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Original Research Article

Colorimetric determination of artemisinin derivatives using 4-nitro-1-naphthalene diazonium tosylate as a coupling reagent

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Abstract

Purpose: *To present a cost-effective colorimetric method for quantifying artemisinin derivatives, and addressing detection limitations due to their weak chromophoric properties.*
Methods: *The method utilizes 4-nitro-1-naphthalenyldiazonium tosylate as coupling reagent, which reacts with acid-decomposed artemisinin derivatives, forming colored azo adducts, which were optimized for analytical wavelength and detector response. Optimal reaction conditions, like temperature and time, were established, with stoichiometric ratios determined for each analyte. Validation confirmed the method's linearity, precision, accuracy, and limits of detection and quantitation.*
Results: *Artemether, artesunate, and dihydroartemisinin, upon acid decomposition, coupled avidly with diazonium reagent, giving reddish adducts with maximum absorbances at 430, 440, and 430 nm, respectively. Overlaid absorption spectra relative to the reagent and analytes revealed a bathochromic effect. Optimum reaction conditions were established at 50 ºC for 15 - 25 min. Assays were linear over 0 - 16 (artemether), 7.33 - 27.33 (artesunate), 6.08 - 16.21 µg/mL (dihydroartemisinin), with limits of detection of 0.21, 0.073, and 0.068 µg/mL, and overall recovery of 100.9 ± 1.7, 101.9 ± 3.3, and 100.4 ± 3.2 %, respectively. The assay results agreed with International Pharmacopoeia methods.*
Conclusion: *This colorimetric approach offers a reliable and sensitive alternative for artemisinin derivative analysis, enhancing antimalarial quality control where sophisticated equipment is inaccessible.*

Keywords: *Artemisinin derivatives, Colorimetric analysis, Diazonium coupling reagent, Azo adduct detection, Antimalarial drug quality control*

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INTRODUCTION

The artemisinin derivatives are antimalarial agents approved for use by the World Health Organization (WHO) and are commonly used in Asia and Sub-Saharan Africa [1]. They are derived from *Artemisia annua* and are effective against *Plasmodium* strains that are resistant to many conventional drugs [2]. There has been a rise in cases of counterfeit drugs, mainly in third-world countries, which have deleterious effects on public health. According to the WHO malaria report in 2020, out of 241 million cases of malaria

recorded, 11 million were pregnant women, and 627,000 deaths were reported, with two-thirds being children under five years old [3]. Studies have shown that the persistent virulence of malaria in developing nations is due to ineffective medicines [3,4]. In some African countries, many antimalarials are expired, substandard, or fake. It has also been said that about 450,000 preventable deaths occur annually due to counterfeit antimalarial usage [4].

Most artemisinin derivatives lack functional groups that readily react with chromogenic reagents. As a result of their poor chromophoric properties, their detection and quantification using standard UV-visible spectrophotometry is problematic. This challenge is apparent in developing nations where sophisticated techniques such as high-performance liquid chromatography (HPLC) are not easily accessible due to high cost and technical demands. Therefore, developing a colorimetric method for the determination of these drugs is highly desirable. Through chemical derivatization, artemisinin derivatives are rendered suitable for colorimetric detection. Diazo coupling, a widely used method in drug analysis, proceeds by chemical derivatization [5]. Chemical derivatization may be used when the analyte absorbs weakly in the UV region, which is often the case with most drugs, when there is interference from irrelevant absorption, when the procedure needs to be made more selective, or when there are financial considerations that favor the use of a colorimetric method over a UV-VIS spectrophotometer.

The use of naphthalene diazonium salt as a derivatization reagent offers a viable approach for developing a simple, cost-effective colorimetric method for artemisinin derivatives. This study therefore, explores reactive, stable, and environment-friendly diazonium ions as chromophoric labels, thereby providing a sensitive method for detecting artemisinin derivatives in pharmaceutical formulations. The diazonium salt, 4-nitro-1-naphthyldiazonium tosylate, has been synthesized and reported in our previous study [6].

Several methods have been reported for the assay of artemisinin derivatives in dosage forms and biological fluids. The International Pharmacopoeia [7-10] describes an HPLC method for the determination of artemether/lumefantrine and artesunate. In addition, a UV spectrophotometric detection of artemether at 254 nm following treatment with a hydrochloric acid/ethanol solution for 5 hours at 55 °C, and an alkalimetric method using sodium hydroxide for artesunate are official. Thomas *et al* [11] reported an HPLC assay for artemether and dihydroartemisinin in plasma, involving solid-phase extraction and acid decomposition prior to chromatographic separation. However, the complexity of the method limits its application as a routine method of analysis. Green *et al* attempted a colorimetric determination of artesunate based on pH-dependent diazonium coupling, but no significant color change was observed for other artemisinin derivatives [12]. Another spectrophotometric method was employed by coupling 4-carboxyl-2,6-dinitrobenzenediazonium (CDNBD) with artemisinin derivatives to yield azo adducts [13]. Although this method seems quite effective, the diazonium reagent could not address the detection at visible wavelengths. Adegoke and Osoye also included a method using *p*-dimethylaminobenzaldehyde (DMAB); this reagent gave a purple color with artemisinin derivatives [14].

In a recent study, Aghayere and Adelusi employed 4-nitrobenzaldehyde for the identification of artesunate in tablet samples with detection at 474 nm [15]. Potassium permanganate has also been employed as a reagent in the spectrophotometric determination of artesunate and dihydroartemisinin tablets, with sensitivity limits reaching 0.51 µg/mL [16]. However, many of these methods are time-consuming, costly, or suitable for just one or two of the artemisinin derivatives at most. Hence, this study is aimed to develop an efficient, robust, low-cost, and sensitive colorimetric method for the quantitative determination of artemisinin derivatives. The application of a more conjugated and reactive naphthalene diazonium salt as a coupling reagent will address the aforementioned limitations, thereby ensuring a cost-effective, sensitive, and rapid method for determining artemisinin derivatives. This approach will be of immense benefit to resource-poor settings, where high cost and technical complexities of official analytical techniques, such as HPLC, limit access to drug quality control. This study also tends to contribute to efforts aimed at combating the growing problem of counterfeit drugs.

EXPERIMENTAL

Reagents

Reference substances of artemether (ART), artesunate (ATS), and dihydroartemisinin (DHA) were purchased from AK Scientific, California, USA. Without additional purification, solvents that were purchased from commercial sources were employed. These include glacial acetic acid

(Abcr), ethyl acetate (JHD), and sulfuric acid (Abcr). Various dosage forms of artemisinin derivatives were purchased from community pharmacies.

Methods

Preparation of stock solutions

Stock solutions of 4-nitro-1-naphthyldiazonium tosylate (2.69 mM) and equimolar concentrations of artemether (ART, 0.008 g), artesunate (ATS, 0.0103 g), and dihydroartemisinin (DHA, 0.0076 g) were prepared in 10 mL of glacial acetic acid. The synthesis of diazonium reagent was as previously described [6].

Coupling reaction

Aliquots (0.1 mL) of ART, ATS, and DHA stock solutions were mixed with 0.5 mL concentrated sulfuric acid in separate test tubes, followed by the addition of 0.5 mL diazonium reagent. The reaction mixtures were incubated at room temperature (5 and 20 min) and 70 °C. Blank determinations were performed using analytes without the reagent and vice versa.

Selection of analytical wavelength

Aliquots (0.1 mL) of ART, ATS, and DHA stock solutions were treated with 0.5 mL conc. Sulfuric acid and 0.5 mL diazonium reagent, followed by incubation at 70 °C for 10 min. The reaction was quenched in an ice bath, diluted to 5 mL with cold water, and extracted with 10 mL of ethyl acetate. Absorbance spectra (250-800 nm) were recorded using an Analytikjena Specord 200 UV-Vis spectrophotometer. Blank samples (analyte-only and reagent-only) were analyzed similarly.

Optimization studies

The azo adduct formation was optimized by varying reaction temperatures (20, 50, 60, 70, and 80 °C) and times (5 and 20 min) using a steepest ascent method. Duplicate samples were analyzed at each condition, followed by cooling in ice, dilution to 5 mL, ethyl acetate extraction, and absorbance measurement at the maximum wavelength. Absorbance was plotted against temperature at both time intervals. The absorbance readings were recorded on a UV-visible spectrophotometer (Beckman DU 640i).

Time optimization was performed by reacting 0.5 mL stock solutions of ART, ATS, and DHA with 0.5 mL conc. Sulfuric acid and 0.5 mL diazonium reagent at 50 °C for varying durations (0 – 30 min). Absorbance at the maximum wavelength was recorded, and the optimal reaction time was determined based on peak absorbance. Measurements were taken in duplicate, and a time-absorbance curve was plotted.

The effect of sulfuric acid volume on azo adduct formation was optimized by reacting 0.5 mL stock solutions of ART, ATS, and DHA with varying acid volumes (0–1 mL). Reactions were carried out at 50 °C for the optimal reaction time, followed by extraction with ethyl acetate. Absorbance at the maximum wavelength was recorded to determine the ideal acid volume.

Determination of stoichiometric ratio

The stoichiometric ratio of the diazonium reagent to artemisinin derivatives was determined using equimolar solutions (2.69 mM) of ART, ATS, and DHA. Varying volumes of diazonium reagent (0 – 1 mL) were reacted with ART, ATS, and DHA (after acid decomposition) at 50 °C for their respective optimal reaction times. Absorbance was measured at 430 nm (ART, DHA) and 440 nm (ATS). Blank corrections were applied by subtracting the combined absorbances of individual components from the final complex. The optimal mole ratio was determined from the peak absorbance of the reagent-analyte complex [17].

Validation studies

Calibration curves were constructed for ART (2.67-16 µg/mL), ATS (14 - 27.33 µg/mL), and DHA (6.08 - 16.21 µg/mL) under optimized conditions. Absorbance was measured in duplicate over three days, and linear regression analysis was performed to obtain slope, intercept, and correlation coefficients.

Precision and accuracy studies

The accuracy and repeatability of the new method for the determination of the artemisinins were assessed by a three-day assessment of triplicate readings. Accuracy was carried out by determining the percentage recoveries and relative errors of three concentrations taken representing the lower, middle, and upper portions of the calibration curves (2.67, 8, and 16 µg/mL for ART, 14, 20.67 and 27.33 µg/mL for ATS and 6.08, 10.13 and 16.21 µg/mL for DHA) from concentrations obtained using the equation of the calibration curve. Likewise, repeatability was assessed using the relative standard deviation of replicate samples. The overall accuracy and precision of the method were recorded as the pooled data for all the recoveries. Limits of detection (LOD) and limits of

quantitation (LOQ) were calculated according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines [18].

Stability of azo adducts in diffuse lights

Standard solutions of the azo adduct containing 0.25 mL of ART (13.33 µg/mL) and 0.5 mL of the diazonium ion were prepared in four sample vials. Aluminum foil was used to wrap only two of the vials, leaving the other two unsecured. Both sets were maintained on the workbench in the lab. For three hours, the extracts' absorbance values at 430 nm were recorded at 30-minute intervals. Similar procedures were repeated using 0.41 mL of ATS (27.33 µg/mL) and 0.33 mL of the diazonium reagent, as well as 0.32 mL of DHA (16.21 µg/mL) and 0.33 mL of the diazonium.

Interference studies

Five milligrams each of magnesium stearate, starch, talc, lactose, gelatin, and a combination of the five excipients were present in different test tubes, and stock solutions (16 µg/mL of ART, 27.33 µg/mL of ATS, and 16.21 µg/mL of DHA) were added to each tube. As previously mentioned, the coupling, extraction, and sample analysis stages were completed.

Dosage form analysis

Different brands of artemisinin derivative tablets and injections were analyzed using the new method and the official method. Uniformity of weight test was carried out on all the brands using 20 tablets.

For artemether, the amount of powdered tablet and injection equivalent to 0.008 g of ART in each case was dispersed into 10 mL of glacial acetic acid. The mixture was placed in a sonicator for 15 min and filtered thereafter (no filtration was required for the injection) with the first 2 mL of the filtrate discarded. A 0.3 mL of the filtrate corresponding to 16 µg/mL with 0.125 mL sulfuric acid was coupled with 0.5 mL of the reagent at the optimized conditions and subjected to the procedures described above. Six replicate samples were prepared for each brand.

The procedure was repeated for ATS and DHA using 0.41 mL and 0.32 mL of filtered sample, respectively. The International Pharmacopoeia [7-10] methods (HPLC for ART tablets, UV spectrophotometry for ART injection, and alkalimetric titration for ATS) were used as reference standards. No official method was available for DHA.

Statistical analysis

Descriptive statistics were used to compare the assay results from the newly developed and official procedures. The official methods and new colorimetric method were compared for equivalence using Student's t-test. A p-value of 0.05 or less was considered significant.

RESULTS

Evidence of coupling reaction

The coupling reaction between acid-decomposed artemisinin derivatives and 4-nitro-1-naphthyldiazonium tosylate produced distinct colored azo adducts. The diazonium reagent is yellow in solution, which remains at room temperature and elevated temperatures. Artemether (ART) upon treatment with conc. Sulfuric acid yields a gold color, but on addition of the diazonium reagent, it gives a brown color at room temperature after about 20 min; however, at 70 ºC, a red color adduct was observed at 5 min, which remains even at 20 min. Artesunate (ATS) and dihydroartemisinin (DHA), in a similar manner upon acid decomposition, gave a gold color which turned red upon the addition of the reagent at an elevated temperature of 70 ºC. Extraction with ethyl acetate yielded a yellow solution in all cases.

Selection of analytical wavelength

The optimal absorption wavelength was determined to ensure significant detection without interference from the reagent or analytes. The optimum absorbance of the ethyl acetate extract of the adduct was found to be 430 nm for ART and DHA and 440 nm for ATS. However, the blank reagent displays maximum absorption at about 383 nm, while the decomposed analytes are at 278 nm for ART and DHA and 279 nm for ATS. The overlaid spectra are presented in Figure 1.

Optimization studies

Temperature optimization indicated that 50 °C produced the highest absorbance for all three derivatives (Figure 2). The absorbance readings of the diazonium-analyte adduct were taken at varying times at the optimized temperature of 50 ºC. It was observed that there was a steady increase in absorbance reading until 10 min and a decline was then observed at 15 min, which

peaked again at 20 min. However, a 25-minute reaction time was adopted as there was no significant difference in absorbance after that. For ATS, the highest absorbance was recorded at 15 min and just a little decline thereafter up until about 30 min; hence, 15 min optimum time was adopted for the coupling reaction. DHA, in a similar manner to ART, had increased absorbance initially but declined after 15 min to increase again thereafter; however, the eventual increase was not significant compared to before the decline, hence 25 min reaction time was adopted for its coupling. A plot of the absorbance against time is shown in Figure 3. The smallest volume of concentrated sulfuric acid (0.125 mL) was sufficient to achieve optimum absorbance. As the amount of acid used was increasing, there was a significant reduction in the absorbance value recorded. The plots of the absorbance recorded against the volume of sulfuric acid used for each of the analytes are shown in Figure 4.

Determination of Stoichiometric ratios

Using the optimized temperature and time for coupling reaction, varying volumes of equimolar concentration of the reagents and decomposed artemisinin derivatives were reacted together in different test tubes to make a volume of 1 mL each. An equal volume of diazonium ion and artemether was found to give the highest absorbance, which is at a mole fraction of 0.5 for the reagent. Absorbance reading decreases with increasing mole/volume of diazonium ion. Thus, a mole ratio of 1:1 was taken as the stoichiometric ratio for subsequent determinations of ART. For ATS and DHA, the highest absorbance in both cases was observed at 0.33 mL of the reagent to 0.67 mL of analytes. Therefore, a mole ratio of reagent to drug compound of 1:2 was taken as the stoichiometric ratio for their subsequent determinations. The plots of the absorbance against the mole ratio of drugs are shown in Figure 5.

Validation studies

Using the various conditions optimized above and the best-fit points obtained from the linearity of response, a calibration curve was prepared for the azo adduct of artemisinin derivatives with the diazonium reagent for three consecutive days. The average determination was prepared and a linear regression equation was obtained. The regression line was fixed using Microsoft Excel® software, the slope, intercept, and correlation coefficient for each drug were obtained and the results are shown in Table 1. The Table also provides the optical parameters, including molar absorptivity, Beer's law limits, absorption maxima, and Sandell's sensitivity values. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the equations 1 and 2 as stated below:

$$LOD = 3.3\delta/s \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$LOQ = 10\delta/s \quad \ldots\ldots\ldots\ldots\ldots \quad (2)$$

where δ is the standard deviation of blank and s is the slope of calibration curve.

Intra-day and inter-day precision and accuracy

Three distinct concentration levels (low, medium, and high) within the drug's working limits were prepared and analyzed in three replicates on the same day (intra-day precision) and in three consecutive days (inter-day precision) in a bid to assess the accuracy and precision (intra- and inter-day precision) of the developed method. Both the intra- and inter-day studies' relative error (RE (%)) and relative standard deviation (RSD (%)) results were adequate and provided a positive evaluation of the approach used daily. The results obtained (presented in Table 2) showed that the accuracy and precision of the proposed method have good repeatability and reproducibility.

Stability of azo adduct in diffuse light

The analytes-diazonium adduct absorbance readings were tracked for three hours, with sets of samples shrouded in aluminum foil and those subjected to diffuse light in the lab, recording their absorbance values every thirty minutes. For up to 180 minutes of exposure, there was no discernible difference in absorbance between sample extracts that were wrapped and those that were not.

Assessment of interference from excipients

A thorough investigation was conducted to establish the influence of excipients under ideal experimental settings, to test the proposed method's selectivity to pharmaceutical samples. The excipients employed include starch, talc, lactose, gelatin, magnesium stearate, and a combination of the five mentioned. For the three artemisinin derivatives, the excipients, except starch and lactose, were found not to obstruct the developed analytical method, and the outcomes were still within the range of 90 % to 110 of % pharmacopeia requirement. The results are presented in Table 3.

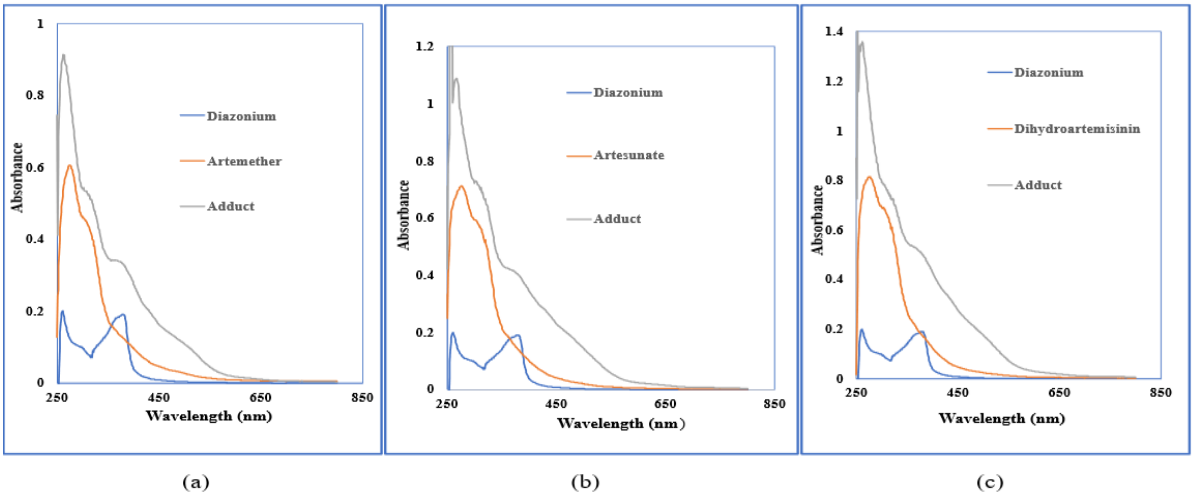


Figure 1: Overlaid absorption of the adduct, diazonium, and artemisinin derivatives -ART (a), ATS (b), and DHA(c)

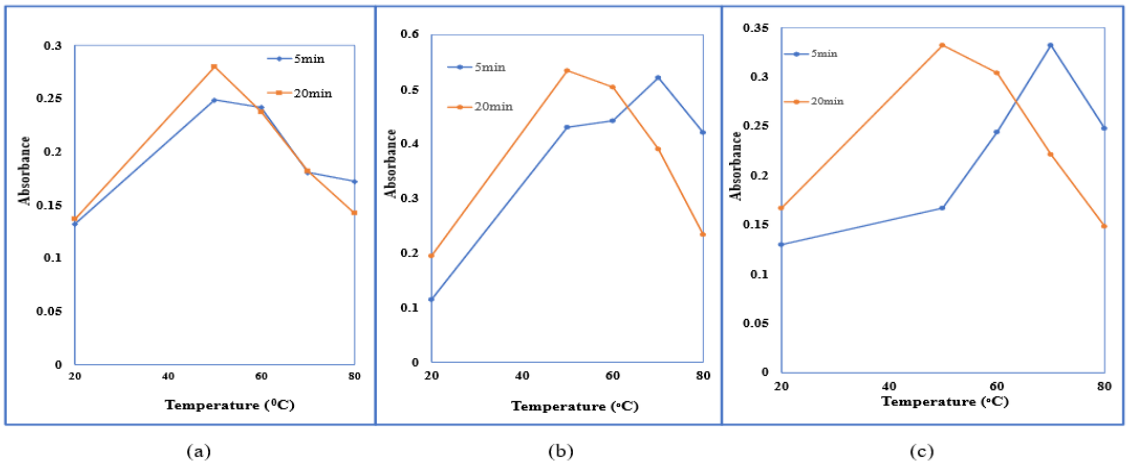


Figure 2: Temperature optimization for diazo coupling of ART (a), ATS (b), and DHA(c)

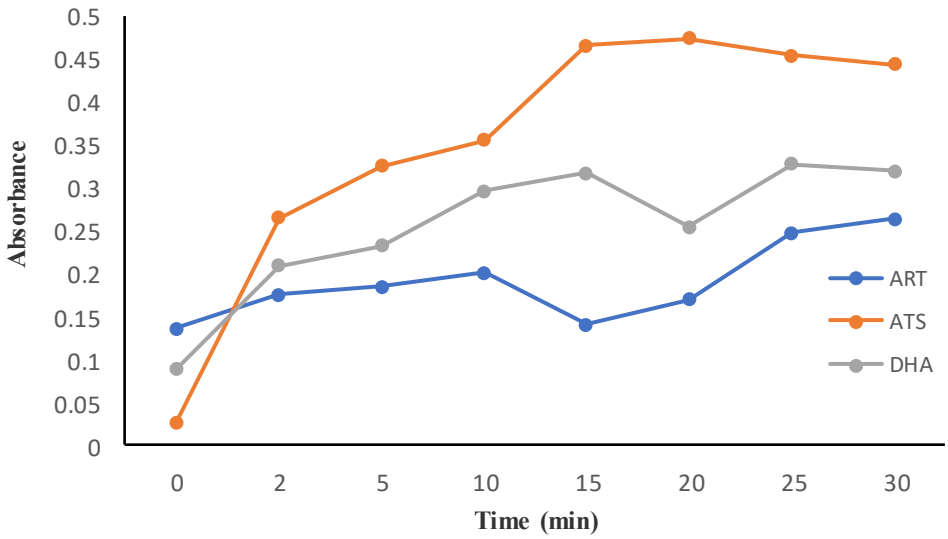


Figure 3: Optimization of time for diazo coupling of ART, ATS, and DHA at 50 ºC

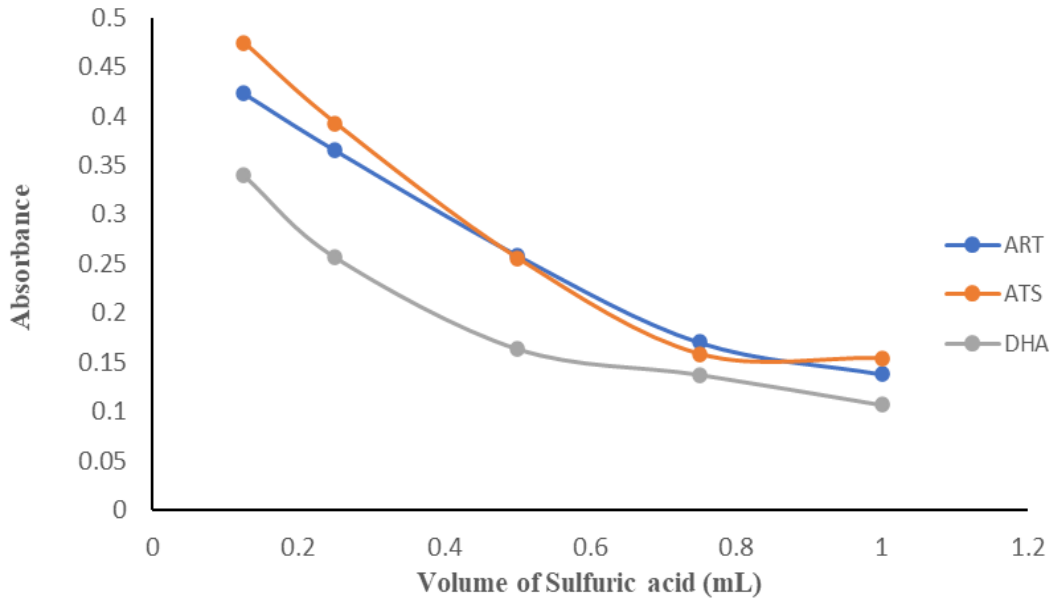


Figure 4: Plot of absorbance against the volume of sulfuric acid used for ART, ATS, and DHA

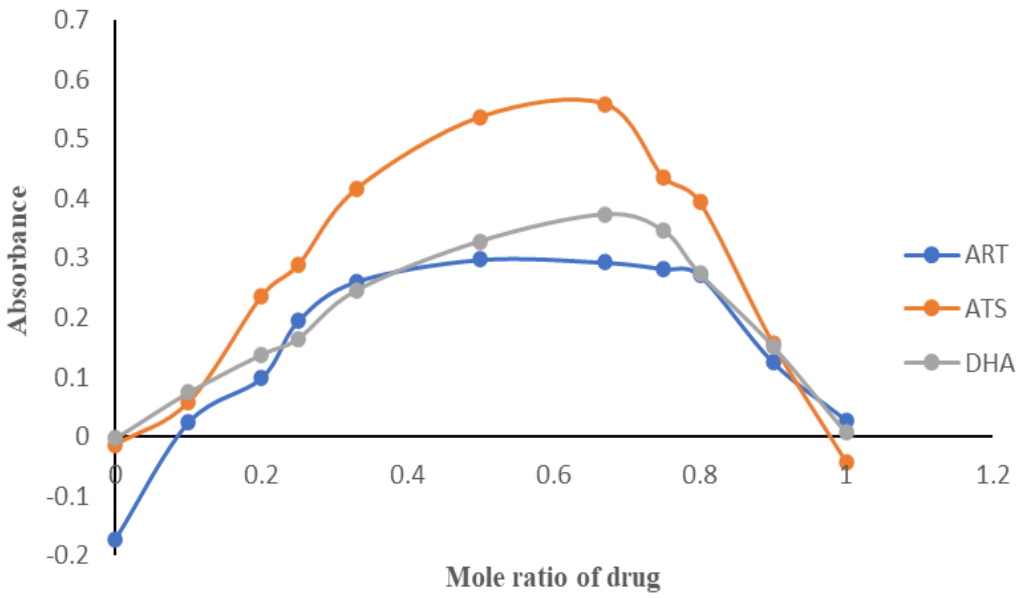


Figure 5: Determination of the stoichiometric ratio of reagent-adduct formation

Table 1: Analytical and validation parameters for azo adducts of analyte derivatives with diazonium salt

Parameter	Artemether	Artesunate	Dihydroartemisinin
λ max (nm)	430	440	430
Beer's law limits (µg/mL)	0 - 16	7.33 – 27.33	6.08-16.21
Limit of detection (µg/mL)	0.21	0.073	0.068
Limit of quantitation (µg/mL)	0.64	0.22	0.205
Molar Absorptivity □, (L/mol/cm)	6.23 x10^3	5.14 x 10^3	4.06 x 10^3
Sandell's sensitivity (µg/cm^2)	0.0437	0.0752	0.0625
Regression equation			
Intercept	-0.0283	0.0092	-0.0238
Slope	0.0229	0.0133	0.016
Correlation coefficient, R^2	0.9952	0.9922	0.996

Table 2: Inter-day and intra-day accuracy and precision for the proposed method

Drug	Conc. taken (µg/mL)	Recovery[a]	RSD (%)	RE (%)
		Inter-day		
Artemether	2.67	101.27	1.3	1.27
	8	102.41	2.3	2.41
	16	98.94	1.4	-1.06
Artesunate	14	100.51	6.89	0.51
	20.67	103.15	1.24	3.15
	27.33	102.13	1.54	2.13
Dihydroartemisinin	6.08	100.37	4.31	0.37
	10.13	100.23	2.75	0.23
	16.21	100.63	2.60	0.63
		Intra-day		
Artemether	2.67	100.26	0.46	0.26
	8	102.67	1.65	2.67
	16	99.95	0.74	-0.05
Artesunate	14	94.98	2.289	-5.02
	20.67	103.25	1.75	3.25
	27.33	99.59	1.02	-0.41
Dihydroartemisinin	6.08	101.63	5.20	1.63
	10.13	101.07	1.65	1.07
	16.21	99.27	2.33	-0.73

[a]n=9; overall recovery for artemether = 100.88 ± 1.72 (RSD%= 1.70), artesunate =101.93 ± 3.26 (RSD%= 3.22), dihydroartemisinin = 100.41 ± 3.23 (RSD% =3.22)

Table 3: Assessment of Interference from Excipients[a]

Excipient	Recovery (% ± SD[b])		
	Artemether	***Artesunate***	***Dihydroartemisinin***
Starch	136.63 ± 8.66	123.95 ± 0.32	139.08 ± 9.71
Talc	104.24 ± 5.58	100.23 ± 0.94	93.01 ± 0.97
Lactose	128.61 ± 6.33	115.46 ± 2.68	131.74 ± 7.72
Gelatin	98.98 ± 1.11	97.77 ± 2.58	100.78 ± 0.59
Mg stearate	105.36 ± 2.44	107.74 ± 3.12	97.53 ± 2.17
Mixture	127.87 ± 0.50	123.14 ± 2.91	132.61 ± 0.64

[a]Concentration spiked = artemether 16 µg/mL, artesunate= 27.33 µg/mL, dihydroartemisinin = 16.21 µg/mL; [b]n=4

Table 4: Determination of artemisinin derivatives in dosage forms using new and IP methods

Drug formulation	New method (% ± SD)[a]	Official method	*P*-value	
			F-test	*t*-test
	Artemether			
Philomether inj	91.42±1.17	96.71±1.52	0.59	0.000083
Shal'artem tab	103.24±3.56	102.05±3.86	0.85	0.24
Arenax tab	95.37±5.01	105.89±9.97	0.25	0.12
Laris tab	94.98±4.47	99.14±3.87	1.33	0.16
Gvither tab	96.73±4.98	100.42±8.61	0.33	0.48
Artesunate				
Camosunate tab	101.94±2.58	101.61±0.90	0.09	0.77
Rekmal inj	106.03±5.21	108.53±0.58	1.37	0.27
Nemal inj	104.51±4.38	100.20±1.16	5.41	0.04
Dihydroartemisinin				
P-Alaxin tab	101.06±2.58	N/A		

Content of Artemether and Artesunate stipulated by the IP, 2020 = 90 – 110%. [a]n = 6

Application of methods to dosage form and statistical analysis

The new colorimetric method developed by coupling 4-nitro-1-naphthyldiazonium tosylate with acid-decomposed artemisinin derivatives was applied to the assay of the same batch of commercially available brands of artemether, artesunate and dihydroartemisinin tablets and artemether and artesunate injectables. The

results were compared with the official method as stated in the International Pharmacopoeia [7-10]. The percentage content observed was within the range of 90-110 % as stated in the reference book. Application of the Student's t-test for accuracy and the F-test for precision allowed for a statistical comparison of the assay results with the reference method; Table 4 presents the results, which at the 95 % confidence level indicated no significant difference in accuracy and precision between the proposed and reference methods.

DISCUSSION

The observed color changes after acid treatment and coupling confirm that the artemisinin derivatives require acid or base treatment to generate reactive methylene centers for colorimetric detection for diazonium coupling [13]. Acid decomposition enables diazonium coupling, forming a colored adduct, which justifies the use of concentrated sulfuric acid in the reaction. Elevated temperature is critical to the coupling of the reagent with artemisinins as it markedly accelerated color development, while minimal acid volumes prevented side reactions that lowers yield.

The bathochromic shifts into the visible region for the adducts, compared to the UV maxima of the decomposed drugs and the reagent, show successful derivatization and this confers specificity of the method relative to detection in the UV regions.

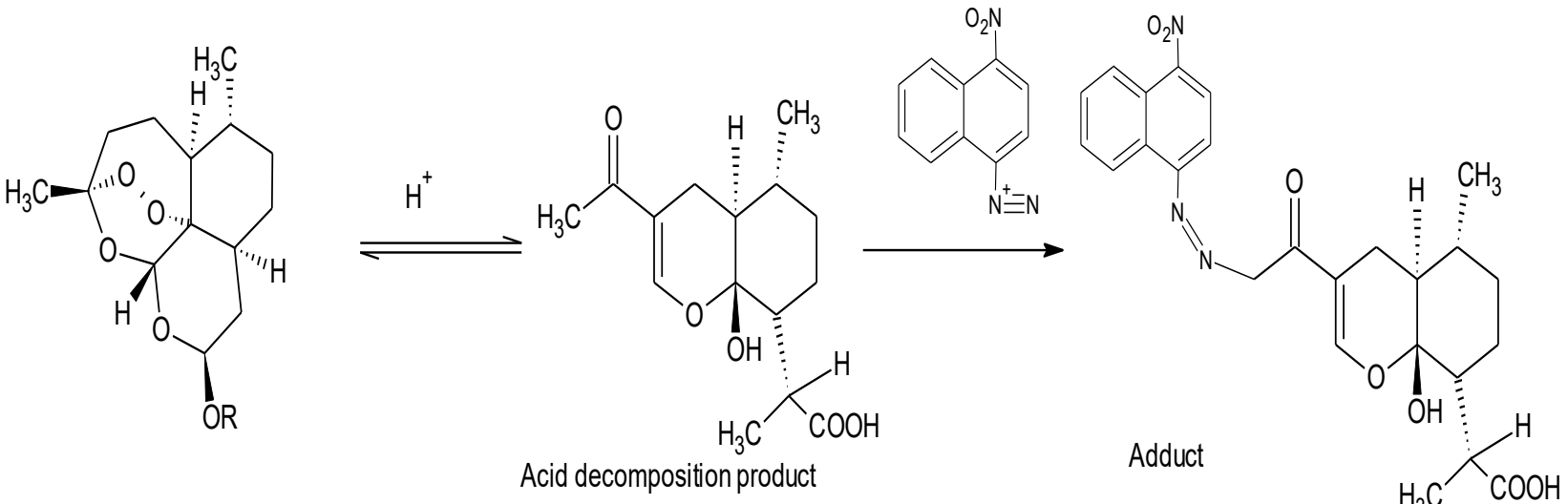
The optimized reaction conditions of 50 °C with reaction times of 15–25 minutes depending on the analyte provided a balance between rate and stability of the chromophore. The absorbance trends with temperature and time suggest that 50 °C is optimal for balancing reaction rate and stability. The time-dependent fluctuations may reflect intermediate formation and degradation. Since the generation of methylene center to react with the reagent is by acid decomposition, it becomes imperative to optimize the volume of acid required to give optimum absorbance reading in the determination of the analytes. In all three cases of the artemisinin derivatives, it was observed that the minutest amount of conc. sulfuric acid is more than sufficient to achieve their acid decomposition prior to coupling with the diazonium reagent. A volume of 0.125 mL of conc. sulfuric acid was thus employed for subsequent determinations in the method development. Minimal acid volumes were adequate, as excess acid may cause competing side reactions, lowering absorbance.

The stoichiometric ratios obtained (1:1 for ART; 1:2 for ATS and DHA) may influence the number of available reactive sites. The mole ratio of 1:1 for diazonium ion and acid-decomposed artemether is expected since only one reactive methylene center should be generated to produce the azo adduct that gave the yellow color. When two species interact at a constant total concentration, the adduct is most absorbing at a point where the species are combined in the proportions that they appear in the complex [19]. A proposed reaction depicting the coupling of the reagent to the analyte, artemisinin derivatives, following their acid decomposition, is presented in Scheme 1.

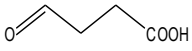
From the results presented in Table 1, the high values of molar absorptivity (ε) and low values of Sandell's sensitivity and LOD indicate the high sensitivity of the new method. The method was found to be more sensitive than similar procedures reported by Adegoke and co-workers [13], where LOD was obtained as 0.55, 0.95, and 0.17 µg/mL for ART, ATS, and DHA, respectively, and by Adegoke and Osoye [14], where LOD was obtained to be 5.93 and 8.49 µg/mL for ATS and DHA, respectively. These indicate suitability for detecting low concentrations in quality control settings.

The stability of the colored complex adducts under ambient light conditions increases the practicality of the method for routine laboratory and field applications. This implies that the magnitude of light going through the solution does not in any way affect absorptivity and hence the stability of the colored complex to diffuse light is assured. As a result, it is advised that artemisinin adduct sample solutions be tested unwrapped and could be left for up to 3 hours.

The interference tests confirm that most common excipients do not affect assay performance, however higher recoveries with starch and lactose confirm that sugars undergo acid-mediated oxidation to species that couple with diazonium salts and hence may require prior removal. These imply that the drugs would require prior extraction before their colorimetric assay may be carried out. The application of the developed method to commercial formulations establishes their equivalence to the official methods and thus, they could be employed for the routine analysis of artemisinin derivatives both in pure and formulated products. This new colorimetric method offers advantages such as low concentrations of the calibration ranges, higher sensitivity, cost-effectiveness, and ease of access to assay equipment.



R = CH_3 = Artemether

R=  = Artesunate

R = H = Dihydroartemisinin

Scheme 1: Proposed coupling reaction scheme of diazonium with artemisinin derivative

CONCLUSION

The procedure developed for the selected antimalarials (artemether, artesunate, and dihydroartemisinin) offers the advantages of accuracy and precision, as well as reduced cost and time of analysis. The use of a colorimeter, which is much more accessible, also confers the benefit of simplicity. It is anticipated that these procedures will be applied for in-process quality control of these drugs and their dosage forms, which will ensure an efficient production process and reduce counterfeiting. The analysis of artemether tablets was achieved in the presence of lumefantrine without interference. Also, this method offers a great advantage over the use of HPLC for routine analysis of artemether/lumefantrine, especially in poor resource economies.

DECLARATIONS

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Ethical approval

None.

Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Use of Artificial intelligence/Large language models

We declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Conflict of interest

No conflict of interest is associated with this work.

Authors contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Aremu Olajire Adegoke, Sunday Olakunle Idowu designed and supervised the study. Material preparation, data collection, and analysis were performed by Oluwatobi Oladayo Olakojo. Eric Beitz and Marc Scherwing carried out some chromatographic analysis and further data interpretation. The first draft of the manuscript was written by Oluwatobi Oladayo Olakojo and Eric Beitz, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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