Naveen S Kotur et al /J. Pharm. Sci. & Res. Vol.4(1), 2012,1672-1675

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Analytical Method Development and Validation for Estimation of Lumifantrine in Pharmaceutical Dosage Forms by HPLC

Naveen S Kotur *[1],Singaravel Sivasamy[2], S.Srinivasan[3]

[1]*Department of Physiology, Shimoga Institute of Medical Sciences, Shimoga, Karnataka.*

[2]*Fathima collage of Pharmacy, kadaiyanallur ,Thirunelvalli Dt,TamilNadu.*

[3]*Department of pharmaceutics ,Krupanidhi college of pharmacy,Bangalore,Karnataka.*

Abstract

A Simple, rapid, sensitive, precise, accurate, stability indicating and reproducible of High Performance Liquid chromatography (HPLC) method has been developed for the estimation of Lumifantrine in pharmaceutical Tablet dosage forms. The HPLC method was carried out using Waters Symmetry C18 (250 X 4.5 mm) analytical column with maintained the column oven temperature 35 °C and isocratic pump mode. The mobile phase compressing of water, acetonitrile and glacial acetic acid in the ratio of 48: 52: 1, v/v/v with delivered the flow rate of 1.2mL /min and the detected the lumifantrine at 276 nm from PDA detector. The retention time of lumifantrine was 4.65 minutes. This method has been validated as per ICH guidelines and the validation data showed that the assay is sensitive, specific and reproducible for the determination of lumifantrine in the dosage form. The method is linear from 10µgmL^{-1} to 100µgmL^{-1} and linear correlation coefficient (R^2) was more than 0.9990. The accuracy of the method by recovery was found between 99.44 and 100.14 %. Mean inter and intraday assay relative standard deviation (RSD) were less than 1.0%. The proposed method provided an accurate and precise analysis of lumifantrine in its Pharmaceutical dosage form.

Key Words: Lumefantrine, Impurity, HPLC, Validation, PDA detector

1. INTRODUCTION

Lumifantrine is chemically (1R, S) -2-Dibutylamino-1- {2, 7-dichloro-9-[(Z)(4-chlorobenzylidene)9H-fluoren-4-yl}-ethanol, molecular formula is $C_{30}H_{32}Cl_3NO$ and molecular weight is 528.9 g per mol. Physically yellow crystalline powder and odourless. practically soluble in water and aqueous acids, free soluble in ethyl acetate, soluble in dichloromethane, slightly soluble in ethanol, chloroform and acetonitrile . Lumifantrine chemical structure given in figure-1.Lumefantrine contains an endoperoxide bridge, which interferes with haeme polymerization, a critical detoxifying pathway for the malarial parasite and secondary action is inhibiting in the nucleic acid and protein synthesis within the parasite. Lumifantrine is anti malarial properties and administered in oral and intravenous route. Markedly lumifantrine available in different pharmaceutical dosage form like tablet, capsule, Dry syrup and injection.

The literature survey 5-10 indicates that Lumefantrine were estimation by UV, TLC, HPTLC and HPLC in different pharmaceutical dosage form. The objective of the proposed study was to assay lumifantrine in its dosage form. This proposed HPLC method is a short Analysis time (≤10min.) coupled with simplicity, and ease of operation warrants use of the given method for analysis of lumifantrine along with its impurity as pharmaceutical dosage form and pure drug substance and chromatographic condition like mobile phase, analytical column, flow rate, column temperature were totally different from above literature

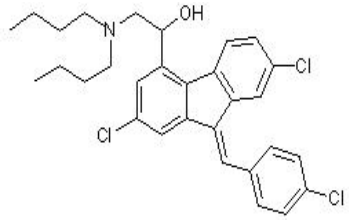


Figure-1: Lumifantrine Chemical Strcture

2. EXPERIMENTAL

2. 1. MATERIALS AND INSTRUMENTS USED

a. **Reagents and materials:** Lumefantrine Reference Standard, Lumefantrine drug and Impurity standard of α, α- (Dibutylamine methyl)-2, 7-dichloro-4-fluorenmethanol [DBA] and Para Chlorobenzalehyde (PCB) were purchased from Ipca Laboratories, Mumbai. The purity of lumifantrine Reference Standard was 99.76 %. Acetonitrile - HPLC Grade, Glacial acetic acid –AR Grade were purchased from Merck Speciality Pvt., Mumbai and water from Milli-Q system and 0.45 micron membrane filter from Millipore.

b. **Instrument:** Dionex HPLC System [Auto sampler with PDA detector] with Chromeleon software, Waters Symmetry C-18, [250 X 4.5 mm], 5 micron particle size analytical column and Ultraviolet sonicator.

2. 2. METHODS

a. Mobile phase Preparation: Prepared a mixture of water, acetonitrile and glacial acetic acid

in the ratio of 48: 52:1, v/v/v. Filtered this solution through 0.45 micron membrane filter and degassed

b. Diluent Preparation: Used Mobile phase as diluents

c. Standard Preparation: 50.0 mg of lumifantrine reference standard was weighed and transferred it into a 100mL volumetric flask. About 50mL of diluent was added and sonicated to dissolve the content. Made up to volume with diluents and mixed well. 10mL of above solution was diluted to 100mL with diluents and mixed well.

d. Sample Preparation: 20 tablets were weighed and crushed the fine powder. The tablets powder equivalent to about 50 mg of lumifantrine was weighed and transferred into a 100mL volumetric flask. About 50mL of diluents was added and sonicated for 25 minutes. Made up to volume of 100mL with diluents and mixed well. Filtered the resulting solution through 0.45 micron membrane filter and collected the filtrate after discarded first few mL of filtrate. 10mL of filtrate was diluted to 100mL with diluents and mixed well.

e. Chromatographic Conditions: Dionex HPLC System [Auto sampler with PDA detector] with Chromeleon software. Freshly prepared water, acetonitrile and glacial acetic acid 48:52:1 (v/v/v) mobile phase and adjust pH to 3 were filtered through 0.45 µ membrane filter and sonicated before use. The chromatographic conditions used for the analysis were given below.

Analytical Column	:	Water Symmetry C18 (250 X 4.6,5µ)
Flow rate	:	1.2mL/min
Column Oven Temperature	:	35°C
Injection Volume	:	20µL
Detection	:	UV@ 276nm
Run Time	:	10 minutes

f. Procedure: A 20µL of blank (Diluent) in single, standard solution in five replicates and sample in two replicates were separately injected into the chromatographic id the % of assay content of lumifantrine.

3. RESULT AND DISCUSSION

3.1 Development and Optimization: The HPLC method, chromatographic conditions like mobile phase composition, injection volume, flow rate, column oven temperature, and detection of wavelength were optimized to be obtained for good separation lumifantrine and lumifantrine related impurities. In order to develop method for estimation of lumifantrine under isocratic conditions, initially various mobile phase compositions that is Different mobile phases with different proportions of organic modifier (acetonitrile) was tried with change of flow rates for good separation of lumifantrine with related impurities and peak parameters. Finally the mobile phase composition of water, acetonitrile and glacial acetic acid in the ratio of 48: 52:1, v/v/v at flow rate 1.2mL/ min was quite satisfactory as mobile phase of water: acetonitrile: Glacial acetic acid (48:52:1,v/v/v) and flow rate 1.2mL/min was preferred. The optimum wavelength of 276 nm was fixed through PDA detector. Finally 20µL of injection volume and column oven temperature at 35°C was optimized and desired due to peak performance. After selecting the best conditions based on peak performance, the run time of the proposed assay was 10 min with isocratic elution. During injection of a standard and sample solution, the retention times were 4.367 and 4.367 min respectively (Fig.2).

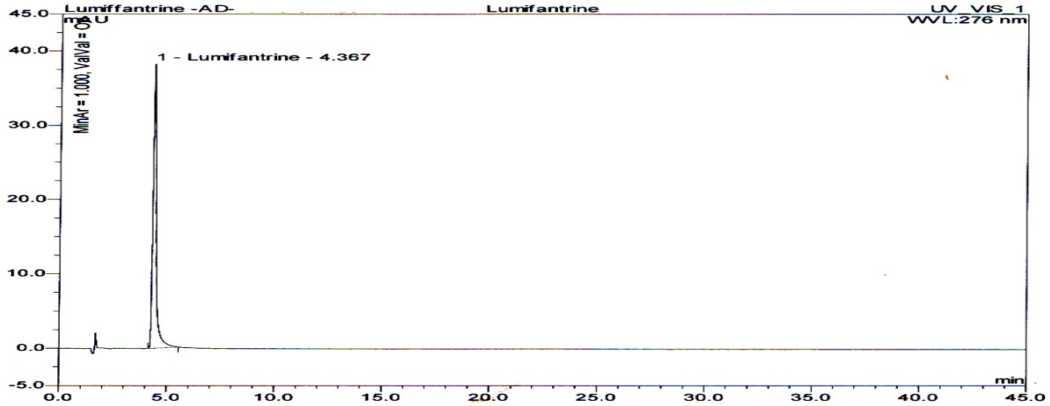
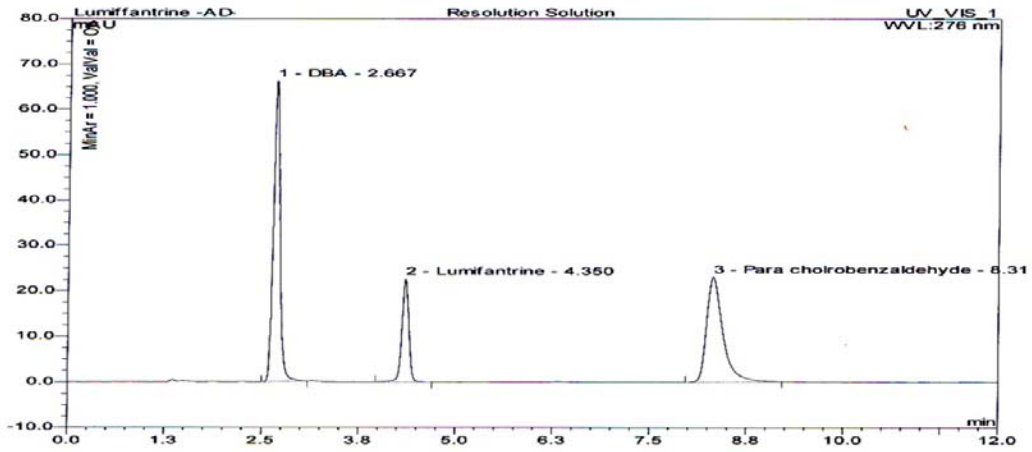


Figure-2A: Lumifantrine Chromatograms

Figure-2B: Lumifantrine Standard and Related Impurities Chromatograms



3.2 System Suitability: System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To determine its efficiency, system suitability tests were established by system precision. System suitability was carried out by 20 µL of freshly prepared standard solution was injected in six replicates and recorded all chromatogram. The system suitability parameters results are shown in Table-1.

Table-1: Lumifantrine Standard System Suitability Result

System Suitability Parameters	Result
Tailing Factor(T)	1.0
Theoretical Plates (N)	7689
% RSD of Retention Time	0.1
% RSD of Area	0.1

3.3 Method Validation:

Method validation was conducted according to published guidelines [14-15]. The validation parameters like linearity & range, accuracy, intraday and inter day (two different days) precision, robustness, solution stability and specificity were validated. Standard and sample solution stability was checked at room temperature (25°C) and result was shown that standard and sample solution is stable up to 24 hours.

3.3.1. Linearity & Range:

Linearity study of detector response for lumifantrine was evaluated in the concentration range of 20 to 100µgmL^{-1}. Linearity solutions were prepared at five concentrations (10.06, 25.04, 50.11, 75.15 and 100.33µg mL^{-1}) and injected in duplicate. Calibration curve was plotted with concentration Vs peak response of lumifantrine and the correlation coefficient was calculated. The linear correlation coefficient (R^2) was 0.9999 and the calibration curve is shown in figure-3.

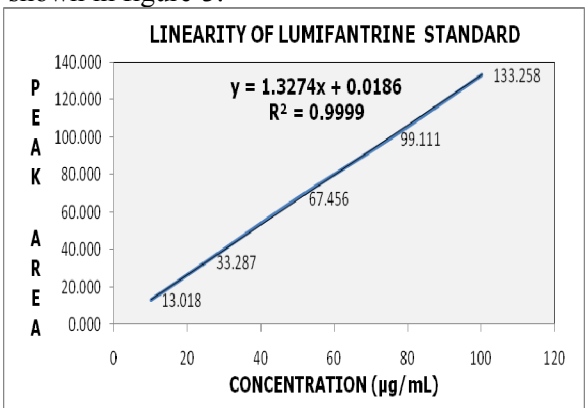


Figure-3: Lumifantrine standard calibration curve

3.3.2. Specificity /Interference Study:

a. Known impurity interference: Lumifantrine with Impurity of α, α-(Dibutylamine methyl)-2, 7-dichloro-4-fluorenmethanol [DBA] and Para Chlorobenzalehyde (PCB) solution was injected. These impurities was well separated from lumifantrine peak .i.e. resolution of these impurities and lumifantrine was more than 3.0 (**figure.2**)

b. Excipients interference: All the inactive ingredients namely like hydroxyl propyl cellulose, microcrystalline cellulose; lactose, magnesium stearate, dicalcium phosphate, sodium lauryl sulphate, crosscarmellose sodium, sodium starch glyoclate, and titanium dioxide were also injected in this proposed method. No peak was found at the retention time of lumifantrine when injected into chromatographic system.

c. Forced degradation studies: Stress studies was established by acid degradation 0.01N HCl with Reflux-30 minutes, alkali degradation 0.1N NaOH with reflux 2 hours, peroxide degradation 3% with reflux 1 hour, Thermal degradation at 105°C -24 hours and UV degradation at 254nm -24 hours that lumifantrine peak was pure in all the stress studies and all degradation peak well separated from principle peak.

3.3.3. Accuracy/Recovery

Accuracy was established by recovery study of the known amount of lumifantrine reference substance added in the sample solutions using three concentration levels covering the specified range from 50% to 150 % of each in triplicate at test concentration (50µg mL^{-1}). The % of recovery was found between 99.41 and 100.33. Result was shown in Table-4.

Table-4: Recovery of lumifantrine

%	Range of % Recovery	Average	% RSD
50	99.59,100.33 & 99.72	99.88	0.3
100	99.86,100.31 & 100.27	100.15	0.2
150	99.41,99.66 & 100.22	99.54	0.3

3.3.4. Precision

The intra and inter day precision were estimated from duplicate injection of six sample solutions prepared at 50.09 µgmL^{-1} of lumifantrine analyzed on two different days. Mean and RSD of % assay were calculated. The results indicate that the method is reproducible. Result was shown in Table -5.

Table-5: Precision Result

Sample No.	Intra day	Inter day
1.	99.95	100.01
2.	100.05	99.92
3.	99.96	99.97
4.	99.90	100.06
5.	99.96	100.02
6.	100.60	99.89
Mean	**100.07**	**99.98**
SD	**0.241**	**0.059**
% RSD	**0.2**	**0.1**

3.3.5. Robustness

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic conditions like flow rate (±10%), mobile phase composition (±5.0% of Organic modifier), detection wavelength (±5nm) and column temperature (± 5°C). The standard solution was injected for each of the changes and system suitability parameters were evaluated. The results were shown that no significant effect due to deliberate changes of chromatographic conditions.

4. CONCLUSION

The proposed HPLC method was developed and validated allows a simple and fast quantitative estimation of lumifantrine in various pharmaceutical dosage form and bulk. A mobile phase composed of water, acetonitrile and glacial acetic acid with a short run time (10 min) and isocratic elution used are advantageous and made the routine analysis easy. This method among the significant advantage and assures that simplicity, specific, accurate, precise and required less time consumption for analysis. This HPLC method can be employed for the routine analysis for assay, dissolution and related substance in pharmaceutical dosage form and bulk drug.

REFERENCES

1. William O Foye, Principles of medicinal chemistry**,** 6, Lippincott Williams and Wilkins, Baltimore, 2008, 1098-1099.
2. Surendra Nath Pandeya., Med. Chem. Vol.2, 3rd ed. 2003; 601-633
3. M Bindschedler, G Lefevre, F Ezzet, N Schaeffer., E. J. of Clinical Pharmacology. 2000; 56:375-381.
4. Agarwal, Suraj P; Ali, Asgar, Ahuja, Shipra., A. J. of Chemistry. 2007; 19: 4407-4414.
5. Zeng. Mei-Yi; Lu, Zhi-Liang, Yang, Song-Cheng; Zhang, Min., Determination of lumefantrine in human plasma by RPHPLC with UV detection, J.C. B & Biomed. Appl.1996; 681(2); 299-306.
6. Mansor, Sharif M; Navaratnam.V; Yahaya Norizah; Nair, N.K. Determination of new anti malarial drug Lumefantrine in blood plasma by HPLC, J.C. B Biomed Appl. 1996; 682(2):321-325.
7. Leonardo K Basco, Jean Bickii and Pascal Ringwald, Invitro activity of Lumefantrine (Benflumetol) against clinical isolates of plasmodium falciparum in Yaounde Cameroon,Antimicrobial agents and Chemotherapy, 42, 9, 1998, 2437-2351.
8. Annerberg.A , Singtoroj.T ., High throughput assay for the determination of lumefantrine in plasma , J.C.B, Analytical tech. Biomed lifesci. 2005; 1822: 330-332.
9. Lindegardha.N, Annerberga.B.A, Blessbornc.D.,Development and validation of a bio analytical method using automated solid-phase extraction and LC-UV for the simultaneous determination of Lumefantrine and its metabolite in plasma. J. Pharm. and Biomed. Analysis. 2005; 37(5):1081-1088.
10. Isabela da Costa Cesar, Fernando Henrique Andrade Nogueina and Gerson Antonio Pianetti, Simultaneous determination of Artemether and Lumefantrine in fixed dose combination tablets by HPLC with UV detection, Journal of Pharmaceutical and biomedical analysis, 48, 3, 2008, 951-954.
11. U.S. Food and Drug Administration; Guidance for Industry, Q3A Impurities in New Drug Substances 2003.
12. U.S. Food and Drug Administration; Guidance for Industry, Q3B Impurities in New Drug Products 2006.
13. United States Pharmacopoeia, US Pharmacopoeia convention, RockVille MD, 31, 2008, 684-687.
14. ICH (1996) Harmonized tripartite guideline: validation of analytical procedures: Methodology.Q2B.
15. ICH (1996) Harmonized tripartite guideline: validation of analytical procedures.Q2A