QbD APPROACH FOR STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF ARTEMETHER AND LUMEFANTRINE IN COMBINED DOSAGE FORM

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RESEARCH ARTICLE

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ABSTRACT

The present paper reports a highly selective, sensitive and robust stability indicating RP-HPLC method using QbD approach, developed and validated for determination of Artemether and Lumefantrine in combined dosage form. The experimental design describes the scouting of the key HPLC method components including mobile phase and pH. Their interrelationships are studied and optimized conditions are obtained for each combination of mobile phase and pH with the help of design expert 10.0 version. Optimal chromatographic conditions were obtained using Phenomenex, Gemini 5u C-18 column combined with a mixture of Acetonitrile: Buffer (35:65 v/v) mobile phase, pH adjusted to 2.5 with buffer H_3PO_4. The flow rate was 1.5ml/min. The injection volume was 10µl. Retention time for Artemether and Lumefantrine were found 4.44 and 7.84 min respectively. The method was linear with 2-6µg/ml and 12-36µg/ml for Artemether and Lumefantrine respectively. The LOD and LOQ were 0.000278 µg/ml and 0.000841 µg/ml respectively. This method found to have a good percentage recovery in forced degradation study using acid, base, oxidation, photolytic, thermal and neutral conditions indicates well separation of both the drugs with other degradation products. All validation parameters were within the acceptance limits.

Keywords: QbD, Artemether, Lumefantrine, HPLC, Stability Indicating Analytical Method.

INTRODUCTION

Quality by Design (QbD) (1-4) is well established in the pharmaceutical industry for manufacturing processes (ICH Q8 for pharmaceutical development and ICH Q11 for development and manufacture of drug substances). QbD is "a systematic approach to development that begins with predefined objectives and emphasizes understanding and control, based on sound science and quality risk management". The outcome of using QbD concepts is a well-understood product and process that consistently delivers its intended performance. The knowledge obtained during development may support the establishment of a design space and determines suitable process controls. This same QbD principle has been applied to the development of analytical methods and is termed "Analytical QbD" (AQbD). A alogous to process QbD, the outcome of AQbD is well understood, fit for purpose, and robust method that consistently delivers the intended performance throughout its lifecycle. High performance liquid chromatography (HPLC) (5-7) is a type of column chromatography used frequently for analytical chemistry and biochemistry. RP-HPLC is the choice for the majority of samples. It consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase. The quality of HPLC methods has become increasingly important in a QbD environment. For the purpose of QbD for HPLC methods, robustness and ruggedness should be verified early in the method development stage to ensure method performance over the lifetime of the product. Otherwise, if a non-robust or non-rugged method is adapted, significant time and resource may be required to redevelop, revalidate and retransfer analytical methods.

The present work is aimed to develop QbD approach to stability indicating analytical method development and validation of Artemether and Lumefantrine by HPLC.

The primary objective of this study was to implement Qbd approach to develop and validate an RPHPLC method that could separate drug from its potential related substances and to establish an in-depth understanding of the method and build in the quality during the method development to ensure optimum method performance over the lifetime of the product.

Artemether ((3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR)- decahydro-10-methoxy-3, 6, 9-trimethyl-3, 12-epoxy- 12Hpyrano [4,3-j]-1, 2-benzo dioxepin) is a semi-synthetic poly-oxygenated-amorphene containing aperoxide bridge that confers potent antimalarial activity. It is the O-methyl ether prodrug of dihydroArtemisinin and a derivative of Artemisinin (qinghaosu), the principal antimalarial constituent of the Chinese herb Artemisia annua (qinghao). Artemether is active against the erythrocytic stage of multidrug-resistant strains of Plasmodium falciparum. Lumefantrine is chemically (9Z)-2,7- dichloro-9-[(4-chlorophenyl) methylene]-a-[(dibutyl amino) methyl]-9H-fluorene- 4-methanol.

Artemether and Lumefantrine are now available in fixed combination products (ACT), which are proven to be highly efficacious for treatment of uncomplicated P. falciparum malaria. Artemether-Lumefantrine (ART-LUM) is the most common ACT used in malaria endemic areas. Artemether has a rapid onset of action and is rapidly eliminated from the plasma (half-life of two to three hours. Lumefantrine is cleared more slowly and has a longer elimination half-life (approximately 4.5 days). The rationale behind this combination is that Artemether initially provides rapid symptomatic relief by reducing the number of parasites present before Lumefantrine eliminates any residual parasites. Artemether & Lumefantrine also reduces gametocyte carriage and thus should have an impact on malaria transmission.

The increasing use of these Artemether Lumefantrine combination anti-malarial products and the intrinsic stability of these products require controlled storage conditions. Therefore, it is important to have a rapid, but robust and stability indicating quantitative method for the simultaneous assay of Artemether and Lumefantrine in fixed dose combination (FDC) products.

Literature survey revealed that though some methods have been developed and reported for estimation of Artemether & Lumefantrine in bulk and in tablet dosage form and in combination also but no method was developed for stability study of Artemether and Lumefantrine in combined dosage form by applying QbD approach using HPLC.

The methods are reported for forced degradation profiling of Artemether by HPLC (1), stability indicating RP-HPLC for Lumefantrine (2). There are various analytical methods UV-spectrophotometric (3), HPTLC (4), HPLC (5-9) are reported for simultaneous estimation of Artemether and Lumefantrine. The literature survey reveals that

The reported method for QbD approaches are QbD approach to analytical method development and validation of Piracetam by HPLC (10) and QbD approach for eberconazole nitrate. (11) The present study was aimed to develop QbD approach based stability indicating HPLC method for Artemether and Lumefantrine in combined dosage form.

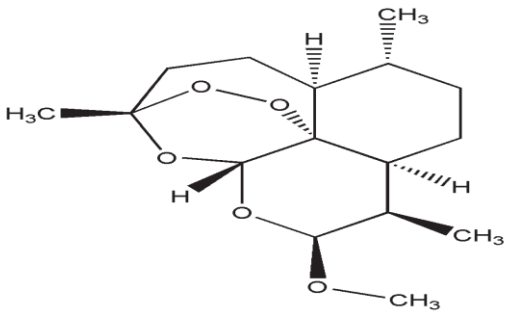

Figure 1: Structure of Artemether

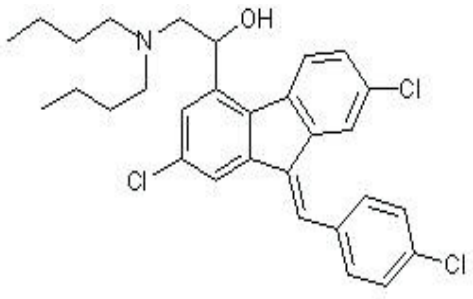

Figure 2: Structure of Lumefantrine

MATERIAL AND METHODS

MATERIALS

Pure sample of Artemether and Lumefantrine was kindly supplied as a gift sample by Ipca

Laboratories. All Chemicals and Reagents used were of Analytical Grade and HPLC Grade.

HPLC METHOD

The HPLC system used was Liquid Chromatography: LC-2010 CHT (Shimadzu) with SPD-M20A Prominence Diode Array Detector (Shimadzu). The column used was Phenomenex, Gemini 5u C-18 column (250 x 4.6mm, 5µm particle size). The optimal composition of mobile phase was composition of Acetonitrile: Buffer (35:65 v/v). The flow rate was set to 1.5 mL/min and wave length was 210 nm.

OPTIMIZATION OF MOBILE PHASE

Preparation of Standard Solution

The standard solution containing Artemether and Lumefantrine equivalent to 40µg/ml of ART and 240µg/ml of Lumefantrine was prepared by accurately weighing 4mg of Artemether and 24mg of Lumefantrine which is transferred to a 100ml volumetric flask. Make up the volume using mobile phase as diluent.

Preparation of Sample Solution

The standard solution containing Artemether and Lumefantrine equivalent to 40µg/ml of Artemether and 240µg/ml of Lumefantrine was prepared by accurately weighing tablet powder equivalent to 4mg of ART and 24mg of LUM which is transferred to a 100ml volumetric flask. Make up the volume using mobile phase as diluent.

Method development by QbD approach

1. Define method intent

The goals of HPLC method development have to be clearly defined, as pharmaceutical QbD is a systemic, scientific, risk based, holistic and proactive approach that begins with predefined objectives and emphasizes product and process understanding and control.

A systematic experimental design is needed to assist with obtaining in- depth method understanding and performing optimization. Here an efficient and comprehensive experimental design based on systematic scouting of two key components of the RP-HPLC method (mobile phase and pH) is presented. It forms a chromatographic database that will assist with method understanding, optimization and selection. In addition, it can be used to evaluate and implement change of the method, should it be needed in the future, for example should the chromatographic column used no longer be commercially available, or an impurity is no longer relevant.

Factorial Design

Central composite statistical screening design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the *in-vitro* release of the drug. A 2-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with Design Expert® (Version 10.0, Stat-Ease Inc., Minneapolis, MN).

$$Y = \beta0 + \beta1A + \beta2B + \beta12AB + \beta11A2 + \beta22B2$$

Where Y is the measured response associated with each factor level combination; β0 is an intercept; β1 to β22 are regression coefficients computed from the observed experimental values of Y from experimental runs; and A and B are the coded levels of independent variables. The terms AB, A2 and B2 represent the interaction and quadratic terms, respectively. The factors were selected based on preliminary study. A pH (A) and mobile phase composition (B) and were selected as independent variables. The Retention time, peak area and peak asymmetry were selected as dependent variables.

2. Perform experimental design

Table 1: Coded Values for Independent Variables

Name of the Factor	Coded values	Level		
		-1	0	1

pH	A	2.5	3	3.5
Mobile phase composition	B	35	40	45

Table 2: Different Batches with their Respective Composition

Batch code	pH(A)	Mobile phase composition (B)
P1	-1	+1
P2	0	0
P3	+1	-1
P4	-1	0
P5	0	0
P6	+1	+1
P7	+1	0
P8	-1	-1
P9	0	-1
P10	0	+1
P11	0	0

3. Evaluate experimental results and select final method conditions

These method conditions were evaluated using the three tiered approach. At the first level, the conditions were evaluated for peaks symmetry, retention time and peaks tailing. This resulted in different chromatographic conditions for API. The best suited experimental conditions shall be optimized using design expert software.

4. Perform risk assessment with robustness and ruggedness evaluation

As the final method is selected against method attributes, it is highly likely that the selected method is reliable and will remain operational over the lifetime of product. Therefore, the evaluation of method robustness and ruggedness to be carried out as the final step of method development is mainly for the method verification and finalization. A risk-based approach based on the QbD principles set out in ICH Q8 and Q9 was applied to the evaluation of method robustness and ruggedness. Structured methodologies for risk assessment, such as Fishbone diagram can be implemented to identify the potential risk of the method due to a small change of method parameters or under a variety of conditions such as different laboratories, analysts, instruments, reagents, days, etc.

5. Define analytical method performance control strategy

As a result of robustness and ruggedness studies, the overall method understanding of method performance under various conditions can be improved and an analytical method performance control strategy along with appropriate system suitability criteria can be defined to manage risk and ensure the method delivers the desirable method attributes. If the risk is high and is hard to manage, it is an opportunity for the analyst to go back to the database described in experimental design to find a more appropriate method and to go through the procedure as described to ensure method robustness and ruggedness.

Analytical method validation

Validation is documented evidence, which provide a high degree of assurance for specific method. Validation is analytical process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirement for intended analytical application.

FORCED DEGRADATION STUDY

ACID DEGRADATION

Preparation of Blank for Acid Degradation

Pipette out 0.1ml 1N HCL and transfer it to a 10ml volumetric flask. Add 5ml diluent to it and keep it on water bath for 2hrs at 60 ^{0}C. After that allow it to cool and then neutralize with 0.1ml 1N NaOH; make up the volume upto 10ml with diluent.

Preparation of Standard Solution of ART for Acid Degradation

Weigh accurately 4mg of ART and transfer it to a 100ml volumetric flask. Add 1ml 1N HCl to it and 25ml diluent; keep it for 2 hrs on water-bath at 60 ^{0}C, after that allow it to cool and then neutralize with 1ml 1N NaOH to stop the degradation further. Now, make up the volume upto 100ml with diluent.

Preparation of Standard Solution of LUM for Acid Degradation

Weigh accurately 24mg of LUM and transfer it to a 100ml volumetric flask. Add 1ml 1N HCL to it and 25ml diluent; keep it for 2 hrs on water-bath at 60^0 C, after that allow it to cool and then neutralize with 1ml 1N NaOH to stop the degradation further. Now, make up the volume upto 100ml with diluent.

Preparation of Sample Solution for Acid Degradation

Weigh accurately tablet powder equivalent to 4mg of ART and 24mg LUM and transfer it to a 100ml volumetric flask. Add 1ml 1N HCl to it and 25ml diluent; keep it for 2 hrs on water-bath at 60 ^{0}C, after that allow it to cool and then neutralize with 1ml 1N NaOH to stop the degradation further. Now, make up the volume upto 100ml with diluent.

BASE DEGRADATION

Preparation of Blank for Base Degradation

Pipette out 0.1ml 1N NaOH and transfer it to a 10ml volumetric flask. Add 5ml diluent to it and keep it on water bath for 2hrs at 60 ^{0}C. After that allow it to cool and then neutralize with 0.1ml 1N HCL; make up the volume upto 10ml with diluent.

Preparation of Standard Solution of ART for Base Degradation

Weigh accurately 4mg of ART and transfer it to a 100ml volumetric flask. Add 1ml 1N HCl to it and 25ml diluent; keep it for 2 hrs on water-bath at 60 ^{0}C, after that allow it to cool and then neutralize with 1ml 1N NaOH to stop the degradation further. Now, make up the volume upto 100ml with diluent.

Preparation of Standard Solution of LUM for Base Degradation

Weigh accurately 24mg of LUM and transfer it to a 100ml volumetric flask. Add 1ml 1N NaOH to it and 25ml diluent; keep it for 2 hrs on water-bath at 60 ^{0}C, after that allow it to cool and then neutralize with 1ml 1N HCL to stop the degradation further. Now, make up the volume upto 100ml with diluent.

Preparation of Sample Solution for Acid Degradation

Weigh accurately tablet powder equivalent to 4mg of ART and 24mg LUM and transfer it to a 100ml volumetric flask. Add 1ml 1N NaOH to it and 25ml diluent; keep it for 2 hrs on water-bath at 60 ^{0}C, after that allow it to cool and then neutralize with 1ml 1N HCL to stop the degradation further. Now, make up the volume upto 100ml with diluent.

OXIDATIVE DEGRADATION

Preparation of Blank for Oxidative Degradation

Pipette out 0.1ml 3% H_2O_2 and transfer it to a 10ml volumetric flask. Add 5ml diluent to it and keep it on water bath for 2hrs at 60 ^{0}C. After that allow it to cool and then make up the volume upto 10ml with diluent.

Preparation of Standard Solution of ART for Oxidative Degradation

Weigh accurately 4mg of ART and transfer it to a 100ml volumetric flask. Add1ml 3%$H_2O_2$to it and 25ml diluent; keep it for 2 hrs on water-bath at 60^0 C temp., after that allow it to cool. Now, make up the volume upto 100ml with diluent.

Preparation of Standard Solution of LUM for Oxidative Degradation

Weigh accurately 24mg of LUM and transfer it to a 100ml volumetric flask. Add 1ml 3% H_2O_2 to it and 25ml diluent; keep it for 2 hrs on water-bath at 60 ^{0}C temp., after that allow it to cool. Now, make up the volume upto 100ml with diluent.

Preparation of Sample Solution for Oxidative Degradation

Weigh accurately tablet powder equivalent to 4mg of ART and 24mg LUM and transfer it to a 100ml volumetric flask. Add 1ml 3% H_2O_2 to it and 25ml diluent; keep it for 2 hrs on water-bath at 60 ^{0}C, after that allow it to cool. Now, make up the volume upto 100ml with diluent.

PHOTO DEGRADATION

Preparation of Blank for Photo Degradation

Pipette out 5ml diluent and keep it under sunlight for 24hrs; now, make up the volume upto 10ml with diluent.

Preparation of Standard Solution of ART for Photo Degradation

Transfer 100mg of ART to a petri dish with a closed lid and expose it to sunlight for 24 hrs; after that weigh 4mg from it and transfer it to a100ml volumetric flask; now, make up the volume upto 100ml with diluent.

Preparation of Standard Solution of LUM for Photo Degradation

Transfer 100mg of LUM to a petri dish with a closed lid and expose it to sunlight for 24 hrs; after that weigh 24mg from it and transfer it to a 100ml volumetric flask; now, make up the volume upto 100ml with diluent.

Preparation of Sample Solution for Photo Degradation

Transfer 100mg of tablet powder to a petri dish with a closed lid and expose it to sunlight for 24 hrs; after that weigh that powder equivalent 4mg ART and 24mg LUM and transfer it to a100ml volumetric flask; now, make up the volume upto 100ml with diluent.

THERMAL DEGRADATION

Preparation of Standard Solution of ART for Thermal Degradation

Transfer small quantity of ART to a petri dish and keep it at 80 ^{0}C in hot air oven for 6 hrs; after that weigh 4mg from it and transfer it to a 100ml volumetric flask; now, make up the volume upto 100ml with diluent.

Preparation of Standard Solution of LUM for Thermal Degradation

Transfer small quantity of ART to a petri dish and keep it at 80 ^{0}C in hot air oven for 6 hrs; after that weigh 24mg from it and transfer it to a 100ml volumetric flask; now, make up the volume upto 100ml with diluent.

Preparation of Sample Solution for Thermal Degradation

Transfer small quantity of tablet powder to a petri dish and keep it at 80 ^{0}C in hot air oven for 4 hrs; after that weigh that powder equivalent to 4mg ART and 24mg LUM and transfer it to a 100ml volumetric flask; now, make up the volume upto 100ml with diluent.

VALIDATION OF PROPOSED HPLC METHOD

The proposed HPLC method was validated as per ICH Q2 (R1) guidelines.

Preparation of Reference Standard Solution

The standard stock solution of Artemether of 40µg/ml was prepared by dissolving 40mg of Artemether in 100ml diluent and Lumefantrine of 240µg/ml was prepared by dissolving 24mg of Lumefantrine in 100ml mobile phase. The standard sub-stock solution of Artemether of concentrations 2, 3, 4, 5 and 6µg/ml and of Lumefantrine of concentrations 12, 18, 24, 30 and 36µg/ml were prepared from above standard solution with diluent.

LINEARITY

The linearity of Artemether and Lumefantrine was determined by analyzing 5 independent levels of calibration curve in the concentration range of 2-6µg/ml for ART and 12-36µg/ml for LUM in terms of slope, intercept and correlation

coefficient values. The calibration curve was prepared by plotting peak area verses concentration and correlation coefficient was determined.

PRECISION

Repeatability

Measure Peak Area of standard stock solution of Artemether and Lumefantrine of 40µg/ml and 240µg/ml respectively at 210nm.The peak area of the solution was measured 6 times and %RSD was calculated.

Intra-Day Precision

Variation of the results within same day is called intra-day precision. The intra-day precision was determined by analyzing Artemether at 3, 4 and 5µg/ml and Lumefantrine at 18, 24 and 30 µg/ml concentrations respectively, three times on same day at interval of 1 hour, simultaneously and %RSD was calculated.

The %RSD should be less than 2.

Inter-Day Precision

Variation of results amongst day is called inter-day precision. Inter-day precision was determined daily by analyzing Artemether at 3, 4 and 5µg/ml and Lumefantrine at 18, 24 and 30µg/ml concentrations respectively, for three days and %RSD was calculated.

The % RSD should be less than 2.

ACCURACY (% RECOVERY)

Accuracy of the method was confirmed by recovery study from marketed formulation at three level (80%, 100% and 120%) of standard addition. Percentage recovery for ART and LUM were found out. Recovery between 98-102 % justifies the accuracy of the method.

LOD & LOQ

The evaluation of the sensitivity of the analytical method was done by lowest limit of detection and lowest limit of quantitation.

LOD and LOQ were measured by the mathematical equation given below.

- **LOD = 3.3** x **σ/S**
- **LOQ = 10** x **σ/S**

Where, **σ** = Standard Deviation of the Response and **S =** Slope

ROBUSTNESS

Robustness of the method was determined by subjecting the method to slight change in method condition:

- Pump Flow Rate
- pH
- Temperature

%RSD was calculated.

SYSTEM SUITABILITY STUDIES

The system suitability was evaluated from standard chromatogram by six replicate injections of Artemether and Lumefantrine. The %RSD, Tailing Factor and Theoretical Plates were calculated for standard solutions.

ASSAY

Preparation for Sample stock solution

20 tablets (each containing 40 mg Artemether and 240mg Lumefantrine) were weighed and powdered. The tablet powder equivalent to 4mg and 24mg of Artemether and Lumefantrine was accurately weighed and transferred to a 100ml volumetric flask, about 25ml of diluent was added and the flask was sonicated for 15 minutes. Filter this solution with Whatman filter paper. (ART-250mg/ml, LUM-2400mg/ml). The volume was made up to the mark with diluent and mixed well.

Working Standard Preparation

Pipette out 1ml from sample stock solution in 10ml volumetric flask and then make up the volume upto 10ml with diluent, (ART-40µg/ml, LUM 240µg/ml)

RESULT AND DISCUSSION

Optimization of Mobile Phase

The mobile phases were optimized with various composition of mobile phase like Acetonitrile:

Water: Triethylamine (60:40:0.2, v/v), Acetonitrile: Buffer (55:45, v/v), (60:40, v/v), (65:35, v/v) to develop a stability indicating method. There were found different chromatograms in which tailing of both drug, tailing with less retention time and satisfactory pick were found. The method was optimized using Phenomenex, Gemini 5u C-18 column (250 x 4.6mm, 5µm particle size) using mobile phase Buffer pH 2.5: Acetonitrile: Buffer (35:65 v/v) The flow rate was 1.5 ml/min and injection volume was 10µl. The detection was carried out at 210nm.

Optimization of various parameters for Analysis of Artemether and Lumefantrine using HPLC (By Using Central Composite Design)

Table 3: Design Summary for optimization

Study Type	Response Surface
Design Type	Central Composite Design
Design Model	Quadratic
Runs	11

Factor	Name	Units	Type	Subtype	Minimum	Maximum
A	pH		Numeric	Continuous	2.5	3.5
B	Mobile Phase	ml	Numeric	Continuous	35	45

Table 4: Evaluation degrees of freedom of design for optimization of analysis of Artemether and Lumefantrine by HPLC.

Response	Name	Units	Analysis	Minimum	Maximum	Ratio	Model
R1	Retention Time	min	Polynomial	6.75	11.55	1.711111	Quadratic
R2	Area	mAU	Polynomial	2007	282266	140.6408	2F1
R3	Peak Assymetry		Polynomial	0	7.63	N/A	Quadratic

Table 5: Obtained solution for optimized formulation

Code	Mobile Phase	pH	Retention time	Area	Peak asymmetry	Desirability
P8	35	2.5	7.31	195977	4.607	0.493

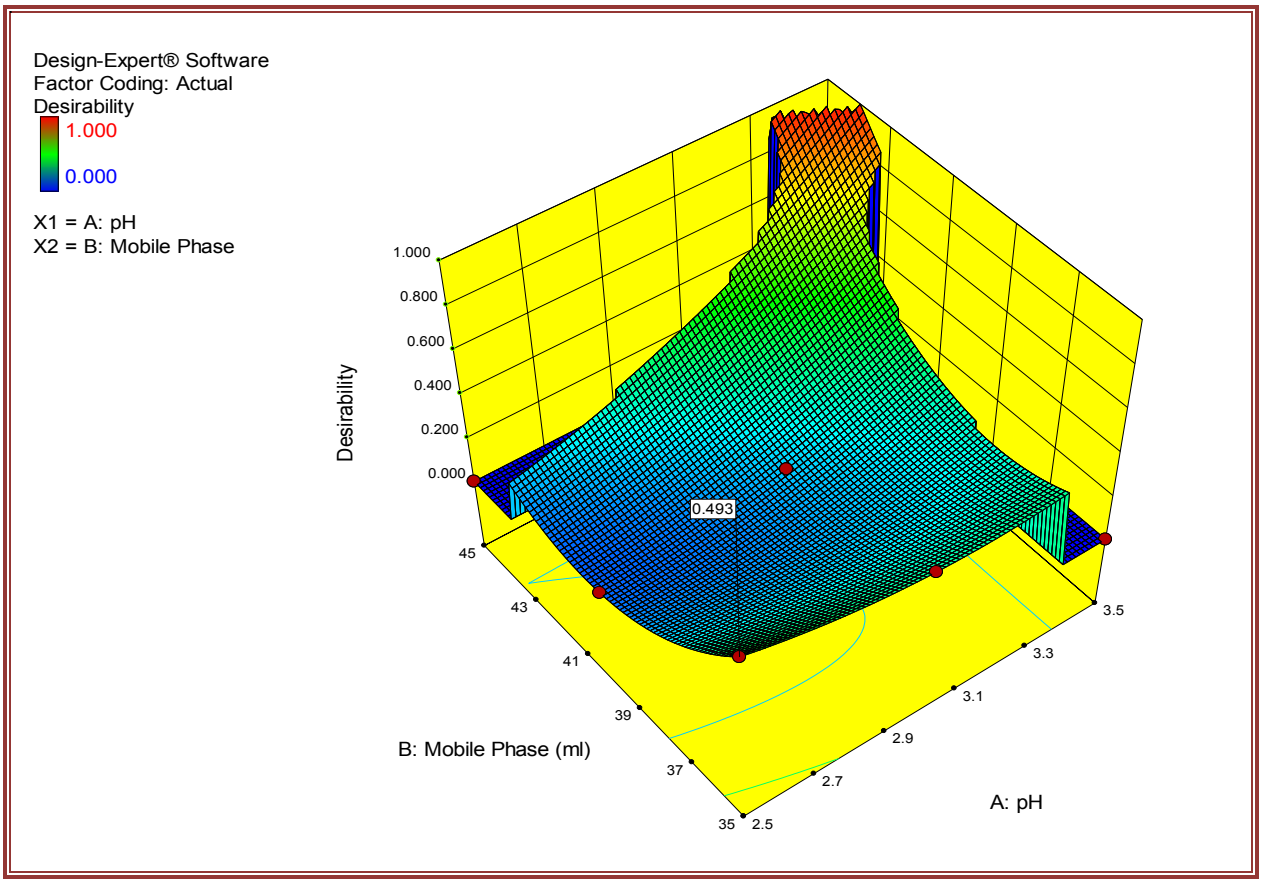

Figure 3: 3D surface plot of desirability for obtaining optimized formulation

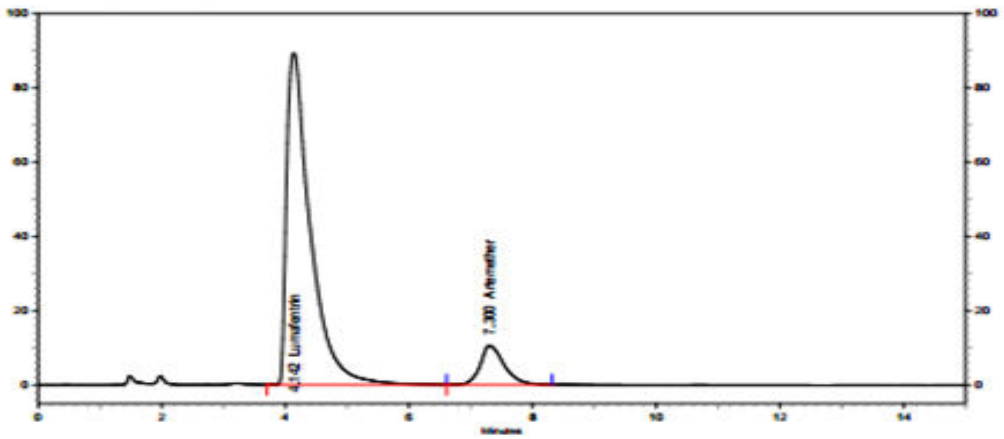

Figure 4: Chromatogram obtained from the optimized formula of Artemether (40µg/ml) and Lumefantrine (240 µg/ml)

Table 6: Condition Predicted From Optimized Formula

Response	Predicted value	Observed value
Retention time	7.31	7.48
Peak Area	195977	282266
Peak Asymmetry (for Artemether)	4.607	4.47

System Suitability Studies

Table 7: System Suitability Test for ART and LUM

Acceptance Criteria	Result	
	ART	LUM
The %RSD for five replication injections of standard preparation for ART and LUM should be NMT 2.0.	0.79	0.37
The Tailing factor for ART and LUM from standard preparation should be NMT 2.0	1.28	1.45
Theoretical Plates for ART and LUM should be NLT 2000	3646.15	2714.80
Resolution	4.47	0.00

FORCED DEGRADATION STUDY

The area of standard for Artemether (40µg/ml) and Lumefantrine (240µg/ml) was 296773 and 2172342, respectively.

Acid Degradation

After refluxing, the drug solution with 1N HCl at 60°C for 2 hrs, the percentage degradation of ART and LUM in acidic condition was found to be 22.55 and 5.41%, respectively.

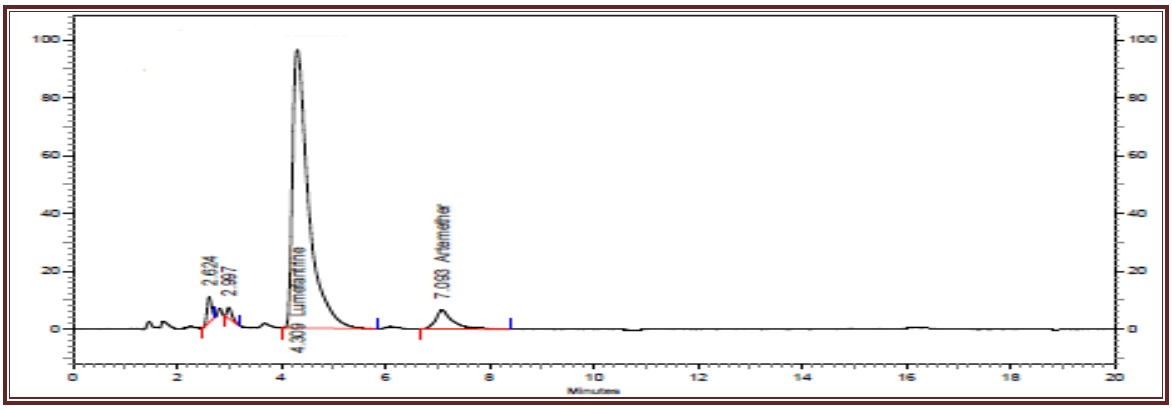

Figure 5: Chromatogram of sample solution of Artemether(40 µg/ml)and Lumefantrine(240 µg/ml) for acid degradation

Base Degradation

After refluxing, the drug solution with 1N NaOH at 60°C for 2 hrs, the percentage degradation of ART and LUM in acidic condition was found to be 5.19and 3.10%, respectively.

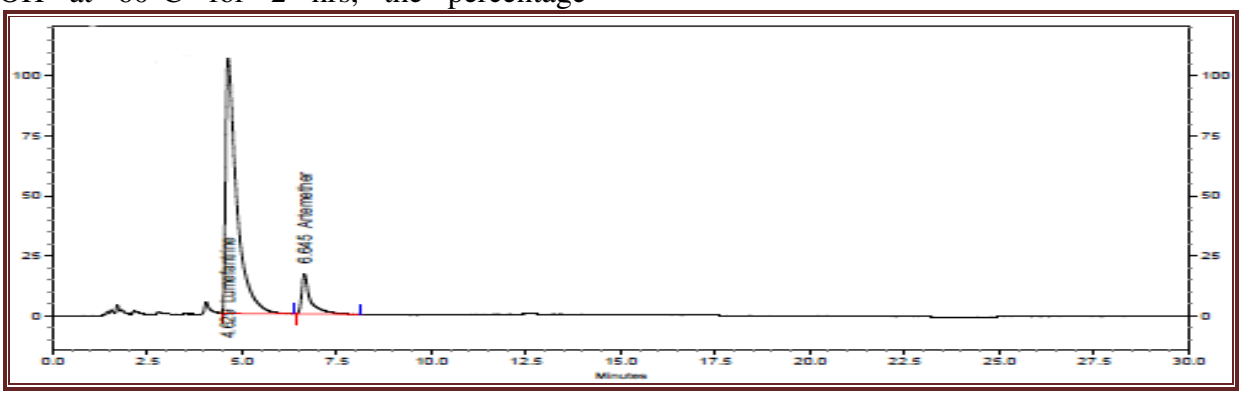

Figure 6: Chromatogram of sample solution of Artemether(40 µg/ml)and Lumefantrine(240 µg/ml) for base degradation

Oxidative Degradation

After refluxing, the drug solution with 3% hydrogen peroxide at 60°C for 2 hrs, the percentage degradation of ART and LUM in acidic condition was found to be 5.68and 7.98%, respectively.

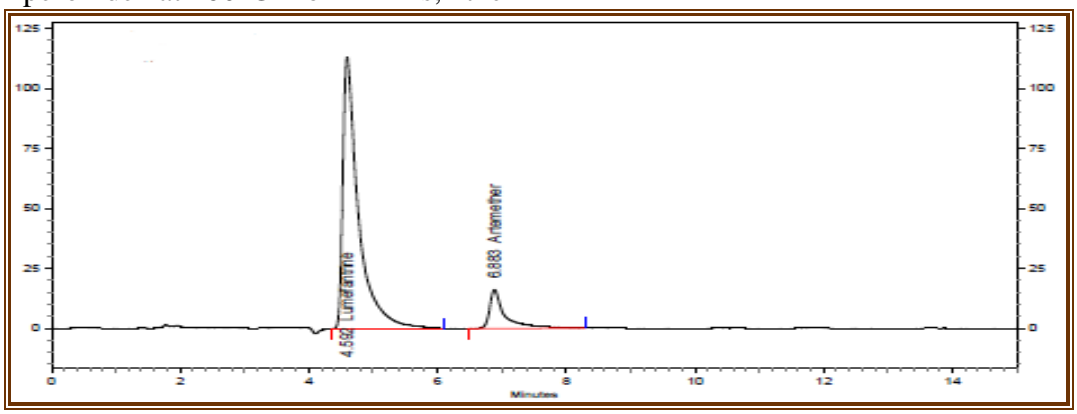

Figure 7: Chromatogram of sample solution for Artemether(40 µg/ml)and Lumefantrine(240 µg/ml) for oxidative degradation

Photolytic Degradation

After exposing the drug powder to direct sunlight in Petri dish covered with lid, the percentage degradation of ART and LUM in Photolytic condition was found to be 5.69and 10.34%, respectively.

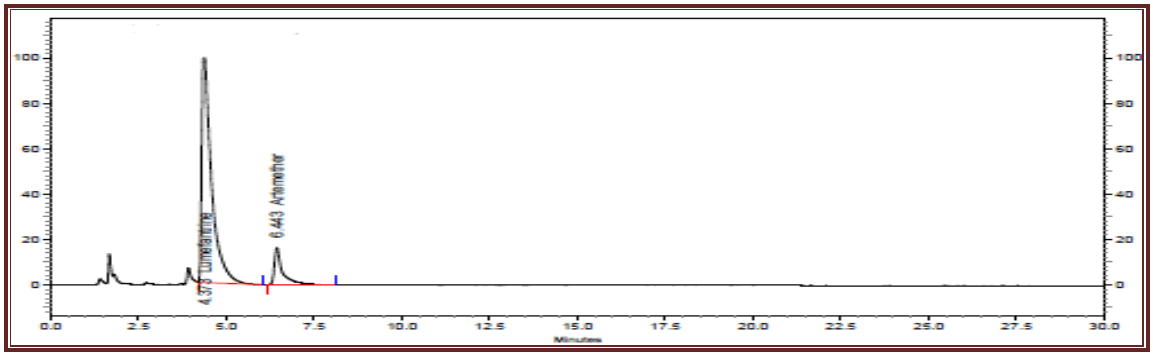

Figure 8: Chromatogram of sample solution of Artemether(40 µg/ml)and Lumefantrine(240 µg/ml) for photolytic degradation

Thermal Degradation

After exposing the drug powder to dry heat at 80°C for 4 hours, the percentage degradation of ART and LUM in Dry Heat condition was found to be 5.50and 9.52%, respectively.

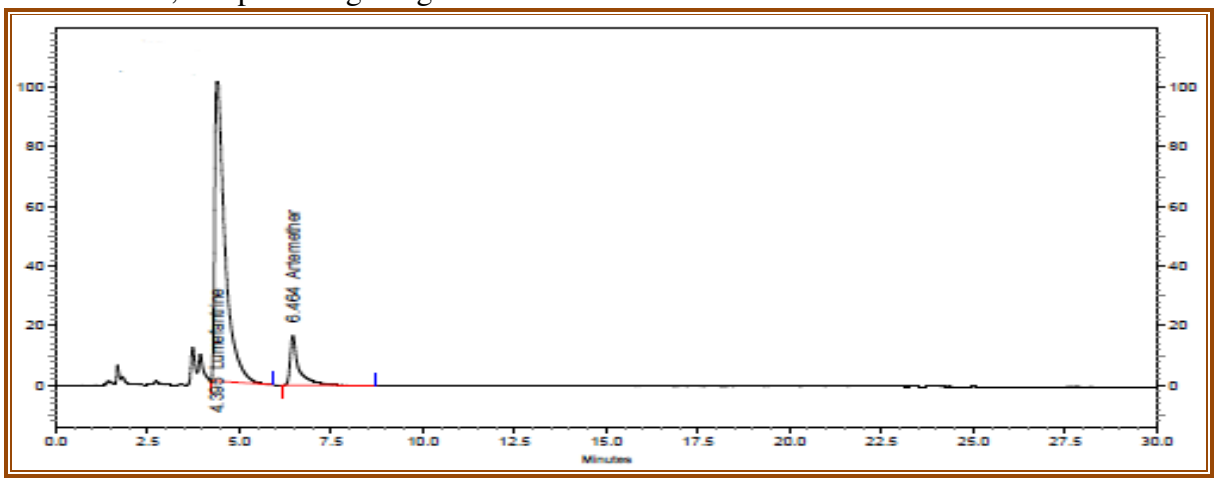

Figure 9: Chromatogram of sample solution of Artemether(40 µg/ml)and Lumefantrine(240 µg/ml) for thermal degradation

Table 8: Summary of Forced Degradation Study

Parameter	Stress Condition	% Degradation of Artemether		% Degradation of Lumefantrine	
		Std	Sample	Std	Sample
Acid Degradation	1N HCl (2hrs reflux at 60^0C)	23.97	22.55	4.00	5.41
Base Degradation	1N NaOH(2hrs reflux at 60^0C)	6.65	5.19	5.62	3.10
Oxidative Degradation	3% H_2O_2 (2hrs reflux at 60^0C)	3.89	5.68	8.38	7.98
Photo Degradation	Photolytic (Sunlight, 24 hrs)	2.18	5.69	8.49	10.34
Thermal Degradation	Dry Heat (80^0C, 6 hrs)	4.88	5.50	8.19	9.52

METHOD VALIDATION

Linearity

The linearity response was determined by analyzing independent levels of Calibration curve in the range of 2-6 µg/ml for ART and 12-36 µg/ml for LUM. The calibration curve was found to be linear in this range.

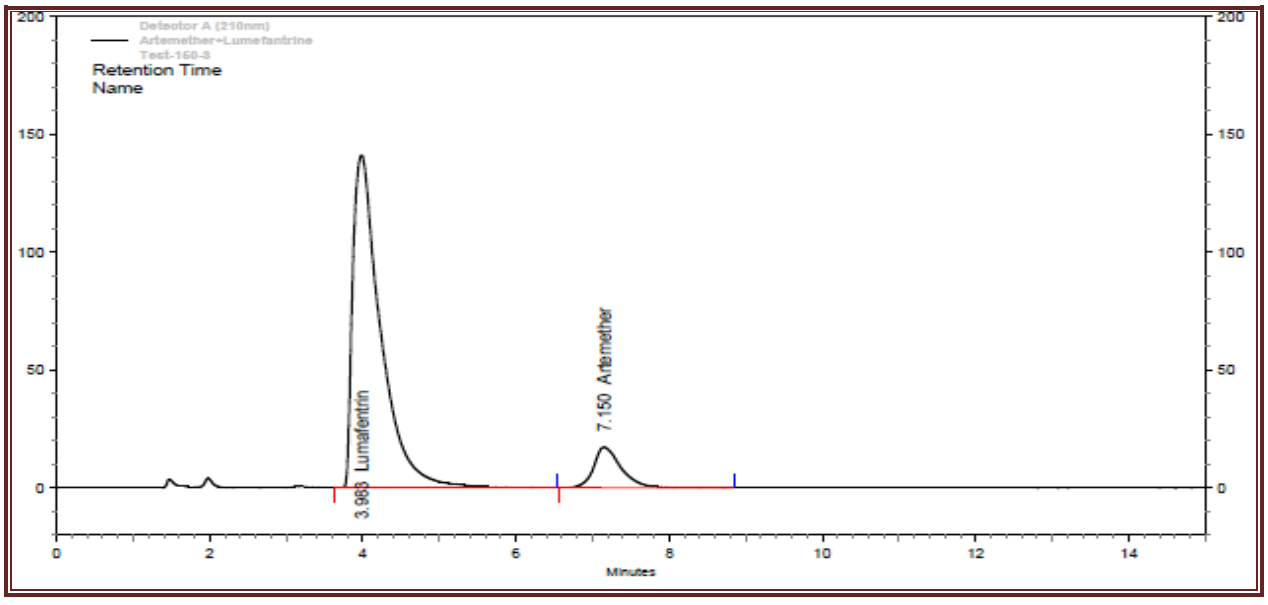

Figure 10: Chromatogram of 5 µg/ml ART and 30 µg/ml LUM

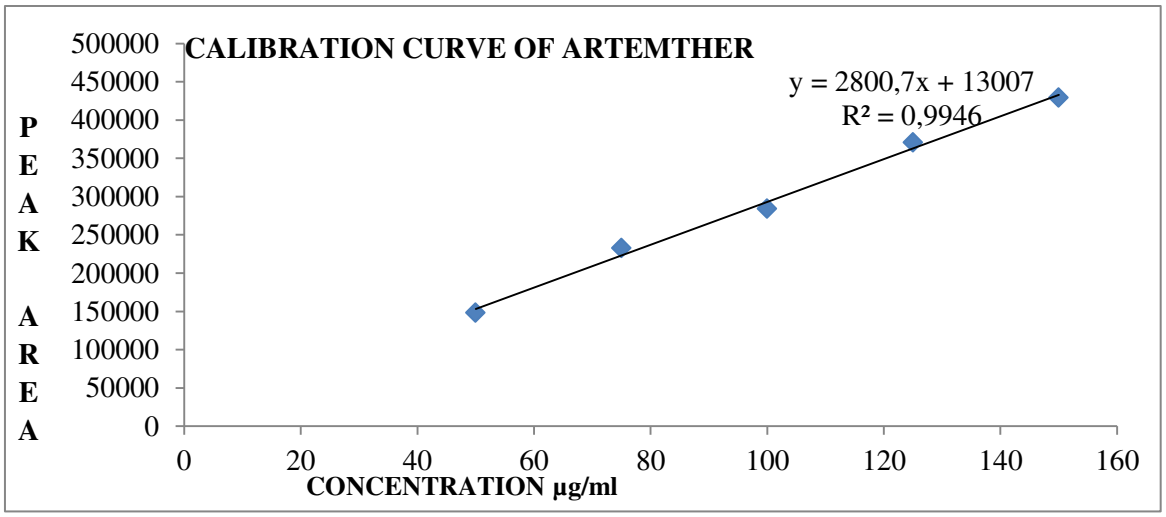

Figure 11: Calibration Curve for Artemether

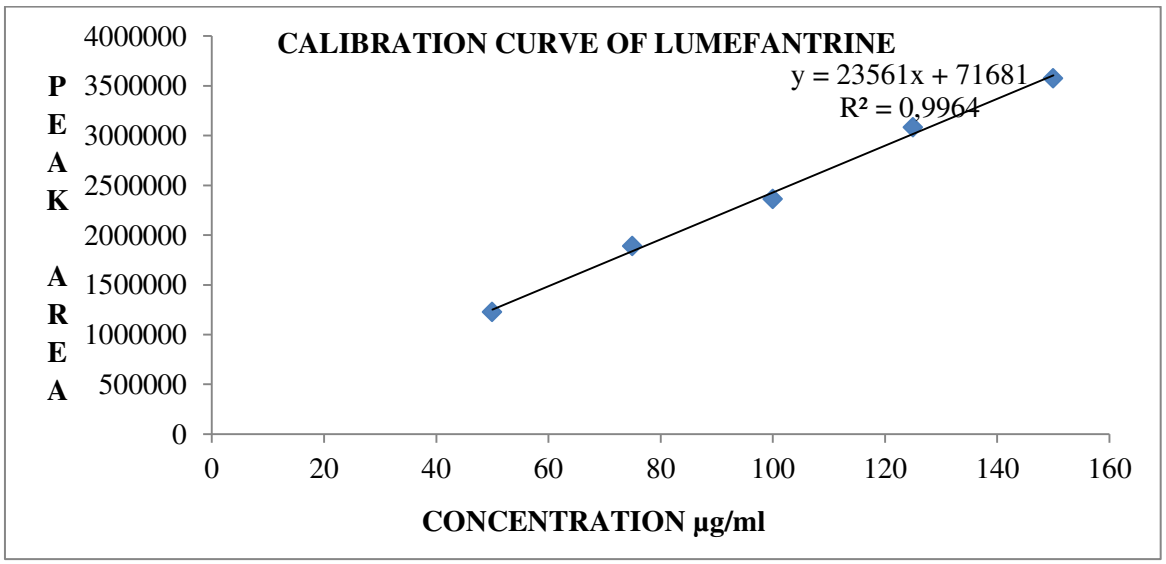

Figure 12: Calibration Curve for Lumefantrine

Table 9: Linearity of Artemether and Lumefantrine

	Artemether		Lumefantrine	
Sr. No.	**Concentration (µg/ml)**	**Peak Area (Mean ± SD); (n=5)**	**Concentration (µg/ml)**	**Peak Area (Mean ± SD); (n=5)**
1	2	1315059.00±16977.49	12	1226898.667±40114.84
2	3	2109405.33 ± 10590.13	18	1890717±3921.80
3	4	2623241.00 ± 1934.25	24	2362688±9794.58
4	5	3408746.33 ± 28983.90	30	3082382±13114.14
5	6	3932050 ± 37621.21	36	3576181±22249.73
Regression line equation		y = 2800.7x + 13007		y = 23561x + 71681
Correlation Coefficient R^2		0.9946		0.9964

LOD and LOQ

The LOD of ART and LUM were found to be 0.601µg/ml and 1.084µg/ml and the LOQ of ART and LUM were found to be 1.153µg/ml and 3.286µg/ml, respectively.

Accuracy

The percentage recovery of ART and LUM were found to be in range 98.20-99.72% and 98.35-99.38%, respectively.

Table 10: Result of Accuracy (%Recovery)

Assay level	Tablet wt. eq. to (mg)		Standard added (mg)		Total drugs recovered (mg)		% Recovery of standard added	
80%	4	24	3.2	19.2	3.164	18.850	98.89	98.95
	4	24	3.2	19.2	3.161	18.980	98.81	99.65
	4	24	3.2	19.2	3.152	18.821	98.52	98.58
Mean± SD							98.74±0.1	98.35±0.44
%RSD							0.20	0.44
100%	4	24	4	24	3.926	23.348	98.17	99.38
	4	24	4	24	3.936	23.117	98.40	98.92
	4	24	4	24	3.921	23.259	98.03	102.46
Mean±SD							98.20±0.18±1.01	99.06±0.54±.1.39
%RSD							0.19	0.55
120%	4	24	4.8	28.8	4.786	28.427	99.72	98.70
	4	24	4.8	28.8	4.794	28.274	99.88	98.17
	4	24	4.8	28.8	4.781	28.295	99.61	98.24
Mean±SD							99.72±0.13	98.70±0.28
%RSD							0.13	0.29

Precision

The %RSD value of ART and LUM for Repeatability was found to be 0.79and 0.37%, for Intra-Day Precision was found to be in range 0.71-1.51% and 0.25-0.43%, for Inter-Day Precision was found to be in range 0.56-1.11% and 0.21-0.43%, respectively. The %RSD value of ART and LUM were found to be less than 2%, which indicates that the developed method is precise.

Table 11: Intra-Day and Inter-Day Precision of ART and LUM

Sr.No	Precision Period	Concentration (µg/ml)		Mean (n=3)	SD (n=3)	%RSD
1	Intraday Precision	ART	3	228499.333	3461.12	1.51
			4	286593.333	2711.60	0.95
			5	371126.667	2648.30	0.71
		LUM	18	1890688.3	5802.82	0.31
			24	2367685.6	5893.64	0.25
			30	3089494.66	13311.07	0.43
2	Interday Precision	ART	3	230228.333	2042.28	0.89
			4	285047.667	3172.90	1.11
			5	370833.666	2069.33	0.56
		LUM	18	1890717	3921.81	0.21
			24	2363295.67	9826.89	0.42

			30	3082382	13114.14	0.43

Robustness

The Robustness of the method was evaluated by A. By changing the flow rate: ± 0.15, B. By changing the Temp. : ± 2.5, C. By changing the pH ± 0.25

Table 12: Results of Robustness for ART and LUM

Sr. No	Parameter	Mean area (n=3)		SD (n=3)		%RSD	
		ART	LUM	ART	LUM	ART	LUM
1	Flow Rate +0.15(1.65 ml/min)	291411.7	2188647.6	1096.64	5921.22	0.38	0.27
2	Flow Rate-0.15 (1.35ml/min)	292262.3	2177514.0	877.42	6181.00	0.30	0.28
3	Temp. +2.5 (27.5^0C)	291224.7	2201052.0	996.51	18136.96	0.34	0.82
4	Temp. -2.5 (22.5^0C)	282768.7	2202572.3	1686.31	9747.02	0.60	0.44
5	pH +0.25 (2.75)	291285.0	2175229.6	1275.14	20109.10	0.44	0.92
6	pH -0.25 (2.25)	293977.0	2200881.6	1103.71	11698.38	0.38	0.53

Assay Analysis

The %Assay was found to be 97.34±0.20for ART and 97.20±1.18 for LUM.

Table 13: Assay of ART and LUM

Sr.no	Drug	Label Claim (mg)	Amount found (mg)	Area of samples	%Assay	%ASSAY±SD	%RSD of assay
1	ART	40	24.56	288226	97.57	97.34±0.20	0.21
2		40	24.60	287379	97.29		
3		40	24.65	287048	97.17		
Average					97.34		
3	LUM	240	67.87	2260629	96.78	97.20±1.18	1.21
4		240	68.01	2248962	96.28		
5		240	68.03	2301483	98.53		
Average					97.20		

CONCLUSION

A reversed phase HPLC method development approach for stability study using QbD principles has been described for Artemether and Lumefantrine. The experimental design describes the scouting of the key HPLC method components including mobile phase and pH. Their interrelationships are studied and optimized conditions are obtained for each combination of mobile phase and pH with the help of design expert 10.0 version. Central composite statistical screening design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the in-vitro release of the drug. A 2-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with Design Expert® (Version 10.0, Stat-Ease Inc., Minneapolis, MN). The factors were selected based on preliminary study. pH (A) and Mobile Phase (B) were selected as independent variables. The Retention time, peak area and peak asymmetry were selected as dependent variables.

All the validated parameters were found within acceptance criteria. The validated method is specific, linear, precise, accurate, robust and rugged for determination based on knowledge of method obtained through the method

development and the results of risk assessment along with robustness and ruggedness studies, detailed analytical method performance control strategy can be defined to manage the risk.

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CONFILCTS OF INTEREST

The authors declare that there are no conflicts of interest.

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