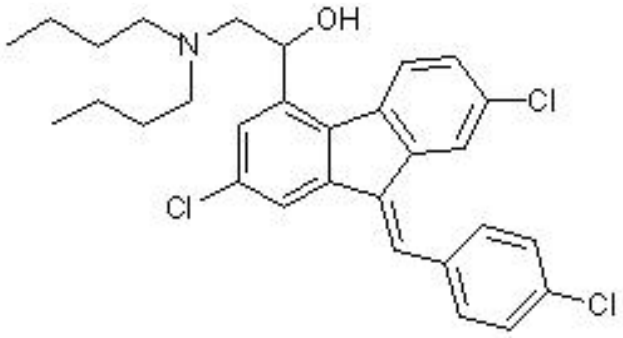

Fig.1b: Lumefantrine $C_{30}H_{32}Cl_3NO$ (1*RS*)-2-(dibutylamino)-1-{(9*Z*)-2,7-dichloro-9-[(4-chlorophenyl) methylidene]-9*H*-fluoren-4-yl}ethanol;

T**able 1:** *In vitro* properties of three fixed-dose sizes (Coartem®, B & C) of artemether-lumefantrine tablets

Dose size (mg)	Assay (%)		% deviation by ± 5 %	Disintegration time (min)	Friability (%)	Hardness (kg/cm^2)
	Artemether	Lumefantrine				
20/120 (Coartem)	93.34±0.76	96.44±0.58	Nil	2.43	0.014±0.0415	4.95 ±0.415
40/240 (B)	98.27±1.58	97.34±1.06	Nil	2.23	0.162 ±0.002	5.30 ±0.11
80/480 (C)	100.78±0.75	93.52 ± 0.82	Nil	2.24	0.186 ±0.062	4.67 ± 0.97

Table 2: Dissolution profile of three dose sizes of artemether-lumefantrine (Coartem®, B & C) at 60 min for artemether and 45min for lumefantrine

Dose size (mg)	Artemether		Lumefantrine	
	Time (min)	% dissolved (Q)	Time (min)	% dissolved (Q)
20/120 (Coartem)	60	38.67± 13.39	45	93.89± 3.90
40/240 (B)	60	32.86 ± 6.84	45	64.391 ± 0.4
80/480 (C)	60	50.74±4.23	45	64.76 ± 2.76

Table 3: Dissolution profile between dose sizes of artemether-lumefantrine in B and C comparing with Coartem® (innovator) using the f1 and f2 factors

Time	f1 Artemether		f1 Lumefantrine	
(min)	B	C	B	C
5	45.11	34.77	58.83	65.28
15	52.95	-14.73	51.28	68.34
30	50.86	-14.73	44.98	40.33
45	46.37	51.09	31.42	31.03
60	15.02	33.82	23.86	27.52
90	36.62	-31.21	45.4	32.15
120	4.96	61.52	36.59	25.77
F1	21.68	60.44	41.52	40.91
Time	f2 Artemether		f2 Lumefantrine	
(min)	B	C	B	C
5	151.125	145.47	185.5	188
15	156.395	128.61	187.5	194
30	161.725	161.825	186	183
45	169.495	162.64	176	175.5
60	140.63	156.51	170.5	173.5
75	159.095	170.365	186	179
90	110.49	159.76	180	172.5
120	132.525	163.89		
F2	158.88	180.19	180.19	180.34

Table 4: Dissolution profile for artemether in three dose sizes of artemether-lumefantrine tablets

Time	% artemether released		
(min)	Coartem	Tab B	Tab C
5	20.88±15.3	11.46±4.6	13.62±8.5
15	22.68±2.7	10.67±1.8	26.02±15.2
30	30.18±10.4	14.83±10.38	14.76±5.9
45	47.34±2.9	25.39±9.2	31.33±2.9
60	38.67±13.3	32.86±6.8	50.74±6.9
75	37.14±7.1	23.54±16.6	14.29±15.2
90	29.23±16.1	27.78±14.05	15.21±13.6
120	22.17±5.01	26.17±14.6	39.13±22.1

Table 5: Dissolution profile for mean lumefantrine released over time in the three FDC of A-L tabs

Time	% lumefantrine released		
(min)	Coartem	Tab B	Tab C
5	79.75±2.7	32.83±1.2	27.69±6.2
15	100.01±4.9	48.72±2.2	31.66±23.2
30	103.50±1.9	56.95±1.9	61.87±5.8
45	93.89±11.5	64.39±0.47	64.76±4.9
60	95.33±11.0	72.58±15.3	69.09±1.6
90	104.88±3.5	57.25±18.7	71.16±2.9
120	97.39±7.6	61.75±4.6	72.29±0.6

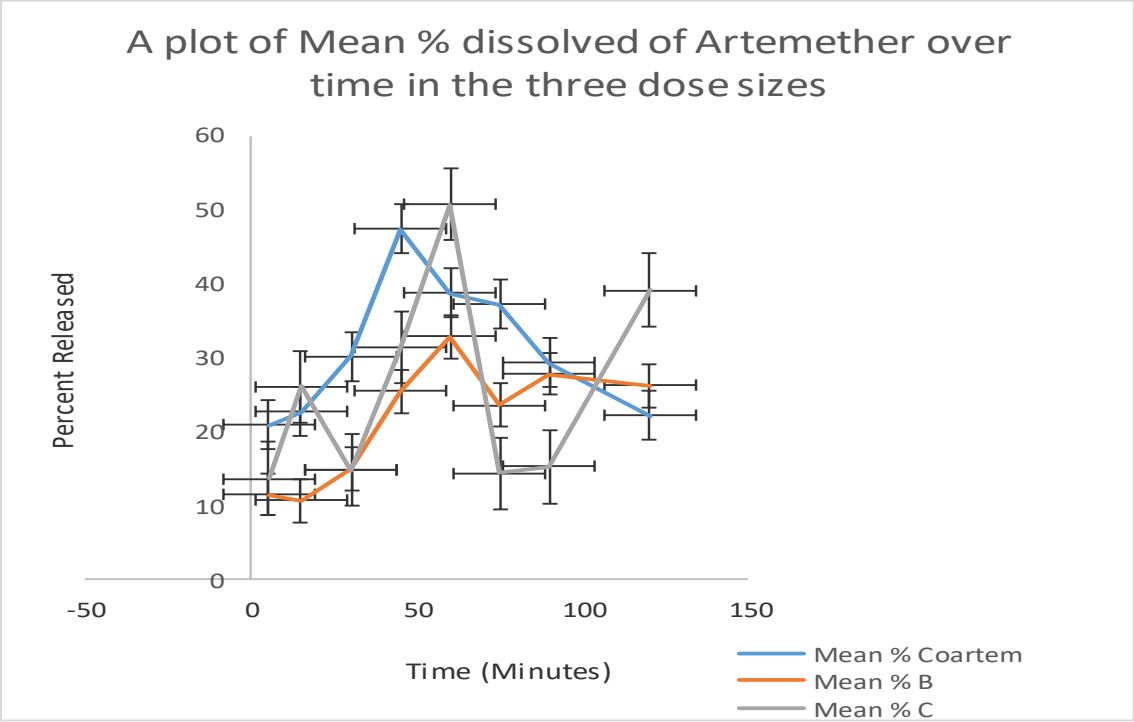

Figure 2: A graph of % mean (n=4) of artemether released versus time (min.) in three dose sizes of A-L tablet

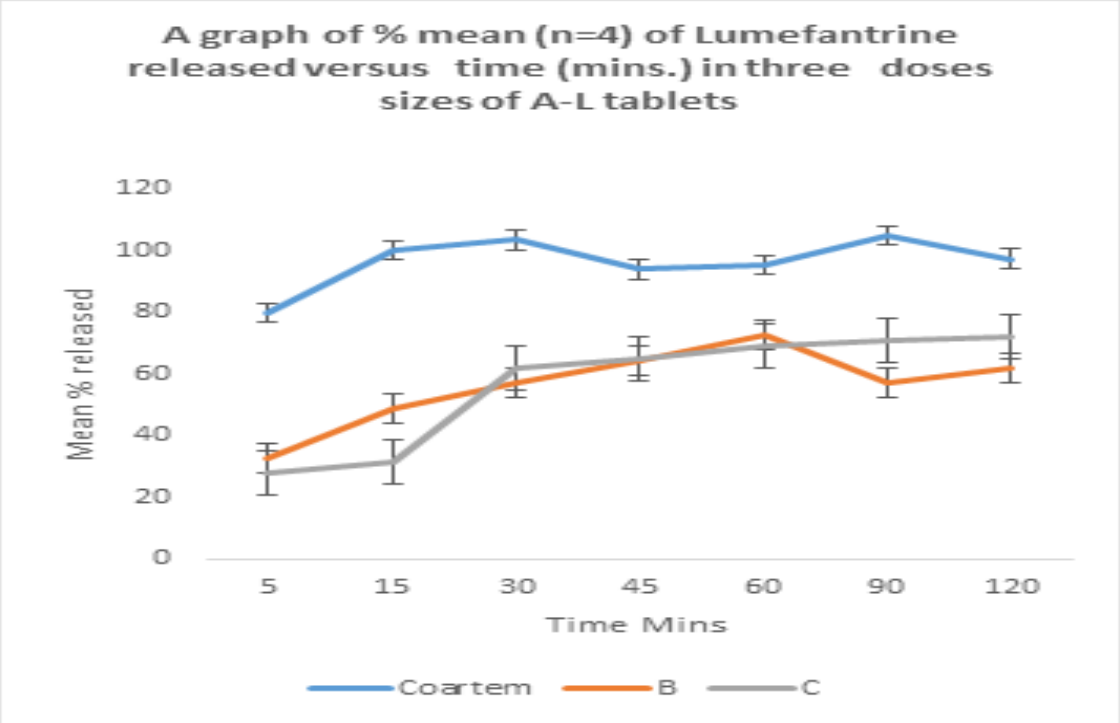

Figure 3: % mean (n=4) of lumefantrine released versus time (min.) in three doses sizes of A-L tablets

Acknowledgement. The authors would like to thank Prof. Silva and Mr. P. D. Ojobor of the CMUL LUTH Nigeria for use of the Dissolution Tester.

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