Original Article

Development and Validation of UV/Visible Spectrophotometric Method for the Estimation of Rifapentine in Bulk and Pharmaceutical Formulations

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ABSTRACT

A simple, accurate, precise and sensitive UV/visible spectrophotometric method were developed for the determination of Rifapentine in bulk and pharmaceutical dosage form. The solvent used was methanol and the wavelength corresponding to maximum absorbance of the drug was found at 478nm. Drug obey's Beers law in the concentration range of 10- 60ug/ml with correlation coefficient 0.9997. The linear regression equation obtained by least square regression method was y=0.0107x-0.0333, where y is the absorbance and x is the concentration of the pure drug solution. The method was validated for several parameters like accuracy, precision as per ICH guidelines. The values of relative standard deviation and % recovery were found to be satisfactory, indicating that the proposed method is precise and accurate and hence can be used for the routine analysis of Rifapentine in bulk and pharmaceutical formulation.

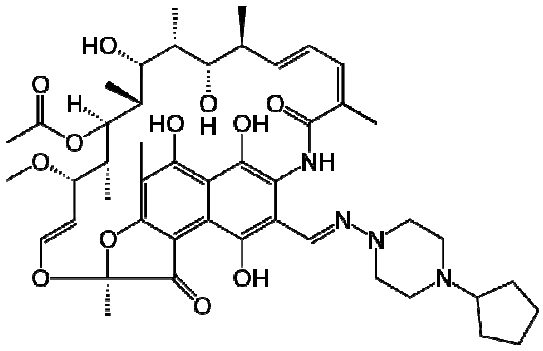
Keywords: Rifapentine, Spectrophotometer, Methanol, Method validation.

INTRODUCTION

Rifapentine is chemically (7S,9E,11S,12R,13S,14R,15R,16R,17S,18S, 19E,21Z)-26-[(E)-N-(4-cyclopentylpiperazi n-1-yl)carboximidoyl]-2,15,17,27,29-penta hydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-6,23-dioxo-8,30-dioxa-24-azatetracyclo[23.3.1.1{4,7}.0{5,28}] triaconta-1,3,5(28),9,19,21,25(29),26-octaen-13-yl acetateor3-[N-(4-Cyclopentyl-1-piperazinyl)formimidoyl] Rifamycinis a piperazinylhydrazone derivative of 3-formyl Rifamycin SV[1]. Rifapentine is a Rifamycin antibiotic and a synthetic derivative of natural products of the bacterium, *Amycolatopsis mediterranei*. The Rifamycinis complex macrocyclic antibiotics that have activity against several bacteria, but most prominently *M. tuberculosis* and several atypical mycobacterial species, probably as a result of inhibition of the DNA-dependent RNA polymerase of mycobacteria. These agents

are considered bactericidal and are active against both intracellular and extracellular organisms. Rifapentine has a longer half-life than rifampin and Rifabutin which allows for once or twice weekly dosing, which is its major advantage. Rifapentine is intermediate between Rifabutin and Rifampin in activity as an inducer of the hepatic microsomal drug-metabolizing P450 enzymes (CYP 1A2, 2C9, 2C19 and 3A4); the relative potencies being: Rifampin (1.0), Rifapentine (0.85) and Rifabutin (0.4). For this reason, use of other medications (such as many antiretroviral agents, oral contraceptives, beta-blockers, benzodiazepines, cyclosporine, macrolide antibiotics and oral anticoagulants) with Rifapentine should be carefully considered and monitored[2-4].

Literature survey reveals that only fewbioanalytical method has been developed[5-7]. The objective of the present work was to develop a simple, sensitive, precise and accurate UV/Visible spectrophotometric method for the determination of Rifapentine in bulk and pharmaceutical formulations.



Rifapentine

MATERIALS AND METHODS

Instrumentation

A] A Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer equipped with Halogen lamp and Deuterium lamp and Silicon photodiode detector with spectral width of 1nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell and.

B] A UV-visible spectrophotometer (Chemitospectroscan UV-2600 Double beam UV-spectrophotometer) with a pair of 1cm matched quartz cell was employed for measuring the absorbance of all the solutions.

Chemicals and reagents

Analytical grade reagent and solvents were used for the study. The pure drug Rifapentine and marketed formulation were obtained as gift sample from Lupin pharmaceutical LTD, Aurangabad.

Preparation of standard stock solutions

Standard stock solutions of Rifapentine were prepared by dissolving accurately weighed 10mg of Rifapentine in 2ml of Methanol in 10ml volumetric flasks. Final volume was made up to 10ml with Methanol to get stock solution containing 1000µg/ml of Rifapentine. Further from 1000µg/ml accurately pipette out 1ml of the stock solution and dilute up to 10ml to get working standard solution containing 100µg/ml of Rifapentine.

Determination of λmax

By appropriate dilution of standard stock solutions of Rifapentine in Methanol, solutions containing 20µg/ml of Rifapentine and was scanned on Chemitospectroscan UV-2600 Double beam UV-spectrophotometer in the range of 400- 800 nm against methanol as blank. Wavelength of maximum absorption was determined for drug. Rifapentine showed maximum absorbance at 478nm (Figure 1).

Preparation of standard calibration curve

From the standard stock solution containing 100µg/ml of Rifapentine serial dilution ranging from 10-60µg/ml were prepared by pipetting out 1. 2, 3, 4, 5 and 6ml of stock solution into 10ml volumetric flask separately and final volume was made up to

10ml with methanol. The absorbance of each solution was measured at 478nm and Calibration curve of the drug was then plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis.Rifapentine followed linearity in the concentration range of 10-60 µg/ml at 478nm. Calibration data of drug at 478 nm is given in (Table 1), whereas the calibration curves are shown in (Figure 2).

Analysis of Marketed Formulation

Assay was performed by using commercial tablets of Rifapentine (Rifapex) containing 150mg of Rifapentine per tablet. The percentage purity of drug was calculated by comparing the absorbance of test solution with standard and the result of assay is the Average of three determinations (Table 2).

Method Validation

The method was validated for several parameters like linearity, accuracy, precision, Ruggedness, Robustness, Limit of detection (LOD), Limit of quantification (LOQ) according to ICH guidelines[8,9].

RESULTS & DISCUSSION

Linearity

The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analysed. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis. The drug showed linearity in the range of 10-60 µg/ml with correlation coefficient 0.9997.

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatability was determined by preparing five replicates of same concentration of the sample and the absorbance was measured.

Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for three different days to determine interday precision.The results were reported as % RSD. The precision result showed a good repeatability with percent relative standard deviation less than 2.

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (50%, 100% and 150%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analyst and also by carrying out the analysis on two different instruments and the respective absorbance was noted and the results was indicated as SD and % RSD.

LOD and LOQ

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOD and LOQ were determined using the following equation.

The detection limit (DL) may be expressed as:

$$LOD = 3.3\ \sigma/S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The quantitation limit (QL) may be expressed as:

LOQ =10 σ/S

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve.

The results obtained from the validation of developed method are summarized in table 3.

CONCLUSION

The linear calibration curve was obtained at concentration range 10-60µg/ml with Correlation Coefficient (0.9997), Slope (0.0107) and Intercept (0.0337). The Limit of detection (LOD) and Limit of quantification (LOQ) found to be 0.347µg/ml and 1.053µg/ml for Rifapentine respectively by the proposed method. The proposed method was reproducible because results obtained with in inter-day and intra-day were in acceptable limit. The results of assay and % recovery were found to be satisfactory, indicating that the proposed method is precise and accurate and hence can be used for the routine analysis of Rifapentine in bulk and pharmaceutical formulation.

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Table 1. Calibration data at 478nm

Sr.No.	Concentration(µg/ml)	Absorbance(n=3)	SD	%RSD
1	10	0.073	0.001	1.3
2	20	0.178	0.00057	0.32
3	30	0.288	0.00057	0.20
4	40	0.395	0.00153	0.38
5	50	0.507	0.00208	0.41
6	60	0.603	0.001	0.16

Table 2: Analysis of marketed formulation

Marketed Formulation	Label Claim. (mg/Tablet)	Percentage Purity (%)	S.D	%R.S.D
Rifapex	150	99.46	0.11	0.1105

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Table 3: Validation parameter

Parameters	Results
λ max (nm)	478nm
Linearity Range (µg/ml)	10-60µg/ml
Slope (m)	0.0107
Intercept (c)	0.0337
Correlation Coefficient	0.9997
Limit of Detection (µg/mL)	0.348µg/ml
Limit of Quantitation (µg/mL)	1.053µg/ml
Precision (%RSD)	
Intra-day precision	0.28
Inter-day precision	0.26
Recovery(%) (n=3)	
50% 100% 150%	99.83% 99.78% 100.27%
Ruggedness	Rugged

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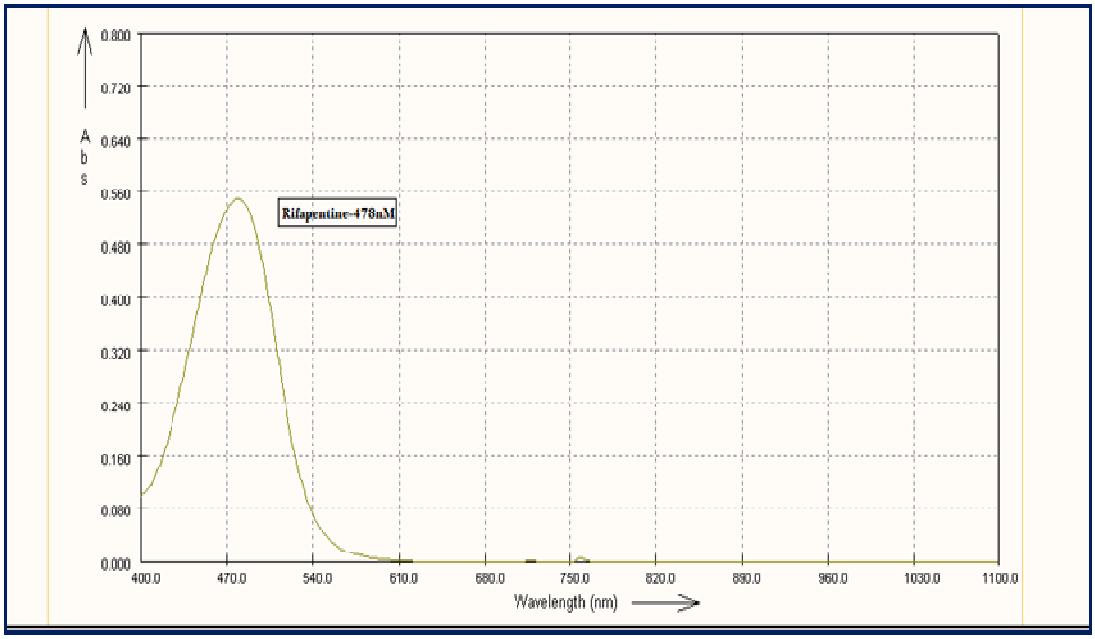

Figure.1. UV/Visible spectrum of drug

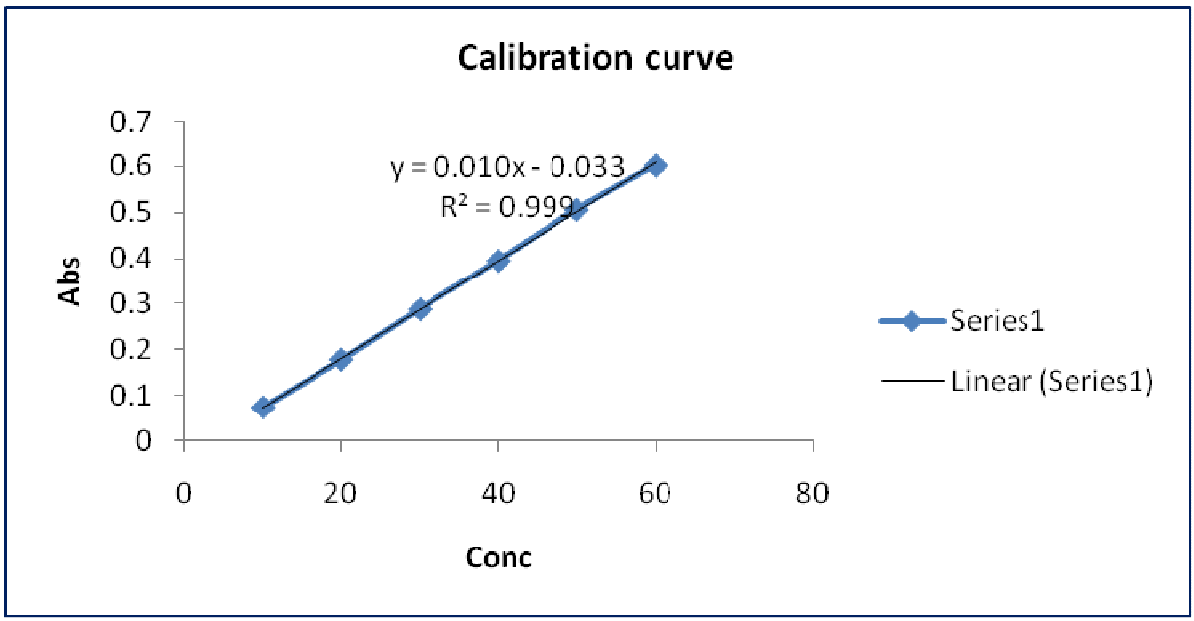

Figure.2. Calibration curve