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Research Article

Development of Analytical Method for Lumefantrine by UV Spectrophotometry

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ABSTRACT

A Simple, sensitive, specific, spectrophotometric method has been developed for the detection of Lumefantrine in pure form and Pharmaceutical formulations. The optimum condition for the analysis of the drug was established. Lumefantrine exhibiting absorption at 234nm and obeyed beers law in the concentration range 8 to 16µg/ml. The lower limit of detection was found to be 4.3×10^{-2} and the limit of quantification to be 13.2×10^{-2}. The regression equation was y = 0.065*x* + 0.02. The precision of the method was found to be 480.96mg at 234nm against the label claim of 480mg . The sample solution was stable up to 24 hours. The assay results were found to be in good agreement with label claim. The proposed method was simple sensitive, precise, quick and useful for routine quality control.

Keywords: Spectrophotometry; Lumefantrine; Determination.

INTRODUCTION

Lumefantrine is an antimalarial drug widely used in malaria endemic areas (Sisowath C et al., 2005). 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 with a moleclar formula $C_{30}H_{32}Cl_3NO$ is an aryl alcohol, antimalarial first synthesized in the 1970's by the Academy of Military Medical Sciences, Beijing, China and registered in China for the treatment of malaria in 1987 (WHO 1990).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 (Colussi D et al., 1999) . The molecular structure hase been presented in fig 1.

Lumefantrine as a drug is commercially available only in a fixed-dose combination with artemether(Coartem® or Riamet®) (Blessborn D et al., 2007) 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.

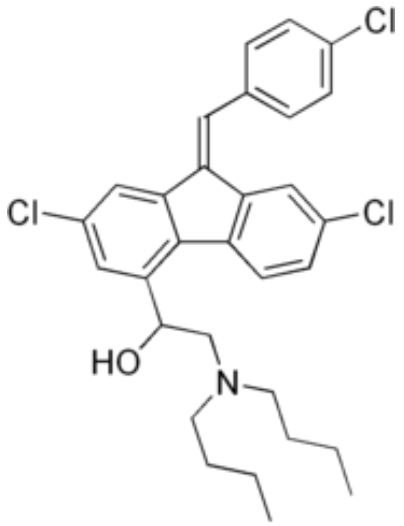

Figure 1: Structure of Lumefantrine

Literature review reveals that the various analytical method like HPLC-UV method (210 nm) for the simultaneous quantitation of artemether and lumefantrine in fixed does combination tablets (César Ida C et al., 2008).Liquid chromatographic method for determination of lumefantrine in capillary blood on sampling paper (da Costa César I et al., 2008) solid-phase extraction and liquid chromatographic method (Annerberg A et al., 2005) in rat plasma by liquid-liquid extraction using LC-MS/MS with electrospray ionization (Lindegårdh N et al., 2005, Wahajuddin, Singh SP et al., 2009, Hodel EM et al., 2009, Isabela da Costa César et al., 2008,).

The aim of the present work is to find out a simple, specific, sensitive, spectrophotometer method developed for the detection of Lumefantrine in pure form and in pharmaceutical formulation.

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EXPERIMENTAL

Instrumentation

A double–beam spectrophotometer shimadzu was used for the detection of absorbance, Mettler Tremedo as Weighing balance and Bronson sonicator, borosil glass apparatus were used for experimental purpose.

Chemicals and Reagents

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.

Procedure

Preparation of stock solution

100mg of mg pure drug was weighed and transferred to a 100ml volumetric flask, 50ml methanol was added to the above flask, dissolved and sonicated for 15 min the volume was made up with the methanol.

Preparation of sample solution

The average weight of the tablets was determined by weighing 10 tablets and these were powdered. Tablet powder equivalent to 50 mg of Lumefantrine 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 methanol and filtered through filter paper. Six replicates of analysis were carried out with sample weighed individually. The average weight of tablet was found to be 103.2mg.

Method validation

Validation of the analytical method for the determination of Lumefantrine in Pure form and in pharmaceutical formulation was carried out as per ICH guidelines.

Linearity

The method was validated according to ICH Q2B guidelines (ICH 1996) for validation of analytical procedures in order to determine the linearity, sensitivity, precision and of the analyte (F.W. Fifield et al., 2000, G.C. Hokanson, A et al.,1994, J.M. Green et al., 1996, Wegscheider et al., 1996, J. Vessman et al., 1996,). For Lumefantrine, five point calibration curves were generated with the appropriate volumes of the working standard solutions for UV methods. The linearity was evaluated by the least-square regression method using unweighted data.

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision and were determined with standard quality control samples (in addition to calibration standards) prepared in triplicate at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements[13]. The intermediate precision was studied by comparing the assays on three different days and the results are documented as the standard deviation and RSD %. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were determined based on the standard deviation of the response and the slope of the corresponding curve using the following equations:

$$LOD = 3.3\ s/m;\ LOQ = 10\ s/m$$

Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability.

Stability

The stability of Lumefantrine in methanol solution was studied by the UV method. Sample solutions were prepared in triplicate and stored at 4 and 25°C for 30, 60, 90, 120min and 24hours. The stability of these solutions was studied by performing the experiment.

RESULTS AND DISCUSSION

Linearity studies were carried out in the concentration range of 8-12 µg/ml and the sample solution is obtained from the stock solution. The readings are obtained by measuring the absorbance at 234nm presented in table 1 and the curve was shown in fig.2.

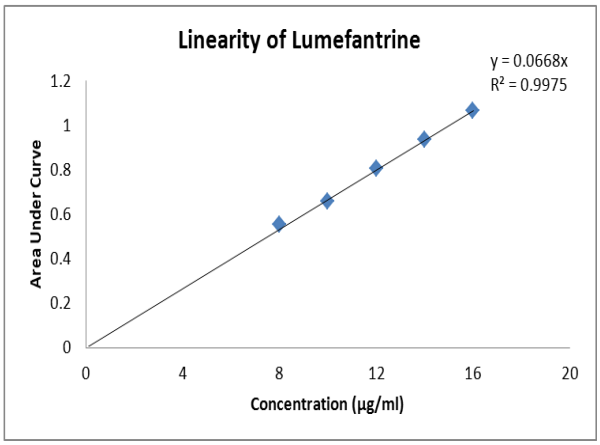

Figure 2: Linearity curve of Lumefantrine

Table 1: Linearity profile of Lumefantrine

Methanol concentration (µg/ml)	Absorbance at 234nm
8	0.5520
10	0.658
12	0.805
14	0.933
16	1.067

Performing replicate analyses of the standard solutions was used to assess the precision and reproducibility of the proposed methods. The selected concentration within the calibration range was prepared in methanol and analyzed with the relevant calibration curves to determine the intra and inter day variability. The intra and inter day precision were determined as the RSD %. The precision, of the results are given in table 2, which demonstrate a good precision and the consolidated recovery data was presented in table 3.

Table 2: Precision data for Lumefantrine in formulation

Sno	Weight (mg)	Absorbance (A^o)	Amount (mg)
1	107.1	0.669	49.03
2	104.5	0.650	48.77
3	105.1	0.678	50.58
4	108.2	0.689	49.93
5	107.6	0.695	50.65
6	106.4	0.691	51.03
		Average	49.99
		SD	0.925

The proposed methods can be successfully applied for Lumefantrine assay in tablet dosage forms without any interference. The assay showed the drug content of this product to be in accordance with the labeled claim 480mg. The values of LOD and LOQ are given in table 3**.**

Table 3: Validation parameters of Lumefantrine

Parameters	Lumefantrine
Measured wavelength (λ_{max})	234
Linearity range, µg/ml	8-16
Slope	0.0652
Intercept	0.02
Recovery (%)	99.89
Correlation coefficient (r)	0.997
LOD, µg/ml	0.0438
LOQ, µg/ml	0.132

The stability of Lumefantrine in methanol solution was studied by the UV method. Sample solutions were prepared in triplicate and stored at 4 and 25°C for 24hrs. The stability of these solutions was studied by performing the experiment. The stability of Lumefantrine in methanol solution was evaluated to verify whether any spontaneous degradation occurs, when the samples were prepared. The stability profile for 24 hrs was studied. The results were expressed as a percentage of the drug remaining. The obtained data showed that the sample solutions were stable up to 24hrs.

CONCLUSION

The developed spectrophotometric method was simple, sensitive, specific, for the determination of Lumefantrine in pure and pharmaceutical formulations. The linearity of Lumefantrine, which obeys beer's law was 8-16µg/ml at 234nm and it shows regression more than 0.99. The precision of the method was found to be 100.05%. It could be precisely detected and quantified at 4.3×10^{-2} and 13.2×10^{-2}respectively. The sample solution was stable up to 24 hrs. The proposed method will be suitable for the analysis of Lumefantrine in pure and tablet dosage form.

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