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Research Article

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Analytical method development and validation of lumefantrine in its bulk dosage form by using RP-HPLC method as per ICH guidelines

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ABSTRACT

An accurate, precise, rapid & economical RP-HPLC method has been developed for the estimation of Lumefantrine as per ICH guideline in pharmaceutical dosage form using ultra violet (UV) detector. Elution was carried out using a mobile phase consisting of Acetonitrile & Methanol (90:10) and flow rate was set on 1.6 ml/min at 235 nm, retention time for Lumefantrine was found to be 1.770 min. The method was found to be linear in the concentration range of 100-500 µg/ml, in the linearity study regression equation was found to be y = 97.17x - 3.660 & correlation coefficient was found to be 0.999. This method was Rugged and Robust in different testing criteria, LOD and LOQ was found to be 10.0 µg / ml & 30.5 µg / ml respectively. Accuracy study was done in 3 different concentration level 50, 100, 150% & % recovery of the method was found to be 100.2%, 100.9%, 100.2% respectively in 3 different levels & mean recovery was 100.4%, so method was accurate. Results of all validation parameter was within the limit as per ICH guideline. So this method can be used for the determination of Bulk Drug as well as Tablet Dosage form easily and the method was precise, economical, and accurate to perform in future.

Key words: mobile phase, Rugged, Robust, Accuracy, Validation

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INTRODUCTION

Lumefantrine is a Anti-Malarial Drug, molecular formula $C_{30}H_{32}Cl_3NO$, IUPAC name 2-Dibutylamino-1-[2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]-ethanol[1]. Mechanism action of drug Involves an interaction with ferrous ions, in the acidic parasite food vacuole, which results in the generation of cytotoxic radical species, mechanism of action of peroxide anti malarials involves interaction of the peroxide-containing drug with heme, a hemoglobin degradation byproduct, derived from proteolysis of hemoglobin, This interaction is believed to result in the formation of a range of potentially toxic oxygen and carbon-centered radicals [2].

According to literature review [3-10] there are very few method reported for the determination of Lumefantrine in different Instrumental techniques, out of these methods only 2 methods were reported in Single Drug by using RP-HPLC.

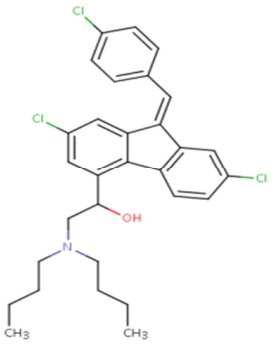

Figure: 1. Shows structure of Lumefantrine

EXPERIMENTAL SECTION

Standard drugs

Lumefantrine was procured from the HETERO Pharma.

Chemicals and reagents

Methanol (FINER chemical LTD), Acetonitrile (Rankem chemicals), Purified water ((Rankem chemicals).

Instruments

HPLC (Analytical technologies), UV (Elico SL-196), Detector (UV detector, Analytical technologies), Column (Hypersil ODS C18, (150 *4.6 mm , 5µ), Software (Analchrome, Clarity), Sonicator (Analytical technologies).

Determination of absorption maxima by UV/Vis Spectrophotometry

Accurately weigh 100 mg of drug in to 100 ml volumetric flask. To this add 90 ml and 10 ml of diluents (acetonitrile 90: 10 methanol) and sonicate it and further make up the volume with diluent. From this take 1 ml and make up to 10ml. The solutions were scanned in the range of 200-400 nm in 1cm cell against blank

Figure: 2. Shows UV spectrum of Lumefantrine

Preparation of mobile phase

Accurately measured 90 ml of acetonitrile, 10 ml of methanol HPLC grade was degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ nylon filter under vacuum filtration.

Diluent

Mobile phase is used as diluent

Standard preparation

Accurately weigh 50 mg of Lumefantrine and transfer in to 50ml volumetric flask. Add about 10ml of solvent mixture sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Transfer 1ml of above solution in to a 10ml volumetric flask and make up the volume with diluent.

Sample preparation
Same as standard preparation.

Optimized chromatographic conditions

Column	-	Hypersil ODS C18 (150*4.6mm), 5 µ
Flow rate	-	1. 6ml/min
Wavelength	-	235nm
Column temperature	-	35c
Injection volume	-	10 µl
Run time	-	5 min

Method validation
The following parameters were considered for the analytical method validation of Lumefantrine in bulk form.

System Suitability
Chromatograph the standard preparations (6 replicate injections) and peak area responses for the analyte peak was measured and the system suitability parameters are evaluated.

Accuracy
For accuracy determination, three different concentrations were prepared separately 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Precision
The standard solution was injected for six times and the area was measured for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Robustness
As part of the Robustness, deliberate change in the temperature and flow rate Variation was made to evaluate the impact on the method.

Linearity and range
Linearity of the analytical method for assay by injecting the linearity solutions prepared in the range of 100µg to 500 µg (33.3% to 166.6%) of test concentration, into the chromatograph, covering minimum 6 different concentrations.

Ruggedness
Establish the ruggedness of the analytical method by using the assay of 6 different sample preparations of same batch by a different analyst using a different HPLC System.

RESULTS AND DISCUSSION

Standard preparation
Accurately weigh 50 mg of Lumefantrine and transfer in to 50ml volumetric flask. Add about 10ml of solvent mixture sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Transfer 1ml of above solution in to a 10ml volumetric flask and make up the volume with diluent.

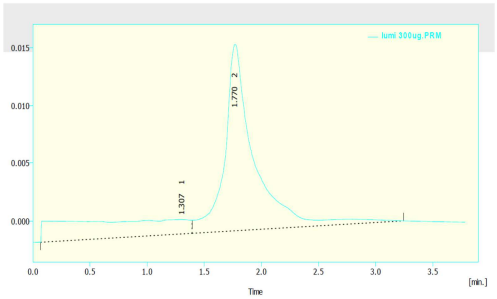

Figure: 3. Shows optimized standard chromatogram of Lumefantrine

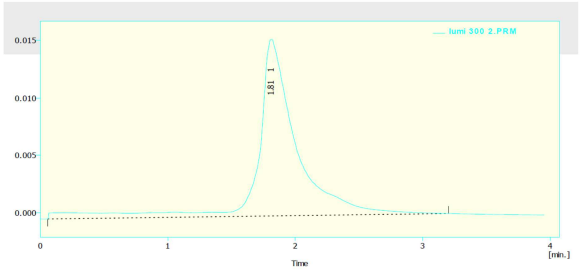

Figure: 4. Shows sample chromatogram of Lumefantrine

Validation

Accuracy

Average recoveries of Lumefantrine are 100.2%, 101.2%, 100.4%, at 50%, 100% & 150% concentrations level respectively. The percentage recoveries of the drug is within the limits 99-102%. So the method is Accurate, accuracy data for Lumefantrine are presented in

Table: 1 .Shows Accuracy results of Lumefantrine

Concentration level	Amount added (mg)	Amount found(mg)	%recovery	Average % recovery
50%	25 mg 25 mg 25 mg	25.5 mg 25.4 mg 24.3 mg	102% 101.6% 97.2%	100.2%
100%	50 mg 50 mg 50 mg	50.5 mg 50.45 mg 50.40 mg	101% 100.9% 100.8%	100.9%
150%	75 mg 75 mg 75 mg	75.5 mg 75.40 mg 74.65 mg	100.6% 100.5% 99.53%	100.2%

Table: 2 .Shows %Recovery of Lumefantrine

Amount added (mg)	Amount found(mg)	Average % recovery
50 mg	50.2 mg	100.4%

Precision

Precision are summarized in **Table No: 3**, respectively. The %RSD values for Precession was less than 2.0%, which indicates that the proposed method is precise.

Table 3 .Shows precision results of Lumefantrine

Sample. no	Peak area of Lumefantrine
Injection 1	284.421
Injection 2	287.884
Injection 3	291.462
Injection 4	291.462
Injection 5	291.514
Injection 6	291.514
Mean	**289.7095**
Standard deviation	**2.964983491**
%RSD	**1.02**

Linearity The response was found linear over a concentration range of 100-500 µg/mL of Lumefantrine. The correlation co-efficient were found to be 0.999 for Lumefantrine**.** So the method is linear, data is presented in **Table: 4.** Linearity curve of Lumefantrine is given in figure: 3.

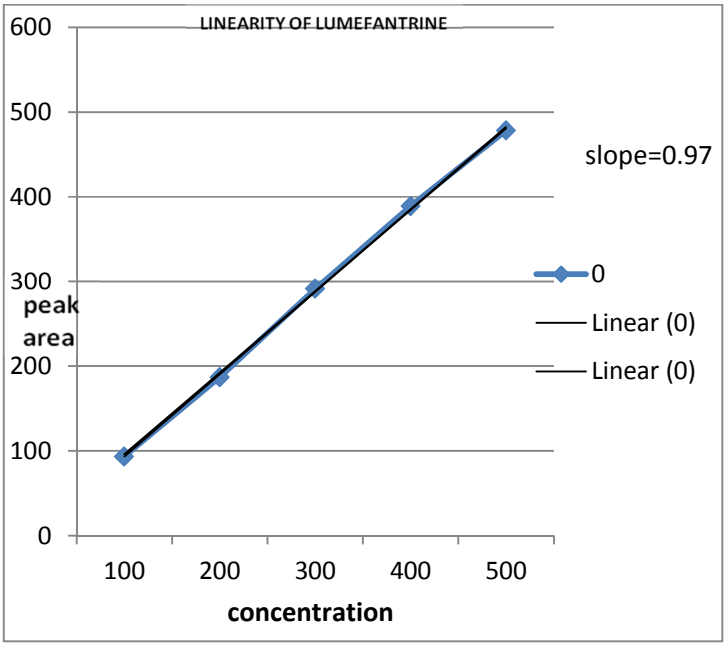

Figure: 5. Shows Calibration graph of Lumefantrine

Table: 4. Shows linearity results of Lumefantrine

%level	Concentration(µg/ml)	Peak area
33	100	93.48
66	200	186.96
100	300	291.514
133	400	389.15
166	500	478.28
Y Intercept		**96.18**
Correlation co-efficient (r^2)		**0.999**
Slope		**0.9**
Linearity range	**100-500**	

Robustness Minor deliberate changes in different experimental parameters such as flow rate (±0.2ml) and temperature (±5° c) did not significantly affect the retention time & peak area of Lumefantrine indicating that the proposed method is robust which is mentioned in **Table – 4 & 5**

Table: 5. Shows robustness results of Lumefantrine (change in flow rate)

S.NO	flow rate	Peak area of Lumefantrine		Average	SD	%RSD
1	1.4ml/min	79.921	78.910	79.4155	0.7	0.88
2	1.6ml/min	291.514	289.512	290.513	1.41	0.48
3	1.8ml/min	389.150	387.45	388.3	1.20	0.30

Table: 6. Shows robustness results of Lumefantrine (change in temperature)

S.NO	Temperature	Peak area of Lumefantrine		Average	SD	%RSD
1	30℃	291.462	285.452.	288.457	1.41	0.48
2	35℃	287.884	285.882	286.883	1.415	0.49
3	40℃	284.421	282.420	283.420	1.414	O.498

Ruggedness

The method is rugged by different analyst, different time intervals and the method did not significantly affect the recoveries, peak area and retention time of all the above drugs indicating that the proposed method is rugged which is mentioned in **Table – 6.**

Table 7. Shows Ruggedness results of Lumefantrine

Name	Peak area of Lumefantrine
Ruggedness-(Day-1)	295.230
Ruggedness-(Day-2)	297.238
Ruggedness-(Day-3)	297.238
Ruggedness-(Day-4)	305.319
Ruggedness-(Day-5)	305.319
Ruggedness-(Day-6)	308.437
Average	**301.4635**
SD	**5.530383088**
% RSD	**1.83**

Limit of Detection (LOD)&LOQ The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected **,** The LOD are calculated from the calibration curve by formula LOD = 3.3 x SD/ b The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision, The LOQ are calculated from the calibration curve by formula LOQ = 10 x SD/ b

Table: 8. Shows LOD & LOQ results of Lumefantrine

Parameters	Lumefantrine
LOD	10.0 µg/ml
LOQ	30.5 µg/ ml

Table: 9. Shows summary of validation parameter Results

S.NO	Parameter	Acceptance criteria	HPLC
1	%recovery	98-102%	100.4
2	Linearity range((µg/ml)	-	100-500(µg/ml)
3	Correlation coefficient	NLT 0.999	0.999
4	No .of Theoretical plates	NLT 2500	3255
5	Precision	%RSD (NMT 2%)	1.02
6	Intermediate precision	%RSD (NMT 2%)	1.83
7	LOD	-	10 µg/ml
8	LOQ	-	30.5 µg/ml

CONCLUSION

Method development & validation of Lumefantrine was done by RP-HPLC method. The estimation was done by using Hypersil C_{18} (4.6 x 150mm, 5µm, Make: Analytical technologies) mobile phase as Acetonitrile, methanol (90:10) at a flow rate 1.6ml/min. The linearity range of Lumefantrine was found to be 100-500 µg/ml. Correlation coefficient value was 0.999, values of % RSD was 1.02 which is within the limit. These results show the method is accurate, precise, sensitive, economic & rugged. The HPLC method is more rapid. The proposed method is successfully applied to the bulk dosage form. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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REFERENCES

[1] Available at: http://en.DBO67O8
[2] Available at http://www.ualberta.ca/82186-77-4
[3] R Prasanna; D Saravanan; J Padmavathy; K Boopathi. *International J of Pharma Res and Development* **2014**, 2(8), 84-90.
[4] FD Naik ; H Chorawala; ZR Dedania, SM Vijyendraswamy, J Surati *J of Pharma Res*, **2014**, 3(4), 70-73.
[5] M Vinodh; M Vinayak; P Pankaj, K Rahul ; M Renita *Malaysian J of Analytical Sci* **2013**, 7 (3), 348 – 358.
[6] NK Gupta; AM Babu ; P Gupta *International J of Pharma Erudition*, **2013**,3(1), 10-17.

[7] NS Kotur ; S Sivasamy, S Srinivasan *J of Pharm. Sci. & Res*, **2012**, 4(1), 1672-1675.
[8] TM. Kalyankar; RB Kakde *International J of Chem Tech Research*, **2011**, 3 (3), 1722-1727.
[9] J Sunil; MS Nath; US Moorthy *International J of Pharmacy and Pharma Sci*, 2(4), **2010**, 93-96.
[10] B Sridhar; KH Rao; TV Srinivas; VS Madhuri; K Madhuri; JV Seshagiri , *International J of Advances in Pharma Sci*, **2010**, 1 (1), 95-99.