

DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ARTEMETHER AND LUMEFANTRINE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive, specific, precise and accurate stability indicating reverse phase liquid chromatographic method was developed for simultaneous determination of Artemether and Lumefantrine in bulk drugs and pharmaceutical formulations. The chromatographic analysis was performed by Microsorb-MV100-5 C-18 column (250 x 4.6mm, 5 μ m), with mobile phase consisting of buffer and acetonitrile in the ratio of 65:35v/v, orthophosphoric acid used as buffer (pH 3.5), at a flow rate of 1 ml/min and eluents monitored at 254nm. 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 2.67 and 4.62 min, respectively. The calibration curves of peak area versus concentration, which was linear from 5-30 μ g/ml for Artemether and 60-210 μ g/ml for Lumefantrine, had regression coefficient (r^2) greater than 0.999 & precise (R.S.D. < 2.0%). The method had the requisite accuracy, precision, and robustness for simultaneous determination of Artemether and Lumefantrine in tablets.

Keywords: Artemether, Lumefantrine, RP-HPLC, Simultaneous determination, Method development

INTRODUCTION

Malaria is the world's most important parasitic infection, ranking among the major health and developmental challenges for the poor countries of the world¹. One of the greatest challenges facing malaria control worldwide is the spread and intensification of parasite resistance to antimalarial drugs. Artemisinin-based combination therapy (ACT) is increasingly being advocated as promising treatment. ACT is based on the use of two drugs with different modes of action: an artemisinin-derivative that causes rapid and

effective reduction of parasite biomass and gametocyte carriage and a partner drug that has a longer duration of action². Artemether (ART) is chemically, (3R,5aS,-6R, 8aS,9R,10S,12R,12aR)-Decahydro-10-methoxy-3,6,9-tri-methyl- 3,12-epoxy-12H-pyrano [4,3-j]-1,2-benzodioxepin and is used as antimalarial agent.(Fig.1A) Lumefantrine (LUM) is chemically, 2, 7-Dichloro-9-[(4-chlorophenyl)methylene]- α -[(dibutylamino)methyl]-9H-fluorene-4-methanol and is used in the treatment of uncomplicated falciparum malaria.(Fig.1B)

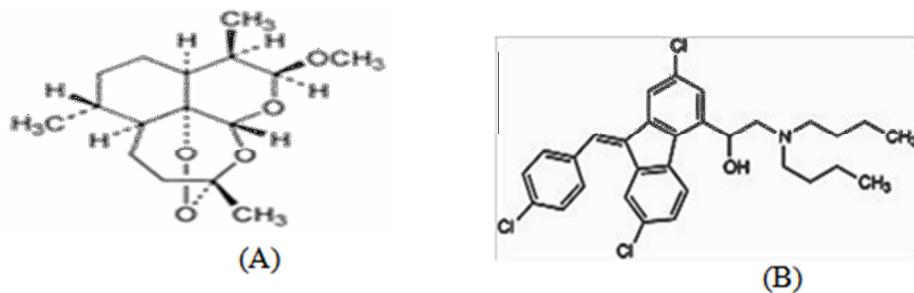


Figure 1
*Chemical structure of Artemether
 (A) and Lumefantrine (B)*

ART-LUM is an ACT widely used nowadays and consists of a registered fixed dose combination of ART (20 mg) and LUM (120 mg) in tablets³. ART has a rapid onset of action and is rapidly eliminated from the plasma (half-life of two to three hours)⁴. LUM is cleared more slowly and has a longer elimination half-life (approximately 4.5 days)⁵. The rationale behind this combination is that ART initially provides rapid symptomatic relief by reducing the number of parasites present before LUM eliminates any residual parasites. ART-LUM also reduces gametocyte carriage and thus should have an impact on malaria transmission⁶. The increasing use of ART-LUM association as an effective treatment for resistant malaria demands the need of analytical methods to simultaneously quantify these drugs in tablets in order to evaluate its quality. Some papers have described the analysis of ART in plasma, based on HPLC with electrochemical⁷⁻⁹ or mass spectrometry detection¹⁰. Few methods are available to assay ART in pharmaceutical products.^{11,12} The quantitative determination of LUM in plasma has been described using HPLC with UV detection.^{13,15} Due to the low molar absorptivity of ART in the UV region (210 nm), and the lower concentration of this drug in the tablets compared to LUM, standard addition of ART was carried out to improve its detection which gives need for the simultaneous quantitation of ART and LUM. The validated method was applied to the analysis of fixed-dose combination tablets containing 40mg ART and 240 mg of LUM. The proposed method is. The objective of the present investigation was to develop & validate simple, accurate, reproducible, stability-indicating and suitable for routine determination of ART and LUM in tablet dosage form with RP-HPLC method.

MATERIALS AND METHODS

ART and LUM reference standards received as gift sample from Ipca Laboratories, Mumbai. Tablets were purchased from local pharmacies which was manufactures by Ipca Laboratories (Mumbai, India) Lumerax-40®. Ultra-pure water was obtained from a Double Distillation Apparatus-BOROSIL. Acetonitrile (HPLC grade) was obtained from E-Merck (India) Ltd, Mumbai, India. All other chemicals used in the analysis were AR grade. The HPLC analyses were carried out on Agilent Technology 1260 infinity with PDA Detector Microsorb-MV 100-5 C-18 column (250 x 4.6mm, 5 μ m). UV detection was performed at 254nm. UV spectra from 200 to 400 nm were online recorded for peak identification. The injection volume of sample was 20 μ L. An isocratic mobile phase containing acetonitrile and 0.01M potassium dihydrogen orthophosphate buffer (65:35), at the pH 3.5 was carried out with the flow rate of 1mL/min. Before the analysis, the mobile phase was degassed using Equiptronics sonicator (Equiptronics EQ 664) and filtered through a 0.45 μ m filter (Gelman Science, India). Sample solutions were also filtered through a filter. The system was equilibrated before making each injection.

Preparation of standard solution

Approximately 10 mg of ART and LUM 30 mg reference standards was accurately weighed and transferred to a 100 ml volumetric flask, 10 ml of acetonitrile was added to ensure the complete solubilisation and the volume was adjusted with the mobile phase. Further dilutions were made to get the final concentration of 5-30 μ g/ml and 60-210 μ g/ml of ART and LUM respectively.

Estimation of ART and LUM from fixed dose combination tablets

To determine the content of ART and LUM in tablets (Lumerax-40®, Label claim: 40mg. and 240mg.), 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 0.45 μ m 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 10 μ g/ml for ART and 60 μ g/ml for LUM. The experiments were performed six times under the optimized chromatographic conditions described prior. The peak areas were measured at 254nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

Method validation

Linearity

It is defined by the correlation coefficient, which must not be less than 0.99, using peak area responses. Linearity for single point standardization should extend to at least 20% beyond the specification range and include the target concentration. This was done by preparing Standard solutions containing 0.16mg/ml of ART and 1mg/ml of LUM was prepared, in triplicate. Aliquots of these solutions were diluted in mobile phase to six different concentrations, corresponding to 5, 10, 15, 20, 25, 30 μ g/ml of ART and 60, 90, 120, 150, 180, 210 μ g/ml of LUM. Calibration curves for concentration versus peak area were plotted for ART and LUM obtained data were subjected to regression analysis using the least squares method with a weighting factor of 1/x

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 six replicates on the same day as well as on three consequent days. The ART and LUM concentrations were determined and the relative standard deviations (RSD) were calculated.

Accuracy

ART and LUM reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (40, 60 and 80 μ g/ml of ART and 40, 60, 80 μ g/ml of LUM). At each level, samples were prepared in triplicate and the recovery percentage was determined.

Specificity

Spectral purities of ART and LUM chromatographic peaks were evaluated using the UV spectra recorded by a UV detector. In addition, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure and injected onto the chromatograph, to evaluate possible interfering peaks.

Robustness

Six sample solutions were prepared and analyzed under the established conditions and by variation of the following analytical parameters: flow rate of the mobile phase (0.8, 1.0 and 1.2ml/min), acetonitrile proportion in mobile phase (75:25, 65:35, 55:45), mobile phase pH (3, 3.5 and 4). The ART and LUM contents were determined for each condition and the obtained data were submitted for statistical analysis.

Detection and quantitation limits

Combined standard solutions were prepared by sequential dilutions and injected onto the chromatograph, at decreasing concentrations, in the range of 0.13–15 μ g/ml of ART and 0.10–11.25 μ g/ml of LUM. The limit of detection was defined as the concentration for which a signal-to-noise ratio of 3 was obtained and, for quantitation limit; a signal-to-noise ratio of 10 was considered.

RESULTS AND DISCUSSION

The chromatographic parameters were initially evaluated using a Microsorb-MV 100-5 C-18 column (250 x 4.6mm, 5 μ m). Under these conditions, the retention factors obtained for ART and LUM were 2.67 and 4.62, respectively. The mobile phase composed of acetonitrile and 0.01M potassium dihydrogen orthophosphate buffer (65:35) promoted an adequate separation, and a short run time (10min), and so, this condition was adopted in subsequent analysis (Fig.2).

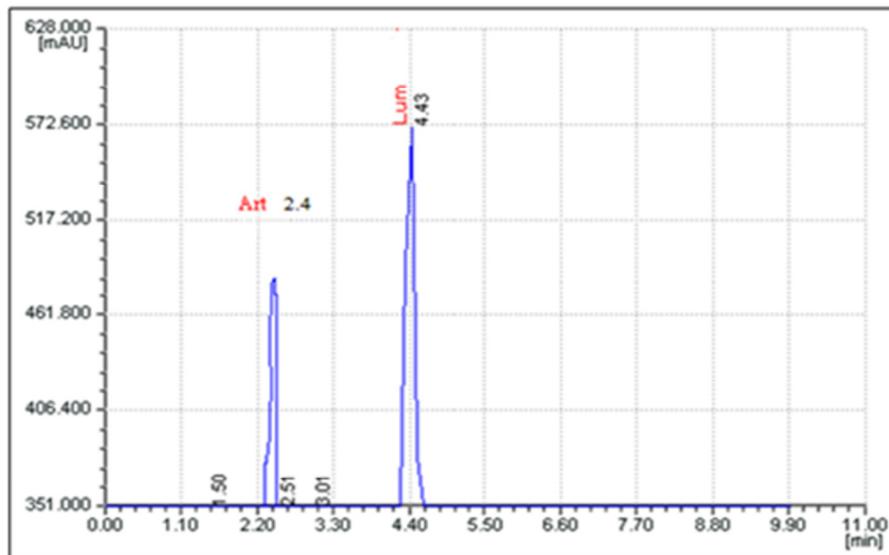


Figure 2
A Typical Chromatogram for ART And LUM

ART shows UV absorption only in the initial wavelengths of the spectrum (200–220 nm), due to the absence of chromophores in its structure. Nevertheless, the ART absorptivity is considerably low in this region, resulting in HPLC-UV methods with poor sensitivity. Hence, a RP HPLC method was developed by means of ART standard addition to the sample solutions. This approach allowed an adequate ART detection and consequently quantitation at 254 nm.

Validation *Linearity*

A linear correlation was found between the peak areas and the concentrations of ART and LUM, in the assayed range. The regression analysis data are presented in Table 1. The regression coefficients (r^2) obtained were higher than 0.99 for both compounds, which attest the linearity of the method.

Table 1
Calibration Curve Data For ART And LUM

Regression Parameters	ART	LUM
Regression coefficient, r^2	0.9963	0.9981
Slope \pm standard error	159.6 \pm 0.2	6427.1 \pm 3.15
Intercept \pm standard error	3665.90 \pm 7.24	5122.8 \pm 5.68
Relative standard error (%)	0.52%	0.27%
Concentration range	5-30	60-210
Number of points	6	6

The values are Mean \pm S.D. of triplicates

Precision

Mean contents of ART and LUM in the intraday precision analysis ($n = 6$) were 20 μ g/ml (R.S.D. = 1.75%) and 120 μ g/ml (R.S.D. = 1.35%), respectively. For the inter-day precision ($n = 18$), the mean contents obtained were 20 μ g/ml (R.S.D. = 1.45%) and 120 μ g/ml (R.S.D. = 1.47%) for ART and LUM, respectively. R.S.D. values lower than 2.0%, assure the precision of the method.

Accuracy

It was investigated by means of addition of ART and LUM reference standards to a mixture of the tablet excipients. ART mean recovery ($n = 9$) was 101.07% (R.S.D. = 0.52%) and LUM mean recovery was 101.58% (R.S.D. = 0.25%), demonstrating the accuracy of the method.

Specificity

Peak purities higher than 99.0% were obtained for ART and LUM in the chromatograms of sample solutions, demonstrating that other compounds did not co-elute with the main peaks. The chromatogram obtained with the mixture of the tablet excipients showed no interfering peaks in the same retention time of ART and LUM.

Robustness

Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of some parameters were introduced. Thus, the method showed to be robust for changes in mobile phase flow rate from 1.1 to 1.5mL/min, acetonitrile: buffer proportion from (75:25, 65:35, 55:45), mobile phase pH (3, 3.5 and 4).

Detection and quantitation limits

According to the determined signal-to-noise ratio, ART and LUM presented limits of detection of 0.600-0.013 μ g/ml and limits of quantitation of 1.95- 0.05 μ g/ml, respectively where the compounds proportion found in the sample solutions injected onto the chromatograph. However, the objective of the method is the quantitation of ART and LUM, so that the values obtained for ART and LUM should be considered as the limit of method sensitivity.

System suitability tests:

To ensure the validity of the analytical procedure, a system suitability test was established. Data from ten injections of 20 μ l of the working standard solution containing 10 μ g/ml for ART and 60 μ g/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).

Table 2
System Suitability Parameters.

Drug Name	ART	LUM
R.T. (min)	2.67	4.62
Resolution	1.35	3.74
Tailing factor	1.02	1.09
Theoretical Plates	4173.6	7652.3

Analysis of fixed dose combination tablets

Samples of fixed dose combination tablets Lumerax-40® containing 40mg of ART and 240mg LUM were analyzed using the validated method. The results obtained were presented in Table 3. All

the analyzed batches presented ART and LUM contents were very close to the labeled amount. The ART content in the tablets samples was 99.25 %, while LUM was 99.65%.

Table 3
Contents of ART and LUM in the Fixed Dose Combination Tablets

Branded Tablet	Tablet formulation	Label claim per tablet (mg)	SD	%RSD	% Drug estimated
Lumerax-40®	ART	40	0.33±0.02	1.45±0.12	99.25
	LUM	240	1.52±0.16	1.46±0.08	99.65

The values are Mean ± S.D. of triplicates

The development of simple and reliable method is essential to assure the identification and quantitative determination of antimalarial drugs, since the problem of substandard antimalarial is well established all over the world. The use of these poor quality drugs might contribute to the development of plasmodium resistance in endemic areas due to the exposition to anti-infective sub therapeutic doses.¹⁶⁻¹⁸ The quality control of the antimalarial pharmaceutical preparations marketed

now a day may help to assure the treatment efficacy and avoid the development of resistance to antimalarial drugs. 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, 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.

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, 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.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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