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Spectrophotometric analysis of artesunate injections available in community pharmacies in Northern and Western Uganda

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ABSTRACT

Aim: The surge in different brands of artesunate injection in Uganda, has raised the need for this study, which aimed at quantifying the actual amount of artesunate in different brands of artesunate injections available in Northern and Western Uganda.

Materials and Methods: The wavelength at maximum absorbance of pure artesunate powder was determined using Ultraviolet-visible spectrophotometer and Beer Lambert's plot was generated. This was validated and used to assay 27 brands of artesunate.

Results: In the spectrophotometric assay method used, Beer Lambert's law was obeyed within the range of 20 µg/ml–140 µg/ml with linear regression equation of $y=0.012+0.030$ and correlation coefficient of (R^2) 0.999 ($n=9$). The limits of detection (sensitivity) and quantification were found to be 0.83 mg/ml and 2.09 mg/ml respectively. About 66.6% (18) and 33.3% (9) had actual artesunate content higher and lower than labeled claim respectively, while 40.7% (11) had deviations from labeled claim that were within acceptable limits.

Conclusion: Most brands of artesunate injection assayed deviated from labeled claim, regional/environmental factor impacted much on the stability of artesunate thus there is need for further screening of the quality of artesunate injection in circulation in view of the therapeutic consequences of substandard artesunate injection.

ARTICLE HISTORY

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KEYWORDS

Artesunate injection; severe malaria treatment; spectrophotometric analysis; drug quality assessment; Uganda malaria burden

PLAIN LANGUAGE SUMMARY

In Uganda, many different brands of artesunate injection are available, but their quality can vary. Artesunate is an important medicine used to treat severe malaria, so it's crucial that each injection contains the correct amount of the drug. This study measured the actual amount of artesunate in 27 brands of injections from Northern and Western Uganda. The researchers used a method called UV spectrophotometry to check the amount of artesunate in each sample. They found that 66.6% of the brands had more artesunate than stated on the label, while 33.3% had less. Only 40.7% of the brands had amounts within the acceptable range of what was labeled. The results suggest that environmental factors might affect the stability of artesunate, which could reduce the drug's effectiveness and impact patient outcomes. The study highlights the need for ongoing monitoring to ensure that artesunate injections are safe and effective for patients who rely on them for life-saving treatment.

ARTICLE HIGHLIGHTS

- Artesunate injection is the WHO-recommended first-line treatment for severe malaria, but drug resistance and quality issues are emerging concerns.
- This study assessed the quality of 27 brands of artesunate injections in Northern and Western Uganda using a validated spectrophotometric method.
- Findings revealed that 66.6% of brands contained more artesunate than labeled, 33.3% had less, and only 40.7% were within acceptable limits.
- Environmental factors, such as heat and humidity, may impact drug stability and contribute to the variability in artesunate content.
- The results emphasize the need for ongoing quality control, post-market surveillance, and supply chain improvements to prevent treatment failure and resistance.
- This research provides critical insights for policymakers, regulatory authorities, and healthcare providers to safeguard patient care and improve malaria treatment outcomes.

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1. Introduction

Malaria is an infection of the red blood cells caused by Plasmodium protozoan parasites, transmitted to humans by the bites of infected female Anopheles mosquito [1]. *Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax, Plasmodium malariae,* and *Plasmodium knowlesi* are the five *Plasmodium* species that cause malaria in humans [2]. The parasite's erythrocytic stage is what causes clinical disease [3]. According to Albalaba and Oo, there is no known disease linked to sporozoites, the parasite's developing liver stage, merozoites expelled from the liver, or gametocytes [4]. The World Malaria Report 2022, states that between 2019 and 2021, the estimated number of cases in Nigeria (4.0 million), Madagascar (2.8 million), Uganda (1.7 million), the Democratic Republic of the Congo (1.6 million), and Angola (1.6 million) increased significantly [5]. Uganda was listed as one of the 17 countries that accounted for nearly 80% of all malaria-related deaths globally in the WHO's 2018 World Malaria Report [5]. Most cases of morbidity and mortality in Uganda are caused by malaria, which is the most serious public health concern in the nation, affecting 95% of the population, especially children under the age of five [6]. According to estimates, 11.3 percent of malaria cases and 3.3 percent of malaria deaths nationwide occurred in 2020 in 11 districts of the Northern Uganda, Lango and Acholi sub-regions [7]. Severe malaria is caused by *P. falciparum* parasite infection that is complicated by significant organ failure or abnormalities in metabolism that cannot be attributed to any other cause [8].

Artesunate injection received FDA approval in 2020, for the treatment of severe malaria [9]. The World Health Organization (WHO) now recommends it as the first-line treatment for severe malaria [10]. Artesunate is derived from artemisinin, a compound extracted from *Artemisia annua*, a plant belonging to the Asteraceae family [11]. It has a molecular formular of $C_{19}H_{28}O_8$, molecular weight of 384.42 g/mol and a melting point of between 135 – 137 °C. Artesunate appears as a white crystalline powder, highly sensitive to heat and light, with limited water solubility. Compared to other artemisinin derivatives such as artemether and artemotil, artesunate has superior physical and chemical properties due to its water solubility, making it suitable for intravenous (IV) and intramuscular (IM) administration [12]. Once administered, it is rapidly converted in vivo into its active metabolite, dihydroartemisinin (DHA), which exerts potent antimalarial effects [13].

Uganda's National Malaria Control Program (NMCP) approved the use of artesunate injection as the first-line treatment for severe malaria in both adults and children in 2011, with policy implementation beginning in 2013 [14]. In cases where artesunate is unavailable, intravenous quinine or intramuscular artemether serve as alternative treatments [15]. However, artesunate has significant advantages over these alternatives. Compared to quinine, which is associated with severe side effects such as hypoglycemia, hyperinsulinemia, cardiac dysrhythmias, abscess formation, and localized injection site pain, artesunate is better tolerated and reduces both mortality and adverse reactions [16]. Additionally, while artemether is an alternative treatment, its poor water solubility and unpredictable absorption in critically ill patients with poor peripheral perfusion limit its effectiveness [15].

Extensive evidence supports the efficacy of artesunate in the treatment of severe malaria, including cases of multidrug-resistant *Plasmodium falciparum* infections [17]. Studies have demonstrated that artesunate injection is superior to both quinine and artemether in reducing mortality and improving patient outcomes [18]. Unlike quinine and artemether, which require slow infusion, intravenous artesunate is administered as a bolus, allowing for rapid parasite clearance and reducing the risk of complications [18]. Artesunate acts quickly by targeting the ring-stage parasites, which are subsequently cleared by the spleen, thereby improving survival rates in both adults and children [19].

Despite its proven effectiveness, emerging reports of artesunate resistance pose a significant concern. Studies have documented clinically confirmed resistance in Rwanda and Northern Uganda [20]. In Gulu, Northern Uganda, a study conducted at St. Mary's Hospital Lacor between 2015 and 2019 identified the independent emergence and local spread of *P. falciparum* strains resistant to artesunate following its widespread use for severe malaria treatment [21]. Additionally, substandard and poor-quality antimalarial medications remain a persistent problem, contributing to treatment failure, drug resistance, and adverse effects [22].

Given the reports of delayed treatment response and potential artesunate resistance in Northern Uganda [20], as well as the lack of comprehensive studies comparing artesunate formulations across different climatic zones in Uganda—where environmental factors may affect drug stability—it is essential to assess the quality of available artesunate injections, thus the primary objectives of this study are (i) to determine the spectrophotometric absorbance maxima (λ-max) for different brands of artesunate injections available in community

pharmacies in Northern and Western Uganda and compare them to pure artesunate and (ii) To evaluate concentration variations among different brands using a validated spectrophotometric method.

2. Materials and methods

2.1. Materials and reagents

The reagents listed below were acquired: pure powdered artesunate (MICRO ORGO CHEM, INDIA), distilled methanol, distilled water freshly prepared from the Biomedical Research Laboratory Kampala International University, Western Campus, Ishaka, 1 M sodium hydroxide solution and twenty-seven (27) different brands of artesunate powder for injection. The equipment used included UV-visible spectrophotometer (BIOBASE BK-UV, China), analytical balance and water bath. Samples used were based on the available brands of artesunate in the market. All brands with their different strengths were represented.

2.2. Determination of λ-max for pure artesunate

10 mg of pure artesunate sample was weighed using an analytical balance and transferred to a conical flask. One (1) ml of distilled methanol was added to the pure artesunate sample in the flask and shaken vigorously for 2 minutes to dissolve all the powder. One (1) ml of the resultant artesunate solution was transferred to a cuvette, placed in spectrophotometer and scanned in the UV-visible spectrum between wavelengths of 190 nm to 1,100 nm to determine the wavelength where maximum absorbance occurred.

2.3. Determination of λ-max for artesunate samples

Three (3) ml of distilled methanol was added to a vial containing 30 mg of artesunate sample and shaken vigorously for 2 minutes to dissolve all the powder. One (1) ml of the resultant artesunate solution was transferred to a cuvette, placed in spectrophotometer and scanned in the UV-visible spectrum between wavelengths of 190 nm to 1,100 nm to determine the wavelength where maximum absorbance occurred. A total of 6 ml and 12 ml of distilled methanol were used for vials containing 60 mg and 120 mg of artesunate respectively. This was repeated for all the samples.

2.4. Optimization of suitable reaction conditions for assay of artesunate

The methods were adapted from previous validated studies, ensuring reliability [23,24]. The methodology includes temperature, time, and solvent variations to ensure reproducibility. After careful study, the reaction conditions were refined. To determine the impact of a specific variable on the effectiveness of the suggested method, variables such as temperature, time, and concentration were changed while others remained constant.

2.5. Temperature variation effect

To study the effect of temperature on absorbance, a solution of known artesunate concentration (1,000 µg/ml) which was made to react with 1 ml of 1 molar sodium hydroxide solution was studied at reaction temperatures of 30, 35, 40, 45, 50, 55, 60, 65 and 70 °C.

2.6. Effect of time

In studying the effect of varying time on absorbance, a constant artesunate concentration (1,000 µg/ml) which was made to react with 1 ml of 1 molar sodium hydroxide solution was studied at a reaction temperature of 60 °C. The absorbance readings were taken at 0, 10, 20, 30, 40, 50 and 60 minutes.

2.7. Preparation of pure artesunate sample and establishment of the wavelength (λmax) at maximum absorbance

This method was adopted from another study conducted elsewhere [23], Ten mg (10 mg) of the pure artesunate powder was weighed in a calibrated analytical balance and transferred to a conical flask. Ten mL (10 mL)

of distilled methanol was added to the drug in the conical flask and shaken vigorously for 1 minute to dissolve it completely, making a stock solution of pure artesunate sample with a concentration of 10 mg/mL. Different aliquots of 20, 30, 40, 50, 60, 80, 100, 120, and 140 µL of the stock solution of artesunate (ART) were pipetted into labeled falcon tubes and the volume of the solution in each falcon tube was made up to 5 ml with distilled methanol. Two (2) ml of one molar sodium hydroxide (1 M NaOH) solution was added to the solution in each falcon tube using a micropipette and shaken vigorously for 1 minute. The volume in each of the falcon tubes was made up to 10 ml with distilled methanol using a micropipette and shaken for 1 minute. The content in each of the falcon tubes was transferred to test tubes with respective labels. The test tubes were sealed and transferred to a water bath heated and maintained at 60 °C for 40 minutes. The test tubes were removed and allowed to cool. One (1) ml of the solution in the test tube labeled "20" was pipetted and transferred to a cuvette and a wavelength scan was done using a UV-visible spectrophotometer to find the wavelength where the peak (maximum) absorbance occurred. Exactly 1 ml of the 20 µL solution was scanned with the UV-visible spectrophotometer to establish the wavelength at maximum absorption (λmax). Then 2 ml each of the different dilutions was collected separately and their triplicate absorbances were recorded at the established λmax. From the results obtained, Beer's plot (Plot of Absorbance against Concentration) was made.

2.8. Preparation of the test artesunate samples

Thirty (30 mg) test samples: Three 3 ml of methanol was transferred into a vial containing 30 mg of artesunate powder and shaken vigorously for 1 minute to dissolve all the artesunate powder in the vial making a stock solution of 10 mg/ml. Thirty (30) µL of the stock solution was pipetted and transferred to a falcon tube, and made up to 5 ml with distilled methanol. Two (2 ml) of 1 M sodium hydroxide solution was added to the solution and the volume made up to 10 ml with distilled methanol and shaken for 1 minute. The solution was transferred to a test tube. The test tube was covered with aluminum foil and heated in a water bath maintained at 60 0C for 40 minutes and removed, cooled, and scanned for absorbance using a UV-spectrophotometer. This was repeated for all the samples labeled 30 mg. This was repeated for 60 mg and 120 mg artesunate samples.

2.9. Color of vial content and clarity of reconstituted solution

This method was adopted from a similar study [24]. Through visual inspection of a small amount of powder placed on a flat white and then black background/surface, the color of the vial content was determined. An ampoule containing the diluent sodium hydroxide set against a white backdrop was used to evaluate the color of the reconstituted solution. Artesunate powder for injection should be presented in a colorless transparent glass vial with a gray halogenated butyl rubber stopper, crimped with an aluminum plastic cap containing white crystalline powder. The reconstituted solution should be colorless. The leaflet placed within the medication package served as instructions for reconstituting the samples. Under enough light, the clarity of the reconstituted solutions was assessed against a black-and-white visual evaluation board. Any white fibers and particles were visible against a black background, while the presence of black particles was visible against a white background.

2.10. Ethical consideration

Ethical approval with registration number KIU-SPRC-002/23, was got from the School of Pharmacy Research Committee, Kampala International University, Western Campus, Ishaka, Bushenyi, Uganda.

2.11. Statistical analysis

Data were entered into an Excel sheet and the individual plots of absorbance against wavelength of standard and test drugs were extracted and the graphs were plotted and superimposed using OriginPro version 2021. Descriptive statistical analysis (mean, standard deviation) and inferential analysis (chi square test) were carried out on the different actual concentration gotten using SPSS version 26.

3. Results

3.1. Characteristics of different artesunate brands analyzed

The characteristics of the eleven artesunate brands, including the pioneer brand that was employed in this investigation, are displayed in Table 1. Every brand had a minimum of six months remaining before the expiration date indicated on the label. The National Drug Authority (NDA), which is in charge of overseeing drug control in Uganda, has registered the majority of the brands of artesunate that are used in these regions of the country. These brands were imported from India.

3.2. Characteristics of pure artesunate used as standard

The reproducible maximum absorbance (λmax) of pure artesunate was determined to be at a wavelength of 243 nm. The absorbance value of pure artesunate obeyed Beer-Lambert's law, demonstrating a linear relationship with concentration. The regression equation for the pure artesunate was $Y = 0.012X - 0.030$, with a correlation coefficient R^2 of 0.999. Other values of selected parameters of pure artesunate are displayed in Table 2. The Beer's plot was followed at lower concentration ranges of 20–140 ug/ml (Figure 1). The limits of detection (sensitivity) and quantification were found to be 0.83 mg/ml and 2.09 mg/ml respectively.

3.3. Qualitative analysis of the sample artesunate brands

A graph plot of absorbance against the wavelength of both pure and sample artesunate shows that the spectra of the test brands followed a similar shape to the pure artesunate sample. The result showed a minor shift in the absorption spectra, no extra peaks was seen among the samples when the individual spectra were superimposed on the pure artesunate. All the samples showed peak at the same wavelength with pure samples. The Absorption spectra for artesunate brands from Northern and Western Uganda as compared to the pure sample are displayed in Figures 2 and 3 respectively.

Table 1. Features of analyzed brands of artesunate in Western and Northern Uganda.

S/N	Brand code-strength	Manufacturing date	Expiry date	Batch number	Manufacturing license No	Country of origin
1	W01-30mg	Mar-22	Feb-25	IP22065	DD/678	India
2	W02-30mg	May-24	Apr-24	IP22127	DD/L/686	India
3	**W03-30mg***	**9/12/2021**	**8/12/2024**	**LA210614**	**7590/06/11**	**China**
4	W04-30mg	Aug-22	Jul-25	2122253	G/28/1078	India
5	W05-60mg	May-23	Apr-26	IP23076	DD/678	India
6	W06-60mg	Jul-22	Jun-25	ARI-072211	16/UA/SC/P-2007	India
7	**W07-60mg***	**25/2/2022**	**24/2/2025**	**LA220432**	**6323/06/08**	**China**
8	W08-60mg	Dec-22	Nov-25	MAI-122201	16/UA/SC/P-2007	India
9	W09-120mg	Aug-22	Jul-25	IP22190	DD/678	India
10	W10-120mg	Sep-22	Aug-24	IP22224	DD/L/686	India
11	**W12-120mg***	**30/06/2022**	**09/06/2025**	**LA220317**	**7401/06/11**	**China**
12	W12-120mg	Aug-22	Jul-24	SD22H005E	G/28/1577	India
13	W13-120mg	Oct-22	Sep-25	EK2015	NDA/MAL/HDP/8359	India
14	N01-30mg	Mar-22	Feb-25	IP22065	DD/678	India
15	N02-30mg	Apr-22	Mar-24	IP22104	DD/L/686	India
16	**N03-30mg***	**9/12/2021**	**8/12/2024**	**LA210163**	**7590/06/11**	**China**
17	N04-30mg	Aug-25	Jul-25	2122252	G/28/1078	India
18	N05-60mg	Jul-22	Jun-25	JFQ042075	28/20/83	India
19	N06-60mg	Jul-22	Jun-25	ARI-072207	16/UA/SC/P-2007	India
20	**N07-60mg***	**6/2/2022**	**5/2/2025**	**LA220347**	**6323/06/08**	**China**
21	N08-60mg	Sep-22	Aug-24	SD22I004E	G/28/1577	India
22	N09-60mg	Dec-22	Nov-25	ED-22027	MB/05/285 & MNB/05/284	India
23	N10-120mg	Aug-22	Jul-25	IP22190	DD/678	India
24	N11-120mg	Sep-22	Aug-24	IP22224	DD/L/686	India
25	**N12-120mg***	**30/06/2022**	**19/06/2025**	**LA220032**	**7401/06/11**	**China**
27	N13-120mg	Oct-22	Sep-25	EK2015	NDA/MAL/HDP/8359	India
27	N14-120mg	Nov-22	Jun-25	DG22K-03	NL-MB/2021/292	Ireland

*Indicates innovator brands.

Table 2. Values of selected parameters of pure artesunate sample.

Parameters	Values
Working wavelength (nm)	243
Linearity range (µl/ml)	20–140
Molar absorptivity	0.012
Limit of detection (µl/ml)	3.33
Limit of quantification (µl/ml)	10.09
Regression equation (Y = mX + C)	Y = 0.012X–0.03
Slope	0.012
Intercept	−0.03
Regression coefficient	0.999

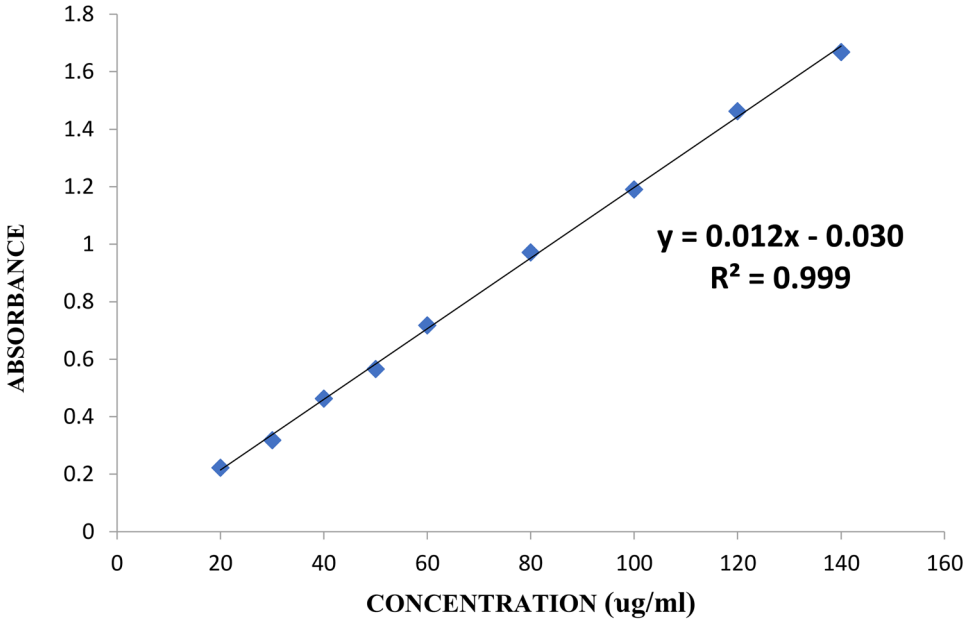

Figure 1. Plot of absorbance of pure artesunate sample against concentration.

3.4. Quantitative analysis of the sample artesunate brands

The actual contents of each test brand were calculated based on the regression equation obtained from the analysis of the pure sample as shown in Table 2. The results showed that 66.6% (18) and 33.3% (9) had actual artesunate content higher and lower than labeled claim respectively, while 40.7% (11) had deviations from labeled claim that were within acceptable limits. The individual actual artesunate contend and percentage deviation found are displayed in Table 3.

A comparative analysis of the actual content of artesunate available in the Northern and Southern region showed a significantly ($p < 0.05$) higher artesunate concentration in western region compared to the Northern region. The mean concentration of 120 mg artesunate from western and Northern region was 130.72 mg and 102.95 mg respectively, ($p = 0.02$). Other mean concentrations and standard deviations are shown in Table 4.

4. Discussion

It is reasonable to suspect that a new drug product's integrity or wholesomeness may be compromised when it is launched to the market under several brand names and frequently with significant price differences, particularly in our local community. This study presents potential regional differences in artesunate content and their implications for drug stability and efficacy. In this study, brands were chosen to reflect the distribution of artesunate brands used in northern and western regions of Uganda. All the brands of artesunate injections collected from Western and Northern Uganda were registered with the National Drug Authority in Uganda except two brands from Northern Uganda coded N07-60mg and N09-120mg. The presence of brands not registered with the National Drug Authority (NDA) in Uganda raises concerns about regulatory oversight. All the brands of artesunate injections had maximum absorbance at a wavelength of 240 nm similar to that of the pure sample of artesunate as shown in Figures 2 and 3. Optimization of suitable reaction conditions for assay of artesunate were ensured. The Beer's plot was obeyed in concentration range of 20 – 140 µg mL-1 as shown in Figure 1,

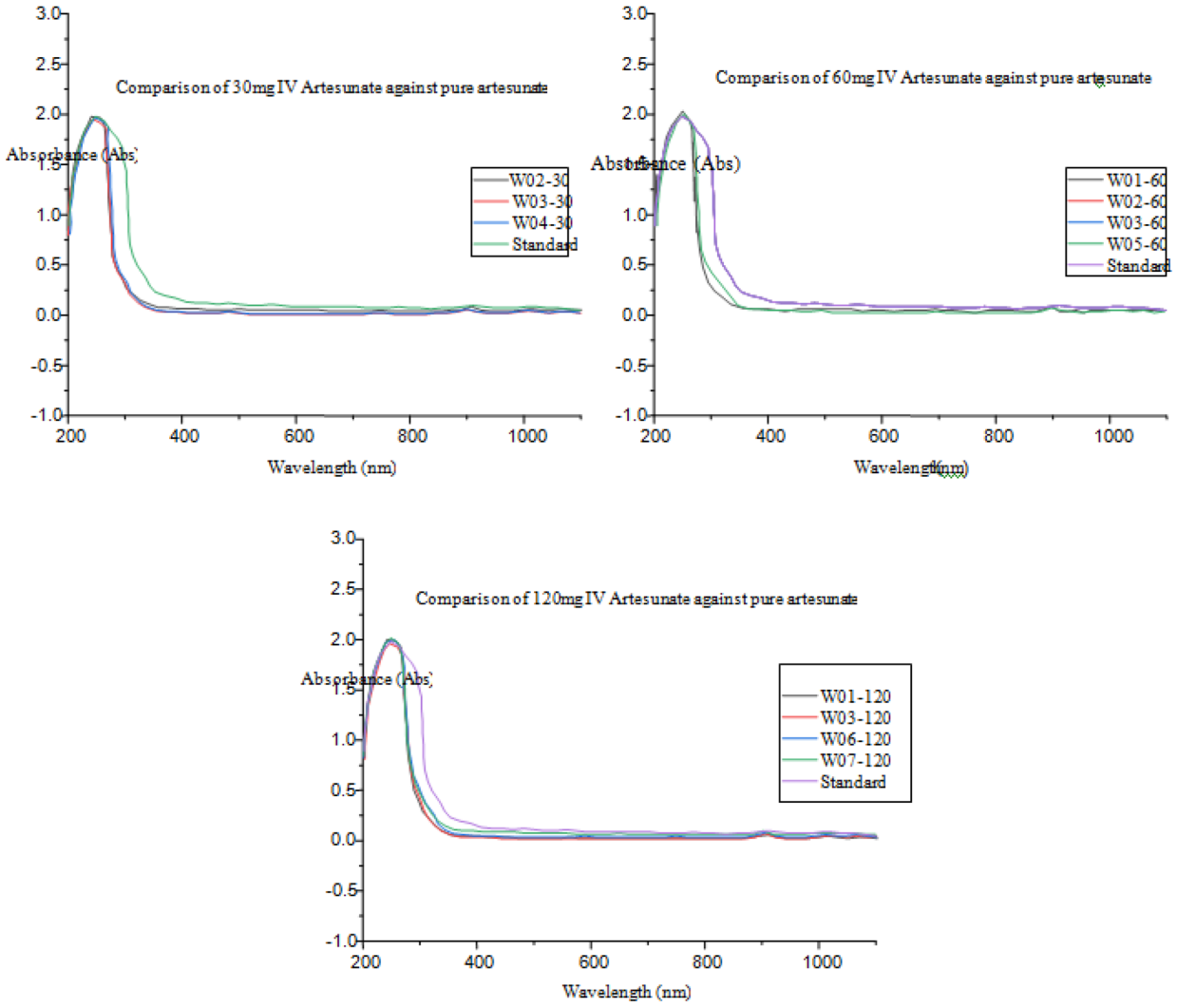

Figure 2. Absorption spectra for artesunate brands from Western Uganda compared to the pure sample. represents artesunate pure sample in plot for comparison of 30mg artesunate against artesunate pure sample. represents artesunate pure sample in plot for comparison of 60mg and 120mg artesunate against artesunate pure sample. Other multiple colors represent the test samples.

with a strong correlation being observed between absorbance and concentration, which satisfies the basis for the method of analysis used. This observation is consistent with another study [25]. To determine the impact of a specific variable on the effectiveness of the suggested method, some variables (such as temperature, time, and concentration) were changed while others remained constant. The absorbance was observed to increase with time and temperature for up to 40 minutes and from 30 to 60 °C respectively. This suggests subjecting artesunate beyond these temperature and time, will lead to its decomposition due to molecular breakdown leading to reduced potency. These observations were consistent with the reports by Attih and colleagues [23].

A qualitative assessment of the test artesunate showed that spectra of the test brands ideally followed a similar shape to the pure artesunate sample with the artesunate samples showing peaks at the same λmax with the pure artesunate. This suggests that the artesunate samples have same active compound with the pure artesunate. Figure 3 showed a slight shift in all the artesunate sample spectra when compared to the standard. This shift may be as a result of presence of slight or low levels of impurities, degradation, or the presence of additional components in the formulation.

4.1. Disparity in label claim among different artesunate brands

Of the 27 samples examined, about half of the brands had label claims that deviated from reality within allowable limits. This calls for serious health concern in Uganda and other African countries. This percentage disparity is close

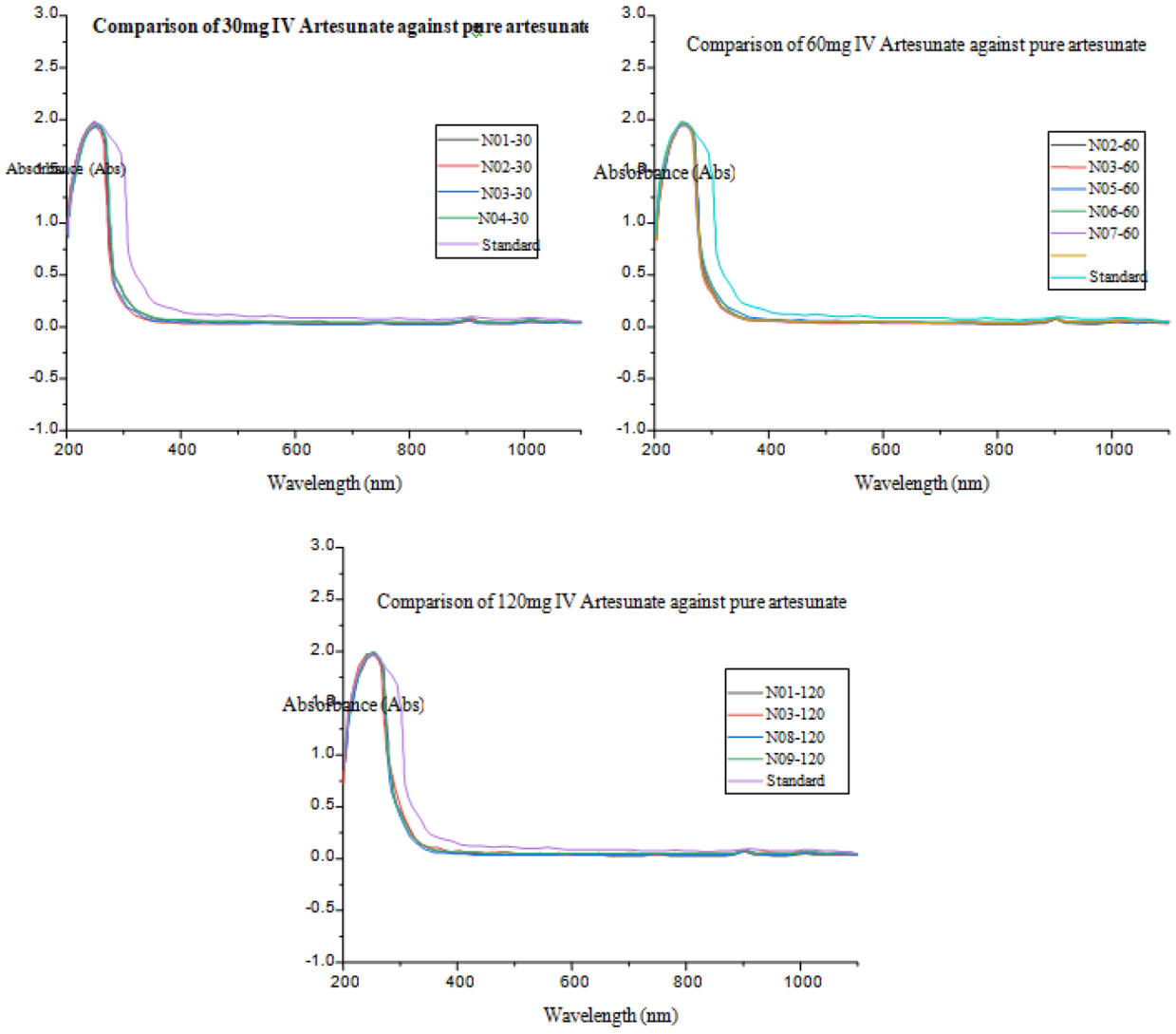

Figure 3. Absorption spectra for artesunate brands from Northern Uganda as compared to the pure sample. represents artesunate pure sample in plot for comparison of 30mg and 120mg artesunate against artesunate pure sample. comparison of 60mg artesunate against artesunate pure sample. Other multiple colors represent the test samples.

to what was reported in a study carried out in Nigeria where approximately 45.6% of the artesunate brands assessed were higher than the label claim [26]. It was observed that one third (7) had actual artesunate content below the label claim, while two third (18) had actual artesunate content above the label claim. A possible reason for this obvious variation between actual content and label claim, may substandard production procedures due to inconsistent formulation, poor quality control, substandard raw material and excipient interaction.

4.2. Regional disparities in artesunate content

There were notable differences between the mean concentrations of artesunate in the two regions studied. The mean concentration in the samples from the Western region was consistently greater than in the samples from the Northern region. Interestingly, the Northern region samples had a significant lower mean concentration (102.95 mg; $p = 0.01$), whilst the Western region has a higher mean concentration of (130.72 mg) and significant at $p = 0.02$. These findings suggest that temperature differences between Northern and Western Uganda may have an impact on the regional variances in artesunate content found in this study. Increased temperature can significantly affect stability, potency and effectiveness due to its light and heat sensitivity. Artesunate is prone to hydrolysis and oxidation which accelerates at higher temperature to hasten artesunate's breakdown, diminishing its effectiveness and potency [27]. In contrast to the relatively temperate western

Table 3. Actual artesunate content of the different artesunate injection.

S/N	Brand code-strength	Absorbance	Actual amount found (mg)	Deviation (mg)	Percentage deviation
1	W01-30mg	0.1080	32.22	2.22	7.40
2	W02-30mg	0.1115	33.33	3.33	11.10
3	**W03-30mg***	**0.1162**	**34.85**	4.85	16.16
4	W04-30mg	0.1503	45.86	15.86	52.86
5	W05-60mg	0.3968	96.86	36.86	61.43
6	W06-60mg	0.2565	60.10	0.1	0.16
7	**W07-60mg***	**0.3301**	**81.70**	21.7	36.16
8	W08-60mg	0.2200	68.30	8.3	13.83
9	W09-120mg	0.6180	150.72	30.72	25.6
10	W10-120mg	0.4769	130.22	10.22	8.51
11	**W11-120mg***	**0.4648**	**127.29**	7.29	6.07
12	W12-120mg	0.4578	125.05	5.05	4.20
13	W13-120mg	0.4399	120.28	0.28	0.23
14	N01-30mg	0.0998	29.57	−0.43	−1.43
15	N02-30mg	0.1027	30.51	0.51	1.7
16	**N03-30mg***	**0.1073**	**31.98**	1.98	6.6
17	N04-30mg	0.0970	28.65	−1.35	−4.5
18	N05-60mg	0.1918	59.23	−0.77	−1.28
19	N06-60mg	0.1792	55.18	−4.82	−8.03
20	**N07-60mg***	**0.1934**	**59.74**	−0.26	−0.43
21	N08-60mg	0.1954	60.41	0.41	0.68
22	N09-60mg	0.2263	70.37	10.37	17.28
23	N10-120mg	0.3263	102.63	−17.37	−14.47
24	N11-120mg	0.3145	98.83	−21.17	−17.64
25	**N12-120mg***	**0.3214**	**101.05**	−18.95	−15.79
26	N13-120mg	0.3324	104.60	−15.4	−12.83
27	N14-120mg	0.3420	107.70	−12.3	−10.25

*Indicates Innovator brand.

Table 4. Comparison of actual concentration of artesunate found in different regions.

Test samples	Mean concentration	Std. Deviation	p-value
West - 30 mg	36.56	8.10	0.02*
North - 30 mg	30.17	2.69	
West - 60 mg	76.74	14.67	0.04*
North - 60 mg	60.98	3.72	
West - 120 mg	130.72	16.34	0.02*
North - 120 mg	102.95	10.07	

*$p < 0.05$.

region, the climate in northern Uganda is hotter, with continuously higher temperatures. The stability, deterioration, and general quality of artesunate injections during storage and distribution may be significantly impacted by certain environmental conditions.

4.3. Supply chain and storage conditions

Artesunate should be stored in cool and dry condition, while transportation in hot climates require temperature-controlled environment to maintain stability. Exposure to light and increased temperature also reduces the expiration date or shelf life of artesunate leading to reduced efficacy and desired therapeutic outcome. The reduced artesunate contents in some samples may be caused by incorrect storage or extended exposure to heat during transportation in Northern Uganda, where ambient temperatures are greater. The storage and transportation of artesunate injections in Northern Uganda may lack adequate temperature control transport system. The lack of strict adherence to recommended storage conditions, particularly in remote or resource-limited settings, could result in thermal degradation of artesunate products. This issue may be less pronounced in Western Uganda, where cooler temperatures naturally mitigate some of the risks associated with heat-sensitive drugs.

4.4. Comparing innovative and generic brands

In terms of consistency, innovator brands fared better. They had showed lesser variations which were within permissible bounds. This suggests that some well-known brands adhere to quality standards better than their generic rivals, which showed more noticeable variability.

4.5. Clinical implications of the research findings

Since the effectiveness of artesunate injection is crucial in the treatment of complicated malaria, the results of this study have significant practical ramifications. The variations in artesunate content found in various brands may have a substantial effect on treatment results, posing problems for both public and individual health.

4.5.1. Increased morbidity and treatment failure

A substantial risk of treatment failure may arise from underdosing of artesunate [28]. Patients in Northern Uganda, where 33.3% of the samples had actual artesunate concentration below the labeled claim, are likely to be disproportionately affected by subtherapeutic doses in hotter regions, which may result in treatment failure and increased morbidity. Subtherapeutic doses may cause delay in parasite clearance in patients, which could result in lifelong disease, serious consequences especially in high-burden areas like Uganda.

4.5.2. Development of drug resistance

In areas where malaria is endemic such as Uganda, artemisinin resistance is already becoming a serious concern [29] especially in northern Uganda, while substandard doses of artesunate may hasten development of resistance [30]. Development of artemisinin derivative resistance is a major challenge in malaria treatment and eradication mandate. It reduces the efficacy of therapy based on artesunate, requiring the use of alternative which are sometimes more costly or less accessible medicines compared to the. Healthcare systems would be under more stress as a result of this growth, particularly in areas with limited resources.

4.5.3. Risk of toxicity and adverse effect

Higher artesunate content as observed in two third of the samples from the western region, can lead to artesunate levels exceeding therapeutic ranges. Even though artesunate is usually well tolerated, adverse effects including hemolysis or neurotoxicity have been reported in patients receiving artesunate medication [31,32]. This implies that adverse events are more likely to occur in susceptible groups, such as children, pregnant women, or people with comorbidities, which may jeopardize patient safety and treatment compliance in western region of Uganda.

4.5.4. Increased financial burden

Increased or decreased levels of artesunate, have the potentials to either expose patient to adverse effects or resistance to artesunate medication respectively. Such events will result in longer hospital stays, more costly second-line medicines, or more doctor visits, thereby constituting financial burden on patients and healthcare systems. Additionally, treating drug-induced toxicities raises healthcare expenses and diverting funds away from other urgent health needs.

4.6. Significance and impact of the study

This study addresses a critical gap in the literature by investigating the quality and concentration variations of artesunate injections in Uganda. Given the emergence of *Plasmodium falciparum* resistance in Northern Uganda [21], ensuring the potency and stability of artesunate formulations is vital to sustaining treatment efficacy and preventing further resistance.

The differences between the Northern and Western regions where samples from Western Uganda exhibiting higher levels of artesunate content, revealed by this study, emphasize inequalities in the supply chain and in the quality control procedures. Public health interventions are made more difficult by these regional differences, necessitating the use of focused tactics to provide fair access to high-quality artesunate injections.

Thus, the study's results can directly inform policy decisions, strengthen pharmacovigilance systems, and optimize malaria treatment outcomes.

5. Result interpretability and recommendations for clinical risk mitigation in the future

The study's results will provide actionable data on artesunate quality, helping regulatory bodies identify substandard or degraded products. This can prevent treatment failures, curb resistance spread, and save lives. Furthermore, the findings could catalyze further research into the impact of storage conditions and supply chain practices on drug integrity.

1. Standardized Quality Assurance: To avoid administration of substandard products to patients, artesunate injections should undergo routine testing and certification prior to distribution.
2. Education of Healthcare Providers and Patients: Healthcare providers should be educated on the need for proper storage of drugs dangers of substandard drugs
3. Manufacturer Collaboration: It is essential to work with pharmaceutical companies to enhance manufacturing procedures and guarantee adherence to global standards.
4. Policy Development: Policymakers should adopt stringent measures to regulate importation, distribution, and sale of artesunate injections, focusing on high-risk regions.

5.1. Novelty and strength of study

This is one of the first studies to compare artesunate formulations across different climatic regions in Uganda, providing unique insights into environmental factors affecting drug stability. While previous studies have evaluated artesunate formulations, administered through oral route, the novelty of this study lies in the fact that it provides a comparative analysis of artesunate injection brands from different climatic regions, assessing potential variations in drug content due to temperature differences. Hence, it highlights disparities in artesunate concentration, identifying regional trends in substandard or possibly counterfeit drugs. Furthermore, the findings contribute to public health concerns by linking artesunate content variations to possible therapeutic failures and drug resistance in malaria treatment, which has not been extensively studied in these specific regions. The use of a validated spectrophotometric technique ensures accurate, reliable data.

5.2. Limitations of the study

Geographical Scope: The study focuses on two regions, which may limit generalizability to other parts of Uganda or sub-Saharan Africa.

Single Analytical Method: While spectrophotometry is robust, incorporating additional techniques (e.g., HPLC) could enhance result validation.

6. Conclusion

More than half of the assayed artesunate injections deviated significantly from the labeled claim. Samples from the western Uganda had consistently higher artesunate content compared to the samples from the northern region. The findings of this work highlight the urgent need to address the diversity in artesunate quality. The relationship between drug stability and environmental factors emphasizes how crucial region-specific approaches are to guaranteeing the quality of artesunate injections. Access to safe and effective antimalarial medications is crucial for both patient care and the accomplishment of more general public health objectives in the battle against malaria. In order to achieve equitable malaria treatment outcomes throughout Uganda, it is imperative that these inequities be addressed.

Author contributions

EE Ayogu and IB wawata conceived and designed the study. EE Ayogu, and EA Ngolryeko carried out the study. JOC Ezeonwumelu and BO Sadiq analyzed and interpreted the data, EE Ayogu drafted the manuscript. JOC Ezeonwumelu and IB wawata revised the manuscript. All authors read and made the final corrections. The authors declare that all data were generated in-house and that no paper mill was used.

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