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A Simple and Rapid Spectrophotometric Method for the Determination of Artesunate in Pharmaceuticals

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Abstract

Simple and rapid spectrophotometric method for the determination of artesunate is described. The method is based on the reaction of H_2O_2, generated by the cleavage of endoperoxide linkage of artesunate and its reaction with potassium iodide to liberate iodine. The liberated iodine bleaches the red colored Safranin O to colorless species and is measured at 521 nm while it oxidizes colorless Variamine blue to violet colored species and is measured at 556 nm. The optimum reaction conditions and other analytical parameters were evaluated. The statistical evaluation of the method is examined by determining intra-day and inter-day precision. The method has been successfully applied to both pure and tablet dosage forms.

Keywords:

Spectrophotometry, Artesunate, Falcigo, Safranin O, Variamine blue

1. Introduction

Malaria is the most widespread of the transmissible diseases and is prevalent among the insect-borne diseases. About forty percent of the world's population is at risk of malarial infection. Every year nearly 100 million people experience malarial infections and over 1 million individuals die. Malaria has essentially been eradicated in most temperate-zone countries. The parasite responsible for the vast majority of fatal malarial infections, Plasmodium falciparum (P. falciparum), can kill patients in a matter of hours. Resistance to antimalarial drugs in P. falciparum represents a major public-health problem, with therapeutic and prophylactic implications. Malaria has traditionally been treated with quinolines such as chloroquine, quinine, mefloquine etc. [1]. Hence several research groups are now working to develop new active compounds as an alternative to chloroquine, especially from artemisinin. Artemisinin is a natural product and is found in the leafy portions of Artemisia annua (qinghao), a plant used by Chinese herbalists since 168 B.C. It is a new class of antimalarials, where the endoperoxide plays an important role. Endoperoxide antimalarials[2-10] based on the ancient Chinese drug Qinghaosu (artemisinin) are currently our major hope in fight against drug-resistant malaria.

Artesunate (ARTS), derivative of artemisinin (ART is an antimalarial drug which possesses bioactivity with less toxicity [11, 12]. Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticide which acts by increasing the oxidant stress

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on the intra-erythrocytic stages of the parasite [13]. 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[4]. Several analytical techniques [14-21] have been used for the determination of artesunate, which rely upon sophisticated and expensive instrumentation and also expensive chemicals. To the best of our knowledge there is no spectrophotometric method for the determination of artesunate in either pharmaceutical formulations or biological fluids. In the present investigation we developed a simple and rapid spectrophotometric method for the determination of artesunate in formulations. The proposed method is simple, accurate and easy to apply to routine use.

2. Experimental

2.1 Apparatus

A Secomam Anthelie NUA 002 UV-VIS Spectrophotometer with 1cm quartz cell was used for the absorbance measurements.

2.2 Materials and Reagents

All solutions were prepared with double distilled water. Chemicals used were of analytical reagent grade.

2.3 Procedure

A 1000 µg mL-1 of artesunate drug solution was prepared in ethanol. The stock solution was diluted appropriately to get the working concentration.

Hydrochloric acid (5 M), Potassium iodide (2 %), Sodium acetate buffer (2 M) were used. A (0.01 %) solution of Safranin O was prepared by dissolving 0.01 g of Safranin O in 50 % ethanol and making up to 100 mL.

A (0.05 %) solution of Variamine blue was prepared by dissolving 0.05 g of Variamine Blue in 25 mL ethanol and making up to 100 mL with distilled water and stored in an amber bottle.

2.3.1 Determination of Artesunate using Safranin O as a reagent

Different aliquots (20.0 – 140.0 µg mL^{-1}) of artesunate were transferred into a series of 10 mL calibrated flasks by means of a micro burette. Then, 1 mL each of 2 % KI and 5 M HCl were added to each flask and the mixture was gently shaken until the appearance of yellow color, indicating the liberation of iodine. To this system, 0.5 mL of 0.01 % Safranin O was added followed by 2 mL of 2 M sodium acetate solution and the reaction mixture were shaken for 5 minutes. The contents were diluted to the mark with distilled water and mixed well. The absorbance of each solution was measured at 521 nm against the corresponding reagent blank. Reagent blank was prepared by replacing the analyte (artesunate) solution with distilled water. The absorbance corresponding to the bleached color, which in turn corresponds to the analyte (artesunate) concentration, was obtained by subtracting the absorbance of the blank solution by that of the test solution.

2.3.2 Determination of Artesunate using Variamine blue as a reagent

Different aliquots (20.0 – 140.0 µg mL^{-1}) of artesunate were transferred into a series of 10 mL calibrated flasks by means of a micro burette. Then, 1 mL each of 2 % KI and 5 M

HCl were added to each flask and the mixture was gently shaken until the appearance of yellow color, indicating the liberation of iodine. To this system, 0.5 mL of 0.05 % Variamine blue was added followed by 2 mL of 2 M sodium acetate solution and the reaction mixture were shaken for 5 minutes. The contents were diluted to the mark with distilled water and mixed well. The absorbance of the colored solution was measured at 556 nm against the corresponding reagent blank.

2.3.3 Analysis of dosage forms

An amount of powdered tablets (Falcigo, Zy. Cadila) equivalent to 50 mg of artesunate was extracted with ethanol and a convenient aliquot was then subjected to analysis using Safranin O and Variamine blue.

3. Results and Discussion

3.1 Absorption Spectra

The absorption spectrum of Safranin O (red colored species) is presented in Fig. 1a and the reaction mechanisms are represented in Scheme 1 and 2a. The method involves the reaction of H_2O_2, generated by the cleavage of endoperoxide linkage of the corresponding drug solution (artesunate) in acidic medium, with potassium iodide to liberate iodine. This liberated iodine bleaches the red colored Safranin O, which were measured at 521 nm. The decrease in absorbance is directly proportional to the analyte concentration.

The absorption spectrum of Variamine blue is presented in Fig. 1b and the reaction mechanisms are represented in Scheme 1 and 2b. This method involves the liberation of iodine by the reaction of H_2O_2, generated by the cleavage of endoperoxide linkage of artesunate in acidic medium, with potassium iodide to liberate iodine. This liberated iodine oxidizes colorless Variamine blue in the presence of acetate buffer to form a violet colored species, which shows a maximum absorbance at 556 nm. This increase in absorbance is directly proportional to the artesunate concentration. The reagent blank has negligible absorbance at this wavelength.

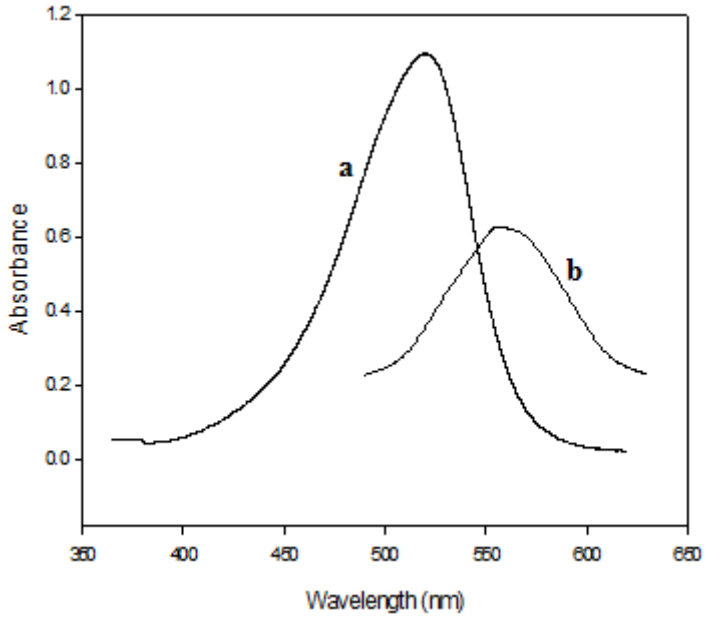
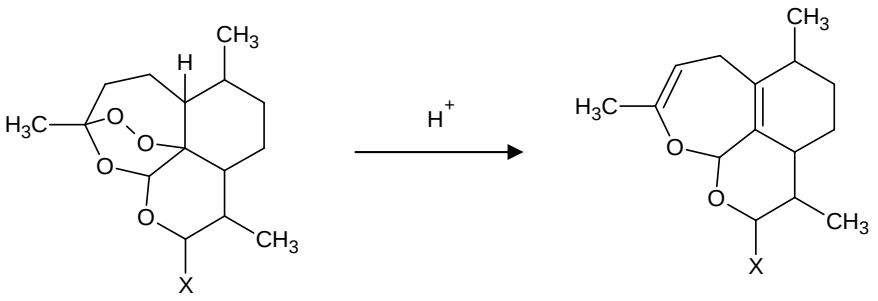
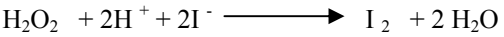


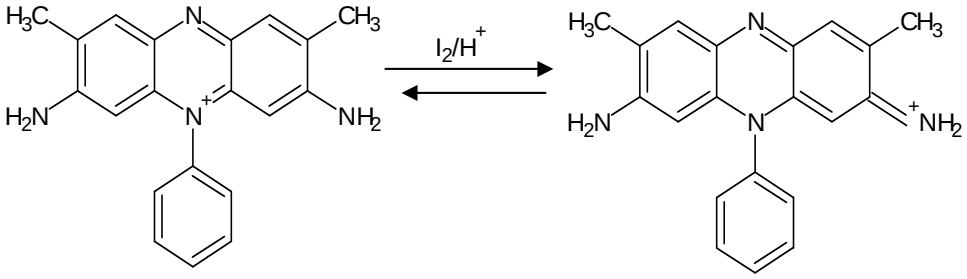
Fig.1. Absorption spectra of a) Safranin O b) Variamine blue



X = OOC-$(CH_2)_2$- COOH (Artesunate) + H_2O_2

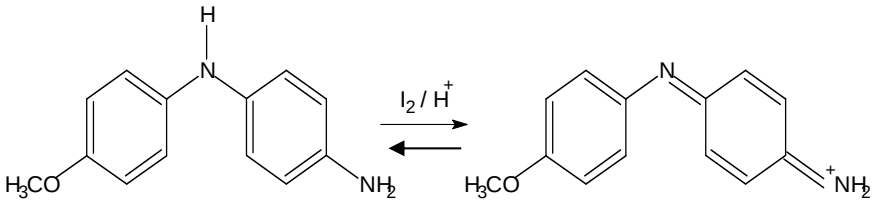
Scheme 1: Reaction of artesunate in acidic medium to form H_2O_2





Safranin O (Red) Safranin O (Leucoform)

Scheme 2a - Reaction between H_2O_2- KI system with Safranin O



Variamine blue (Leucoform) Variamine blue (Violet)

Scheme 2b - Reaction between H_2O_2- KI system with Variamine blue

3.2 Effect of reagent concentration

The oxidation of iodide to iodine was effective in the pH range 1.0 - 1.5, which could be maintained by adding 1 mL of 5 mol L^{-1} HCl in a final volume of 10 mL. The liberation of iodine from potassium iodide in an acidic medium was quantitative. The appearance of yellow color indicates the liberation of iodine. It was found that 1 mL of each 2 % potassium iodide and 5 mol L^{-1} HCl were sufficient for the liberation of iodine from iodide by artesunate. This liberated iodine is sufficient to bleach the red colored Safranin O to the leucoform and also to oxidize colorless Variamine blue to colored species. Constant and maximum absorbance values were obtained in the pH range of 4.0 - 5.0. This could be achieved by adding 2 mL of 2 M acetate buffer solution to a total volume of 10 mL. Maximum absorbance was obtained

instantaneously and required no heating under the reaction conditions. Under the optimum reaction conditions, the color system was stable for a period of over 4 hr.

3.3 Analytical data

The adherence to Beer's law was studied by measuring the absorbance values of solutions varying analyte concentration. A linear relation was found between absorbance at λ max and concentration ranges given in Table 1. Regression analysis of Beer's law data using the method of least squares were made to evaluate the slope (b), intercept (a) and correlation coefficients (R), for each system of artesunate and are also presented in Table 1. Sensitivity parameters such as molar absorptivity, detection limit and quantification limit are also compiled in Table 1. The limit of detection (DL= 3.3σ/s; where s is the slope of the calibration curve of the artesunate and σ is the standard deviation of the reagent blank) and the limit of quantitation (QL= 103σ/s; where s is the slope of the calibration curve of the artesunate and σ is the standard deviation of the reagent blank) were calculated according to ICH guidelines. The accuracy of the method was established by analyzing the pure drug at three levels (within working limits) and the precision was ascertained by calculating the relative standard deviation of five replicate determinations on the same solution containing the drug at three levels and are presented in Table 2a and 2b.

Table 1. Analytical Parameters

Parameters	**Method I**	**Method II**
λ_{max} (nm)	521	556
Beer's law limits ($\mu g\ mL^{-1}$)	20.0-140.0	20.0- 140.0
Molar absorptivity ($L\ mol^{-1}cm^{-1}$)	0.1803×10^4	0.2551×10^4
Limit of detection** ($\mu g\ mL^{-1}$)	0.0043	0.4496
Limit of quantification** ($\mu g\ mL^{-1}$)	0.0128	1.3625
Regression Equation*	Y= a + bX	Y= a + bX
Slope (b)	0.0526	0.0734
Intercept (a)	0.0014	0.0057
Correlation coefficient (R)	0.9995	0.9998

* Y is the absorbance and X is the concentration in $\mu g\ mL^{-1}$
** Calculated according to ICH- Guidelines.

Table 2. Evaluation of accuracy and precision.

	Amount taken ($\mu g\ mL^{-1}$)	Amount found* ($\mu g\ mL^{-1}$)	RSD (%)	RE ($\mu g\ mL^{-1}$)	SD (%)
a. *Using Safranin O (SO)*	40.0	40.02	0.35	0.02	0.05
	60.0	60.02	0.40	0.03	0.05
	80.0	80.02	0.30	0.02	0.03
b. *Using Variamine blue (VB)*	40.0	40.01	0.25	0.02	0.05
	60.0	60.02	0.27	0.02	0.04
	80.0	80.02	0.25	0.04	0.05

* Mean value of five determinations. RE - Relative Error;
SD - Standard Deviation; RSD - Relative Standard Deviation

3.4 Interference study

In the pharmaceutical analysis, it is important to test the selectivity towards the excipients and fillers added to the pharmaceutical preparations. Several species which can occur in the real samples together with drug were investigated. The level of interference was considered acceptable. Commonly encountered excipients such as starch, glucose did not interfere in the determination.

3.5 Method Validation

3.5.1 Accuracy and precision

The proposed method is applied to the assay of artesunate in one brand of tablets (Falcigo, Zy. Cadila) and the results are compiled in Table 3. The accuracy and reliability of the proposed method are further established by performing recovery studies. To a fixed amount of drug in dosage forms pure artesunate solution at three different levels (within the working limit) was analyzed, each being repeated five times. The relative error and relative standard deviation indicate the high accuracy and precision for the method (Table 2). For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for five days with all solutions being prepared afresh each day. The day-to-day relative standard deviation values represent the best appraisal of the method in routine use.

3.6 Applications

The proposed methods were applied to the assay of artesunate (Falcigo, Zy. Cadila) tablets and the results are compiled in Table 3. The accuracy and reliability of the methods were further established by recovery studies.

Table 3. Results of assay of formulations by the proposed method

Brand name of tablet	Labeled amount (mg)	Found*± SD	
		Using SO (mg)	Using VB (mg)
Falcigo**	50	49.73 ± 0.03	49.01 ± 0.02

* Mean value of five determinations (n = 5)
** Manufactured by Zy. Cadila

4. Conclusions

Simple and rapid determination of artesunate using two different reagents have been developed .The method is easier and cheaper to perform compared to many existing methods and do not entail any stringent experimental variables which affect the reliability of results. The ingredients usually present in the pharmaceutical formulations of artesunate do not interfere in the proposed method. The method thus can be used in the routine determination of artesunate in pure and in dosage forms.

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