Journal of Science and Practice of Pharmacy
December 2019; 6 (1): 298-302
Available at http://www.jsppharm.org **doi: https://doi.org/10.47227/jsppharm/v6i1.3**
ISSN: 2449-0458 (print); 2449-0466 (electronic)

Original Research Article

Development of colorimetric method for the assay of artesunate using 4-nitrobenzaldehyde

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Abstract

Purpose: Artesunate is one of the artemisinin derivatives that is commonly used in combination with some anti-malarials as artemisinin-based combination therapies. High performance liquid chromatographic technique is the required standard assay method for artesunate, but this is not readily available or affordable in most developing countries. This study was carried out to develop a simple, accurate and cost-effective assay method for quantitative determination of artesunate.
Methods: The method is an indirect colorimetric assay, which was developed from the formation of yellow coloured product due to the reaction between acid decomposed product of artesunate with 4-nitrobenzaldehyde.
Results: The wavelength of maximum absorption for the yellow coloured product was 474 nm. The Beer's law was obeyed at the range of 20-100 µg/ml artesunate concentration with a linear coefficient of 0.9996. The molar absorptivity was 2.3183×103 mol-1 cm^{-1}. The limit of detection and quantification were 0.0753 µg/ml and 0.2283 µg/ml, respectively. The method required water as diluent. The result obtained from recovery study confirmed that there was no interference from pharmaceutical excipients. Five brands of artesunate tablets were assayed using the developed method.
Conclusion: The results compared favourably well with those obtained using the official method described in the international pharmacopoeia. The developed method is useful for the quantitative determination of artesunate in tablets and raw pharmaceutical material.

Keywords: Artemisinin, quantitative determination, in-direct colorimetric assay, molar absorptivity

Indexing: Index Copernicus, African Index Medicus

Introduction

Malaria is a life-threatening transmissible disease caused by plasmodium through the bite of infected female anopheles mosquitoes. It is a disease with a major negative effect on economic development. In 2017, an estimated 219 million cases and 435,000 deaths from malaria occurred worldwide. Malaria is a major health problem in Nigeria. She accounted for 19% of global malaria deaths [1]. In 2015 alone, it was estimated that about 50% of the population had at least one episode of malaria while children under the age of five had an average of 2-4 attacks [2].

Artesunate is one of the most widely used artemisinin derivatives that possess rapid action against plasmodium falciparum malaria [3-5]. It is commonly used in artemisinin-based combination therapies (ACTs) as standard treatment against uncomplicated cases of malaria [6,7]. It is a first line drug of choice for the treatment of severe malaria in injectable dosage form [8].

Most of the reported quantitative assay method for artesunate required the use of high-performance liquid chromatography (HPLC) which is the standard method [9-12]. This is not readily available or affordable in most developing countries. It is difficult to quantify artesunate by standard spectrophotometric method because it does not absorb significant light at wavelengths in the region of ultra violet and visible range. It also does not possess chemical groups that can make it react with reagents to produce colour complex or compound. However, there are few reported spectrophotometric methods but the methods

require expensive reagents and chemicals [3,13]. In some cases, the methods are less sensitive and cumbersome [14-16]. Acid or base decomposed product of artesunate is known to possess reactive compounds [17]. The developed colorimetric assay is based on the generation of reactive methylene centres in acid decomposed artesunate which reacts with 4-nitrobenzaldehyde to produce a yellow coloured compound.

Methods

Materials

Artesunate, 4-nitrobenzaldehyde and acetonitrile were obtained from Sigma-Aldrich, USA. Other reagents include; concentrated sulphuric acid, ethyl acetate and acetone (BDH, Poole England) and methanol and ethanol (JHD, China). The reagents were of analytical grade.

Wavelength scanning was recorded on T80 Double Beam UV/Visible Spectrophotometer (PG Instruments UK) with 1cm quartz cell cuvette and all weighing was done using Ohaus digital weighing balance (Ohaus Corps. USA) with sensitivity of 0.0001 g.

The reaction was carried out in a temperature-controlled Optima T 100 regulatory digital water bath (Grant instruments UK).

Preparation of 10 M concentration of sulphuric acid solution

A volume of 271.7 ml of concentrated sulphuric acid (18.4 M) was diluted in distilled water and made to 500 ml in a volumetric flask to obtain a concentration of 10 M sulphuric acid solution.

Preparation of the 4-nitrobenzaldehyde solution in 10 M Sulphuric acid

Exactly 0.2000 g of 4-nitrobenzyladehyde was weighed and transferred into a 100 ml volumetric flask. It was dissolved in 1.0 ml of methanol and made up to 100 ml volume with 10 M sulphuric acid. The solution was used for investigating the formation of a coloured product and for wavelength scanning. Thereafter, the developed method for assay of artesunate was carried out using a freshly prepared solution.

Preparation of standard solution of artesunate

Exactly 0.1000 g of pure artesunate powder was accurately weighed and transferred into a 100 mL volumetric flask. Then, 50 ml methanol was added and the powder was dissolved by gentle agitation of the flask. The solution was made to 100 ml volume with additional methanol to obtain a 1 mg/ml stock solution of artesunate solution. Then, 2.5 ml of the stock solution was transferred into 25 ml volumetric flask and made to volume with methanol to obtain a solution with a concentration 100 µg/ml. This solution was used for investigating formation of a coloured product and wavelength determination. From the remaining part of the stock solution, solutions with concentrations of 100 µg/ml, 80 µg/ml, 60 µg/ml, 40 µg/ml and 20 µg/ml were prepared and used to develop the standard calibration curve and the colorimetric assay method.

Preparation of solution of artesunate tablets of different brands

The different brands of artesunate tablets were weighed and reduced to powder. The weight of the powdered artesunate tablet equivalent to 0.1 g of artesunate was weighed and transferred into a 200 ml conical flask. Then, 100 ml of methanol was added. The flask was agitated for about 10 minutes. The mixture was filtered into a 100 ml volumetric flask and made to volume with methanol.

Determination of wavelength of maximum absorption

Exactly 1.0 ml solution of the 4-nitrobenzaldehyde (0.2% w/v) in 10 M surphuric acid was reacted with 1.0 ml artesunate (0.1% w/v) in 10 ml volumetric flask. This produced an immediate yellow coloured compound. It was made to 10 ml with methanol after heating at 80 °C for 10 minutes. Wavelength scanning of the solution of the 4-nitrobenzaldehydes in 10 M sulphuric acid and the reaction product (coloured compound) was recorded by scanning from 200 nm to 800 nm using the UV/Visible Spectrophotometer. The appropriate wavelength of maximum absorption (λmax) of the compound was selected.

Optimization studies

Optimal conditions for the reaction between acid decomposed product of artesunate and 4-nitrobenzaldehyde was determined by a method described by Aghayere *et al* [18]. However, there was a slight modification of the method. The 3,4,5 trimethoxybenzaldehyde was replaced with 4-nitrobenzaldehyde. Briefly, the conditions required for optimal colour formation were determined by the studies of the different factors that affect the reaction. The factors were temperature, time, acid concentrations, concentration of 4-nitrobenzaldehyde and diluent used.

Stability of coloured compound

Artesunate was assayed at three different concentrations of 20 µg/ml, 40 µg/ml and 100 µg/ml using 0.2% 4-nitrobenzaldehyde in 10 M sulphuric acid solution. The absorbance of the yellow coloured compound was then observed for three hours at thirty

minutes interval at 474 nm in order to determine the colour stability.

Development of spectrophotometric assay method

Exactly 1.0 ml 4-nitrobenzaldehyde (0.2% w/v) in 10 M sulphuric acid was measured and transferred to 10 ml volumetric flask. Then, 1.0 ml artesunate (100 µg/ml) in methanol was measured and transferred into the flask. This gave an immediate yellow coloured compound which was heated at 80 °C for 10 minutes. It was made to 10 ml with water. The absorbance was read at 474 nm which was the wavelength of maximum absorbance using the UV/Visible spectrophotometer against the blank. A serial dilution which produced final solutions of artesunate (80, 60, 40 and 20) µg/ml was prepared. The procedure was repeated. Their absorbances were read. The Beer's plot was obtained. The limits of detection and quantification were computed using the current International Council for Harmonization Guidelines [19]. Interference from excipient in the developed method was evaluated using standard addition method.

Application of the developed method to analyse artesunate in tablets

The developed method was applied for the quantitative determination of artesunate in five (5) brands of artesunate tablets obtained from pharmacies in Benin City, Nigeria.

Statistical analysis

All the determinations carried out in this study were done in five determinations while the results were presented as Mean ± Standard Deviation (S.D). The results from the assay obtained using the official and the developed colorimetric methods were analyzed by unpaired t-test and $P < 0.05$ was considered significant

Results

The optimization studies showed that the optimal conditions for the reaction was 10 minutes at a temperature of 80 °C in 10 M concentration sulphuric acid using 2% w/v 4-nitrobenzaldehyde. Water was the best diluent for the developed method. The coloured compound was stable for three hours duration when the colour stability was carried out using three concentrations of artesunate in the laboratory at optimal conditions. The result obtained from recovery studies confirmed that there was no interference from pharmaceutical excipients. The spectra shown in Figures 1 and 2 were obtained from scanning solution of 0.2% w/v 4-nitrobenzaldehyde in 10 M sulphuric acid and the product of reaction of artesunate (0.1%w/v) with 4-nitrobenzaldehyde (0.2%) in 10 M sulphuric acid, respectively.

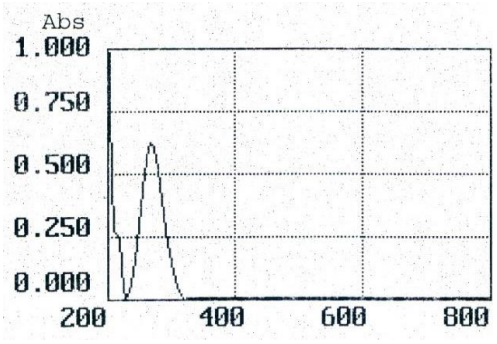

Figure 1: The absorption spectrum of 0.2% w/v 4-nitrobenzaldehyde in 10 M sulphuric acid

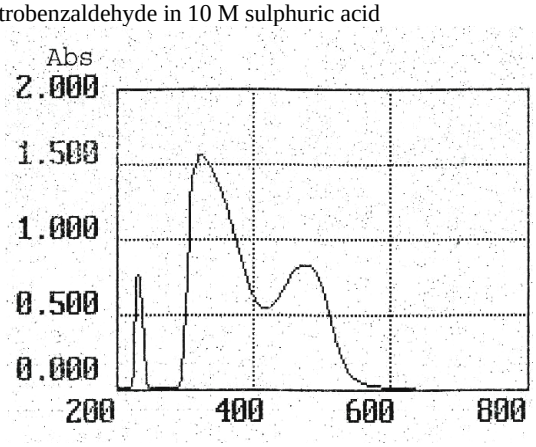

Figure 2: The absorption spectrum of the product of reaction of artesunate (0.1%w/v) with 4-nitrobenzaldehyde (0.2%) in 10 M sulphuric acid

Discussion

This study showed the formation of a yellow coloured product as a result of the reaction between acid decomposed product of artesunate and 4-nitrobenzaldehyde. Acid decomposed product of artesunate is known to possess reactive methylene centre that readily releases a proton (Figure 3) [3,15].

An observation of the spectra in Figures 1 and 2 recorded from wavelength scanning showed that artesunate reacted with 4-nitrobenzaldehyde in acid to form a new compound. The yellow coloured compound produced a spectrum with pronounced bathochromic shift and the wavelength of maximum absorption was 474 nm (Figure 2).

The molar absorptivity obtained was (2.3183 ± 0.011) x 103 $mol^{-1}cm^{-1}$ (Table 1). The value was higher than those reported in studies in which dimethylaminobenzaldehyde ((9.153 ± 0.59) × 102 $mol^{-1}cm^{-1}$) and vanillin (1.19 × 101 $mol^{-1}cm^{-1}$) were used [15,16]. Hence, the method is more sensitive. It is simple and required readily available laboratory reagents that are eco-friendly. Water was required as diluent in the developed method. The study also revealed that there was no interference from commonly used tablet excipients.

Table 1: Analytical parameters for the developed colorimetric method of artesunate using 4-nitrobenzaldehyde

Parameter	Value
Wavelength of maximal absorption (λ max) (nm)	474
Beer's law limits (µg/ml)	20-100
Molar absorptivity (mol^{-1} cm^{-1})	(2.3183 ± 0.011) x 10^3
Coefficient of determination (r^2)	0.9996
Slope (m)	0.0067
Intercept (c)	0.0001
Limit of detection (LOD) (µg/ml)	0.0753
Limit of quantitation (LOQ) (µg/ml)	0.2283

Table 2: Result of percentage drug content of five brands of artesunate tablets applying the official method and the developed method

Brand code	Strength (mg)	Official method	Developed method
A	50	98.66 ± 1.94	99.55 ± 1.37
B	50	93.54 ± 1.72	93.78 ± 1.27
C	100	78.16 ± 1.90	78.05 ± 1.51
D	100	85.85 ± 1.72	84.37 ± 1.48
E	200	101.22 ± 1.82	100.59 ± 1.44

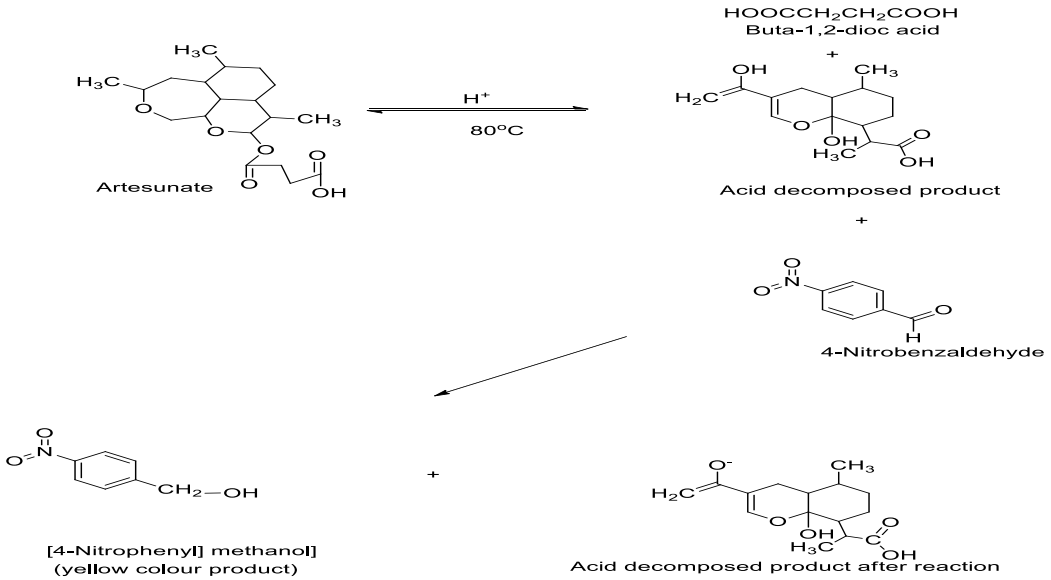

Figure 3: Probable reaction scheme for spectrophotometric assay method of artesunate using 4-nitrobenzaldehyde

The developed colorimetric method was applied in the determination of five (5) brands of artesunate. There was no significant difference in the result obtained when compared with that of the official method (Table 2). $p > 0.05$. The method is as accurate as the official Pharmacopoeia method. The result revealed that three brands met the specification while two failed. The result further, re-affirms the report of substandard artesunate tablets in the Nigerian pharmaceutical market [13,16,20].

Conclusion

The developed method is simple, accurate and affordable for the analysis of artesunate in tablets and raw pharmaceutical materials. It can be used as alternative method for the quantitative determination of artesunate in tablet dosage form and pharmaceutical raw material, where sophisticated facilities like HPLC are not affordable and only limited range of chemicals and reagents are available.

Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. GEA and SAA Conceived and designed the study. GEA performed the experiments. GEA and SAA wrote the manuscript and approved it for publication

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