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Method Development And Validation Of Lumifantrine By Uv-Spectroscopy

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PHARMACEUTICAL ANALYSIS

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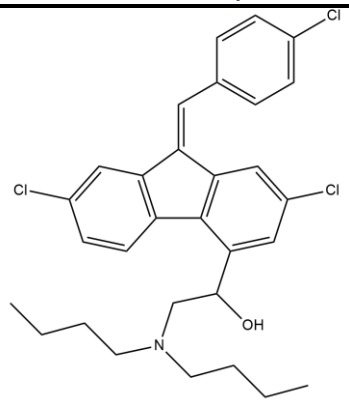
Abstract: A new UV Spectroscopic method for the determination of Lumefantrine, a novel therapeutic agent, was developed and validated in accordance with ICH guidelines. The method was optimized using a UV-visible spectrophotometer, with a wavelength range of 200–400 nm, and Lumefantrine was detected at a specific absorbance peak at 303nm. The method was validated for linearity, accuracy, precision, ruggedness, robustness, LOD, LOQ. Calibration curves were constructed over a concentration range of 2-10µg/ml, and the method demonstrated excellent linearity with a correlation coefficient (r^2) of 0.999. The accuracy and precision of the method were within acceptable limits, with relative standard deviations of 2.976 and 2.559 for both intra-day and inter-day variations. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 6.81 and 20.6. ensuring high sensitivity. The method was successfully applied to the analysis of Lumefantrine in pharmaceutical formulations, demonstrating its reliability and robustness. This validated UV Spectroscopic method provides a simple, rapid, and cost-effective approach for the routine analysis of Lumefantrine in quality control settings.

Index Terms - precision, UV- spectroscopy, wavelength, lumefantrine, validation.

I. INTRODUCTION

Chemically lumefantrine is: (±)-2,7-Dichloro-9-((Z)-p-chlorobenzylidene)-α-((dibutylamino)methyl)fluorene-4methanol with a molecular formula $C_{30}H_{32}Cl_3NO$ and molecular weight of 528.939g/mol is a antimalarial drug that belongs to the chemical class of aryl amino alcohols. It is also a member of the fluorene class of compounds. Lumefantrine is an antimalarial agent used in combination with artemether for the treatment of acute uncomplicated malaria caused by Plasmodium falciparum. It is administrated by oral route. Sparingly soluble in water, it is more soluble in non-polar and aprotic organic solvent, fastly dissolved in DMF, slightly soluble in ethanol, freely soluble in methanol, also soluble in toluene. Lumefantrine's mechanism of action involves binding to hemin, a product of hemoglobin breakdown, and inhibiting the parasite's ability to detoxify it. This leads to a buildup of toxic heme, which kills the parasite. Structure of lumefantrine is shown in fig 1



2-(dibutylamino)-1-[(9Z)-2,7-dichloro-9-[(4-chlorophenyl)methylidene]fluoren-4-yl]ethanol

Fig 1: structure of lumefantrine

Karajgi S. R.[et al]: A new area under curve uv spectrophotometric method has been developed for the simultaneous determination of Artemether and Lumefantrine has been developed. Rabia Habib[et al]: This paper describes the development and validation of a high-performance liquid chromatography (HPLCUV)method for the simultaneous quantitative determination of artemether and lumefantrine in fixed dose combination tablets. R. K. Sangeetha [al]: A HPTLC, UV spectrophotometric and ion pair extraction method were developed and validated for the estimation of lumefantrine in tablets. The parameters linearity, precision, specificity, robustness, limit of detection and limit of quantitation were studied according to "The International Conference on Harmonization Guidelines" for validation of analytical procedures. Kidanemariam Desta[et al]: Methods reported including the official methods for determination of chloroquine in tablet samples use carcinogenic organic solvents. In this study, UV-Vis spectrometry using water as a solvent was developed for determination of chloroquine phosphate in pharmaceutical tablet dosage forms. D.Snigdha[etal]: A simple, reproducible and efficient spectroscopic method development and validation of hydroxy chloroquine sulfate (HCS) in tablet dosage form. The drug was determined by using methanol as a solvent for this study, which is determined by spectrophotometrically at 224-nm.

From above literature survey many methods are available for the simultaneous estimation of Lumefantrine by UV spectroscopy has been reported, but a few methods are reported for the individual's estimation of Lumefantrine in its bulk and formulations by UV spectroscopy. Hence the present work has made an attempt for Method Development and Validation of Lumefantrine by UV spectroscopy as per ICH guidelines.

Experimental method:

Instruments used:

UV - VIS Spectrophotometer double beam SYSTRONICS and LAB INDIA 3000+, Analytical balance Shimadzu High Precision Electronic balance.

Chemicals used:

Ammonium chloride, ammonium hydroxide, ethanol, methanol, distilled water, acetonitrile.

SELECTION OF SUITABLE WAVELENGTH (λmax):

The UV spectrum of lumefantrine was obtained by scanning the sample in UV visible spectrophotometer at a wavelength range of 200-400nm with methanol. Drug shows maximum absorbance at 303nm, Spectram was shown in figure2

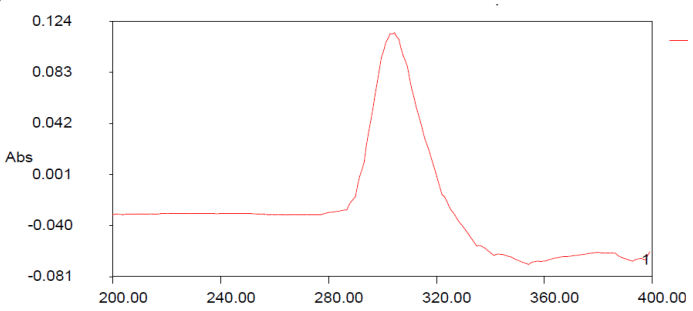


Fig 2 Spectrum of lumefantrine by uv-vis spectrophotometer

Preparation of Solutions Standard Solutions:

Preparation of Lumefantrine stock solutions:

Accurately weighed quantity of 25 mg lumefantrine was transferred into a 25 ml volumetric flask, and dissolved in small amount of selected solvent and made up to 25 ml.

Preparation of dilutions from working standard solution:

Working standard solutions of Lumefantrine of different volumes such as 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml of solutions were taken in 5 different 10ml volumetric flasks and made up to the mark with the solvent to give the following concentrations of 2µg/ml, 4 µg/ml, 6µg/ml, 8µg/ml,10µg/ml.

Sample Preparation:

Accurately 10 tablets of lumefantrine were taken, weighed and powdered. The amount equivalent to the 25mg of Lumefantrine pure form was weighed and taken in a 25 ml volumetric flask, and then some quantity of solvent was added. And then the solution was shaken for 15 minutes, for complete dissolution of the sample and then the volume was made up to 25ml with the solvent. The solution was filtered and then the obtained filtrate is used for the analysis

Procedure For Assay:

An equivalent amount of 10mg of Lumefantrine pure drug and test sample drug powdered were weighed separately and solutions for same concentrations were prepared. By scanning the sample separately at the absorbance maxima wavelength and the % purity can be estimated by the following formula.

% purity= (Test Absorbance)/ (standard Absorbance) X 100

Method validation linearity:

Solutions of test were prepared by diluting the 0.2ml,0.4ml,0.6ml,0.8ml, and 1ml of working standard solution in 5 different volumetric flasks with the solvent to give concentrations of 2µg/ml,4µg/ml,6µg/ml,8µg/ml, and 10ug/ml. These solutions were scanned by a spectrophotometer at a wavelength of 303nm

Precision:

Preparation of solution:

To prepare the required solution of 10µg/ml, Dilution was done from a working standard solution of 100µg/ml by taking 1ml of above solution and make up into the 10ml with the solvent used. This solution was then scanned in a UV-visible spectrophotometer.

Procedure:

The prepared standard sample solutions were scanned for absorbance for five replicates separately. The percentage RSD for absorbing of five replicates was found to be within the limits

Accuracy:

Accuracy of the proposed method was confirmed by studying percentage recovery of six different concentration of sample of 80%,100%,120%, in accordance with ICH guidelines but standard addition method. a known amount of standard drug solution was added to the tablet powder to make the final concentrations and absorbance was noted and the percentage drug recovery was measured by using the formula.

%Recovery =[A-B/C]x100

Robustness:

Small changes do not affect the parameters of the assay

For determining the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample.

A] Effect of wavelength:

Robustness of assay method was carried out with variation of detection of wavelength Standard solution was prepared and the analysis was performed as per the proposed test method and system suitability parameters were evaluated.

Results and discussion:

Linearity studies were carried out in the concentration in the concentration range of 2-10µg/ml. the readings are obtained by measuring absorbance at 303nm. Results were discussed in table 1 and the curve was shown in fig3

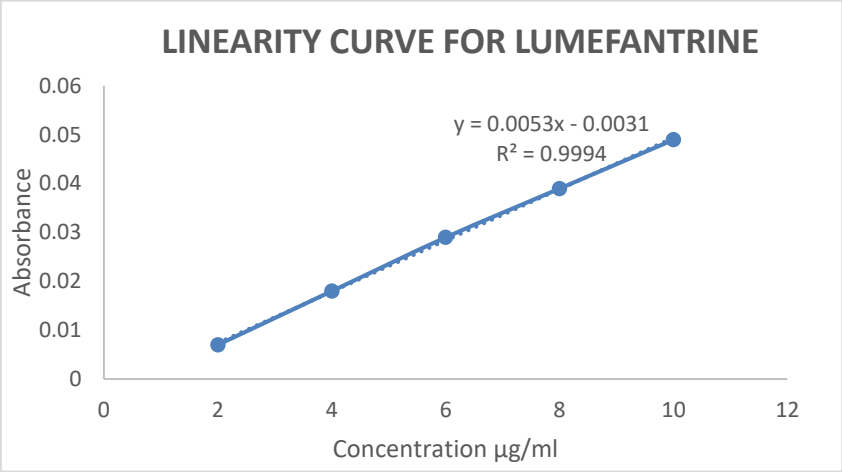


Fig 3: Linearity graph for Lumifantrine

Table 1: Linearity values of lumifantraine

Concentration[µg]	Absorbance
2	**0.007**
4	**0.018**
6	**0.029**
8	**0.039**
10	**0.049**

Precision:

The interday and intraday of the test methods were done by performing on five replicates of same concentration within a day and consequent days on same time and the values were discussed in table 5 and table 6 from the acceptance criteria the percentage RSD were calculated.

Table 5: System precision for Lumifantrine [intraday]

Concentration[µg/ml]	Absorbance
10µg/ml	1.04
10µg/ml	1.05
10µg/ml	1.02
10µg/ml	1.02
10µg/ml	1.03
Average	1.0242
Standard deviation	0.00475
%RSD	0.463751

Table 6: System precision for Lumifantrine [interday]

Concentration[µg/ml]	Absorbance
10µg/ml	1.05
10µg/ml	1.03
10µg/ml	1.04
10µg/ml	1.04
Average	1.04
Standard deviation	0.006325
%RSD	0.60813

Accuracy:

Accuracy of proposed method was confirmed by studying percentage recovery of six different concentrations of sample 80%,100%,120%. The percentage recovery of lumifantrine was found to be 100%,100%, and 93.33% respectively. The above values explain that the method is Accurate. the results were discussed in table7

Table 7: % RECOVERY STUDIES

S. No	Tablet Powder taken [mg]	Percentage [%]	Amount of pure drug added [mg]	Total amount of Drug recovered [mg]	Percentage drug recovery [%]
1.	25	50	12.5	12.5	100
2.	25	100	25	25	100
3.	25	150	37.5	35	93.33

Robustness:

Small changes do not affect the parameters of the assay

For determining the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample.

A] Effect of wavelength:

Robustness of assay method was carried out with variation of detection of wavelength Standard solution was prepared and the analysis was performed as per the proposed test method and system suitability parameters were evaluated. discussed in table 8.

Table 8: Effect of Wavelength

Wavelength[nm]	Absorbance
302	0.019
303	0.020
304	0.019
Average	0.0193
S.D	0.0005
%RSD	2.98

Ruggedness: The effect of variation in RSD values from one analyst to other analyst was observed.

Table 9: Ruggedness parameters (Analyst)

Concentration	Absorbance by Analyst 1	Absorbance by Analyst 2
10µg/ml	0.020	0.017
10µg/ml	0.020	0.017
10µg/ml	0.020	0.018
10µg/ml	0.020	0.017
10µg/ml	0.020	0.017
Average	0.020	0.01725
SD	0	0.0005
%RSD	0	2.89

The absorbance values for the given sample concentration were found similar by both the analysis.

Table 10: Ruggedness parameters (instrument)

Concentration	Absorbance by Instrument 1	Absorbance by Instrument 2
10µg/ml	0.020	0.021
10µg/ml	0.020	0.022
10µg/ml	0.020	0.022
10µg/ml	0.020	0.021
10µg/ml	0.020	0.021
Average	0.020	0.0214
SD	0	0.00054
%RSD	0	2.559

Limit of detection and limit of quantification:

Detection and quantification limits were calculated by the method based on the standard deviation and slope of the calibration plot using the formula,

LOD = (σ x 3.3) / S

LOQ = (σ x 10) / S

Where,

σ = standard deviation of absorbance

S = slope of the calibration plot

LOD = (σ x 3.3) / S

= [0.002066*3.3]/0.001

= 6.81 µg/ml

LOQ = (σ x 10)/S

= [0.002066*10]/0.001

= 20.6μg/ml

Where,

σ = standard deviation of absorbance

S = slop of the calibration plot

Table 11: LOD and LOQ Parameters:

S.NO	Concentration of Solution in μg/ml	Absorbance of the solution At 225nm
1.	0.001	0.003
2.	0.002	0.004
3.	0.003	0.006
4.	0.01	0.006
5.	0.02	0.002
6.	0.03	0.003
7.	0.1	0.003
8.	0.2	0.008
Average	-	0.004375
Standard deviation	-	0.002066

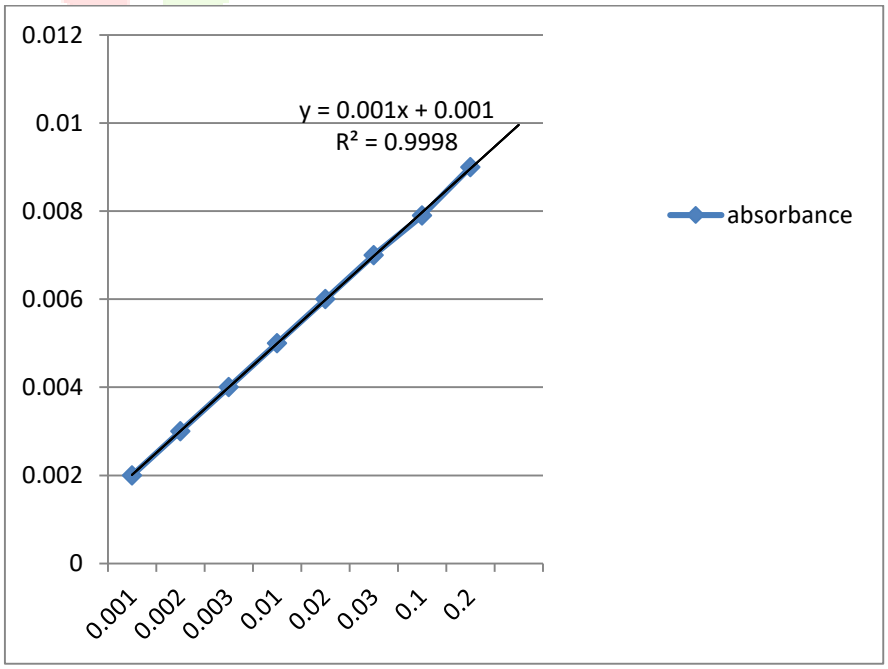


Fig 4 : Absorbance of the solution at 303nm

Conclusion:

From the reported literature, there are few methods established for the determination of lumefantrine. Present work concluded that, to develop and validate a new UV-VIS spectro-photometric method for the estimation of lumefantrine in bulk and pharmaceutical dosage forms .The scope and objective of the present work is to develop a simple, rapid, accurate, sensitive, and economical method than the existing methods.

In the present method development for the drug Lab India Double beam UV-VIS spectrophotometer (UV-3000+) is used with the 1cm thickness of the quartz cuvettes as sample holder. Analytical grade methanol is selected as a solvent for optimised method and absorbance is measured at 303 nm.

The linearity of lumefantrine was observed in the Range of 2-10 µg/ml. The slope and correlation coefficient values of lumefantrine were found to be 0.052 and 0.999 which indicates the excellent correlation between Response factor Vs. concentration of standard solution. The precision of developed method was studied intraday precision and interday precision The percentage RSD values for precision were found to be within the acceptable limits, which reveal that the developed method was precise. The developed method was found to be Rugged and also validated for Rubustness.

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