Annals of Phytomedicine 13(1): 709-714, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.73

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

OriginalArticle : Open Access

Analytical method development and validation of Artemether by RP-HPLC

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Article Info

Article history
Received 8 April 2024
Revised 25 May 2024
Accepted 26 May 2024
Published Online 30 June 2024

Keywords
Artemether
Plasmodium falciparum
Specificity
Robustness
Validation parameters
ICH guidelines

Abstract

The reverse phase-high performance liquid chromatography (RP-HPLC) method, known for its simplicity, accuracy, and reliability, was employed for the estimation and validation of the Artemether assay. Artemether exhibits maximum absorbance at 210 nm and inhibits the erythrocytic phases of *Plasmodium falciparum* by impeding nucleic acid and protein synthesis. The separation was achieved using a waters symmetry column (150 x 3.9 mm, 5 µm). The mobile phase consisted of a mixture of 450 volumes of phosphate buffer and 550 volumes of acetonitrile, which was filtered through a nylon filter 0.45 µm and subjected to 10 min of sonication. Chromatographic conditions included a flow rate of 1.3 ml/min in isocratic elution mode. Following ICH guidelines, the method underwent validation for precision, linearity, specificity, and robustness. The calibration curve demonstrated linearity within the concentration range of 60-160 mcg/ml for Artemether, with a correlation coefficient of 0.99956. This developed method was successfully to the (HPLC) analysis to estimate the validated Artemether tablets.

1. Introduction

Artemether inhibits protein and nucleic acid production, targeting the erythrocytic phases of *plasmodium falciparum*, making it a primary treatment for malaria, particularly infections caused by *P. falciparum* (Epstein *et al.,* 2007; Subhamalar *et al.,* 2023). Often combined with another antimalarial drug like lumefantrine to enhance effectiveness and mitigate drug resistance, the Artemether molecular formula is $C_{16}H_{26}O_5$, it needs to be purged using an inert gas (Guinovart *et al.,* 2006). It dissolves in ethanol, DMSO, and DMF at approximately 16, 10, and 20 mg/ml, respectively, but only partially dissolves in aqueous solutions (Sunil *et al.,* 2010). While its primary approved usage is for malaria treatment, Artemether may be used off-label for other illnesses. High-performance liquid chromatography (HPLC), known for its sensitivity, selectivity, and precision, is widely employed for the quantitative assessment of pharmaceutical compounds (Jean-Pierre Mufusama *et al.,* 2018; Raghavi *et al.,* 2023). Reverse-phase HPLC is particularly beneficial for analyzing hydrophobic substances like Artemether tablets and their related compounds, achieving effective separation and quantification through interaction between a non-polar stationary phase and non-polar analytes dissolved in a polar mobile phase (Pawan *et al.,* 2010). Quality and consistency of pharmaceutical formulations rely on the development and validation of a reliable (RP-HPLC) method for quantifying Artemether and its associated compounds (Ripandeep Kaur *et al.,* 2021). Such a technique should accurately differentiate Artemether from contaminants and breakdown products with high specificity, accuracy, and precision (Bhupinder Singh 2021; Dhritimoni Devi and Sumithra, 2023). Validation in compliance with regulatory standards demonstrates suitability for regular stability testing and quality control analysis. It represents partial validation of an RP-HPLC technique for quantifying Artemether and its associated compounds, ensuring pharmaceutical quality control laboratories and researchers have a dependable analytical instrument through meticulous optimization and validation of parameters (Suleman *et al.,* 2013).

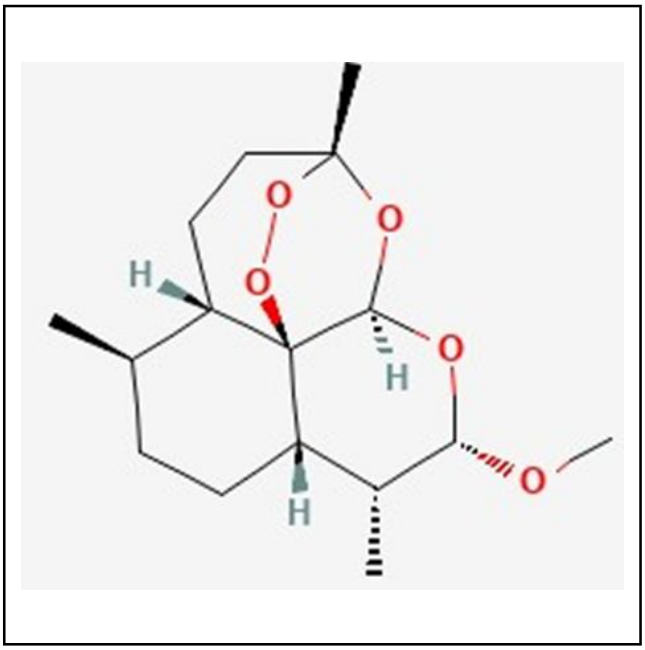

Figure 1: Chemical structure of Artemether.

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RP-HPLC is characterized by a non-polar stationary phase and an aqueous or slightly polar mobile phase. During the method development process, the parameters vary based on the mobile phase, the wavelength at which an asymmetric peak can be formed, column size, and equipment characteristics (Vimal Raj and Sumithra, 2023). It is therefore essential to develop a method that is validated for the specific instrument and column. To demonstrate that the research data obtained from the instrument is reliable for assessing the product, a method must be developed and validated (Tayade *et al.,* 2007; Sumithra *et al.,* 2023). The RP-HPLC process is critical to the discovery, development, and manufacture of pharmaceuticals. Liquid formulations have more advantages over other dosing types. Numerous research investigations have been carried out to establish the appropriate dosage forms of Artemether tablets and their interactions with other drugs. However, it is not common to be raising the dose of a single agent can help to reduce the side effects of the individual drugs (Zubairi *et al.,* 2013).

2. Materials and Methods

2.1 Chemicals and reagents

Ethanol and acetonitrile are used as per HPLC grade and 1-hexane sulphonic acid sodium salt anhydrous, and sodium phosphate monobasic anhydrous, are used as per analytical research grade.

2.2 Standard and sample

Artemether tablet is used as the sample. For standard, it was a gift sample and it was checked. Melting point at 86-88°C.

2.3 Instrument and chromatographic conditions

A high-performance liquid chromatography system, analytical balance, ultra sonicator, pH meter, and vacuum oven were used. The HPLC system with the DAD detector, and water symmetry, (150 x 3.9 mm, 5 µm) was used to achieve chromatographic separation. column oven temperature 25°C. The flow rate is 1.3 ml/min, and the Sample volume injection of the drug was 20 µl, and the detection was detected at 210 nm.

2.4 Mobile phase preparation

2.4.1 Selection of mobile phase

To determine the optimal conditions, various mobile phase compositions were tested by running Artemether in different ratios with the chromatographic system. Among the tested mobile phases, it was found that acetonitrile and phosphate buffer offered the most effective separation of the two components compared to others. Different combinations of the selected mobile phase at various pH levels and flow rates were experimented with the pKa value for these species are approximately 2.15. Ultimately, acetonitrile and phosphate buffer with a pH of 2.3 were selected as the mobile phase in the ratio of 45:55v/v and it less for an environmental condition composition for chromatographic separation of Artemether.

2.4.2 Buffer solution

Weighed and dissolved 2.75 g of sodium phosphate monobasic anhydrous and 5.65 g of sodium salt anhydrous of 1-hexane sulphonic acid in 1000 ml of filtered water, thoroughly mixing. Used diluted phosphoric acid to bring the pH down to 2.3.

2.4.3 Dilute orthophosphoric acid

Dispensed 6.9 ml of orthophosphoric acid into a 100 ml volumetric flask using a pipette, then filled the flask with water.

2.4.4 Mobile phase

A solution was prepared by combining 450 volumes of phosphate buffer with 550 volumes of acetonitrile. The resulting mixture underwent filtration through a 0.45 µm nylon filter and was subsequently subjected to ten minutes of sonication.

2.4.5 Diluent

Mixed buffer, acetonitrile, and ethanol in proportions of 100:300:100. After passing through a nylon filter of 0.45 µm, the solution underwent sonication for 10 min.

2.4.6 Preparation for blank

Use diluent as blank.

2.5 Preparation of solution

2.5.1 Preparation for standard solution

The Artemether working standard, weighing precisely 80 mg, was transmitted to a 250 ml volumetric flask. Following this, 100 ml of diluent was added, and it sonicated for 15 min to ensure dissolution. After cooling, the solution was diluted to volume using diluent and thoroughly mixed. The initial two millilitres of the filtrate were discarded before passing the solution through a nylon filter of 0.45 µm and collecting it in a vial.

2.5.2 Preparation for placebo solution

The procedure commenced with the precise measurement of 29.8267 g of the placebo designated for Artemether, which was then transferred into a 250 ml volumetric flask. Following this, 120 ml of diluent was added to the flask, and manual shaking ensued for 30 min. Subsequently, a 20 min sonication process with intermittent shaking was conducted to facilitate dissolution. After cooling the solution, it was further diluted to the volumetric flask's capacity with additional diluent. The mixture underwent filtration using a 0.45 µm nylon filter to eliminate any particulate matter or impurities. Once filtered, the resulting sample solution was cautiously transferred into an HPLC vial for subsequent analysis. Notably, the initial two millilitres of the filtrate were discarded to eliminate any residual contaminants or impurities.

2.5.3 Preparation for sample solution

The Artemether tablet was dissolved in water according to the labelled instructions. Next, precisely 80 mg (29.64 g) of Artemether material was weighed and transmitted into individual 250 mL volumetric flasks. After adding 120 ml of diluent to each flask, they were mechanically shaken for 30 min. Subsequently, sonication was performed for 20 min to intermittent shaking. The result was filtered through a nylon filter of 0.45 µm. The sample solution was then collected into an HPLC vial, discarding the initial two millilitres of the filtrate.

3. Results

The goal of achieving precision, accuracy, robustness, and specificity was achieved by optimizing and validating the analytical technique in compliance with the most recent ICH guidelines.

3.1 Specificity

The ability of the analytical method to distinguish between the analytes and the other components of the sample matrix is known as its specificity.

Table 1: Specificity analysis of Artemether by RP-HPLC

Name of the solution	Obtained result	Criteria for acceptance
Blank	Absence of peak detected at the excepted retention time of principal peak	There should be no peaks detected at Artemether retention time.
Placebo	No peaks were detected at the retention time of the principal peak.	No peaks are present at the retention time specific to the Artemether.
Sample	Peak detected at expected retention time	No peaks are present at the retention time specific to the Artemether.

3.2 Linearity and range

Linearity is the ability of an analytical procedure to yield test results that, within a given range, are precisely proportionate to the concentration of analyte in the sample.

To determine the linearity of the procedure, five test concentrations, ranging from 60% to 160% of working concentrations, are conducted in compliance with the protocol. The concentrations of 60%, 80%, 100%, 120%, and 160% were used to create the standard solutions in proportion to the 100% working concentration. For every concentration, the HPLC apparatus was filled with three identical injections. Area and concentration are used to produce a graph.

Table 2: Linearity report for Artemether by RP-HPLC

Parameter	Obtained result of Artemether	Criteria for acceptance
Tailing factor derived from five replicative injections	1.03	NMT 3.0
Theoretical plates derived from five replicative injections	9061	NLT 2000
RSD % of the area for five standard injections	0.030	NMT 2.0%

Table 3: Linearity data of Artemether by RP-HPLC

% Level concentration in linearity	Artemether concentration (mcg/ml)	Peak area in (area under curve-1)	Peak area in (area under curve-2)	Average area
60	193.0	1792	1792	1792
80	253.0	2377	2375	2376
100	315.0	2907	2905	2906
120	381.0	3390	3393	3392
160	504.0	4557	4556	4557

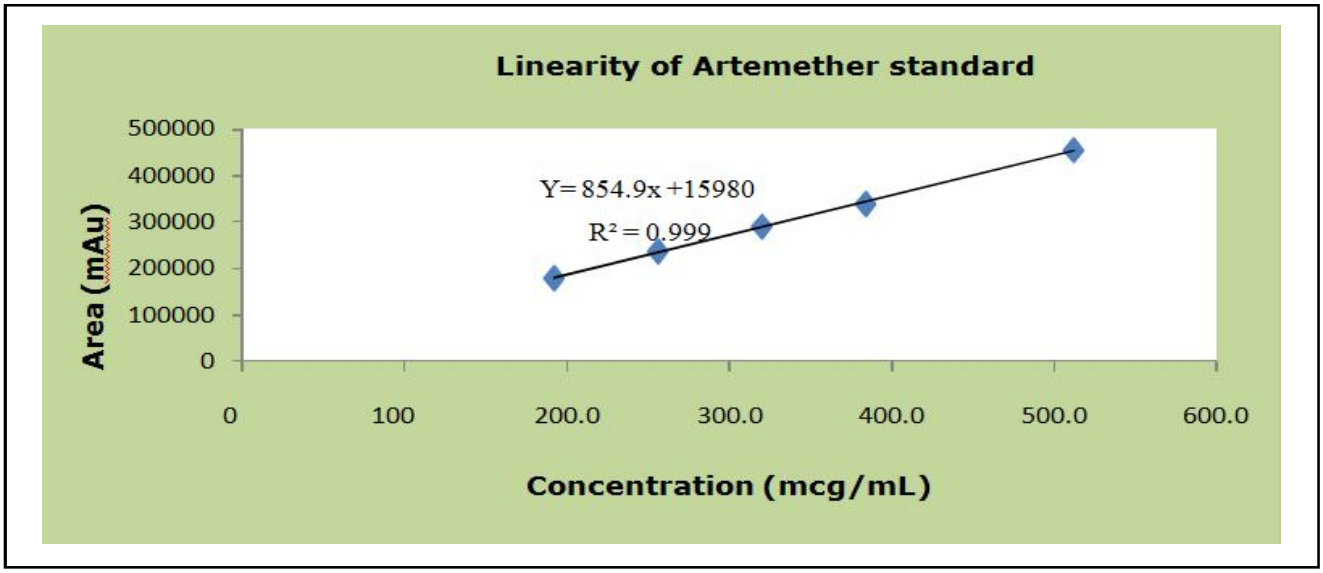

Figure. 4: Linearity graph for Artemether.

Table 4: Linearity analysis of Artemether by RP-HPLC

Parameter	Obtained results of Artemether	Criteria for acceptance
Correlation coefficient	0.9995	NLT 0.998
Intercept	15980.01	Informative
The slope of the regression line	854.93	Informative

3.3 Precision

The degree of agreement (or scatter) between a set of measurements made by regularly taking samples from the same homogeneous sample under predefined conditions is known as precision.

3.3.1 System precision

Table 5: System precision analysis of Artemether by RP-HPLC

Injection	Retention time in min	Peak area in (area under curve)	Tailing factor	Theoretical plate
1	26.8	2891	1.04	9380
2	26.7	2895	1.05	9390
3	26.7	2888	1.02	9391
4	26.8	2894	1.02	9402
5	26.8	2893	1.01	9418
6	26.8	2893	1.01	9399
Mean	26.8	2892	1.04	9396
Std. dev	0.052	289.56	NMT 3.0%	NLT 2000
RSD%	0.2	0.100		
Limit	NMT 1.0%	NMT 2.0%		

3.3.2 Method precision

Table 6: Method precision analysis of Artemether by RP-HPLC

Samples area			Obtained results		Criteria for
Sample-1	Sample-2	Average	Quantity of the drug present in (mg)	% of drug	acceptance
2868	2871	2869	180.4	100.2	**98.0% - 102.0%**
2853	2853	2853	178.7	99.3	
2863	2861	2862	176.9	98.3	
2859	2857	2858	181.5	100.8	
2878	2878	2878	183.4	101.9	
2891	2888	2889	180.4	100.2	
Mean	100.1				
Std. dev	1.242				
RSD	1.240				**NMT 2.0%**

Table 7: Accuracy analysis of Artemether by RP-HPLC

% Level in accuracy	Artemether in (mg)	Artemether % recovered in (mg)	Recovered %	Recovered of mean	Criteria for acceptance
50%	0.0598	0.0600	100.13	100.01%	
	0.0610	0.0607	99.80		
	0.0602	0.0604	100.10		
100%	0.1181	0.1240	101.87	100.67%	**98.0-102.0%**
	0.1228	0.1269	101.26		
	0.1181	0.1145	98.90		
150%	0.4787	0.4816	100.60	100.55%	
	0.4871	0.4950	101.62		
	0.4819	0.4791	99.43		

3.4 Accuracy

The accuracy of an analytical procedure is determined by the degree of agreement between a value found and a value recognized as either a conventional true value or an acceptable reference value. The analytical method of the accuracy 50%, 100%, and 150% of the level of accuracy and its level of average % recovered was found to be in the range of 98.0%-102.0%.

3.5 Robustness

The ability of an analytical procedure to tolerate small but deliberate changes in technique parameters is measured by its robustness, which represents its dependability under ordinary operating conditions. The parameters such as the mobile phase, wavelength, and flow rate were studied and it was six replicates were used to study the robustness the %RSD of the peak areas was reported of each parameter was found to be 0.9%.

Table 8: Robustness analysis of Artemether by RP-HPLC

Parameter	Obtained results		RSD%	Criteria for acceptance
	Artemether in mg	Artemether in %		
Robustness wavelength 212 nm	180.3	100.1	0.9%	**98.0 - 102.0 %**
Robustness wavelength 208 nm	179.5	99.7		
Robustness flow rate 2.2 ml	178.3	99.0	0.8%	
Robustness flow rate 1.8 ml	181.2	100.7		
Robustness mobile phase +5%	178.6	99.2	0.9%	
Robustness mobile phase -5%	179.9	99.9		

4. Discussion

The method developed for Artemether exhibits commendable precision, accuracy, specificity, and linearity based on thorough validation studies. Specificity is purity of peak without any interference of demonstrate and there will not be blank interference in the Artemether and peak by this assay method. In linearity relation between concentrations to detector, hence it is concluded that the range of concentrations, 60% to 160% with respect to 100% working concentration for assay method is linear for Artemether. In the system precision method is reproducibility and reputability the parameters are well within the desirable limits it indicates the prescribed method is suitable to perform the estimation of Artemether, there was no deviation in given method. In the accuracy to identify the % recovery and %level of accuracy 50%, 100%, 150% results pass the criteria for acceptance. The robustness of chromatographic condition slightly changes and remains unaffected and the parameter like flowrate, mobile phase, temperature and wavelengths the %RSD of the peak areas was reported of each parameter was found to be 0.9%.

5. Conclusion

The method developed for Artemether exhibits commendable precision, accuracy, specificity, and linearity based on thorough validation studies. These findings support its suitability for the quantitative analysis of Artemether in pharmaceutical formulations or biological samples. With robust validation results, this method provides a reliable tool for researchers and analysts, ensuring the accurate determination of Artemether content in various contexts such as pharmaceutical quality control.

Acknowledgments

The authors are very much thankful to the Principal and Management, Vels Institute of Science Technology and Advanced Studies (VISTAS), Pallavaram, Chennai for providing fulfillment of all facilities, and also especially thank my guide and co-guide for their valuable guidance.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **M. Sumithra and S. Surya (2024). Analytical method development and validation of Artemether by RP-HPLC. Ann. Phytomed., 13(1):709-714. http://dx.doi.org/10.54085/ap.2024.13.1.73.**