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Development and validation of UV-visible spectrophotometric method for estimation of rifapentine in bulk and dosage form

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ABSTRACT

The aim of present work is to develop and validate simple, sensitive and specific spectrophotometric method for the determination of rifapentin, an anti-tubercular drug in pure form and in pharmaceutical formulations. UV-VISIBLE spectrophotometric method, which is based on measurement of absorption at maximum wavelength in 0.1N HCl, was found to be at 478 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. The optimum conditions for analysis of the drug were established. The drug obeyed the Beer's law and showed good correlation. Beer's law was obeyed in concentration range 5-50 µg/ml having line equation y = 0.004x + 0.024 with correlation coefficient of 0.999. The results of analysis were validated by recovery studies. The method was found to be simple, accurate, precise, economical and robust.

Keywords: Accuracy, Rifapentin**,** Recovery, UV-VISIBLE spectrophotometric method.

INTRODUCTION

Rifapentine is a semisynthetic rifamycin derivative from the piperazinyl hydrazone class with a microbiologic profile similar to that of rifampin.[1] Its structure differs from that of rifampin by the presence of a cyclopentyl ring instead of a methyl group at the piperazinyl moiety.[2] The chemical formula of rifapentine is rifamycin, 3-[[(4-cyclopentyl- 1-piperazinyl)imino] methyl]. Its molecular formula is $C_{47}H_{64}N_4O_{12}$ and its molecular weight is approximately 877 Da. [3]

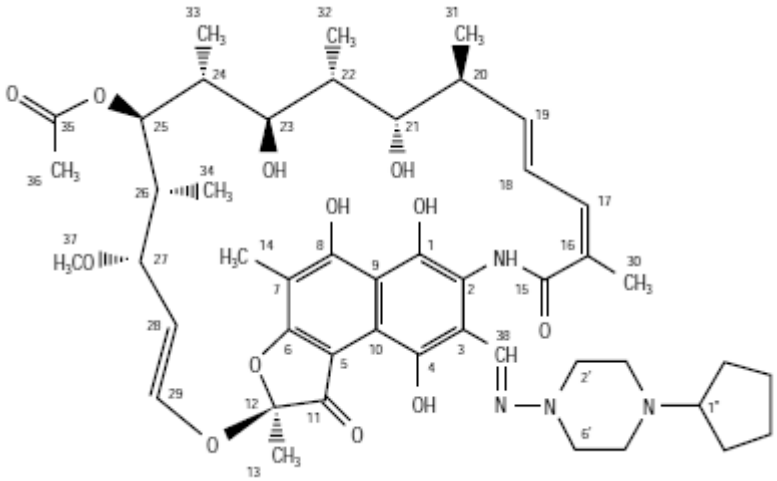

Figure I: Chemical structure of Rifapentin [4]

Rifapentine was first synthesized in 1965 by the same company that produced rifampin. The drug was approved by the Food and Drug Administration (FDA) in June 1998. It is synthesized in one step from rifampicine.[5]

Rifapentine is approved for the treatment of tuberculosis in the US. The drug is also used in China. Rifapentine has a long half-life which allows for once-weekly administration. When administered twice weekly during the intensive phase and once weekly during the continuation phase, rifapentine has demonstrated efficacy in the treatment of pulmonary tuberculosis in immunocompetent patients. [6, 7]

A suitable and validated method is to be available for the analysis of drug in the bulk, dosage forms and in biological samples. If a suitable method, for specific need, is not available then it becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples.

The present study was undertaken to develop and validate a simple, sensitive, accurate, precise, and reproducible UV spectrophotometric method for determination of Rifapentine.

MATERIALS AND METHODS

Apparatus:
Chemito UV 2600 spectrophotometer with 1 cm matched quartz cells were used for the absorbance measurements connected to computer and loaded with UV Probe software. Ohaus weighing balance and bath sonicator, borosil glass apparatus were used for experimental purpose.

Chemicals and reagents:
Rifapentine pure drug and Tablet RIFAPEX was obtained as a gift sample from Lupin Pharmaceuticals Ltd. Aurangabad. HCL was purchased from S.D. Fine (P) Ltd. Mumbai. All chemicals and reagents used were of analytical grade.

Preparation of standard and test solutions
Rifapentine standard stock solution:
10 mg Rifapentine was accurately weighed and dissolved in 10 ml 0.1N HCL then transferred to a 100 ml volumetric flask sonicate it for 5 min, finally volume was made up to the mark with 0.1N HCL to make 100µg/ml stock solution.

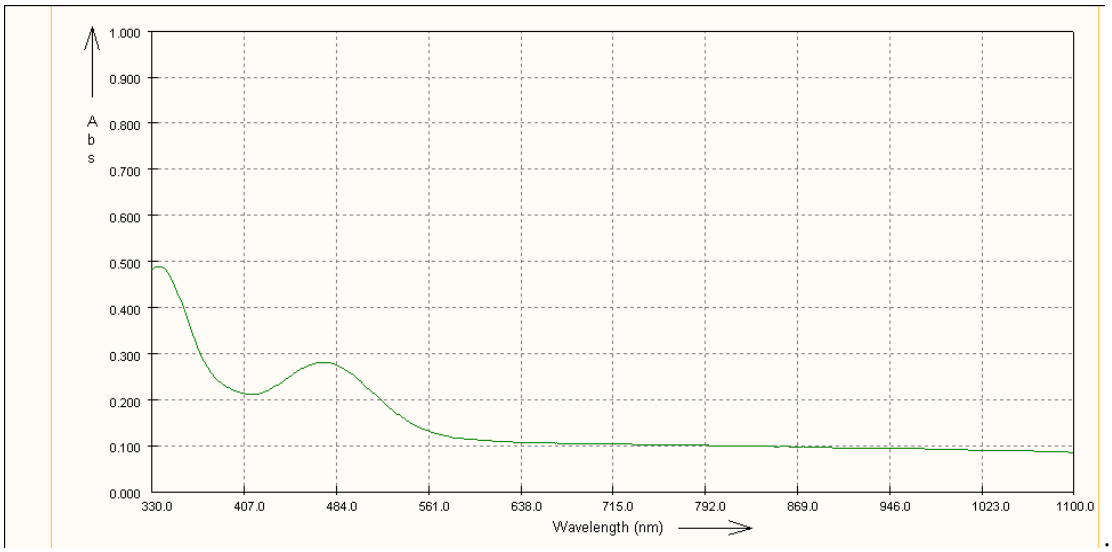

Figure II: Absorption spectrum of Rifapentine showing maximum absorbance at 478nm

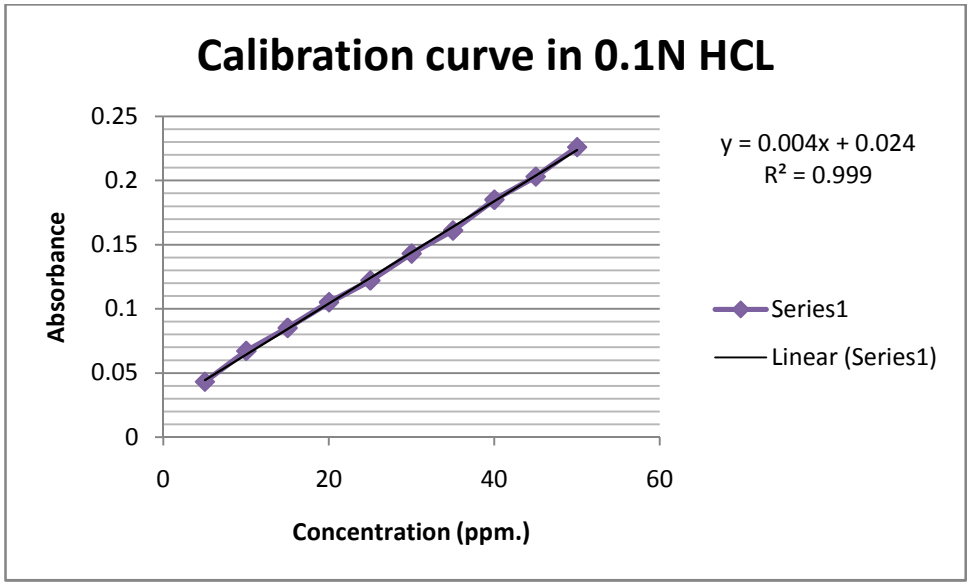

Figure III: Standard calibration curve for analysis of Rifapentine at 478 nm

Procedure for calibration curve:-
The standard solutions were prepared by the proper dilution of the primary stock solution with 0.1N HCL to obtain working standards. All the measurements were performed at room temperature. The absorbance of the solutions containing Rifapentine was determined in the UV-VISIBLE range 200-800 nm using an appropriate blank. The λmax was found to be 478 nm. The spectrum of Rifapentine was as shown in figure II. For linearity study, dilutions were made for Rifapentine in the range of 5 to 50 µg/ml concentrations were prepared by diluting the stock solution with 0.1N HCL. The calibration curve was established at this wavelength by plotting graph between absorbance and concentration. The standard calibration was as shown in figure III.

Preparation of sample solution:
The proposed method was successfully applied for the determination of Rifapentine in tablet dosage form.

Ten tablets were weighed and powdered. The amounts of tablet powder equivalent to 15 mg of Rifapentine was weighed accurately and transferred to 5 ml 0.1N HCL and kept for 5 min in sonicator and volume was made up to mark with 0.1N HCL in 100ml Volumetric flask. The solution was then filtered through Whatmann filter paper # 41. This filtrate was diluted suitably with 0.1N HCL to get the solution of 15 µg/ml concentration. The absorbance was measured against blank. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is given in Table I.

Table I: Determinations of Active Ingredients in Tablets

Sample	Label claimed (mg)	Amount found(mg) per tablet	% label claim *
Rifapentine	150	149.51±0.089	99.71±0.599

(Average of Three Determinations)*

Validation of method parameters
Precision:
Assay of method precision (intra-day precision) was evaluated by carrying out three independent assays of test samples of Rifapentine. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, systems and different days in the same laboratory.

Linearity:
The aliquots of concentration ranging 2-80 µg/ml were prepared in triplicate, but linearity was found to be between 5-50 µg/ml concentrations. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis.

Accuracy (recovery test) [8]**:**
The accuracy of the method is the closeness of the measured value to the true value for the sample. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed by preparing of concentration 15 µg/ml of Rifapentine standard solution.

Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The % recovery of the added pure drug was calculated as % recovery = [(*Ct–Cs*)/*Ca*] x 100, where *Ct* is the total drug concentration measured after standard addition; *Cs*, drug concentration in the formulation sample; *Ca*, drug concentration added to formulation. The results were as shown in Table II.

Table II - Results of Recovery

Sample	Amount added µg/ml	Amount added % µg/ml	% Recovery ± SD
Rifapex	12	11.93±0.03	99.42±0.25
Rifapex	15	15.02±0.045	100.1±0.3
Rifapex	18	17.96±0.142	99.78±0.78

Limit of detection (LOD) and limit of quantification (LOQ):
The LOD and LOQ of Rifapentine were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. LOD and LOQ values were calculated using the relation,

LOD=3.3δ /S
LOQ=10 δ /S

Where, δ= standard deviation of residuals from the curve; S=slope of the curve

Table III - Regression and validation parameters of Rifapentine

Sr. No.	Parameter	Result
Regression Parameters		
1	Slope	**0.004**
2	Intercept	**0.024**
3	Standard Regression Equation	**y = 0.004x + 0.024**
4	Correlation Coefficient (R^2)	**0.999**
5	Residual standard deviation	**0.003985**
Validation Parameters		
1	Absorption maxima(nm)	**478**
2	Molar absorptivity	**3964.912**
3	A(1%, 1 cm)	**43.6**
4	LOD (µg/ml)	**3.28**
5	LOQ (µg/ml)	**9.96**
6	Linearity range (µg/ml)	**5-50**
7	Accuracy(% Recovery ±SD)	**99.77±0.34**

RESULTS AND DISCUSSION

The development of a simple, rapid, sensitive, and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations, the cost of materials and labor. Rifapentine is a UV-VISIBLE absorbing molecule with specific chromospheres in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV-VISIBLE spectrophotometric method. The absorption spectrum of Rifapentine in 0.1N HCL was shown in Figure II. Calibration curve data was constructed in the range of the concentrations of 2-80µg/ml, but Beer's law obeyed in concentration range of 5-50 µg/ml. The regression equation was found to be y = 0.004x + 0.024. The correlation coefficient (r^2) of the standard curve was found to be greater than 0.999. The stock solutions and working standards were made in 0.1N HCL. The λmax of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV-VISIBLE region. Performing replicate analyses of the standard solutions was used to assess the accuracy, precision, and reproducibility of the proposed method. The selected concentration within the calibration range was prepared in 0.1N HCL and analyzed with the relevant calibration curve to determine the intra and inter day variability. The proposed method can be successfully applied for assay in tablet dosage forms without any interference. The assay showed that the drug content of this product to be in accordance with the labeled claim (Table I). The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method (Table II). The obtained results demonstrate the validity and accuracy of the proposed method for the determination of drug in tablet (Table III). In order to check the accuracy and precision of the developed method and to prove the absence of interference by excipients, recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of all drugs. It was found that the sample solution was stable up to 20 hrs in which no decomposition was observed. These results reveal that the developed method

have an adequate precision and accuracy, and consequently, can be applied to the determination of Rifapentine tablet in pharmaceuticals without any interference from the excipients.

CONCLUSION

A spectrophotometric method for quantifying Rifapentine in formulation samples has been developed and validated. The assay is selective, precise, accurate and linear over the concentration range studied. LOD was approximately 2.15µg/ml in formulation and the LOQ was found to be 6.52µg/ml. The sample solution was stable for 20 hr. In summary, the proposed method can be used for the drug analysis in routine quality control.

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