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Research Article

Simultaneous Estimation of Artemether and Lumefantrine by RP-HPLC Method development in pharmaceutical tablet dosage form

Gupta N.K.*, Babu A.M.[1] and Pramila Gupta [2]

*****Department of Pharmaceutical Sciences, NIMS University. Jaipur -303121. India.
[1]School of Pharmacy and Health science. International medical university, Kuala Lumpur-57000. Malaysia.
[2]M.S.J.Govt. College, Bharatpur-321001. Rajasthan

The chromatographic analysis was performed by Hypersil BDS C_{18} ,250 × 4.6 mm, 5 µ particle size with mobile phase consisting of buffer and acetonitrile in the ratio of 50:50v/v, orthophosphoric acid used as buffer (pH 3.0 ± 0.6), at a flow rate of 1.5 ml/min and eluents monitored at 215nm. The method was validated for linearity, accuracy, precision, robustness and application for assay as per ICH guidelines. The retention times of artemether and lumefantrine were 2.464 and 6.236 min, respectively. The calibration curves of peak area versus concentration, which was linear from 4-24µg/ml for artemether and 24-144µg/ml for lumefantrine, had regression coefficient (r^2) greater than 0.999. The method had the requisite accuracy, precision, and robustness for simultaneous determination of artemether and lumefantrine in tablets. The proposed method is simple, economical, accurate and precise, and could be successfully employed in routine quality control for the simultaneous analysis of artemether and lumefantrine in tablets.

Keywords: ART (Artemether), LUM (Lumefantrine), RP-HPLC (Reverse phase –High performance liquid chromatography), ICH (International Conference on Harmonization), µ (Micron).

INTRODUCTION

Chemical name of artemether is ; (3R,5aS,6R,8aS,9R,10S,12R,12aR)-decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2benzodioxepine[1].

Artemether is concentrated in the food vacuole. It then splits its endoperoxide bridge as it interacts with haem, blocking conversion to haemozoin, destroying existing haemozoin and releasing haem and a cluster of free radicals into the parasite[2, 3].

***Address for correspondence**
nirmalgupta1712@rediffmail.com

Lumefantrine is (±)-2-dibutylamino-1-[2, 7-dichloro-9-(4-chlorobenzylidene)-9Hfluorene-4-yl] ethanol.[4]

Lumefantrine is thought to interfere with the haem polymerisation process, a critical detoxicifying pathway for the malaria parasite. Lumefantrine and artemether combination therapy is indicated for the treatment of acute uncomplicated malaria caused by *Plasmodium falciparum*, including malaria acquired in chloroquine-resistant areas. May also be used to treat uncomplicated malaria when the *Plasmodium* species has not been

identified. Indicated for use in adults and children greater than 5 kg.[5, 6]

Some methods which are available in literature are for the simultaneous estimation of Artemether and Lumefantrine[7-14].

The aim of this work is to develop an accurate, specific, repeatable, and validated method for simultaneous determination of both Artemether and Lumefantrine in bulk and tablet formulations.

EXPERIMENTAL

Materials

Pure Artemether (ART) and Lumefantrine (LUM) were used as working standards, gifted from Balaji drugs. Pontasahib (H.P). India. Tablets containing 20mg. of ART and 120mg. of LUM were purchased from market of Themis pharma, India and used within their shelf life period. Acetonitrile and water (HPLC-grade) were purchased from Merck, India. All other chemicals and reagents employed were of analytical grade, and purchased from Merck and Ranbaxy, India.

Instrumentation

A Shimadzu HPLC system consisting of a LC-2010 CHT binary gradient pump, an inbuilt auto sampler, a column oven and dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data were acquired through the Empower-2 software. The column used was Hypersil BDS symmetry C_{18}, 250×4.6 mm, 5µm. A Bandline sonerex sonicator was used for enhancing the dissolution of the compounds.

Optimized chromatographic conditions

The chromatography elution was carried out in the isocratic mode using a mobile phase consisting of buffer and acetonitrile in a ratio of 50:50 v/v, buffer (pH 3.0 $\pm$ 0.6 adjusted with orthophosphoric acid). The analysis performed at ambient temperature using a flow rate of 1.5 ml/min with a run time of 5 min. The eluent was monitored using DAD at a wavelength of 215 nm. The mobile phase was filtered through whatman filter paper No.41 prior to use.

Preparation of stock and standard solutions

A stock solution of ART and LUM (500µg/ml) were prepared by taking accurately weighed 50 mg. of ART and LUM as reference standard in 100 ml volumetric flask containing 50 ml of acetonitrile and then the volume was made up to the mark with acetonitrile . The stock solution is protected from light using aluminum foil. Aliquots of the standard stock solution of ART and LUM were transferred, using A-grade bulb pipette into 10 ml volumetric flasks and solutions were made up to the mark with the mobile phase to give the final concentrations of 4-

24µg/ml and 24-144µg/ml of ART and LUM respectively.

Estimation of Artemether and Lumefantrine from tablets

To determine the content of ART and LUM in tablets (Label claim: 20mg. and 120mg.), 20 tablets were taken and the contents were weighed. An aliquot of powder equivalent to the weight of one tablet was accurately weighed and transferred to 50 ml volumetric flask and was dissolved in 25 ml of acetonitrile and volume was made up to the mark with acetonitrile. The flask was sonicated for 20 minutes to affect complete dissolution. The solution filtered through a 0.45µm millipore filter. A suitable aliquot of the filtered solution was transferred into a 100 ml volumetric flask and made up to the volume with the mobile phase to yield the concentration of 10µg/ml for ART and 60µg/ml for LUM.

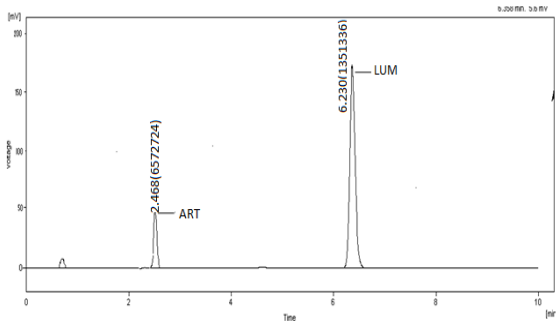

Fig. 1: A typical Chromatogram of Marketed formulation of 'ART' and 'LUM'

The experiments were performed six times under the optimized chromatographic conditions described prior. The peak areas were measured at 215nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

Method validation

Linearity: By appropriate aliquots of the standard ART and LUM solution with the mobile phase, six working solutions ranging between 4-24µg/ml and 24-144µg/ml were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of ART and LUM to obtain the calibration curve.

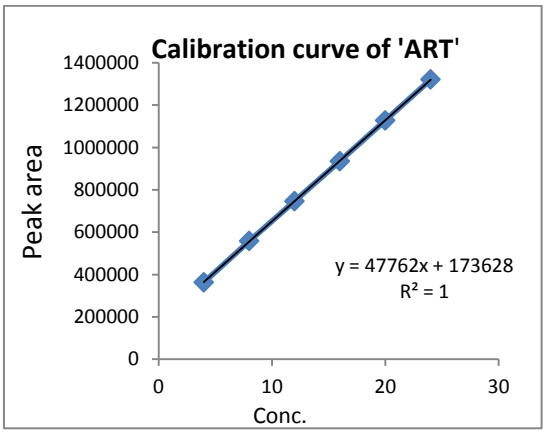

Fig. 2: Linearity curve of ART

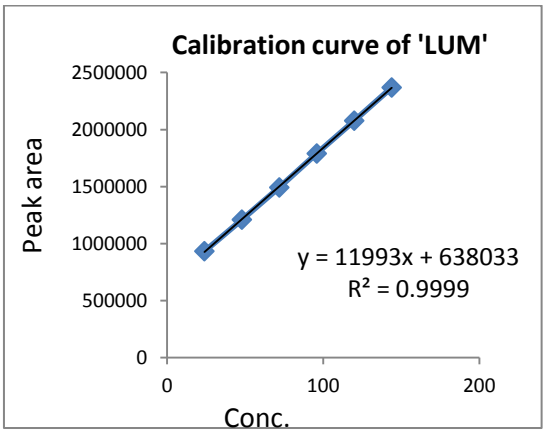

Fig. 3: Linearity curve of LUM

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Table 1: Linearity data and their analytical performances for Artemether and Lumefantrine

Drugs	Conc. µg/ml	Peak area	Linear Range	Correlation co-efficient	Slope	Intercept
ART	4	363500	4-24µg/ml	1.0	47762	17362
	8	558846				
	12	746533				
	16	935406				
	20	1127776				
	24	1321699				
LUM	24	933685	24-144 µg/ml	0.999	11993	63803
	48	1210387				
	72	1492460				
	96	1789840				
	120	2077678				
	144	2368677				

Accuracy: Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of ART and LUM to which known amounts of standard ART and LUM, corresponding to 80,100 and 120% of label claim were added. The accuracy expressed as the percentage of analyte recovered by the proposed method.

Precision: Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of ART and LUM at all concentration in linear range respectively. Determinations were performed with three replicates on the same day as well as on three consequent days.

Reproducibility: The reproducibility of the method was checked by determining precision on a same instrument, the analysis being performed by another person in the same laboratory. It was analyzing the samples of ART and LUM at different concentration in between 4-24µg/ml and 24-144µg/ml in triplicate respectively and calculates the amount of drug present in the sample.

Robustness: The robustness of the method was performed by deliberately changing the chromatographic conditions. The organic strength and buffer pH were varied

by ±2% and 0.2 units, respectively.

System suitability tests: To ensure the validity of the analytical procedure, a system suitability test was established. Data from ten injections of 20µl of the working standard solution containing 10µg/ml for ART and 60µg/ml for LUM were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time.

Table 2: System suitability parameters.

Parameter	ART	LUM
Retention time (min.)	2.464	6.236
Resolution	12.34	2.092
No. of Theoretical plates	10283	8038
Tailing factor	1.655	1.389

Limit of detection and the limit of quantification:

Limit of detection (LOD) and limit of quantification (LOQ) was calculated, based on the ICH guidelines.

RESULTS AND DISCUSSIONS

A RP-HPLC method was proposed as a suitable method for the estimation of ART and LUM in the tablet dosage forms. The best chromatographic conditions were adequately selected. The selection of mobile phase and flow rate was made on the basis of peak shape, baseline drift, time required for analysis, and the mobile phase consisted of acetonitrile and buffer (pH 3± 0.6, adjusted with orthophosphoric acid) in the ratio of 50:50 v/v at a flow rate of 1.5ml/min and analyzed at 215nm. The retention time observed (2.464 for ART and 6.236 for LUM) allows a rapid determination of these drugs. In Figure 1, a typical chromatogram obtained under these conditions is shown.

The calibration plot of peak area against concentration was linear in the range of 4-24µg/ml and 24-144µg/ml for ART and LUM respectively. The linear regression data for the calibration curves were indicative of a good linear relationship between peak area and concentration over a wide range (Table 1). The correlation coefficient was indicative of high significance. The LOD and LOQ were found to be 0.0166µg/ml and 0.0552µg/ml and 0.0071µg/ml and 0.0237µg/ml for ART and LUM respectively.

The accuracy was assessed from three replicates containing a concentration range of 80, 100 and 120%. The recovery of the method determined by spiking a previously analyzed test solution with standard ART and LUM solution, and the recovery values were found to be in the range of 99.75-100.20% and 99.8-100.30% respectively. The values of % recovery and %COV were indicated that the method is accurate.

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The precision of the method was assessed in accordance with ICH guidelines. The low %COV (<2) values indicate that the method is precise. Reproducibility of the method was performed in the same laboratory on a same instrument which was performed by another analyst. The assay values and low %COV (<2) values indicate that the method is reproducible.

Table 3: Estimation of amount present in tablet dosage form

Brand name	Tablet Formulation	Label Claim per Tablet (mg)	% Label claim estimated (Mean) N=5	SD	%COV	% Drug estimated
Lumether	ART	20	19.96	0.5946	0.5962	99.72
	LUM	120	119.98	0.1950	0.1951	100.04

The robustness was determined by analyzing the same sample under a variety of conditions. The factors consider being variations in the pH (0.2 units) and strength of acetonitrile (±2%).The results and the experimental range of the selected variables, together with the optimized conditions. There were no significant changes in the chromatography pattern when the above modifications were made in the experimental conditions, showing that the method is robust. The system suitability tests were also carried out to evaluate the reproducibility of the system for the analysis to be performed. The results of system suitability tests are given in Table 2, showing that the parameters are within the suitable range. The proposed method was applied to the analysis of marketed formulations and the results obtained are given in Table 3. The blank solution was prepared containing the components indicated in tablet dosage form except the active ingredient. No interference was observed from the tablet excipients. The ART and LUM content was found to be 99.72% and 100.04% respectively.

CONCLUSION

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of ART and LUM from its tablet dosage form. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, lack of extraction procedures. All these factors make this method suitable for quantification of ART and LUM in tablet dosage forms. The method can be successfully used for routine analysis of ART and LUM in bulk drugs and pharmaceutical dosage forms without interference.

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