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METHOD DEVELOPMENT AND VALIDATION OF RIFAMPICIN BULK AND MARKETED CAPSULE BY SIMPLE UV SPECTROPHOTOMETRIC ANALYSIS

B. Ravi Shankar[*1], R. Rajesh[1], K. Ramya[1]

[1*]Department of Pharmaceutical Analysis, Acharya and BM Reddy College of Pharmacy, Bengaluru, Karnataka, India.

ABSTRACT

A simple, specific, accurate, precise and reproducible method has been developed and validated for the determination of Rifampicin in bulk and capsule by UV spectrophotometric method. This includes the detection of wave length for bulk and marketed at 337 nm. Rifampicin follows the beers law over the concentration range of 5-13µg/ml. The percentage recoveries for both the bulk and marketed capsule were found to be nearly 98-100% representing the accuracy of the proposed methods. Validation of the proposed methods was carried out for its accuracy, precision, and specificity according to ICH guidelines. The proposed and developed method can be successfully applied in routine laboratory analysis for the determination of Rifampicin individually.

KEYWORDS

Rifampicin, UV spectroscopy and Validation.

Author for Correspondence:

Ravi Shankar B,

Department of Pharmaceutical Analysis,

Acharya and BM Reddy College of Pharmacy,

Soldevanahalli, Hesaraghatta,

Bengaluru, Karnataka, India.

Email: bravishankar91@gmail.com

INTRODUCTION

Rifampicin is an antibiotic also known as 'rifampin' used to treat a bacterial infections[1]. It was first sold in 1971 and isolated in 1957[2]. Rifampicin is a generic medicine obtained from *Amycolatopsis rifamycinica*[3]. It is a basic health medicine as listed in the who list of Essential Medicines. When rifampin is used in combination with pyrazinamide and other 1st line drugs (MDT) tuberculosis treatment duration can be reduced to six months[4]**.** Chemically it is (12Z, 14E, 24E)- (2S, 16S, 17S, 18R, 19R, 20R, 21S, 22R, 23S)-1,2 -dihydro- 5, 6, 9, 17, 19- pentahydroxy, 23 -methoxy- 2, 4, 12, 16, 18, 20, 22 heptamethyl -8- (4-methylpiperazin-1yliminomethyl) -1, 11-dioxo 2, 7 (epoxypentadeca-

1, 11, 13 trienimino) napthol [2,1-b] furan -21-yl acetate[5,6]. Literature survey revealed many UV spectrophotometric studies for determination of rifampicin in combination with other drugs and in biological fluids[7,10], but no validated UV spectrophotometric method was reported for the estimation of rifampicin alone. Thus an attempt is made to develop a simple, precise and accurate validated method by UV spectrophotometer for the determination of rifampicin in bulk and marketed dosage form.

MATERIALS AND METHODS

Materials

Rifampicin was obtained as gift sample by Lupin ltd, Ahmedabad, India. The pharmaceutical marketed formulation R-cin capsule manufactured by Lupin Ltd, Ahmedabad, India was procured from local market. Dilutions are made with methanol of AR grade and were purchased from Karnataka fine chemicals, Bengaluru, India.

Selection of common solvents

Methanol of analytical reagent was selected as common solvent for developing spectra of the drug. The selection was made after performing solubility of the drug in different solvents.

Preparation of standard stock solution

Weight accurately about 10mg of rifampicin was transferred into a 10 ml volumetric flask, dissolved with sufficient volume of methanol. The volume was made up to 10 ml with a methanol to get a concentration 1000 µg/ml (Stock I). From the stock solution 1ml was further diluted to 10 ml with a methanol to get a concentration 100 µg/ml (Stock II). Further dilution is done by taking 1ml of stock II and making up the volume to 10 ml (10 µg/ml).

Preparation of sample solution

Ten capsules (R-cin-150mg) were weighed and powdered. Capsule powder equivalent to 10mg is transferred into a 10 ml volumetric flask, dissolved with methanol. The volume was made up to 10 ml with a methanol to get a concentration 1000 µg/ml (Stock I). From the stock solution 1ml was further diluted in a 10 ml volumetric flask with a methanol to get a concentration 100 µg/ml. (Stock II). Further dilution is done by taking 1ml of stock II and making up the volume to 10 ml (10 µg/ml).

Selection of Wavelength λ_{max}

The wavelength of rifampicin was selected based on the maximum absorbance of the drug at particular wavelength using stock solution of 10 µg/ml concentration. Then scanned using the wavelength range from 200 nm to 800 nm. Rifampicin showed absorbance maxima at 337nm.

Assay of Capsule formulation

The average weight of 10 capsules with weight equivalent to 10mg was transferred to 10 ml of volumetric flask. The contents were dissolved by diluting with 10 ml of methanol. The solution was further diluted to 100 ml to give concentration of 10 µg/ml of rifampicin. The sample is now scanned in the range of 200-800 nm and the absorbance was measured at 337 nm. The results so obtained were calculated for the percentage purity and are shown in the Table No.1.

METHOD VALIDATION

The method is developed and validated as per ICH guidelines[11] for validation of analytical procedures to determine the linearity, precision, accuracy, LOD and LOQ, robustness for the analyte.

Linearity

The measurement of linearity is measured by evaluating different concentrations of the standard solutions of rifampicin. The above method is verified for the beers range for the results obtained within the concentration range of 5-13 µg/ml. as shown in the Figure No.4.

Sensitivity

The LOD (limit of detection) and LOQ (limit of quantification) were calculated for the rifampicin by using the following equation LOD=3.3σ /S, LOQ=10 σ /S, where σ is the standard deviation of y intercept for the calibration (n=5) and S is the slope of regression coefficient.

Precision

The precision for the proposed developed method was performed by carrying out the analysis of the five analytes (n=5) taken. The low value of relative standard deviation showed that the method is precise.

Intra-day precision

It was done by taking the solutions of same analyte three times within a day at intervals of 1hr. The percentage RSD is shown in the Table No.2.

Inter-day precision

It was done by taking the solutions of same analyte on alternate days till 3rd day. The percentage RSD is shown in the Table No.3.

Accuracy

To measure the accuracy of the developed method recovery studies have been performed by using standard addition method at 80, 100 and 120% levels. The percentage recovery was calculated from the total amount of the drug found.

RESULTS AND DISCUSSION

The proposed method for the estimation of rifampicin in the marketed formulation was found to be simple, precise, and reproducible. Beers law was obeyed for the concentration range of 5-13 µg/ml. The correlation coefficient showed good linear relationship for the rifampicin with R^2 value 0.9967. The assay results for the capsule by proposed method were very close with the label claim with low relative standard deviation suggesting that the method developed is highly precise. In order to check the accuracy of developed method known quantity of the standard drug rifampicin is added in three different levels mentioned to its pre-determined capsule sample and analyzed by the developed methods. The mean percentage recoveries were found in the range of 98-100% which showed no interference of the excipients. From the capsule formation. The results of optimized validated parameters such as beers law, correlation coefficient, slope, intercept, LOD and LOQ values were summarized in the Table No.4.

Table No.1: Assay results of capsule formulation

S.No	Formulation	Drug	Label claim mg/cap	Amount found mg/cap	% Label claim
1	R-cin	Rifampicin	150	151.305	100.87

Table No.2: Results of sensitivity of the method

S.No	Name of the drug	LOD (µg/ml)	LOQ (µg/ml)
1	Rifampicin	1.653	5.007

Table No.3: Results for precision of the method

S.No	Concentration (µg/ml)	Intra-day	Inter-day
1	13	0.186	0.395

Table No.4: Results for accuracy of the method

S.No	% Amount added levels	Label claim (mg)	% Recovery	% Mean recovery
1	80	150	98.88	98.97
2	100	150	98.50	
3	120	150	99.54	

Table No.5: Optimized parameters of validation for the proposed method

S.No	Parameter	Results
1	λ max (nm)	337
2	Beers law limit (µg/ml)	5-13
3	Correlation coefficient (r^2)	0.9967
4	Regression equation (y = a + bC) Slope (b) Intercept (a)	y = 0.0304x + 0.0049 0.0049 0.0304
5	Inter-day Precision (% RSD)	0.186
6	Intra-day Precision (% RSD)	0.395
7	Limit of Detection (LOD) (µg/ml)	1.653
8	Limit of Quantification (LOQ) (µg/ml)	5.007

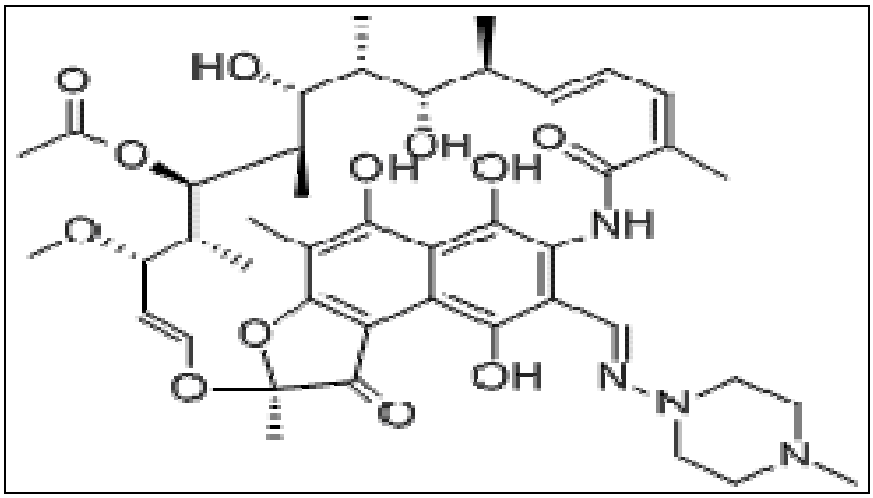

Figure No.1: Structure of Rifampicin

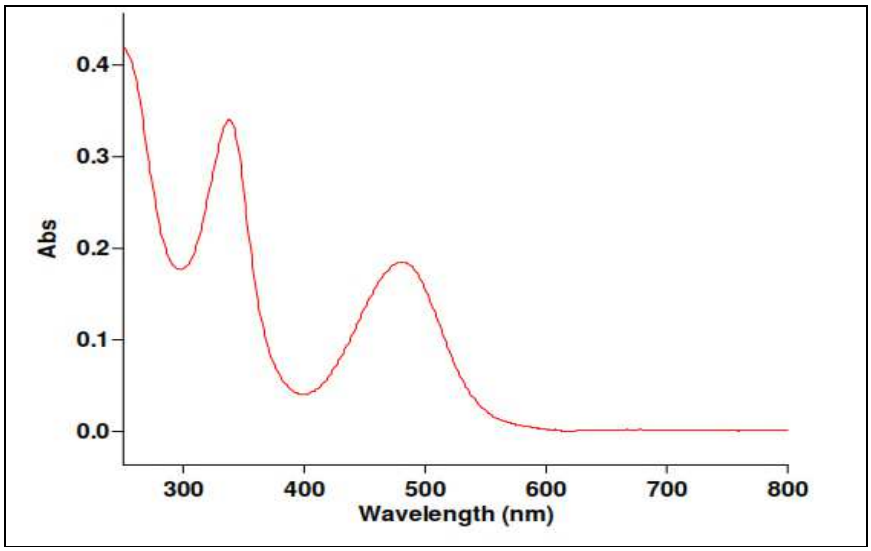

Figure No.2: Absorption spectrum of rifampicin in methanol (10 µg/ml) showing λ_{max} 337 nm

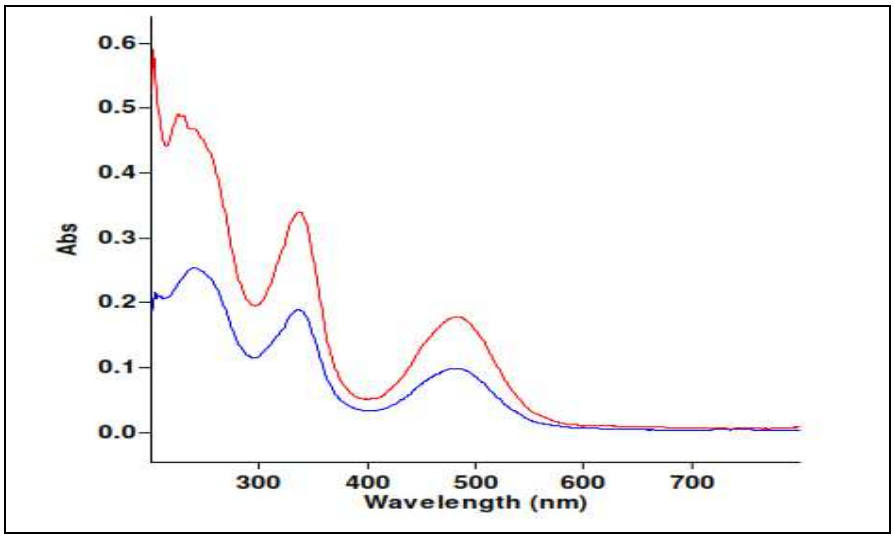

Figure No.3: Absorption spectrum of rifampicin bulk and capsule in methanol (10 µg/ml) showing λ_{max} 337 nm

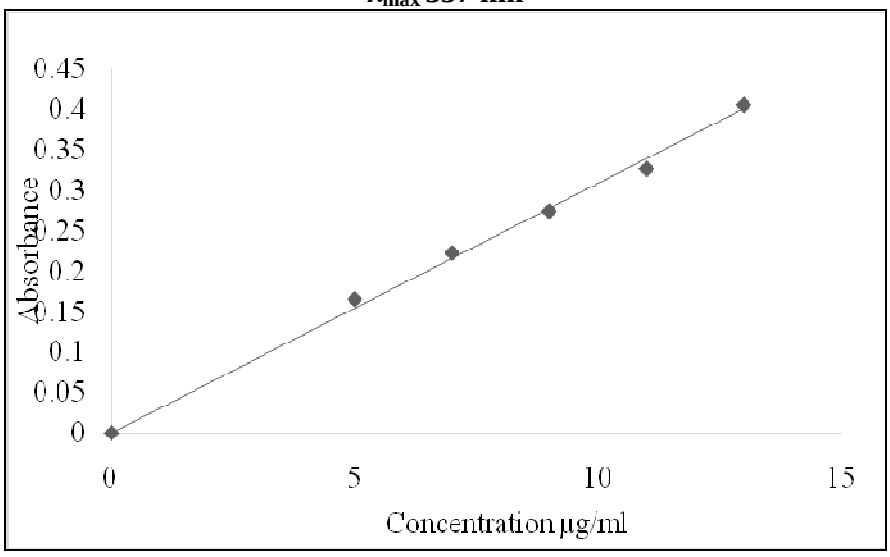

Figure No.4: Calibration plot for rifampicin in methanol (5-13 µg/ml)

CONCLUSION

A simple, precise, accurate method was developed to make the analytical method economical and make it acceptable for performing the routine laboratory quality control analysis. The developed method is validated as per ICH guidelines.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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