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Original Article

THE DEVELOPMENT AND VALIDATION OF A FAST STABILITY INDICATING RP-HPLC METHOD FOR QUANTIFICATION OF LUMEFANTRINE AND ITS ORGANIC IMPURITIES USING CENTRAL COMPOSITE EXPERIMENTAL DESIGN

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ABSTRACT

Objective: The objective of the present study was to develop and validate a stability indicating RP-HPLC method for Lumefantrine (LF) and its organic impurities using a central composite design (CCD).

Methods: A specific, simple quality control friendly isocratic elution method using reverse phase HPLC was developed for quantification of Lumefantrine (LF) and its organic impurities at a wavelength of 265 nm. The chromatographic separation was achieved on the column of Thermo Hypersil ODS C18 (150x4.6 mm, 3µ) with a buffer containing 0.1percent formic acid and acetonitrile 10:90 v/v as a mobile phase with a flow rate of 1.6 ml/min at 35 °C with a run time of 10 min. Based on the preliminary trials, CCD was employed to check the effect of independent variables such as Acetonitrile ratio (A), Flow rate (B), and Column oven temperature (C). While resolution between Lumefantrine (LF) and Impurity-A (X1), Impurity-A and Impurity-B (X2), and Plate count of Lumefantrine (LF) (X3) were considered as dependent variables and statistical evaluation performed by using design expert software. The optimized conditions were validated as per ICH guidelines.

Results: The retention time of LF and its organic impurities were 1.9 min, 3.0, 4.5, and 6.4 min, respectively. Design space was established and desirability was found. LOD and LOQ for the Lumefantrine (LF) and its impurities were established with respect to test concentration. The plotted calibration curves were linear with a regression coefficient of R2>0.99, indicating that the linearity was within the limit. As a part of method validation, the parameters like Specificity with forced degradation, Linearity, Precision, Accuracy, Ruggedness, and Robustness were determined and the results were found to be within the allowable limits.

Conclusion: The method developed and validated was found to be suitable for routine analysis and to be used for the measurement of Lumefantrine and its impurities. Since there is no stability indicating the RP-HPLC method with design space was reported in the literature, there is a need to develop quantitative methods under different conditions to achieve improvement in specificity and selectivity.

Keywords: Central composite design, Organic impurities, RP-HPLC, Forced degradation, Validation, Lumefantrine

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INTRODUCTION

Lumefantrine (LF) is an antimalarial agent that demonstrates synergistic anti-malarial activity with Artemether [1, 2]. Malaria is a serious and sometimes fatal protozoan disease caused by malaria parasites (*Plasmodium vivax, ovale, falciparum and malariae*) and transmitted by the female *Anopheles* mosquitoes [3-5].

Lumefantrine (LF) is chemically, 2,7-Dichloro-9-[(4-chlorophenyl) methylene]-α-[(dibutylamino) methyl]-9H-fluorene-4-methanol and is a racemic aromatic fluorene derivative (fig. 1).

LF is first synthesized and registered in China for the treatment of malaria [6, 7]. The molecular formula is $C_{30}H_{32}Cl_3NO$ and the molecular weight is 528.9 g per mol. physically yellow crystalline powder and odorless. Practically soluble in water and aqueous acids, freely soluble in ethyl acetate, soluble in dichloromethane, and slightly soluble in ethanol, chloroform, and acetonitrile [8].

The tablet dosage forms with a combination of Artemether and Lumefantrine (10 mg+60 mg, 20 mg+120 mg, 40 mg+240 mg, and 80 mg+480 mg) are available. The mechanism is that Artemether will rapidly reduce parasitemia, resulting in symptomatic relief and Lumefantrine will eliminate the remaining parasites [9]. Lumefantrine contains an endoperoxide bridge, which interferes with heme polymerization, a critical detoxifying pathway for the malarial parasite, and secondary action is inhibiting the nucleic acid and protein synthesis within the parasite [10].

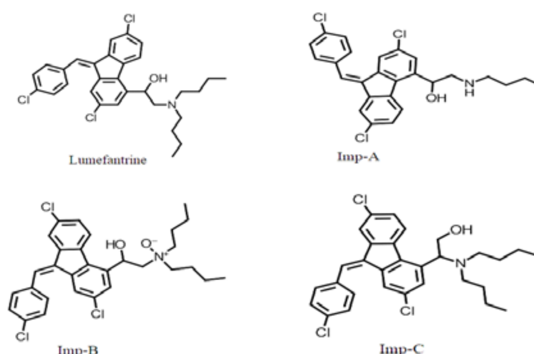

Fig. 1: Chemical structures of lumefantrine (LF) and its impurities

Determinations of drug impurity and drug degradation products are very important from both pharmacological and toxicological perspectives [11, 12]. The primary objective of this study was to implement the DOE approach to develop and validate a RP-HPLC method that could separate the drug from its potential organic impurities 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. Thin layer chromatographic methods have been reported for the determination of DHA, Artemeisin, alpha artemether, impurity A in artemether active substance, and artemether and lumefantrine tablets in International Pharmacopoeia. A GC-FID method is also reported for the identification and determination of Lumefantrine and its impurities in active drug substance [13]. Analytical procedures have been reported for the assay of lumefantrine in different samples, using HPLC-UV [14-16]. Furthermore, an LC/MS/MS bio-analytical method for the quantification of lumefantrine in human plasma has been developed [17].

A simple isocratic stability indicating RP-HPLC method for quantification of LF and its organic impurities in bulk and tablets are currently not available in official monographs and available methods described in the literature need relatively long run times, and do not contain design space information. Consequently, developing an appropriate method for LF product is crucial. The present study was therefore aimed to develop and validate a QC-relevant, efficient, robust, and specific method for quantification of LF and its impurities in bulk and tablets. Hence, the present study reports a rapid, economical, precise, and accurate method for the assay of Lumefantrine and its degradants.

MATERIALS AND METHODS

Chemicals

Lumefantrine standard and its impurities were obtained as a gift sample from Micro labs Ltd. (Banglore, India), and Trifluoro acetic acid (TPA), Hydrogen peroxide (30% w/v), were procured from Loba Chemie, India. Acetonitrile (HPLC Grade), Sodium hydroxide (NaOH), and Hydrochloric Acid (HCl), were purchased from Merck Ltd., India. Double distilled water was used throughout the analysis.

The instrumentation

Analysis was performed on Acquity UPLC (Waters) consisting of a binary solvent delivery system (pump) with a diode array detector, Column oven, and a rheodyne injector with 20 µl loop Separations was achieved on Thermo Hypersil ODS C18 column (150 mmX4.6 mm, 3 µm). Data collection and analysis were performed with Empower-3 software. Stress degradation studies were assisted with a thermostatic Water Bath with a digital controller. All weighing operations for the present analysis were carried out with the help of RADWAG analytical balance. Ultrasonication of samples was performed using Ultra sonicator; Powersonic Pvt. Ltd., India.

Software

Design Expert (Version 11 Stat-Ease Inc., USA) trial version statistical software was used to create an experimental design, analyze the data generated in the experiments, and optimize the RP-HPLC method.

Preparation of buffer

1 ml of Trifluoro acetic acid was dissolved in 1 L of HPLC grade water.

Preparation of mobile phase

Mixed buffer and acetonitrile in the ratio of 10:90 v/v.

Optimization of mobile phase

Different trials have been done, different buffers and different mobile phases were used to develop the method. In all trails, peaks are not separated properly. Finally, for the proposed method, all the peaks are separated and the entire suitability conditions are within the limit.

Sample solution

The sample solution was prepared from Lumefantrine tablets. The quantity of pulverized tablet mass equivalent to 50 mg Lumefantrine was transferred into 200 ml volumetric flask containing 140 ml of mobile phase, after ultrasonication for 20 min; volume was made up to the mark to get the concentration of 250 µg/ml. The resulting solution was filtered through a 0.45 µm filter (Rankem). The resulting solutions were subjected to the proposed method for further analysis [18].

Impurity standard stock solution

Weighed accurately 5 mg each of Impurity-A, Impurity-B, and Impurity-C into a 50 ml volumetric flask, 35 ml of diluent was added, sonicated to dissolve, and made up to the volume with diluent. Mixed well.

Spiked standard solution

Weighed accurately 50 mg of Lumefantrine sample into a 200 ml volumetric flask, added 140 ml of diluent, sonicated for 20 min, and also added 1 ml of impurity standard stock solution and made up to the mark with diluent. Filtered through 0.45µ syringe filter [19].

Spiked sample solution

Transferred equivalent to 50 mg of lumefantrine sample into a 200 ml volumetric flask, added 140 ml of diluent, sonicated for 20 min, and also added 1 ml of impurity standard stock solution and made up to the mark with diluent. Filtered through 0.45µ syringe filter.

Method development using experimental design

Response surface methodology with Central composite design (CCD) was applied to optimize the chromatographic parameters and to evaluate the main effect, interaction effects, and quadratic effects of the factors on the Resolution between critical pairs and Theoretical plates of the drug. The factors like acetonitrile ratio in Mobile phase composition (A), Flow rate (B), and Column temperature (C) were selected based on the initial experiments conducted with prior scientific knowledge about the molecule. The nominal value for all three factors, A, B, and C were 90%, 1.6 ml/min, and 35 °C respectively. The design matrix contains three levels (at low, medium, and high) with 17 experiments including 3 center points.

The models were employed to design response surface and contour plots (2D) which allowed the prediction of the optimal response. The ANOVA model was used to assess the importance of the selected factors. The experiments were conducted in random order to eliminate ascending or descending forms in the residuals and to minimize bias in the chromatographic data.

RESULTS AND DISCUSSION

The main analytical challenge during the development of a new method was to separate active pharma ingredients and impurities. In order to provide good performance, the chromatographic conditions were optimized using CCD.

Method optimization with statistical data analysis and model validation

The spiked sample was injected in all the experiments as per CCD design with independent variables like % Acetonitrile from 80% to 98% v/v (A), the flow rate from 1.2 ml/min to 1.9 ml/min (B), and the column temperature from 30 °C to 45 °C (C). The dependent variables are the Resolution between LF and Impurity-A (X1), the Resolution between Impurity-A and Impurity-B (X2), and Theoretical plates for Lumefantrine (LF) (X3).

The results were tabulated in table 1 and table 2. The results met the criteria which describe that the model was significant ($p<0.05$), lack of fit was a non-significant value ($p>0.05$), the coefficients of determination (R^2) was above 0.7 and the difference in value between adjusted R^2 (Adj. R^2) and predicted R^2 (Pred. R2) below 0.2, reveals the good closeness between the predicted and experimental results, indicating the model is accurate. Based on statistical data obtained in table 2, the overall equation model meets the requirements to be used in predicting optimal conditions.

The below equations describe the effect of coded factors on responses:

Resolution between LF and Imp-A (A)=+11.5431-2.45365A-0.012526B-0.035166C…

Resolution between Imp-A and Imp-B (B)=+5.33-0.4730A-0.7472A^2

LF Plate count(C) =+3161.81-360.64A-376.21A^2

It indicates that the responses A, B, and C were increased with the decrease of the Acetonitrile ratio in the mobile phase. The response A was increased with the decrease in Flow rate and Column temperature. The effect of Flow rate and Column temperature on the B and C responses was statistically not significant. All the responses met the regulatory requirements like resolution should be more than 2.0 and plate count should be not less than 2000. It indicates, that the deviation from the target method setting parameters did not affect the method performance. The contour plot is very useful for studying the interaction effects of factors on the response (fig. 2A, 2B, and fig. 2C), and the plots were shown no curvature displaying a linear effect of factors on responses.

Table 1: Coded levels and matrix for central composite design (CCD)

Run	Factor 1 acetonitrile ratio (A)	Factor 2 Flow rate (B)	Factor 3 column oven temperature (C)	Response 1 resolution between LF and Imp-A (X1)	Response 2 resolution between Imp-A and Imp-B (X2)	Response 3 LF plate count (X3)
1	85	1.8	30	3.5	3.9	2260
2	85	1.4	40	4.4	4.1	3260
3	90	1.2	35	4.6	4.4	2560
4	90	1.6	35	4.2	5.5	3100
5	85	1.4	30	4.8	4.6	2710
6	95	1.8	40	2.9	3.3	2050
7	90	1.6	45	4.2	6.2	3210
8	85	1.8	40	3.3	3.5	2210
9	90	1.6	35	4.3	5.4	2815
10	90	1.9	35	3.4	3.7	2120
11	98	1.6	35	4.2	6.2	3910
12	90	1.6	35	4.2	5.4	3200
13	80	1.6	35	4.5	6.4	3420
14	95	1.4	30	4.2	3.9	2850
15	95	1.8	30	3.1	3.2	2350
16	95	1.4	40	4.3	4	3090
17	90	1.6	30	4.5	6.5	3455

Table 2: ANOVA regression analysis for models and responses

Response	Mean	SD	%CV	R^2	Adjusted R^2	Predicted R^2	Adequate precision	F	P
Resolution between LF and Imp-A (X1)	4.04	0.3116	7.72	0.8544	0.7299	0.5613	11.3651	13.31	0.0003
Resolution between Imp-A and Imp-B (X2)	4.72	0.9006	19.09	0.8625	0.7439	0.5862	6.3197	6.02	0.013
LF plate count (X3)	2857.06	328.04	11.48	0.8753	0.7661	0.6299	10.068	14.56	0.0004

Where, SD; standard deviation, F; Fischer's ratio

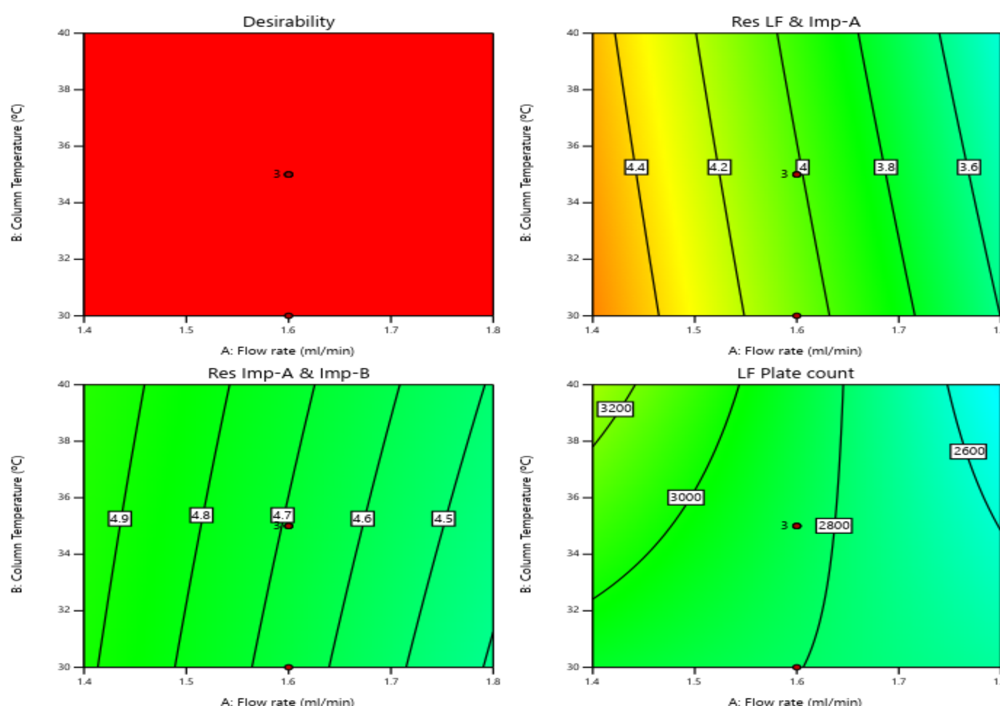

Fig. 2A: Contour plots of the effect of factors on responses (X1, X2 and X3)

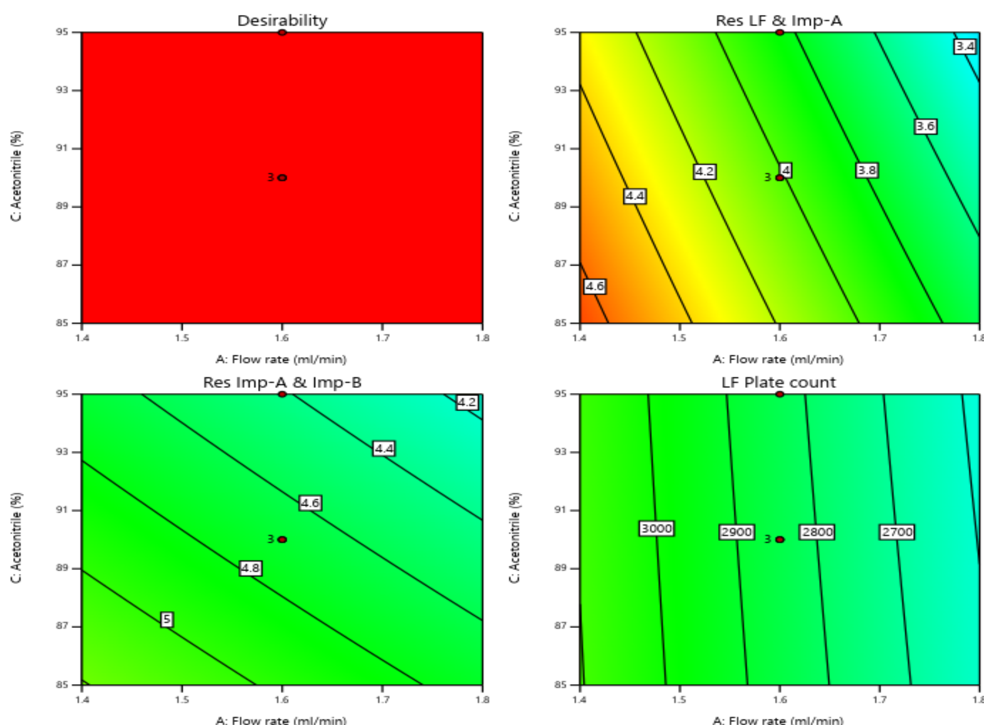

Fig. 2B: Contour plots of the effect of factors on responses (X1, X2 and X3)

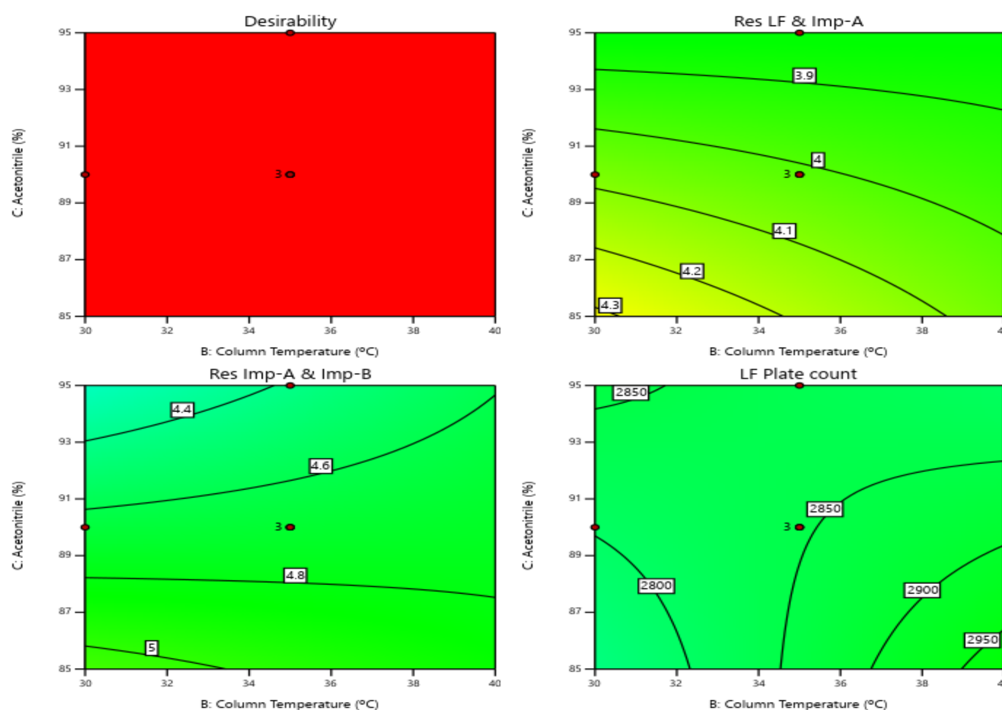

Fig. 2C: Contour plots of the effect of factors on responses (X1, X2, and X3)

Optimized condition obtained

The selected chromatographic conditions of RP-HPLC i.e. mobile phase buffer and acetonitrile 10:90, flow rate (1.6 ml/min), and column temperature 35 °C were optimal.

Design space

Establishing a design space for the method is the main goal when developing a method. The design space of the method was shown in fig. 3 and it revealed that the optimized method parameters are well within the method operatable design region (MODR) under robustness conditions. It was shown that the developed method provided assurance of quality.

Method validation

The optimized RP-HPLC validated [20] method according to ICH guidelines in terms of system suitability, specificity with forced degradation, linearity, accuracy, precision, ruggedness, and robustness.

System suitability

Instrument suitability [21] was performed by injecting a spiked standard solution containing 250 µg/ml of Lumefantrine, and 0.5 µg/ml of each Impurity-A, Impurity-B, and Impurity-C in six replicates. The results show that the instrument fitness parameter is within the limit provided by ICH. The results were shown in table 3.

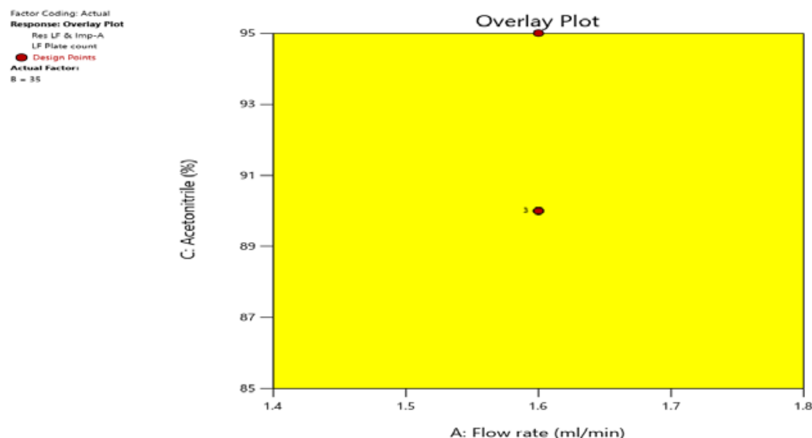

Fig. 3: Design space of selected chromatographic method conditions

Table 3: System suitability results of lumefantrine (LF) and its impurities

System suitability parameter	LF	Imp-A	Imp-B	Imp-C
USP Plate count	2548	7301	8177	8063
USP Tailing	1.0	1.02	1.01	0.99
USP Resolution	-	4.2	5.4	5.5
% RSD of Peak area	1.30	1.11	0.82	0.76
Retention Time	1.94	3.04	4.51	6.40

Values are expressed as mean, n=6

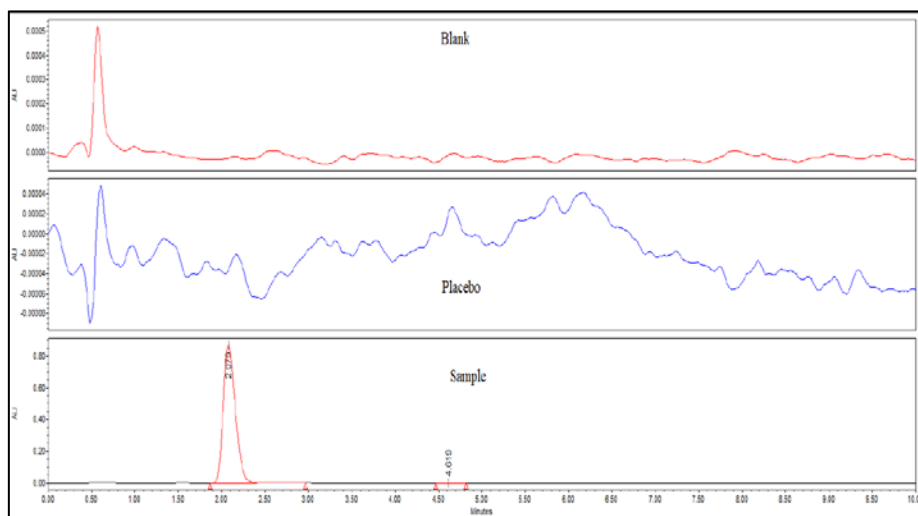

Fig. 4: Blank, placebo and sample chromatogram

Specificity

In this parameter by using the method, placebo, sample, and standard solutions were analyzed individually to examine the interference [22]. Fig. 4 shows that no interference was observed at the retention time of Lumefantrine and its related substances from blank and placebo. Hence the method was found to be specific.

Linearity

Linearity was demonstrated by plotting a calibration curve of the peak area against its respective concentration [23]. From this calibration curve, it was noticed that the curve was linear between the range of 25-375 µg/ml of lumefantrine and 0.05-0.75 µg/ml of each Impurity-A, Impurity-B, and Impurity-C. The regression equations for the calibration curve were Y=91375x+38614 (R=0.999) for lumefantrine and Y= 89247x+1111.1 (R=0.997) for Impurity-A and Y=107879x+1673.6 (R=0.999) for Impurity-B and Y=76999x+331.39 (R=0.999) for Impurity-C. Linearity plots were shown in fig. 5 and results were shown in table 4. The method was found to be linear.

Accuracy

The accuracy [24] of the developed method was evaluated by measuring the recovery experiments at three levels (50 percent, 100

percent, and 150 percent). APIs with concentrations of 125, 250, and 375 µg/ml of Lumefantrine and 0.25-0.75 µg/ml of each Impurity-A, Impurity-B, and Impurity-C were prepared. For each spike stage, the test solution was injected three times and the test was performed according to the test procedure. The recovery results were similar to 100% and also the RSD values were less than±2% for LF. Accuracy results have been shown in table 5 and the method was found to be accurate.

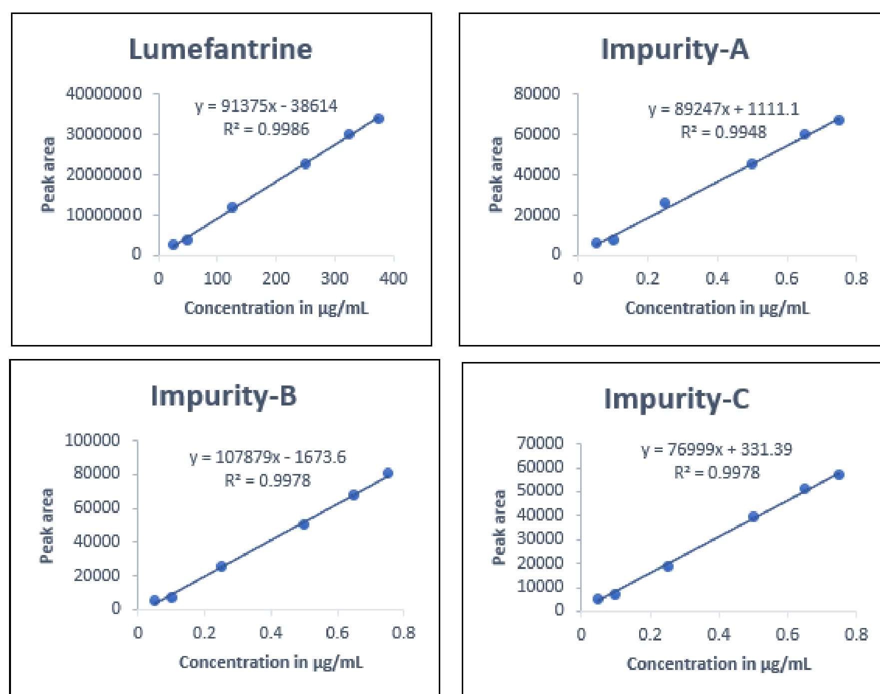

Fig. 5: Linearity plots

Table 4: Linearity results of lumefantrine (LF) and its impurities

Linearity	Lumefantrine		Impurity-A		Impurity-B		Impurity-C	
	Concn (µg/ml)	Peak area	Concn (µg/ml)	Peak area	Concn (µg/ml)	Peak Area	Concn (µg/ml)	Peak area
Linearity-1	25	2741000	0.05	5820	0.05	5469	0.05	5157
Linearity-2	50	3690500	0.1	7507	0.1	7425	0.1	7212
Linearity-3	125	11846500	0.25	26481	0.25	25918	0.25	18680
Linearity-4	250	22579500	0.5	45174	0.5	50735	0.5	39569
Linearity-5	325	29897000	0.65	59942	0.65	67905	0.65	51535
Linearity-6	375	34095000	0.75	67010	0.75	80628	0.75	56932
Slope	91374.94		89246.81		107879.07		76998.56	
Intercept	-38613.81		1111.06		-1673.64		331.39	
CC	0.999		0.997		0.999		0.999	
Bias	-0.2		2.5		-3.3		0.8	

Table 5: Accuracy results

S. No.	% Level	% Recovery			
		Lumefantrine	Imp-A	Imp-B	Imp-C
1	50	100.2	94.5	94.1	95.6
2	100	100.9	99.5	104.6	98.3
3	150	100.2	97.4	102.9	97.1
Mean		100.4	97.1	100.5	97.0
SD		0.404	2.511	5.636	1.353
%RSD		0.4	2.6	5.6	1.4

Values are expressed as mean±SD, Number of experiments, n= 3

Precision

The precision of the analytical technique is the degree of proximity of the sequence of measurements obtained from multiple homogeneous mixture samplings. The precision of the method was evaluated by injection of six individual spiked sample preparations of Lumefantrine and its related substances.

Intraday precision

Six replicates of a sample solution containing Lumefantrine and related substances were analyzed on the same day [25]. Peak areas were calculated, which were used to calculate mean, SD, and %RSD values. The method was found to be precise. Method precision results were shown in table 6 and the sample chromatogram was shown in fig. 6.

Table 6: Intraday precision results

S. No.	% Assay	% Impurities		
	Lumefantrine	Imp-A	Imp-B	Imp-C
1	101.0	0.197	0.197	0.205
2	100.9	0.201	0.188	0.204
3	100.8	0.202	0.199	0.204
4	100.6	0.204	0.208	0.209
5	100.5	0.195	0.206	0.205
6	99.5	0.191	0.207	0.208
Mean	100.6	0.198	0.201	0.206
SD	0.547	0.005	0.008	0.002
%RSD	0.5	2.5	3.8	1.0

Values are expressed as mean±SD, Number of experiments, n= 6

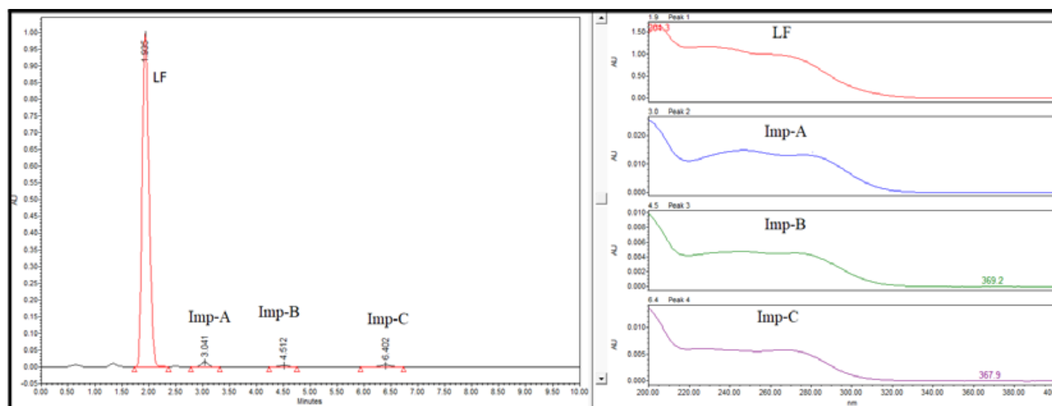

Fig. 6: Spiked sample chromatogram and spectra of LF and its impurities

Intermediate precision

Six replicates of the spiked sample solutions were analyzed by a different lot of columns, chemicals, and different tools were checked on separate days. The average percent of RSD values has been determined.

Inter-day precision

Six replicates of a sample solution containing lumefantrine and its related substances were analyzed on a different day [26]. % Assay and % impurities were calculated, which were used to calculate mean, SD, and %RSD values. The present method was found to be precise as the RSD values were less than 2% for assay and 5% for impurities and also, the percentage assay values were close to 100%. The results are given in table 7. The method was found to be rugged.

LOD and LOQ

LOD and LOQ were determined separately using the s/n technique. The LOD and LOQ of the compound were measured using the developed RP-HPLC method by injecting lower and lower concentrations of the standard and impurity solutions. The LOD and LOQ concentrations and their s/n values of Lumefantrine and its impurities were represented in table 8. This method is validated as per the ICH guidelines [27, 28].

Table 7: Inter-day precision results

S. No.	% Assay	% Impurities		
	LF	Imp-A	Imp-B	Imp-C
1	98.5	0.205	0.193	0.215
2	98.8	0.193	0.198	0.194
3	98.6	0.199	0.207	0.201
4	98.2	0.204	0.209	0.189
5	97.8	0.204	0.191	0.211
6	98.0	0.196	0.196	0.196
Mean	98.3	0.200	0.199	0.201
SD	0.382	0.005	0.007	0.010
%RSD	0.4	2.5	3.7	5.0

Values are expressed as mean±SD, Number of experiments, n= 6

Table 8: LOD and LOQ results

Name	LOD Conc (µg/ml)	s/n	LOQ Conc (µg/ml)	s/n
Lumefantrine	7.576	7.6	25	13.5
Imp-A	0.015	4.4	0.05	12.2
Imp-B	0.015	5.3	0.05	14.8
Imp-C	0.015	4.8	0.05	13.4

Robustness

The conditions of the experiment were designed to measure the robustness [29] of the intentionally changed conditions such as flow rate, Column temperature, and Mobile phase in organic percentage. Robustness results for lumefantrine and its impurities were found to be within the limit and results were tabulated in table 9 [30]. The method was found to be robust.

Table 9: Robustness results

Parameter name	% Assay	Resolution		
	LF	LF and Imp-A	Imp-A and Imp-B	Imp-B and Imp-C
Flow rate (1.6 ml/min)	99.4±0.15	4.5±0.18	6.4±0.26	6.4±0.15
Flow rate (1.8 ml/min)	98.9±0.21	4.2±0.22	6.2±0.32	6.4±0.25
Column temperature (30 °C)	99.4±0.31	4.5±0.11	6.5±0.45	6.4±0.09
Column temperature (40 °C)	99.1±0.25	4.2±0.16	6.2±0.47	6.7±0.11
Organic (+10%)	99.6±0.43	3.4±0.15	3.7±0.15	4.5±0.33
Organic (-10%)	100.5±0.96	4.2±0.34	5.5±0.21	5.4±0.49

Values are expressed as mean±SD, Number of experiments, n= 3

Table 10: Stability results

Stability	% Difference from the initial area	
	LF-standard	LF-sample
Day-1 at RT	0.95±0.01	1.30±0.02
Day-2 at RT	1.03±0.03	1.53±0.01
Day-1 at 2-8 °C	0.82±0.02	0.92±0.01
Day-2 at 2-8 °C	0.85±0.03	1.03±0.01

Values are expressed as mean±SD, Number of experiments, n= 3

Stability

The standard and sample solutions were kept at room temperature and at 2-8 °C for up to 48 h. These solutions were analyzed at 24 h and 48 h intervals and calculated the % difference from the initial area [31]. No major variations were found and verified that the solutions were stable up to 48 h. There is no effect in storage conditions for lumefantrine and its related impurities. Stability results were tabulated in table 10.

Degradation studies

The optimized LC method was used to study the degradation [32] behavior of the drug under various stress conditions. Stress studies were carried out as per ICH Q1A (R2) recommendations for hydrolysis, oxidation, thermal (dry heat stress), and Q1B recommendations for photolysis. Forced degradation experiments have been performed to establish that the developed method was acceptable for degradation materials [33, 34].

Acid degradation

Acidic hydrolytic stress was induced by adding 1N HCl and refluxing at 60 °C for 12 h in a thermostatic water bath. The solution was subjected to neutralization using 1N NaOH solution. 3.8% of lumefantrine impurity-A was observed.

Alkali degradation

Base hydrolytic stress was induced by adding 1N NaOH and refluxing at 60 °C for 12 h in a thermostatic water bath. The solution was subjected to neutralization using 1N HCl solution. 4.7% of Lumefantrine impurity-A was observed.

Peroxide degradation

Oxidative stress was induced by adding hydrogen peroxide (30% v/v). The solution was kept in dark at room temperature for 12 h to avoid any degradation by the combination of exposed light and oxidative stressors. 53.4 % of Lumefantrine impurity-B was observed.

Thermal degradation

Thermal stress study was conducted by placing the sample in a hot air oven at 105 °C for 7 d. significant degradation was not observed.

Hydrolysis degradation

Hydrolysis degradation was done by using HPLC water and no degradation was observed.

Photolytic degradation

Photolysis was carried out by exposing samples directly to sunlight for 7 d and no degradation was observed.

The chromatograms and purity plots of major degradation conditions are shown in fig. 7A and 7B. The degradation results were shown in table 11. Amass balance of above 97% was observed in all the stress conditions [35, 36]. The purity plots revealed that the purity angle is less than the purity threshold, hence the method was found to be specific.

Table 11: Forced degradation results

Degradation condition	% Assay	% Degradation			Mass balance (%)
	LF	Imp-A	Imp-B	Imp-C	
Control sample	98.1	0.046	0.049	0.026	NA
Acid degradation	94.0	3.788	0.056	0.040	99.7
Alkali degradation	93.0	4.702	0.043	0.030	99.6
Peroxide degradation	43.7	0.044	53.43	0.029	99.0
Hydrolysis degradation	98.4	0.049	0.281	0.051	100.6
Thermal degradation	98.1	0.039	0.874	0.034	100.9
Photolytic degradation	97.0	0.046	0.301	0.030	99.2

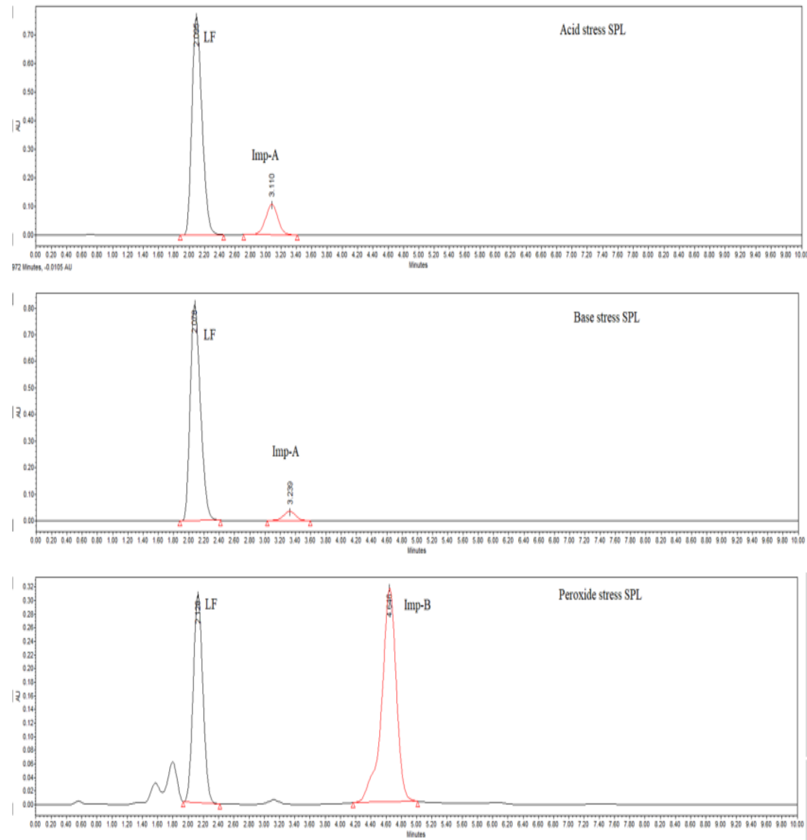

Fig. 7A: Chromatograms of degradation samples

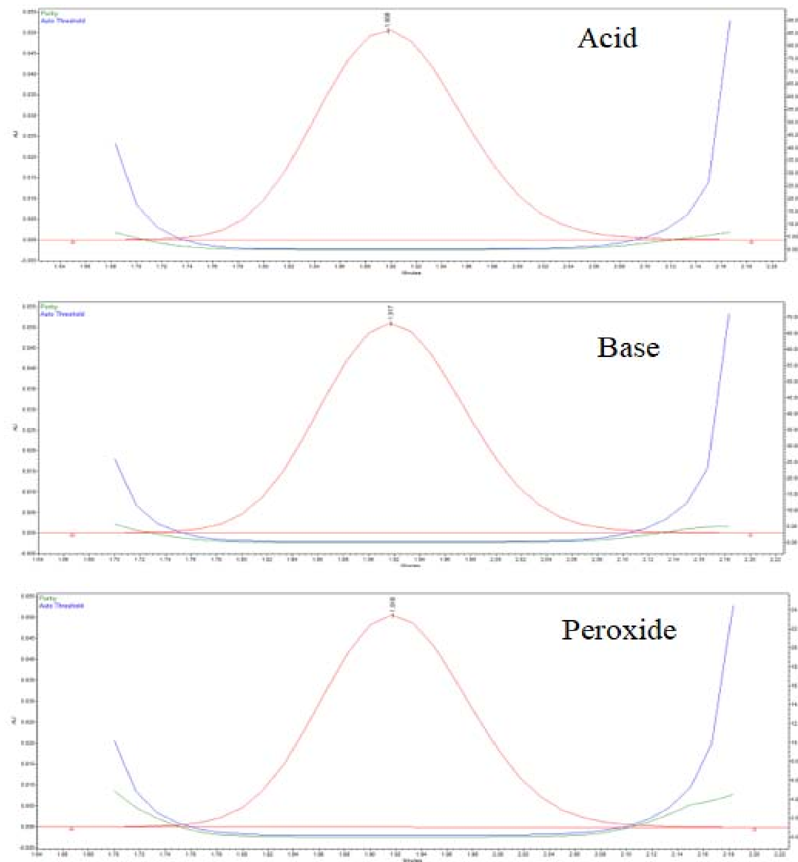

Fig. 7B: Purity plots of degradation samples

Suleman S (2015) reported the GC-FID method for quality control analysis of LF and its impurities in marketed formulations [13]. It was observed that Suleman S [13] had an excessive retention time of about 26 min, which increased the time for analysis and the cost of study. Singh P (2017) [14] and Sukanya B (2019) [15] did not extend their approach to the estimation of impurities in formulations, nor did they conduct design of experiments on LF and its impurities.

The developed method had a run time of 10 min and was shown design space for the estimation of LF and its impurities, it revealed that the optimized method parameters are well within the method operatable design region (MODR) under robustness conditions. It was shown that the developed method provided assurance of quality.

Plate count, resolution, tailing factor, and % RSD values measured during system suitability criteria testing were found in the limits of ICH acceptance criteria, which proves the suitability of the developed method [21]. The specificity/selectivity of the method was verified to confirm that there was no interference from inactive ingredients of formulations, mobile phase solvents, or/and stress degradants. Interference at RT of LF and its impurities has not been noticed, which proves selectivity [22]. Linear regression assessment was used to determine the graph's linearity. The linearity findings demonstrated good linearity of the method [23]. The recovery of known quantities of analyte and impurities which were spiked in placebo matrices or formulations was used to check accuracy. Recovery of LF and impurities (%) was near to 100 percent, proving the accuracy of the presently developed method [24]. During the precision evaluation, RSD was less than 2.0% for active and less than 5% for impurities, demonstrating good precision of the method [25]. Values of LOD and LOQ prove good sensitivity for analyzing LF and its impurities [27]. During the robustness study, it was observed that system suitability execution values were inside the required limits [29]. The standard and sample solutions were stable at room temperature and in refrigerator condition for two days in the selected diluent. It proved the ruggedness of the method [31]. LF degradation was more in the peroxide stress condition than that in the hydrolytic stress condition. The degradation analysis also proves the stability indicating power of the method [34].

CONCLUSION

The developed method using the CCD approach was a simple, fast stability indicating method and showed good results for Lumefantrine and its three impurities with a run time of 10 min. The utilization of Hypersil ODS C18 column within the present work has shown better elution of analytes with good resolution, improved plate count, and tailing. The main advantages of the method over the USP method are the use of the simplest mobile phase, optimum flow rate, low system pressure, and lower column length with simultaneous estimation of degradation products in isocratic mode. The proposed method was found to be simple, precise, accurate, linear, robust, and rapid for the simultaneous determination and quantification of Lumefantrine and its impurities. The forced degradation revealed that the drug product is highly sensitive to oxidation conditions and moderately sensitive to hydrolytic conditions. The drug product is photostable and thermal stable. The sample recovery was in good agreement with their respective label claims suggesting non-interference within the estimation. Hence, the technique is often easily and conveniently adopted for routine analysis of Lumefantrine in the dosage form.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

The author declares that there have been no conflicts of interest.

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