



Development and Validation of UV-Visible Spectrophotometric Method for Estimation of Rifabutin in Bulk and Formulation

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Abstract

Aim: To develop and validate a simple, precise and cost-effective UV- visible spectrophotometric method for the estimation of Rifabutin according to the ICH Q2 (R1) guideline. **Methods:** Spiked Rifabutin solution was scanned over UV-Visible range for its wavelength of maximum absorbance. Various calibration standards of Rifabutin were prepared and absorbance was recorded at wavelength of maximum absorbance. Calibration curve of concentration vs. absorbance was plotted and linearity and range was calculated. Various analytical method validation parameters viz. accuracy, precision, LOD, LOQ, robustness and ruggedness were calculated using QC standards. In order to check the performance, pre-validated method was used to estimate the Rifabutin content of marketed formulation. **Results:** The wavelength of maximum absorbance for Rifabutin was found to be 237 nm. The correlation coefficient over the concentration range of 1-20 µg/mL was found to be 0.999. Developed UV method was found to be precise during the intra-day and inter-day study and showed percent relative standard deviation in the range of 0.61 to 1.86 & 0.27 to 1.60 respectively. The total percent recovery of Rifabutin was found to be 99.18 to 99.93 %. Developed method was found to be robust and rugged for the intended use. Developed UV-Visible method was successfully used for the estimation of Rifabutin content in marketed formulation of reputed Indian pharmaceutical industry. **Conclusion:** A simple, precise and cost-effective UV- visible spectrometry method for the estimation of Rifabutin was developed. The said method was developed using economical percentage of organic phase in aqueous media as solvent. Said validated UV- visible method can be efficiently used for the estimation of Rifabutin in bulk as well as formulation.

Keywords

Rifabutin, UV- visible spectrometry, Validation

INTRODUCTION

Rifabutin is an approved broad-spectrum antibiotic used for the treatment of tuberculosis [1-2]. It has a molecular formula $C_{46}H_{62}N_4O_{11}$ and its molecular weight is 847.019 g/mol [3-4]. Rifabutin

(Figure 1) is a drug of choice in case of patients who cannot tolerate Rifampicin [5-6]. Rifabutin has fewer drug interactions which makes it preferred anti-TB drug for the patients with HIV infection [7-8].

In spite of therapeutic vitality and extensive utility of Rifabutin, there are limited scientific reports that demonstrate the development and validation of analytical methods for its estimation in bulk and formulation. Therefore, considering the therapeutic importance of the Rifabutin and the need of simple yet precise and robust analytical method for the

same, it was envisaged that development of UV-Visible spectrophotometric method for the determination of Rifabutin in bulk and the formulation by using co-solvent system consisting economic percentage of organic solvent will be worth.

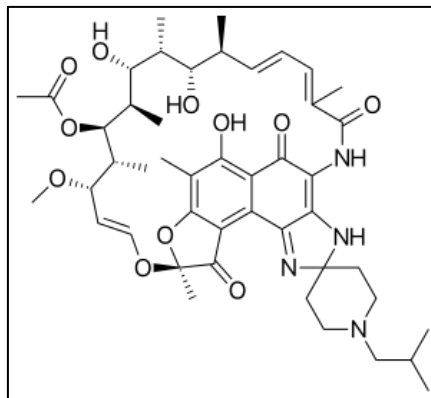


Fig. 1: Chemical structure of Rifabutin

EXPERIMENTAL

Instruments and reagents

A double beam UV-Visible spectrometer (UV-530, Jasco) with spectra manager software were used for the analysis. Quartz cells having 3 cm length with 1 cm path length were used for spectral measurement. Weighing balance (Essae, Vibra HT) with internal calibration mode was used for the accurate weighing purpose. Rifabutin was obtained as gift sample from LUPIN Pharma Ltd, Aurangabad. Methanol was purchase from Merck. All the chemicals of analytical grade were used for the proposed study.

Preparation of working standard drug solution

The standard Rifabutin (5mg) was accurately weighed and transferred into the (5ml) Volumetric Flask and dissolved properly and diluted up to the mark with the mixture of Water: Methanol (40:60 v/v) to achieve a final concentration of 1000 µg/mL (S-1). Further S-1 was properly diluted using mobile phase to achieve a 100 µg/mL (S-2) solution.

Determination of wavelength of maximum absorbance (λ_{max})

The S-2 was scanned utilizing full output mode with medium scanning speed for a whole range of UV/VIS Spectrophotometer, the ranging from 800-200 nm with a co-solvent system as a blank. After acquiring the spectrum, λ_{max} was identified. The above method was repeated thrice.

Preparation of calibration curve

The Calibration curve was prepared by using S-2 to accomplish the seven-diverse calibration standard representing 1, 2, 4, 8, 12, 16, 20 µg/mL strength. An

absorbance of every calibration standard was estimated at λ_{max} 237nm using fixed wavelength measurement mode. The calibration curves representing concentration vs. absorbance was plotted utilizing in Microsoft Excel 2016. Previously mentioned technique was rehashed multiple time with the goal that reproducible outcomes can be obtained

Method Validation

Developed UV method for the estimation of Rifabutin was validated in terms of parameters like linearity, range, precision, robustness, ruggedness, accuracy, limit of quantification (LOQ) and limit of detection (LOD) using predefined calibration standards as portrayed below [9-10].

Linearity and range

Linearity of the proposed UV method was established using seven different calibration standards. Based on analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy

The accuracy of the proposed UV method was evaluated using recovery studies after standard addition of analyte of interest. Three different solutions of Rifabutin were prepared in triplicate at

level of 80%, 100% and 120% of its predefined concentration (1, 10, 20 µg/mL). In predefined concentrations, different amount of rifabutin was included (standard addition method) and accuracy was determined based on percent recovery. For calculating the percent recovery, following equation was utilized

$$\%RC = \frac{(SPS - S/SP) \times 100}{S}$$

Where,

% RC = Percent recovery

SPS = Amount found in the spiked sample

SP = Amount added to the sample

S = Amount found in the sample

Intra-day precision and Inter-day precision

Precision of the assay method was assessed in terms of repeatability by carrying out seven independent assays of rifabutin test arrangement and the % RSD of measurement (intra-day). Intermediate precision of the method was checked by performing same methodology on three consecutive days.

Robustness

Robustness the developed UV method was obtained utilizing different percentage of methanol in a co-solvent system. Methanol percentage in a co-solvent system was purposefully changed 45 and 55%. Rifabutin (4 µg/mL) was prepared utilizing above mentioned co-solvent system independently, (n=5) and sample was analyzed at λ_{max} 237 nm for Rifabutin content. The result was determined in terms of % RSD.

Ruggedness

Ruggedness, the UV/VIS method was carried out by analyzing triplicate sample of Rifabutin utilizing two distinct instruments (BA-UV-2600, Bioage and V-530, Jasco). The result was depicted in terms of % RSD.

Limit of Quantification (LOQ)

In UV method development LOQ was determined by utilizing the following equation.

$$LOQ = 10 \times SD/S$$

Where, S= slope

SD= Standard deviation of Y-intercepts

Limit of Detection (LOD)

In UV method development LOD was determined by utilizing the following equation

$$LOD = 3.3 \times SD/S$$

Where, SD= Standard deviation of Y-intercepts

S= Slope

Estimation of Rifabutin content in marketed formulation

Developed and pre-validated UV-Visible method was successfully used for estimation of Rifabutin content in marketed formulation. For the study, Ributin capsule were purchased from local market of Aurangabad and contents of capsule were collected and suitable dilution were made using pre-optimized co-solvent system. Prepared samples were analyzed using pre-validated UV-method & results were reported in terms of average percent assay.

RESULTS AND DISCUSSION

Method development and optimization

Identification of wavelength of maximum absorbance is prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for Rifabutin solution (10 µg/mL) was carried out using full scan mode of UV-Visible spectrophotometer (Figure 2). Full scan was processed using UV software and the λ_{max} was identified with the help of software. It was found to be 237 nm for Rifabutin.

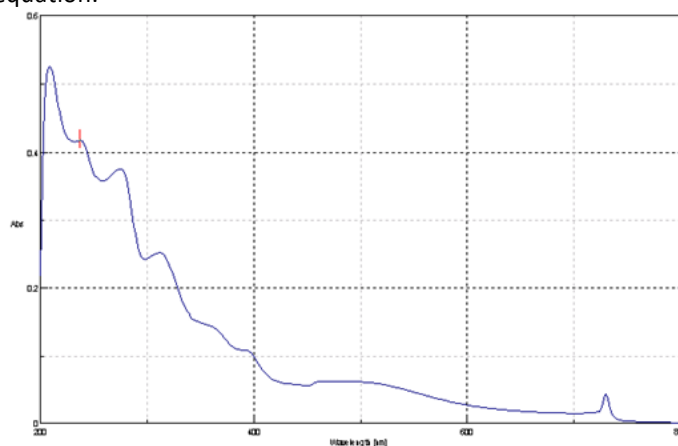


Fig. 2: UV-Visible spectra of Rifabutin

Preparation of calibration curve

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and an equation stating correlation between concentration and the response. As compare to graphical method, above stated method is widely accepted and reproducible in nature. Considering the utility of quantitative analysis of Rifabutin, calibration curve for Rifabutin was developed using seven different calibration standards. The absorbance of different calibration standards at 237 nm was recorded using fixed wavelength mode of UV-Visible spectrophotometer. Calibration curve was repeated three times and reported as shown in Table 1.

Method Validation

Linearity and Range

Proposed method is to be used for its optimum performance. Considering the importance of linearity and the range, seven-point calibration curve of Rifabutin covering a range of 1-20 µg/ml was developed. Details of concentrations and the respective mean absorbance values are depicted in Table 1. Calibration curve when subjected to least square regression analysis yielded an equation; $y = 0.0503x + 0.0031$ with correlation coefficient 0.999 as shown in Figure 3. From the linearity study, it was revealed that, developed UV method was linear in the pre-defined concentration range of calibration standards.

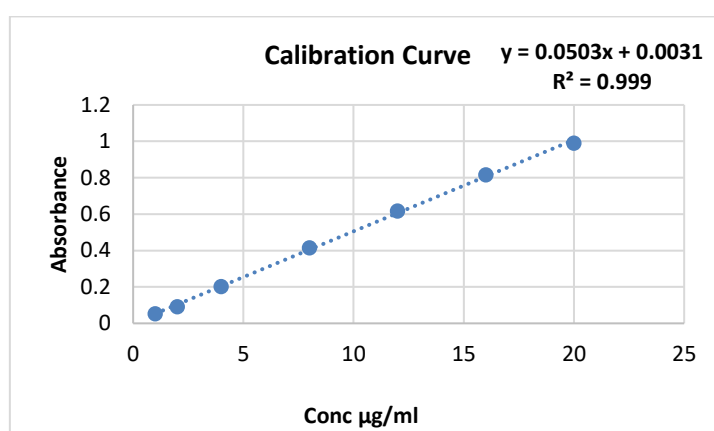


Fig. 3: Calibration curve for Rifabutin

Table1: Results of calibration curve at 237 nm

Standard	Conc (µg/ml)	Absorbance ±
CAL STD-1	1	0.0521 ±0.0021
CAL STD-2	2	0.0924 ±0.0012
CAL STD-3	4	0.2017 ±0.0035
CAL STD-4	8	0.4161 ±0.0042
CAL STD-5	12	0.6177 ±0.0018
CAL STD-6	16	0.8169 ±0.0058
CAL STD-7	20	0.9906 ±0.0042

Accuracy

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. In case of UV method for Rifabutin, accuracy was established using recovery studies. At 80 % standard addition, mean recovery of

Rifabutin was found to be 99.93% whereas at 100 and 120 % standard addition, it was found to be 99.45 and 99.18% respectively. % RSD was found to be less than 2 for the Rifabutin recovery studies as shown in Table 2. From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 99.18 to 99.93% and the % RSD was well below 2%.

Table No 2: Accuracy data of UV method for Rifabutin

Sr No.	Concentration (%)	Original level (µg/mL)	Amount added (µg/mL)	% Recovery	Mean % Recovery	% RSD
1	80	1	0.8	100.81	99.93	1.32
2	80	1	0.8	100.58		
3	80	1	0.8	98.40		
4	100	10	10	99.97	99.45	0.46
5	100	10	10	99.11		
6	100	10	10	99.25		
7	120	20	24	100.02	99.18	0.72
8	120	20	24	98.73		
9	120	20	24	98.79		

Precision

Precision is a measure of degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of developed UV method was established at 1, 10 and

20µg/ml levels of Rifabutin. The results in terms of mean absorbance values, percent assay and % RSD for the intra- and inter-day precision study are demonstrated in Table 3 and Table 4 respectively. % RSD values of intra-day precision study were found to be in between 0.61 and 1.86 whereas those of inter-day precision study were in between 0.27 and 1.60. Overall, % RSD values of less than 2 showed the precision of developed UV method.

Table No 3: Intra-day precision data of UV method for Rifabutin

S No.	Conc. (µg/mL)	Morning			Afternoon			Evening		
		Mean	% Assay	%RSD	Mean	% Assay	%RSD	Mean	% Assay	%RSD
1	1	0.0500	100.40	1.55	0.0504	101.22	1.48	0.0496	99.67	1.86
2	10	0.5016	100.73	0.93	0.4906	98.51	1.51	0.4956	99.51	1.69
3	20	0.9841	98.81	1.04	0.9782	98.21	0.61	0.9780	98.19	0.62

Table No 4: Inter-day precision data of UV method for Rifabutin

S No.	Conc. (µg/mL)	Day 1			Day 2			Day 3		
		Mean	% Assay	%RSD	Mean	% Assay	%RSD	Mean	% Assay	%RSD
1	1	0.0500	100.43	0.92	0.0498	100.17	1.48	0.0500	100.45	1.24
2	10	0.4959	99.58	0.38	0.4910	98.60	1.60	0.4913	98.65	0.99
3	20	0.9801	98.40	0.27	0.9807	98.46	0.65	0.9815	98.54	0.55

Robustness

Robustness of analytical method is the ability of a method to resist the change in its performance in spite of small, deliberate change in method parameters. It is an important parameter of analytical method as a small, un-intentional change in method parameters like solvent composition; pH etc. may occur during routine use and may hamper the performance of said method. It is expected that such change should not alter the performance of the

analytical method. Therefore, robust analytical method is preferred. Robustness of proposed UV method was established by modifying the composition of co-solvent system. Change in water and methanol percentage (55 to 65 %) in co-solvent system did not affect the method performance. % RSD values were found to be in between 0.93 and 0.99 as shown in Table 5. % RSD values below 2 showed that proposed UV method is robust in nature.

Table 5: Robustness data of UV method for Rifabutin

Sr. No.	Concentration (µg/mL)	% Methanol	Absorbance	% RSD
1	4	55	0.2235	0.9873
2	4	65	0.2118	0.9314

Ruggedness

Ruggedness of analytical method is the ability of a method to resist the change in its performance in spite of influential environmental factors like temperature. Rugged analytical methods are preferred as these methods are free from impact of environmental factors. In order to establish the

ruggedness of proposed UV method, Rifabutin solution was analyzed at three different temperature conditions. Sample analysis and data processing resulted into % RSD values between 0.91 and 0.98. Results revealed that proposed UV method was rugged as it showed % RSD values less than 2 as shown in Table 6.

Table 6: Ruggedness data of UV method for Rifabutin

S. No.	Concentration (µg/mL)	Instrument Model & Make	Absorbance	% RSD
1	4	V-530, Jasco	0.2001	0.9870
2	4	BA-UV-2600, Bio-age	0.1971	0.9148

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

LOQ represents the lowermost concentration that can be analyzed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard. LOD and LOQ of proposed UV method was

found to be 0.815 and 2.47µg/ml respectively as shown in Table 7. Lower LOQ value indicated that proposed method would be suitable for analyzing the samples containing even small quantities of Rifabutin.

Table No 7: LOD & LOQ data for UV method for Rifabutin

1	LOD	0.815 µg/mL
2	LOQ	2.47 µg/mL

Estimation of Rifabutin

The developed UV method was successfully applied for the estimation of Rifabutin content in Ributin150mg USP. Average percent assay of Rifabutin capsule was found to be 100.01 %.

CONCLUSION

A simple, accurate and precise UV-Visible spectrophotometric method for the estimation of Rifabutin was developed and validated. The Proposed method was found to be robust and rugged in nature and was successfully used for the estimation of Rifabutin.

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