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Original Research Article

Pharmacokinetic and Bioanalytical Consideration of the Salivary Concentration of Artesunate and Dihydroartemisinin in Nigerian Volunteer Subjects: Using Spectrophotometric Methods

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Abstract

This study evaluates the use of saliva as a biological fluid for UV-visible spectrophotometric analysis of artemisinin derivatives: artesunate and dihydroartemisinin in Nigerian subjects. The absorbance of artesunate and dihydroartemisinin saliva samples was measured using the UV-VIS spectrophotometer. The samples were analysed with and without coupling to benzene diazonium chloride (chromogenic agent). The saliva samples collected from the subjects at various time intervals after the administration of artesunate and dihydroartemisinin at λmax of 318nm and 312nm, respectively, showed significant accuracy and sensitivity of the method. A standard curve of artesunate gave a straight line with a standard deviation of 0.008178, with LOD and LOQ values of 35.80µg/mL and 108.485µg/mL, respectively. The standard curve of dihydroartemisinin gave a straight line with a standard deviation of 0.0000940, with LOD and LOQ values of 4.094µg/mL and 12.405µg/mL, respectively. Due to its ease of obtainment and greater patient acceptability, saliva sampling analysis using spectrophotometric methods is beneficial in the pharmacokinetic study of artesunate and dihydroartemisinin.

Keywords: Artesunate, Bioanalysis, Dihydroartemisinin, Pharmacokinetics, Saliva, Spectrophotometry

INTRODUCTION

Artemisinin is a tetracyclic 1,2,4-trioxane isolate of *Artemisia annua* L. and is currently the World Health Organization's recommended first-line agent against malaria due to *Plasmodium falciparum* (WHO, 2021). To date, artemisinin and its derivatives are the most relevant medicine for malarial treatment (Khanal, 2021), being effective against nearly all asexual and sexual parasite stages. Notably, the parent drug artemisinin has poor solubility in both oil or water phases, thus, limiting its route of administration to either orally or rectally; this led to the synthesis of its derivatives such as dihydroartemisinin (by a reduction of the carbonyl group of artemisinin), water-soluble artesunate (water-soluble artemisinin derivative) and artemether and arteether (which are oil-soluble artemisinin), for a greater anti-malarial activity (Cui and Su, 2009). Of these derivatives of artemisinin, dihydroartemisinin is an active metabolite of artemisinin and all its derivatives as well as an anti-malarial itself. Figure 1

Pharmacokinetics (PK) evaluations are useful in

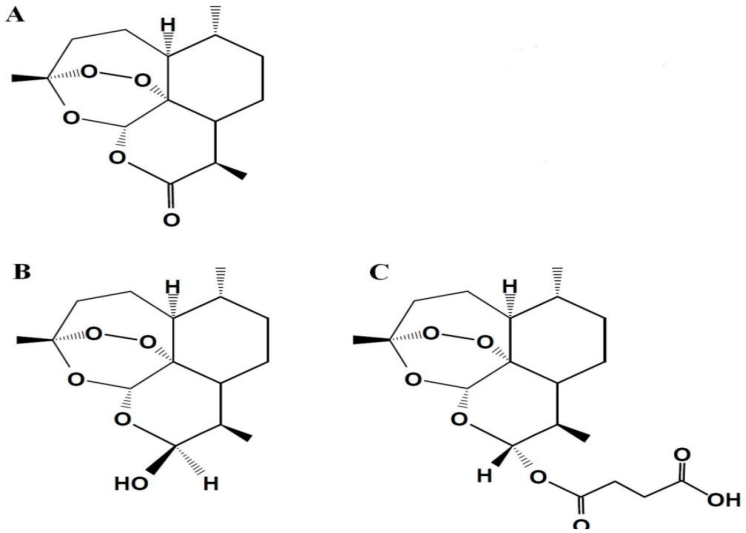

Figure 1. Structure of Artemisinin (A), Dihydroartemisinin (B), Artesunate (C)

pharmacological studies either to access the effect of a drug and the time course of its activities in the body (Toomula *et al*., 2011), and to show the therapeutic response of a drug. These studies are carried out by measuring the concentration of the drug substance in physiological fluids, like blood, plasma, serum, and on occasion, saliva (Malangu, 2018); using various analytical methods. According to Saravanan *et al.* (2021), there are about thirty-three known analytical methods being utilized for studies on analysis of artesunate done in bulk, formulations (pharmaceutical) and biological fluids. While, White (2013), had reported that the concentration profile (pharmacokinetics) of antimalarial in the blood is an effective way to determine the therapeutic response in malaria; *Gordi et al.* (2000), had highlighted cultural, ethical and practical (requiring expertise) challenges in the use of blood samples for PK studies of artemisinin.

So far, saliva has been reported to be an effective sampling method for both pharmacological tests, with benefits of increased procedure speed and better patient compliance (Bolani *et al.,* 2021), ease in collection and handling of samples (Gordi *et al.,* 2000) and a non-invasive replacement of plasma sampling for therapeutic drug monitoring (Kim *et al.,* 2020; Idkaidek, 2017). The aim of this study is to evaluate the use of saliva as a suitable biological fluid for UV-visible spectrophotometric analysis of artemisinin derivatives: artesunate and dihydroartemisinin in Nigerian subjects.

MATERIAL AND METHODS

Materials

All chemicals were of analytical grade. Stock solutions of artesunate (ARTESUNATE tablet manufactured by Mekophar Chemical Pharmaceutical Joint Stock Co.) and dihydroartemisinin (CODISIN® tablets manufactured by Adams Pharmaceutical (Anhui) Co. Ltd.) were prepared from drugs purchased from Prolimsa Pharmacy, Uyo, Nigeria. They were freshly prepared and diluted with methanol before use.

Methods

Study Design

Five healthy Nigerian subjects aged 21 to 24 years were enrolled in this study, conducted at the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria. The study protocol was approved by the Research Ethics Committee at the University of Uyo, Nigeria. The aim of the study, potential discomfort, and adverse effects were explained to the volunteers. None of the volunteers had recently (< 3 months) taken any other prescribed or over-the-counter medication or taken any artemisinin-related drug. Participants were recruited voluntarily.

Study Drug

The participant's saliva was stimulated by chewing inert rubber bands. Pre-dose saliva samples were collected to ensure the absence of analytical interference. The subjects were divided into three groups: subjects A and B ingested 2 x 50mg tablets of artesunate (ARTESUNATE®) each; subjects C and D ingested 2 x 60mg tablets of dihydroartemisinin (CODISIN®) each, and subject E

ingested 2 x 50mg tablets of artesunate (ARTESUNATE®).

Saliva Sample Collection

The subjects were monitored and 5mL of saliva samples were collected at the following times post-dose: 30, 60, 90, 120, 150, 180, 210, and 240 minutes into labelled sample bottles (Sterile®). Samples were analysed immediately using the UV-VIS spectrophotometer. Saliva samples obtained from subjects A and B were analysed at wavelength 318nm; samples obtained from subjects C and D were analysed at wavelength 312nm; while the saliva sample obtained from subject E was analysed at wavelength 312nm to confirm the metabolite (dihydroartemisinin) of the administered drug artesunate. Stimulated saliva samples (using inert rubber bands) were collected (as much as possible during *in-vitro* testing and 5mL each during *in-vivo* testing) from each subject into a sterile sample bottle (Sterile®). Saliva samples collected from healthy, volunteer male and female individuals were used for analysis immediately after collection to minimize microbial proliferation or distortion of salivary components.

Drug Analysis

Extraction of Artesunate from Pharmaceutical Formulation (Artesunate®) and Dihydroartemisinin from Pharmaceutical Formulation (Codisin®)

Five tablets of ARTESUNATE® and CODISIN® respectively, were weighed using the analytical balance and the average weight of the tablets was calculated. Thereafter, twelve tablets of ARTESUNATE® (50mg Artesunate) were crushed into powder in a porcelain mortar. The powder obtained was transferred into a beaker and 100mL of dichloromethane was added with continuous stirring. This stood for 30 minutes with occasional stirring. The mixture was filtered using Whatman size 1 filter paper into a beaker and the residue was rinsed with 50mL of dichloromethane to make up the filtrate volume to about 150mL. The filtrate was then evaporated under room conditions to obtain a dried powder of artesunate. About ten tablets of CODISIN® (60mg Dihydroartemisinin) were crushed into powder in a porcelain mortar. The powder obtained was transferred into a beaker and 100mL of chloroform was added with continuous stirring. This stood for 30 minutes with occasional stirring and the mixture was filtered using Whatman filter paper (size 1) into a beaker. The residue was rinsed with 50mL of chloroform to make up the volume of the filtrate to about 150mL. The filtrate was then evaporated under room conditions to obtain dried crystals of dihydroartemisinin.

Solubility Test

20mg each of the extracted artesunate and dihydroartemisinin was weighed and put into different test tubes containing 5mL each of distilled water, acetone, chloroform, dichloromethane, ethanol, methanol, 40% sodium hydroxide, 0.9% sodium chloride and saliva. The mixture was allowed to stand for 5 minutes and the solubility of artesunate and dihydroartemisinin in each solvent was recorded.

Preparation of Stock Solutions

To 30mg of artesunate weighed into a beaker, a measured 30mL of methanol was added to dissolve it. Then 30mL of fresh saliva was added to it to obtain a stock solution of 1mg/mL artesunate (dissolved with methanol) in saliva. The same procedure was repeated for a weighed 30mg of dihydroartemisinin.

Benzene Diazonium Chloride (Diazotization)

As described in Maas (2001), a mixture of 5mL distilled water and 5mL hydrochloric acid was added to 2mL of aniline in a volumetric flask and cooled in an ice bath. A solution of 1.5g of sodium nitrite in 2mL of water was added gradually to the solution and the temperature was maintained at less than 4°C in the ice bath. 5mg of artesunate and dihydroartemisinin were incubated in 40% sodium hydroxide (5mL) for 5 hours. A solution of each product was obtained at a concentration of 1mg/mL. Freshly prepared benzene diazonium chloride was used to couple the two products. Also, stock solutions of artesunate and dihydroartemisinin were incubated in 40% sodium hydroxide for 5 hours. The alkalinity of the solution was confirmed with a litmus paper. Freshly prepared benzene diazonium chloride was used to couple the two stock solutions.

UV-VIS Spectrophotometric Analysis

UV-VIS Spectrophotometric Analysis of Artesunate and Dihydroartemisinin in Methanol

About 10mg of artesunate and dihydroartemisinin were accurately weighed and dissolved in 10mL of methanol in separate beakers to obtain a 1mg/mL solution. Exactly 5mL of the 1mg/mL solutions were scanned using the UV-VIS spectrophotometer. The absorbance of the solutions was scanned at different wavelengths to

establish the wavelength of maximum absorption (λmax). A graph of absorbance against wavelength was plotted for both artesunate and dihydroartemisinin to obtain the absorption spectrum. This was done to establish the absorption spectrum of artesunate and dihydroartemisinin in the solvent (methanol) without the interference of the biological fluid (saliva).

UV-VIS Spectrophotometric Analysis of Artesunate (Dissolved in Methanol) In Saliva and Dihydro-artemisinin (Dissolved in Methanol) In Saliva

Exactly 5mL of the stock solutions [1mg/mL artesunate (dissolved in methanol) in saliva and 1mg/mL dihydroartemisinin (dissolved in methanol) in saliva] were scanned using the UV-VIS spectrophotometer respectively. The absorbance of the solutions was scanned at different wavelengths to establish the wavelength of maximum absorption (λmax). A graph of absorbance against wavelength was plotted to obtain the absorption spectrum. A 5mL graduated pipette was used to obtain 1mL, 2mL, 4mL, 6mL, and 8mL of the stock solution into five separate labelled clean test tubes. Then 9mL, 8mL, 6mL, 4mL and 2mL volumes of freshly collected saliva was added to the test tubes respectively to obtain diluted concentrations of 0.1mg/mL, 0.2mg/mL, 0.4mg/mL, 0.6mg/mL and 0.8mg/mL solutions respectively. Then 5mL of each of the different dilutions was analysed and their triplicate absorbances were recorded at the established maximum wavelength (λmax). From the results obtained, Beer's plot was generated and the limits of detection and quantitation were established using the relationship: limit of detection (sensitivity) in mg/mL = 3.3Sy/m and limit of quantitation in mg/mL = 10Sy/m where Sy is the standard deviation computed from the intercept on the absorbance axis and m is the slope of the graph.

UV-VIS Spectrophotometric Analysis of Artesunate (Dissolved in Methanol) in Saliva Coupled to Benzene Diazonium Chloride and Dihydroartemisinin (Dissolved in Methanol) in Saliva Coupled to Benzene Diazonium Chloride

Exactly 5mL of the respective stock solutions [1mg/mL artesunate (dissolved in methanol) in saliva and 1mg/mL dihydroartemisinin (dissolved in methanol) in an alkaline medium (40% NaOH) coupled to benzene diazonium chloride was scanned using the UV-VIS spectrophotometer. The absorbance of the solution was scanned at different wavelengths to establish the wavelength of maximum absorption (λmax). A graph of absorbance against wavelength was plotted to obtain the absorption spectrum.

A 5mL graduated pipette was used to obtain 1mL, 2mL, 4mL, 6mL, and 8mL of the coupled stock solution into five separate labelled clean test tubes. Then 9mL, 8mL, 6mL, 4mL and 2mL volumes of freshly collected saliva was added to the test tubes respectively to obtain diluted concentrations of 0.1mg/mL, 0.2mg/mL, 0.4mg/mL, 0.6mg/mL and 0.8mg/mL solutions respectively. Then 5mL of each of the different dilutions was analysed and their triplicate absorbances were recorded at the established maximum wavelength (λmax). From the results obtained, Beer's plot was generated, and the limits of detection and quantitation were established using the relationship: limit of detection (sensitivity) in mg/mL = 3.3Sy/m and limit of quantitation in mg/mL = 10Sy/m where Sy is the standard deviation computed from the intercept on the absorbance axis and m is the slope of the graph.

UV-VIS Spectrophotometric Analysis of Saliva Samples Obtained from Test Subjects to Determine Artesunate and Dihydroartemisinin Concentrations.

The subjects were monitored and 5mL of saliva samples were collected at the following times post dose: 30, 60, 90, 120, 150, 180, 210, and 240 minutes into labelled sample bottles (Sterile®). Samples were analysed immediately using the UV-VIS spectrophotometer. Saliva samples obtained from subjects A and B were analysed at wavelength 318nm; samples obtained from subjects C and D were analysed at wavelength 312nm; while the saliva sample obtained from subject E was analysed at wavelength 312nm to confirm the metabolite (dihydroartemisinin) of the administered drug artesunate.

RESULTS AND DISCUSSION

The two artemisinins studied had very similar physicochemical characteristics in that they were soluble in many similar organic solvents, and, coupled to the diazonium dye. In the solubility test, artesunate was soluble in acetone, dichloromethane, ethanol, methanol; sparingly soluble in chloroform, water, 40% sodium hydroxide, 0.9% sodium chloride, and saliva. Whereas, dihydroartemisinin was soluble in acetone, chloroform, ethanol, methanol; sparingly soluble in 40% sodium hydroxide but practically insoluble in water, dichloromethane, 0.9% sodium chloride, and saliva. According to Cayman (2014), the in-vitro dissolution of artesunate and dihydroartemisinin in biological fluids such as saliva, normal saline (0.9% NaCl), and physiological buffers such as phosphate buffer saline (pH 7.2), phosphate buffer (pH 7.4) (plasma pH) and phosphate buffer 6.8 (saliva pH), was practically impossible and is only possible by first dissolving the

Table 1. *Result of UV-VIS spectrophotometric analysis of 1mg/ml each of drug samples in methanol*

S/N	Wavelength (nm)	Absorbance of Artesunate	Absorbance Of Dihydroartemisinin
1	200	-0.005	-0.005
2	220	-0.019	-0.006
3	240	-0.009	-0.007
4	260	-0.010	0.003
5	280	-0.013	-0.003
6	300	0.033	0.150
7	305	0.036	0.153
8	320	0.030	0.130
9	340	0.021	0.118
10	360	0.015	0.090
11	380	0.011	0.072
12	400	0.005	0.061

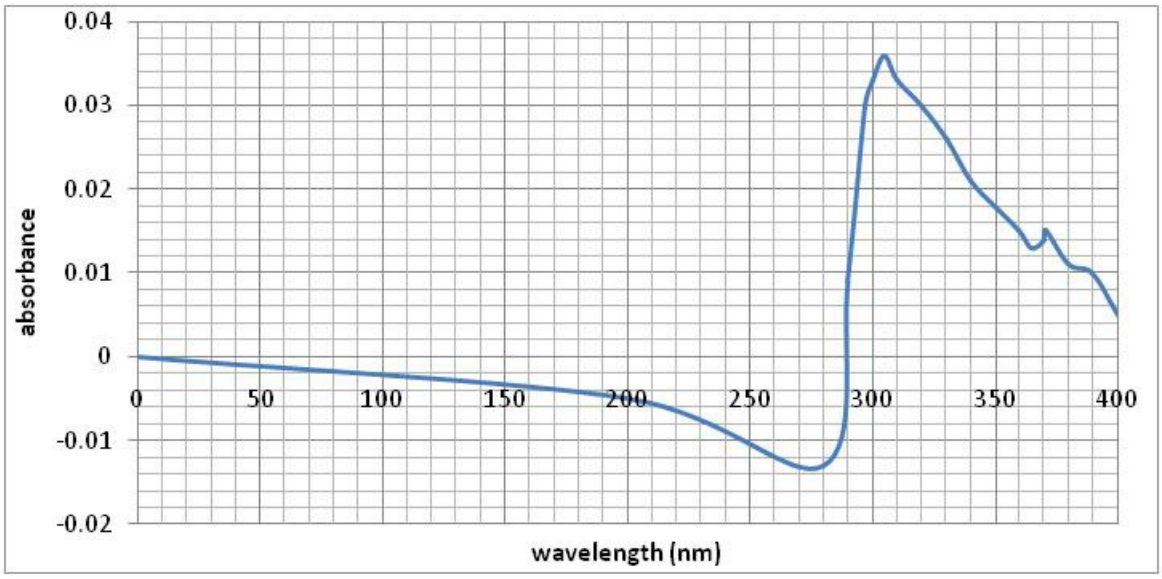

Figure 2. Result of UV-VIS spectrophotometric absorbance of 1mg/ml artesunate in methanol at different wavelengths (200, 240, 260, 280, 300, 305, 320, 340, 360, 380, and 400nm respectively)

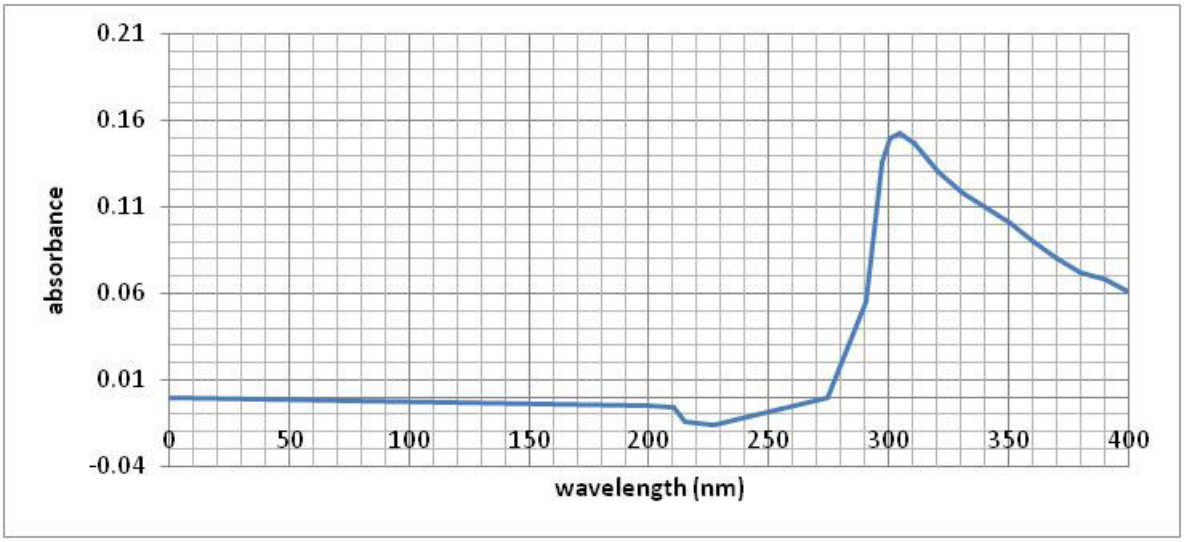

Figure 3. Result of UV-VISspectrophotometric absorbance of 1mg/ml dihydroartemisinin methanol at different wavelengths (200, 240, 260, 280, 300, 305, 320, 340, 360, 380, and 400nm respectively)

Table 2. *Result of theUV-VISspectrophotometric analysis of the stock solution of artesunate and dihydroartemisinin in an alkaline medium coupled with benzene diazonium chloride*

S/N	Artesunate		Dihydroartemisinin	
	Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance
1	200	0.006	200	0.007
2	220	0.003	220	0.003
3	240	0.010	240	0.493
4	260	0.006	260	0.291
5	280	0.012	265	0.276
6	300	0.030	280	0.503
7	320	0.440	297	0.820
8	335	0.565	300	0.879
9	340	0.547	320	1.347
10	360	0.240	329	1.388
11	380	0.025	340	1.324
12	395	0.010	360	0.825
13	400	0.010	380	0.264
14	420	0.076	399	0.186
15	440	0.279	420	0.490
16	460	0.777	440	0.948
17	470	0.113	460	1.428
18	481	1.305	469	1.570
19	500	1.104	480	1.495
20	-	-	500	1.104

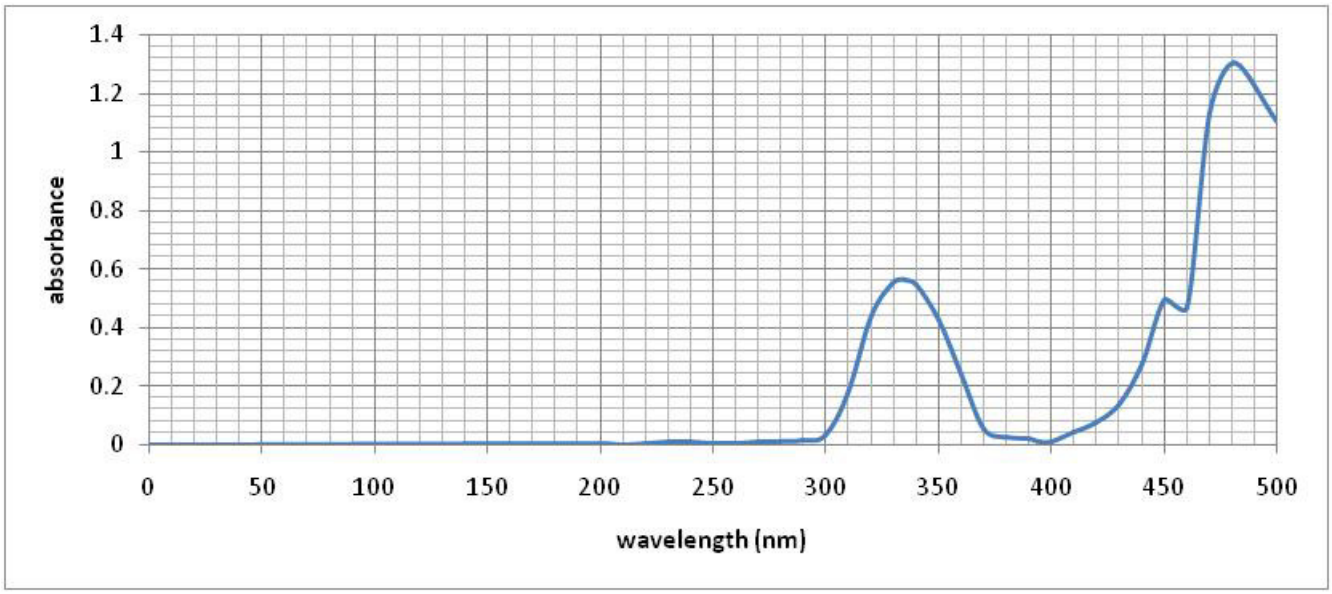

Figure 4. *Result of the UV-VISspectrophotometric analysis of the stock solution of artesunate in an alkaline medium coupled with benzene diazonium chloride*

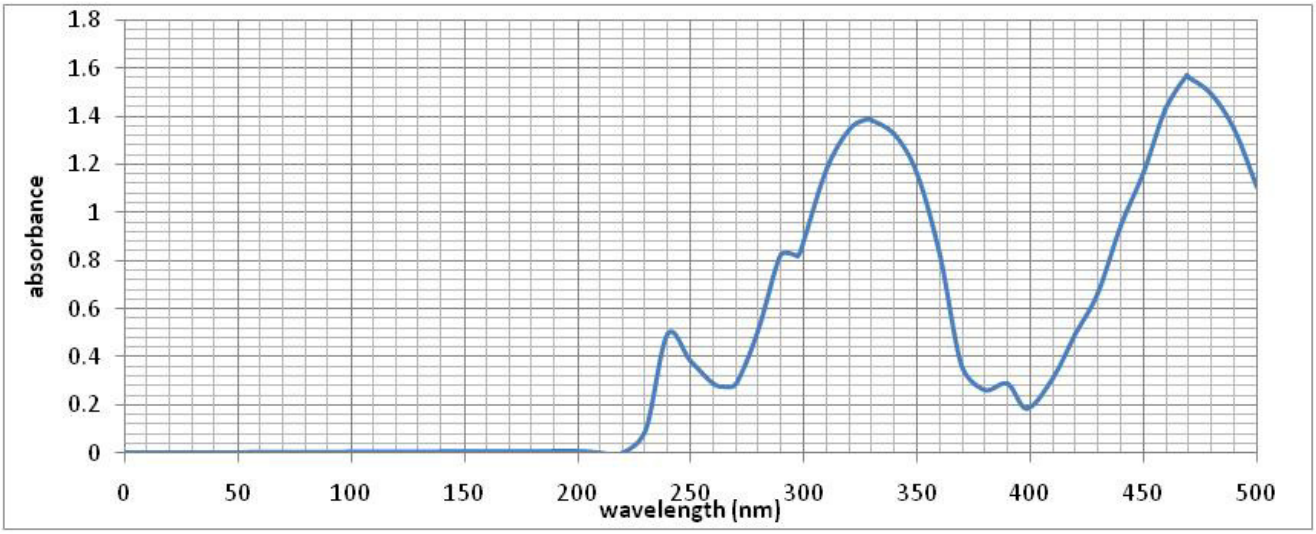

Figure 5. *Result of the UV-vis spectrophotometric analysis of dihydroartemisinin in an alkaline medium coupled with benzene diazonium chloride*

Table 3. *Linearity and Sensitivity Data*

Parameters	Result	
	Artesunate	**Dihydroartemisinin**
λmax (nm)	481	496
Beer's Law Linearity Range (mg/ml)	0.1-0.8	0.1-0.8
Regression Equation	y= 1.4523x – 0.0238	y= 1.8431x- 0.0029
Intercept (X)	-0.023	-0.002
Slope (B)	1.452	1.843
Correlation Coefficient (R)	0.998	0.999
Limit of Detection	0.04923 mg/mL	0.04441 mg/mL
Limit of Quantification	0.14917 mg/mL	0.134563 mg/mL

Table 4. *Result of UV-VIS spectrophotometric analysis of the stock solution of artesunate and dihydroartemisinin respectively*

S/N	Wavelength (nm)	Absorbance of Artesunate	Absorbance Of Dihydroartemisinin
1	200	0.005	0.005
2	220	0.000	0.003
3	240	0.000	0.017
4	260	0.003	0.008
5	270	0.002	0.166
6	297	0.202	0.261
7	300	0.331	0.427
8	318	0.608	0.415
9	340	0.601	0.376
10	360	0.538	0.324
11	380	0.471	0.271
12	400	0.477	0.274
13	405	0.470	0.262
14	420	0.487	0.243
15	440	0.468	0.227
16	460	0.449	0.213
17	480	0.431	0.198
18	500	0.414	0.184
19	520	0.402	0.174
20	550	0.377	0.164
21	570	0.364	0.155
22	630	0.327	0.141
23	650	0.321	0.124

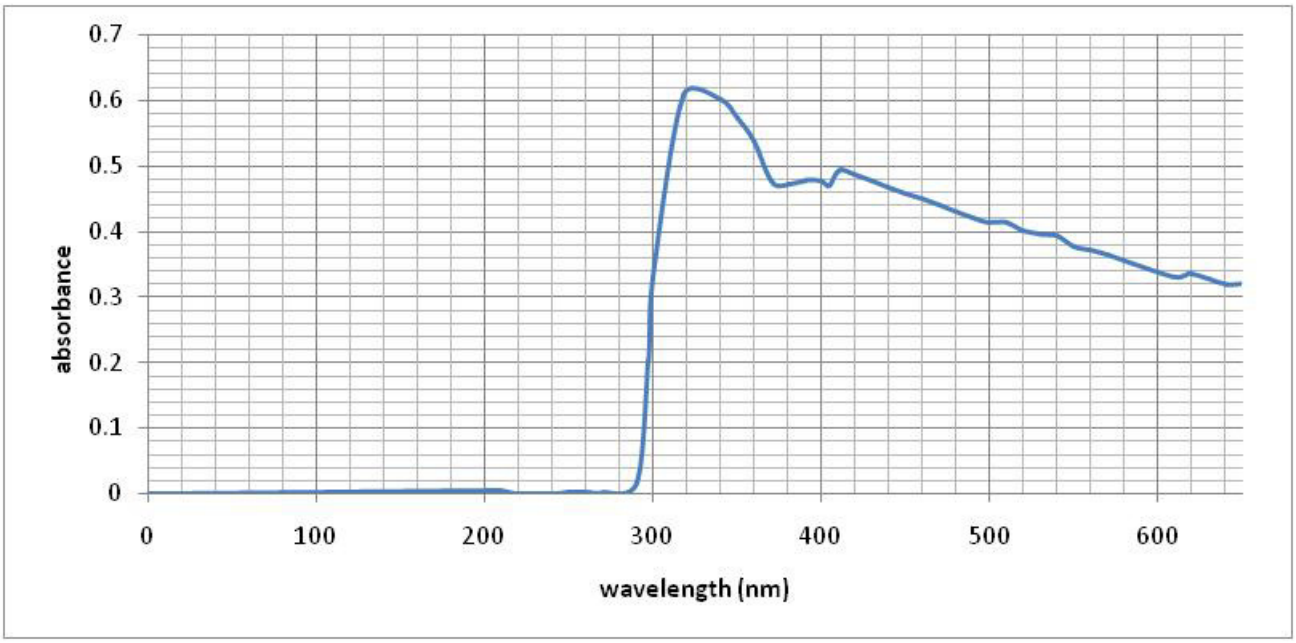

Figure 6. *Result of UV-VIS spectrophotometric analysis of the stock solution of artesunate*

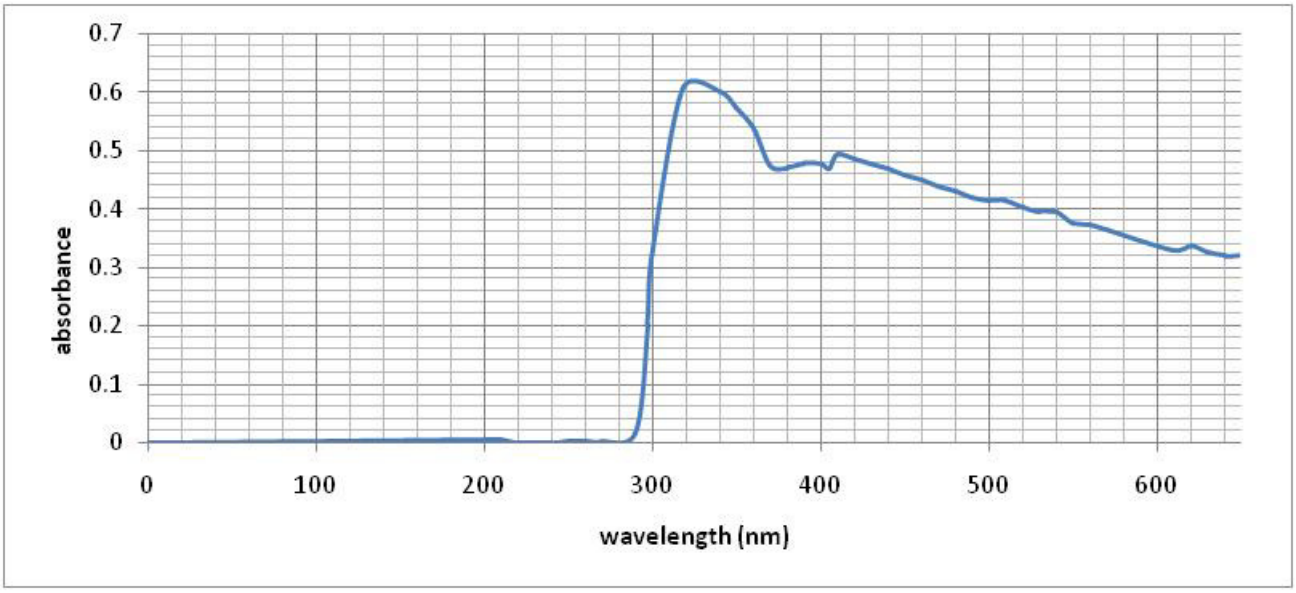

Figure 7. *Result of UV-VIS spectrophotometric analysis of the stock solution of dihydroartemisinin*

Table 5. *Linearity and Sensitivity Data*

Parameters	Result	
	Artesunate	**Dihydroartemisinin**
λmax (nm)	318	312
Beer's Law Linearity Range (mg/ml)	0.1-0.8	0.1-0.8
Regression Equation	y= 0.7134x- 0.0132	y= 0.4689x +0.0104
Intercept (X)	0.013	0.010
Slope (B)	0.713	0.458
Correlation Coefficient (R)	0.998	0.998
Limit of Detection	0.03580mg/mL	0.004094mg/mL
Limit of Quantification	0.108485mg/mL	0.012405mg/mL

Table 6. *Result of UV-VIS spectrophotometric absorbances obtained from saliva samples after administration of 100mg artesunate (2 tablets of artesunate®) collected at 30 minutes intervals for 4 hours at 318nm*

S/N	Time (MINS)	Subject A		Subject B	
		Absorbance	**Concentration (µg/mL)**	**Absorbance**	**Concentration (µg/mL)**
1	30	-0.713	---	-0.998	---
2	60	-1.076	---	-0.134	---
3	90	-1.020	---	-0.589	---
4	120	-0.333	---	-0.298	---
5	150	-0.103	---	-0.408	---
6	180	-1.093	---	-0.787	---
7	210	-0.707	---	-1.339	---
8	240	-0.219	---	-1.107	---

Table 7. *Result of UV-VIS spectrophotometric absorbances obtained from saliva samples after administration of 120mg dihydroartemisinin (2 tablets of CODISIN®) collected at 30 minutes intervals for 4 hours at 312nm*

S/N	Time (MINS)	Subject C		Subject D	
		Absorbance	Concentration (µg/mL)	Absorbance	Concentration (µg/mL)
1	30	0.130	262.0	0.297	627.0
2	60	-0.606	---	0.054	96.0
3	90	-0.340	---	0.025	3.03
4	120	0.258	541.0	0.331	701.0
5	150	-0.812	---	-0.442	---
6	180	0.105	207.0	0.805	---
7	210	-0.820	---	-0.843	---
8	240	-0.163		-0.634	---

Table 8. *Result of UV-VIS spectrophotometric absorbances obtained from saliva samples after administration of 100mg artesunate (2 tablets of artesunate®) collected at 30 minutes intervals for 4 hours at 312nm*

S/N	Subject E		
	Time (MINS)	Absorbance	Concentration (µg/mL)
1	30	0.714	358.0
2	60	0.025	33.0
3	90	0.103	203.0
4	120	0.268	563.0
5	150	0.052	1838.0
6	180	0.869	1876.0
7	210	0.538	1153.0
8	240	0.863	1862.0

samples in ethanol or methanol before adding the physiological fluid or buffer in line with the Cayman catalogue for artesunate (Cayman, 2014).

The benzene diazonium chloride was prepared freshly

according to the procedure detailed in Maas (2001). It was coupled with the stock solutions of artesunate and dihydroartemisinin, both in an alkaline medium (40% NaOH) and both produced orange-coloured solutions instantly. A colour change from colourless to orange was observed signifying the coupling of the benzene diazonium chloride by the electron-rich stock solutions of artesunate and dihydroartemisinin in an alkaline medium, respectively. This coupling of benzene diazonium chloride is through its azo end to the "C_1" of the artemisinins. While variamine blue and safranin O are the most chromogenic agents recommended in the literature (USP, 2014.), benzene diazonium chloride was used based on the chemistry of the reacting media, principle of the method, and its availability in the country. This reagent is easily produced and is an affordable, and readily available chromogenic agent as the findings of Etim *et al*. (2016).

Accordingly, the first UV-VIS spectrophotometric analysis of this work involved the development of the UV-VIS spectra of the absorbance of 1mg/mL solution of artesunate and dihydroartemisinin in methanol against different wavelengths which gave maximum wavelength of absorption (λmax) at 305nm and 304nm for artesunate and dihydroartemisinin respectively (Table 1). This was done to observe the spectral behaviour of the compounds in the dissolving solvent (methanol), and it was found out that methanol did not interfere with the combination of the drugs with saliva nor its absorbance in other spectrophotometric procedures developed. Following the coupling of the stock solutions of artesunate and dihydroartemisinin to a chromogenic agent (benzene diazonium chloride) quantitative analysis using UV-VIS spectrophotometry provided the UV-VIS spectra of the coupled stock solutions against different wavelengths which gave maximum wavelength of absorbance (λmax) at 481nm and 469nm for artesunate and dihydroartemisinin respectively. The absorbances of artesunate and dihydroartemisinin obeyed Beer-Lambert Law, which implies that the unknown concentrations of artesunate and dihydroartemisinin in saliva samples can be extrapolated from the calibration graphs of the coupled (with benzene diazonium chloride) stock solutions of artesunate and dihydroartemisinin (in an alkaline medium) respectively.

The method was validated according to The Inter-

national Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Useguidelines (ICH, 2014). And the results of the experiments on both drugs yielded a calibration curve with correlation coefficients (R^2) of 0.998 and 0.999 for artesunate and dihydroartemisinin, respectively (Table 3). These values are within the acceptable limit set by the ICH (0.95-0.999) (ICH, 2014). Statistically, the errors in the concentration of the coupled artesunate and dihydroartemisinin solutions were obtained from their standard deviations which were 0.000588 and 0.011939, respectively (Table 3). When solutions of artesunate and dihydroartemisinin, coupled with benzene diazonium chloride, with known concentrations of 0.3mg/ml each was used to test for the accuracy of the calibration graphs, as seen in Table 3, it gave an average absorbance of 0.412 and 0.549 for artesunate and dihydroartemisinin, respectively, whereas, the absorbance interpolated from the graph gave an absorbance of 0.413 and 0.551 for artesunate and dihydroartemisinin, respectively. This implies that both graphs are suitable reference standards in the qualitative analysis of artesunate and dihydroartemisinin in saliva. The proposed method exhibited good limits of detection (LOD) and quantitation (LOQ) with values of 0.04923 mg/mL (49.23µg/mL), respectively, for artesunate. While the limit of detection (LOD) and quantitation (LOQ) for dihydroartemisinin was also good with values of 0.04441mg/mL (44.41µg/mL) and 0.134563mg/mL (134.563µg/mL), respectively (Table 3). These data are comparable to earlier results obtained from other costlier and relatively unavailable procedures used by Birgersson and others (Birgersson *et al*., 2014).

Following the qualitative analysis of the stock solutions of artesunate and dihydroartemisinin using UV-VIS spectrophotometry, the UV-VIS spectra were developed for each solution against different wavelengths which gave maximum wavelength of absorbance (λmax) at 318nm and 312nm for artesunate and Dihydroartemisinin, respectively.

UV-VIS spectrophotometric analysis of different concentrations (mg/mL) of artesunate and dihydroartemisinin stock solutions at a constant wavelength of 318nm and 312nm, respectively, showed that the absorbance increased as their concentrations increased, thus, obeying Beer-Lambert Law. This implies that the unknown concentrations of artesunate and dihydroartemisinin in saliva samples can be extrapolated from the calibration graphs of the stock solution of artesunate and dihydroartemisinin, respectively. The results of the experiments on both drugs yielded a calibration curve with correlation coefficients (R^2) of 0.998 and 0.998 for artesunate and Dihydroartemisinin, (Table 5). These values are within the acceptable limit set by the ICH (0.95-0.999). Statistically, the errors in the concentration of artesunate and dihydroartemisinin solutions were obtained from their standard deviations, which are 0.008178 and 0.0000940, respectively. When saliva samples containing known concentrations (0.3mg/mL) of artesunate and dihydroartemisinin were used to test for the accuracy of the calibration graph, it gave an average absorbance of 0.231 and 0.147 for artesunate and dihydroartemisinin, respectively, whereas, the absorbance interpolated from the graphs gave an absorbance of 0.227 and 0.147 for artesunate and dihydroartemisinin, respectively. Thus, both graphs served as reference standards in the qualitative and quantitative analysis of artesunate and Dihydro-artemisinin in saliva samples respectively; the proposed method exhibited good limits of detection (LOD) and quantitation (LOQ) with values of 0.03580mg/mL (35.8µg/mL) and 0.108485mg/mL (108.485µg/mL), respectively for artesunate. The limit of detection (LOD) and quantitation (LOQ) for dihydroartemisinin were also good with values of 0.004094mg/mL (4.094µg/mL) and 0.012405mg/mL (12.405µg/mL), respectively (Table 5). These data are comparable to earlier results obtained by the method of Birgersson and others (Birgersson *et al*., 2014).

Regarding the pharmacokinetic considerations**,** no detectable concentrations of artesunate could be quantified from the saliva samples obtained from subjects' “A” and “B” at any particular time interval as indicated by the negative absorbances (Table 7). This is because the parent compound (artesunate) has a very short half-life (<10 minutes) and must have been metabolized (hydrolysed) to dihydroartemisinin, which is also responsible for the antimalaria activity of the drug. The metabolite (dihydroartemisinin) of artesunate was confirmed in saliva samples from subject “E” by analysing spectrophoto-metrically at wavelength 312nm (λmax used in the analysis of dihydroartemisinin) in contrast to 318nm which was the λmax used in the analysis of artesunate (Table 8). The metabolite was seen to exhibit somewhat time-dependent kinetics. The concentrations were seen to increase with time and stabilize relatively after two hours. Concentrations of dihydroartemisinin in saliva samples obtained from subjects' “C” and “D” were seen to relatively decrease as the time increased. The pharmacokinetics in the different subjects were variable, although the drug could not be detected after 2-3 hours, showing its clearance from the saliva (Table 7).

High saliva concentrations were detected in samples taken at 30 minutes from the subjects, most probably due to residual concentrations of the drug in the mouth after intake of the tablets. From studies carried out by Gordi (2000), salivary concentrations of the artemisinins serve as an alternative body fluid for pharmacokinetic studies of the artemisinin compounds and it correlates with the concentrations in the unbound plasma fractions; the overall absolute differences in artemisinin concentrations in saliva and unbound venous and capillary plasma were

negligible and non-significant (Gordi, 2000).

CONCLUSION

The use of saliva served as a cost-effective analytical procedure for the quantification of artesunate and dihydroartemisinin. The results obtained from this research shows that both artesunate and dihydro-artemisinin concentrations can be quantified from saliva samples using a very simple, yet accurate and rapid method such as UV-VIS spectrophotometry, either by analysing the saliva samples directly or by coupling with benzene diazonium chloride; although, analysing the sample directly is preferred and more accurate.

RECOMMENDATIONS

i. Other derivatives of artemisinin and other drugs known to shunt into the saliva can be analysed using the method proposed in this study.
ii. Analysing saliva samples by the method proposed in this study can be used to replace venepuncture sampling in the pharmacokinetic assay and therapeutic drug monitoring of artesunate and dihydroartemisinin.
iii. The results obtained from this research can be used to study the pharmacokinetic behaviour of new drug derivatives from the artemisinin group of compounds.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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