International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.3, pp 1722-1727, July-Sept 2011

Reversed-Phase Liquid Chromatographic Method for Simultaneous Determination of Artemether and Lumefantrine in Pharmaceutical Preparation

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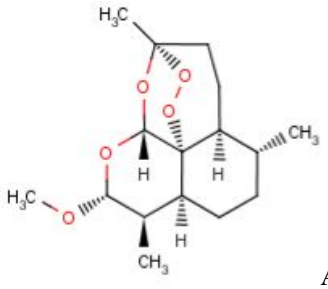
Abstract: A simple, rapid, precise and accurate reversed phase high performance liquid chromatographic method has been developed for the simultaneous determination of Artemether in combination with Lumefantrine. This method uses a Hypersil ODS C_{18} (250mm×4.6mm×5µ particle Size) analytical column, a mobile phase of methanol: 0.05 % trifluroacetic acid with triethylamine buffer pH 2.8 adjusted with orthophosphoric acid in ratio (80:20 v/v). The instrumental settings are a flow rate of 1.5 ml/min and PDA detector wavelength at 210 nm**.** The retention times for Artemether and Lumefantrine are 6.15 min and 11.31min, respectively. The method is validated and shown to be linear. The linearity range for Artemether and Lumefantrine are 20-120 & 120-720 µg/ml respectively. The Percentage recovery for Artemether and Lumefantrine are ranged between 99.50–101.16 and 99.78–101.21 respectively. The correlation coefficients of Artemether and Lumefantrine are 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 analysis of Artemether and Lumefantrine as a bulk drug and in pharmaceutical formulation without any interference from the excipients.

Key words - Artemether, Lumefantrine, Validation and RP-HPLC.

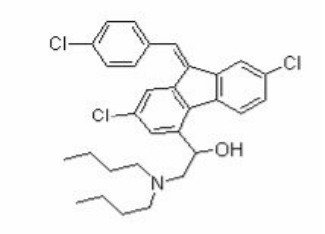
Introduction

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-benzodioxepin[1] and is used as antimalarial agent. Lumefantrine is chemically 2, 7-Dichloro-9-[(4-chlorophenyl) methylene]-α-[(dibutylamino) methyl]-9H-fluorene-4-methanol[2] 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 [11-15] and no methods has yet 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. The method described is rapid, precise,

and accurate and can be used for routine analysis of tablets. It was validated as per ICH norm. [16]



A



B

Figure 1.: Structures of Antimalarial Drugs: A- Artemether and B- Lumefantrine

Experimental

Instrumentation

The LC system was from Perkin Elmer Quaternary pump Series 200 and was comprised of auto sampler injector; and an Intelligence PDA detector connected to the Total Chrome Navigator version 6.3. For controlling the instrumentation as well as processing the data generated was used.

Material and reagents

Artemether API and Lumefantrine API were obtained as gift sample from Ajantha Pharmaceutical Ltd (Mumbai, Maharashtra, India). Acetonitrile (HPLC grade), triethylamine (AR grade), methanol (HPLC grade), trifluroacetic Acid (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.

Chromatographic conditions

The isocratic mobile phase consists of methanol: 0.05 % trifluroacetic acid with triethylamine buffer pH 2.8 adjusted with orthophosphoric acid in ratio 80:20 v/v, flowing through the column at a constant flow rate of 1.5 ml/min. A Hypersil ODS C_{18} column (250mm × 4.6mm, 5 µ) was used as the stationary phase. ART and LUM have different λ max but considering the chromatographic parameter, sensitivity, and selectivity of the method for these drugs, 210 nm was selected as the detection wavelength for PDA detector. The injection volume was 20 µl.

Mobile phase

The mobile phase consisted of methanol: 0.05 % trifluroacetic acid with triethylamine buffer pH 2.8 adjusted with orthophosphoric acidin ratio (80:20 v/v). The buffer used in the mobile phase consisted of 2.5 ml Triethylamine transfer to 100 ml volumetric flask and make up the volume up to 100 ml with 0.05% trifluroacetic acid. The mobile phase was premixed and filtered through a 0.45-µm membrane filter and degassed.

Standard preparation

Artemether

Accurately, about 20 mg of standard ART was weighed and transferred to separate 100 ml volumetric flasks. The drug was dissolved in 50 ml of methanol and 1 ml orthophosphoric acid with shaking and then volume made up to the mark with methanol to obtain standard stock solutions of each drug of concentration 200 µg/ml. The stock solutions were filtered through a 0.45 µ membrane filter paper.

Lumefantrine

Accurately, about 100 mg of standard LUM was weighed and transferred to separate 100 ml volumetric flasks. The drug was dissolved in 50 ml of methanol and 1 ml orthophosphoric acid with shaking and then volume made up to the mark with methanol to obtain standard stock solutions of each drug of concentration 1000 µg/ml. The stock solutions were filtered through a 0.45 µ membrane filter paper.

Calibration curve solutions

From the mentioned stock solutions of ART and LUM calibration curve solutions containing 20 µg/ml to 120 µg/ml of ART and 120 µg/ml to 720 µg/ml of LUM in each calibration level were prepared.

Preparation of sample solutions

Twenty tablets were weighed and finely powdered. A quantity equivalent to one tablet containing 80 mg of ART and 480 mg of LUM was transferred in a 100 mL volumetric flask and Add 25ml methanol with 1 ml of Orthophosphoric acid. The contents were sonicated for 20 min with methanol to dissolve the active ingredients and the volume was made up to 100 ml with methanol and filtered through 0.45µm membrane filter.

Results and Discussion

Optimization of chromatographic conditions

The chromatographic method was optimized by Different experiments were performed to achieve the adequate retentions and resolution for the peaks of ART and LUM. To set the adequate retentions and resolution, the effects of the mobile phase components, changes in ionic strength were studied, initially methanol and water in different ratios were tried. But ART gave broad peak shape While LUM gave no peak, so water was replaced by potassium dihydrogen buffer (0.2 M) and mixture of methanol and potassium dihydrogen phosphate buffer in different ratios (78:22) were tried. It was found that both peak shows broad peaks finally methanol: 0.05% trifluoroacetic acid with triethylamine buffer of pH 2.8 adjusted with orthophosphoric acid in ratio (80:20 v/v) gave acceptable retention time (6.15 min for ART and 11.31 min for LUM) and good resolution for ART and LUM was found to be 6.88 at the flow rate of 1.5 ml/min. gave adequate retentions and resolution, and the chromatographic run was 15 min.

Validation of the method

Specificity

The specificity of the method was checked by a peak purity test of the sample preparation done by PDA detector. The peak purity for ART and LUM was found to be 999. The result of the peak purity analysis shows that the peaks of the analytes were pure and also the formation excipients were not interfering with the analyte peaks.

Calibration and linearity

The standard solutions containing 20 µg/ml to 120 µg/ml of ART and 120 µg/ml to 720 µg/ml of LUM in each linearity level were prepared. Linearity solutions were injected in triplicate. In the simultaneous determination, the calibration graphs were found to be linear for both the analytes in the mentioned concentrations. The coefficient of correlation was found to be 0.999 and 0.999 for ART and LUM, respectively.

Precision (repeatability)

The precision of the method was studied by determining the concentrations of each ingredient in the tablets six times. In the precision study, % relative standard deviation of the ART and LUM were found to be 0.657 and 0.247 respectively. The results of precision study indicate that the method is reproducible.

Accuracy (recovery test)

The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the pure drug. The recovery was done at three levels: 80%, 100%, and 120% of the label claim. Three samples were prepared for each recovery level. The recovery values for ART and LUM ranged from 99.50–101.16 and 99.78–101.21, respectively (**Table I**). The average recovery of three levels for ART and LUM were 100.24 and 100.27 respectively.

Table I. Results of the Recovery Tests for the Drugs

Table I. Results of the Recovery Tests for the Drugs (n = 3)						
Level of addition (%)	**Amount added (mg)**		**Recovery(%)***		**Average Recovery†**	
	ART	**LUM**	**ART**	**LUM**	**ART**	**LUM**
80	64	384	99.50±0.579	99.78±0.304	100.24	100.27
100	80	480	100.06±0.122	99.81±0.127		
120	96	576	101.16±0.277	101.21±0.143		
* RSD shown in parenthesis. † Average recovery = average of three levels, nine determinat						

Table II. Assay Results of Active Ingredients in Tablets

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Set	**Ingredients**	**Label claim (mg)**	**Found (mg) †**	**% Label claim ± %RSD**
Precision	ART	80	80.04	100.05 ± 0.657
	LUM	480	478.43	99.67 ± 0.247
Intermediate precision	ART	80	79.38	99.22 ± 0.204
	LUM	480	480.79	100.16 ± 0.115
† Average of six analyses				

Table III. System Suitability Parameters

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Parameters	**ART**	**LUM**
Retention time (min)	6.15	11.31
Tailing Factor	0.970	1.45
Theoretical Plates	12991.72	61407.69
Resolution	6.88	

Intermediate precision

Intermediate precision of the method was done by analyzing the sample six times on different days, by different chemists, using different analytical column of the make, and different HPLC systems. The percentage assay was calculated using the calibration curve. The assay results are shown in Table II.

Determination of the limits of detection and Quantitation

For determining the limits of detection (LOD) and quantitation (LOQ), the method based on the residual standard deviation (SD) of a regression line and slope was adopted. To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analytes in the range of the detection and quantitation limits. The LOD for ART and LUM were 0.0019 and 0.00047 µg/ml and the LOQ were 0.0060 and 0.0014 µg/ml respectively.

System suitability

For system suitability studies, five replicate injections of mixed standard solutions were injected, and the parameters like RSD of peak area ratio, column efficiency, resolution, and tailing factor of the peaks were calculated. Results are shown in Table III.

Robustness

To evaluate robustness of the developed method, few parameters were deliberately varied. These parameters included variation of flow rate, percentage of methanol in the mobile phase, pH of buffer and temperature. Each factor selected was changed at three levels (-1, 0, +1). One factor was changed at one time to estimate the effect. The results are shown in Table IV.

Table IV. Summary of Robustness Study

Chromatographic changes							
Flow Rate (ml/min)	**Level**	**RT**		**Tailing factor**		**% drug Found**	
		ART	**LUM**	**ART**	**LUM**	**ART**	**LUM**
1.3	-1	6.85	11.75	0.942	1.438	100.09	100.06
1.5	0	6.15	11.31	0.978	1.451	99.94	100.01
1.7	+1	5.27	10.25	0.938	1.448	100.05	99.97
% of MEOH in the mobile phase (v/v)	**Level**	**RT**		**Tailing factor**		**% drug Found**	
		ART	**LUM**	**ART**	**LUM**	**ART**	**LUM**
78	-1	6.84	11.75	0.961	1.496	100.21	100.88
80	0	6.15	11.31	0.978	1.451	99.94	100.01
82	+1	5.77	10.58	0.95	1.464	99.97	99.42
Temperature	**Level**	**RT**		**Tailing factor**		**% drug Found**	
		ART	**LUM**	**ART**	**LUM**	**ART**	**LUM**
33	-1	6.64	11.74	0.955	1.452	100.20	99.97
35	0	6.15	11.31	0.978	1.451	99.94	100.01
37	+1	6.04	10.54	0.954	1.479	100.04	100.27
pH	**Level**	**RT**		**Tailing factor**		**% drug Found**	
		ART	**LUM**	**ART**	**LUM**	**ART**	**LUM**
2.6	-1	6.17	11.38	0.549	1.533	99.67	99.91
2.8	0	6.15	11.31	0.978	1.451	99.94	100.01
3.0	+1	6.14	11.28	0.950	1.490	99.89	99.90

* Mean of three levels (n = 3)

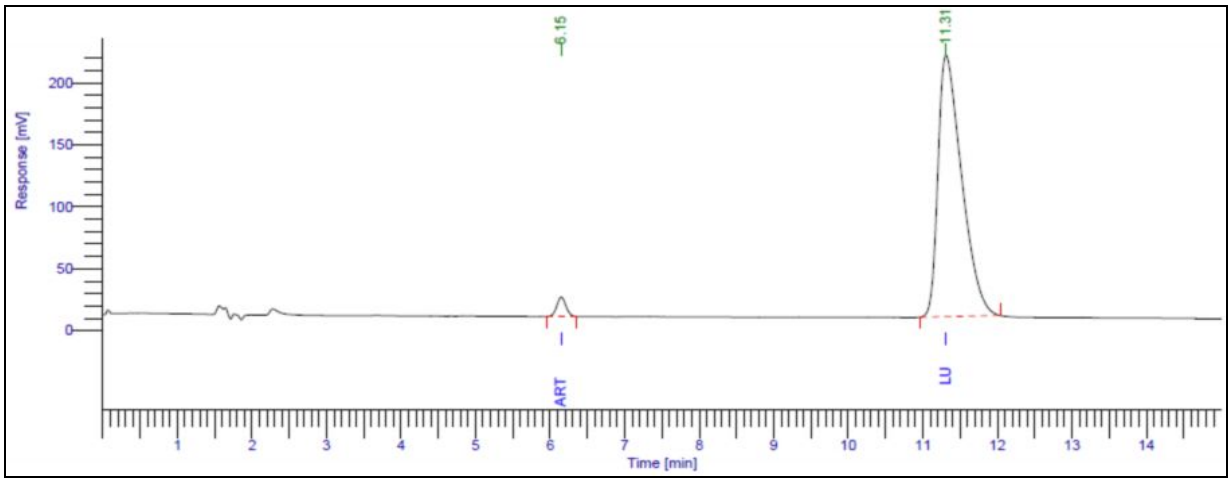

Figure 2. A typical chromatogram of Artemether and Lumefantrine

Determination of active ingredients in tablets

The contents of two drugs in tablets were determined by the proposed method using a calibration curve. The determinations were done in two sets, one for precision and the second for intermediate precision, and six samples were prepared for each set. The results are shown in table III. The chromatogram of the tablet sample is shown in figure 2**.**

Conclusion

The proposed RP–HPLC method enables simultaneous determination of ART & LUM enabling good separation and resolution of the chromatographic peaks. This is the first reported method for simultaneous quantitative analysis of ART & LUM, and is a significant advance in chromatographic analysis of such pharmaceutical mixtures. The method is suitable for qualitative and quantitative analysis of these pharmaceutical products. The results obtained are in a good agreement with the declared contents. Statistical analysis showed the method is accurate and precise. There was no interference from excipients in the tablets.

Acknowledgements

The authors are grateful to the School of Pharmacy S.R.T.M. University Nanded, Maharashtra State, India for providing the facilities for this research work.

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