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Research Article

In-Vitro Bioequivalence Analysis of some Artemether -Lumefantrine-Based Combination Formulations utilized in Nigeria

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Abstract

Bioequivalence analysis is a scientific method of establishing the uniformity in weight, rate of dissolution, disintegration, friability, safety, quality, and standard of varying pharmacokinetics of the same medication active component manufactured by various companies.

Aim: The study's goal was to assess the in-vitro bioequivalence of five different brands of artemether/lumefantrine-based ACT solid dose formulations and compare them to an innovator brand as well as British pharmacopoeia standards.

Method: All the tests including uniformity of weight, dissolution rate, friability, and UV/Visible spectroscopy was conducted following the guidelines stated in the British pharmacopeia.

Results: Individual tablets of the standard innovator product had a mean weight of 0.243g, with a variation and percentage standard deviation of 0.0024 and 1.08%, respectively. Weight, weight deviation, and percent standard deviation for the other brands were 0.308, 0.0023, and 1.0% (L2), 0.342g, -0.0012, and -0.5% (L3), 0.351g, -0.0012, and -0.5% (L4), and 0.308g, 0.0023, and 1.0% (L5), respectively. Thus, SD had 0.4% friability, L2 had 0.4% friability, L3 had 0.7% friability, L4 had 0.46% friability, and L5 had 0.13% friability. The artemether component of L3 dissolved more quickly than the other brands in 5 minutes, followed by L5, L2, the standard medication SD, and L4 with a longer disintegration and dissolving time. The medicine was released at 5 minutes and 90 minutes in the standard innovator brand (SD), 5 minutes and 10 minutes (L2), 10 minutes and 5 minutes (L3), 10/90 minutes and 45 minutes (L4), and 60 minutes and 10/15 minutes (L5), respectively. The samples were evaluated using TLC and compared to a standard using the British Pharmacopeia, and they were all found to be pure.

Conclusion: Based on the criteria provided in the British Pharmacopoeia, all assessed brands were found to be bioequivalent within the standard limits. Proprietary excipients and evident discrepancies in dissolution, disintegration, friability, and weight variation would eventually influence the pharmacokinetic characteristics of these products when administered.

Keywords: Artemether; lumefantrin; antimalaria; bioavailability; artemisinin-based combination treatment (ACT)

Introduction

Malaria is a serious public health issue in 97 tropical and subtropical nations and territories. Annually, around 214 million cases of malaria occur worldwide, with 3.2 billion people at risk [1]. According to the WHO, there will be 247 million new cases of malaria in 2021, resulting in 619,000 fatalities [2]. Children under the age of five are the most vulnerable, accounting for 67% of malaria fatalities globally in 2019 [3]. Every year, around 125 million preg-

nant women are at risk of infection; in Sub-Saharan Africa, maternal malaria is related with up to 200,000 projected child fatalities [4]. As a result, there has been a significant increase in interest in research and innovation in diagnostic tools, medications and vaccines, and the creation of malaria control strategies during the last decade. As a result, worldwide malaria incidence rates plummeted by 30% between 2000 and 2013, and by 34% in Africa [5]. Nigeria has the world's highest malaria burden, with roughly 51 million cases and 207,000 fatalities recorded each year (nearly 30% of Africa's overall malaria burden), and 97% of the whole population is at risk of infection. Plasmodium falciparum and mosquitoes such as Anopheles gambiae, A. funestus, A. arabiensis, and A. moucheti cause malaria.

Since 2008, Nigeria's National Malaria Control Program (NMCP) has implemented a specific plan with the goal of attaining at least 80% coverage of long-lasting coated mosquito nets (LLINs) by 2013, in addition to other measures such as 20% of houses in the designated regions receiving indoor residual spraying (IRS) as well as treatment with two doses of intermittent preventive therapy (IPT) for 100% of women who are pregnant who visit a health facility. As a result of these efforts, the number of homes with at least one LLIN increased from 5% in 2008 to more than 70% by 2010. Although past studies have shown that malaria is prevalent across Nigeria, there has been little study on people's knowledge, attitudes, and practices (KAP) concerning malaria in the majority of the country, particularly in Southern Nigeria, which includes Bayelsa State [6]. In 2004, the approved treatment regimen was artemether-lumefantrine (AL) or artesunate with amodiaquine [7]. Certain types of malaria infections are treated with a combination of artemether and lumefantrine. Artemether is produced from the Chinese plant Artemisia annua [8].

Artemether's antimalarial activities result from interactions with parasite transport proteins, impairment of parasite mitochondrial activity, suppression of angiogenesis, and modification of host immunological function [9-13]. Artemether is converted in the body to the active metabolite dihydroartemisinin. It operates against P. falciparum erythrocytic stages by decreasing nucleic acid and protein production. Artemether has a quick start of effect and is quickly eliminated from the body. Artemether is supposed to give quick symptom relief by lowering the quantity of malarial parasites. Artemether is used in conjunction with lumefantrine to boost effectiveness, which is known as artemisinin-based combination treatment (ACT). Lumefantrine has a substantially longer half-life of around 4.5 days, is 99.7% protein bound, and is extensively metabolized in the liver, predominantly by CYP3A4, to desbutyl-lumefantrine. It is thought to eliminate leftover parasites by interacting with ferriprotoporphyrin IX (heme) or ferrous ions in the acidic parasite feeding vacuole, resulting in the production of cytotoxic radical species [14]. Lumefantrine is a blood schizonticide that kills Plasmodium falciparum erythrocytic stages. It is believed that combining lumefantrine and artemether resulted in cooperative antimalarial clearance effects.

Artemether has a quick start of effect and is quickly eliminated from the body. As a result, it is expected to give immediate symptom alleviation by lowering the quantity of malarial parasites. Lumefantrine has a substantially longer half-life and is thought to kill any remaining parasites. According to the available evidence, lumefantrine inhibits the creation of -hematin by establishing a complex with hemin, as well as nucleic acid and protein synthesis [15]. The interaction of the peroxide-containing medication with heme, a hemoglobin breakdown byproduct formed from hemoglobin proteolysis, is the commonly acknowledged mode of action of peroxide antimalarials. This interaction is thought to produce a variety of potentially harmful oxygen and carbon-centered radicals [16] (Figure 1&2). The availability of low bioavailability anti-malarial generic medications marketed in Sub-Saharan Africa raises concerns about individuals obtaining therapeutic concentrations after taking such products [17]. A prior study compared the bioavailability of one generic tablet formulation to the product of an innovator.

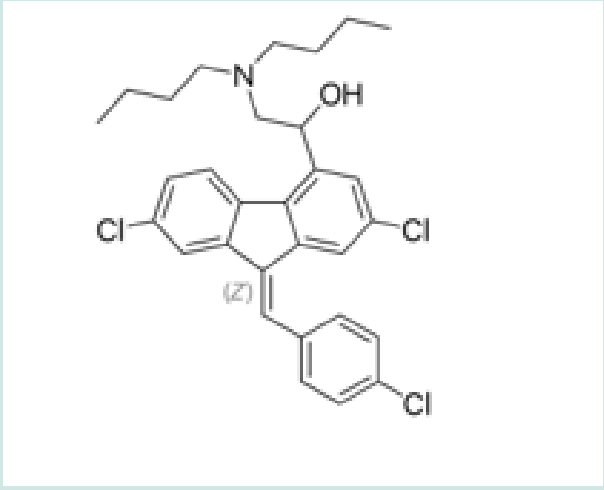

Figure 1: Structure of lumefantrine.

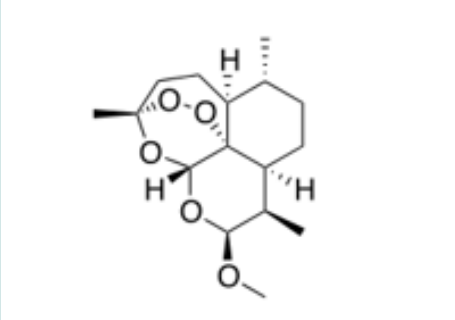

Figure 2: Structure of artemether.

Both included artemether and lumefantrine in fixed-dose combination tablet forms. Artefan®, the most widely available generic, was collected for bioavailability comparison with SD®. (Novartis innovator product). A randomized, two-treatment cross-over study was conducted on 18 healthy Tanzanian black male volunteers. Artefan® (test) and SD® formulations were administered to each participant, followed by a 42-day drug-free washout period. Up to 168 hours after a single dose of each medication was delivered orally, serial blood samples were obtained. HPLC with UV detection was used to quantify lumefantrine plasma levels. The two drugs' bioequivalence was established using US Food and Drug Administration (FDA) standards. Artefan®, manufactured in India, was the most widely available generic in pharmacies. All eighteen volunteers' finished the experiment, and both the test and reference pill formulations were well tolerated. In terms of pharmacokinetic metrics, there were no statistically significant changes between the two formulations with a P value > 0.05.

Regulators acknowledge the use of in-vitro bioequivalence techniques as a critical enabler for the effective development of complicated generic drug formulations. The US Food and Drug Administration (FDA) and other authorities have provided product-specific guidelines emphasizing the need of determining physicochemical equivalence as part of demonstrating bioequivalence of a test generic product with a reference-listed drug (RLD) product. This method can dramatically cut the time to market for new generics by eliminating the necessity for clinical endpoint studies [18]. Previous studies on the physicochemical characterization tools, combined with available knowledge and experience of methods validation as well as transfer requirements, can assist in defining an appropriate analytical strategy to support change management, ensuring that product performance and safety are maintained. As a result, the current study will compare the in-vitro bioequivalence of four different brands of artemether-lumefantrine-based combination solid dosage formulation to an innovator brand as the standard (SD) using weight uniformity, dissolution, disintegration, friability tests, thin layer chromatography, and UV spectroscopic analysis.

Methods

Sample Collection

Five different brands of artemether-lumefantrine (AL) combination tablet formulations were obtained from reputable community pharmacies in Amassoma and Yenagoa cities of Bayelsa state, Nigeria. The samples were labeled SD (Standard - innovator), L2, L3, L4, and L5 respectively. Two packs each of SD and L4 were bought from Denson Pharmacy, Opolo Yenagoa, two packs each of L5, and L2, were obtained from Keto-Divine Pharmacy in Amassoma, Wilberforce Island, and a brand of L3 was purchased from Everplus Pharmacy, Amassoma, Wilberforce Island, all in Bayelsa State, Nigeria. Prior to the more stringent quantitative testing, visual inspection of packaging and dose form was used to quickly determine the quality of the samples. The package was examined to ensure that the active ingredients and strength, expiration date, batch number, manufacturer, and country of origin were correctly and legibly labeled. All processes were meticulously carried out in accordance with British Pharmacopoeia (BP) standards [19].

Uniformity of weight

Twenty (20) pills were randomly selected from each sample and weighed separately before being weighed combined on an analytical balance. Each sample's average weight was established. The divergence of each individual pill in each sample from the sample's average weight was also calculated.

Disintegration

Two (2) tablets each from the five samples of artemether/lumefantrine were isolated for this study using the Disintegration time machine to using 50 rpm at 37°C in purified water to measure the time of breaking into smaller units as a result of the solvent medium and subsequent loss of cohesiveness.

Dissolution rate determination

The rate of release of artemether/lumefantrine was determined in 1000 milliliters of purified water and 1000 milliliters of 0.1 M hydrochloric acid containing 1% of benzalkonium chloride. Dissolution tests were carried out according to BP (British Pharmacopeia) standards.

Friability tests

Twenty tablets were weighed from each brand and subjected to abrasion using a tablet friability tester using 25 revolutions per minute (rpm). The tablets were weighed and compared with their

initial weights and values recorded for each.

Content of active pharmaceutical ingredient

Before the TLC, all the samples were observed for absorbance in the UV spectroscopy, and readings were noted. The active pharmaceutical ingredients were determined using thin layer chromatography (TLC); whereby three solvents namely Toluene, acetic acid, and ethyl acetate were prepared in different ratios, 5 TLC plates were provided, with each a baseline and solvent front marked, a microcapillary tube was used to spot the sample on the plate at the baseline. The plate was put in the TLC chamber and observed till the solvent moved up to the solvent front.

Discussion

In terms of weight uniformity, SD(SD) has the lowest mean weight of the test samples and L4 has the highest mean weight. Individual tablets of the standard innovator product had a mean weight of 0.243g, with a variation and percentage standard deviation of 0.0024 and 1.08%, respectively. Weight, weight deviation, and percent standard deviation for the other brands were 0.308, 0.0023, and 1.0% (L2), 0.342g, -0.0012, and -0.5% (L3), 0.351g, -0.0012, and -0.5% (L4), and 0.308g, 0.0023, and 1.0% (L5), respectively (Table 1).

Table 1: Weights uniformity of SD and test Samples.

Sample	Weight of tablets	Weight deviatio (x̄–x)	(x̄–x)^2	% Standard deviation
SD	0.243 ± 0.003	0.0024 ± 0.001	6.61592E-06 ± 4.98313E-06	1.084025 ± 0.66
L2	0.308 ± 0.015	0.0023 ± 0.015	0.00023 ± 0.0006	1.004 ± 5.406
L3	0.342 ± 0.011	-0.0012 ± 0.0114	0.00013 ± 0.00045	-0.499 ± 2.994
L4	0.351 ± 0.011	-0.0012 ± 0.0114	0.00013 ± 0.00045	-0.499 ± 2.994
L5	0.308 ± 0.015	0.0023 ± 0.0152	0.00023 ± 0.00059	1.0044 ± 5.406

Due to the excipients that were used, L3 had the highest percentage of weight loss in the friability test, whereas L5 had the lowest percentage. After the friability test, the weight of the SD and other samples decreased significantly from 4.865 - 4.845g (SD), 6.156 - 6.132g (L2), 6.886 - 6.837g (L3), 7.021 - 6.989g (L4), and 6.782 - 6.773g. (L5). Thus, SD had 0.4% friability, L2 had 0.4% friability, L3 had 0.7% friability, L4 had 0.46% friability, and L5 had 0.13% friability (Table 2).

Table 2: Friability test results.

Friability Test	SD	L2	L3	L4	L5
Initial weight (g)	4.865	6.156	6.886	7.021	6.782
New weight (g)	4.845	6.132	6.837	6.989	6.773
Percentage friability (%)	0.411	0.399	0.712	0.4557	0.133

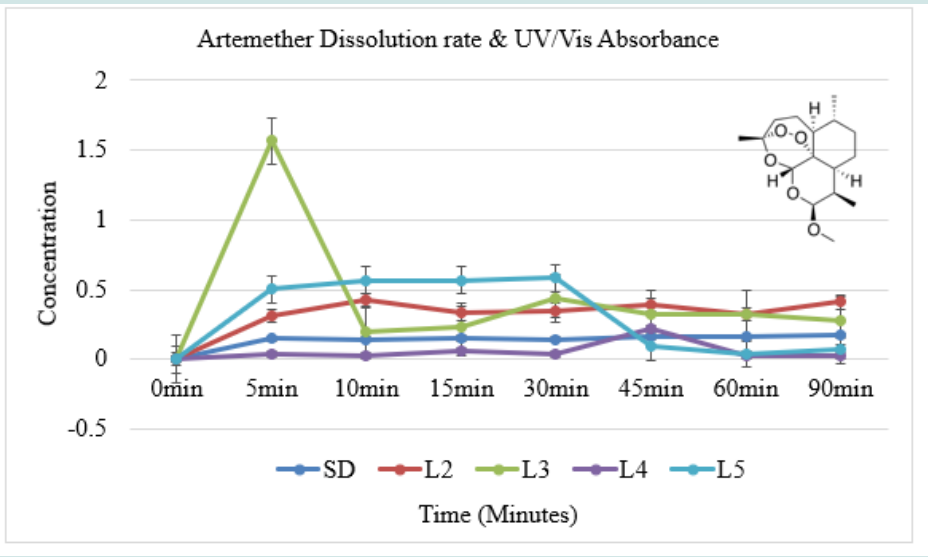

Figure 3: Dissolution rate and UV Absorbance of artemether component.

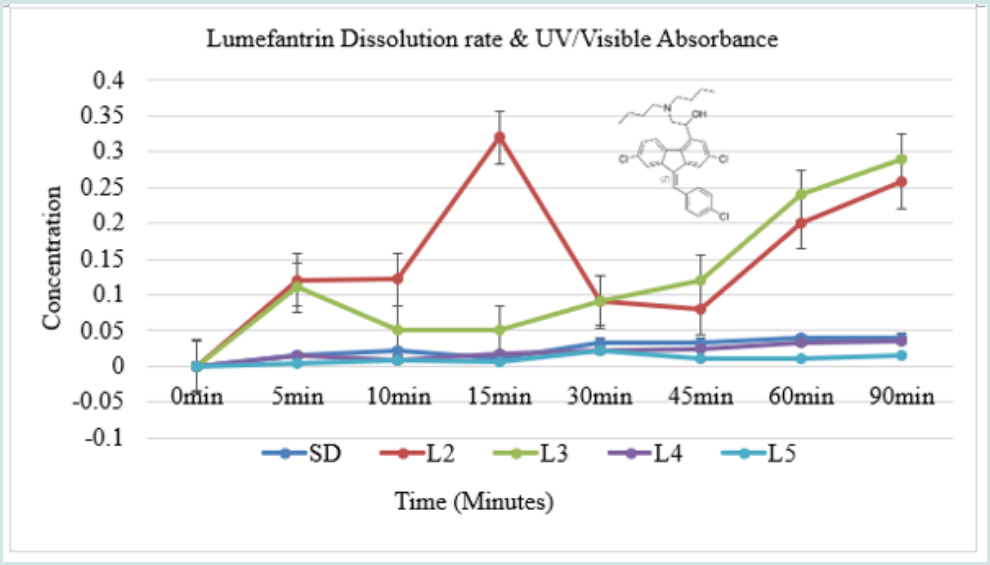

Figure 4: Dissolution rate and UV Absorbance of lumefantrin component.

The pattern of drug release was found to be varied between the two substances (artemether and lumefantrin). The artemether component of L3 dissolved more quickly than the other brands in 5 minutes, followed by L5, L2, the standard medication SD, and L4 with a longer disintegration and dissolving time. (Figure 3). Furthermore, L3 demonstrated rapid dissolve after only 10 minutes in the dissolution chamber, whereas other ALs shown marked dissolution after 30 minutes in the veego VDA-6DR USP standard dissolving machine. L2 dissolved more easily at 15 and 90 minutes for the lumefantrine component, followed by L3, which dissolved faster at 90 minutes than L2 and SD. When these two formulations are supplied to patients, this will eventually impact the beginning of effect. The dissolving rates of the innovator brands and other samples (L4 and L5) were comparable (Figure 4). The fastest dissolution rates were 90 minutes (SD), 45 minutes (L4), and 30 minutes (L5). The higher the UV/Visible absorbance, the faster the dissolving rate. It is also worth noting that L2 was found to have a stronger covering throughout the investigation, which makes the tablet resistant to fracture and dissolve if not much effort and time is put in.

The dissolving test for immediate-release tablets has been reported to pass if the tablet's dose form has more than 70% release in 45 minutes [20]. The lowest release of drug was observed at 5 minutes and highest at 90 minutes in the standard innovator brand (SD), 5 minutes and 10 minutes (L2); 10 minutes and 5 minutes (L3); 10/90 minutes and 45 minutes (L4); 60 minutes and 10/15 minutes (L5), respectively for all brands (Table 3-5). The samples were analyzed using TLC and they were compared to a standard using the British pharmacopeia, and they all proved to be pure samples. The SD product had an Rf of 0.85, compared to 0.91 (L2), 0.57 (L3), 0.61 (L4), and 0.95 (L5), respectively (Figure 5). There was a comparative disintegration time among the samples analyzed compared to the SD, with the standard having a disintegration time of 14.8 minutes, which is approximately 15 minutes. This is also in line with BP specifications of disintegration time of tablets (13 – 17 minutes). The other sample had disintegration time ranging from 14.6 mins (L2), 14.0 mins (L3), 14.5 mins, (L4) and 14.4 mins (L5), respectively. The method of assay is simple, effective, sensitive, accurate, and economical. It can be adopted for the routine quality control of artemether/lumefantrine combination formulations, and these in vitro studies can be efficiently used to evaluate the quality of commercial tablets. The results were in conformation with the specifications outline in the British Pharmacopeia.

Table 3: Drug release time from both components.

Time (Minutes)	SD		L2		L3		L4		L5	
	Ar	Lu	Ar	Lu	Ar	Lu	Ar	Lu	Ar	Lu
0	0	0	0	0	0	0	0	0	0	0
5	0.148	0.015	0.312	0.120	1.565	0.110	0.033	0.016	0.504	0.004
10	0.144	0.021	0.423	0.122	0.194	0.050	0.025	0.008	0.567	0.008
15	0.149	0.010	0.330	0.320	0.232	0.050	0.054	0.018	0.567	0.006
30	0.145	0.034	0.350	0.090	0.434	0.092	0.034	0.021	0.582	0.021
45	0.160	0.034	0.390	0.080	0.326	0.120	0.222	0.025	0.089	–0.01

60	0.160	0.039	0.320	0.201	0.323	0.240	0.030	0.033	0.036	0.010
90	0.169	0.040	0.410	0.258	0.279	0.290	0.025	0.035	0.067	0.016

Table 4: Disintegration time.

S/N	Disintegration time (Minutes)				
	SD	L2	L3	L4	L5
1	15.5	15.0	13.9	15.5	15.6
2	15.0	15.5	13.0	14.0	14.0
3	14.8	14.0	14.0	14.6	14.0
4	15.2	15.0	14.0	13.6	15.0
5	14.0	13.9	14.5	14.7	15.5
6	14.0	15.7	14.0	15.0	14.0
7	15.0	15.0	14.3	15.0	13.0
8	15.5	14.0	13.0	15.7	14.0
9	15.5	13.8	13.0	13.5	13.0
10	14.5	14.4	15.0	13.9	13.5
11	14.7	13.0	14.0	14.6	14.8
12	14.0	15.0	15.0	13.2	15.0
13	15.0	14.0	14.5	14.0	14.0
14	15.0	16.0	15.6	13.0	15.0
15	14.5	15.5	13.5	16.0	15.0
16	13.9	13.5	14.0	15.5	14.7
17	13.9	13.0	13.0	15.4	15.4
18	14.5	15.4	14.6	15.1	15.5
19	15.5	15.0	13.6	13.5	13.0
20	15.0	15.7	13.2	14.0	14.6
∑ (Total)	295.0	292.4	279.7	289.8	288.6
Mean ± STD	14.8 ± 0.6	14.6 ± 0.9	14.0 ± 0.7	14.5 ± 0.9	14.4 ± 0.8

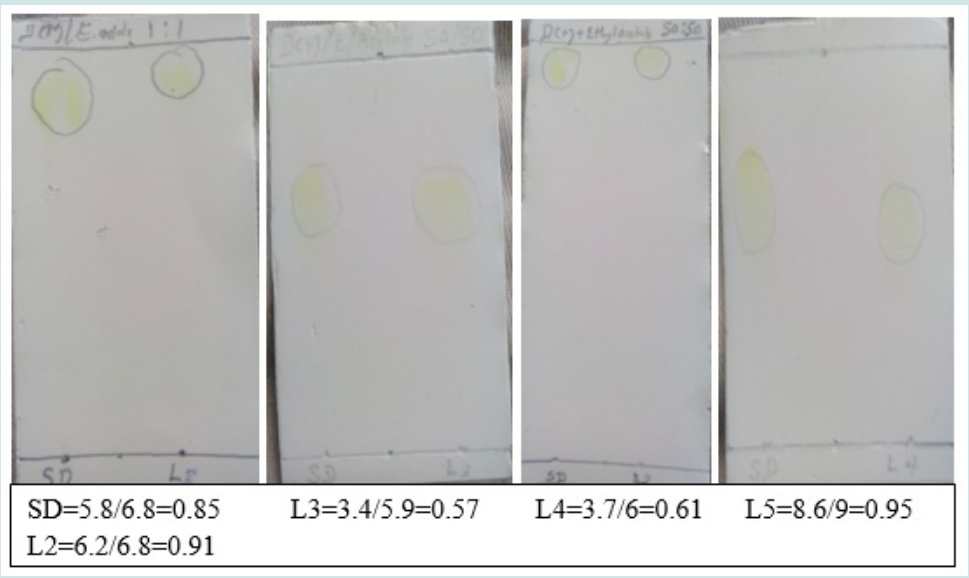

Figure 5: TLC chromatogram of analyzed samples.

Conclusion

All of the AL tablets formulations used in the study were consistent in terms of hardness and weight fluctuation, and they satisfied BP friability requirements. As a result, all of the AL brands examined had consistent weight and adequate physical stability to retain physical integrity over time, as well as the capacity to tolerate mechanical shocks experienced during manufacture, packing, shipping, and dispensing. The approach does not necessitate any drug pretreatment and may compete with other current methods in the regular quality control analysis of artemether/lumefantrine (AL) combination formulations.

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