A simple and precise method for quantitative analysis of lumefantrine by planar chromatography

Abstract

A simple, precise and sensitive high performance thin layer chromatographic (HPTLC) method has been developed and validated for drug of choice Lumefantrine in treatment of malaria (*P. falciparum*). Silica gel 60 F254 HPTLC precoated plates were used for quantitative analytical purpose. Methanol water 9.5 + 0.5 (v/v) was used as the solvent system. Densitometric scanning was carried out with deuterium lamp set at detection wavelength of 266 nm. The response to lumefantrine concentration was linear in the concentration range of 1.25-12.50 µg/ml. The suitability of the method developed and validated was in accordance with the requirements of the ICH guidelines (Q2B). Thus the validated method can be further applied to quantitative analysis of lumefantrine in commercial pharmaceutical dosage form. The proposed method is simple, sensitive, precise and accurate, confirming its pharmaceutical application in routine quality control analysis.

Key words: Densitometric estimation, HPTLC, lumefantrine

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INTRODUCTION

Lumefantrine[1] is a dichlorobenzylidine derivative effective for the treatment of various types of malaria. Chemically lumefantrine is 2-Dibutylamino-1-[2, 7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]-Ethanol (racemate) [Figure 1]. The antimalarial agent is active against multi-drug resistant strains of *Plasmodium falciparum*. In combination with artemether, the drug is also used for the treatment of uncomplicated *falciparum* malaria. It has primary action as blood schizontocidal and secondary action as inhibition of nucleic acid and protein synthesis within the malarial parasite thus having a longer duration of anti malarial action.[2-4] Thus, today lumefantrine is a drug of choice in antimalarial treatment against *P. faliciparum*.[5] Therefore, development of an appropriate analytical procedure for the quantitative analysis of lumefantrine is of considerable importance to pharmaceutical industry.

Considering simplicity, accuracy, cost-effectiveness and rapidity of HPTLC it is a preferred method by the industry over various conventional as well as state of the art chromatographic methods. Literature survey reveals that there are very few analytical methods reported for the estimation of lumefantrine. The reported methods involving HPLC[6-9] are basically for analysis of lumefantrine from complex matrices like human plasma. And also there are no HPTLC methods reported for routine analysis of lumefantrine. Literature survey further revealed that the drug is not officially recognized in any pharmacopoeia, thus development of a chromatographic method namely HPTLC is of great importance and having a wide application in the routine quality control analysis.[10]

Hence, an attempt has been made to develop HPTLC method for the analysis of lumefantrine which would be highly sensitive, simple, precise and accurate with good resolution and reproducibility.

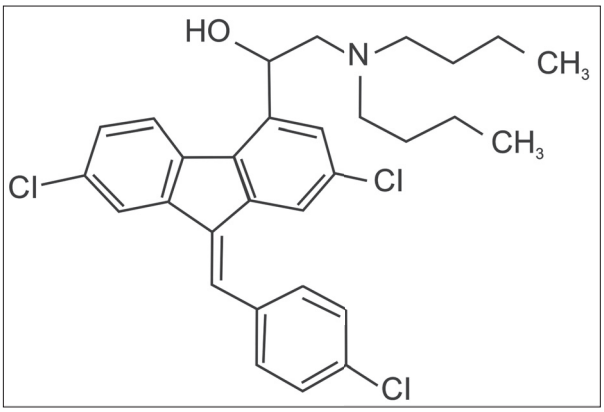

Figure 1: Structure of lumefantrine

MATERIALS AND METHODS

Chemicals and reagents

The analytically pure sample of lumefantrine working standard was obtained from Benzochem Lifesciences Pvt. Pharmaceutical Ltd., Mumbai, India. HPLC grade Methanol was purchased from Merck (Darmstadt, Germany). Distilled water was obtained from a Millipore (USA) Milli Q water-purification system.

Equipment

The HPTLC system employed in the method development and validation was Camag Linomat IV applicator (Muttenz, Switzerland), a Camag twin trough chamber of appropriate size, Camag TLC scanner III, WINCATS (version 3.17) software as data integrator and a Hamilton syringe (Switzerland) of 100µl capacity.

Preparation of stock and working standard solutions of Lumefantrine

10 mg of working standard of lumefantrine was accurately weighed and dissolved in 10 ml methanol and sonicated for 5 min. to give a stock solution of 1 mg/ml. 1 ml of this solution was diluted to 10 ml with methanol to get a working standard solution of 100 µg/ml. Further dilutions were made in methanol for preparation of calibration curve in the concentration range of (1.25-12.50 µg/ml).

Chromatography

Chromatography was performed on 10 cm × 20 cm aluminum backed silica gel 60 F_{254} HPTLC plates (Merck, Darmstadt, Germany). Before use the plates were washed with methanol then dried in an oven at 50°C for 5 min. Samples were applied as 6-mm bands, 6 mm apart and 1 cm from edge of the plate, by spraying at a rate of 15 s/ µl by means of a Camag (Muttenz Switzerland) Linomat IV automatic sample applicator equipped with a 100 µl syringe (Hamilton, Reno, Nevada, USA) under the flow of nitrogen gas. Ascending development of the plate, migration distance 80 mm, was performed at 25 ± 2°C and 40–50% relative humidity with methanol: water 9.5 + 0.5 (v/v), as mobile phase in a Camag twin-trough chamber previously saturated for 10 min. The average development time was 20 min. After development the plate was dried at 50°C in an oven for 5 min. Densitometric scanning at 266 nm was then performed with a Camag TLC Scanner equipped with WINCATS software, version 3.17, using a deuterium light source; the slit dimensions were 6.00 mm × 0.45 mm.

Method validation

The method was further validated in accordance with the requirements of the ICH guidelines (Q2B).[11]

Linearity and range for lumefantrine

Working standard solutions of lumefantrine in the concentration range 1.250-12.500 µg/ml were applied, to prewashed HPTLC plates. The plate was developed, dried, and scanned using the optimized conditions described above. The densitograms were then acquired and the peak areas were recorded for each concentration of lumefantrine. A calibration plot was constructed by plotting peak area against amount of lumefantrine (1.250-12.500 µg/ml). The calibration plot for lumefantrine was linear in this concentration range with a correlation coefficient (r) of 0.999 and the slope was 1.4837 ± 0.108 (n = 6). RSD of lumefantrine peak area for solutions of the same concentration were less than 2%, indicating there was no statistically significant variation. Validation results are summarized in Table 1.

System suitability

According to the USP 23 method 621, system-suitability tests are an integral part of a chromatographic analysis and should be used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis. To ascertain the effectiveness of the method developed in this study, system suitability tests were performed on freshly prepared standard stock solutions of lumefantrine.

Sensitivity

The sensitivity of measurement of lumefantrine by use of the proposed method was estimated in terms

Table 1: Validation parameters

Parameters	Values
Detection limit (ng/ml)	0.416 µg/ml
Quantitation limit (ng/ml)	1.250 µg/ml
Calibration range (µg/ml)	1.250-12.500
Slope	1.4837 ± 0.108
Correlation coefficient (r)	0.999

of the limit of quantitation (LOQ) and the lowest concentration detected under the chromatographic conditions as the limit of detection (LOD). The LOQ and LOD were calculated by the use of the equations LOD = 3 × N/B and LOQ = 10 × N/B where N is the standard deviation of the peak areas of the drug (n = 3), taken as a measure of the noise, and B is the slope of the corresponding calibration plot. The limit of detection (LOD) was 0.416 μg and the limit of quantification (LOQ) was 1.250 μg.

Precision

Validation of the method for precision was measured by using standard solutions containing lumefantrine at concentrations covering the entire calibration range. Further the method was validated for instrumental precision, intraday precision, and interday precision. Instrumental precision was studied by repeated analysis (n = 10), of lumefantrine standard solution (6.250 μg/ml) on the same day. The RSD for instrumental precision was 0.10 %. The precision of the method in terms of intraday variation (%CV) was determined by analyzing lumefantrine standard solutions in the range (1.250-12.500 μg/ml) three times on the same day. Interday precision (%CV) was assessed by analyzing these solutions (1.250-12.500 μg/ml) on three different days over a period of one week. The results of the precision studies are shown in Table 2.

Accuracy

The accuracy of the method was established by use of standard addition method i.e., measurement of recovery at three different concentration levels. 80%, 100%, and 120% of the standard drug solutions were added to the solution of known concentration and the percentage recovery was then determined. The results are summarized in Table 2.

Specificity

The optimized mobile phase gave a very good resolution of lumefantrine, indicating the specificity of the method. A typical absorbance spectrum of the drug is shown in [Figure 2]. The optimized solvent system yielded a symmetrical peak for the drug with R_F 0.59 [Figure 3]. To achieve the best detection sensitivity, wavelength 266 nm was selected for detection.

Ruggedness and robustness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating conditions from instrument to instrument and from analyst to analyst. Robustness is a measure of the capacity of a method to remain unaffected by small but deliberate variations in the method conditions. The %RSD was recorded less than 2%, thus indicating reliability of the method.

Reproducibility

The repeatability of sample application was assessed by spotting drug solution (10 μl) seven times on an HPTLC plate then development of plate and recording

Table 2: Summary of precision and accuracy

Actual concentration (μg/ml)	Precision: Measured concentration (μg/ml) ± S.D.; RSD (%)		Accuracy (%)	
	Intraday	Interday	Intraday	Interday
3.75	3.74 ± 0.012; 0.32	3.76 ± 0.044; 1.17	99.73	100.27
6.250	6.25 ± 0.019; 0.30	6.26 ± 0.032; 0.51	99.84	100.16
12.50	12.47 ± 0.223; 1.79	12.64 ± 0.186; 1.47	99.76	101.12

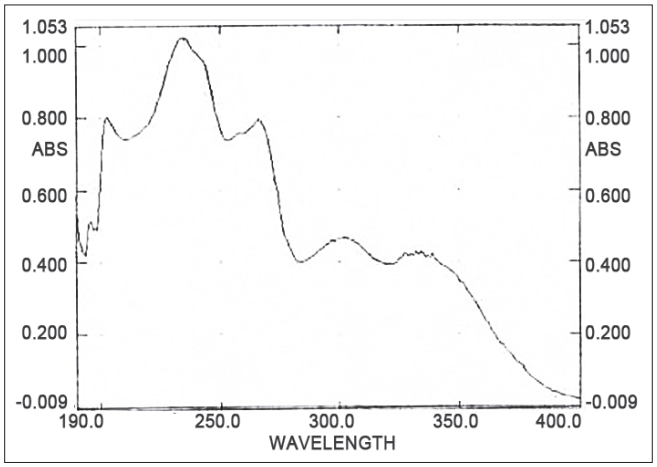

Figure 2: Absorption spectrum of lumefantrine scanned at 190-400 nm

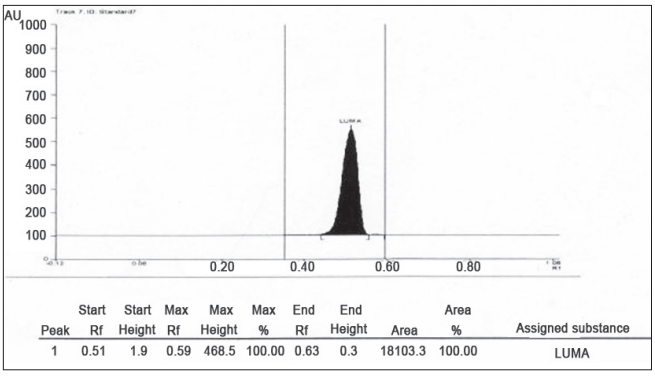

Figure 3: Representative chromatogram of standard lumefantrine at 266 nm

the peak height and area for the spots. The %RSD for peak height and peak area values of lumefantrine were found to be 0.49% and 0.98%, respectively. Repeatability of measurement of peak height and area were determined by spotting 10-µl standard drug solution on the HPTLC plate and developing the plate. The separated spot was scanned seven times without changing the position of the plate. %RSD for measurement of peak height and peak area of lumefantrine was 0.53 and 1.05, respectively.

Stability studies

To test the stability of drugs on the HPTLC plates, analyte was tested against freshly prepared solutions. No decomposition of the drug as observed during chromatogram development. No decrease in the concentration of drug on the plate was observed within three hours.

RESULTS AND DISCUSSION

The chemical nature and solubility of the drug plays an important role in development and optimization of a chromatographic method.[10] Lumefantrine is practically insoluble in water sparingly soluble in strong acid and soluble in methanol and strong base. The calibrator solutions were prepared in methanol. Various mobile phases were tried during HPTLC method development of lumefantrine. Methanol: ethyl acetate (9 + 1) % v/v was used as the mobile phase after optimizing a series of mobile phases, but it was observed that the spot moved along with the solvent front and thus was not retained on the HPTLC plate. The polarity of mobile phase was modified by changing the proportion of ethyl acetate. But good resolution was not obtained. Thus, it was observed that as the polarity of the mobile phase is increased, the R_F increased but slight tailing was observed. Finally, after several trials, the mobile phase was optimized to methanol: water (9.5 + 0.5) % v/v, which resulted good separation and resolution. Also there was no interference around the drug R_F. Hence the mobile phase selected was methanol: water (9.5 + 0.5) % v/v. Densitometric scanning was carried out using Camag TLC Scanner in the ultra-violet mode at 266 nm. The 266 nm wavelength was due to presence of chromophoric group α, β-unsaturated dienes of fluorine in the structure of lumefantrine.

The spectroscopic techniques were used to confirm the identity of lumefantrine. The IR spectra, showed strong absorption band at 3404.67 cm^{-1} (OH), 2953.28 cm^{-1} (aliphatic and aromatic CH), 1757.31 cm^{-1} (-C=C-),

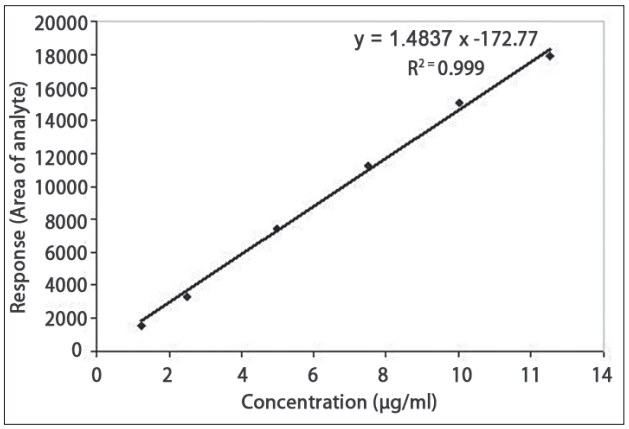

Figure 4: Calibration curve

933 cm^{-1} (alkanes) and 696.37-373.22 cm^{-1} (Cl). Thus, IR spectra confirmed the presence of these functional groups in the structure of lumefantrine. The mass spectrum showed a sharp molecular ion peak at 528.0 m/z in Q1 MS (m/z, parent ion) parameter at negative polarity confirming the molecular weight of lumefantrine. The NMR spectra observed triplet at 0.943-0.989 (methyl protons of alkyl chain); a multiplet at 1.372-1.498 (methylene protons of alkyl chains); a multiplet at 2.449-2.909 (methylene protons of alkyl chain); broad singlet at 4.573 (OH proton); and multiplet at 7.314-7.733 (aromatic proton), thus confirming identity of lumefantrine.

The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, intraday and interday precision, repeatability of measurement of peak area as well as repeatability of sample application [Table 1]. The method was found to be linear in the range of 1.250-12.500 µg/ml, with correlation coefficient of 0.999 [Figure 4]. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.416 µg and 1.250 µg. The percent RSD of intraday and interday precision of lumefantrine was 0.30-1.79% [Table 2]. The value indicates that the method is precise. The accuracy values obtained in the range 99.73-100.27% for drug are indicative of excellent accuracy and recovery. There was no evidence of peaks or any other interfering co eluting peaks at the R_F of standard (0.59) [Figure 3]. This indicates that the method is specific. Stability studies were carried out for standard. It was found to be stable in sample solution, prior to development and after development.

CONCLUSION

The proposed HPTLC method is rapid, specific, precise and accurate. The promising validation results

of the optimized method confirm the application of the method, in routine analysis of lumefantrine in bulk drugs and pharmaceutical dosage forms. Hence, we conclude that the method discussed above is of considerable importance to the pharmaceutical industry with wide application in quantitative estimation of lumefantrine.

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