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Research Article

HPLC Method Development and Validation for Determination of Lumefantrine in Pharmaceutical Dosage Forms

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Abstract

A simple, precise and rapid HPLC method was developed for estimation of Lumefantrine in pure and pharmaceutical dosage forms. The chromatographic separation was conducted on Shimadzu (Prominence LC 20 UFLC XR) connected with PDA detector; using column C18; Waters, (300 x 3.9 mm, 10 μm). The isocratic mobile phase consisted of ion pair reagent: Acetonitrile in ratio of (35 : 65 v/v). Ion pair reagent is composed of 5.65 g of sodium hexanesulfonate and 2.75 g of sodium dihydrogen phosphate in 900 ml of water, adjusted to pH 2.3 with Phosphoric acid 85%, diluted to 1000 ml with water and filtered through 0.45 µm filter. The mobile phase was delivered to the system at a flow rate of 2 ml/min. An injection volume of 20 μl was used for Lumefantrine. The detection was carried out by PDA detector 342 nm. The calibration curve of Lumefantrine in mobile phase was linear with correlation coefficient (r^2) = 0.99946; over a concentration range of 60 – 1200 mg/l; with a retention time of 3.686 minutes. The percentage recovery of Lumefantrine was 100.029%. The relative standard deviation (RSD) was found to be less than 2. The proposed method was validated and successfully applied for determination of Lumefantrine in tablet dosage form.

Keywords: HPLC, Lumefantrine, PDA detector, Method Validation, Dosage form, Antimalarial

1.Introduction

Lumefantrine is an antimalarial, which is chemically (1R, S)-2-Dibutylamino-1-{2,7-dichloro-9-[(Z)(4-chlorobenzylidene)-9H-fluoren-4-yl}-ethanol (racemate); as shown in (Fig.1).

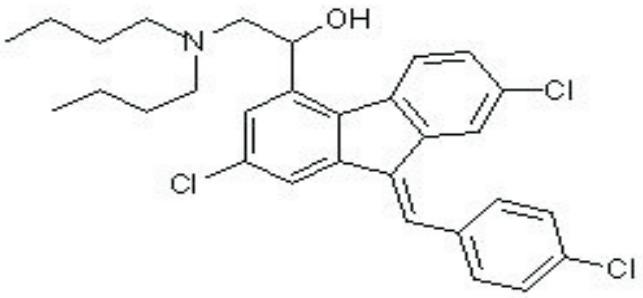

Fig. 1: Chemical structure of Lumefantrine

Lumefantrine, artemether derivative, is an antimalarial agent active against multi-drug resistant strains of Plasmodia falciparum, effective for the treatment of various types of malaria. Lumefantrine has marked blood schizontocidal activity against a wide range of Plasmodia [1-3]. Lumefantrine is a compound with molecular formula $C_{30}H_{32}C_{l3}NO$ and molecular weight 528.9 g/mol. It is a yellow crystalline powder; that is practically insoluble in water and aqueous acids. Few methods have been developed to analyze Lumefantrine alone or in combinations in different dosage forms using HPLC. Literature survey reveals that few analytical methods have been reported for the estimation of Lumefantrine from bulk drug [4-13].

2. Materials and Methods

2.1. Materials

All chemicals and reagents used were HPLC grade. Pure standards of Lumefantrine were obtained from Hetero Drugs, Hyderabad. Ortho-phosphoric was HPLC grade from Fluka chemicals. Acetonitrile was Isocratic HPLC grade from Fisher chemicals. 1-Propanol HPLC grade was from Caledon. 1-hexanesulfonic acid sodium salt was HPLC grade from Tedia. Sodium dihydrogen phosphate was purchased from local market.

2.2. Analytical procedure for determination of Lumefantrine in tablet dosage form

2.2.1. Chromatographic condition

Shimadzu LC prominence 20 (UFLC XR) connected with PDA detector was used. Shimadzu Lab solutions software was used for data acquisition. Column C18 (Waters) (300 x 3.9 mm, 10 μm) was used as a stationary phase. The mobile phase was isocratic consisted of ion pair reagent : Acetonitrile in ratio of (35 : 65 v/v) (ion pair reagent is composed of 5.65 g of Sodium hexanesulfonate and 2.75 g of Sodium dihydrogen phosphate in 900 ml of water, adjust the PH to 2.3 with Phosphoric acid 85%) delivered to the system at a flow rate of 2 ml/min. An injection volume of 20 μl was used for Lumefantrine. The detection was carried out by UV detector 342 nm, run time was 6 minutes. The column was maintained at ambient temperature.

2.2.2. Preparation of solvent

Mixing 200 ml of ion pair reagent, 60 ml of water and 200 ml of 1-Propanol and diluting to 1000 ml with Acetonitrile.

2.2.3. Preparation of stock and working standard solution

A 120 mg of Lumefantrine working standard was weighed and transferred into a 100 ml clean and dry volumetric flask. 85 ml of the solvent (previously stated) was added; then was sonicated for 30 minutes until Lumefantrine was dissolved and diluted to volume with diluents to give concentration range of 60 – 1200 mg/L.

2.2.4. Analytical method validation

a) Selectivity: It provides an indication of the selectivity of the procedure. The method is to be selective, if the main peak retention time is well resoluted from any other peak by resolution of minimum 2. This could be done by injecting placebo and comparing it with that of standard and the test samples. The peak purity was ascertained by using of PDA scanning.

b) Linearity: It is defined by the correlation coefficient, which should be found N.L.T 0.99, using peak area responses. Linearity for single point standardization should extend to at least 20% beyond the specification range and include the target concentration. This was performed by preparing six different concentrations, and then making

three replicates of each concentration. The linear working range was determined from the constructed standard calibration curve.

c) Intraday Precision: This study was conducted by performing multiple analysis on a suitable number of portions of a homogeneous sample. This was performed by assaying multiple aliquots with the same concentration starting from the first step to the final step of analysis. The analytical precision of the method was determined by the relative standard deviation.

d) Inter-day Reproducibility (Method Ruggedness): It is the degree of reproducibility determined by analysis of samples from homogeneous lot of materials, under different but typical test conditions. The method is to be rugged, at any item if the pooled %RSD of the total number of replicates that have been made in this item is within the acceptance criteria. Three replicates of a single sample of powder material are used for each determination. On the first day, three replicates while on the second day, three replicates; then finally on third day, another replicates of freshly prepared test from the same sample are analyzed, using the same conditions.

e) Accuracy and Recovery: Accuracy was evaluated by spiking standard solution. The measurements are made at a concentration of standard mix, which is found to be the target concentration, and at suitable intervals around this point. The test samples was spiked with known quantities of standard Lumefantrine using three determinations over six concentrations level covering the specified range (i.e. six concentrations and three replicates). Relative recoveries of Lumefantrine used in the standards were evaluated by comparing their peak area with those obtained from the calibration curve equation.

3. Results and Discussion

The proposed HPLC method required fewer reagents and materials, and it is simple and very rapid. This method could be used in quality control test in pharmaceutical industries. The chromatogram of Lumefantrine is shown in Fig. 2 (Retention time: 3.686 minutes).

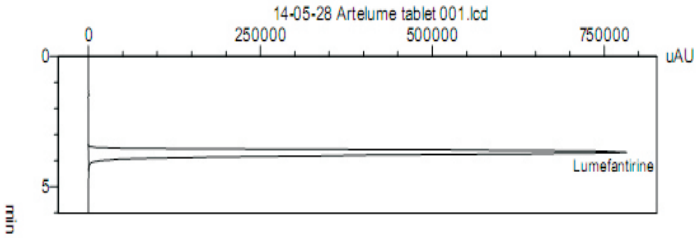

Fig. 2: HPLC chromatogram of Lumefantrine

3.1. Specificity

The PDA chromatograms of the Lumefantrine in standard and sample were recorded. In the chromatograms of the formulations, some additional peaks were observed which may be due to excipients present in the formulations. These peaks however did not interfere with the standard peaks, which demonstrate that the assay method is specific. Furthermore, the purity of the peaks was studied by peak purity studies. The results revealed that the peak is free from interferences, which shows that the HPLC method is specific.

3.2. Linearity

The response for the detector was determined to be linear over the range of 60-1200 mg/L (60, 120, 480, 600, 960 and 1200) for Lumefantrine as shown in Fig. 3.

Each of the concentrations was injected in triplicate to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value

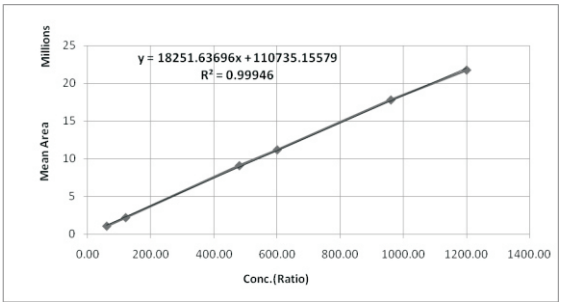

Fig.3: Calibration curve of Standard Lumefantrine

calculated in the statistical study. They were represented by the linear regression equation

Lumefantrine = 18252 X + 110735, $r^2 = 0.99946$

Slopes and intercepts were obtained by using regression equation (Y = mx + c) and least square treatment of the results used to confirm linearity of the method developed.

3.3. Quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of Lumefantrine found to be 10 mg/L. The LOQ of Lumefantrine found to be 30 mg/L.

3.4. Solution Stability

In this study, the mobile phase, the standard solutions, and the sample solution were subjected to long term (3 days) stability studies. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention, and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions

3.5. System suitability

The resolution, capacity factor, theoretical plates/meter, R_t values and peak symmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations System suitability parameters might be fall within ± 3% standard deviation range during routine performance of the method.

4. Conclusion

This method is simple, specific, precise, selective, and easy to perform and requires short time to analyze the samples. Low limit of quantification and limit of detection makes this method suitable for use in quality control. This method enables determination of Lumefantrine because of good separation and resolution of the chromatographic peaks. The method was found to be accurate, precise, linear, and rugged.

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