

# HPLC-UV method for simultaneous quantitation of artemether and lumefantrine in fixed dose combination orodispersible tablet formulation

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**Abstract:** This paper describes the development and validation of a high performance liquid chromatography (HPLC-UV) method for the simultaneous quantitative determination of artemether and lumefantrine in fixed dose combination tablets. Chromatographic quantitation was carried out on a C-18 column Mediterrania Sea 18 (250×4.6 mm i.d.; 5 µm particle size) using a mobile phase consisting of 80:20 v/v mixture of acetonitrile and 0.05 % trifluoroacetic acid with final pH adjusted to 2.35 at flow rate of 1 ml/minute. The eluents was detected using photo diode array detector at wavelength of 210 nm for artemether and 286 nm for lumefantrine. The retention times were ~5.8 mins for artemether and ~7.3 mins for lumefantrine. The newly developed method was validated and was found linear ( $r^2 > 0.99$ ), precise (R.S.D. <2.0 %), accurate, specific and robust. The artemether contents in the tablet formulation varied from 99.026 % to 99.347 %, while lumefantrine contents were 99.546–99.728 %.

**Keywords:** Artemether, lumefantrine, HPLC-UV with photo diode array detector, analysis, validation.

## INTRODUCTION

Malaria, the world's most important parasitic infection is considered among the major health and developmental challenges in the poor countries of the world (Guerin, Olliaro *et al.*, 2002). World Health Organization (W.H.O) has recommended the artemisinin-based combination therapy (ACT) as first line therapy for treating the falciparum malaria in Endemic areas. This therapy is based on the combination of an Artemisinin derivative and slowly-eliminated antimalarial Lumefantrine (W.H.O., 2001).

Artemether (fig. 1) is chemically known as 3*R*,5*aS*,6*R*,8*aS*,9*R*,10*S*,12*R*, 12*aR*)- decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4, 3-*j*]-1,2-benzodioxepin with a molecular formula C<sub>16</sub>H<sub>26</sub>O<sub>5</sub> and molecular weight of 298.4 g mol<sup>-1</sup>. Artemether is a white crystalline powder, practically insoluble in water, very soluble in dichloromethane & acetone and freely soluble in ethyl acetate and dehydrated ethanol.

Lumefantrine (fig. 1) is chemically (1*R*, 5*S*)-2-dibutylamino-1-ethanol with molecular formula C<sub>30</sub>H<sub>32</sub>Cl<sub>3</sub>NO and molecular weight of 528.9 g mol<sup>-1</sup>. Lumefantrine is a yellow crystalline powder practically insoluble in water and aqueous acids.

The increasing use of artemether-lumefantrine combination as an effective treatment for resistant malaria requires the need of analytical methods for the quantification of these drugs in pharmaceutical dosage

forms. Several methods have been reported for determining the presence of either artemether (Arun and Smith, 2011a, Karbwang *et al.*, 1997, Navaratnam *et al.*, 1995, Sioufi *et al.*, 1997, Yu *et al.*, 2006, Gauducheau *et al.*, 2002) and lumefantrine (Singtoroj *et al.*, 2005, Van Vugt *et al.*, 2000, Annerberg *et al.*, 2005, Navaratnam *et al.*, 1996, Singh *et al.*, 2009, Lu *et al.*, 1996), however only few methods are available for the simultaneous quantification of both artemether and lumefantrine in biologicals and formulations (César and Pianetti, 2009, Hodel *et al.*, 2009). The Artemether detection through UV detection is not adequate due to its low sensitivity; certain method has been developed on reverse phase HPLC-UV for simultaneous quantification of artemether and lumefantrine. The applications of such methods are limited in routine practice due to long chromatographic retention times (Belew *et al.*, 2020, Sridhar *et al.*, 2010).

In light of reported literature, this study was conducted to develop and validate analytical method for simultaneous determination of artemether and lumefantrine in a fixed dose combination tablets containing 20 mg artemether and 120 mg lumefantrine.

## MATERIALS AND METHODS

### Chemicals and Reagents

Artemether and Lumefantrine were received as gift samples from Mangalam Drugs and Organics Ltd (Mumbai, India). Acetonitrile (HPLC grade) was purchased from Fischer Scientific (Pittsburgh, PA, USA) and triethylamine and ortho-phosphoric acid were obtained from Merck (Darmstadt, Germany).

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### **Instrumentation and Chromatographic Conditions**

The separation of both drugs and the analyses of eluents were performed on Agilent 1200 system (Palo Alto, CA, USA), composed of a quaternary pump, autosampler, photo diode array detector (DAD) while data acquisition was performed through HP ChemStation software. A Mediterrania Sea 18 (250×4.6 mm i.d.; 5 µm particle size) from Teknokroma, Barcelona, Spain was used as a stationary phase for the separation of drugs. A mixture of acetonitrile and 0.05% trifluoroacetic acid 80:20 (v/v) was used as mobile phase. The mobile phase pH was adjusted to 2.35 with ortho-phosphoric acid. The mobile phase was degassed in ultrasonicator (Ultrasonic LC-10 H, Elma, Germany) for 15-20 mins before the separation of the drug was carried out under ambient conditions at a flow rate of 1 mL/min. The detection of eluents was undertaken by setting the UV spectra from 190 to 400 nm for peak identification. The peaks were monitored at wavelength of 210 nm and 286 nm for artemether and lumefantrine respectively. The separation of the analytes was evaluated in different proportions of mobile phase solvents. The retention factor (k) and resolution (R) were calculated for each condition.

### **Preparation of Artemether–lumefantrine standard solution**

Approximately 20 mg of artemether and 120 mg of lumefantrine reference standards were accurately weighed on weighing balance. The weighed quantity was transferred to a 100 ml volumetric flask. 7 ml Chloroform was added to the flask to ensure complete solubilization, followed by the addition of 80 ml of acetonitrile. The volume was filled up to the mark with 0.05% trifluoroacetic acid. The resulting solution contained 200 µg/ml of artemether and 1200 µg/ml of lumefantrine. The solution was filtered through 0.45 µm membrane filter.

### **Preparation of Sample Solution**

The tablets were weighed and crushed to a finely powdered state. An accurately weighed portion of the powder, equivalent to about 20 mg of artemether and 120 mg of lumefantrine, was transferred to a 100ml volumetric flask followed by the addition of 7ml of chloroform. The solution was sonicated for 15 min and diluted with acetonitrile to volume upto 100ml. The solution was filtered through 0.45µm membrane filter. Different batches of test formulations and one batch of reference were analyzed using the validated method. Chloroform was added to ensure the complete solubilization of the samples. Six replicates of each batch were assayed for the analysis.

The placebo solution was prepared by weighing all the excipients separately, mixed with the diluent and finally filtered through 0.45 µm membrane filter.

### **Method Validation**

The currently reported HPLC-UV analytical procedure for simultaneous determination of artemether and lumefantrine in different formulations was evaluated and validated according to International Conference on Harmonization (ICH) guidelines. The parameters for method validation as per ICH guidelines included system, suitability and specificity, linearity, accuracy, precision, reproducibility, robustness and range (ICH, T., 1996).

### **System Suitability and Specificity**

The system suitability for the analysis was determined by running 5 replicates of both the blank (mobile phase only) and standard solutions. The area under the curves of each replicates in the standard solution were calculated for percentage relative standard deviation % RSD ( $\leq 2\%$ ), theoretical plates ( $\geq 1000$ ) and tailing factor ( $\leq 2.0$ ) as per USP.

To evaluate the system specificity for the analytes, placebo (formulation constituents without active drug ingredients), blank (mobile phase only) and standard solutions were injected separately and respective chromatograms were observed for any interference between the active drug ingredients and excipients present in the finished formulation.

### **Linearity and Accuracy**

System linearity and Accuracy was calculated for 5 concentrations (50%, 75%, 100%, 125% and 150%) of standard solutions of both artemether and lumefantrine prepared by diluting the standard solution and run on HPLC. The accuracy of the system was determined by evaluating the percent recovery of these concentrations in quintuplets.

### **Precision and Intermediate Precision**

The precision of the system was carried out by comparing the percentage assay of six independently prepared samples with standard solution of both artemether and lumefantrine at final concentration (200 µg/ml artemether and 1200µg/ml lumefantrine).

The intermediate precision of the systems was carried out by performing the assay tests each on two separate but similar instruments by two individual analysts on two different days. All the other procedures and factors were kept same as mentioned above for precision testing.

### **Robustness**

To test the capacity of this newly developed analytical procedure to withstand small changes in the analytical method, samples of standard solution were run at different mobile phase flow rates (0.8, 1.0 and 1.2 ml/min) and mobile phases with different pH values (pH 2.25, 2.35 and 2.45).

**Range**

To determine the range of this analytical procedure, various concentrations prepared for linearity testing were analyzed to determine highest and lowest quantifiable concentration with acceptable accuracy and precision.

**Solution Stability**

To determine the solution stability of standard and the sample preparation, the solution was stored at ambient temperature and tested after 24, and 48 hrs. The responses obtained after analysis of standard and sample solutions were assessed in comparison with freshly prepared solutions. The stability study of the stored standard and

sample solutions were performed and found to be stable for up to 48 hrs.

**RESULTS**

**Analytical Method Validation**

The validation of this newly developed analytical procedure for simultaneous quantitative determination of artemether and lumefantrine was successfully established following the ICH guideline. The validation of this procedure, included all the parameters as described in USP for validation of compendial methods under category-I. (ICH.T., 1996).

**Table 1:** System Suitability Parameters for quantitation of Artemether and Lumefantrine

Analyte	Peak area of analytes after replicate injections (n=5)					Mean	%RSD	Theoretical plates	Tailing factor
Artemether	1486804	1495328	1489702	1498602	1499814	1494050	0.377	2657	1.007
Lumefantrine	41008445	40885646	40958604	40905349	40982496	40948108	0.126	2605	0.973

\* USP-NF 29

**Table 2:** Nominal concentrations of calibration standards for determination of linearity

Analyte	Nominal concentration					r <sup>2</sup>
	50%	75%	100%	125%	150%	
Artemether (µg/ml)	100	150	200	250	300	0.997
Lumefantrine (µg/ml)	600	900	1200	1500	1800	0.996

**Table 3:** Analytical Recovery of Artemether and Lumefantrine

Analyte	Mean recovery of analytes at different concentrations			Mean	SD	%RSD
	50%	100%	150%			
Artemether	99.142	99.038	99.347	99.176	0.157	0.158
Lumefantrine	99.594	99.553	99.546	99.564	0.719	0.722

**Table 4:** Results of analyst variability for determination of Artemether and Lumefantrine

Analyte	Mean % accuracy			
	Analyst 1		Analyst 2	
	Mean	%RSD	Mean	%RSD
(n=6)				
Artemether	99.085	0.370	99.194	0.438
Lumefantrine	99.077	0.291	99.062	0.228

**Table 5:** Analytical method robustness of Artemether and Lumefantrine

Analyte	Mean accuracy at three different flow rates of mobile phase			Mean accuracy at three different pH of mobile phase		
	0.8 ml/min	1 ml/min	1.2ml/min	pH 2.25	pH 2.35	pH 2.45
(n=3)						
Artemether	99.179	99.112	99.178	99.067	99.034	99.648
Lumefantrine	99.155	99.184	100.178	99.039	99.096	99.1

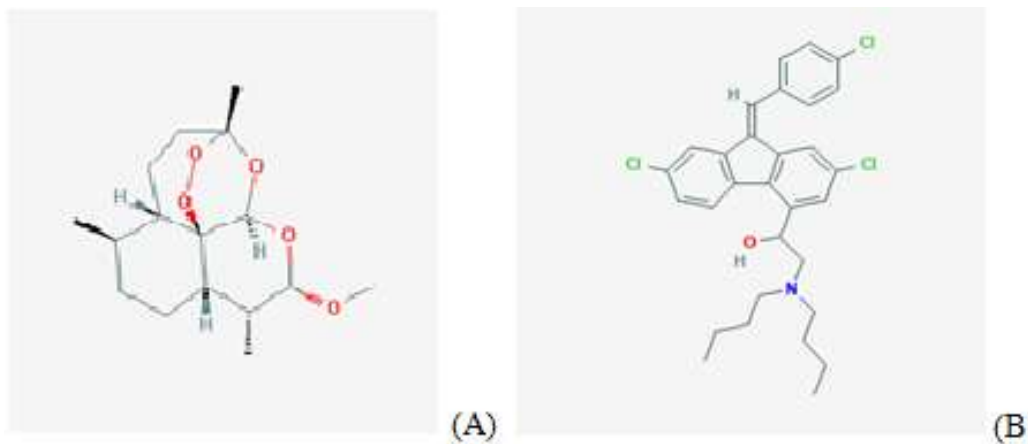


Fig. 1: Structural formula of Artemether (A) and Lumefantrine (B).

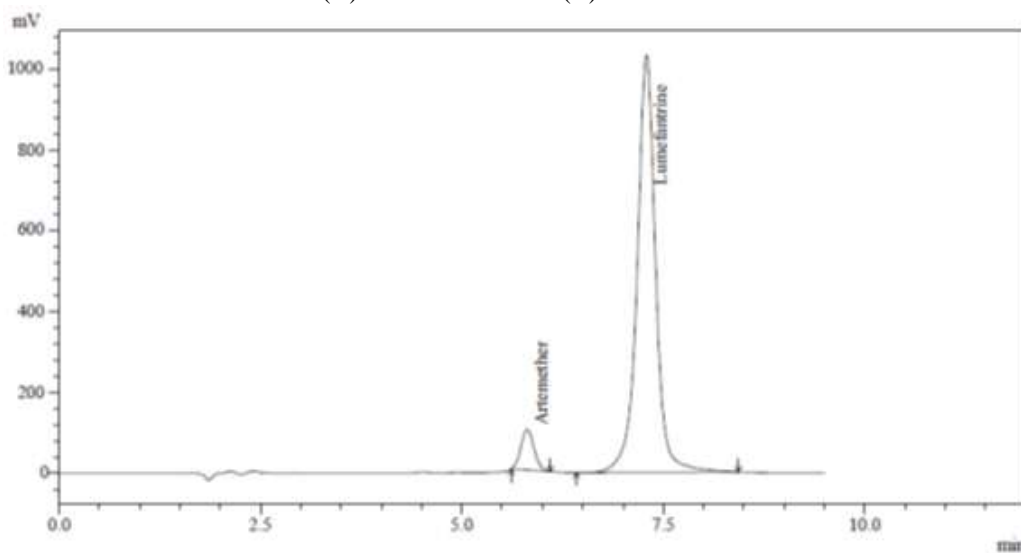


Fig. 2: HPLC-UV Chromatogram showing resolution of peaks of Standard solution of Artemether and Lumefantrine.

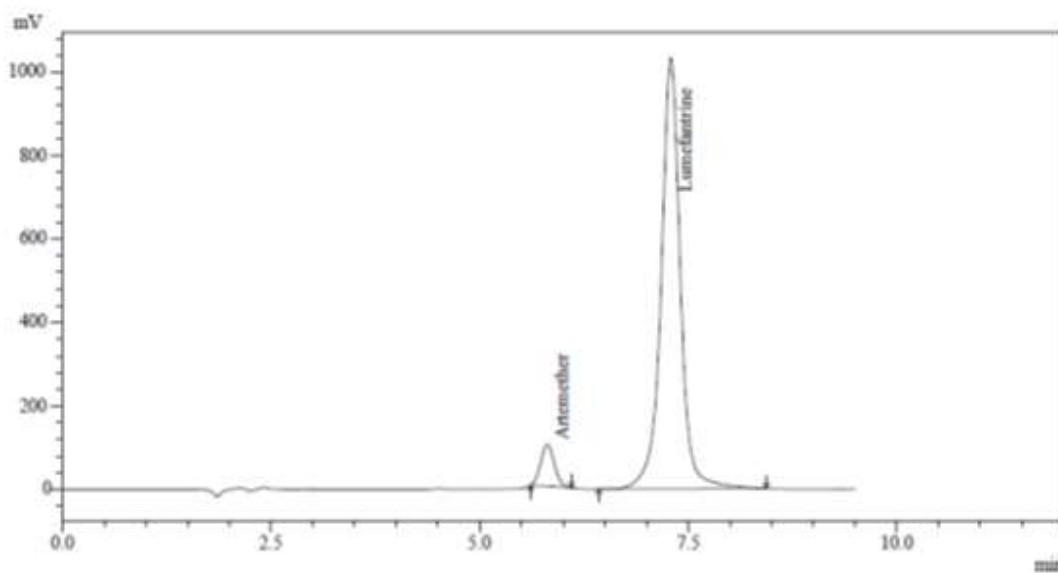


Fig. 3: HPLC-UV Chromatogram showing resolution of peaks of Sample solution of Artemether and Lumefantrine.

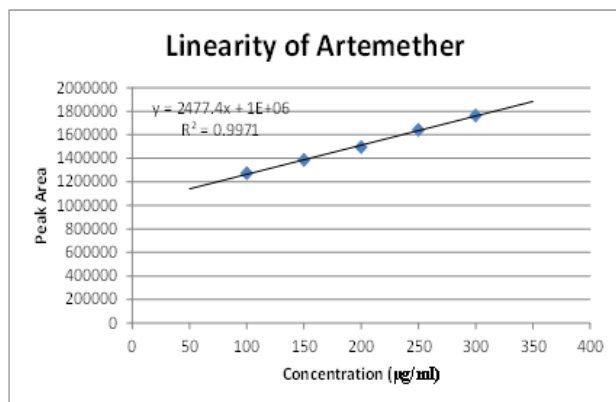


Fig. 4: Graph showing Linearity of Artemether

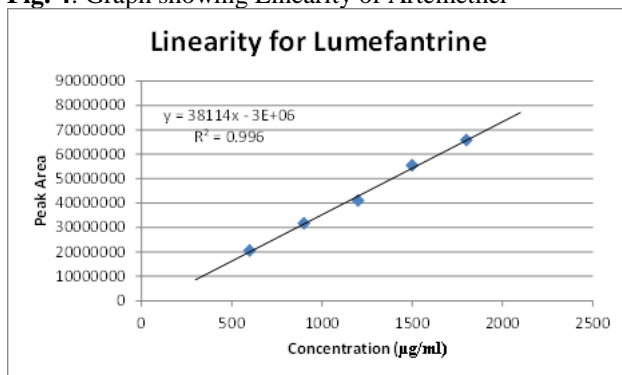


Fig. 5: Graph showing Linearity of Lumefantrine

#### System Suitability and Specificity

System suitability of the newly developed analytical procedure was performed and results were evaluated by calculating % RSD for peak areas, theoretical plates and tailing factor of quintuplets runs of the standard solution. All the parameters observed were satisfactory as per USP requirements for % RSD ( $\leq 2\%$ ), theoretical plates ( $\geq 1000$ ) and tailing factor ( $\leq 2.0$ ). Parameters calculated for system suitability of artemether and lumefantrine is shown in table 1.

Excipient interference was evaluated by chromatographic analysis of analytes, placebo (excipients without active ingredients), standard solution and blank when run on HPLC with photo diode array detector. There was no chromatographic interference observed in the developed method due to any additive material found in the formulation (fig. 2 and 3).

#### Linearity and Accuracy

Linearity Testing was performed to estimate the degree of proportionality between the concentration of analytes and their respective amounts recovered after analyses. Five working concentrations (50%, 75%, 100%, 125% and 150%) of standard solution were run on HPLC and their respective amounts recovered after analysis were found to be linearly correlated ( $R^2 \geq 0.995$ ) for both artemether and lumefantrine which were all above the USP limit of  $> 0.99$  (fig. 4, 5 and table 2).

The accuracy of the method was determined by quintuplets run of three different concentrations (50 %, 100 % and 150 %) of sample solution. The system was found to be very accurate as the mean recovery of both artemether and lumefantrine obtained were found well within the range of 98 % to 102 % with % RSD calculated  $\leq 2.0$  as per USP requirements (table 3).

#### Precision and Intermediate Precision

The newly established Analytical method was highly precise as the % RSD of mean recovery of 6 independently prepared standard solutions were 0.404% and 0.260% for artemether and lumefantrine respectively (table 4). These values of % RSD were well within the range defined in USP  $\leq 2.0\%$ .

Intermediate precision of the newly developed method was also observed to be satisfactory as the % RSD of 3 different concentrations of standard solution by two individual analysts on two different days were found very well within the range specified in USP  $\leq 2.0\%$ .

#### Robustness

The slight deliberate changes in pH of mobile phase and Flow rate did not impart any significant difference in the recovery of eluents (table 5). The % RSD of mean recovery of the artemether and lumefantrine was found to be within the acceptable range of  $\leq 2\%$ .

#### Range

The range for this newly developed analytical procedure was established after evaluating the accuracy, precision and linearity for the highest and lowest possible concentrations. The parameters evaluated were found to be satisfactory within the limits as per pharmacopoeial standards. Therefore, concentration ranges calculated for the artemether and lumefantrine were 100µg/ml to 300 µg/ml and 600µg/ml to 1800µg/ml respectively (table 2). This suggests that the newly developed analytical procedure is valid over a wide range of concentrations for the objective drugs.

#### Solution Stability

Solution Stability was assessed by preparing the standard and the sample solutions and kept at ambient temperature. The stored samples were tested after 24 and 48 hrs. The responses of both the standard and sample solutions were evaluated by comparing with freshly prepared solutions. The results stability study obtained were found to be stable for up to 48 hrs.

### STATISTICAL ANALYSIS

During the course of the study, all the statistical analysis was performed using the Microsoft Office Excel version 2016.

## DISCUSSION

To develop a robust HPLC based analytical method for simultaneous determination of artemether and lumefantrine, preliminary investigations were made in the light of reported literature (Arun and Smith, 2011b, Kalyankar and Kakde, 2011, Shrivastava, Issarani *et al.*, 2010, Sunil, Nath *et al.*, 2010). Initially the analytical method used consisted of buffer and acetonitrile in ratio of 50:50 as mobile phase. The buffer solution was prepared using 0.01 M potassium dihydrogen orthophosphate with pH adjusted to 3.00 with orthophosphoric acid. Sunil *et al* employed these chromatographic conditions in their reported method but it suffered from unacceptably high tailing factor for lumefantrine peak i.e. 3.6 the samples of artemether and lumefantrine were run on HPLC at flow rate of 1 ml/min with injection volume 50 µL setting the wavelength at 210 nm. Arun, R *et al.*, 2011 and Sridhar *et al.*, 2010 respectively selected wavelengths of 254 nm and 303 nm for the quantitation of the two analytes(Arun and Smith, 2011b, Debrah, Nettey *et al.*, 2016, Sridhar, Rao *et al.*, 2010). They reported accurate quantitation of artemether at this wavelength which seems contrary to its very low molar absorptivity value as reported by Cesar *et al*(César, de Aquino Ribeiro *et al.*, 2011). The chromatographic column used was symmetry C18 (250×4.6 mm i.d.; 5 µm particle size). Artemether and Lumefantrine eluted at 4 and 5 minutes respectively. The peak areas of the eluents was noted and observed that lumefantrine eluted with sharp peak while artemether with a broader peak. Therefore, the mobile phase composition was changed to buffer and acetonitrile in ratio of 40:60 (Buffer: ACN). No significant difference in the peak shape was observed.

The mobile phase composition was changed replacing buffer with 0.05 % trifluoroacetic acid. The ratio studied with this mobile composition of 0.05 % trifluoroacetic acid and acetonitrile was 50:50, 40:60, 30:70 & 20:80. The flow rate was set at 1ml/min. The standard and sample solutions of artemether and lumefantrine were run separately on HPLC at optimized concentrations. The peak areas of the eluents were then monitored at different wavelengths in the range of 190 nm to 400 nm using diode array detector. Finally, the optimum wavelength of 210 nm for artemether and 286 nm for lumefantrine was selected where the peak areas of both the drugs were quantified accurately with satisfactory resolution. Kalyankar, T.M., 2011 selected a wavelength of 210 nm for detection of both analytes which in our case exhibited very high absorbance of lumefantrine to overload the UV detector which is contrasting to these previously reported results. For the appropriate composition of mobile phase it was observed that at higher ratio of 0.05 % trifluoroacetic acid in the mobile phase (acetonitrile: 0.05 % trifluoroacetic acid, 80:20), the analyte peaks sharpened and hence was selected as optimum

composition for mobile phase with retention times of ~5.8 mins for artemether and ~7.3 mins for lumefantrine in the standard and sample solutions (see figure 2 and 3).

## CONCLUSION

This study was conducted to develop a new facile HPLC based analytical method for the simultaneous determination of artemether (20 mg) and lumefantrine (120 mg) in a newly developed formulation. Various advantages were offered by this method which includes easily constitutable mobile phase and shorter run time with high resolution of the analytes's peaks. This newly developed analytical method has been validated according to parameters provided in ICH guidelines. The method has been found to be very simple and convenient to perform; sensitive and specific for the objective drugs. Moreover the method is accurate, precise and robust over a wide range of analytes's concentration. Therefore, in the light of the study, the proposed method can be used for routine simultaneous analysis of combined formulation of artemether and lumefantrine in any analytical setting of either a pharmaceutical industry or research organization or any academic institution which houses an HPLC-UV instrument.

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