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RP-HPLC Method for The Simultaneous Estimation of Lumefantrine and Artemether in Pure Form and Tablet Dosage Form

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Abstract
A new, simple, accurate, precise and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the separation and quantification of Lumefantrine and Artemether in pure form and tablet dosage form. The determination was carried out using Symmetry C18 ODS (4.6mm×250mm, 5µm) particle size as a stationary phase and mobile phase comprised of Methanol: TEA Buffer (36:64v/v) and the pH of tri ethyl amine buffer adjusted to pH-4.2 using orthophosphoric acid. The flow rate was maintained at 1.0 ml/min and the eluent was monitored at 296nm.The retention time of Lumefantrine and Artemether were 2.249 min and 5.430min respectively. The method was validated in terms of linearity, precision, accuracy, specificity and robustness. The method was linear and for precision studies; RSD for RIS AND HPD were 0.02 and 0.04 respectively. The percentage recoveries for both drugs from their tablets were 100.2203 and 100.60% respectively. The method precision for the determination of assay was below 2.0 %RSD. The method is useful in the quality control of pharmaceutical formulations.

Keywords
Lumefantrine and Artemether, Method Development, Validation, Accuracy.

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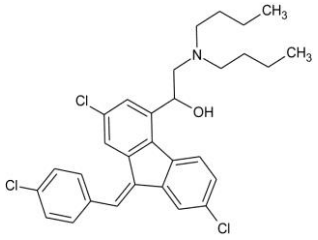
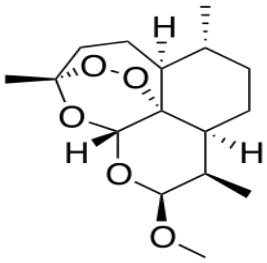
INTRODUCTION
Lumefantrine is an antimalarial agent used to treat acute uncomplicated malaria. It is administered in combination with artemether for improved efficacy. This combination therapy exerts its effects against the erythrocytic stages of Plasmodium spp. and may be used to treat infections caused by P. falciparum and unidentified Plasmodium species, including infections acquired in chloroquine-resistant areas. Its chemical name is 2-(dibutylamino)-1-[(9Z)-2,7-dichloro-9-[(4-chlorophenyl) methylidene]-9H-fluoren-4-yl] ethan-1-ol and having molecular weight of 528.94 gm/mole. Lumefantrine is freely soluble in DMF, chloroform and ethyl acetate, soluble in dichloromethane, slightly soluble in ethanol and methanol, and insoluble in water. The exact mechanism by which lumefantrine exerts its antimalarial effect is unknown. However, available data suggest that lumefantrine inhibits the formation of β-hematin by forming a complex with hemin and inhibits nucleic acid and protein synthesis (Tripati K et al 2010, Suhas Sahebrao Khandave et al 2010, Arun R et al 2010, Srivasthava et al 2010).

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. Its chemical name is (1R, 4S, 5R, 8S, 9R, 10S, 12R, 13R)-10-methoxy-1, 5, 9-trimethyl-11, 14, 15, 16-tetraoxatetracyclo [10.3.1.0^4, ^{13}.0^8, ^{13}] hexadecane and having molecular weight of 298.3746 gm/mole. Artemether is freely soluble in acetone, soluble in methanol and ethanol, and practically insoluble in water. Artemether is soluble in organic solvents such as ethanol, DMSO, and dimethyl form amide, which should be purged with an inert gas. Artemether and lumefantrine are both highly bound to human serum proteins in vitro (95.4% and 99.7%, respectively). Dihydro artemisinin is also bound to human serum proteins (47% to 76%) (Singh Pratiksha et al 2017, Tripathi KD et al 2010 and Martindale 2009).



Lumefatrine



Artemether

METHODS AND MATERIALS

Instrument used: WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.

HPLC METHOD DEVELOPMENT:

Preparation of standard solution: Accurately weigh and transfer 10 mg of Lumefantrine and Artemether working standard into a 10 ml of clean dry volumetric flasks add about 7 ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 1ml of the Lumefantrine and 0.2 ml of the Artemether stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer (pH-4.2) in proportion 36:64 v/v respectively.

Optimization of Column: The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry C18 ODS (4.6 mm×250 mm, 5 µm) particle size was found to be ideal as it gave good peak shape and resolution at 1 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature: Ambient
Column: Symmetry C18 ODS (4.6 mm×250 mm, 5 µm) particle size
Mobile phase: Methanol: TEA Buffer (pH-4.2) (36:64 v/v)
Flow rate: 1ml/min
Wavelength: 296 nm
Injection volume: 20 µl
Run time: 10 min

METHOD VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Triethylamine (TEA) buffer (pH-4.2): Dissolve 1.5 ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.2. Filter and sonicate the solution by vacuum filtration and ultra-sonication.

Preparation of mobile phase: Accurately measured 360 ml (36%) of Methanol and 640 ml of buffer (64%) a were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY: Accurately weigh and transfer 10 mg of Lumefantrine and 10 mg of Artemether working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1 ml of the Lumefantrine and 0.2 ml of the Artemether stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

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

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Lumefantrine and 10mg of Artemether working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1 ml of the Lumefantrine and 0.2 ml of the Artemether stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Lumefantrine and Artemether sample into a 10 mL clean dry volumetric flask and add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1 ml of the Lumefantrine and 0.3 ml of the Artemether stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Lumefantrine and 10 mg of Artemether working standard into a 10ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (60 ppm of Lumefantrine and 10ppm of Artemether):

Pipette out 0.6 ml of Lumefantrine and 0.1 ml of Artemether stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (80 ppm of Lumefantrine & 15ppm of Artemether):

Pipette out 0.8 ml of Lumefantrine and 0.15 ml of Artemether stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (100 ppm of Lumefantrine and 20ppm of Artemether):

Pipette out 1 ml of Lumefantrine and 0.2 ml of Artemether stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (120 ppm of Lumefantrine and 25ppm of Artemether):

Pipette out 1.2 ml of Lumefantrine and 0.25 ml of Artemether stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (140 ppm of Lumefantrine and 30ppm of Artemether):

Pipette out 1.4 ml of Lumefantrine and 0.3 ml of Artemether stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

REPEATABILITY

Preparation of Lumefantrine and Artemether Product Solution for Precision:

Accurately weigh and transfer 10 mg of Lumefantrine and 10 mg of Artemether working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1 ml of the Lumefantrine and 0.2 ml of the Artemether stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1: The standard solution was injected for six times and measured the area for all six injections in

HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
DAY 2: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
Accuracy:
For preparation of 50% Standard stock solution: Accurately weigh and transfer 10 mg of Lumefantrine and 10 mg of Artemether working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.5 ml of the Lumefantrine and 0.1 ml of the Artemether stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.
For preparation of 100% Standard stock solution: Accurately weigh and transfer 10 mg of Lumefantrine and 10 mg of Artemether working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1 ml of the Lumefantrine and 0.2 ml of the Artemether stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.
For preparation of 150% Standard stock solution: Accurately weigh and transfer 10 mg of Lumefantrine and 10 mg of Artemether working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1.5 ml of the Lumefantrine and 0.3 ml of the Artemether stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.
Procedure: Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Lumefantrine and Artemether and calculate the individual recovery and mean recovery values.
ROBUSTNESS:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.
For preparation of Standard solution: Accurately weigh and transfer 10 mg of Lumefantrine and 10 mg of Artemether working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1 ml of the Lumefantrine and 0.2 ml of the Artemether stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.
Effect of Variation of flow conditions: The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1 ml/min, remaining conditions are same. 20 µl of the above sample was injected and chromatograms were recorded.
Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. Methanol: TEA Buffer was taken in the ratio and 41:59, 31:69 instead (36:64), remaining conditions are same. 20 µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)
Mobile phase: Methanol: TEA Buffer (pH-4.2) (36:64 v/v)
Column: Symmetry C18 ODS (4.6 mm×250 mm, 5 µm) particle size
Flow rate: 1 ml/min
Wavelength: 296 nm
Column temp: Ambient
Injection Volume: 20 µl
Run time: 10 minutes

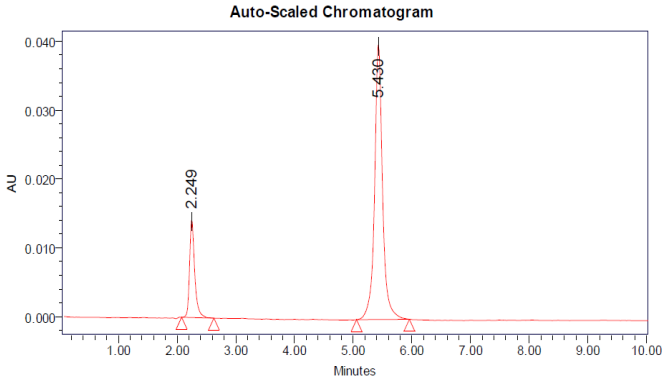


Fig1-: Optimized Chromatogram
Table1: - Peak Results for Optimized Chromatogram

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Lumefantrine	2.249	87584	1653		1.46	6258
2	Artemether	5.430	465872	26532	11.35	1.28	8697

Observation: From the above chromatogram it was observed that the Lumefantrine and Artemether peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

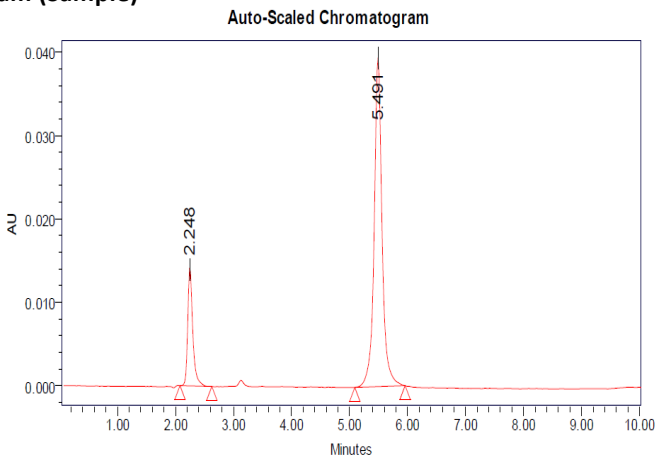


Figure2-: Optimized Chromatogram (Sample)

Table2-: Optimized Chromatogram (Sample)

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Lumefantrine	2.248	88598	1758		1.48	6378
2	Artemether	5.491	475985	27854	11.38	1.29	8759

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Table3-: Results of system suitability for Lumefantrine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lumefantrine	2.247	87589	1658	6235	1.46
2	Lumefantrine	2.246	87596	1625	6248	1.46
3	Lumefantrine	2.248	87584	1689	6294	1.47
4	Lumefantrine	2.252	87598	1675	6247	1.46
5	Lumefantrine	2.248	87659	1635	6285	1.47
Mean			87605.2			
Std. Dev			30.58921			
% RSD			0.034917			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table4: Results of system suitability for Artemether

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution

1	Artemether	5.452	465847	26584	8659	1.28	
2	Artemether	5.484	465789	26985	8695	1.29	
3	Artemether	5.491	465536	26534	8634	1.28	11.36
4	Artemether	5.482	465898	26485	8657	1.29	
5	Artemether	5.491	465826	26854	8695	1.28	
Mean			465779.2				
Std. Dev			141.5475				
% RSD			0.030389				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitated Lumefantrine and Artemether in drug product.

Table5-: Peak results for assay standard

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Lumefantrine	2.256	87586	1654		1.46	6258
2	Artemether	5.427	465875	26536	11.36	1.29	8659
3	Lumefantrine	2.249	87569	1659		1.45	6259
4	Artemether	5.430	465869	26587	11.37	1.29	8695
5	Lumefantrine	2.248	87589	1648		1.46	6287
6	Artemether	5.443	465789	26534	11.36	1.28	8675

Table6-: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Lumefantrine	2.247	88574	1758		1.46	6356	1
2	Artemether	5.452	478598	27854	11.39	1.27	8764	1
3	Lumefantrine	2.246	88569	1769		1.45	6385	2
4	Artemether	5.461	478568	27698	11.38	1.27	8798	2
5	Lumefantrine	2.243	88689	1759		1.46	6395	3
6	Artemether	5.466	478598	27854	11.38	1.28	8759	3

The % purity of Lumefantrine and Artemether in pharmaceutical dosage form was found to be 99.86 %.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Table7-: Chromatographic Data for Linearity Study of Lumefantrine and Artemether:

Concentration µg/ml	Average Peak Area	Concentration µg/ml	Average Peak Area
60	56985	10	244854
80	74587	15	358568
100	92584	20	465872
120	109865	25	572594
140	128564	30	685787

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table8-: Results of Repeatability for Lumefantrine:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lumefantrine	2.269	87598	1658	6295	1.46
2	Lumefantrine	2.255	87569	1659	6287	1.47
3	Lumefantrine	2.252	87546	1685	6259	1.46
4	Lumefantrine	2.267	87526	1698	6284	1.47
5	Lumefantrine	2.260	87598	1638	6295	1.46
Mean			87567.4			
Std. Dev			31.8088			
% RSD			0.036325			

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table9-: Results of method precision for Artemether:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Artemether	5.274	465879	26584	8695	1.28	
2	Artemether	5.266	468598	26598	8675	1.29	
3	Artemether	5.265	465748	26875	8692	1.28	11.36
4	Artemether	5.278	465987	26985	8635	1.29	
5	Artemether	5.305	465851	26874	8697	1.28	
Mean		5.319	466412.6				
Std. Dev			1224.635				
% RSD			0.262565				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Table10-: Results of Intermediate precision for Lumefantrine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lumefantrine	2.248	89856	27859	8759	1.46
2	Lumefantrine	2.245	89574	27965	8769	1.48
3	Lumefantrine	2.242	89565	27485	8745	1.47
4	Lumefantrine	2.239	89675	27698	8726	1.48
5	Lumefantrine	2.243	89754	27895	8796	1.48
6	Lumefantrine	2.246	89645	27854	8759	1.47
Mean			89678.17			
Std. Dev			111.4835			
% RSD			0.124315			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

Table11: Results of Intermediate precision for Artemether

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Artemether	5.284	485978	27895	8798	1.29	
2	Artemether	5.293	486985	27584	8769	1.30	
3	Artemether	5.306	485745	27698	8746	1.29	11.38
4	Artemether	5.319	486986	27451	8764	1.30	
5	Artemether	5.346	487514	27635	8792	1.29	
6	Artemether	5.352	489652	27985	8746	1.30	
Mean			487143.3				
Std. Dev			1399.569				
% RSD			0.287301				

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Table12-: Results of Intermediate precision Day 2 for Lumefantrine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lumefantrine	2.255	86985	1859	6589	1.49
2	Lumefantrine	2.260	86458	1836	6582	1.48
3	Lumefantrine	2.242	86985	1874	6541	1.49
4	Lumefantrine	2.245	86524	1896	6529	1.48
5	Lumefantrine	2.260	86245	1842	6569	1.49
6	Lumefantrine	2.255	86924	1896	6583	1.48
Mean			86686.83			
Std. Dev			318.7949			
% RSD			0.367755			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

Table13-: Results of Intermediate precision for Artemether

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Artemether	5.266	479856	28956	8795	1.29	
2	Artemether	5.265	478754	28754	8769	1.30	
3	Artemether	5.306	472685	28698	8749	1.29	11.38
4	Artemether	5.293	477844	28547	8745	1.30	
5	Artemether	5.265	476985	28654	8725	1.29	
6	Artemether	5.266	478755	28869	8749	1.30	
Mean			477479.8				
Std. Dev			2540.221				
% RSD			0.532006				

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table14-: Results of Accuracy for concentration-50%

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Lumefantrine	2.251	46785	1258		1.26	3596	1
2	Artemether	5.466	238989	21584	11.39	1.06	4872	1
3	Lumefantrine	2.251	46671	1269		1.27	3652	2
4	Artemether	5.447	238785	21698	11.38	1.07	4896	2
5	Lumefantrine	2.252	46682	1263		1.26	3698	3
6	Artemether	5.425	238754	21875	11.38	1.06	4875	3

Table15-: Results of Accuracy for concentration-100%

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Lumefantrine	2.261	92658	1758		1.48	6352	1
2	Artemether	5.416	465985	26598	11.39	1.29	8796	1
3	Lumefantrine	2.261	92695	1699		1.49	6395	2
4	Artemether	5.395	465874	26854	11.38	1.30	8754	2
5	Lumefantrine	2.267	92557	1725		1.49	6387	3
6	Artemether	5.382	465742	26985	11.39	1.30	8759	3

Accuracy 150%:

Table16-: Results of Accuracy for concentration-150%

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Lumefantrine	2.271	138234	1986		1.68	8568	1
2	Artemether	5.368	693487	32658	12.68	1.37	9935	1
3	Lumefantrine	2.272	138154	1985		1.69	8547	2
4	Artemether	5.354	692548	32698	12.84	1.37	9857	2
5	Lumefantrine	2.273	138213	1968		1.68	8535	3
6	Artemether	5.339	692547	32698	12.98	1.36	9968	3

Table17-: The accuracy results for Lumefantrine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	46712.67	50	50.110	100.22	
100%	92636.67	100	100.333	100.333	100.2203%
150%	138200.3	150	150.162	100.108	

Table18-: The accuracy results for Artemether

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	238842.7	10	10.096	100.96	
100%	465867	20	20.100	100.50	100.60%
150%	692860.7	30	30.102	100.34	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION
Result: Lumefantrine: 0.9µg/ml
Artemether: 1.4µg/ml
LIMIT OF QUANTITATION
Result: Lumefantrine: 2.7µg/ml
Artemether: 4.2µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Lumefantrine and Artemether. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Lumefantrine and Artemether were injected by changing the conditions of chromatography. There

was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

Table19-: Results for Robustness Lumefantrine:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	87584	2.249	6258	1.46
Less Flow rate of 0.9 mL/min	92658	2.505	6168	1.45
More Flow rate of 1.1 mL/min	84541	2.046	6098	1.43
Less organic phase	86985	2.505	6224	1.42
More organic phase	84575	2.046	6198	1.45

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table20-: Results for Robustness Artemether:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	465872	5.430	8697	1.28
Less Flow rate of 0.9 mL/min	498545	5.599	8956	1.26
More Flow rate of 1.1 mL/min	458488	4.576	8425	1.25
Less organic phase	426587	5.599	8264	1.27
More organic phase	436586	4.576	8198	1.24

Acceptance criteria: The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Lumefantrine and Artemether in bulk drug and pharmaceutical dosage forms. Artemether is freely soluble in acetone, soluble in methanol and ethanol, and practically insoluble in water. Artemether is soluble in organic solvents such as ethanol, DMSO, and dimethyl form amide, which should be purged with an inert gas. Methanol: TEA Buffer (pH-4.2) (36:64 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Lumefantrine and Artemether in bulk drug and in Pharmaceutical dosage forms.

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