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ANALYTICAL METHOD DEVELOPMENT VALIDATION FOR SIMULTANEOUS DETERMINATION OF ARTEMETHER AND LUMEFANTRINE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A new simple, accurate, economic, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Artemether and Lumefantrine, in its pure form as well as in pharmaceutical dosage form. Chromatography was carried out on X bridge C18 (4.6×150mm) 5 µ column using a mixture of Methanol: Phosphate Buffer pH-3.6 (30:70v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 260nm. The retention time of the Artemether and Lumefantrine was 2.669, 3.855±0.02min respectively. The method produce linear responses in the concentration range of 10-50µg/ml of Artemether and 10-50µg/ml of Lumefantrine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

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

1. INTRODUCTION

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 Plasmodium falciparum and unidentified Plasmodium species, including infections acquired in chloroquine-resistant areas. 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]-9Hfluorene-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.

2. MATERIALS AND METHODS

2.1 Materials- Artemether and Lumefantrine were procured from Sura labs, Telangana. Water and Methanol for HPLC was procured from LICHROSOLV (MERCK). Anhydrous di hydrogen phosphate, Phosphate Buffer and Citric Acid were purchased, from Merck.

2.2 Instrumentation- Chromatographic conditions were developed for the analytical technique using Waters HPLC with auto sampler and PDA detector 996 model. The column was X bridge C18 with dimension 4.6mm×150mm length and particle size packing 5µm.

2.3 Preparation of mobile phase- Accurately measured 300 ml (30%) of Methanol and 700 ml of Phosphate buffer (70%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

2.4. System Suitability- Accurately weigh and transfer 10 mg of Artemether and Lumefantrine 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 out 0.6ml of Artemether and Lumefantrine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

2.5. Linearity- Accurately weigh and transfer 10 mg of Artemether and 10mg of Lumefantrine working standard into a 10 ml and 10 ml of clean dry volumetric flasks add about 10ml and 10 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution, 1000ppm). Solutions were prepared containing 10ppm, 20ppm, 30ppm, 40ppm, 50ppm, concentrations of Artemether and 10ppm, 20ppm,

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30ppm, 40ppm, 50ppm, concentrations of Lumefantrine. Inject each level into the chromatographic system and measure the peak area.

2.6. Precision- Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of An analytical method is usually expressed as the standard deviation correlative standard deviation (coefficient of variation) of series of measurements.

2.7. Accuracy- Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150 % of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For 50%, 150% concentration five sets and for 100% three sets were prepared and injected.

2.8. Robustness- The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), variation of mobile phase i.e. i.e. Methanol: Phosphate buffer pH-3.6 was taken in the ratio and 35:65, 25:75 instead 30:70, remaining conditions are same

2.9. Limit of detection (LOD) and Limit of quantification (LOQ)- LOD and LOQ was calculated from linear curve using formulae LOD=3.3*σ/slope, LOQ=10*σ/slope (Where σ=the standard deviation of the response and S= Slope of calibration curve).

3. RESULTS AND DISCUSSION

Several mobile phase compositions were tried to resolve the peak of Artemether and Lumefantrine. The mobile phase containing Methanol: Phosphate Buffer pH3.6 (30:70v/v) was found ideal to resolve the peak of Artemether and Lumefantrine. Retention time of Artemether and Lumefantrine were 2.669and 3.855min respectively. System suitability parameters were evaluated and results shown in (Table-2), which were within acceptance criteria. Result of assay is shown in Table3. Results of intraday and interday precision were shown in the (Table-4&5). LOD and LOQ values were placed in Table-6. The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and mobile phase. The result obtained implies method is robust for routine qualitative analysis (Table-7).

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 Phosphate buffer was pH 3.6 and the mobile phase was optimized with consists of Methanol: Phosphate buffer (pH-3) mixed in the ratio of 30:70 % v/ v. An Xbridge column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions

Table 1 - Observations of sample Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Artemether	2.669	988374	128892		1.6	3581.0
2	Lumefantrine	3.855	5364316	562226	1.8	1.3	4676.7

Table 2-: Results of system suitability parameters for Artemether and Lumefantrine

S. No	Name	Retention ime(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Artemether	2.669	979845	129657		1.7	3853
2	Lumefantrine	3.855	5356494	587453	1.9	1.8	4797

Table 3-: Results of Assay

S. No.	Name of Compound	% Purity
1	Artemether	98%
2	Lumefantrine	97%

Table 4 -: Results of Intermediate precision for Artemether

S. no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Artemether	2.669	978986	128871	3687	1.6
2	Artemether	2.529	975687	128360	3653	1.6
3	Artemether	2.669	969875	128475	3535	1.6
4	Artemether	2.569	975488	128694	3684	1.6

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5	Artemether	2.569	978544	128363	3599	1.6
6	Artemether	2.669	976899	128248	3537	1.6
Mean			975913			
Std. Dev			3286.897			
% RSD			0.336802			

Table 4a-: Results of Intermediate precision for Lumefantrine

S. No	Name	Rt	Area	Height	USP plate count	USP Resolution
1	Lumefantrine	3.845	5352142	563657	4686	1.8
2	Lumefantrine	3.795	5365848	564585	4666	1.8
3	Lumefantrine	3.855	5378413	563653	4653	1.8
4	Lumefantrine	3.840	5378544	563548	4642	1.8
5	Lumefantrine	3.855	5363597	565812	4660	1.8
6	Lumefantrine	3.855	5386878	562540	4659	1.8
Mean			5370903			
Std. Dev			12656.43			
% RSD			0.235648			

Table 5-: Results of method precision for Artemether

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Artemether	2.669	876857	128232	3654	1.6
2	Artemether	2.659	877854	129853	3542	1.6
3	Artemether	2.671	875474	128146	3636	1.6
4	Artemether	2.669	876589	129612	3596	1.6
5	Artemether	2.669	875213	128323	3697	1.6
Mean			876397.4			
Std. Dev			1075.302			
% RSD			0.122696			

Table 5a-: Results of method precision for Lumefantrine

Sno	Name	Rt	Area	Height	USP plate count	USP Resolution
1	Lumefantrine	3.855	4378559	465622	4676	1.7
2	Lumefantrine	3.842	4386231	464586	4697	1.7
3	Lumefantrine	3.850	4385411	463652	4683	1.7
4	Lumefantrine	3.845	4369874	463543	4762	1.7
5	Lumefantrine	3.855	4389745	478548	4955	1.7
Mean			4381964			
Std. Dev			7880.279			
% RSD			0.179834			

Table 6: LOD and LOQ

S. No.	Name of Compound	LOD (µg/ml)	LOQ (µg/ml)
1	Artemether	1.3	3.9
2	Lumefantrine	1.2	4.6

Table 7a-: Robustness –System suitability results for Artemether

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4788.4	1.5
2	*Actual	3552.0	1.5
3	10% more	4636.6	1.5

Table 7b-: Robustness System suitability results for Lumefantrine

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5864.8	1.4
2	*Actual	4677.7	1.4
3	10% more	5343.4	1.4

4. CONCLUSION

The proposed RP-HPLC method was used for the simultaneous estimation of Artemether and Lumefantrine was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, in vitro dissolution study of combinational dosage formulations containing Artemether and Lumefantrine.

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