WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 10, 749-767. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF ARTEMETHER & LUMEFANTRINE IN PHARMACEUTICAL DOSAGE FORMS

A. Rajesh[1]* and K. Swapna[2]

[1]Hindu College of Pharmacy, Guntur-522 002.

[2]MLR Institute of Pharmacy, Hyderabad-500 043.

Article Received on
09 July 2017,

Revised on 29 July 2017,
Accepted on 19 August 2017

DOI: 10.20959/wjpr201710-9352

***Corresponding Author**
Dr. A. Rajesh
Hindu College of Pharmacy,
Guntur-522 002.

ABSTRACT

The developed method was validated for parameters such as System suitability, Precision, Accuracy, Linearity and Robustness for the assay of Artemether + Lumefantrine. Hence the method is suitable, linear, precise, accurate and robust for the assay of Artemether and Lumefantrine. Precision and Accuracy are the major control parameters of the whole validation procedure were within the acceptable limits. The experimental data makes a relevant contribution to the understanding of validation parameters.The present work shows a validated, highly sensitive and selective method for determination of Artemether (20mg) + Lumefantrine (120mg) in tablet dosage forms.

Key Word: Precision , Validation, Dosage Forms

INTRODUCTION

Chromatography is a technique by which the components in a sample, carried by the liquid or gaseous phase, are resolved by sorption-desorption steps on the stati onary phase.[1-3] HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.Speed (many analysis can be accomplished in 20 min or less), Greater sensitivity (various detectors can be employed) Improved resolution (wide variety of stationary phases), Reusable columns (expensive columns but can be used for many analysis), Ideal for the substances of low viscosity, Easy sample recovery, handling and maintenance. Instrumentation leads itself to

automation and quantification (less time and less labour), Precise and reproducible, Integrator itself does calculations. A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modeling.[4]

The selection of the column in HPLC is somewhat similar to the selection of columns in G.C, in the sense that, in the adsorption and partition modes, the separation mechanism is based on inductive forces, dipole-dipole interactions and hydrogen bond formation. In case of ion-exchange chromatography, the separation is based on the differences in the charge, size of the ions generated by the sample molecules and the nature of ionisable group on the stationary phase.[5] One approach is to use an isocratic mobile phase of some average organic solvent strength (50%). A better alternative is to use a very strong mobile phase first (80-100%) then reduce% B as necessary. The initial separation with 100% B results in rapid elution of the entire sample, but few groups will separate. Decreasing the solvent strength shows the rapid separation of all components with a much longer run time, with a broadening of latter bands and reduced retention sensitivity.[6,7]

Method validation can be defined as (ICH*)* "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".[8]

Artemether is fast acting drug with a short half-life. Lumefantrine acts slowly and has a longer half-life. Artemether rapidly reduces parasite biomass and quickly resolves clinical symptoms, whilst the long-acting activity of Lumefantrine is thought to prevent recrudescence. This dual effect also appears to reduce the selective pressure on the parasite to develop resistance. The antimalarial activity of the combination of Lumefantrine and Artemether is greater than that of either substance alone.[9-12] A detailed review of literature showed that different methods have been developed for detecting Artemether and Lumefantrine in pharmaceutical formulations and in human plasma, but these methods are costly and require sophisticated equipments.

So the present study is designed to develop an assay method for the simultaneous estimation of these drugs by RP-HPLC in the tablet formulation. This method will be validated for various parameters like accuracy, precision, system suitability, linearity and robustness as per

ICH guidelines. Solubility of Artemether and Lumefantrine in Purified water, 0.1N HCl, 0.1N HCl + 1%Tween80, pH 4.5 Acetate buffer, pH 4.5 Acetate buffer + 2% SLS, pH 6.8 Phosphate buffer, pH 7.4 Phosphate buffer will be studied. Through Review of Literature initial chromatographic conditions for estimation of Artemether (20mg) + Lumefantrine (120mg) in tablet dosage form were established and optimized. Assay method for the estimation Artemether and Lumefantrine will be developed. Developed assay method is subjected to validation for various parameters like Accuracy, Precision, System suitability, Linearity and Robustness as per ICH guidelines.

Experimental Part

Assay Method Development

The objective of this experiment was to optimize the assay method for simultaneous estimation of Artemether and Lumefantrine based on the literature survey made and the methods given in official pharmacopoeias. So here the trials mentioned describes how the optimization was done.

Buffer preparation: Dissolve accurately 1.36g of Potassium dihydrogen Orthophosphate in 900ml of Milli-Q water. Adjust the pH to 3.0 with Ortho phosphoric acid and make up the volume to 1000ml with Milli-Q water and then filter through 0.45µm nylon membrane filter and degas.

Mobile phase: Buffer and Acetonitrile were mixed in the ratio of 40: 60.

Diluent: Mobile phase.

Chromatographic conditions

Flow rate	**:**	1.5ml/min
Column	**:**	Symmetry C_{18}, 250 x 4.6 mm, 5µ
Detector wave length	**:**	Dual i.e, 210 and 303nm
Column temperature	**:**	Ambient
Injection volume	**:**	20µl
Run time	**:**	20 mins

Observation

The peak shapes of Artemether and Lumefantrine were good and also optimum resolution was obtained.

Optimized Method For Assay

Buffer preparation: Dissolve 1.36gm of potassium dihydrogen orthophosphate in 900ml of Milli-Q water. Adjust the pH to 3.0 with Ortho phosphoric acid. Makeup the volume to 1000ml and filter through 0.45µm nylon membrane filter and degas.

Mobile phase: Prepare a degassed mixture of Buffer and Acetonitrile in the ratio of 40:60% v/v.

Diluent: Buffer:Acetonitrile (40:60).

Chromatographic conditions

Flow rate	**:**	1.5 ml/min
Column	**:**	Symmetry C_{18}, 250 x 4.6 mm, 5µ
Detector wave length	**:**	Dual i.e, 210 and 303nm
Column temperature	**:**	Ambient
Injection volume	**:**	20µl
Run time	**:**	20 mins

OBSERVATION

S. No	Name of the peak	Retention time(min)
1.	Artemether	13.895
2.	Lumefantrine	7.135

The two peaks were well resolved with good peak shape and symmetry.

CONCLUSION

Hence this method was finalized for the simultaneous estimation of Artemether and Lumefantrine.

Evaluation of System Suitability

The column efficiency as determined for Lumefantrine and Artemether peaks is not less than 5000 USP plate count and the tailing factor for Lumefantrine and Artemether peaks is not more than 4.5 and 2.0 respectively.

The relative standard deviation for the peak areas of the five replicate injections is not more than 2.0%.

Validation Of The Assay Method

The following experimental design is drawn in order to prove the test method is capable to yield consistent, reliable and reproducible results within the pre-determined acceptance limits. Acceptance criteria for above validation parameters are specified in individual experimental design. Observations and results are recorded in individual method validation data sheets. Summarize the findings of the method validation and draw interference. Based on the interpretation of the results in method validation, draw the conclusion.

The following parameters have been validated.

1. System suitability 2. Precision3. Accuracy 4. Linearity 5. Robustness.

System suitability

A Standard solution was prepared by using, Artemether and Lumefantrine working standards as per test method and was injected ten times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from ten replicate injections for Artemether and Lumefantrine retention times and peak areas.

Acceptance criteria

1. The % RSD for the retention times of principal peak from 10 replicate injections of each Standard solution should be not more than 2.0%
2. The % RSD for the peak area responses of principal peak from 10 replicate injections of each standard Solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Artemether and Lumefantrine peaks is not less than 1500.
4. The Tailing factor (T) for the Artemether peak is NMT 2.0 and for Lumefantrine peak is not more than 4.0.

PRECISION

a. System precision: Standard solution prepared as per test method and injected five times.
b. Method precision: Prepared six sample preparations individually using a batch of tablets of Artemether and Lumefantrine tablets (20/120mg) as per the test method and injected each solution.

Acceptance criteria

The % relative standard deviation of individual Artemether and Lumefantrine from the six samples should be not more than 2.0%.

The assay of Artemether and Lumefantrine should be not less than 95.0% and not more than 105.0%.

ACCURACY (RECOVERY)

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Artemether and Lumefantrine into each volumetric flask for each spike level to get the concentration of Artemether and Lumefantrine equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Artemether and Lumefantrine was calculated.

Separately inject the blank, placebo, Artemether and Lumefantrine in to the chromatograph.

Acceptance criteria

The mean % recovery of the Artemether and Lumefantrine at each level should be not less than 95.0% and not more than 105.0%.

LINEARITY OF TEST METHOD

A Series of solutions are prepared using Artemether and Lumefantrine working standard at concentration levels from 50% to 150% of target concentration (50%, 75%, 100%, 125% and 150%). Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

Acceptance criteria

Correlation Coefficient should be not less than 0.9990.

% of y- Intercept should be ±2.0.

% of RSD for level 1 and Level 6 should be not more than 2.0%.

ROBUSTNESS

i) Effect of variation in mobile phase composition

A study was conducted to determine the effect of variation in Organic phase composition in mobile phase. Standard solution prepared as per the test method was injected into the HPLC system using two mobile phases. The system suitability parameters were evaluated and found to be within the limits for mobile phase having 95% and 110% of method highest organic phase. Artemether and Lumefantrine blend solution at target concentration was chromatographed using mobile phase having 90% and 110% of the method organic phase.

Artemether and Lumefantrine were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having 100% of the organic phase.
From the study it was established that the allowable variation in mobile phase composition is 90% to 110% of the method highest organic phase of mobile phase.

Acceptance criteria

The Tailing Factor of Artemether standard should be NMT 2.0 and Lumefantrine standard should be NMT 4.0 for Variation in Organic Phase.

ii) Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.4ml/min and 1.6ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.4ml/min and 1.6ml/min flow.

Artemether and Lumefantrine were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.5ml/min.

From the above study it was established that the allowable variation in flow rates is 1.4ml/min and 1.6ml/min.

Acceptance criteria

The Tailing Factor of Artemether standard should be NMT 2.0 and Lumefantrine standard should be NMT 4.0 for Variation in Flow.

iii) Effect of variation of temperature

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 30ºC temperature. The system suitability parameters were evaluated and found to be with in the limits for a temperature change of 30ºc.

Similarly sample solution was chromatographed at 30ºC temperature. Artemether and Lumefantrine were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having ambient temperature.

Acceptance criteria

The Tailing Factor Artemether and Lumefantrine standard and sample solutions should be NMT 2.0 and 4.0 respectively for Variation in temperature.

iv) Effect of variation of pH

A study was conducted to determine the effect of variation in pH. Standard and sample solutions were prepared as per the test method and injected into the HPLC system using pH 2.8 and 3.2. The system suitability parameters were evaluated and found to be within the limits for pH 2.8 and 3.2.

Artemether and Lumefantrine were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having pH3.0.

From the above study it was established that the allowable variation in pH 2.8 and 3.2.

Acceptance criteria

The Tailing Factor of Artemether and Lumefantrine standard should be NMT 2.0 and 4.0 respectively for Variation in pH.

RESULTS AND DISCUSSION

SOLUBILITY RESULTS

The solubility of Artemether in mg/ml in all medias can be calculated by the following formula.

Table 1: Lumefantrine.

S.No.	Media	Sample wt. in mg	Sample absorbance	Standard absorbance	Solubility mg/ml
1	Purified water	25.39	0.671	0.453	3.90
2	0.1N HCl	70.81	0.516	0.461	6.95
3	0.1N HCl + 1%Tween80	400	0.482	0.487	38.83
4	pH 4.5 Acetate buffer	150.40	0.513	0.437	18.42
5	pH 4.5 Acetate buffer + 2%SLS	170	0.401	0.479	13.14
6	pH 6.8 Phosphate buffer	25.0	0.627	0.452	3.58
7	pH 7.4 Phosphate buffer	25.0	0.678	0.462	3.791

From the above observation, it has been observed that Lumefantrine showed acceptable solubility in all media exhibiting higher solubility in 0.1N HCl + Tween80.

Table 2: Artemether.

S.No.	Media	Sample wt.	Sample absorbance	Standard absorbance	Solubility mg/ml
1	Purified water	20.16	0.536	0.325	3.26
2	0.1N HCl	70.90	0.473	0.391	9.69
3	0.1N HCl + 1%Tween80	70.00	0.459	0.345	10.49
4	pH 4.5 Acetate buffer	251.1	0.391	0.309	29.93
5	pH 4.5 Acetate buffer + 2%SLS	520	0.394	0.325	57.35
6	pH 6.8 Phosphate buffer	20.00	0.531	0.384	2.73
7	pH 7.4 Phosphate buffer	20.16	0.592	0.371	3.14

From the above observation it has been observed that Artemether showed acceptable solubility in all media exhibiting higher solubility in pH 4.5 Acetate buffer + 2%SLS.

ASSAY RESULTS

LUMEFANTRINE

Table 3.

S.NO.	Std. areas of Lumefantrine
1	6327565
2	6327565

3	6328224
4	6342690
5	6329931
Mean	6331195
Std.dev.	6498.33
%RSD	0.10

At = 5929391

As = 6331195

Aw = 266.12

Std Wt. = 24.85

Sample Wt = 52.39

P = 100

Table 4.

%Assay of Lumefantrine							**% Assay**
Sample-1	5929391	24.85	100	266.12	100	100	98.51
	6331195	100	52.39	120	100		
Sample-2	6165393	24.85	100	266.12	100	100	101.6
	6331195	100	52.83	120	100		
Sample-3	6117776	24.85	100	266.12	100	100	101.3
	6331195	100	52.57	120	100		

ARTEMETHER

Table 5.

S.NO.	**Std.areas of Artemether**
1	23201
2	22524
3	22831
4	22926
5	22909
Mean	22878.2
Std.dev.	242.45
%RSD	1.06

At = 22023

As = 22878

Aw = 266.12

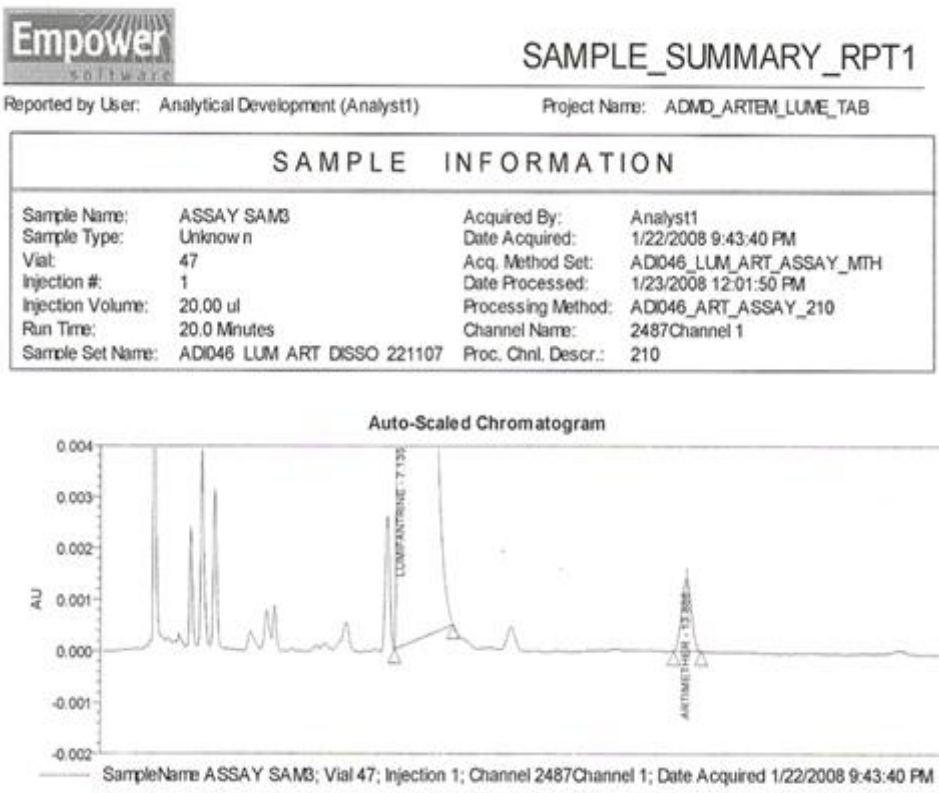
Std Wt. = 40.82

Sample Wt = 52.08

P = 98.56

Table 6.

%Assay of Artemether								**% Assay**
Sample-1	22023	40.82	5	100	266.12	98.56	100	98.95
	22878	50	100	52.08	20	100		
Sample-2	22003	40.82	5	100	266.12	98.56	100	98.99
	22878	50	100	52.01	20	100		
Sample-3	22016	40.82	5	100	266.12	98.56	100	98.84
	22878	50	100	52.12	20	100		



	Sample Name	Name	Vial	RT	Area
1.	Assay sam 3	Artimether	47	13.886	22016

Fig. 1 Assay of Drugs.

Validation Data

Table 7: System Suitability I) Lumefantrine.

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	7.23	6327565	1979.6	3.5
2	7.26	6327661	1975.3	3.5
3	7.22	6327489	1988.9	3.6
4	7.20	6327375	1995.1	3.6
5	7.17	6327240	2000.2	3.6
6	7.21	6327517	1991.3	3.5
7	7.28	6327698	1962.0	3.4
8	7.19	6327330	1998.5	3.6
9	7.24	6327575	1972.4	3.6
10	7.25	6327620	1993.6	3.5
Mean	7.225	6327507	1985.69	3.54
SD	0.034	149.6	---	---
% RSD	0.46	0.002	---	---

Table 8: Artemether.

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	13.882	23201	17815	1.0
2	13.890	22524	17927.4	1.0
3	13.888	22831	17578.2	1.1
4	13.878	22926	18225.3	1.0
5	13.887	22909	17820.3	1.1
6	13.881	23192	17760.2	1.1
7	13.886	22789	17585.4	1.0
8	13.883	22288	17770.6	1.1
9	13.889	22891	17935.2	1.2
10	13.885	22916	17790.3	1.0
Mean	13.88	22846.7	17815.88	1.06
SD	0.004	275.18	---	---
% RSD	0.03	1.20	---	---

Acceptance criteria

1. The % RSD for the retention times of Lumefantrine + Artemether peaks from 10 replicate injections of each Standard solution should be not more than 2.0%.
2. The % RSD for the peak area responses of Lumefantrine + Artemether peaks from 10 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Lumefantrine and Artemether peaks is not less than 1500.
4. The Tailing factor (T) for the Lumefantrine peak is not more than 4.0 and Artemether peak is not more than 2.0.

Observations

1. The % RSD for the retention times of Lumefantrine peak from 10 replicate injections of each Standard solution is 0.46 & the %RSD for the retention times of Artemether peak from 10 replicate injections of each Standard solution is 0.03.
2. The % RSD for the peak area response of Lumefantrine peak from 10 replicate injections of each Standard solution is 0.002 & the %RSD for the peak area response of Artemether peak from 10 replicate injections of each Standard Solution is 1.20.
3. The number of theoretical plates (N) for Lumefantrine is 1985.69 & for Artemether is 17815.9.
4. The Tailing factor (T) for Lumefantrine peak is 3.54 & for Artemether peak is 1.06.

Table 9: System Precision.

	Injection	Peak Areas of Artemether	Peak Areas of Lumefantrine
Concentration 100%	1	23201	6327565
	2	22524	6327565
	3	22831	6328224
	4	22926	6342690
	5	22909	6329931
Statistical Analysis	**Mean**	22878	6331195
	SD	242.51	6498.34
	% RSD	1.06	0.10

Table 10: Method Precision.

Tablet ID	% Assay			Statistical Analysis	
	ARTE	LUME		ARTE	LUME
1	99.6	98.9	Mean	99.26	99.11
2	97.8	99.6			
3	100.3	97.8	SD	0.79	0.73
4	99.8	100.1			
5	98.9	98.8	%RSD	0.79	0.74
6	99.2	99.5	100.3		

Acceptance criteria

1. The %RSD for the peak area responses of Lumefantrine and Artemether from the five replicate injections of Standard solution should not be more than 2.0%.
2. The %RSD for % Assay of Lumefantrine and Artemether of 6 units should not be more than 5.0.

Observation

1. %RSD for the peak area responses of Lumefantrine peak from the five replicate injections of Standard solution is 0.10 & the % RSD for the peak area responses of Artemether peak from 5 replicate injections of Standard solution is 1.06.
2. The %RSD for %Assay of Lumefantrine of 6 units is 0.74 and the %RSD for %Assay of Artemether of 6 units is 0.79.

Table 11: Lumefantrine Accuracy (Recovery).

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	12.24	12.17	99.42	**MEAN**	98.46
50% Sample 2	12.06	12.02	99.66	**SD**	0.1833
50% Sample 3	12.88	12.79	99.30	**%RSD**	0.184
100 % Sample 1	24.34	24.28	99.75	**MEAN**	98.76
100% Sample 2	24.15	24.09	99.75	**SD**	0.023
100% Sample 3	23.86	23.81	99.79	**%RSD**	0.0231
150% Sample 1	36.51	36.49	99.94	**MEAN**	99.86
150% Sample 2	35.98	35.90	99.77	**SD**	0.087
150% Sample 3	36.46	36.42	99.89	**%RSD**	0.0874

Table 12: Artemether.

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	2.17	2.14	98.8	**MEAN**	99.03
50% Sample 2	2.10	2.08	99.1	**SD**	0.16
50% Sample 3	2.80	2.77	99.2	**%RSD**	0.162
100 % Sample 1	4.12	4.06	98.6	**MEAN**	98.63
100% Sample 2	4.23	4.17	98.6	**SD**	0.04
100% Sample 3	4.7	4.63	98.7	**%RSD**	0.04
150% Sample 1	6.25	6.18	98.9	**MEAN**	99.09
150% Sample 2	6.31	6.25	99.1	**SD**	0.16
150% Sample 3	6.28	6.23	99.3	**%RSD**	0.161

Acceptance criteria: The mean % recovery of the Artemether and Lumefantrine at each level should not be less than 95.0.

Observation: The mean % recovery levels were found to be not less than 95.0.

Table 13: Linearity Lumefantrine.

Linearity Level	Concentration ppm	Average Area	% of RSD	Statistical Analysis	
L1-50%	123.35	3163782	0.003	Slope	25649
L2-75%	185.02	4745673	--	y-Intercept	-50.703
L3-100%	246.7	6327565	--	% of y- Intercept	0.405
L4-125%	308.37	7909456	--	Correlation Coefficient	0.999996
L5-150%	370.05	9491347	0.001	R2	1.0

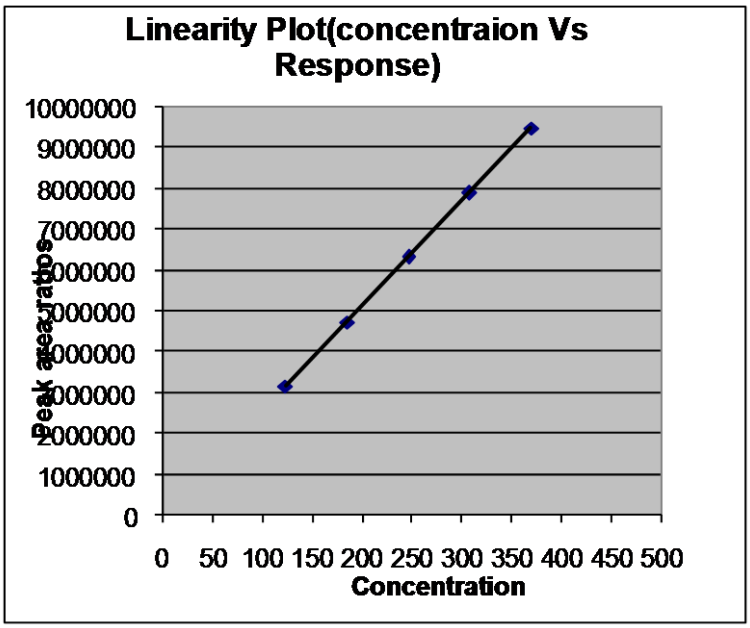


Fig 2: Lumefantrine graph.

Table 14: Artemether.

Linearity Level	Concentration ppm	Average Area	% of RSD	Statistical Analysis	
L1-50%	20.32	11439	0.785	Slope	5719.5
L2-75%	30.48	17158	--	y-Intercept	-5719.3
L3-100%	40.64	22878	--	% of y- Intercept	0.625
L4-125%	50.8	28597	--	Correlation Coefficient	0.999998
L5-150%	60.96	34317	0.246	R2	1.0

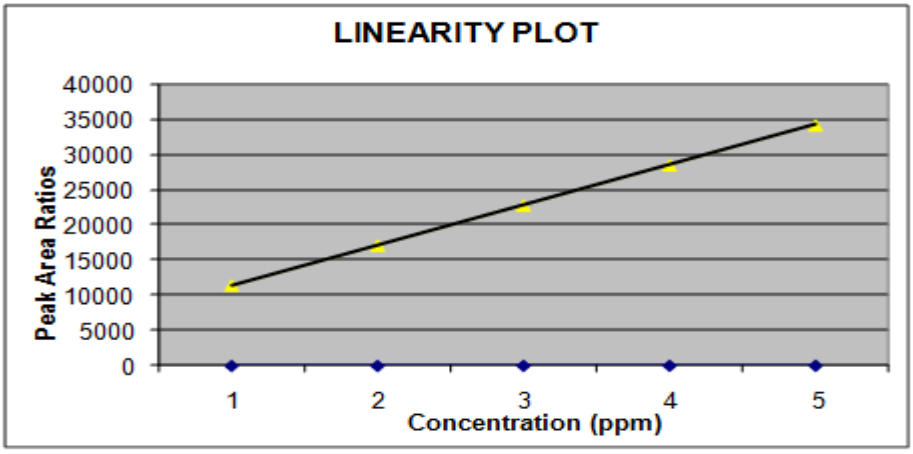


Fig 3: Artemether Graph.

Acceptance criteria

1. The linearity regression coefficient should be more than 0.999.
2. % of y- Intercept should be ±2.0.
3. % RSD for level 1 and Level 6 should be not more than 2.0%.

Observation

1. The linearity regression coefficient for Lumefantrine is 0.999996 & for Artemether is 0.999998.
2. The % y-intercept of Lumefantrine is 0.405 & for Artemether is 0.625.
3. The % RSD of peak area response of Lumefantrine peaks for level 1 and level 6 are 0.003 and 0.001 respectively & the % RSD of peak area response of Artemether peaks for level 1 and level 6 are 0.785 and 0.246 respectively.

Table 15: Robustness.

Parameters	Optimum range	Conditions in procedure	Remarks
Mobile phase composition (% Of Acetonitrile)	10% variations in gradient conditions	Isocratic	Does not have any effect on system suitability.
Flow rate ml/min	0.9-1.1	1.0	Does not have any effect on system suitability.
Temperature	25-30^{o}C	Ambient	Does not have any effect on system suitability.
PH of mobile phase	2.8-3.2	3.0	Does not have any effect on system suitability.

Acceptance criteria

1. The Tailing Factor of Artemether standard should not be more than 2.0 and Lumefantrine standard should not be more than 4.0 for Variation in Organic Phase.

2. The Tailing Factor of Artemether standard should not be more than 2.0 and Lumefantrine standard should not be more than 4.0 for Variation in Flow.
3. The Tailing Factor Artemether and Lumefantrine standard and sample solutions should not be more than 2.0 and 4.0 respectively for Variation in temperature.
4. The Tailing Factor of a Artemether and Lumefantrine standard should not be more than 2.0 and 4.0 respectively for Variation in pH.

OBSERVATION

1. **Effect of variation in mobile phase composition:** The tailing factor for Artemether and Lumefantrine are found to be within the limits.
2. **Effect of variation of flow rate:** The tailing factor for Artemether and Lumefantrine are found to be within the limits.
3. **Effect of variation of temperature:** The tailing factor for Artemether and Lumefantrine are found to be within the limits.
4. **Effect of variation of pH:** The tailing factor for Artemether and Lumefantrine are found to be within the limits.

CONCLUSION

The chromatographic method developed for the test procedure of assay for Artemether (20mg) + Lumefantrine (120mg) in tablet dosage forms were simple, reliable, sensitive and less time consuming. The advantage of the present test procedures is that it does not require any complicated mobile phase and it is simple isocratic method. The present method can be confidently used for rapid and precise estimation of Artemether (20mg) + Lumefantrine (120mg). Especially this procedure can be a major interest in analytical pharmacy, since it offers a distinct quality control in the test procedures of assay of pharmaceutical dosage forms. The methods developed may be recommended for routine and quality control analysis of the investigated drug. The observed values are within the acceptance criteria for the validation of stability indicating HPLC method for the assay of Artemether & Lumefantrine. The developed method was validated for parameters such as System suitability, Precision, Accuracy, Linearity and Robustness for the assay of Artemether + Lumefantrine. Hence the method is suitable, linear, precise, accurate and robust for the assay of Artemether and Lumefantrine. Precision and Accuracy are the major control parameters of the whole validation procedure were within the acceptable limits. The experimental data makes a relevant contribution to the understanding of validation parameters. The present work shows

a validated, highly sensitive and selective method for determination of Artemether (20mg) + Lumefantrine (120mg) in tablet dosage forms.

REFERENCES

1. H.H. Willard; L.L.Merit; F.A. Dean & F.A.Settle, "Instrumental methods of analysis", 7th edition, CBS Publishers, New Delhi, 580-613.
2. B.K. Sharma, "Instrumental Methods of Chemical Analysis", 19th edition, 2000.
3. G.R.Chatwal & Sham K Anand, "Instrumental methods of chemical analysis", 9th edition, 2004.
4. R.J. Hamilton and Swell, "Introduction to HPLC", 2nd edition, 1984; 2-9.
5. Craig S. Young and Raymond J. Weigand, "An efficient approach to column selection in HPLC Method Development", Altech Associates, www.alltech web.com
6. Lloyd R. Snyder, Joseph J. Kirkland, Joseph L. Glajesh, "Practical HPLC Method Development", 2nd Edn, 1997; 1-14.
7. Ahuja Satinder, Rasmussen Henrik, "HPLC method development for pharmaceuticals", Vol.8, Apr. 2007; 510.
8. "Asean Guidelines for Validation of Analytical Procedures", Adopted from ICH Guidelines, ICH Q2A-27th Oct. 1994; ICH Q2B-6th Nov.1996.
9. Surendra Nath Pandeya, "A Text book of Medicinal Chemistry", Vol.2, 3rd edition, 2003; 601-633.
10. White NJ, Van Vugt M, Ezzet F, "Clinical Pharmacokinetics and Pharmacodynamics of Artemether-Lumefantrine", Journal of Pharmacology, 1997; 37: 105-125.
11. M Bindschedler, G Lefevre, F Ezzet, N Schaeffer, European Journal of Clinical Pharmacology, 2000; 56: 375-381.
12. Agarwal, Suraj P; Ali, Asgar, Ahuja, Shipra, Asian Journal of Chemistry, 2007; 19: 4407-4414.
13. Ziyou; Chen, Zufen, Yaowu Fenxi zazhi, "Simultaneous determination of ß-artemether and its metabolite and urine by a HPLC-MS using Electrospray ionization", Journal of Pharmaceutical analysis, 2000; 20(3): 178-179.
14. Zhi-Liang, Yang, Song-Cheng; Zhang, Min; "Determination of lumefantrine in human plasma by RP-HPLC with UV detection", Journal of Chromatography B & Biomedical Applications, 1996; 681(2): 299-306.