Development and validation of stability indicating assay method for estimation of lumefantrine in bulk and tablet dosage form

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Simple, precise, accurate, sensitive, economical, and rapid stability indicating method was developed for the estimation of Lumefantrine in bulk and tablets. Chromatographic analysis was performed on A Hibar C18 (4.6 × 250 mm, 5 μm) column and mobile phase made up of acetonitrile: methanol (50:50 v/v); used for this study. The flow rate of the mobile phase was to 1.2 ml/min; the temperature of the column was adjusted to 40°C and UV analysis was carried out at 234 nm. The degradation studies were performed and the analytical method was validated as per ICH Q2R1 guideline. The Retention time of Lumefantrine was found to be 8.8 min. The developed method was found to be linear in the concentration range of 10-60 μg/ml. The value of the correlation coefficient between peak area and concentration was found to be 0.995. The value of % RSD was found to be within prescribed limits for precision studies which indicate reproducibility of the method. The values of LOD and LOQ were obtained at 14.54 and 44.07 μg/ml, respectively. The results of degradation studies indicate that the drug was found to be stable in acidic, basic, neutral, photolytic, and neutral conditions while degraded in oxidation condition.

INTRODUCTION

Lumefantrine belongs to arylamine alcohols group, which is indicated for treatment of a patient suffering from malaria. Lumefantrine has specific schizonticidal action against a wide group of plasmodia present in blood, including P. falciparum which is resistant to chloroquine. (Arun and Smith, 2010) Lumefantrine was approved by FDA in combination with Artemether (Coartem) on April 8, 2009 for the prevention of malaria caused by Plasmodium falciparum. (Khuda et al., 2014) Artemether and lumefantrine have combined action; the short period antimalarial action of artemether bringing about a quick decrease of
parasite biomass over the initial treatment, and the more extended acting lumefantrine giving supported antimalarial action to kill the left-over parasites in blood. (da Costa César et al., 2008b) Literature study explains that some various analytical methods have been available for the determination of lumefantrine and Artemether combination by using UV spectrophotometer, HPLC and tandem mass spectrometry. (Khalil et al., 2011) One stability indicating assay (SIAM) method for lumefantrine has been reported but the run time of this method is about 30 min. (Lindegårdh et al., 2005) Quality control department in pharmaceutical industry has to analyse lot of samples and HPLC system can analyse one sample at a time. (Vinodh et al., 2013) In this context, less time of analysis is needed. (da Costa César et al., 2008a) Therefore, a SIAM has been developed, which has less run time (15 min). (Huang et al., 2010) This paper presents simple, rapid, accurate and precise stability indicating method for determination of lumefantrine and structure of lumefantrine as shown in Figure 1. (Suleman et al., 2013)

MATERIALS AND METHODS

Chemicals and Reagents

The pure working standard of Lumefantrine was procured from Coral Laboratories Ltd, Daman. Commercially available Lumefantrine tablet 120 mg was procured from local market. Acetonitrile and Methanol were procured from Loba Chemie Pvt. Ltd, Mumbai.

Instruments

The method was performed on Shimadzu LC 2010 CHT (Japan). Analytical balance AX-200 (Shimadzu Japan), UV-Visible spectrophotometer UV-1800 (Shimadzu, Japan), Ultrasoundator USB-40 (Spectra Lab), Hot Air Oven BTI (Bio Technics India), Column Hibar C18 (4.6 × 250 mm, 5 μm) and LC solution software were used for this work.

Preparation of standard stock solution

The stock solution of standard drug was prepared by weighing 10 mg of Lumefantrine accurately. Weighed drug was transferred to 100 ml volumetric flask with 50 ml methanol and sonicated for 15 minutes. Then diluted with methanol to yield the solution of 100 μg/ml of Lumefantrine. (Patil et al., 2009)

Preparation of working standards

Working standards (10-60 μg/ml) of Lumefantrine were prepared in methanol by suitable dilutions of the standard stock solution. (Verbeken et al., 2011)

Selection of detection wavelength

Working standard (10 μg/ml) was analysed in UV range (400 to 200 nm) by using UV spectrophotometer against methanol as a blank (Silva et al., 2015)

Validation of method

The developed method was validated for accuracy, precision, linearity, range and robustness parameters as per ICH Q2R1 guideline. (Ippolito et al., 2018)

Linearity and range

Working standards were injected in the concentration of 10-60 μg/ml under the optimized chromatographic conditions and peak areas were calculated at detection wavelength 234 nm. Linear regression data as well as calibration curve were shown in table under results and discussion section. (Goversder et al., 2015)

Precision

Intermediate precision was carried out with three concentrations of Lumefantrine with three replicates and Repeatability study was carried out with nine replicates and the values of % relative standard deviation (% RSD) for both parameters.

Robustness

Robustness of the optimized method was studied by altering flow rate (± 1 ml/min), change in wavelength (± 1 nm) and change in temperature (± 1°C) during analysis. The sample was injected in triplicate for every condition and % RSD was calculated for each condition.

Limit of detection and Limit of quantitation

Six sets of concentrations were prepared between 10-60 μg/ml and the corresponding areas of these sets were measured. Calibration curves were plotted for each set.

Accuracy

The accuracy of the developed method was studied by calculating percentage recovery of drug by standard addition method. Percent recovery of Lumefantrine was determined at 80%, 100%, and 120% of the target concentration in triplicate.

Forced degradation studies

To evaluate the stability, Lumefantrine was subjected to forced degradation under the condition of acid, base, neutral hydrolysis, heat, photolysis and oxidation as per ICH guidelines. (Guideline, 2005)

Acid hydrolysis

200 mg of Lumefantrine was weighed accurately in 250 ml round bottom flask with 100 ml of 0.1N hydrochloric acid (HCl) and 100 ml methanol. This
mixture was refluxed at 80°C. After every 1h, 5 ml of refluxed sample was withdrawn and neutralized with 5 ml of 0.1N sodium hydroxide and diluted 25 times with mobile phase.

**Alkaline hydrolysis**

200 mg of Lumefantrine was weighed accurately in 250 ml round bottom flask containing 100 ml of 0.1N sodium hydroxide (NaOH) and 100 ml Methanol. This mixture was refluxed at 80°C. After every 1h, 5 ml of refluxed sample was withdrawn and neutralized with 5 ml of 0.1N hydrochloric acid (HCl) and diluted 25 times with mobile phase. (Bakshi and Singh, 2002)

**Neutral Hydrolysis**

200 mg of pure Lumefantrine was transferred in 250 ml round bottom flask and 100 ml of methanol was added to make solution. 100 ml HPLC grade water was added to this solution and refluxed at 80°C for about 1 h. Sample (0.2 ml) was withdrawn and diluted 10 times with mobile phase. (Guideline, 2003)

**Dry Heat Degradation**

100 mg of pure Lumefantrine packed in the vial, kept in oven at 80°C for 1h. After 1h 10mg of drug was dissolved in 10 ml Methanol and sonicated for 20 min. Further 0.2 ml of the above sample solution was diluted to 10ml with mobile phase. The chromatogram obtained after 5 h of dry heat degradation.

**Photolytic Degradation**

10mg of pure Lumefantrine were weighed in 10 ml volumetric flask and volume was adjusted with diluents. The solution was kept in sunlight for 1h. Sample (0.2 ml) was withdrawn and diluted 10 times with diluents.

**Oxidative hydrolysis**

200 mg of pure Lumefantrine were transferred in 250 ml round bottom flask and 200 ml of 3% hydrogen peroxide was added to make the solution. This reaction mixture was refluxed at 80°C for about 1 h. Sample (0.2 ml) was withdrawn and diluted 10 times with mobile phase.

**RESULTS AND DISCUSSION**

**Optimization of chromatographic conditions**

Optimized chromatographic condition and system suitability parameters are selected as shown in Table 1. Detection wavelength 234 nm was selected as wavelength maxima (Amax) from UV absorption spectrum of the drug as shown in Figure 2. Different columns and mobile phases as well as their compositions were analysed to find the best chromatographic conditions for development of HPLC method for estimation of Lumefantrine as shown in Figure 3. Finally, A Hibar C18 (4.6×250 mm, 5μm) column at 40°C and mobile phase containing acetonitrile: Methanol (50:50) at flow rate 1.2 ml/min was selected. The retention time of Lumefantrine was found to be 8.8 min.
Table 1: Optimized chromatographic condition and system suitability parameters

| Parameters        | Details                                             |
|-------------------|-----------------------------------------------------|
| Mobile phase      | Acetonitrile: Methanol (50:50 V/V)                  |
| Column            | Hibar C18(4.6×250mm), 5μm                          |
| Flow rate         | 1.2 ml/min                                          |
| Detection         | UV at 234 nm                                        |
| Injection volume  | 40μl                                               |
| Runtime           | 15 min                                              |
| Retention time    | 8.8 min                                             |
| Diluent           | Acetonitrile: Methanol (50:50 V/V)                  |

Table 2: Results for linearity and range

| Parameters        | Lumefantrine                                      |
|-------------------|---------------------------------------------------|
| Linearity range   | 10-60 μg/ml                                       |
| Coefficient of correlation | 0.9915                                        |
| Slope             | 16367                                             |

Table 3: Intermediate precision and Repeatability studies for Lumefantrine

| Precision         | Amount μg/ml | Area          | Mean Area ±SD | % RSD    |
|-------------------|--------------|---------------|---------------|----------|
|                   | 10           | 2453141       | 2453141±46907.34 | 0.19     |
| Intermediate      | 10           | 2359924       |               |          |
| Precision         | 10           | 2415689       |               |          |
| (n=9)             | 20           | 3585423       | 3566810±29263.29 | 0.82     |
|                   | 20           | 3581927       |               |          |
|                   | 20           | 3533080       |               |          |
|                   | 30           | 5973609       | 5914583±51127.67 | 0.86     |
|                   | 30           | 5886064       |               |          |
|                   | 30           | 5884076       |               |          |
| Repeatability     | 20           | 3531222       | 3559281.556±30737 | 0.86 %  |
| Intraday precision| 20           | 3526031       |               |          |
| (n=9)             | 20           | 3535397       |               |          |
|                   | 20           | 3582423       |               |          |
|                   | 20           | 3591707       |               |          |
|                   | 20           | 3603092       |               |          |
|                   | 20           | 3532224       |               |          |
|                   | 20           | 3546049       |               |          |
|                   | 20           | 3585389       |               |          |

Figure 4: Calibration Curve of Lumefantrine

areas and concentrations as shown in fig. 4. Therefore, the developed method was found linear in the concentration range of 10-60 μg/ml and result for linearity and range is shown in Table 2.

Precision

The method is precise and the %RSD values were within an acceptable limit as shown in Table 3.

Robustness

The value of % RSD was obtained within acceptance
Table 4: Robustness studies for Lumefantrine

| Factor                  | Level | Lumefantrine Mean tR ± SD | Peak Area Mean peak area ± SD |
|-------------------------|-------|---------------------------|------------------------------|
| A: Change in Wavelength of detection |       |                           |                              |
| 233 nm                  | -1    | 8.78 ± 0.05               | 3461724.6 ± 48224.2          |
| 234 nm                  | 1     | 8.76 ± 0.04               | 3510697.3 ± 44142.2         |
| 235 nm                  | +1    | 8.76 ± 0.1                | 3474234 ± 27935.76         |
| B: Change in composition of flow rate |       |                           |                              |
| 1.1 ml/min              | -1    | 9.15 ± 0.07               | 3833974.3 ± 67197.3         |
| 1.2 ml/min              | 1     | 8.79 ± 0.08               | 3515717 ± 18239.2          |
| 1.3 ml/min              | +1    | 8.55 ± 0.06               | 3723672.3 ± 55828.6        |
| C: Change in temperature of oven |       |                           |                              |
| 39                      | -1    | 8.92 ± 0.04               | 3666622 ± 2334.3            |
| 40                      | 1     | 8.76 ± 0.1                | 3389068.66 ± 34233         |
| 41                      | +1    | 7.71 ± 0.07               | 3695386 ± 24133.9          |

Table 5: Results of Accuracy of Lumefantrine

| Drug        | Amount Added ug/ml | Amount Recovered ug/ml | % Recovery | Mean % Recovery ± %RSD |
|-------------|--------------------|------------------------|------------|------------------------|
| Lumefantrine| 45                 | 37.33                  | 82.3       | 81.42 ± 0.97 ± 1.19    |
|             |                    | 36.72                  | 81.6       |                         |
|             |                    | 36.17                  | 80.4       |                         |
|             | 50                 | 53.07                  | 106.09     | 105.33 ± 1.15 ± 1.14   |
|             |                    | 52.46                  | 104.76     |                         |
|             |                    | 53.07                  | 106.55     |                         |
|             | 55                 | 68.30                  | 124.18     | 123.5 ± 1.40 ± 1.09    |
|             |                    | 68.46                  | 124.44     |                         |
|             |                    | 67.00                  | 121.88     |                         |

Results for robustness shown in Table 4.

Limit of detection (LOD) and limit of quantitation (LOQ)
The values of LOD & LOQ were found to be 14.54 µg/ml and 44.07 µg/ml; respectively that indicated the method is sensitive.

Accuracy
The value of mean % recovery and % RSD at each level was found within acceptance criteria that indicate the method is accurate. Results of accuracy of lumefantrine are shown in Table 5.

Forced degradation study
At first, 0.1 N HCl solutions was taken for degradation of Lumefantrine at 80 °C for 1 hour and the chromatogram indicated that the drug was stable in acidic condition. Then, 0.1 N NaOH solution was used at 80 °C for 1 hour and the chromatogram indicated that the drug was stable. In neutral degradation water was used at 80 °C for 1 hour and the chromatogram indicated that the drug was stable in basic condition. Chromatogram obtained by dry heat degradation suggested that no degradation of Lumefantrine, when exposed to 80 °C for 5 hours. Chromatogram obtained by photolytic degradation suggested that no degradation of Lumefantrine, when exposed to 80 °C for 5 hours. The results of degradation studies shows that the drug was stable in acidic, basic, neutral, dry heat and photolytic condition and degraded in oxidative conditions. Degradation chromatograms of Lumefantrine in 0.1 N HCl, in 0.1 N NaOH, in water at 80 °C after 1 hour, Dry heat and Photolytic degradation after 5 hours and in 30% H2O2 at 80 °C after 1 hour as shown in Figure 5.
CONCLUSIONS

The method was successfully validated as per ICH-Q2(R1) guideline. It can be concluded from validation results that developed HPLC method is simple, accurate, precise and sensitive. Results of stress testing indicate that the method is stability indicating. The developed method is rapid than the reported method. Results suggest that this analytical method can be efficiently used for routine analysis of Lumefantrine in bulk and tablets and to check the stability of bulk sample.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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