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ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND DETERMINATION OF ARTESUNATE IN PHARMACEUTICAL TABLET FORMULATIONS

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ABSTRACT

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). Artesunate (ARTS) is the most widely used of the artemisinin derivatives. It is an antimalarial agent. It is a water-soluble hemisuccinate derivative of artemisinin. In Sudan, artesunate tablet is the commonest artemisinin product in the market and is available in various strengths from both local and foreign manufacturers. The quality of these antimalarials if not properly safeguarded could lead to therapeutic failure in patients and the development of drug resistance. A simple and sensitive spectrophotometric method is developed for the determination of Artesunate (ARTS) in tablet formulations. Due to difficulty to detect and identify Artesunate by standard spectrophotometric methods, since it absorbs light only at low wavelengths, has a relatively low molar extinction coefficient, and has no distinct UV-Visible spectrum or fluorescent properties. In order to assay ART by developed UV method, it is necessary to involve it in a reaction process that would break the endo-peroxide ring and introduce a least one double bond in the molecule and this can be achieved by reacting it with sodium hydroxide for generation of chromophore needed for UV Spectroscopy. 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 used to evaluate the quality of four brands (X1-X4) of artesunate in Sudan drug market, The accuracy and validity of the method was validated, and the results evaluated by performing recovery studies via standard addition method. Artesunate was determined spectrophotometrically at λmax 289. Beer's law was obeyed in the range of 0.02-0.1mg/ml, with regression coefficient of 0.999. The limit of detection (LOD) and limit of quantification (LOQ) determined as per the current ICH guidelines were found to be 0.01mg/ml and 0.02mg/ml respectively. The accuracy and precision was expressed. The validated proposed method was used to assay ARTS in commercial tablets brands and the results showed good congruence with the reference methods (titration). The accuracy and validity of the proposed method was evaluated by performing recovery studies via standard addition method, the results showed excellent recoveries with no appreciable interference from excipients.

KEYWORDS: *Artesunate, determination, dihydroartemisinin, dosage form, spectrophotometric.*

INTRODUCTION

The quality of commercially available drugs varies greatly among countries. Due to lack of regulations and poor quality control practices in some countries, the amount of the active ingredient can be inconsistent. Poor formulation techniques can affect the release of active ingredients from a tablet, with some tablets releasing very little if any drug. Malaria, over the decades, is still one of the most severe infectious diseases globally which is widespread mainly in the tropical and subtropical regions. It kills more people each year than any other infectious diseases except AIDS and tuberculosis. Although it is difficult to obtain an exact figure of the malaria cases, the World Health Organization (WHO) estimates that malaria is responsible for over 300 million clinical cases and over one million deaths annually. About 40% of the global population is estimated to be at risk. The disease is caused by single-celled protozoan parasites of the genus *Plasmodium*. Four species infect humans by entering the bloodstream. The most serious forms of the disease are caused by *Plasmodium falciparum* and *Plasmodium vivax*, and the other related species are *Plasmodium ovale* and *Plasmodium malariae*. These groups of human-pathogenic

Plasmodium species are usually referred to as malaria parasites. Malaria parasites are transmitted by female anopheles mosquitoes. The parasites multiply within red blood cells, causing symptoms similar to regular influenza that include headache, fever, anemia, chills, flu-like illness, and in severe cases, coma (cerebral malaria) and death. Malaria has been treated, over the years, with numerous drugs. Cheap and effective drugs such as chloroquine (CQ) and sulphadoxine/ pyrimethamine (SP) was the antimalarial drug of choice for many years in most parts of the world. However, resistance of *Plasmodium falciparum* and *Plasmodium vivax* to CQ and SP has spread recently in the endemic areas, making the drug ineffective against the most dangerous Plasmodium strains in many affected regions of the world. The emergence and rapid spread of *P. falciparum* resistance to commonly used antimalarial drugs poses a serious challenge to the effectiveness of early diagnosis and prompt treatment as a priority strategy within current malaria control efforts.[1,2] Due to the high resistance of P. falciparum, there has been the urgent need for drug combination therapy. 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.[3,4] This situation is more pronounced in the tropics where the incidence of malaria remains a serious burden with high infant and maternal mortality.[5,6] Moreover, the World Health Organization (WHO) has warned that the artemisinins must be jealously guarded through combination with other known antimalarials of diverse classes.[7] Following the WHO's adoption of the new malaria policy, advocating the use of artemisinin-combination therapy (ACT), 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. Artemisinin is obtained from the extracts of the plant *Artemisia annua*, with several derivatives; dihydroartemisinin, its methyl ether (artemether), its ethyl ether (arteether) and its hemisuccinate ester (artesunate) are known as more effective than its parent material artemisinin. These are rapidly gaining grounds as antimalarials that are used for the treatment of severe and uncomplicated multidrug-resistance *falciparum* malaria. Since 2001 the World Health Organization has recommended using artemisinin-based combination therapy (ACT) as first-line treatment for uncomplicated malaria in areas experiencing resistance to older medications. Artesunate (ARTS) is the most widely used of the artemisinin derivatives.[8] Artesunate (figure 2) is an antimalarial agent. It is a water-soluble hemisuccinate derivative of artemisinin.

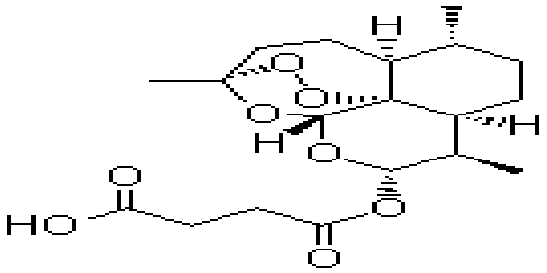

Figure 1: Artesunate chemical structure.

Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides, active against the ring stage of the parasite. Artesunate (ARTS) is powerful especially in the treatment of advanced and potentially lethal cases of *P. falciparum* infection. It is a sesquiterpene with an unusual endoperoxide linkage structurally unrelated to other known antimalarials. Artesunate is ideal for the treatment of severe malaria, including cerebral malaria. It is also active against chloroquine and mefloquine resistant strains of *P. falciparum*. ARS was developed as a pro-drug for the treatment of both uncomplicated and severe P. *falciparum* malaria. It is available in both enteral and parenteral formulations. It is more potent than artemisinin and is active by virtue of the endoperoxide. Their activity against strains of the parasite that had become resistant to conventional chloroquine therapy and the ability due to its lipophilic structure, to cross the blood brain barrier, it was particularly effective for the deadly cerebral malaria. Whether employed as mono-therapy or as a component of ACT, artesunate plays a pivotal role in the global fight against malaria, hence there is the need to guarantee its quality and eliminate any form of fakery or counterfeiting. Already there have been several reports cataloguing the distribution and use of counterfeit and substandard artesunate tablets in many South-East Asian countries.[9-14] At present, few analytical procedures exist for the analysis of artemisinins and it is derivatives. These are the indirect colorimetric assay (ICA)[15] and the high per- formance liquid chromatography (HPLC).[16] Unfortunately, these methods have their demerits which greatly impair their functionality especially in sub Saharan Africa. It is either the problem of non-avail- ability 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. In Sudan, artesunate tablet is the commonest artemisinin product in the market and is available in various strengths from both local and foreign manufacturers. The quality of these antimalarials if not properly safeguarded could lead to therapeutic failure in patients and the development of drug resistance.

Making attempts in this study to develop a rapid, sensitive, selective, accurate and cost- effective method. The proposed method is the objective of the present

investigation was to determine and evaluate the quality of artesunate tablets and validated against existing pharmacopoeial methods and standards.

MATERIALS AND REAGENTS

The following drug materials were procured; pure artesunate powder (Shanghai Pharmaceutical Co. Ltd., Sudan), four different brands of artesunate coded as: X1–X4. The brands under study were selected based on frequency of prescription; other materials include ethanol, sodium hydroxide, Potassium biphathalate, phenolphthalein/ethanol indicator, Distilled water.

Apparatus

Water SG Ultra Purification System (UK).
Ultra Sonicator (China).
I.R Spectrophotometer (Shimazdu) Japan.
Glassware (Isolable) Germany.
Double Beam UV Spectrophotometer, (T80) PGT80, England.
Sensitive Balance (Kern) Germany.
IR moisture balance, (Kern) Germany.
Centrifuge (Braun) UK.
Dissolution tester, Model D-6534, China.
Disintegration Tester, British Model DIST1.

Sample Collection

Different brands of artesunate studied were selected based on frequency of prescription, use and availability in hospital and community pharmacy shelves. Drugs were obtained from pharmacies located in four different major towns in Sudan. The towns were selected to ensure adequate geographical spread. All the brands used were registered and, all the artesunate tablet brands sampled had a remaining shelf life of at least one year at the time of sampling.

Table 1: Feature of selected brands of Artesunate in Sudan drug market.

Brand Code-strength	Country of origin
X1-100mg	Sudan
X2-100mg	Sudan
X3-100mg	China
X4-50mg	China

Methods of Analysis

Compendial Method

0.25g of Artesunate was accurately weighed and dissolved in 25 ml of neutralized ethanol and titrated with 0.05M sodium hydroxide using 2 drops of phenolphthalein/ethanol as indicator.

Each mL of sodium hydroxide (0.05M) is equivalent to 19.22mg of $C_{19}H_{28}O_8$ (artesunate).

Developed and Non-Compendial Method

Preparation of standard solution of Artesunate

The standard stock solution was prepared by dissolving one hundred milligrams (100 mg) of pure artesunate powder in 30ml purified water and Sonicated up to 30 minutes for completion the dissolution, filtrate, add 10ml 1M NaoH then complete volume up to 100ml with purified water to make final concentration of 1mg/ml(100mg/100 ml). Different aliquots were taken from stock solution and diluted to prepare series concentrations from 0.02 - 0.1mg/ml (Calibration range). Warm the standard solutions to 50 ± 1 ºC for 45 min cool to room temperature immediately. Absorbance was measured at 289 nm against blank. The calibration curve was prepared by plotting absorbance versus concentration of Artesunate.

Absolute drug content of the different brands

Twenty tablets of each brand were weighed and mean weight calculated. They were crushed and weights equivalent to labeled concentration of each brand was weighed out into a 100 ml volumetric flask containing about 50 ml of purified water. The flasks were agitated to achieve complete dissolution. Then 10ml sodium hydroxide 1M was added and complete the volume .The solution was filtered into new clean dry 100 ml volumetric flasks. All the solutions were scanned to check for possible interaction of other constituents present in the tablets. An appropriate dilution was made and the working solution of drug (0.05mg) was prepared from standard stock solution. The absorbance of working solution was measured and amount of Artesunate was calculated from the calibration curve. The same process was repeated three times and average value recorded.

RESULTS

Assay of Artesunate

Compendial method of assay

Table 2: Titration data for the assay of artesunate brands.

Sample	Weight (gm)
X1	0.795
X2	0.690
X3	0.718
X4	0.695

Table 3: Burette reading for titration.

Sample	1st Determination	2nd Determination	3rd Determination
X1	5.2ml	5.3ml	5.5ml
X2	5.9ml	6ml	6.2ml
X3	6.7ml	6.6ml	6.8ml
X4	3.1ml	3ml	3.2ml

Calculation of percentage purity

Content purity of drug was calculated using the correlation of each mL of 0.05M sodium hydroxide reacted being equivalent to 19.22mg of C18H28O8 (artesunate). The percentage purity calculated from actual and experimental weights of artesunate.

Table 4: The content% of Artesunate in different brands of Artesunate tablets sampled (mean of 3 measurements).

Sample	Content purity % (average)	Content uniformity requirements Ph. nt.
X1	%98.18	Pass
X2	%98.05	Pass
X3	%107.18	Pass
X4	%101.36	Pass

Table 5: Results of samples assay in Compendial (titration) method.

Sample	Labeled amount (mg)	Found*	% RSD
X1	100	98.18 ± 2.76	2.81
X2	100	98.05 ± 1.65	1.70
X3	100	107.18± 1.02	0.95
X4	50	50.68 ± 2.27	2.24

* Mean value ± standard deviation of three determinations.
RSD; relative standard deviation.

Proposed Spectrophotometric method

Simple and rapid spectrophotometric method for the determination of Artesunate is described. The method is based on the reaction of Artesunate with a mixture of 1M sodium hydroxide and water.

The absorbance maxima (λmax) were found to be 289 nm and were reproducible and the absorbance of standard Artesunate shown in Table below.

Table 6: Absorbance data of standard Artesunate.

STD Artesunate Concentration 1mg/ml	Concentration	Absorbance at 289 nm
1	0.02mg	0.061
2	0.04mg	0.122
3	0.06mg	0.186
4	0.08mg	0.249
5	0.1mg	0.312

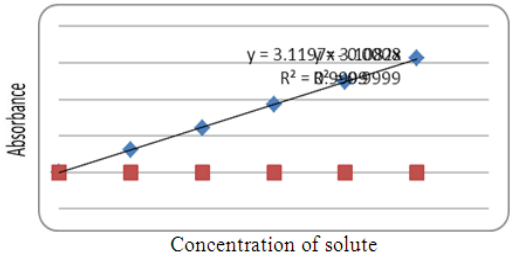

Figure 2: Calibration Graph for UV analysis of Artesunate.

Table 7: Data for UV analysis of Artesunate in tablets.

Brand code-Strength	Absorbance	Content %*	Amount found (mg) *	% RSD
X1 -100mg	0.154	99.1± 0.66	99.1± 0.66	0.67
X2 -100mg	0.146	93.9±2.56	93.9± 2.56	2.73
X3 -100mg	0.154	99.1± 1.53	99.1± 1.53	1.54
X4 -50mg	0.145	93.3± 0.76	53.6± 0.76	0.81

* Mean value ± standard deviation of three determinations
RSD; relative standard deviation

Method Validation

Table 8: Data for Calibration Curve of Artesunate.

Sr. No.	Parameters	In water/1M NaoH
1	Limit of Detection	0.01 mg/ml
2	Limit of Quantitation	0.02 mg/ml
3	Regression Equation	Y=3.108X
4	Correlation Coefficient	0.999
5	Slope	3.108
6	Beer's law limit (mg/ml)	0.02- 0.1
7	Absorbance maximum (λ max) in nm	289

Table 9: Evaluation of Accuracy and Precision of UV Spectrophotometric method.

Precision of an analytical method is usually expressed as the relative standard deviation (% RSD of the assay results).

RSD = [(standard deviation)/ (average)] × 100

Sample	Absorbance	Mean	RSD%
X1	0.152	0.154	0.92
	0.153		
	0.155		
	0.155		
	0.155		
X2	0.140	0.146	2.62
	0.144		
	0.149		
	0.147		
	0.149		
X3	0.152	0.154	1.49
	0.158		
	0.155		
	0.154		
	0.153		
X4	0.145	0.145	0.79
	0.146		
	0.145		
	0.144		
	0.147		

Linearity and Range

The prepared aliquots (0.02-0.1) were scanned for absorbance at 289 nm The absorbance range was found to be 0.145- 0.154 .These solutions obeyed Beer-Lambert's law in above concentration range with regression of 0.999.

Pharmacopoeial Assessments

The quality parameters associated with pharmaceutical products are always assured through quality control methods of analysis. Quality procedures are pertinent to ensuring that drugs or medicines reaching patients are safe, efficacious and potent. Quality control measures allows for the development of rapid, sensitive, selective, accurate and cost- effective method of analyzing pharmaceuticals. New methods developed are validated against existing pharmacopoeial methods and standards.

Assay of Artesunate by standard method

Firstly sodium hydroxide should be standardized with potassium biphathalate to assure its molarity, also ethanol required neutralization by titrated with NaoH to avoided burette reading errors.

Upon analyzing the artesunate brands with the titration method, a mean percentage was observed , 98.18%, 98.05% , 107.18%, and 101.36% for X1,X2, X3,and X4respectively (Table 5). All samples did meet the Ph. Int. requirement of containing not less than 90.0% and not more than 110.0% of the amount stated on the label.

Proposed UV-method

The proposed method exhibited good levels of detection and quantitation with values of 0.01 and 0.02 mg/ml respectively. And the other tablet excipients usually present during compounding are not likely to interfere with the absorption spectrum of Artesunate. 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; $Y= 3.108X$ and the correlation coefficient (R2) of 0.999. The Beer's plot was obeyed in concentration range of 0.02- 0.1(20- 100 mg% of the tablet) (Fig. 2). There is good correlation between absorbance and concentration, which is the basis of this method of analysis. The application of the method to the four brands showed that all brands had values within the range specified in the IP (90–110%) (Table 7).

DISCUSSION

Assay of Artesunate by titration method

Quantitative analysis using titration procedure has been reported for the chemical content determination of various drugs in official books. The method was able to detect an apparently fake brand of Artesunate tablets and confirming the result obtained from the Spectrophotometric method. Titration method is widely used in determining the identity, purity, efficacy, stability and content of drugs. This method is still widely used in official compendial assays, because of their robustness, cheapness and capability of high precision. Quantitative analysis using titration measurements

(standard method) of the studied brands showed all of the tablet brands passing the WHO International Pharmacopoeia requirement (Table 5) which specifies Artesunate tablets to contain not less than 90.0 % and not more than 110.0 % of the amount of artesunate indicated on the label.[17] Accordingly all the samples pass the International Pharmacopoeia (90-110%) content requirements of Artesunate. On the whole, Artesunate tablets manufactured in Sudan were of good quality. Even though no counterfeit Artesunate tablets was observed in the results of this method. Fortunately, there is no counterfeit or substandard Artesunate tablets was observed in this studied brands, Because the use of substandard Artesunate tablets in treatment would result in sub-therapeutic levels of the drug in patients, leading to treatment failure and possible development of drug resistance. There is, therefore, the need for drug regulatory bodies in Sudan and other African countries to be vigilant and undertake routine assessment of the quality of Artesunate and other Artemisinin products on the market in order to flush out and to overcome developing of counterfeit and substandard ones.

Proposed UV-Spectrophotomeric Method

ART being an Artemisinin derivative is a sesquiterpene lactone with an unusual peroxide bridge is difficult to detect and identify by standard spectrophotometric methods, since it absorbs light only at low wavelengths, has a relatively low molar extinction coefficient, and has no distinct UV-Visible spectrum or fluorescent properties.[18,19] Since analysis is an important development of any dosage form, it necessary to have a simple, precise, accurate and sensitive method for assay of any drug product both as a bulk and in its formulation.[20] Simple UV method has become necessary for the assay of this drug because, UV unlike HPLC is simple, rapid and readily available in malaria endemic areas of the world. This will also help to checkmate influx of fake and adulterated products into the drug market and reduce the burden of malaria.[21] As an alternative to existing standard methods, we propose a procedure to determine Artesunate drug substance based on UV spectrophotometry.

The aim of this work was to develop and modify USP method for dissolution rate study of Artesunate and used for individual analysis of tablets and fulfilling the requirements of analytical quality necessary to be applied to the content uniformity tests indicated by the IP, for finished pharmaceutical products.[17] In order to assay ART by UV method, it is necessary to involve it in a reaction process that would break the endo -peroxide ring and introduce a least one double bond in the molecule and this can be achieved by reacting it with sodium hydroxide. The addition of sodium hydroxide is aimed to generate chromophore through reaction process that involves breaking the endoperoxide ring. The development of reliable and affordable procedures for the assay of drug substances either as pure drug or in formulation remains a major research area in today's pharmaceutical care and practice. Finally, content uniformity test of commercial brands individually processed as indicated in (Table 7) were found to contain between 93.3 and 99.1% of the nominal value, meeting therefore, with the requirement. 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. And our results do seem to agree with the compendial and standard (Titration) method of assay.

CONCLUSION

The results obtained from the present study show that UV absorption of Artesunate could be employed for the assay of the drug, especially in the absence of high technology equipment that are not easily available in most developing countries.

The proposed method is an alternative to determine Artesunate in pharmaceutical dosage forms. Its advantages over other existing methods are its simplicity, fastness, low-cost and non-polluting conditions.

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