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Simultaneous UV Spectrophotometric Method for the Estimation of Lumefantrine in Pharmaceutical Dosage Forms

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Abstract: Three simple, rapid, accurate, precise and cost-effective methods, I; formation and solving of simultaneous equation method, II; absorbance ratio method, III; Dual Wavelength Method have been developed for simultaneous estimation of Lumefantrine in tablet dosage form. Beer's law was obeyed in concentration range 5-35 µg/ml for Lumefantrin for all the proposed methods. The sampling wavelengths for methods I, II and III, selected for both the drugs were 252nm, 268nm and 296nm, on trial and error basis using 0.01 N NaOH solutions as solvent. For methods I, II, III seven mixed standards solutions with concentration of Lumefantrin in the µg/ml of 5:35, 10:30, 15:25, 20:20, 25:15, 30:10 and 35:5, were prepared by diluting appropriate volumes of standard stock solutions for all proposed three methods. Results of analysis for six methods were tested and validated for various parameters according to ICH guidelines.

Key words: Lumefantrine · Simultaneous equation · Absorbance ratio · Dual Wavelength

INTRODUCTION

Lumefantrine is an antimalarial drug widely used in malaria endemic areas [1]. Many studies have demonstrated that it is highly effective in the treatment of resistant *P.falciparum* malaria, resulting in high cure rates and prevention against reinfection. Lumefantrine also named benflumetol and chemically (9z)-2, 7-dichloro-9-((4-chlorophenyl) methylene)-a-((dibutylamino) methyl)-9H-fluorene-4-methanol. The compound is a yellow powder that is poorly soluble in water, oils and most organic solvents, but soluble in unsaturated fatty acids and acidified organic solvents. Lumefantrine is extensively bound (˜99%) to plasma proteins, mainly high density lipoproteins [2]. Lumefantrine is having following side effects such as cough, diarrhea, dizziness, fatigue, headache, loss of appetite, nausea, vomiting and weakness. The exact mechanism of Lumefantrine is not well defined. Literatures review reveals that the various analytical method like HPLC-UV method [3] for the simultaneous quantitation [4], Liquid chromatographic [4], solid-phase extraction and liquid chromatographic method [5], liquid-liquid extraction using LC-MS/MS with electrospray ionization [6-7]. The present groups of authors have already reported UV Method development different pharmaceutical dosage form [8-22].

Experimental

Instrumentation and Reagents: UV/visible double beam spectrophotometer (Shimadzu Model, 1700) was employed with spectral bandwidth of 1nm and wavelength accuracy of ±0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). Lumefantrine working standard was supplied by M/S Orchid chemicals and Pharmaceuticals Chennai. Marketed sample for the analysis which bought from local pharmacies Lumefantrine (2mg/tablet) was manufactured by The Medicare Ltd., Haridwar, India. All other chemicals used in the analysis were AR grade.

Preparation of Standard Stock Solution and Sample Solution: Standard stock solution of Lumefantrin having concentration 100 µg/ml prepared by dissolving separately 20 mg of each drug in 100 ml volumetric flask using 0.01N NaOH solution. Beer's law was obeyed in concentration range 5-35 µg/ml for Lumefantrin for all the proposed methods. The sampling wavelengths for methods I, II and III, selected for both the drugs were 252nm, 268nm and 296nm, on trial and error basis using 0.01 N NaOH solutions as solvent. For methods I, II, III seven mixed standards solutions with concentration of Lumefantrin in the µg/ml of 5:35, 10:30, 15:25, 20:20, 25:15, 30:10 and 35:5, were prepared by diluting appropriate volumes of standard stock solutions.

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The average weight of the tablets was determined by weighing twenty tablets and these were powdered. Tablet powder equivalent to 20 mg of Lumefantrin was weighed and transferred to a 100ml volumetric flask. About 20ml of methanol was added and sonicated for 15min for complete dissolution of drugs; the volume was made up with 0.01NaOH and filtered through filter paper. Six replicates of analysis were carried out with sample weighed individually.

Method I: Simultaneous Equation Method: Simultaneous equation method [23] of analysis was based on the absorption of drug (Lumefantrin) at the wavelength maximum of the each other. Wavelengths were selected for the development of the simultaneous equations was 252 nm, λ max of Lumefantrin respectively. The absorbances of both the drugs were measured at 252 nm. The absorptivity values E (1%, 1cm) determined for Lumefantrin at 252 nm were 271.1 respective values. For These values were means of six estimations.

The concentration of drug in mixture was calculated by, using following equations

$$C\text{Lumefantrin} = \frac{(A_2 ay_1 - A_1 ay_2)}{(ax_2.ay_1 - ax_1.ay_2)} \quad (1)$$

Where A_1 and A_2 were the absorbances of sample at 252 nm, a_{x1} and a_{x2} were the absorptivity E (1%, 1cm) of Lumefantrin at 271.1 nm; a_{y1} and a_{y2} were the absorptivity of Lumefantrin at 290.8 nm.

Method II: Absorbance Ratio Method: Absorbance ratio method [24-25] of analysis was based on the absorbance's at selected wavelengths, one of which is an isobestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Fig. 1) 285 nm (Isobestic point) and 268 nm (λmax of Lumefantrin) were selected for the formation of Q absorbance equation (Eqn. 2). The absorbances at 285 nm and 268 nm for Lumefantrin were measured. The absorptivity values of each drug at both wavelengths were determined which was the mean of six independent values. The absorbances and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

$$C\text{Lumefantrin} = \frac{(Q_M - Q_Y)}{(Q_X - Q_Y)} . \frac{A_1}{ax_1} \quad (2)$$

Q_M, Q_X and Q_Y were obtained as bellow:

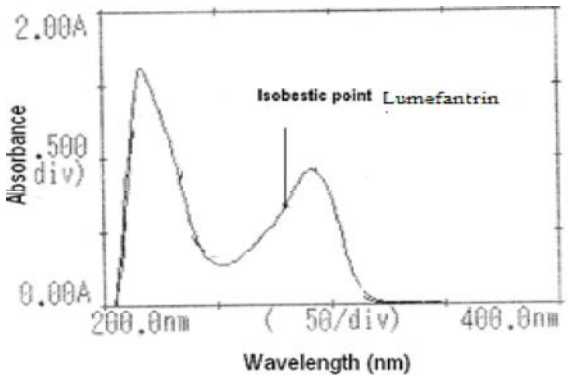

Fig. 1: Overlain spectra of Lumefantrin along with isobestic point.

$$Q_M = \frac{A_2}{A_1},\ Q_X = \frac{ax_2}{ax_1},\ Q_Y = \frac{ay_2}{ay_1}$$

Where A_1 and A_2 were the absorbance of the sample at 268 nm, a_{X1} and a_{X2} were the absorptivity of Lumefantrin at 285 nm respectively and a_{y1} and a_{y2} were the absorptivity.

Method III: Dual Wavelength Method: In this method [26] two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for one drug at a time. The spectrum of Lumefantrin showed that the absorbance of Lumefantrin is identical at 296 nm (λ_1) and 303 nm (λ_2), so these two wavelengths were selected for the analysis of Lumefantrin. All the solutions of series were scanned to ensure that absorbance difference between λ_1 and λ_2 is zero.

Validation of the Developed Methods: The developed methods for the simultaneous estimation of of Lumefantrin were validated as per ICH guidelines[27-28].

Linearity: Appropriate dilutions of standard stock solutions were assayed as per the developed methods. To establish linearity of the all proposed six methods, six separate series of solutions of Lumefantrin (5-35 µg/ml in 0.01N NaOH) were prepared from the stock solutions and analyzed.

Accuracy: To check the accuracy of proposed method, recovery studies were carried out from the pre-analyzed sample at three deferent level of standard addition 80%, 100% and 120% of the level claim. The results of the recovery studies are given in (Table 1).

Table 1: Recovery study of Lumefantrin

Method	Drug	% mean recovery	S.D.	% R.S.D.	S.E.
I	LF	100.13	0.3265	0.1659	0.1216
II	LF	101.93	0.3277	0.2154	0.3654
III	LF	99.87	0.3245	0.4232	0.6654

LF- Lumefantrin [b] Average of three determinations, S.D.: Standard deviation, R.S.D.: Relative standard deviation, S.E.: Standard error

Table 2: Result of commercial formulation analysis

Method	Drug	Label claim (mg/capsule)	% of label claim estimated[a]	S.D.	% R.S.D	S.E.
I	LF	2	99.80	0.1887	0.821	0.653
II	LF	2	99.84	0.2743	0.324	0.043
III	LF	2	99.72	0.4328	0.213	0.098

LF- Lumefantrin [a] Average of six determinations, S.D.:Standard deviation, R.S.D.:Relative standard deviation, S.E.: Standard error

Table 3: Interdays, intraday data of commercial samples of Lumefantrin and LOQ, LOD data for Lumefantrin

Method	Drug	% RSD Interdays	% RSD Intraday	LOD (µg/ml)	LOQ (µg/ml)
I	LF	0.1493	0.1493	0.1153	0.3607
II	LF	0.2435	0.4532	0.0272	0.8270
III	LF	0.4152	0.6853	0.1049	0.3179

LF- Lumefantrin R.S.D. is relative standard deviation, LOD is least of detection and LOQ is least of quantitation.

Precision

Repeatability: To the check of degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times at all concentrations in linear range. The standard deviation, relative standard deviation and standard error were calculated. The results of statistical evaluation are reported in (Table 2).

Intermediate Precision (Intra-day and Inter-day Precision): The Intra and Inter-day precision was determined by assay of the sample solution on the same day and different days at different time intervals respectively. The results of the same are presented in (Table 3).

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ of Lumefantrin by the proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation. The results of the same are shown in (Table 3).

RESULTS AND DISCUSSION

The linear regression equations obtained were; absorbance at 252 nm= [0.076×conc. in µg/ml]+0.23 (Methods I, II and III for Lumefantrin, r^2 = 0.9994,0.9989,0.9990), 268 nm Methods I,II and III for Lumefantrin = [0.0262×conc. in µg/ml]+0.0116 (Method I for DS, r^2 = 0.9995,0.9982,0.9997), 285= [0.0332×conc. in µg/ml]+0.0154 (Method II for Lumefantrin, r^2 = 0.9991), Linearity range for Lumefantrin estimation were found to be 5-35 µg/ml at their respective selected wavelengths for all proposed methods. The validity and reliability of proposed method was assessed by recovery studies by standard addition method. The means of %recovery (%RSD) were found to be low values (<2.0) for all the six proposed methods (Table 1). These results revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed analytical methods. Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicated the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, RSD and standard error was calculated of Lumefantrin. The results of statically evaluation are given (Table 2). Intermediate precision study expresses within laboratory variation in different days. In intermediate precision study, %RSD values were not more than 2.0% in all the cases (Table 3). RSD values found for both the analytical methods were well within the acceptable range indicating that these all methods have excellent repeatability and intermediate precision. From data (standard deviation of y-intercept of regression equation and slope of calibration curve), it was possible to calculate the detection and quantitation limits. For method I, the LOD, LOQ values for Lumefantrin was found to be 0. 782, 1.465 (µg/ml) respectively; for method II, 0.436, 1.214 (µg/ml) respectively; for method III, 0.241,

0.362 (µg/ml) respectively. Formulation assay values of Lumefantrin for method I, II and III was found to be 99.97 %, 98.65% and 98.91 respectively with standard deviation<1.0. Table 1. This methods utilizes the active analogue principle that lies at the spectroscopic method [8-22].

CONCLUSION

The proposed validated three Spectrophotometric methods are simple, rapid, accurate, precise and inexpensive and hence can be used for the routine analysis of Lumefantrin in dosage forms. The sample recovery for all three methods was in good agreement with their respective label claims, which suggested non interference of formulation additives in estimation. The developed Spectrophotometric method was simple, sensitive and specific, for the determination in pure and pharmaceutical formulations.

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