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Quantitative estimation of rifapentine using UV-spectrophotometry - Area under curve technique in bulk and tablets

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ABSTRACT

A simple, precise and economical UV-Spectrophotometric method has been established for the quantification of Rifapentine in bulk drug and tablets. In this method Area under Curve (AUC) was integrated in the wavelength range of 307 -350 nm. Rifapentine obeyed linearity in the concentration range of 4 - 24 µg/mL with $r^2 > 0.9994$. Calibration curves were constructed using instrument response between chosen wavelengths and concentrations of analyte in the solution. The proposed method was effectively executed for the quantitative estimation of Rifapentine in in-house prepared tablets and % amount of Rifapentine was found to be 98.94 %. The proposed method was validated for precision, accuracy and ruggedness as per ICH Guidelines[7].

KEYWORDS

Rifapentine;
Area under curve;
UV- spectrophotometry;
Validation.

INTRODUCTION

Rifapentine is chemically 3-[N-(4-Cyclopentyl - l-piperazinylinterminidoyl] rifamycin[1]. It is a rifamycin antibiotic that is similar in structure and activity to rifampin and rifabutin and that is used in combination with other agents as therapy of tuberculosis, particularly in once-weekly regimens. Rifapentine is associated with transient and asymptomatic elevations in serum amino transferase and is a likely cause of clinically apparent acute liver injury.

Literature survey revealed that few analytical methods for rifapentine such as RP-HPLC methods in human plasma[3], human serum[4] have been established for the determination of rifapentine in biological fluids.

The objective of the present work is to establish simple, precise and accurate 'Zero order UV-Spectrophotometric method using AUC technique for determination of rifapentine in bulk and in pharmaceutical formulation. Further the developed method has to be validated as per the ICH guidelines[5].

EXPERIMENTAL WORK

Chemicals

Rifapentine supplied as a gift sample by Lupine Pharmaceuticals Ltd, Aurangabad. Methanol (A.R.Grade) was purchased from Merck Ltd., Worli Mumbai, India. In- house tablets containing 150 mg of

rifapentine was prepared.

Instrumentation

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; band width (Δλ):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Selection of common solvent

Methanol of analytical reagent grade was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility in different solvents.

Preparation of stock standard solution and selection of wavelengths

A stock standard solution of rifapentine was prepared by dissolving 10 mg of drug in methanol to obtain concentrations 100 µg/mL. After appropriate dilutions, 10 µg/mL rifapentine was scanned in the UV-region i.e. 800 – 200 nm. Rifapentine showed maximum absorbance at 334 nm. In zero order UV - Spectrophotometric method, two wavelengths 307 nm to 350 nm were selected for determination of area under curve [AUC]

Study of linearity curves

An aliquot portions 0.4-2.4 mL were transferred into series of six separate 10 mL volumetric flasks and volume was adjusted to mark with methanol to obtain concentration of 4 – 24 µg/mL. The AUC between the two selected wavelengths (307- 350) were determined and calibration curves were constructed by plotting concentration *versus* AUC between the selected wavelengths.

Preparation of in-house tablet formulation

In- house tablets containing 150 mg of rifapentine were prepaired by using some commonly used ingredients as sodium starch glycolate as super disintigrant and microcrystalline cellulose as an excipient employing direct compression technique.

Preparation of sample solution

For the estimation of rifapentine twenty in house tablets were selected randomely containing 150 mg of drug were weighed and crushed into fine powder. A quantity of powder drug equivalent to one tablet was transferred into 100 mL volumetric flask, containing 10 mL methanol and volume was made up to mark using methanol. Then filtered through Whatman filter paper (No. 41). From, it further dilutions were prepaired and AUC were recorded in between the selected wavelengths and the concentrations were determined using respective linear regression equation. The analysis procedure was repeated five times, with tablet formulation. The responses were measured and concentration in the sample was determined by comparing the response of sample with that of the standard.

METHOD

Area under curve

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of area with respect to the wavelength between the two selected wavelengths 307 and 350. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. The spectrum obtained of zero order derivatives was used to calculate AUC. The calibration curve was constructed by plotting concentration (4-24 mg/mL) ver sus AUC.

Validation of method

The proposed method was validated as per ICH guidelines[7]

Linearity

For the method, calibration curve was prepared on 3 different days. The calibration curve was constructed by plotting the response (y) versus the theoretical concentrations of standards (x), by using linear regression analysis. Linearity was expressed as a correlation coefficient; the value must be > 0.999.

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Precision

The intraday and inter day precision of the proposed Spectrophotometric method was determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of rifapentine for area under curve 8.0, 12.0, and 16.0 µg/mL and the results are reported in terms of percent relative standard deviation.

Accuracy

The accuracy of the method was determined by calculating recoveries of rifapentine by the method of standard additions. The study was performed by spiking three known amount concentration of rifapentine (6.4, 8.0, and 9.4 µg/mL; ranging from 80% to 120%) into a prequantified sample solution (8 µg/mL). Three samples were prepared at each of these concentrations. The recovery of added drug was estimated by measuring the response and by fitting these values to the straight line equation of calibration curve.

Specificity

Results of tablet solution showed that there is no interference of excipients when compared with the working standard solution. Thus, the method was said to be specific.

TABLE 1: Optical characteristics of rifapentine

Parameters	Rifapentine
Beer-Lambert's range (µg/mL)	4-24
λ max (nm)/ wave length range (nm)	334
Slope	0.305
Intercept	0.032
Correlation coefficient	0.999
Limit of detection (µg/mL)	0.24
Limit of quantitation (µg/mL)	0.72

TABLE 2: Assay results of rifapentine tablets.

Rifapentine in house tablets	lable claim tablet	% Recovery	% RSD
Tables	150mg	98.94%	1.192

***Average of three determinations**

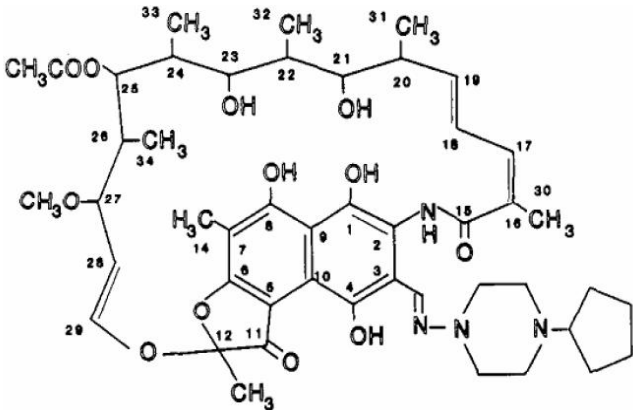

Figure 1 : Chemical structure of rifapentine

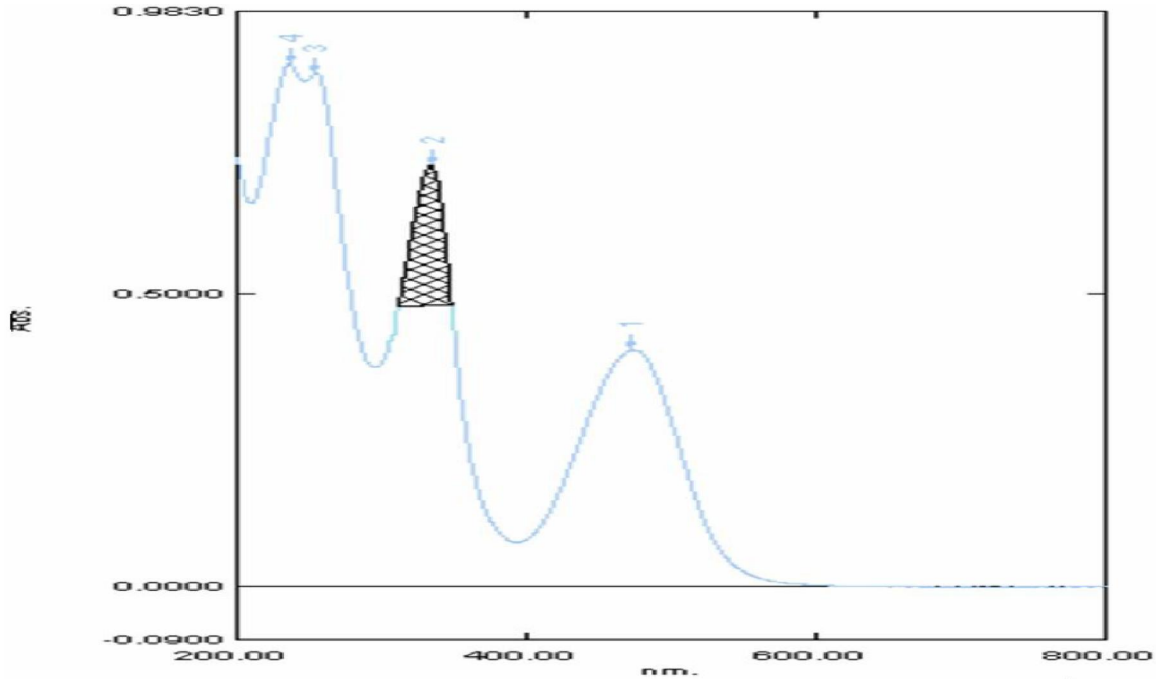

Figure 2 : Area under curve spectrum of rifapentine

Ruggedness

Ruggedness of the proposed method was determined by analyzing aliquots from homogenous slot (8.0µg/mL) in different laboratories by different analysts using similar operational and environmental conditions. The results are reported in terms of percent relative standard deviation.

RESULTS AND DISCUSSION

The molecular structure of the rifapentine is presented in Figure 1 In methanol, Rifapentine showed maximum absorbance at 334 nm. Figure 2 shows the absorption spectrum of rifapentine in Methanol for the method. Optical characteristics of rifapentine were calculated by the proposed methods and presented in TABLE 1.

The intra-day and inter-day precision values (%RSD) were calculated (TABLE 3) and lying in the acceptable limits (d"2%) for rifapentine. The accuracy of rifapentine which was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120% were found to be in the acceptable limits (d"2%) (TABLE 4). This indicates that there was no interference from the excipients present in the dosage form. Ruggedness of proposed method was determined with the help of two different analysts and results were evaluated by calculating the %RSD value and lying within the range (TABLE 5).

TABLE 3: Precision

Conc. µg/ml	Intraday		Interday	
	% Recovery	% RSD	% Recovery	% RSD
8	99.23	0.59	100.60	0.68
12	100.45	1.35	100.48	0.67
16	101.33	0.68	100.02	0.92

TABLE 4: Accuracy

Nominal Value %	Initial amt	Added amt	% recovery	%RSD
80	8	6.4	99.89	1.26
100	8	8	101.12	1.27
120	8	9.6	99.32	0.86

TABLE 5: Ruggedness

Analyst	Amount found of Rifapentine [%]	%RSD [n]
I	98.78	0.56
II	99.27	1.19

n= no. of estimations

CONCLUSION

Method that was developed for the determination of Rifapentine based on different analytical techniques, UV-Spectrophotometric, AUC method. The method was validated and found to be simple, sensitive, accurate, and precise. Hence, the method can be used successfully for routine analysis of pharmaceutical dosage form of Rifapentine. The proposed Spectrophotometric method will not replace the presently known methods available for the analysis of Rifapentine. However, it can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis.

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