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Research Article

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Spectrophtometric method for the determination of artenimol using Rhodamine B as a new reagent

B. S. Virupaxappa[1]*, K. H. Shivaprasad[1] and M. S. Latha[2]

[1]Department of Industrial Chemistry, VSK University Bellary, Karnataka, (INDIA)
[2]Department of Chemistry, GM Institute of Technology, Davangere, Karnataka, (INDIA)

ABSTRACT

A Simple sensitive and rapid spectrophotometric method for the determination of Artenimol is described. The method is based on the reaction of H_2O_2, generated by the cleavage of endoperoxide linkage of Artenimol and its reaction with potassium iodide to liberate iodine. The liberated iodine bleaches the colored Rhodamine B to colorless species and is measured at 550 nm. The Beer's law is obeyed in the range of 33 – 274 µg/ml for Artenimol using Rhodamine B as reagent. The Sandell sensitivity, detection limit and quantization limit were also calculated. The optimum reaction conditions and other analytical parameters were evaluated.

Keywords: Spectrophotometry, Artenimol, and Rhodamine B.

INTRODUCTION

Malaria is one of the most widespread infectious diseases in the world. There are 300–500 million clinical cases each year, and between one and three million deaths, mostly children (and pregnant women) are attributable to this disease [1]. Because of the rapidly developing resistance of the malaria parasite *Plasmodium falciparum* to currently used alkaloidal drugs such as quinine and chloroquine, new non alkaloidal artemisinin type antimalarial drugs (artemisinin and its derivatives) have become increasingly important. Artemisinin is a sesquiterpene endoperoxide which is isolated from the herb of the Chinese medicinal plant *Artemisia annua* [2]. Artemisinin is a potent antimalarial drug against the resistant strains of *P. falciparum* [3]-[4]. Though the mechanism of action of the artemisinin type antimalarial drugs is not completely understood, there is growing evidence supporting the idea that the initial key step is the reductive cleavage of O–O bond of the endoperoxide group. This reaction presumably works by hemin, leading to oxygen and then carbon-centred radicals that subsequently lead to the biologically relevant damage to the malarial parasite [5], [6] and [7].

Since artemisinin shows low solubility and poor oral bioavailability [8] – [9], derivatizations of artemisinin were carried out and yielded different semi synthetic antimalarial drugs such as artemether and sodium artesunate. Artemether (decahydro-10-methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano [4.3-j]-1, 2-benzodioxepin) is more active than the parent compound artemisinin [10]. Artemether is practically insoluble in water, very soluble in dichloromethane and acetone, freely soluble in ethyl acetate and dehydrated ethanol and shows a higher stability when dissolved in oils. The antimalarial action of artemether appears like artemisinin to be mediated by the generation of free radicals from the Endoperoxy Bridge of the drug. This endoperoxy bridge is essential for antimalarial activity because experiments with compounds having one oxygen instead of two showed no

activity [11]. Artesunate is an antimalarial drug. It is a semi-synthetic derivative of artemisinin. Its primary use is as an intermediate in the preparation of experimental artemisinin-derived antimalarial drugs. Malaria has traditionally been treated with Quinolines such as chloroquine, quinine, mefloquine, etc. It is useful mainly in the treatment of uncomplicated *P. falciparum* malaria that is resistant to other antimalarial drugs [12]. Both for quality assurance and consumer safety the quantification of artemether in its commercial pharmaceutical products is particularly important. Suggested methods of determining the quantity of artemether are complex chromatographic (HPLC, TLC scanning) and NMR methods [13] – [15]. The analyses of artemether in tablets and/or capsules is till now carried out by using TLC, HPLC, TLC scanning techniques and one spectrophotometric method [16] –[19].The purpose of the present study was to develop and validate an analytical method for the determination of artemether. The method ought to be not time-consuming and simple and therefore suitable in routine work. Since artemether contains the electrochemically active peroxide (–O–O–) group it can be reduced easily at various electrodes [20-25].

EXPERIMENTAL SECTION

Apparatus: A Peltier Accessory (Temperature controlled) Varian Cary 50 model UV- Vis spectrophotometer equipped with 1 cm quartz cell was used for all spectral measurements. Systronics pH meter were used for the accurate pH determinations.

Reagents: All solutions were prepared with doubly distilled water. Chemicals used were of analytical reagent grade.

Artenimol: A 1000 µg ml^{-1} standard Artenimol drug (Fig 1) solution was first prepared by dissolving 0.1 g in ethanol and diluting to the mark in 100 ml calibrated flask. The stock solution was diluted approximately to get the working concentration. Hydrochloric acid (5M), potassium iodide (2%), sodium acetate buffer (2M) were used. A 0.05% solution of Rhodamine B (RB) was used.

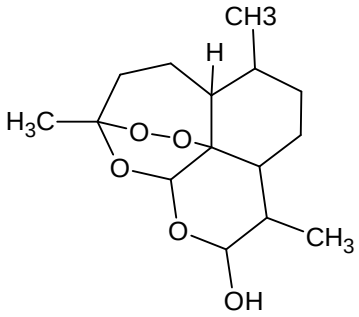


Fig 1: Structure of studied Artenimol drug.

Procedure

Determination of Artenimol using Rhodamine B as reagent: Different aliquots (33.0 – 274.0 µg ml^{-1}) of Artenimol were transferred into a series of 10 ml calibrated flasks by means of micro burette. Then, 1 ml each of 2% KI and 5M 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% RB is added followed by 2ml of 2M sodium acetate solution and the reaction mixture was shaken for 5 min. The contents were diluted to the mark with distilled water and mixed well. The absorbance of the colored solution was measured at 550 nm against the corresponding reagent blank and the absorbance corresponding to the bleached color, which in turn corresponds to the drug concentration, was obtained by subtracting the absorbance of the blank solution by that of the test solution and measured at 550 nm.

RESULTS AND DISCUSSION

Absorption spectra

The method involves the reaction of H_2O_2, with Artenimol in acidic medium, and its reaction with potassium iodide to liberate iodine. This liberated iodine bleaches the colored RB to colorless leucoform, which was

measured at 550 nm and are represented in scheme 1. The decrease or increases in absorbance are directly proportional to the analyte concentration. The spectrum spectrum of Rhodamine B is presented in Fig 2.

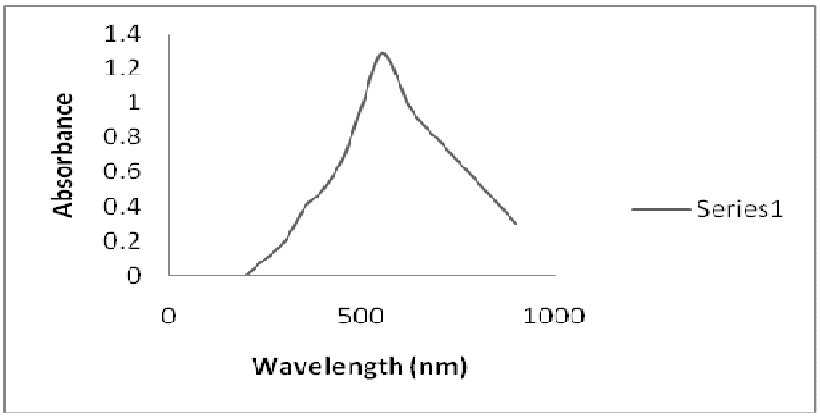
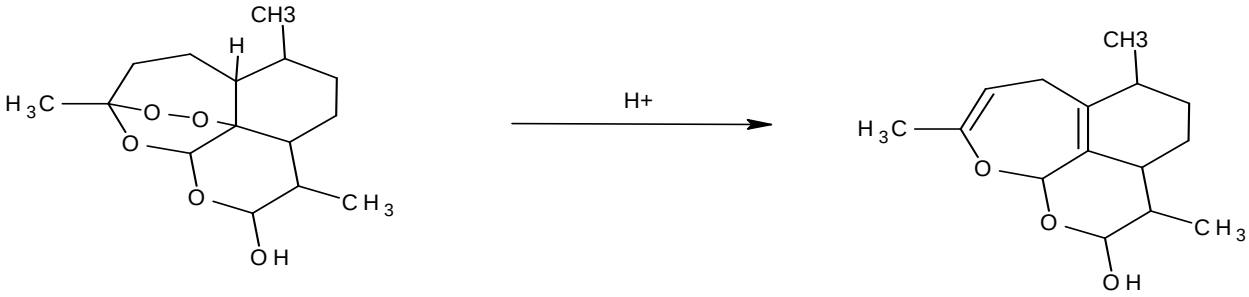
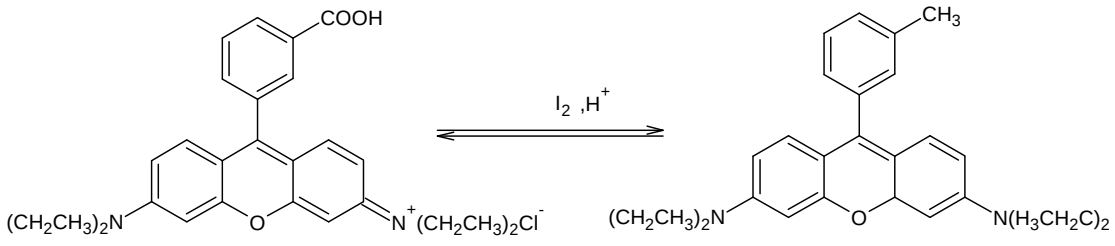


Fig 2. Absorption spectrum of Rhodamine B Scheme1.



Reaction between H_2O_2-KI system and Rhodamine B

$$H_2O_2 + 2H^+ + 2I \longleftrightarrow I_2 + 2H_2O$$



Rhodamine B (colored) Rhodamine B (colorless)

Optimization of reagent

The oxidation of iodide to iodine was effective in the pH range of 1.2 -1.8 which could be maintained by adding 1ml of 5 ml 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 M HCl were sufficient for the liberation of iodine from iodide by Artenimol. This liberated iodine is sufficient to bleach the blue colored Rhodamine B to the colorless leucoform. Constant and maximum absorbance values were obtained in the pH range of 4. This could be achieved by adding 2ml of 2M acetate buffer solution to a total volume of 10 ml.

Analytical data

A linear relation was found between absorbance at λ_{max} and concentration ranges given in (Table 1). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficients (R), for each system of Artenimol, which are also presented in Table 1. Sensitivity parameters such as Sandell sensitivity, Limit of detection and quantification were calculated according to ICH guidelines. The accuracy of the method was established by analyzing the pure drug at the 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 (Table. 1).The Beer's law is obeyed in the range 33 – 274 µg/ ml and is shown in Fig 3.

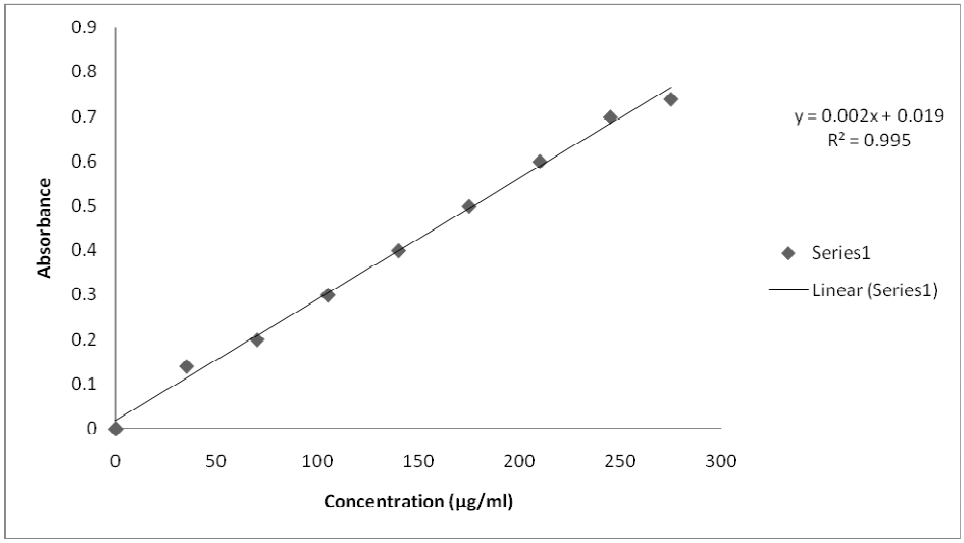


Fig 3. Adherence to Beer lambert's law

Table1. Analytical parameters

Parameters	Artenimol
λ max (nm)	550
The Beer's law limits (µg/ml)	33.0-275.0
The Sandell sensitivity (µg/ml)	0.000114
Limits of detection (µg/ml)	0.3371
Limits of quantification (µg/ml)	1.2012
Regression Equation	y = a + bx 0.0065
Slope(b)	0.0089
Intercept(a)	0.0048
Correlation coefficient (R)	0.9991

Table 2 .Analysis of Artenimol in pharmaceutical formulations (Found values[a] ± SD% and comparison with the official method).

Drug	Labelled	Found (X ± SD) Proposed method
Cotecxin	60 mg/Tab	60.2 ± 0.55 t = 0.43, F = 0.89

Interference study

In the pharmaceutical analysis, it is important to test the selectivity towards the recipients 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, lactose did not interfere in the determination.

Accuracy and precision

The accuracy of the proposed methods was established by analyzing the pure drug at four levels (within the working limits) and precision was ascertained by calculating the relative standard deviation (RSD) of five replicate determinations. The relative error and relative standard deviation values which are less than 1% indicate the high accuracy and precision of the methods (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 four 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.

CONCLUSION

Simple and rapid method for the determination of Artenimol using Rhodamine B reagent have been developed. The method is simple and easy to perform compared to many other methods and do not involves rigorous experimental conditions which affect the reliability of results. The methods can thus be used for the determination of Artenimol in pure and dosage forms.

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