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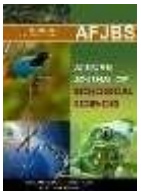

Analytical method development and validation of Lumefantrine by RP-HPLC

P. Shanmugasundaram*, G. Nithish kumar1

[1*,2]Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, Tamil Nadu, India

*[1]Corresponding Author: P. Shanmugasundaram

*Dean. Associate Professor Department of Pharmaceutical Analysis School of Pharmaceutical sciences, Vels University (VISTAS), Chennai, Tamilnadu, India. Email: dean.sps@velsuniv.ac.in Tel.: +91 984012657

ABSTRACT

A reversed-phase high-performance liquid chromatography (RP-HPLC) utilizing a Waters Symmetry column (150 x 3.9mm, 5µm) emerged as a reliable and straightforward approach for accurately estimating and validating Lumefantrine assay. Lumefantrine, which exhibits maximum absorbance at 380 nm, interacts with hemin generated during hemoglobin breakdown, thereby impeding detoxification to crystalline malaria pigment.The mobile phase, consisting of a mixture of 450 parts buffer and 550 parts acetonitrile, underwent preparation, filtration through a 0.45 µm nylon filter, and subjected to 10 min of sonication. Chromatographic separation was executed at a flow rate of 1.3 ml/min via Isocratic elution Mode. Following the International Council for Harmonisation (ICH) guidelines, the method underwent validation for precision, accuracy, linearity, specificity, and robustness. The calibration curve displayed linearity within the concentration range of 60-160 mcg/ml, boasting a correlation coefficient of 0.99911. This HPLC analysis method proved effective in estimating and validating Lumefantrine tablets without compromise on accuracy or reliability.

Keyword: Lumefantrine, Hemoglobin, Mobile phase, Precision, Specificity, Robustness, Linearity, Method Development, Validation, ICH guidelines.

1. Introduction

Lumefantrine, when combined with artemether, serves as a primary treatment for uncomplicated malaria caused by Plasmodium falciparum, particularly in regions where resistance to other antimalarial drugs has emerged (Epstein et al., 2007; Subhamalar et al., 2023). With a molecular formula of $C_{30}H_{32}Cl_3NO$, Lumefantrine exhibits poor solubility in water, approximately 0.0074 mg/ml at 25°C. However, its solubility improves in organic solvents such as ethanol and methanol

(Guinovart et al., 2006). High performance liquid chromatography (HPLC) is extensively utilized for the quantitative analysis of pharmaceutical substances due to its sensitivity, selectivity, and precision (Sunil et al., 2010). Reverse-phase HPLC (RP-HPLC) proves especially advantageous for hydrophobic compounds like
lumefantrine and related molecules (Jean-Pierre Mufusama et al., 2018; Raghavi et al., 2023). RP-HPLC employs a non-polar stationary phase that interacts with non-polar analytes dissolved in a polar mobile phase, facilitating effective separation and quantification (Pawan et al., 2010). The development and validation of a reliable RP-HPLC method are vital for ensuring the quality and consistency of pharmaceutical formulations containing lumefantrine (Ripandeep Kaur et al., 2021). Such a method must demonstrate high specificity, accuracy, and precision in distinguishing lumefantrine from contaminants and breakdown products (Bhupinder Singh 2021). Additionally, it should be validated according to regulatory standards to establish suitability for routine stability testing and quality control analysis. This article presents the development and partial validation of an RP-HPLC technique for quantifying lumefantrine and its associated compounds. By optimizing technique parameters and validating key aspects, pharmaceutical quality control laboratories and researchers involved in lumefantrine tablet formulation can rely on this analytical tool to deliver accurate and trustworthy results (Suleman et al., 2013).

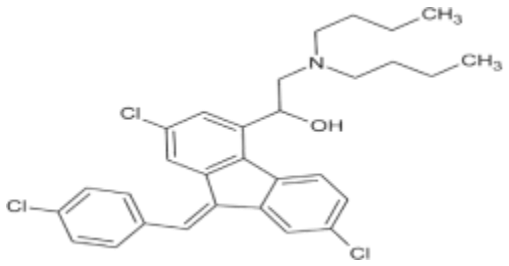

Figure. 1: Chemical structure for lumefantrine.

Material and method

Chemical and reagent

Ethanol and acetonitrile is used as per HPLC grade and anhydrous sodium salt of hexane sulfonic acid, anhydrous monobasic sodium phosphate, sodium phosphate monobasic anhydrous, are used as per analytical research grade.

Standard and sample

Lumefantrine tablet sample is used as the sample. For standard it was a gift sample and it checked.

Instrumental and chromatographic condition

A suite of laboratory equipment was utilized in the experiment, including a high-performance liquid chromatography (HPLC) system, an analytical balance, an ultrasonicator, a pH meter, and a vacuum oven. The HPLC system featured a diode array detector (DAD) and employed a waters Symmetry column with dimensions of (150 x 3.9 mm) and a particle size of 5 µm for chromatographic separation. The mobile phase flow at a rate of 1.3 ml/min and 20 µl of a drug sample was injected for analysis. Detection of compounds was conducted at a wavelength of 380 nm. The analytical balance ensured precise measurements, while the ultrasonicator facilitated sample preparation by degassing solvents and dispersing particles. The pH meter enabled the monitoring and adjustment of solution acidity or alkalinity, crucial for maintaining experimental conditions. Additionally, the vacuum oven provided a controlled environment for drying heat-sensitive materials under reduced pressure, preventing thermal degradation of the sample.

Preparations

Selection of mobile phase

A range of mobile phase compositions was investigated to optimize the conditions for simultaneous determination of lumefantrine. These compositions included methanol: water, acetonitrile: water, acetonitrile, pH 6.8, and acetonitrile pH 2.3 phosphate buffer. Among these options, the combination of acetonitrile and phosphate buffer provided the most effective separation compared to other mobile phases. During the experimentation, different proportions of acetonitrile and phosphate buffer were tested, along with variations in pH levels and flow rates After careful evaluation, it was concluded that the optimal mobile phase composition for chromatographic separation of lumefantrine was acetonitrile combined with phosphate buffer at pH 2.3. This selection was based on its superior ability to achieve the desired level of separation efficiency and resolution for lumefantrine from other components in the sample.

Buffer solution

A solution was prepared by dissolving 5.65 g of 1-hexane sulfonic acid sodium salt anhydrous and 2.75 g of sodium phosphate monobasic anhydrous in 1000 ml of purified water, ensuring complete mixing. Subsequently, the pH of the solution was adjusted to 2.3 ml using dilute phosphoric acid.

Mobile phase

A mixture was prepared by combining 450 volumes of buffer with 550 volumes of acetonitrile, ensuring thorough mixing. The resulting solution was then filtered through a 0.45 µm nylon filter and subjected to sonication for ten minutes.

Diluent

Buffer, ethanol, and acetonitrile were combined proportions of 100:100:300. The resulting mixture was passed through a 0.45 µm nylon filter and subjected to sonication for ten minutes. Diluent was used as blank.

Preparation of Solutions

Standard solution

Weigh accurately out 30 mg of the working standard for lumefantrine were added, then the mixture was put into a 250 ml volumetric flask. 100 ml of diluent was added and subjected the mixture to sonication for 15 min until fully dissolved. Upon cooling, the solution was diluted to the mark with diluent, thoroughly mixed, and filtered through a nylon filter 0.45 µm.

Subsequently, the filtered solution was collected in HPLC vial, with the initial 2 ml of the filtrate discarded.

Placebo

Accurately measured 1.8391 g of lumefantrine placebo (considering a density of 1.1215 g/ml) and transferred it into individual 250 ml volumetric flasks. Added 120 ml of diluent to each flask and mechanically shook them 30 min, followed by 20 min of sonication with intermittent shaking until complete dissolution. After cooling, each solution was brought to volume with diluent. The solutions were then combined and filtered through a nylon filter 0.45 µm. After discarding the first 2 ml of the filtrate, the resultant sample solution was collected in a vial.

Sample

Lumefantrine tablets were dissolved in water to reach the specified volume indicated on the label. Approximately 30 mg of lumefantrine was accurately weighed and transmitted into individual 250 ml volumetric flasks. To each flask, 120 ml of diluent was added, and the mixture was mechanically shaken for 30 min. Subsequently, sonication was performed for 20 min with intermittent shaked until complete dissolution. After cooling, each solution was diluted to the mark with diluent.

Following this, the solutions from the individual volumetric flasks were combined and filtered through a 0.45 µm nylon filter. The initial 2 ml of the filtrate were discarded, and the resultant sample solution was collected in an vial for further analysis.

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.

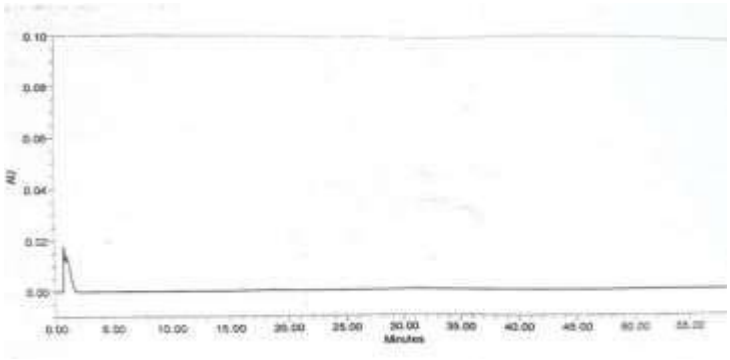

Figure. 2: Blank chromatogram.

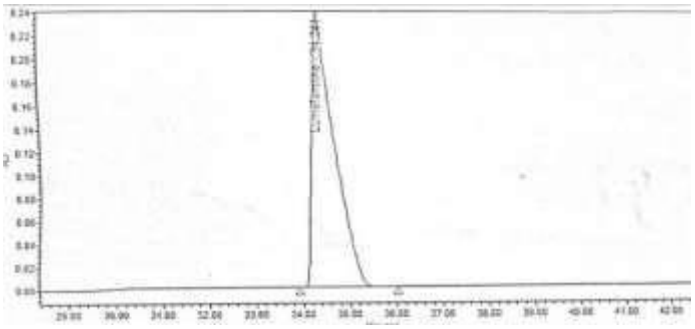

Figure. 3: Standard chromatogram.

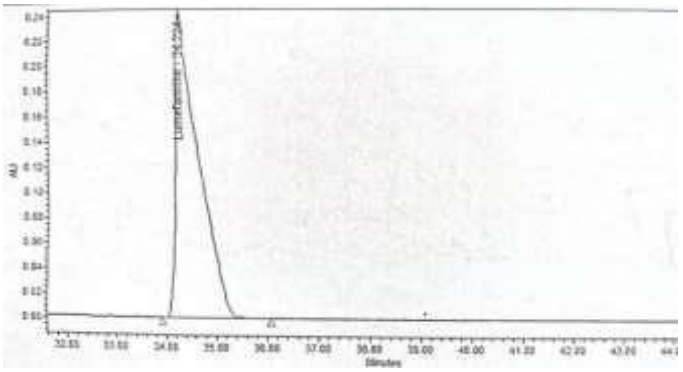

Figure. 4: Sample chromatogram.

Specificity

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

Table No 1: Specificity result

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 lumefantrine's retention time.
Placebo	No peaks detected at the Retention time of the principal peak.	No peaks should be present at the retention time specific to lumefantrine.
Sample	Peak detected at the expected Retention time.	Peak should be present athe retention time specific to lumefantrine.

Linearity

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 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

Parameter	Obtained result	Criteria for acceptance
	Lumefantrine	
Tailing factor derived from five replicative injections	2.28	NMT 3.0
Theoretical plates derived from five replicative injections	4096	NLT - 2000
% RSD for five std injection	0.027	NMT - 2.0%

Table 3: Linearity data

% Level concentration in linearity	Lumefantrine concentration mcg/ml	Peak area in (AUC)-I	Peak area in (AUC)- II	Avg area
60	72 .0	2979	2977	2978
80	96 .0	3983	3979	3981
100	120.0	4876	4886	4881
120	144.0	5662	5663	5663
160	192.0	7694	7724	7709

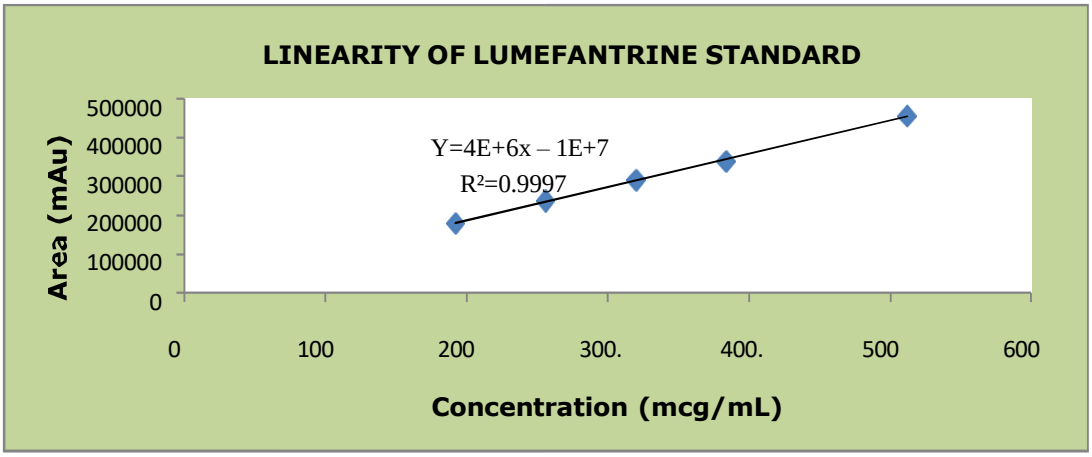

Figure. 5: Linearity Graph Obtained for Lumefantrine.

Table. 4: Linearity result

Parameter	Obtained results	Criteria for acceptance
	Lumefantrine	
Correlation coefficient	0.9997	NLT 0.998
Intercept	190449.3	Informative
Slope of regression line	38882.0	Informative

Precision

System precision

Precision refers to the level of agreement or consistency between multiple measurements obtained from the same homogeneous sample under predefined conditions. It reflects the degree of scatter or variability among these measurements.

Table- 5: System precision result

Injection	Retention	Area	Tailing factor	Theoretical plate
1	33.95	4972	2.33	3836
2	34.23	4911	2.31	3854
3	34.35	4913	2.29	3848
4	34.28	4927	2.35	3841
5	34.33	4932	2.30	3834
6	34.16	4935	2.34	3845
Mean	34.21	4933	2.32	3843
Std. dev	0.009	2440.94	NMT 3.0%	NLT 2000
%RSD	0.071	0.48		
Limit	NMT 1%	NMT 2%		

Method precision

The extent of consistency or scatter observed among a series of measurements obtained by consistently sampling from a uniform sample under predetermined conditions is termed precision.

Table- 6: Method precision result

Samples area			Obtained results		Criteria for acceptance
Sample-I	Sample-II	Avg	Number of drug present in (mg)	Drug in%	
4464	4472	4468	1087.8	100.7	98.0% - 102.0%
4456	4462	4459	1073.7	99.4	
4429	4429	4429	1066.5	98.7	
4456	4458	4459	1079.1	99.9	
4489	4495	4492	1081.6	100.1	
4441	4443	4442	1075.6	99.5	
			Mean	1077.4	
			Std. dev	7.296	NMT 2.0%
			RSD	0.677	

Accuracy

The accuracy of an analytical procedure is assessed by evaluating the extent of agreement between the obtained value and a known true value or an accepted reference value.

Table No 7: Accuracy results

Level % in accuracy	Lumefantrine in (mg)	Lumefantrine recover in(mg)	% Recovered	% Recovered mean	Criteria for acceptance
50%	0.0632	0.0640	100.26	100.66%	102.0% -98.0%
	0.0644	0.0646	100.35		
	0.0640	0.0642	100.37		
	0.1241	0.1251	100.77		
100%	0.1213	0.1221	100.66	100.66%	
	0.1225	0.1232	100.54		
150%	0.1870	0.1901	101.68	101.59%	
	0.1838	0.1867	101.60		
	0.1917	0.1946	101.50		

Robustness

The robustness of an analytical procedure gauges its capability to withstand minor, intentional variations in technique parameters. It reflects its reliability under typical operating conditions.

Table 8: Robustness result

Parameter	Obtained results		Criteria for acceptance
	Lumefantrine in mg	Lumefantrine in%	
Robustness wavelength 212 nm	1085.5	100.4	98.0% - 102%
Robustness wavelength 208 nm	1083.2	100.3	
Robustness flow rate 2.2 ml	1083.5	100.2	
Robustness flow-rate 1.8 ml	1077.6	99.6	
Robustness Mobile phase +5%	1079.2	99.9	
Robustness mobile phase -5%	1086.2	100.5	

3. 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 lumefantrine 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 lumefantrine. 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 lumefantrine there was no deviation in given method. In the accuracy to identify the % recovery and %level of accuracy 50%, 100%, 150% results passes the criteria for acceptance. The robustness of chromatographic condition slightly change and remains unaffected and the parameter like flowrate, mobile phase, temperature and wavelength of the peak areas was reported.

4. Conclusion

The lumefantrine developed method exhibits a strong and reliable analytical performance. The validation results attest to the method's compliance with regulatory standards in terms of accuracy, precision, specificity, and linearity. The quality and integrity of results in pharmaceutical applications can be guaranteed by using this method with confidence for the quantitative measurement of lumefantrine in different pharmaceutical formulations or biological samples.

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

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