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PHARMACEUTICAL EVALUATION AND QUALITY ASSESSMENT OF ARTESUNATE TABLETS AVAILABLE IN PHARMACIES IN SUDAN

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ABSTRACT

Quality control (QC) is a process employed to ensure a certain level of quality in a product. Drug analysts play an indirect but very important role in creating the basis for highly efficient drug therapy by giving analytical support to synthetic, biotechnological, pharmacological, pharmaceutical technology, clinical, etc... Much more important is the role of drug analysts in other area, in securing the highest possible safety for drug therapy. 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. The disease is caused by single-celled protozoan parasites of the genus *Plasmodium*. Artesunate is a sesquiterpene with an unusual endoperoxide linkage structurally unrelated to other known antimalarials. Artesunate (hemisuccinate ester) its artemisinin derivative 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. In this study, the pharmaceutical evaluation and quality assessment of commercial Atesunate tablets that are available in the Sudanese market was performed according to the Pharmacopoeial standard method. The study sought to ascertain the presence or otherwise of counterfeit and substandard artesunate tablets in Sudan. Artesunate tablets were purchased from pharmacies in Khartoum for the study. The mechanical properties of the tablets were evaluated, namely: uniformity of weight, breaking strength, friability, tablet diameter and rate of disintegration in aqueous medium. Titration method was used to determine the presence of artesunate and to assay the tablets. None of the artesunate tablets sampled was found to be a counterfeit or substandard. Most of the brands had acceptable mechanical properties in terms of mass uniformity, hardness, friability, tablet diameter and disintegration time. However, the artesunate content of the tablets was observed (98.18%- 107.18%). Quantitative analysis using titration measurements (standard method) of the studied brands showed all of the tablet brands passing the WHO International Pharmacopoeia requirement which specifies Artesunate tablets to contain not less than 90.0 % and not more than 110.0 % of the amount of artesunate. Accordingly, all Artesunate tablets manufactured in Sudan were of good quality. 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.

KEYWORDS: *Artesunate tablets, Artemisinins, Titration method, Counterfeit drugs, substandard drugs, Malaria.*

INTRODUCTION

Quality control (QC) is a process employed to ensure a certain level of quality in a product. The basic goal of quality control is to ensure that the products, or processes provided meet specific requirements and are dependable, satisfactory, and fiscally sound.[1] Quality control (QC) originated from the industrial goal of producing each batch of product with consistently high quality, detecting and correcting process errors that result in defective products. Therefore, QC is an essential component of a laboratory QA program, because it provides a mechanism for monitoring the quality of laboratory results.[1] Quality control is integral to all modern industrial process and the pharmaceutical is no

exception. Often, quality control is confused with quality assurance (QA). Though the two are very similar, there are some basic differences. Quality control is concerned with the product, while quality assurance is process–oriented. Two issues of fundamental importance in drug therapy are efficacy and safety. Drug analysts play an indirect but very important role in creating the basis for highly efficient drug therapy by giving analytical support to synthetic, biotechnological, pharmacological, pharmaceutical technology, clinical, etc... Much more important is the role of drug analysts in other area, in securing the highest possible safety for drug therapy. 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. Malaria is not just a disease commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development.[2] 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, anaemia, chills, flu-like illness, and in severe cases, coma (cerebral malaria) and death.

Artesunate (hemisuccinate ester) and other artemisinin (obtained from the extracts of the plant Artemisia annua) derivatives such as; dihydroartemisinin, its methyl ether (artemether), its ethyl ether (arteether) 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. In sudan Artesunate-SP combination is most common.[3]

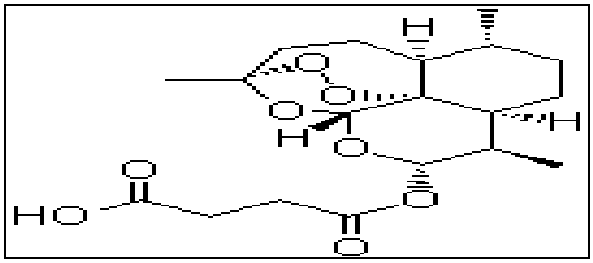

Figure 1: Artesunate chemical structure.

Artesunate (*figure 1*) is an antimalarial agent. It is a water-soluble hemisuccinate derivative of artemisinin. Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides, active against the ring stage of the parasite. Artesunate (ARS) is powerful especially in the treatment of advanced and potentially lethal cases of P. falciparum infection.

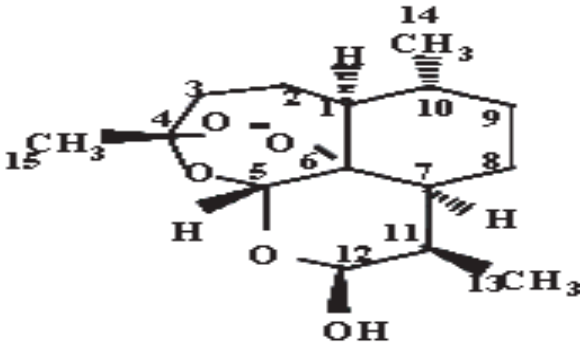

Figure 2: Chemical structure of dihydroartemisinin.

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.

Artesunate exist as a fine, white crystalline powder, very slightly soluble in water, freely soluble in methanol. It has chemical designation is (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, and its molecular formula is C19H28O8.

Artesunate (ARS) act as a weak acid with a pH of an aqueous suspension containing 10 mg/g to be 3.5 – 4.5, and a pKa value of 4.6 for the hydroxyl group. ARS is formulated for oral, parenteral, (intravenous and intramuscular) and rectal administration. Combination

therapy is only in oral dosage forms.[4] Many African countries lack the resources to employ advanced techniques such as high performance liquid chromatography; mass spectrometry and Raman spectroscopy[5] which can be used to ascertain the authenticity of artemisinin products in the market and are therefore vulnerable to counterfeiting and substandard drugs. Also, artesunate and other artemisinins, being generally expensive drugs, are potential targets of counterfeiting. As a result of the above factors, Africa has been cited as the next possible destination for counterfeit and substandard artemisinins.[6] Already, cases of counterfeit and substandard artemisinins have been reported in African countries such as Tanzania, Cameroon,[7] Kenya and the Democratic Republic of Congo.[8]

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. The objective of this study was, therefore, to evaluate the quality of artesunate tablets available in community and wholesale pharmacies in Khartoum, Sudan. The study also sought to determine the existence or otherwise of counterfeit or fake artesunate tablets in the study area.

MATERIALS AND METHODS

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.
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 Qualitative and Quantitative Analysis

Pharmacopial Assessment

Weight Variation

Using the sensitive balance, twenty tablets of each brand was measured one by one; the net weight was then calculated and divided by 20 to obtain the mean weight of the twenty tablets.

Friability and Hardness

The breaking strength of twenty randomly selected tablets was determined using a Schleuniger hardness tester (Schleuniger Co., Switzerland). Twenty tablets were weighed and subjected to abrasion at 25rpm. The tablets were weighed after five minutes and the weight compared to the initial weight. According to USP, the tablets should not lose more than 1% of their total weight.

$$\% \text{ Loss} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Disintegration

The disintegration times of six randomly selected tablets was determined in distilled water at 37 ± 0.5 °C using an Erweka tablet disintegration tester (Type ZT3/1, Heusenstamm, Germany). Until no particle remained on the basket of the system. The time taking for each of the six tablets tested in each of the brand was recorded.

Dissolution

The dissolution tests were carried out using the basket method according to US Pharmacopoeia (USP) guidelines, operated at 100 rpm in a dissolution bath containing 100 ml water, with sink condition maintained at a temperature of 37 ± 0.5ºC.

Standard Solution: Transfer 10mg of pure Artesunate to 250ml volumetric flask; dissolve in 200 ml of medium and 25ml of 1N sodium hydroxide. Dilute with medium to volume.

Sample Solution: Pass aportion of the solution under test through a suitable filter of 0,45mm pore size. Transfer 20ml of filtrate to a 25ml volumetric flask, add 2.5ml of 1N NaOH solution, and dilute with medium to the volume. Absorbance reading determined at 289 nm using UV Spectrophotometer against the blank (2.5ml of 1N NaOH to a 25ml volumetric flask, and dilute with medium to volume). Warm the standard solution and sample solution to 50 ± 1ºC for 45 min, cool to room temperature immediately, and determine the absorbance of the solutions, using blank to zero the

spectrophotometer calculate the percentage of C19H28O8 dissolved. The practical concentration of dissolved Artesunate calculated from the corresponding absorbance of each sample as:

Result = (Au/As) X (Cs/L) XVX100

Where
Au=Absorbance of sample solution.
As=Absorbance of standard solution.
Cs=Concentration of the standard solution (mg/ml).
L=label claim (mg/tablet).
V=Volume of the medium.

Quantitative Analysis

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 C19H28O8 (artesunate).

RESULTS

Uniformity of weight, Friability and Hardness determinations

Table 2A: Uniformity of weight, Friability and Hardness determinations of four brands of Artesunate tablets.

Sample	Uniformity of weight (gm)*	Friability (%)	Hardness (Mean Crushing strength) (Kg/cm2) *
X1	0.361 ± 0.008	0.08	4.96 ± 0.65
X2	0.251 ± 0.003	0.35	5.06 ± 0.62
X3	0. 272 ± 0.030	0.30	4.35 ± 0.41
X4	0. 264 ± 0.002	0.31	4.40 ± 0.23

* Mean value ± standard deviation

Disintegration, Dissolution rate% and Diameter test

Table 2B: Disintegration, Dissolution rate% and Diameter test for four brands of Artesunate tablets.

Sample	Disintegration Time (minutes)*	% Dissolution at 30 minutes C30 (Mean value)	Tablets Diameter (cm)
X1	15:00 ± 0.50	88.39	1.1
X2	05:00 ± 0.50	94.70	0.9
X3	06:20 ± 0.29	103.70	0.9
X4	06: 50 ± 0.50	108.97	0.9

* Mean value ± standard deviation

Assay of Artesunate using compendial Titration method of assay

Table 3: Titration data for the assay of artesunate brands.

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

Table 4: 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 5: The content% of Artesunate in different brands of Artesunate tablets sampled (mean of 3 measurements).

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

Table 6: 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.

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. The uniformity of weight determinations for all the brands gave values which complies with the official books specification for weight uniformity, as none of the brands deviated by up to 10% from the mean value (Table 2A). Similarly, the friability results for all the brands also complied with official specification; all the brands gave a weight loss of less than the official specification of 1%w/w (Table 2A).

Also the mean crushing strength which is an indication of the hardness of the tablets showed that the average value of all brands are acceptable, brand X2 gave the highest crushing strength of 5.06 kg/cm2 (Table 2A). All the brands passed the disintegration test (Table 2B). The B.P. 1988 specifies 15 minutes which are (15min, 5min, 6min, and 6min for X1, X2, X3, and X4 respectively).

In-vitro Dissolution Test

The dissolution profiles for brands X1, X2, X3, and X4 indicate that all the brands complies with the IP specifications as not less than 60% labeled content should dissolved at 30 minutes, the average value for each one which are 88.39%,94.7%,103.7%,108.97% for X1,X2,X3,and X4 respectively (Table 2B).

Assay of Artesunate by standard (Titration) method

Firstly sodium hydroxide should be standardized with potassium biphathalate to assure its molarity, also ethanol required neutralization by titrated with NaoH to avoid 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 6). 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.

DICUSSION

Biopharmaceutical determinations of tablets

All the brands used were within their shelf life as at the time of the study. Four different brands of Artesunate tablets obtained from different retail pharmacy were subjected to a number of tests in order to assess their quality. The assessments involved the use of both qualitative and quantitative methods of evaluation. The qualitative methods of evaluation includes tablet description i.e. color, size and shape, which were carried out by visual observation, while quantitative evaluations used are uniformity of weight, friability, hardness, disintegration and dissolution tests as well as chemical content determination.

Most of the tablets possessed appropriate mechanical properties in terms of weight uniformity, breaking strength, friability and disintegration time. The different brands of artesunate tablets showed acceptable weight uniformity with weight deviations less than 10 %w/w (Table 2A).

The average breaking strength of the tablets was >4 kg/cm2 (Table 2A) The breaking strength is used to characterize the hardness of tablets. Tablets with breaking strength values >4 kg/cm2 have acceptable hardness.

All the brands gave less than 1% w/w loss in weight with the friability test determination; these results (Table 2A) comply with the official specification of 1%w/w (B. P. 1998). This showed that all the brands could withstand abrasion without loss of tablet integrity. Also all brand tablets show uniform diameter.

All the brands passed the disintegration test. The B.P. 1988 specifies 15 minutes (Table 2B) and the ability of brands to disintegrate within this time limit is an indication that the drug will show good disintegration in the gastrointestinal tract. Hence, the tablet may readily be broken down to facilitate release of content into the

system. This usually has a direct effect on the dissolution and bioavailability of the drugs, thus all the artesunate brands passed the friability and disintegration tests. It could thus be inferred that all the artesunate brands studied could withstand abrasion without loss of tablet integrity and could disintegrate readily in aqueous medium, especially in the gastrointestinal tract (GIT). Although, the crushing strength is not an official method of assessing tablet quality, it is still useful in assessing the integrity of tablet dosage forms.

Dissolution

The obtained dissolution content at 30 minutes indicate that all brands passed the dissolution test, The disintegration time of all brands may definitely indicate that the drug would be released into the dissolution medium easily, with observation that The dissolution profiles for brands X1and X2 indicate that the brands released >80% of the active ingredient within 30 min (Table 2B). Similarly, the dissolution profiles of X3, and X4 as shown in (Table 2B) indicate that the brands released >100% of the active ingredient within 30 min. Our results based on the in vitro dissolution show that significant variation exists in the bioavailability of artesunate from the four brands of artesunate tablets. Dissolution rate has been reported to have a direct bearing on the bioavailability profile of tablet dosage forms as it can be used to predict the drug release pattern in vivo, and it usually affected by disintegration rate of dosage. Accordingly, low crushing strength and very low disintegration values of brands X3 and X4 may justify the high release% obtained from their dissolution tests.

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. 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 6) 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.[9] Accordingly all the samples pass the International Pharmacopoeia (90-110%) content requirements of Artesunate.

CONCLUSION

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.

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