



## AN INDIRECT SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ARTEMETHER IN PURE AND FIXED DOSE FORMULATIONS

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### ABSTRACT

The increasing use of artemether-lumefantrine combination as an effective treatment for resistant malaria demands the need for analytical methods for the assay of these drugs in tablets and pediatric dosage forms. Some UV spectrophotometric methods have been developed for quantification of artemether in various formulations and biological fluids; the laborious heating required in these methods necessitates the need to develop a simpler spectrophotometric method for the quantification of artemether. We report herein, a method developed by reacting artemether solution in methanol with concentrated HCl for 30 minutes to obtain an  $\alpha,\beta$ -unsaturated ketone with  $\lambda_{max}$  of 260 nm. IR measurement of the  $\alpha,\beta$ -unsaturated ketone obtained revealed a strong band at  $1675\text{ cm}^{-1}$  which is consistent with the range ( $1685 - 1666\text{ cm}^{-1}$ ) for such type of ketones. The method was successfully applied in the assay of artemether in the standard powder and five brands of artemether-lumefantrine suspensions. The method obeyed Beer's law in the range  $20 - 120\text{ }\mu\text{g/ml}$  with regression equation  $A = 0.01C + 0.193$  and correlation coefficient ( $r$ ) 0.9987. The precision (% CV), accuracy (% Er), recovery, detection and quantification limits were found to be 2.0 %, 2.67 %, 97 %,  $0.14\text{ }\mu\text{g/ml}$  and  $0.58\text{ }\mu\text{g/ml}$  respectively. The content of artemether in all the samples assayed was within the official specification (98 – 101.6 %). No significant difference ( $P < 0.05$ ) was observed when the developed method was compared with the International Pharmacopoeial method (IP, 2008) for determination of artemether. The results of the study indicate that the proposed method can be used for quantitative determination of artemether in pure and pharmaceutical dosage forms.

**KEYWORDS:** Artemether, UV spectrophotometry, Conc. HCl, decomposition product

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### INTRODUCTION

Artemether (3R,5aS,6R,8aS,9R,10S,12R,12aR)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin) is a semi synthetic polyoxygenated amorphous compound containing a peroxide bridge that confers potent antimalarial activity. It is the o-methyl ether pro-drug of dihydroartemisinin and a derivative of artemisinin (qinghaosu), the principal antimalarial constituent of the Chinese herb *Artemisia annua* [1].

Artemisinin based combination therapy (ACT) is now the mainstay in malaria treatment despite

reports of gradual development of resistance. ACT is based on the use of two drugs with different modes of action. The rationale is that the short-acting but highly potent artemisinin derivative delivers a rapid and effective reduction in parasite biomass, with the remaining parasites being removed by the intrinsically less active but more slowly eliminated partner drug [2].

World Health Organization (WHO) recommends this drug as first line therapy for falciparum malaria in endemic areas [3]. The increasing use of artemether-lumefantrine combination as an effective

treatment for resistant malaria demands the need of analytical methods for the quality control of these drugs in tablets and suspensions [4].

The available UV Spectrophotometric methods for the analysis of artemether make use of its HCl decomposition product ( $\alpha$ ,  $\beta$  - unsaturated decalone) that absorbs at a wavelength of 254nm [5]. Though this product absorbs strongly at the said wavelength, its formation from artemether requires very vigorous conditions.

The International pharmacopoeia (IP) method for the assay of artemether requires heating in a water-bath at 55 °C for 5 hours before taking its UV absorbance at 254 nm [6]. The method reported by [7] requires heating at 60 °C for three hours before taking the absorbance at 254 nm. A colorimetric method for the assay of artemether and other artemisinins based on the reaction of their acid decomposition product with a dye yielded a coloured derivative which absorbs at 420 nm [8]. The method requires a period of at least one hour for the formation of the product prior to reaction with the dye. Another method developed for the assay of artemether requires heating at 80 °C for 20 minutes [9].

The present study is aimed at developing and validating a sensitive, less time demanding and non-thermal spectrophotometric method for the determination of artemether in pure and pharmaceutical dosage forms.

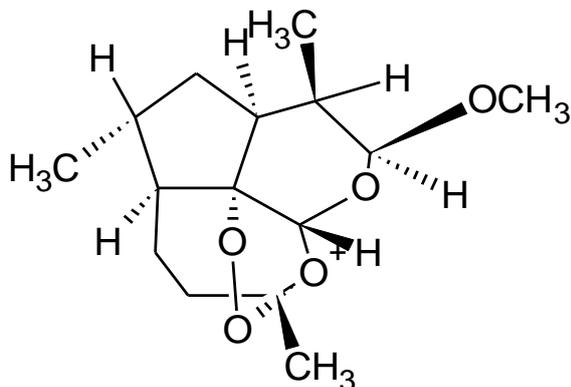


Figure 1: Structure of Artemether

## MATERIALS AND METHOD

### Materials

A double scanning UV/VIS spectrophotometer (Helios Zeta, Model 164617) was used for analyzing artemether content via measurement of its

decomposition product. Mettler AE 240 digital analytical balance (Mettler Instruments Limited, Switzerland). Standard artemether powder was generously provided by Green Life Pharmaceutical limited, five different brands of artemether-lumefantrine powders for suspension were purchased from pharmacy shops in Zaria, Nigeria and given codes (A,B,C,D and E). All the reagents and chemicals used were of analytical grade.

## METHODS

### Identification and assay of artemether (official methods)

Artemether was identified and assayed in both the reference standard and powder for suspension sampled using official methods [6].

### Preparation of standard stock solution of artemether in methanol

A standard solution of artemether was prepared by accurately weighing and dissolving 20mg of pure artemether powder in 20ml of methanol to obtain a concentration 1 mg/ml solution.

### Method development

The method is based on reacting a solution of artemether in methanol with concentrated HCl to obtain an  $\alpha$ ,  $\beta$ -unsaturated ketone decomposition product. From the stock solution, 1 ml was taken and diluted to 10 ml with methanol in a volumetric flask. A portion of this dilute solution (1 ml) was pipetted and transferred to a test tube, thereafter; 1 ml of concentrated HCl acid was added and stoppered. The solution was left to stand for 30 minutes to for the decomposition of artemether into the  $\alpha$ , $\beta$ -unsaturated ketone. IR measurement of the  $\alpha$ , $\beta$ -unsaturated ketone obtained revealed a strong band at 1675  $\text{cm}^{-1}$  which is consistent with the range (1685 – 1666  $\text{cm}^{-1}$ ) for such type of ketones. The solution was then scanned in the spectrophotometer in the range 200 to 600 nm and the maximum absorption was observed at 260nm.

### Preparation of calibration curve

From the stock solution, different aliquots (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) were transferred into series of 10 ml volumetric flask and the volume made up to the mark with methanol to obtain solutions of concentration 20, 40, 60, 80, 100 and 120  $\mu\text{g/ml}$  (0.2-1.2 mg/ml). From each solution, 1 ml was transferred into series of test tubes and then 1 ml of

concentrated HCl acid added to each and stoppered. The solutions were left to stand for 30 minutes and their respective absorbances were determined at 260 nm against the reagent blank. A plot of absorbance against the corresponding concentration gave the calibration curve

### Validation of method

The method was checked for its precision, accuracy, % recovery, limit of detection and limit of quantitation according to ICH guidelines [10].

### Application of the developed method in assay of artemether

For the assay of the content of artemether in the standard and artemether/lumefantrine powders for suspension, each brand (A, B, C, D and E) was weighed and the weight recorded. An amount of powder equivalent to 45 mg artemether from each sample was weighed and transferred to a 100 ml volumetric flask containing about 30 ml of methanol. The volume was made up to the mark with methanol and the content shaken for 10 minutes, then filtered through a Whatman filter paper no. 41. From the filtrate, 1 ml was transferred to a separate 10 ml volumetric flask and each was made up to the mark with methanol to obtain a concentration of 45 µg/ml. A quantity (1 ml) of each of the solutions were transferred separately into five different test tubes and 1 ml of concentrated HCl acid added and left to stand for 30 mins. The absorbance of the contents of each test tube was measured at 260 nm.

The assay results of the proposed method were statistically compared with that of the International Pharmacopoeial method.

## RESULTS

The UV spectrum of the acid decomposition product of artemether when scanned between 200 and 600 nm shows a  $\lambda$  max at 260 nm (Figure 2).

**Table 1; Calibration parameters of the developed method**

Parameter	Values
Beers law range (µg/ml)	20 – 120
Regression equation	$A = Cy + x$
Slope (y)	0.01 0
Intercept (x)	0.193
Correlation coefficient (r)	0.9987

$A =$  absorbance,  $C =$  concentration

The accuracy, precision, percentage recovery, as well as detection and quantitation limits for the developed method are shown in Table 2. Satisfactory percentage recovery, detection and quantitation limits were achieved for artemether in the developed method.

**Table 2; Validation parameters of the developed method**

Parameter	Values
Precision (% CV)	2
Accuracy (% Er)	2.67
Percentage recovery (%)	97
Detection Limit (µg/ml)	0.14
Quantitation Limit (µg/ml)	0.58

Note: CV = coefficient of variation, Er = relative error,  $\lambda_{max}$  = wavelength of maximum absorption

### Assay of artemether in artemether/lumefantrine powders for suspension

The assay results of artemether in the artemether/lumefantrine suspensions for both the developed method and the International Pharmacopoeial method are shown in Table 3. The results indicate that the content of artemether in all the five different brands were within the official limits of 98 – 102.0 % [6].

**Table 3: Assay of artemether in artemether/lumefantrine suspensions using the developed method and IP method**

Brands	Percentage (%±SEM) Drug Content Assayed IP Method (n = 3)	Developed Method (n = 3)
A	101.6±0.35	101±0.35
B	101.6±0.25	99±0.05
C	98±0.8	98.4±0.22
D	99±0.22	98±0.21
E	99.0±0.01	99.8±0.01
Standard	100±0	100.03±0.0

No statistically significant difference between the means ( $P < 0.05$ )

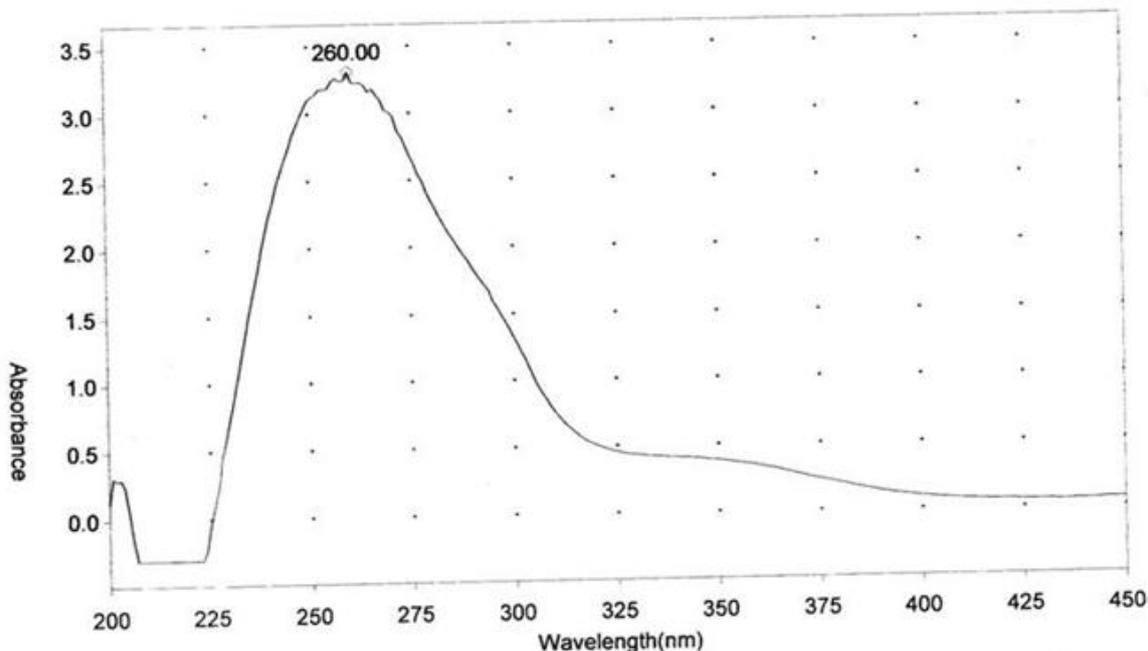


Figure 2: UV Absorption spectrum of decomposition product of artemether

The calibration curve (Figure 3) obeyed the Beer-Lambert's law in the concentration range of 20 – 120 µg/ml.

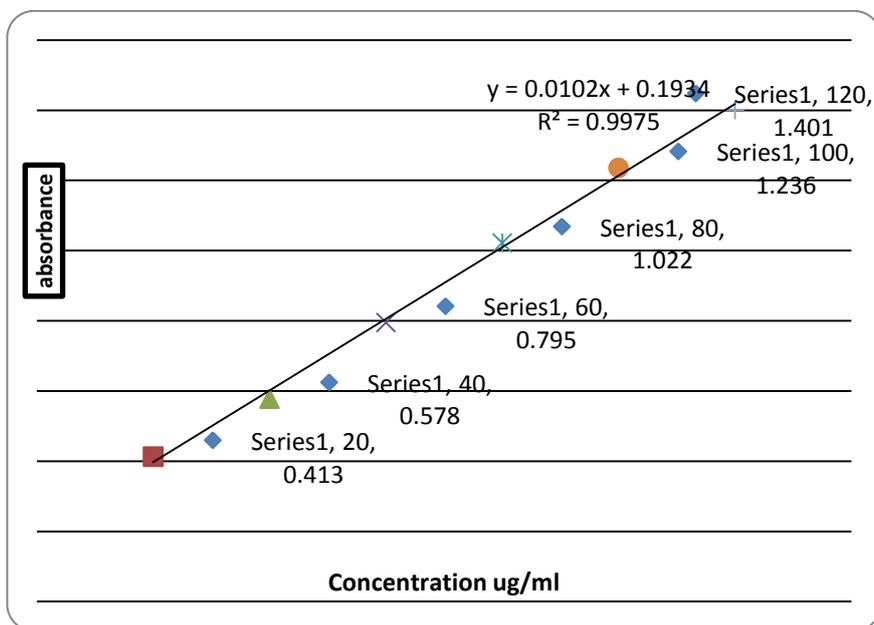


Figure 3: Calibration curve

The linear relationship between absorbance (A) and concentration (C in µg/ml) gives the regression equation  $A = Cy + x$ , with a coefficients of correlation (r) 9.9987 (Table 1).

## DISCUSSION

The coefficient of determination ( $r$ ) for the calibration curve of the developed method clearly shows the direct proportional relationship and high correlation between the absorbance (A) and the respective concentrations (C) used for these determinations (Table 1).

The accuracy (%  $Er$ ) obtained for the developed method is within the acceptable range (1–5 %) for moderately accurate procedure [11]. A percentage accuracy of 8 and 10 was reported by [8] in a colorimetric method. The relatively low percentage coefficient of variation (% CV) which is within the acceptable limit (< 15 % CV) shows the precision of the method has high precision. A % CV of 3–10.4 % was reported for a GC-MS selected ion monitoring method for determination of artemether [12]. This shows that the precision of the developed method is satisfactory. The percentage recovery for the developed method was 97 % and thus, satisfactory. Detection limit (DL) and quantitation limit (QL) for the developed method were 0.14 and 0.58  $\mu\text{g/ml}$  respectively. The DL and QL of this new method are better than the DL and QL of 8 and 25  $\mu\text{g/ml}$  respectively reported by [13] for a HPLC method for determination of artemether.

The most significant advantage the developed method has over the other reported methods [6,7,8,9] for the analysis of artemether is that it does not require any heating. It also has a comparatively short reaction time of only 30 minutes.

The results for assay of artemether in the standard powder and in brands A, B, C, D, and Eartemether/lumefantrine suspensions were within the official limits of 98 – 102 % of the stated amount [6] and is line with results obtained by [9] in a similar study. Statistical analysis revealed no statistically significant difference ( $P < 0.05$ ) between the means of content of artemether assayed by the developed method and the International Pharmacopoeial method. This shows that the method can be used interchangeably with the IP method in routine analysis of artemether.

## CONCLUSION

An improved (requiring no heating) spectrophotometric method for determination of artemether in pure and dosage forms within a relatively short time (30 minutes) was developed and validated. The method, which can be used interchangeably with IP method, was successfully

applied in the assay of fixed dose combination artemether/lumefantrine powder for suspension.

## CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

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