RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LUMEFANTRINE IN BULK DRUG

ABSTRACT

Lumefantrine is an antimalarial agent used to treat acute uncomplicated malaria. It is administered in combination with artemether for improved efficacy. This combination therapy exerts its effects against the erythrocytic stages of *Plasmodium spp.* and may be used to treat infections caused by *P. falciparum* and unidentified *Plasmodium* species, including infections acquired in chloroquine-resistant areas. A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of lumefantrine in bulk drug. The separation was achieved on Thermo C_{18} analytical column (250 mm × 4.6 mm i.d., 5.0µm) using 10mM KH_2PO_4: acetonitrile (pH adjust 3.0 with OPA) in the ratio 20:80 v/v as mobile phase and at a flow rate of 1.0 ml/min. Detection was carried out using a UV detector at 240nm. The total chromatographic analysis time per sample was about 6.0min with lumefantrine eluting at retention time of about 3.225 ± 0.001min. The method was validated for accuracy, precision, specificity, linearity and sensitivity. Validation studies demonstrated that this HPLC method is simple, specific, rapid, reliable and reproducible. The standard curve was linear over the concentration range of 5-25µg/ml with r^2 close to one (0.999). The limit of detection (LOD) and limit of quantitation (LOQ) obtained for lumefantrine were 0.25µg/ml and 0.75µg/ml respectively. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of lumefantrine in bulk drugs.

Key words: Lumefantrine, RP-HPLC, ICH guidelines, Antimalarial agent

INTRODUCTION

Malaria is endemic throughout most of the tropics where approximately 3 billion people, living in 108 countries are exposed. Approximately 243 million people annually develop symptomatic malaria [1]. Most of these can be attributed to *Plasmodium falciparum*, but *Plasmodium vivax* and *Plasmodium knowlesi* can also cause severe diseases. An estimated 3.3 billion people were at risk of malaria in 2010 with populations living in sub- Saharan Africa having the highest risk of acquiring malaria and children under five years of age and pregnant women being most severely affected [2,3]. Malaria case management remains a vital component of malaria control strategies. This entails early diagnosis and prompt treatment with effective anti-malarial medicines [4]. The World Health Organization (WHO) has recommended that all antimalarials should consist of a combination of an artemisinin derivative with a co-drug such as lumefantrine, amodiaquine or mefloquine; most malaria

endemic countries have now adopted artemisinin-based anti-malarial combination therapy (ACT) as first-line treatment of *P. falciparum* malaria in place of chloroquine, quinine and sulphadoxine-pyrimethamine fixed dose combinations [5]. Lumefantrine also named benflumetol and chemically (9z)-2,7-dichloro-9-[(4-chlorophenyl) methylene]-a-[(dibutylamino)methyl]-9H-fluorene-4-methanol, 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 [6]. 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 with molecular formula $C_{30}H_{32}Cl_3NO$ and molecular weight of 528.9 g mol^{-1}. Lumefantrine is extensively bound (>99%) to plasma proteins, mainly high density lipoproteins [7]. Lumefantrine as a drug is commercially available only in a fixed-dose combination with artemether [8]. This combination is well tolerated and highly effective and now becoming the most recommended first-line treatment for uncomplicated *falciparum* malaria. Literature survey reveals that few analytical methods have been reported for the estimation of lumefantrine from bulk drug, biological fluids and pharmaceutical dosage forms [9-19]. This paper describes the development and validation of reliable, simple, robust, time and money saving reversed phase HPLC method, using PDA detection, for the estimation of lumefantrine in bulk drugs. The developed method validated according to ICH guidelines [20].

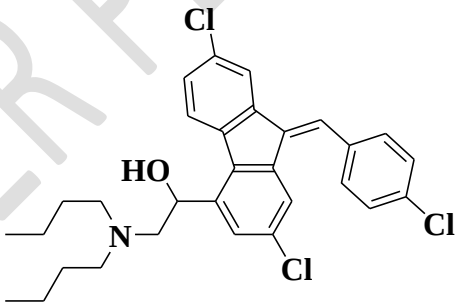


Figure 1 Chemical structure of lumefantrine

MATERIALS AND METHODS

Instrumentation

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data. Weighing was done on a Digital Micro Balance (CX-265) manufactured by Citizen Scale (I) Pvt. Ltd.

Reagents and chemicals

Analytically pure sample of lumefantrine was a generous gift from Mylan Pharmaceuticals Private Limited Hyderabad, India along with their analytical reports. Potassium di hydrogen phosphates (AR grade), disodium hydrogen phosphate (AR grade), OPA and acetonitrile (HPLC Grade) was purchased from E. Merck Ltd. Worli, Mumbai, India. All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house.

Diluents

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials, 0.1 N HCl was used as diluents.

Selection of mobile phase

Initially to estimate lumefantrine simultaneously, number of mobile phases in different ratios was tried. Taking into consideration the system suitability parameter like RT, tailing factor, number of theoretical plates and HETP, the mobile phase was found to be most suitable for analysis was 10mM KH_2PO_4: acetonitrile (pH 3.0 with orthophosphoric acid) in the ratio 20:80 v/v run as isocratic system. The mobile phase was filtered through 0.45 m filter paper and then degassed by Sonication. Flow rate employed for analysis was 1 ml/min.

Chromatographic conditions

The isocratic mobile phase consisted of 10mM KH_2PO_4: acetonitrile (pH 3.0 with orthophosphoric acid) in the ratio 20:80 v/v, flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 240.0 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Preparation of stock solution

Accurately weighed 10 mg API of lumefantrine was transferred into 10 ml volumetric flask separately and added 5ml of 0.1 N HCl as diluents, sonicated for 20 minutes and volume was made up to 10ml with 0.1 N HCl to get concentration of solution 1000µg/ml (Stock-A)

Preparation of sub stock solution

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (0.1 N HCl) to give concentration of 100µg/ml of lumefantrine respectively (Stock-B).

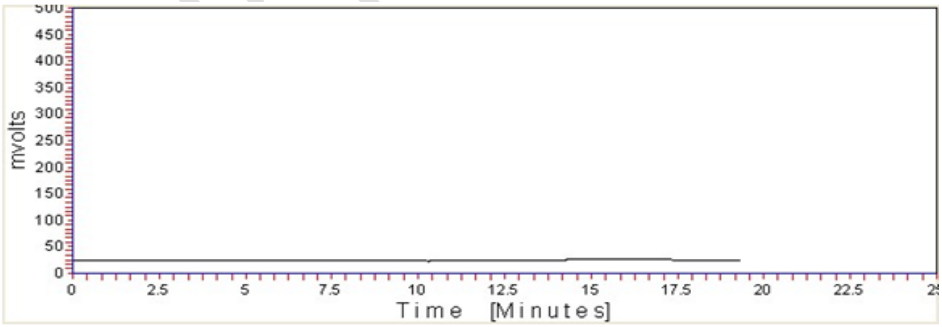
Preparation of different solution

0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (0.1 N HCl). This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml, for lumefantrine.

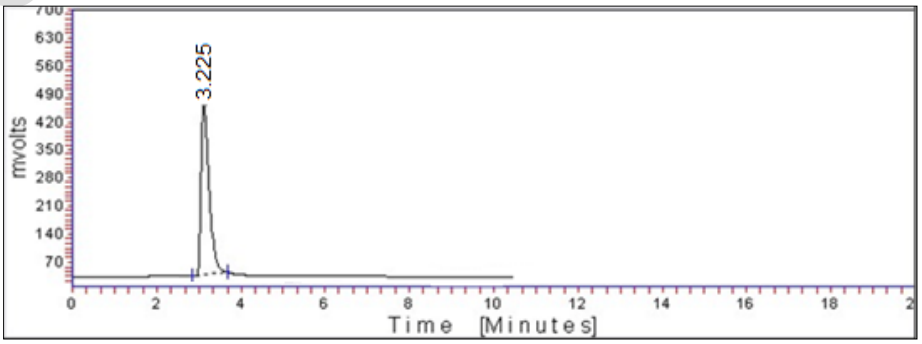
RESULTS AND DISCUSSION

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of 10mM KH_2PO_4: acetonitrile (pH 3.0 with orthophosphoric acid) in the ratio 20:80 v/v was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 min were studied. A flow rate of 1 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using a reversed-phase C_{18} column, the retention times for lumefantrine was observed to be 3.225 ± 0.001min. Total time of analysis was less than 6 min. The maximum absorption of lumefantrine was detected at 240nm and this wavelength was chosen for the analysis. Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components Fig. 2.



(A)



(B)

Figure 2 Chromatograms of (A) Blank mobile phase (B) lumefantrine (15µg/ml) as reference substances

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for lumefantrine was 2366.7.

Table 1 Results of system suitability parameters

Parameters	Lumefantrine
AUC*	286.7
No. of Theoretical Plates	2366.7
Tailing Factor*	1.2
Retention time*	3.225
Calibration range (µg/ml)	5-25

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 5-25µg/ml for lumefantrine. The linearity was represented by a linear regression equation as follows:

Y (lumefantrine) = 29.96conc-4.805 (r^2 = 0.999)

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to reanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed. The value of percentage RSD was found less than 2 (0.280, 0.168 and 0.245) show good recovery at all three level 80, 100 and 120% respectively. Each level was made in triplicate Table 2.

Table 2 Results of recovery study

% Level	% Mean±SD*
	Lumefantrine
80%	99.61±0.279
100%	99.83±0.168
120%	99.66±0.244

***** Value of three replicate and three concentrations.

Precision

Repeatability

Five dilutions in three replicates were analyzed in the same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in Table 3.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variations and results were found within acceptable limits (RSD < 2) as shown in Table 3.

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 10mM KH_2PO_4: acetonitrile (20:80 % v/v), to (15: 85% V/V) and method is found robust as RSD is again found < 2.0 Table 3.

Table 3 Statistical data for precision and robustness

Statistical parameter	Lumefantrine		
	Mean*	**S.D***	**R.S.D***
Repeatability	99.352	0.067	0.067
Intermediate Precision (I) (A day to day)	99.304	0.078	0.079
(II) Analyst to Analyst	99.679	0.044	0.044
Robustness	99.375	0.061	0.061

*Mean of 15 determinations (three replicates at five concentration level)

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve Table 4.

Table 4 LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Lumefantrine	0.25	0.75

CONCLUSION

The proposed HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of lumefantrine by HPLC in pharmaceutical dosage form. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of lumefantrine with no interference from other formulation excipients. The proposed method was highly reproducible, reliable, rapid, robust and specific. Therefore, a high percentage of recovery and the run time of less than seven minutes allow its application for the routine determination of lumefantrine in the pharmaceutical dosage form.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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