

Original Article

ANALYTICAL METHOD DEVELOPMENT & VALIDATION OF ARTEMETHER IN BULK DRUG BY RP- HPLC METHOD AS PER ICH GUIDELINES

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ABSTRACT

Objective: An accurate, precise, rapid & economical RP-HPLC method was developed for the estimation of Artemether as per International Conference on Harmonization (ICH) guideline in pharmaceutical dosage form using ultraviolet (UV) detector.

Methods: Elution was carried out using a mobile phase consisting of Acetonitrile & Methanol (50:50 v/v) and the flow rate was set to 1.6 ml/min at 216 nm, retention time for Artemether was found to be 1.330 min.

Results: The method was found to be linear in the concentration range of 100-600 µg/ml, in the linearity study regression equation was found to be $y = 0.199x - 1.133$ & correlation coefficient was found to be 0.999. This method was Rugged and Robust in different testing criteria, LOD and LOQ were found to be 23.037 µg/ml, 69.809 µg/ml respectively. Accuracy study was done in 3 different concentration level i. e 50, 100, 150% & % recovery of the method was found to be 99.4%, 100.4%, 99.7% respectively in 3 different levels & mean recovery was 99.8%, so method was accurate.

Conclusion: Results of all validation parameters were within the limits as per ICH guidelines.

Keywords: HPLC, Validation, Method Development, Artemether, Accuracy, Precision.

INTRODUCTION

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 *Plasmodium falciparum* and unidentified *Plasmodium* species, including infections acquired in chloroquine-resistant areas. IUPAC Name: (1R,4S,5R,8S,9R,10S,12R,13R)-10-methoxy-1,5,9-trimethyl-11,14,15,16 tetra oxatetracyclo[10.3.1.0^{4,13}.0^{8,13}]hexadecane[1]. It Involves an interaction with ferriprotoporphyrin IX ("heme"), or ferrous ions, in the acidic parasite food vacuole, which results in the generation of cytotoxic radical species. The generally accepted mechanism of action of endo peroxide antimalarials involves interaction of the endo peroxide-containing drug with heme, a hemoglobin degradation byproduct, derived from proteolysis of hemoglobin. This interaction is believed to result in the formation of a range of potentially toxic oxygen and carbon-centered radicals[2]. According to literature review [3-16] there are very few method reported for the determination of Artemether in different instrumental techniques, out of these methods only one method was reported in single Drug by using RP- HPLC. The objective or need of the proposed method is to develop simple and accurate methods for the determination of Artemether by RP-HPLC methods in pharmaceutical dosage forms.

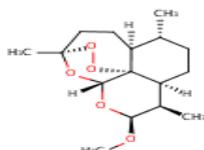


Fig. 1: It Shows structure of Artemether

MATERIALS AND METHODS

Standard drugs

Artemether was procured from the Hetero Pharma, Hyderabad.

Chemicals and reagents

Methanol (Finer chemical Ltd.), Acetonitrile (Rankem chemicals), Purified water ((Rankem chemicals).

Instruments

HPLC (Analytical technologies), UV (Elico SL-196), Detector (UV detector, Analytical technologies), Column (Hypersil ODS C18, (150 *4.6 mm, 5µ), Software (Analchrome, Clarity), Sonicator (Analytical technologies).

Preparation of mobile phase

Accurately measured 50 ml of Acetonitrile mixed with 50 ml of Methanol HPLC grade was degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ nylon filter under vacuum filtration.

Diluent

Mobile phase was used as diluents.

Standard preparation

Accurately weighed 25 mg of Artemether was transferred in to 25 ml volumetric flask and about 10 ml of the solvent mixture was added to dissolve the compound. The solution was cooled after sonication to room temperature and diluted to the mark with the solvent mixture. From this 3 ml was transferred to 10 ml volumetric flask and made up the mark and it was used as working sample to get 300 µg/ml.

Sample preparation

Sample prepared as same as standard preparation.

Method development

Wavelength selection

The test sample was scanned for its absorbance maxima using UV spectrophotometer from 400 nm to 200 nm. The λ max was found to be 216 nm. All scans to Artemether were done using this wavelength.

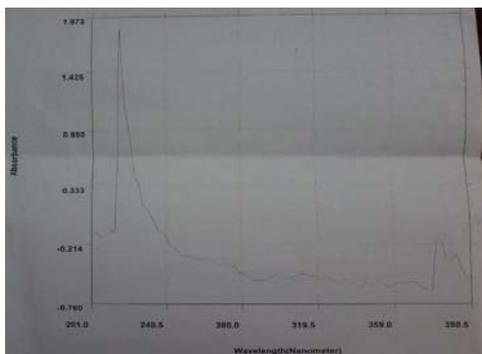


Fig. 2: It Shows UV spectrum of Artemether standard

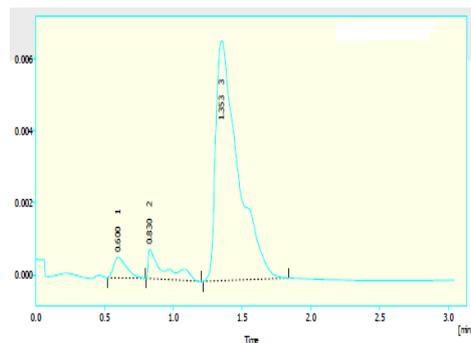


Fig. 3: It Shows Chromatogram of Artemether standard

Optimized chromatographic conditions

Column - Hypersil ODS C18 (150*4.6 mm), 5 μ
 Flow rate - 1.6 ml/min
 Wavelength - 216 nm
 Column temperature - 35c
 Injection volume - 10 μl
 Run time - 5 min

Method validation

The following parameters were considered for the analytical method validation of Artemether in bulk form.

System Suitability

Chromatograph standard preparations (6 replicate injections) and peak area responses for the analyte peak was measured and the system suitability parameters were evaluated.

Accuracy

For accuracy determination, three different concentrations were prepared separately i. e. 50%, 100% and 150% for the analyte and chromatograms were recorded for the same.

Precision

The standard solution was injected for six times and the area was measured for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Robustness

As part of the robustness, deliberate change in the temperature and flow rate variation was made to evaluate the impact on the method.

Linearity and range

Linearity of the analytical method for assay by injecting the linearity solutions prepared in the range of 100μg to 600 μg (33.3% to 200%) of test concentration, into the chromatograph, covering minimum 6 different concentrations.

Ruggedness

Establish the ruggedness of the analytical method by using the assay of 6 different sample preparations of the same batch by a different analyst using a different HPLC system.

RESULTS AND DISCUSSION

Standard preparation

The dissolved standard sample was run in 10 μl aliquots using RP HPLC and the Chromatogram of Artemether was shown in Figs 3 & 4.

And the Artemether’s retention time as a peak was found at 1.33 min during its elution in solvent mixture medium of CAN and Me (50:50v/v). The procedure was used for samples also.

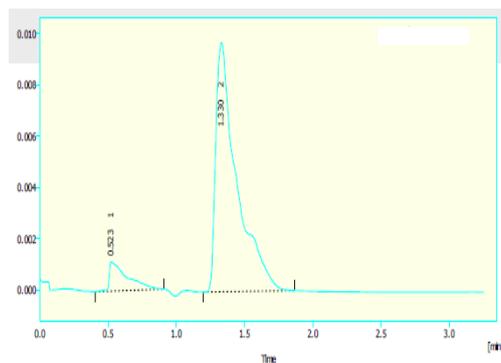


Fig. 4: It Shows Chromatogram of Artemether sample

Validation

Accuracy

Average recoveries of Artemether are 99.4%, 100.4%, 99.7%, at 50%, 100% & 150% concentrations level respectively, with mean percentage recoveries of the drug is 99.8 % which is within the limits 98-102%. So the method is accurate, accuracy data for Artemether are presented in the table 1.

Table 1: Shows Accuracy results of Artemether

Concentration level	Amount added (mg)	Amount found (mg)	%recovery	Average % recovery
50%	12.5 mg	12.5 mg	100%	99.4%
	12.5 mg	12.4 mg	99.2%	
	12.5 mg	12.3 mg	98.4%	
100%	25 mg	25.0 mg	100%	100.4%
	25 mg	24.9mg	99.6%	
	25 mg	25.6 mg	102.4%	
150%	37.5 mg	37.5 mg	100%	99.7%
	37.5 mg	37.6 mg	100.2%	
	37.5 mg	37.3 mg	99.4%	

Table 2: Shows % Recovery of Artemether

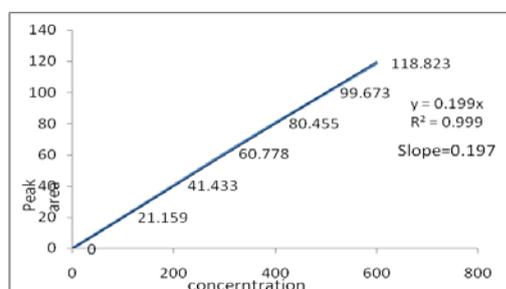
Amount added (mg)	Amount found (mg)	Average % recovery
25 mg	24.95 mg	99.8%

Precision

Precision are summarized in Table 1 , respectively. The %RSD values for precession was less than 2.0%, which indicates that the proposed method is precise.

Table 3: Shows precision results of Artemether

Sample No.	Peak area of Artemether
Injection 1	74.913
Injection 2	72.197
Injection 3	72.943
Injection 4	76.015
Injection 5	74.198
Injection 6	73.602
Mean	73.978
Standard deviation	1.375249505
%RSD	1.8

**Fig. 5: It Shows Calibration graph of Artemether****Linearity**

The response was found linear over a concentration range of 100-600 µg/ml of Artemether. The correlation co-efficient were found to

be 0.999 for Artemether. So the method is linear, data is presented in Table: 4. Linearity curve of Artemether is given in Fig.5:

Table 4: Shows linearity results of Artemether

% level	Concentration (µg/ml)	Peak area
33	100	21.159
66	200	41.433
100	300	60.778
133	400	80.455
166	500	99.673
200	600	118.823
Y Intercept		1.133
Correlation co-efficient (r ²)		0.999
Slope		0.197
Linearity range	100-600	

Robustness

Minor deliberate changes in different experimental parameters such as flow rate (± 0.2 ml) and temperature (± 5 c) did not significantly affect the retention time & peak area of Artemether indicating that the proposed method is robust which is mentioned in table - 5 & 6.

Ruggedness

The method is rugged by different time intervals and the method did not significantly affect the recoveries, peak area and retention time of all the above drugs indicating that the proposed method is rugged which is mentioned in table 7.

Table 5: Shows robustness results of Artemether

S. No.	Flow rate	Peak area of Artemether		Average	SD	%RSD
1	1.4 ml/min	207.377	206.535	206.956	0.5953	0.28
2	1.6 ml/min	103.778	104.889	104.333	0.7855	0.75
3	1.8 ml/min	50.593	50.315	50.454	0.1965	0.38

Table 6: Shows robustness results of Artemether

S. No.	Temperature	Peak area of Artemether		Average	SD	%RSD
1	30°C	66.015	65.432	65.723	0.4122	0.63
2	35°C	103.778	104.889	104.333	0.7855	0.75
3	40°C	60.123	61.521	60.822	0.9885	1.62

Table 7: Shows Ruggedness results of Artemether

Name	Peak area of Artemether
Ruggedness-(Day-1)	84.758
Ruggedness-(Day-2)	84.642
Ruggedness-(Day-3)	85.466
Ruggedness-(Day-4)	85.937
Ruggedness-(Day-5)	84.591
Ruggedness-(Day-6)	85.159
Average	85.0921
SD	0.533562899
% RSD	0.62

LOD (limit of detection)

The **limit of detection** is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected, The LOD are calculated by formula $LOD = 3.3 \times SD / b$ where, SD- standard deviation of the peak area of the drugs, b -is slope of the corresponding calibration curve. LOD for Artemether was 23.037µg/ml.

LOQ

The limit of quantification is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision, The LOQ are calculated by formula $LOQ = 10 \times SD / b$ where, SD- standard deviation of the peak area of the drugs, b -is slope of the corresponding calibration curve. LOQ for Artemether was 69.809 µg/ml.

Table 8: Shows LOD & LOQ results of Artemether

Parameters	Artemether
LOD	23.037 μ g/ml
LOQ	69.809 μ g/ml

From the results of all validation parameter it is shown that all the values are within the limits according to ICH guideline. Summary of the validation parameter is given below in table 9.

Table 9: Shows summary of validation parameter Results

S. No.	Parameter	Acceptance criteria	HPLC
1	% recovery	98-102%	99.8%
2	Linearity range(μ g/ml)	-	100-600(μ g/ml)
3	Correlation coefficient	NLT 0.999	0.999
4	No. of Theoretical plates	NLT 2500	3457
5	Precision	% RSD NMT 2%	1.80
6	Intermediate precision	% RSD NMT 2%	0.62
7	LOD	-	23.037(μ g/ml)
8	LOQ	-	69.809(μ g/ml)

CONCLUSION

Method development & validation of Artemether was done by RP-HPLC method. The estimation was done by using Hypersil C₁₈ (4.6 x 150 mm, 5 μ m, Make: Analytical technologies). Mobile phase was used as Acetonitrile and Methanol in (50:50) ratio at a flow rate 1.6 ml/min, retention time was 1.33 min. at λ_{max} 216 nm. The linearity range of Artemether was found to be within 100-600 μ g/ml. Mean recovery was 99.8 %, which is within 98-102%. Correlation coefficient value was 0.999, % RSD was 1.02 % which is within the limit. These results show the method is accurate, precise, sensitive, economic & rugged. The HPLC method is more rapid. The proposed method can be successfully applied to estimate bulk drug & Tablet dosage form. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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CONFLICT OF INTERESTS

Declared None

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