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Research Article

HPLC Method Development And Validation For Determination Of Artemether In Pharmaceutical Dosage Form

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ABSTRACT

A simple, rapid and precise and accurate method was developed for the estimation of Artemether in bulk & tablet dosage form. Analysis was performed on a Phenomenax C18, 250 x 4.6 mm column 82:21 Acetonitrile : water, the mobile phase filtered through to 0.45 µm membrane filter degassed. as mobile phase, a flow rate 1.0 mL/min, column temperature 30°C and UV detection at 216 nm. ART were well resolved on the stationary phase and the retention time for Artemether was 4.509 min. The calibration curves were linear in the concentration range of 50-500 µg/mL for ART. Intra-and inter-day relative standard deviations for both the components were <2.0%. The percentage recoveries obtained for ART within the limit.

INTRODUCTION

Development of simple and reproducible analytical methods for estimation of drugs is very important part of quality control and assurance. Chemically Artemether is 3R,5aS,6R,8aS,9R,10S,12R,12aR)-10-methoxy-3,6,9-trimethyldecahydro-12H-3,12-epoxy[1,2]dioxepino[4,3-i]-2-benzopyran.

Artemether is an antimalarial agent used to treat acute uncomplicated malaria. It is administered in combination with lumefantrine for improved efficacy. This combination therapy exerts its effects against the erythrocytic stages of Plasmodium spp. and may be used to treat infections caused by P. falciparum and unidentified Plasmodium species, including infections acquired in chloroquine-resistant areas.. The structure is shown in fig.1

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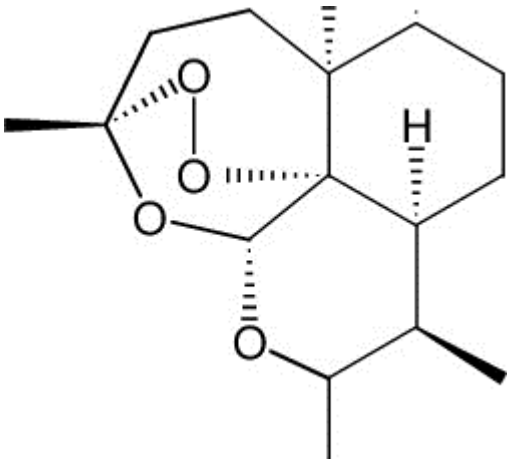


Figure No.1: Structure of Arthemeter

MATERIAL AND METHODS

Apparatus and software

1. HPLC(Agilent) computer loaded EZ Chrom Elite software was used for all the HPLC measurements. The column was a Phenomenax C18, 250 x 4.6 mm column used. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm.

Reagents and materials

Artemether (99.5% purity) was received as gift samples from Micro Orgo Chem. HPLC grade Acetonitrile & Water.

Standard Preparation (Stock Solution):

Preparation of Artemether standard solution Approximately 20 mg of artemether reference standards were accurately weighed on weighing balance. The weighed quantity was transferred to a 100 ml volumetric flask. 7 ml Chloroform was added to the flask to ensure complete solubilization, followed by the addition of 80 ml of acetonitrile. The volume was filled up to the mark with 0.05% trifluoroacetic acid. The resulting solution contained 200 µg/ml of artemether. The solution was filtered through 0.45 µm membrane filter.

Preparation of Sample Solution:

The tablets were weighed and crushed to a finely powdered state. An accurately weighed portion of the powder, equivalent to about 20 mg of artemether, was transferred to a 100ml volumetric flask followed by the addition of 7ml of chloroform. The solution was sonicated for 15 min and addition of 80 ml of acetonitrile. The volume was filled up to the mark with 0.05% trifluoroacetic acid. The solution was filtered through 0.45µm membrane filter.Different batches of test formulations and one batch of reference were analyzed using the validated method. Chloroform was added to ensure the complete solubilization of the samples. Six replicates of each batch were assayed for the analysis.

Selection of Chromatographic Condition:

Proper selection of the method depends up on the nature of the sample, its molecular weight and solubility. The drugs selected in the present study. Thus normal Phase HPLC was selected for the initial separation because of its simplicity, suitability, ruggedness and its wider usage.

Initial Separation Condition:

The mobile phase selected to elute the drug from the stationary phase was methanol & acetonitrile and HPLC grade water, because of its favorable UV transmittance

Preparation of buffer:

0.1g of hexane sulphonic acid was weighed into a 100 ml beaker, dissolved and diluted to 100 ml with HPLC grade water, the flask was shaken until the particles get dissolved and volume was made up to the mark with HPLC grade water. The pH was adjust to 4 with orthophosphoric acid.

Preparation of Mobile Phase:

Acetonitrile HPLC (82%) & Water (21%) were mixed andfiltered through to 0.45 µm membrane filter & degrassed.

Diluent Preparation:

Acetonitrile was used as diluent.

Preparation of Standard Solution: An accurately weighed of 20 mg of artemether was transferred into 100 ml volumetric flask and add 7 ml of Chloroform, addition of 80 ml of acetonitrile. The volume was filled up to 0.05 % of trifluoroacetic

acid. The resulting solution concentration of 200 µg/ml.

OPTIMIZED METHOD

Optimized method for the estimation of Artemether by HPLC was finally achieved by using the following chromatographic condition.

Table No. 1 Optimizes Method

Chromatographic Condition	
Mobile Phase	82:21 Acetonitrile : water, the mobile phase filtered through to 0.45 µm membrane filter & degrassed.
Column	Phenomenax C18, 250 x 4.6 mm
Flow Rate	1.0 ml/min
Wavelength	216 nm
Injection volume	20 µl

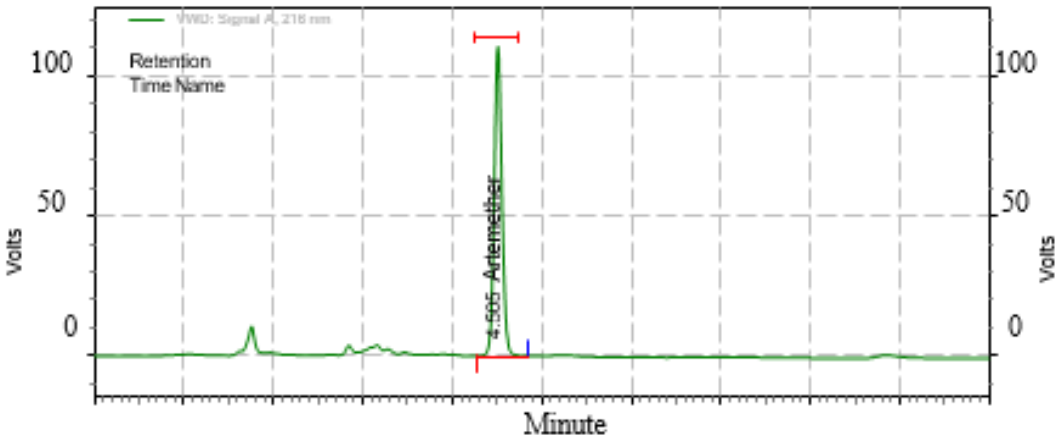


Figure 2. Chromatogram for Optimized Method

RESULT & DISCUSSIONS

VALIDATION:

1. System suitability

Sample solution of Artemether was injected three times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the %RSD of retention times, tailing factor, theoretical plates and peak areas from three replicate injections.

Table No. 2 System Suitability Parameter of Artemether

Injection	Retention Time	Mean Peak area (n=3)	Tailing Factor
1	4.509	11534248	1.22
2	4.504	11439070	1.25
3	4.509	11192819	1.20
4	4.509	11804974	1.22
5	4.509	11568910	1.20
Mean	4.508	11508004.2	1.218
SD	0.0022	221744.75	0.020494
%RSD	0.05	1.93	1.68%

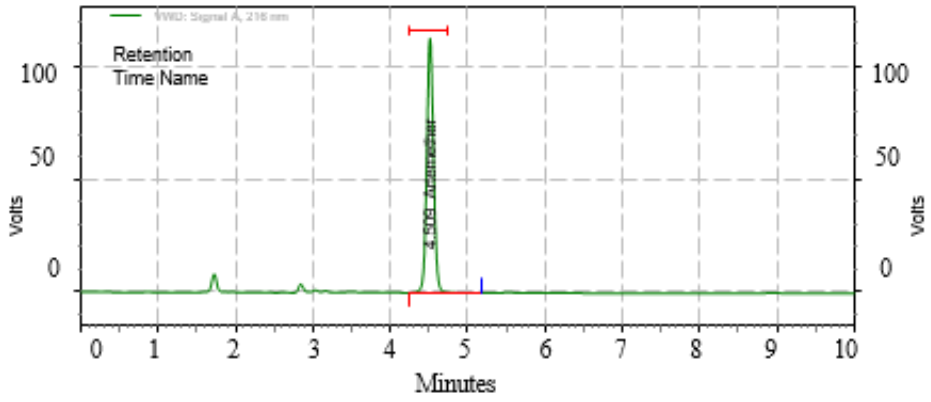


Figure 3. Chromatogram for System suitability

2. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. A particular analytical procedure's lack of specificity may be addressed by additional supporting analytical techniques.

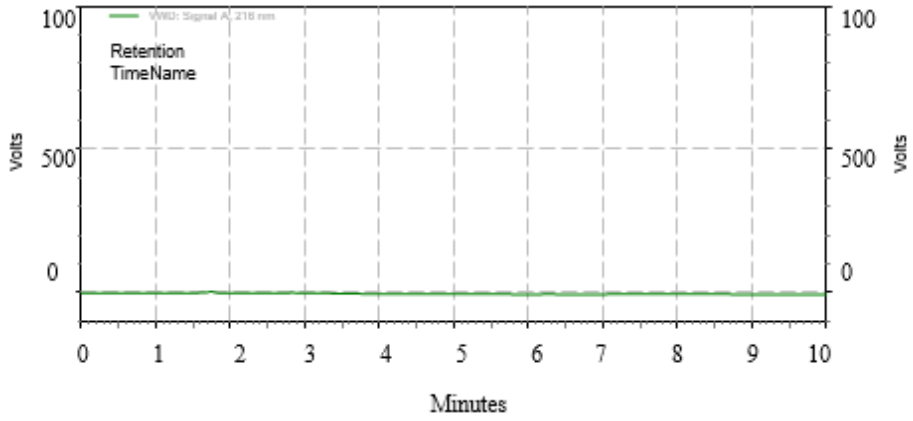


Figure 4. Chromatogram For without sample (Blank)

3. Linearity & Range Studies:

Six different concentration ranging 50% to 500% of artemether labelled claim for linearity standard solution ranging 50µg/ml to 500µg/ml. The calibration curve obtained by plotting peak area against concentration and injected under optimized chromatographic condition.

Table No. 3 Concentration and mean peak area of Artemether for Linearity Study

Sr. No.	Description	Concentration in µg/ml	Mean Area ±%RSD
1.	Linearity 50%	50	2000
2.	Linearity 100%	100	5000
3.	Linearity 200%	200	11000
4.	Linearity 300%	300	17000
5.	Linearity 400%	400	23000
6.	Linearity 500%	500	30000
Regression Equation		**y=61.589x-1243.8**	
Correlation Coefficient(R^2)		**R^2=0.9992**	

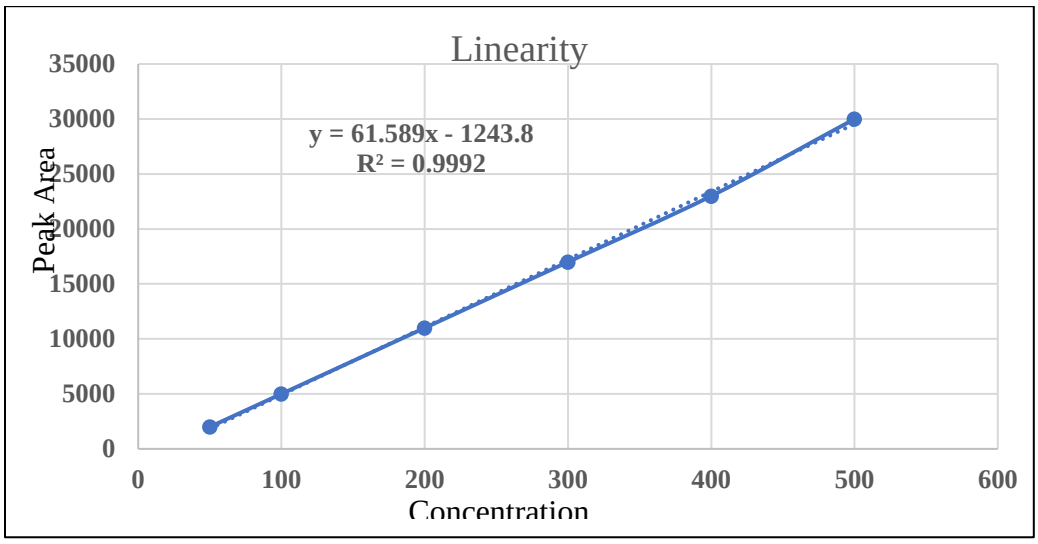
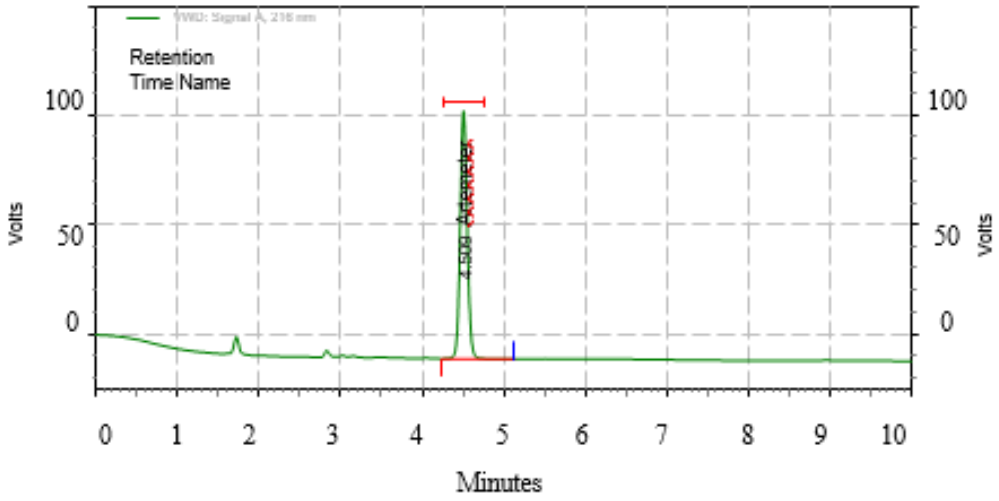


Figure 5. Linearity Studies Artemether

4. Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.



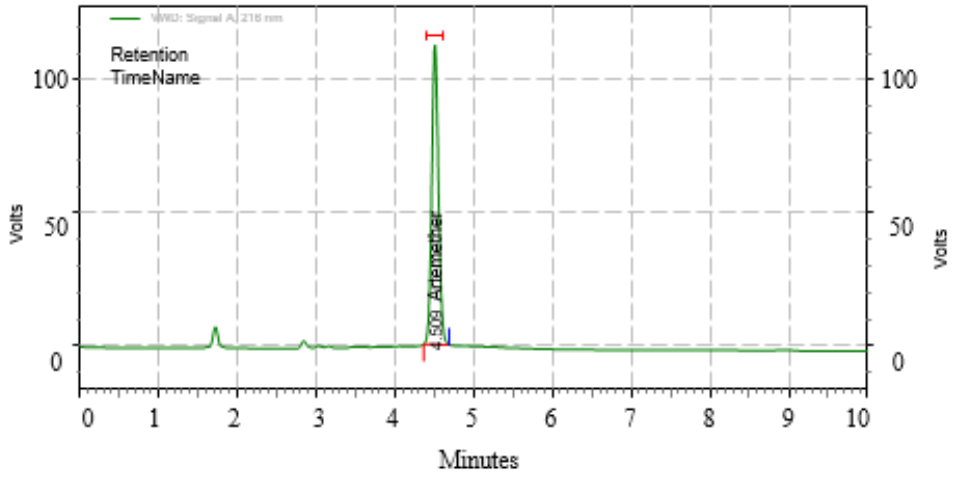
VWD: Signal A, 216 nm Results

Retention Time	Area	Area %	Height	Name
4.509	11605870	100.00	1897747	**Artemether**

Figure 6. Chromatogram for Precision

5. Accuracy (Recovery)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.



VWD: Signal A, 216 nm Results

Retention Time	Area	Area %	Height	Name
4.509	11387499	100.00	1860521	Artemether

Figure 7. Chromatogram for Accuracy (Recovery)

6.Robustness

Robustness studies were carried out using slight modifications in chromatographic such as:

1) Change in flow rate by ±10%
2) Change in pH by ±0.2
3) Change in wavelength by ±2

Table No. 4 Robustness study observation of Artemether with changing the Flow rate

Sr. No.	Altered Condition	R.T.	Peak area	Tailing factor
1	Flow rate (0.9ml/min)	4.504	11439070	1.25
2	Flow rate (1.1ml/min)	4.509	11444273	1.27
Average	--	**4.5065**	**11441671.5**	**1.26**
STD DEV	--	**0.003536**	**3679.076583**	**0.014142**
%RSD	--	**0.078454**	**0.032155062**	**1.122392**

Table No. 5 Robustness study observation of Artemether with changing the pH

Sr. No.	Altered Condition	R.T.	Peak area	Tailing factor
1	pH 4.2	4.502	11292819	1.24
2	pH 3.9	4.504	11144974	1.22
Average	--	**4.503**	**11218896.5**	**1.23**
STD DEV	--	**0.001414214**	**104542.202**	**0.01414214**
%RSD	--	**0.031406031**	**0.93184033**	**1.14976712**

Table No. 6 Robustness study observation of Artemether with changing the Wavelength

Sr. No.	Altered Condition	R.T.	Peak area	Tailing factor
1	Wavelength 214nm	4.505	11605870	1.24
2	Wavelength 218nm	4.507	11534248	1.23
Average	--	**4.506**	**11570059**	**1.235**
STD DEV	--	**0.001414214**	**50644.4019**	**0.00707107**
%RSD	--	**0.031385121**	**0.43771948**	**0.5725561**

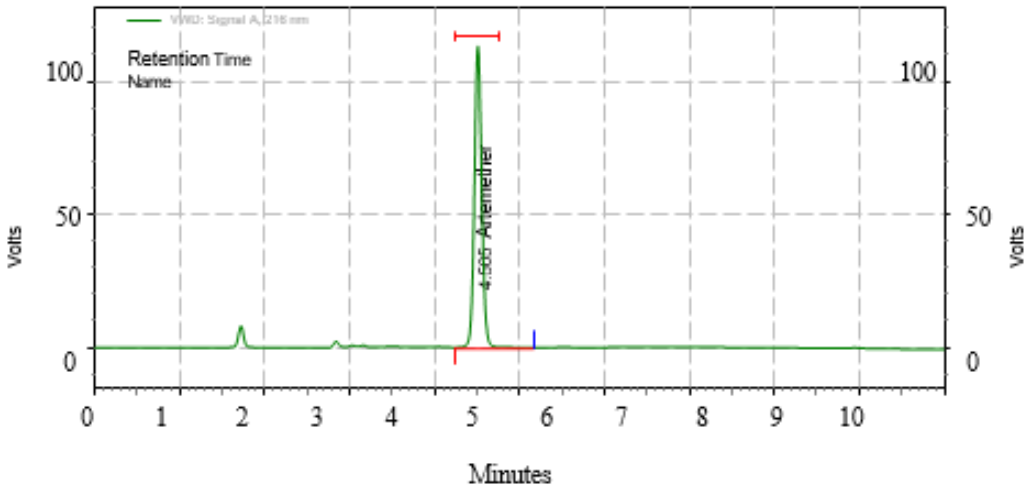


Figure 8. By changing the wavelength by 214nm

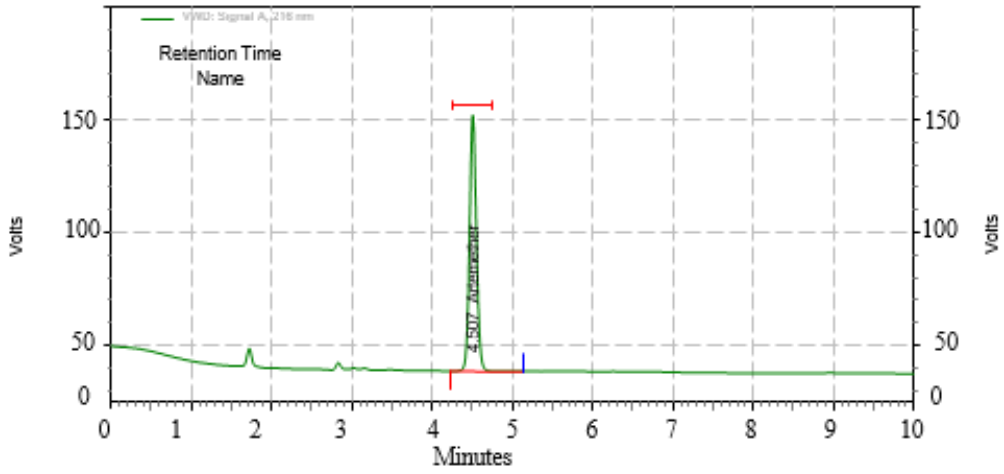


Figure 9. By changing the wavelength by 218nm

SUMMARY & CONCLUSION

The developed HPLC technique is simple, precise and accurate. As the drug is sensitive to degradation, selectivity is an important validation parameter. Statistical analysis proves that the method is reproducible and selective for the analysis of Artemether in pharmaceutical dosage forms. It can be used to determine the purity of the drug available from various sources. This study was conducted to develop a new facile HPLC based analytical method for the determination of artemether (20 mg) in a newly developed

formulation. Various advantages were offered by this method which includes easily constitutable mobile phase and shorter run time with high resolution of the analytes's peaks. This newly developed analytical method has been validated according to parameters provided in ICH guidelines. The method has been found to be very simple and convenient to perform; sensitive and specific for the objective drugs. Moreover the method is accurate, precise and robust over a wide range of analytes's concentration. Therefore, in the light of the study, the proposed method can be used for analysis of formulation of artemether in any analytical setting of either a pharmaceutical industry or research organization or any academic institution which houses an HPLC instrument.

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