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Research Articles

Evidence for the spectroscopic determination of Artesunate in dosage form

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Abstract

Background & objectives: Resistance to conventional antimalarials triggered off new policies to circumvent the devastating consequences of malaria especially in the trans-Saharan Africa. The use of artemisinin-based combinations as first line drug in treatment of uncomplicated malaria was then advocated and adopted by the World Health Organization (WHO). In Nigeria, this new policy has witnessed a surge in the number of circulating brands of such combinations. Unfortunately, at present, there are no "on-the-spot" cheap and reliable assay procedures for artesunate-based combinations. This is what the present research aims to achieve.

Methods: Ultraviolet absorption spectroscopy was used to establish the wavelength of maximum absorbance for pure powder of artesunate and then the Beer's plot generated. This was validated and used to assay nine brands (X1–X9) of artesunate in Nigerian drug market.

Results: Distinctive ultraviolet absorption at 287 nm of pure sample of Artesunate in simulated intestinal fluid (SIF) afforded a simple, precise and the most reliable method for the analysis of nine different brands of Artesunate marketed in Nigeria. SIF does not have any appreciable absorption in the ultraviolet region. This simple method yielded a Beer's plot for Artesunate with high correlation (R^2) of 0.9972± 0.00016 and was reproducible. The Beer's plot was obeyed in concentration range of 10–200 mg%. The limits of detection (sensitivity) and quantitation were found to be 0.471 mg/ml and 1.27 mg/ml respectively. The results showed that only four out of the nine brands assayed had deviations from label claims that were within acceptable limits.

Interpretation & conclusion: Based on these convincing data, simple ultraviolet spectroscopy at 287 nm could be used to assay artesunate in formulations.

Key words Artesunate – determination – dosage form – evidence – spectrophotometric – tablet

Introduction

In recent times, emergence of resistant *Plasmodium* sp to many of the cheap and readily available antimalarials has resulted in the continued use and dependence of artemisinins and its based combination[1,2]. This situation is more pronounced in the tropics where the incidence of malaria remains a serious burden with high infant and maternal mortality[3,4]. Moreover, the World Health Organization (WHO)

has warned that the artemisinins must be jealously guarded through combination with other known antimalarials of diverse classes[5]. Following the WHO's adoption of the new malaria policy, advocating the use of artemisinin-combination therapy, many drug manufacturing companies have embarked on the production of artemisinin-based combination regimens, a situation that led to proliferation of diverse brands in the market. In Nigeria, there are more than 10 brands of this drug currently being marketed. These diverse brands have varying prices, most times wide enough to suggest compromise of standard. The suspicion grows thicker in Africa where corrupt and sharp practices are prevalent. Besides, it places the Pharmacists and other clinicians in a difficult situation about choice of a suitable brand or interchangeability amongst brands to make[6].

At present, few analytical procedures exist for the analysis of artemisinins and derivates. These are the indirect colorimetric assay (ICA)[7] and the high performance liquid chromatography (HPLC)[8]. Unfortunately, these methods have their demerits which greatly impair their functionality especially in sub-Saharan Africa. It is either the problem of non-availability of specific reagents (like the Fast TR red salt used in the indirect colorimetric assay) and equipment needed for the assay procedure or that of excessive cost of the method. It is, therefore, necessary to develop other reliable and reproducible methods for the analysis of artesunate and its derivatives. This will checkmate the influx of fake and adulterated products that will aggravate the incidence and burden of malaria in the tropics.

Recently, we reported the *in vitro* bioequivalence studies of these nine brands of artesunate. Results obtained in the study show that there were significant differences in the dissolution profiles of the brands, with one brand performing abysmally poor[6]. In this work, spectrophotometric quantification of artesunate, a derivative of artemisinin is described. Artesunate is a semi-synthetic derivative of artemisinin, a naturally occurring sesquiterpene endoperoxide. It is indicated chemically as 3R,5aS,6R,8aS,9R,-10S,-12R,12aR)-decahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin-10-ol hydrogen succinate (Fig. 1). The chemical formula is $C_{19}H_{28}O_8$ with molecular weight of 384.42 (Fig. 1). It is a white crystalline powder with melting point range of 132–135°C and slightly soluble in water[9]. Artesunate is an antimalaria agent and a hemi-succinate derivative of dihydroartemisinin. It is activated *in vivo* by hydrolysis, to dihydroartemisinin, the active metabolite of the drug. Artemisinin is a sesquiterpene lactone isolated from *Artemisia annua,* a herb that has been used traditionally in China for many years in the treatment of malaria[10–12].

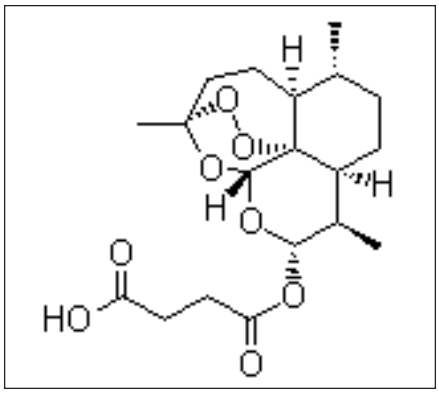

Fig. 1: Chemical structure of artesunate [Chemical name: 3R,5aS,6R,8aS,9R,10S,12R,12aR)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin-10-ol hydrogen succinate; Molecular formula: $C_{19}H_{28}O_8$; Molecular weight: 384.42]

Material & Methods

The following drug materials were procured; pure artesunate powder (Shanghai Sinoki International Trading Co. Ltd., China), nine different brands of artesunate coded as: X1–X9. The brands under study were selected based on frequency of prescription, use and availability in tertiary hospitals and pharmacies located in different regions of Nigeria. Other materials include sodium hydroxide (May & Baker, England), monobasic potassium phosphate (Sigma

Aldrich, Germany). Distilled water used was freshly prepared in Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria. The equipments used are: Electronic balance (Metler Toledo, P31- Min 0.01 g) and UV-visible spectrophotometer (Model UNICO 2100).

Preparation of simulated intestinal fluid (SIF): Exactly 40 g of sodium hydroxide was dissolved in 2 L of distilled water and to this was added 34 g of monobasic potassium phosphate and shaken for complete homogenization. The solution was made up to 5 L with distilled water.

Preparation of standard solution of Artesunate: One hundred milligrams (100 mg) of pure artesunate powder was accurately weighed and dissolved in 50 ml of SIF. Then, 10, 20, 40, 60, 80, 100, 120, 140, 160 and 200 mg% were prepared accordingly from the stock. Exactly 5 ml of the 10 mg% solution was scanned with the UV-Visible spectrophotometer to establish the wavelength of maximum absorption (λmax). Then 5 ml of the different dilutions were collected separately and their triplicate absorbances recorded at the established λmax. From the results obtained, Beer's plot was generated and the limits of detection and quantitation established using the relationship; Limit of detection (sensitivity) in mg/ml = 10Q/S and limit of quantitation in mg/ml = 3Q/S where, Q is the standard deviation computed from the intercepts on absorbance axis and S is the slope of the individual graphs.

Absolute drug content of the different brands: Five tablets of each brand were weighed and mean weight calculated. They were crushed and three different mean weights were weighed out each into a 100 ml volumetric flask containing about 50 ml of SIF. The flasks were agitated for not less than 30 mins to achieve complete dissolution. The solution was filtered into new clean dry 100 ml volumetric flasks and made up to volume with fresh SIF. All the solutions were scanned to check for possible interaction of other constituents present in the tablets. An appropriate dilution was made and then the absorbances obtained at the λmax from where drug concentrations were calculated. The same process was repeated five times and average value recorded. The limits of quantitation and detection of the proposed assay method were computed as described above.

Results

The features of the nine brands of artesunate used in this study are depicted in Table 1. It is worthy of note that none of the brands was expired at the time of

Table 1. Features of the selected brands of artesunate in Nigeria drug market

S.No.	Brand code-strength	Manufacturing date	Expiry date	Batch No.	NAFDAC No.	Country of origin
1	X1-50 mg	10/2005	09/2008	MP-004	04-4213	India
2	X2-100 mg	10/2005	09/2008	90-05	04-3723	India
3	X3-100 mg	03/2004	03/2007	00-808	04-3127	Belgium
4	X4-50 mg	10/2004	10/2007	0170904	04-3394	Vietnam
5	X5-50 mg	02/2006	01/2008	K60123	04-6964	India
6	X6-100 mg	01/2006	10/2009	26001	04-3496	Nigeria
7	X7-50 mg	11/2005	10/2008	191105	04-6031	China
8	X8-200 mg	02/2006	01/2008	AB-247	04-3727	Switzerland
9	X9-50 mg	05/2006	04/2008	113-010	04-6397	India

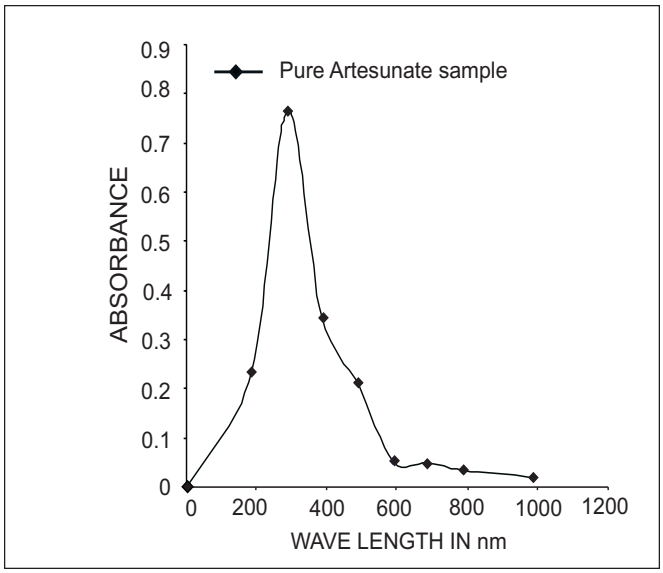

Fig. 2: UV/Visible scan of Artesunate in simulated intestinal fluid (SIF)

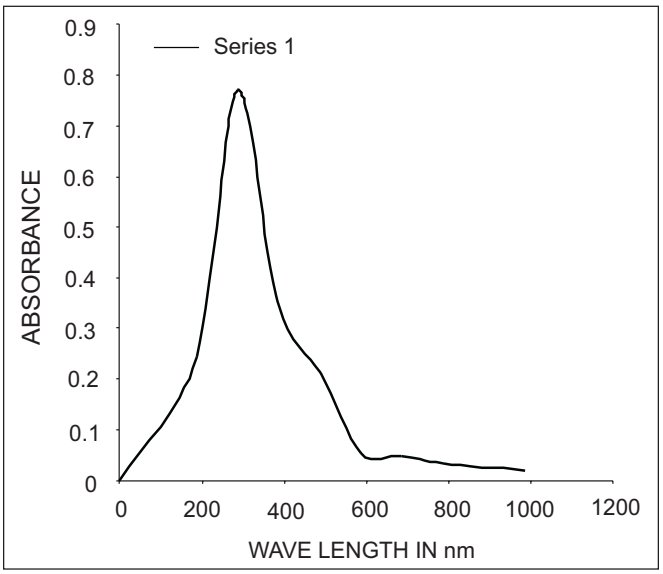

Fig. 3: Absorbance spectrum of market brands of Artesunate in SIF

study. Obviously, most of them had more than six months before their labeled expiry dates. The Table also shows that most of the artesunate used in Nigeria are claimed to have come in from India and are registered by the National Agency for Food Drug Administration and Control (NAFDAC), an agency charged with the control of food and drugs in Nigeria. The absorbance maxima (λmax) was found to be 287 nm (Fig. 2) and was reproducible. The recovery experiment following the established Beer-Lambert's plot afforded the absolute drug contents for the different brands as shown in Table 2. The proposed method exhibited good levels of detection and quantitation with values of 0.471 and 1.27 mg/ml respectively. These data are comparable to earlier results from other costlier and relatively unavailable procedure[7,8]. In summary, these findings show that artesunate is UV-active and could thus be analyzed by this method. The other tablet excipients usually present during compounding are not likely to inter-

Table 2. Absolute drug contents of the brands

S.No.	Brand code-strength	Absorbance	Amount found (mg)	Deviation (mg)	Deviation (%)
1	X1–50 mg	1.32	92.8 ± 3.56	42.8	85.6
2	X2–100 mg	1.87	131 ± 11.33	31	31
3	X3–100 mg	1.44	101 ± 7.31	1	1
4	X4–50 mg	2.71	190 ± 10.12	140	280
5	X5–50 mg	0.68	47.7 ± 1.51	2.3	4.6
6	X6–100 mg	1.92	135 ± 9.53	35	35
7	X7–50 mg	1.12	78.7 ± 5.72	28.7	57.4
8	X8–200 mg	2.69	189 ± 13.75	11	5.5
9	X9–50 mg	0.78	55.1 ± 2.13	5.1	10.2

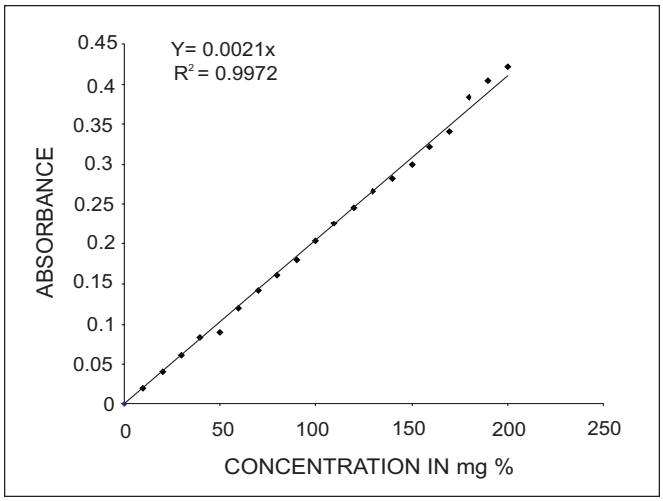

Fig. 4: Beer's plot of Artesunate in SIF at 287 nm

fere with the absorption spectrum of artesunate (Fig. 3). This was evidenced by uniform and reproducible UV-absorption spectrum for both pure drug and that in dosage form. The regression equation for the Beer-Lambert's plot of pure artesunate was found to be; Y=0.0021X and the correlation coefficient (R^2) of 0.9972. The Beer's plot was obeyed in concentration range of 10–200 mg% (Fig. 4). There is good correlation between absorbance and concentration, which is the basis of this method of analysis.

Discussion

The development of reliable and affordable procedures for the assay of drug substances either as pure drug or in combination remains a major research area in today's pharmaceutical care and practice. In situation where a new drug product is introduced into the market under different brand names and most times with wide discrepancies in cost, a suspicion of impairment of integrity or wholesomeness of the product is justifiable especially in our local environment. The selection of the brands used in this study was done in such a manner as to represent a good geographical spread of the different drug markets in Nigeria. Out of the nine brands of artesunate assayed by this proposed procedure, five brands namely; X1, X2, X4, X6 and X7 showed a very wide and unacceptable quantity of active contents against the label claims—X1: 92.8 ± 3.56 mg versus label claim of 50 mg; X2: 131 ± 11.33 versus 100 mg label claim; X4: 190 ± 10.12 versus 50 mg label claim; X6: 135 ± 9.53 versus 100 mg label claim; and X7: 78.7 ± 5.72 versus 50 mg label claim (Table 1). This aberration represents approximately, 45.6% of the total brands surveyed, a situation that evokes concern in the Health care system in Nigeria, Asia and other parts of sub-Saharan Africa. While it may be difficult at this time, to associate this gross formulation aberration to any definite reason, it is certain that the observed wide deviation could be attributed to poor production processes. It appears that some manufacturers of artesunate do not consider quality of their products. The excess weight of active ingredients could possibly precipitate overdosing or poisoning. Consequently, companies and their products should be closely monitored to ensure that every product reaching to the final consumer is of optimal quality. Here the pharmacists and doctors can play an important role. Surprisingly, the entire nine brands assayed were bearing NAFDAC registration numbers (as could be seen in Table 1), a seal that indicates an approval of a substance for use in Nigeria. However, from experiences so far and supported by the present data, the presence of registration number on a product in Nigeria may not serve as a full guarantee on its overall quality until its quality assurance is done. There is the need to rule out fakery of such numbers and this is one of the purposes of this research. Interestingly, in terms of absolute drug content, only X3, X5, X8 and X9 are considered acceptable with respect to the deviation from claimed active ingredient weigh in the tablet.

We have recently reported an *In vitro* bioequivalence study of these nine brands and data show that there were variations in dissolution, disintegration time and other parameters including *in vivo* bioavailabilty[6]. The existence of a single distinctive ultraviolet absorption band for artesunate at 287 nm in SIF is an evidence for its assay by this method. Moreover, solutions prepared from the tablet brands exhibited

similar absorption spectrum, an indication that the tablet excipients and manufacturing methods did not affect its absorption. The generated detection and quantification limits are an indication that this proposed method is sensitive and consequently, only small quantity of artesunate is needed for assay. Our results do not seem to agree with previous authors who claimed that artesunate cannot easily be analyzed by ultraviolet spectrophotometry. Unfortunately, the solvent environments used by these authors were not indicated since we are aware that very high pH environments decompose artesunte; which is the basis of the indirect colorimetric assay developed by the previous workers[7]. In this work, it is worthy of note that the pH of SIF (pH, 6.8) is fairly acidic and would protect the basic chemical nucleus of artesunate without breaking the lactone ring (Fig. 1). Strongly basic medium decomposes artesunate and this procedure has been suggested as a means of indirect assay for artesunate. Finally, this simple experiment could be used to assay artesunate in pure form or in dosage form especially in the developing countries where sophisticated equipments are lacking.

Conclusion

The development of simple and cost-effective analytical protocols for artemisinin and its derivatives in both pure form and in dosage form has become very important in the face of increasing supply and demand for this antimalarial agent. The results obtained from the present study show that UV absorption of artesunate could be employed for the assay of the drug especially in poorly equipped laboratories like those found in most developing countries. The proposed method is sensitive and reproducible.

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