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VALIDATED ANALYTICAL METHODS FOR THE ESTIMATION OF LUMEFANTRINE IN TABLETS

***R. K. Sangeetha[1], M. Gandhimathi[1] T. Sudhakaran[1] and M. S. Prakash[2]**

[1]College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, 395, Sarojini Naidu Road, Siddhapudur, Coimbatore-641 044, India.

[2]K. M. College of Pharmacy, Uthangudi, Melur Road, Madurai-651107, India.

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ABSTRACT:

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. The HPTLC method was performed using precoated silica gel plates $GF60_{254}$ with mobile phase ratio consisting of methanol : chloroform : ammonia (8:2:0.05 v/v/v). Densitometric scanning was performed at 268 nm. The Rf value was found to be 0.66. The linearity range were in the concentration range of 100- 500 ng/spot, 2-16 µg/ml, 5-25 µg/ml for HPTLC (method 1), UV spectrophotometric (method 2) and ion pair extraction method (method 3). The intraday precision and Interday precision were found to be less than 2 for all the three methods. The limit of detection and limit of quantitation were found to be 25 ng/spot and 50 ng/spot for method 1, 0.5 µg/ml and 1 µg/ml for method 2 and 2µg/ml and 4 µg/ml for method 3. Statistical analysis by student's t-test showed no significant difference between the results obtained by methods ($p = 0.19497$). The proposed methods are highly sensitive, precise and accurate and can be used for the routine quantitation of lumefantrine in tablets.

KEYWORDS: Lumefantrine, UV Method, HPTLC, Ion pair method.

***Corresponding Author:**
Rapilane Krishnamurthi Sangeetha
College of Pharmacy,
Sri Ramakrishna Institute of Paramedical Sciences, 395,
Sarojini Naidu road, Siddhapudur,
Coimbatore-641 044, India.
Email: geetkaran@gmail.com

INTRODUCTION

Lumefantrine (fig. 1) is chemically (9Z)-2,7-Dichloro-9-[(4-chlorophenyl)methylene]-α-[(dibutylamino) methyl]-9H-fluorene-4-methanol with a molecular weight of 528.95 and molecular formula of $C_{30}H_{32}Cl_3NO$. It is official in USP 2009[82186-77-4]. Lumefantrine belongs to the class of aryl amino alcohols used in the treatment of uncomplicated falciparum malaria[1]. Lumefantrine is also known as Benflumetol. The reported methods include HPLC assay, solid phase extraction method, UV in combination dosage form.

MATERIALS AND METHODS

The reagents methanol, chloroform, ammonia, water and HCl of analytical grade were procured from E. Merck, India. Lumefantrine RS (99.12 %) was procured from commercial market. The formulation was procured commercially from the local market in India.

The high performance thin layer chromatography was performed on Camag HPTLC system with TLC scanner 3, WinCATS software and Linomat 5 as applicator. The samples were spotted in the form of bands of width 6 mm with Hamilton syringe on precoated silica gel aluminium plate $60F_{254}$ Camag twin trough chamber was used for plate development. Densitometric scanning was performed using TLC scanner with deuterium lamp as light source. The HPTLC, UV and Ion pair extraction method was performed using UV-visible

spectrophotometer (Model Shimadzu 1201) at 268 nm and 420 nm respectively using one cm quartz cell and one cm glass cell.

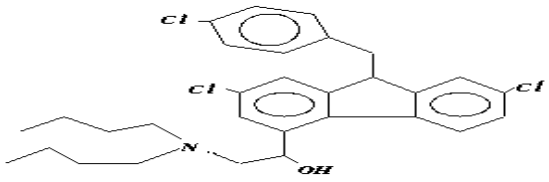

Fig. 1: Lumefantrine

The methods were validated according to the ICH guidelines for validation of analytical procedures. Student's *t*-test was used to verify the validation of method (2) and method (3). The standard deviation was found to be 0.17682 and 0.2805 respectively with a p- value of 0.19497.

EXPERIMENTAL

Preparation of Standard solution:

HPTLC: The standard stock solution was prepared using chloroform to give a concentration of 1 mg/ml. Further dilutions were made with the same to give a concentration of 10µg/ml of Lumefantrine. Preparations of solutions were made at a temperature of 8^0C and care was taken to avoid the vaporization of the solvent.

UV spectrophotometric method: The standard solution was prepared using 0.1M methanolic sulphuric acid to give a concentration of 1mg/ml subjected to further dilution to give a concentration of 10µg/ml with the same.

Ion pair extraction method: 10mg of the pure drug was weighed in a 10 ml volumetric flask and volume made up with chloroform. This solution was further diluted with the same to give a concentration of 10µg/ml. The solution was transferred into a 100 ml separating flask and to this 2 ml of Potassium hydrogen phthalate buffer of pH 4.2 followed by addition of 2ml of dye 0.5 % bromocresol green. The ion pair was extracted well by shaking the separating flask and the lower chloroform layer was collected.

Preparation of sample solution:

HPTLC: Sample solution was prepared by dissolving 10 mg equivalent of tablet powder with chloroform in a 10 ml volumetric flask. The volume was made with the same. The solution is filtered through Whattman filter paper and further diluted to give a concentration of 10µg/ml.

UV method: Tablet powder equivalent to 10 mg was weighed into 10 ml volumetric flask and dissolved in 0.1 M methanolic sulphuric acid and made up the volume with the same. The solution was further diluted to give a concentration of 10 µg/ml of drug lumefantrine.

Ion Pair exchange method: The amount of powder equivalent to 10 mg was weighed in a 10 ml volumetric flask and volume made up with chloroform. This solution was further diluted with the same to give a concentration of 10µg/ml. The solution was transferred into a 100 ml separating flask and to this 2 ml of Potassium hydrogen phthalate buffer of pH 4.2 followed by addition of 2 ml of dye 0.5% bromocresol green. The ion pair was extracted well by shaking the separating flask and the lower chloroform layer was collected.

Method Validation:

Linearity: Linearity was assessed by analyzing solutions in the range of 100-500 ng/spot by spotting in a linear ascending order for HPTLC method, in the range of 2-16 µg/ml in triplicate for UV spectroscopic method and in the range of 5-25 µg/ml in triplicate for ion pair extraction method. The statistical evaluation was performed for regression line.

Precision: Intraday precision was evaluated with 6 samples prepared as described in the sample preparation section during the same day by the same analyst. Interday precision studies were studied by comparing the results obtained on three different days in a week by same analyst. The % RSD was determined.

Accuracy: A recovery studies were performed by adding known amount of lumefantrine reference substance to sample solution. The sample solution with added reference substance was compared with standard solution. Sample solution was prepared as described in sample solution preparation section. The solutions with two concentrations of 50 and 100 % were analyzed for the recovery studies. The results were average of 5 studies.

Limit of detection and limit of quantitation: Limit of detection and limit of quantitation were calculated using the standard deviation values.

Specificity: A Placebo solution was analyzed to evaluate the specificity of the method. The placebo solution was prepared by using the excipients without the drug lumefantrine. The solution was analyzed against a freshly prepared standard solution. The % RSD was calculated.

Robustness: For HPTLC method the robustness was evaluated by variations in the proportions of mobile phase of methanol: chloroform: ammonia and chamber saturation time. The effects on Rf value and peak area were tested.

RESULTS AND DISCUSSION

HPTLC method: High performance thin later chromatography method has been widely used for the quantitative analysis of various drugs. In the present method HPTLC was used for the quantitation of

Lumefantrine in tablets. Precoated silica $G_{60}F_{254}$ was used. The parameters such as mobile ratio, chamber saturation period were fixed after testing with various ratios of different mobile phase system. The reported methods include HPLC with use of phosphate buffer and the present method is an alternative to quantitative analysis of Lumefantrine in tablets.

The drug is freely soluble in chloroform, ethyl acetate and dimethyl formamide, partially soluble in methanol. The mobile phase ratio was fixed depending on the solubility of the drug. Various ratios of mobile phase of chloroform, methanol and ethyl acetate were tested. Tailing was observed in all the ratios due to the high basic nature of the drug. To overcome tailing effect ammonia was added. Peaks obtained were of good symmetry with acceptable Rf values of 0.66. Figure 2 shows typical chromatogram obtained from the analysis of the standard and sample lumefantrine. As shown in the figure, lumefantrine has a symmetrical peak well separated from the solvent front.

For the specificity of the drug an assay method should show the separation and quantization of drug from the physical mixture of the drug and excipients. The excipients were also tested for the interference studies which showed no interference from the excipients showing the proposed method is specific.

The robustness of the method was evaluated by modifying the ratio of mobile phase and chamber saturation time. There were no significant alterations in the symmetry of the peak. The results are shown in table 1.

To assess the linearity, a standard calibration curve was constructed by plotting concentration versus peak area. The curve showed good linearity over the concentration range of 100-500 ng/ml, with the correlation coefficient of 0.9973.

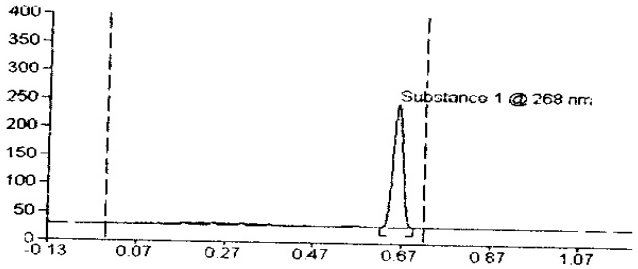

Fig. 2: Chromatogram of Lumefantrine

The validity of the assay was verified by means of ANOVA which showed that there was no deviation from linearity (P=0.19497). The LOQ was found to be 50 ng/spot and LOD was found to be 25 ng/spot indicating high sensitivity of the method.

The precision of the method was determined by repeatability (intraday) and intermediate (interday) precision and was expressed as the RSD if the results. The results presented in the Table 2 indicated good repeatability and low interday variability. The accuracy of the method was shown by the recovery range of 80-120%.

UV method: The proposed UV method is quite simple and economical for the quantization of lumefantrine from tablet dosage form without any time consuming sample preparation. Moreover the spectrophotometric method involves simple instrumentation compared with instrumental techniques. The absorption spectrum of lumefantrine in 0.1 M methanolic sulphuric acid shows a maximum absorbance at 268 nm. This wavelength was used for all measurements.

Table 1: Results of the determination of lumefantrine in tablets by the proposed methods

Method	Sample	% label claim Day 1	% label claim Day 2	% label claim Day 3
HPTLC	1	99.54	99.62	100.09
	2	100.11	100.16	100.48
	3	99.85	98.93	99.69
	4	99.74	100.03	100.22
	5	99.98	100.16	100.03
	6	100.20	100.44	100.34
	Mean(n=6) Intraday(RSD)		Mean(n=18) Interday (RSD)	
UV	1	99.97	99.67	99.74
	2	100.14	99.59	100.95
	3	99.88	99.82	99.96
	4	99.95	99.94	99.81
	5	99.48	99.76	99.76
	6	100.08	99.86	99.90
	Mean(n=6) Intraday(RSD)		Mean(n=18) Interday(RSD)	
Ion pair extraction	1	100.25	100.25	99.63
	2	99.68	100.40	99.86
	3	99.90	100.6	99.35
	4	98.99	100.7	99.54
	5	100.02	99.47	99.58
	6	100.42	100.53	99.87
	Mean(n=6) Intraday(RSD)		Mean(n=18) Interday(RSD)	

The specificity test demonstrated that there was no interference from any of the excipients. The spectrum obtained did not show any of the peak other than drug peak. The standard calibration curve shows the linearity in the range of 2-16 µg/ml with a correlation coefficient of 0.9988.

The validity of the assay was verified by means of ANOVA which shows that there is no deviation from linearity. The limit of detection and limit of quantization was found to be 0.5µg/ml and 1.0µg/ml respectively. The low values indicted sensitivity of the proposed method.

The precision of the method was determined by repeatability (intraday) and intermediate (interday) precision and was expressed as the RSD if the results.

The results presented in the Table 2 indicated good repeatability and low interday variability. The accuracy of the method was shown by the recovery range of 80-120 %.

Ion pair extraction method: Ion pair extraction method is a simple, economical and time saving method for the quantification of lumefantrine in tablets. The method was based don the extraction of the drug with the dye bromocresol green at a pH of 4.2 using potassium hydrogen phthalate as buffer. The extraction was dependent on the pH and pKa of the drug. The experimental parameters varied were the pH and the dye concentration. The yellow chromogen obtained showed a maximum absorbance at a wavelength of 420 nm.The specificity test demonstrated that there was no interference from any of the excipients. The standard calibration curve shows the linearity in the range of 4-20 µg/ml with a correlation coefficient of 9984.

The validity of the assay was verified by means of ANOVA which shows that there is no deviation from linearity .The limit of detection and limit of quantization was found to be 4µg/ml and 2µg/ml respectively. The low values indicated sensitivity of the proposed method.

The precision of the method was determined by repeatability (intraday) and intermediate (interday) precision and was expressed as the RSD if the results. The results presented in the Table 2 indicated good repeatability and low interday variability. The accuracy of the method was shown by the recovery range 50 % level.

Comparison between HPTLC, UV and Ion Pair Extraction Methods: The proposed analytical methods were compared using statistical values. The student t test revealed no significant differences between the experimental values obtained with three methods. The results show that the three methods are equivalent for the quantitative analysis of lumefantrine in tablets.

Table 2: Experimental value obtained in the recovery test for Lumefantrine in tablets by proposed methods

Method	Sample conc-entration	Concentration of added standard	Recovery ± RSD %
HPTLC (mg/ml)	300	150	449.57 ± 0.8147
	300	300	448.92 ± 0.5641
	300	375	449.19 ± 0.6224
UV (µg/ml)	10	2.5	449.75 ± 0.7743
	10	5.0	448.91 ± 0.2144
	10	10.0	449.18 ± 0.6652
Ion pair extraction(µg/ml)	10	2.5	449.24 ± 0.2356
	10	5.0	448.65 ± 0.3147
	10	10.0	449.51 ± 0.6621

CONCLUSION

The developed HPTLC, UV and Ion pair extraction method for the determination of Lumefantrine in tablets was found to be specific, linear, accurate, sensitive and precise. Therefore the methods can be used for the routine analysis of lumefantrine in tablets.

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