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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMIFANTRINE IN PURE AND MARKETED FORMULATION

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ABSTRACT

A simple, rapid, precise and accurate reversed phase high performance liquid chromatographic method has been developed for the simultaneous determination of Artemether in combination with Lumefantrine. This method uses a Hypersil ODS C18(4.6×150mm,5µ particle Size) analytical column, a mobile phase of methanol: ammonium acetate buffer pH 3 adjusted with orthophosphoric acid in ratio(65:35 v/v). The instrumental settings are a flow rate of 1.2 ml/min and PDA detector wavelength at 256 nm**.** The retention times for Artemether and Lumefantrine were 2.8 min and 3.8 min, respectively. The method is validated and shown to be linear. The linearity range for Artemether and Lumefantrine were 10-50µg/ml & 60-300µg/ml respectively. The Percentage recovery for Artemether and Lumefantrine are ranged between 99–102 and 99–102 respectively. The correlation coefficients of Artemether and Lumefantrine were 0.999, and 0.999, respectively**.** The relative standard deviation for six replicates is always less than 2%. The Statistical analysis proves that the method is suitable for routine analysis of Artemether and Lumefantrine as a bulk drug and in pharmaceutical formulation.

Keywords: Artemether, Lumefantrine, RP-HPLC and Validation.

INTRODUCTION

Artemether is chemically (3R,5aS,-6R,8aS,9R,10S,12R,12aR)-Decahydro-10-methoxy-3,6,9 trimethyl- 3,12-epoxy-12H-pyrano [4,3-j]-1,2-benzodioxepin1 and is used as antimalarial agent. Lumefantrine is chemically 2, 7-Dichloro-9-[(4-chlorophenyl) methylene]-α-[(dibutylamino) methyl]-9H-fluorene-4-methanol2 and is used in the treatment of uncomplicated falciparum malaria. Both of these drugs available in combined tablet dosage form with lable claim of Artemether 80 mg and Lumefantrine 480 mg per tablet. The review of literature reveals that there were analytical methods of two drugs individually or in combinations with other drugs has also been reported in pharmaceutical dosage forms and even in biological samples and very few methods has been reported for combination of these two drugs. It was essential to develop a chromatographic method for simultaneous estimation of two drugs in a tablet formulation. Theto dissolve method described is rapid, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH norm [1,2].

MATERIALS AND METHODS

Artemether API and Lumefantrine API were obtained as gift sample from Ajantha Pharmaceutical Ltd (Mumbai, Maharashtra, India). Methanol (HPLC grade), Ammonium acetate (AR grade), orthophosphoric acid (AR grade) were obtained from Rankem Pvt. Ltd. Delhi, India. The 0.45 µm membrane filter was used throughout the experiment. The tablets of ART in combination with LUM (Lumerax) were purchased from Local market. Double distilled water was used throughout the experiment. Other chemicals used in the experiment were of analytical or HPLC grade [3-6].

Preparation of standard solution

10 mg of Artemether and 10mg of Lumifantrine were accurately weighed and transferred into a 10 ml clean

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dry volumetric flask, about 7 ml of diluent was added, sonicated it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution) Further 0.3 and 1.8 ml were pipetted out from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to give a concentration of 30 µg/ml and 180 µg/ml respectively. The stock solutions were filtered through a 0.45µ membrane filter paper [7-10].

Preparation of sample solution

10 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 10 mg of active ingredient present in Artemether and Lumifantrine was transferred into a 10 ml clean dry volumetric flask, 7 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 1000 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 0.3 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as per standard solution. The solution was filtered through 0.45 µm filter before injecting into HPLC system [11].

METHOD VALIDATION

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH guidelines, the validation parameters were

SYSTEM SUITABILITY

Sample solution of Artemether and Lumefantrine were injected three times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard Chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from three replicate injections [12].

LINEARITY

Preparation of sample stock solution

About 10 mg ofArtemether and 60 mg of Lumefantrine samples were weighed in to 10 ml volumetric flask, it was dissolved with diluent and the volume was made up to the mark with same diluent (1000µg/ml of Artemether and 6000µg/ml ofLumefantrine) [13].

Preparation of Level – I (10µg/ml of Artemether&60µg/ml of Lumefantrine)

0.1ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–II (20 µg/ml of Artemether&120 µg/ml of Lumefantrine)

0.2ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–III (30 µg/ml of Artemether&180 µg/ml of Lumefantrine)

0.3ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–IV (40 µg/ml of Artemether&240 µg/ml of Lumefantrine)

0.4ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–V (50 µg/ml of Artemether& 300 µg/ml of Lumefantrine).

0.5ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent. 10µl of each level were injected into the system and recorded the peak response.

PRECISION

The precision of the method was checked by repeated injected sample solution of Artemether(30 µg/ml) and lumifantrine(180 µg/ml)

ACCURACY

Assay was performed in triplicate for various concentrations of Artemether and Lumefantrine equivalent to 50, 100, and 150 % of the standard amount was injected into the HPLC system as per the test procedure.

Preparation of Standard stock solution

10 mg of Artemether and 10mg of Lumefantrine accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicated to dissolve it completely and volume was made up to the mark with the same solvent to give the concentration of 1000 µg/ml. (Stock solution)

ROBUSTNESS

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and mobile phase composition, temperature variations which may differ but the responses were still within the specified limits of the assay.

Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. The flow rate was varied at 1.0 ml/min to 1.4 ml/min. Standard solution 30 ppm Artemether and 180 ppm Lumefantrinewere prepared and analysed using the varied flow rates along with method flow rate.The results are summarized on evaluation of the

above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%. The method is robust only in less flow condition. The effect of variation of flow rate was evaluated.

LIMIT OF DETECTION

The limit of detection was checked by signal to noise ratio.

For Artemether

The prepared solution of 0.004 µg/ml Artemether was checked by repeated injected sample solution.

For Lumefantrine

The prepared solution of 0.006 µg/ml lumifantrine was checked by repeated injected sample solution.

LIMIT OF QUANTIFICATION

The limit of quantification was checked by signal to noise ratio.

For Artemether

The prepared solution of 0.015µg/ml lumifantrine was checked by repeated injected sample solution.

For Lumefantrine

The prepared solution of 10 µg/ml lumifantrine was checked by repeated injected sample solution.

RESULTS AND DISCUSSION

Method Validation

Table 1. System Suitability

Arthemeter			Lumifantrine		
Injection	**R_t**	**Peak Area**	**Injection**	**R_t**	**Peak Area**
1	2.799	304728	1	3.863	1111263
2	2.799	301592	2	3.861	1153869
3	2.813	294803	3	3.886	1112110
Mean	300226		Mean	1136953	
%RSD	1.0		%RSD	1.2	

Table 2. Linearity

Artemether				Lumefantrine			
S.No	**Linearity Level**	**Concentration**	**Area**	**S.No**	**Linearity Level**	**Concentration**	**Area**
1	I	10 ppm	222407	1	I	60 ppm	1606125
2	II	20ppm	276578	2	II	120ppm	1878367
3	III	30 ppm	334892	3	III	180ppm	2204843
4	IV	40 ppm	394409	4	IV	240 ppm	2511642
5	V	50 ppm	451762	5	V	300 ppm	2835708
Correlation coefficient			0.9998				0.998

Table 3. Calibration parameters for Artemether and Lumefantrine

Parameter	Results for Artemether	Results for Lumifantrine
Slope	5765.4	5094.07
Intercept	163047	1269204
Correlation co-efficient	0.9998	0.998

PRECISION

Table 4. Sample Chromatogram values for Reproducibility

Artemether			Lumefantrine		
S.No	**Park Area**	**R_t**	**S.No**	**Park Area**	**R_t**
1	368013	2.808	1	2321302	3.880
2	372552	2.808	2	2308016	3.880
3	367873	2.808	3	2326058	3.880
4	375555	2.808	4	2334897	3.880
5	374843	2.808	5	2326143	3.880
Avg	371767		Avg	9845.8	
SD	3663.5		SD	0.42	
%RSD	0.99		%RSD	0.99	

Table 5. Sample Chromatogram values for intermediate Precision

Artemether			Lumifantrine		
S.No	**Park Area**	**R_t**	**S.No**	**Park Area**	**R_t**
1	377409	2.808	1	2268108	3.882
2	371977	2.808	2	2275775	3.882
3	376191	2.808	3	2254168	3.882
4	372169	2.808	4	2285916	3.882
5	378930	2.808	5	2296220	3.882
Mean	375335		Mean	2276037	
SD	3132.9		SD	16171.8	
%RSD	0.83		%RSD	0.71	

Table 6. Accuracy

Artemether						Lumifantrine					
Sample No.	**Spike Level**	**Amount (µg/ml) added**	**Amount (µg/ml) found**	**% Recovery**	**Mean % Recovery**	**Sample No.**	**Spike Level**	**Amount (µg/ml) added**	**Amount (µg/ml) found**	**% Recovery**	**Mean % Recovery**
1	50%	5	4.9	98%	100%	1	50%	5	4.9	98%	100%
		5	5.1	102%				5	5.1	102%	
		5	5	100%				5	5	100%	
2	100%	10	9.88	98.8%	99.13%	2	100%	5	5	100%	99.31%
		10	9.91	99.1%				10	9.88	98.8%	
		10	9.95	99.5%				10	9.91	99.1%	
3	150%	15	14.89	99.2%	99.69%	3	150%	10	9.95	99.5%	99.89%
		15	14.86	99.0%				15	14.89	99.2%	
		15	14.82	99.79%				15	14.86	99.0%	

ROBUSTNESS

Table 7. Robustness (Effect of variation in flow rate)

Artemether						Lumifantrine					
Flow rate	**R_t**	**Area**	**Height**	**Plate count**	**Tailing**	**Flow rate**	**R_t**	**Area**	**Height**	**Plate count**	**Tailing**
Less flow	3.091	421480	45332	2741.1	1.71	Less Flow	4.274	2558248	234950	4162	1.57
More flow	2.553	343858	43270	2543.2	1.58	More Flow	3.538	2084296	225397	3921.4	1.48

Table 8.Effect of variation in mobile phase composition

Artemether						Lumifantrine					
Mobie phase	**R_t**	**Area**	**Height**	**Plate count**	**Tailing**	**Mobie phase**	**R_t**	**Area**	**Height**	**Plate count**	**Tailing**
Less organic	3.301	372832	39645	2980.4	1.60	Less organic	4.344	2244995	211957	4457.1	1.44
More organic	2.469	380129	48101	2423.5	1.64	More organic	3.508	2303836	245935	3712.3	1.56

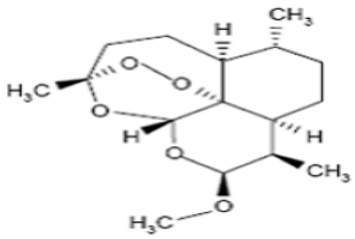
Table 9. Limit of Detection

Artemether			Lumifantrine		
Baseline noise(µV)	**Signal obtained (µV)**	**S/N ratio**	**Baseline noise(µV)**	**Signal obtained (µV)**	**S/N ratio**
48 µV	141µV	2.941	48 µV	134µV	2.808

Table 10. Limit of Quantification

Artemether			Lumifantrine		
Baseline noise(µV)	**Signal obtained (µV)**	**S/N ratio**	**Baseline noise(µV)**	**Signal obtained (µV)**	**S/N ratio**
48 µV	470µV	9.79	48 µV	498µV	10.37

Fig 1. A. Artemether



B. Lumifantrine

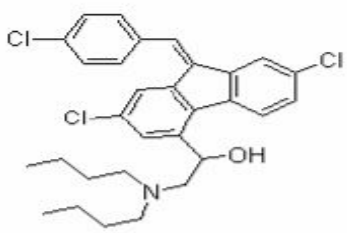
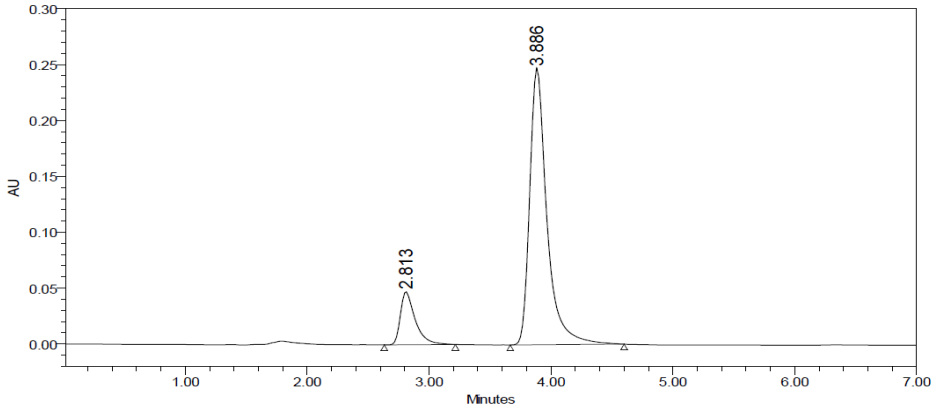


Fig 1. Chromatograms for System suitability



SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Artemether and Lumefantrine was done by RP-HPLC. The Ammonium acetate buffer was p^H 3 and the mobile phase was optimized with consists of Methanol: Ammonium acetate buffer mixed in the ratio of 65:35 % v/ v. A $C1_8$ column C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.2 ml/min. the linearity range of Artemether and Lumefantrine were found to be from 10-50 µg/ml. of Artemether and 60-300µg/ml of Lumefantrine. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Artemether and Lumefantrine. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. 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 INTEREST:

The authors declare that they have no conflict of interest.

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