# METHOD DEVELOPMENT, VALIDATION, AND ESTIMATION OF RIFAPENTINE IN BULK AND TABLET DOSAGE FORM BY USING UV-SPECTROPHOTOMETRY AND RP-HPLC METHOD

**AUTHORS:**

**Pasupati Nath Tiwari, M. Pharm (pharmaceutical analysis) Karnataka College of Pharmacy, Bangalore-64, India**

**Dr. C. Sreedhar, HOD of Pharmaceutical analysis, Karnataka College of Pharmacy, Bangalore-64**

**Ayesha Rehman, M. Pharm (pharmaceutical analysis) Karnataka College of Pharmacy, Bangalore-64, India**

**Ram Autar Bishwakarma, B. pharmacy, Aditya College of Pharmacy, Bangalore-64, India**

**ABSTRACT:**

A simple, accurate, precise and sensitive reverse phase high performance liquid chromatography method was developed for estimation of Rifapentine in bulk and pharmaceutical dosage form. For HPLC method, Column: C18 (4.6ID x 250mm) in isocratic mode with mobile phase containing Acetonitrile: 0.01M KH2PO4 buffer pH (6.0) in ratio of 80: 20 v/v was used. The flow rate was 0.8 ml/min with injection volume of 20µl and effluent was monitored at 478nm. Retention time was found to be 5.00 ± 0.1 minute. 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.

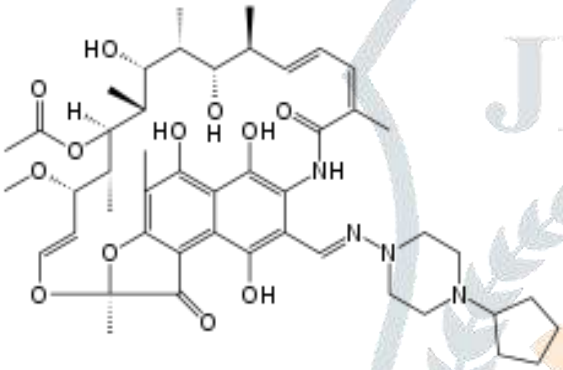
**KEYWORDS**: Rifapentine, RP-HPLC, Area under- curve, UV-Spectrophometry, Method Validation.

## 1. INTRODUCTION:

Rifapentine 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 or twice weekly regimens. Rifapentine is associated with transient and asymptomatic elevations in serum aminotransferase and is a likely cause of clinically apparent acute liver injury **[ 1,2].** Rifapentine is an antibiotic drug used in the treatment of tuberculosis. It inhibits DNA-dependent RNA polymerase activity in susceptible cells. Specifically, it interacts with bacterial RNA polymerase but does not inhibit

the mammalian enzyme. The antimicrobial spectrum of Rifapentine strongly resembles that of its homologue rifapin, with a remarkably greater therapeutic efficacy against Mycobacterium tuberculosis and Mycobacterium lepraein experimental infection **[3,4].** The drug has an advantage of five time's longer half-life than rifampicin and it is recommended for use in intermittent therapy. Literature survey reveals that only bio-analytical method has been developed for the estimation of Rifapentine in blood, plasma, serum etc . The objective of the present work was to develop and validate both UV and RP-HPLC method for the estimation of Rifapentine in bulk and Pharmaceutical formulations. **[6]**

## 2. DRUG PROFILE:



**Other Names:** 3{[(4-cyclopentyl-1-piperazinyl)imino]methyl}rifamycin

**Route of administration:** by Oral

**Chemical Formula:** $C_{47}H_{64}N_4O_{12}$

**Molar Mass:** 877.031 g/molg.mol$^{-1}$

**Melting point:** 179 to 180 $^{o}$C

Rifapentine was approved for medical use in the United States in 1998. It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system. **[5]**

## 3. INTRUSMENTATION:

### 3.1 RP-HPLC:

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 the solutions. HPLC Binary Gradient system 3000series, equipped with Serial dual plunger, UV/Visible detector and InertsilC18, (4.6 ID×250mm) column with HPLC work station software was employed for the analysis. **[6]**

### 3.2 UV:

A double beam UV-VIS spectrophotometer (UV- 2450,Shimadzu,Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 10 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 (fî):10.0 nm; spectral slit width: 1nm. All weights were taken on electronic balance (Model ShimadzuAUX 120). **[7]**

## 4. CHEMICALS AND REAGENT:

### 4.1 RP-HPLC:

All chemicals and solvents of HPLC grade were taken. HPLC grade water was obtained from Milli-QRO water purification system.

#### 4.1.1. Preparation of 0.01M Potassium phosphate buffer:

For the preparation of 0.01M Potassium dihydrogen phosphate buffer, Dissolve 1.36gm of Potassium dihydrogen phosphate in small amount of distilled water in 1000ml capacity volumetric flask and makeup the final volume with distilled water. And adjust the pH of the resulting solution to 6.0 with 1M KOH solution.

**[6]**

#### 4.1.2. Preparation of Mobile phase:

Mobile phase is composition of Acetonitrile and 0.01M Potassium dihydrogen phosphate buffer, pH (6.0) with concentration ratio of (80:20). For the preparation of mobile phase 160 ml of acetonitrile is mixed carefully with 40 ml of 0.01M Potassium dihydrogen Phosphate buffer, pH (6.0) and the resulting mixture is subjected to sonication for 5 min for degasing. The mobile phase prepared for RP-HPLC analysis was filtered from 0.45Mm membrane filter. **[6]**

### 4.1.3. Preparation of Stock solution:

Standard stock solutions of Rifapentine was prepared by dissolving accurately weighed 10mg of Rifapentine in 2ml of above mentioned mobile phase in 10ml volumetric flasks. Final volume was made up to 10ml with mobile phase to get stock solution containing 1000µg/ml of Rifapentine. **[6]**

### 4.1.4. Selection of Analytical Wavelength:

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

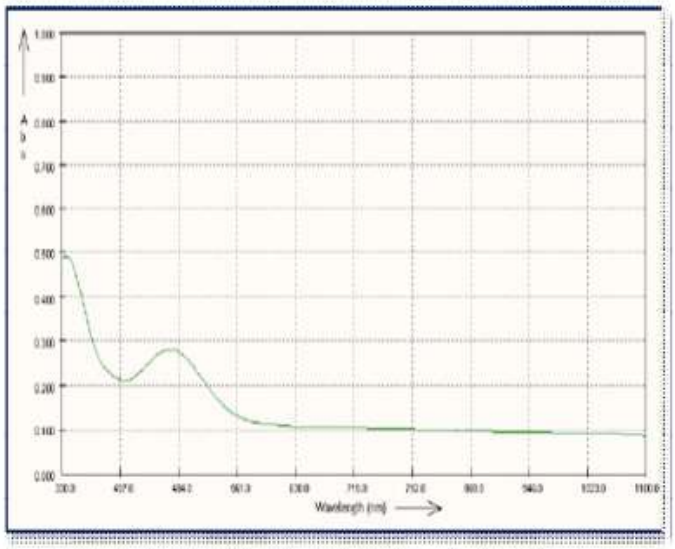


**Figure 1.** Absorbance Spectrum of drug

**[6]**

### 4.1.5. Optimization of RP-HPLC Method:

The HPLC procedure was optimized with the view to develop assay method for the estimation of Rifapentine in bulk and marketed formulation. Various combination of mobile phase was tried such as methanol: 0.01M Potassium dihydrogen phosphate buffer, acetonitrile: 0.01M Potassium dihydrogen phosphate buffer of different pH etc. It was found that Acetonitrile and Potassium dihydrogen phosphate buffer (pH6) in the ratio 80:20 v/v, at flow rate of 0.8ml/min gives good resolution of peak with minimum tailing as compared to other mobile phases (Figure 2).

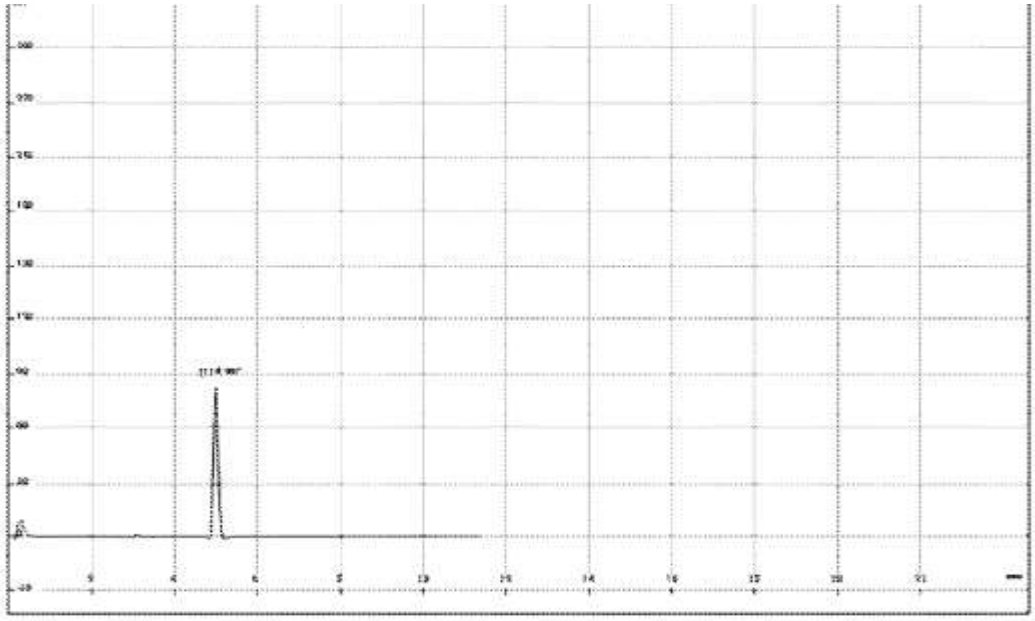


**Figure 2.** Chromatogram of RIFA (20µl/ml)

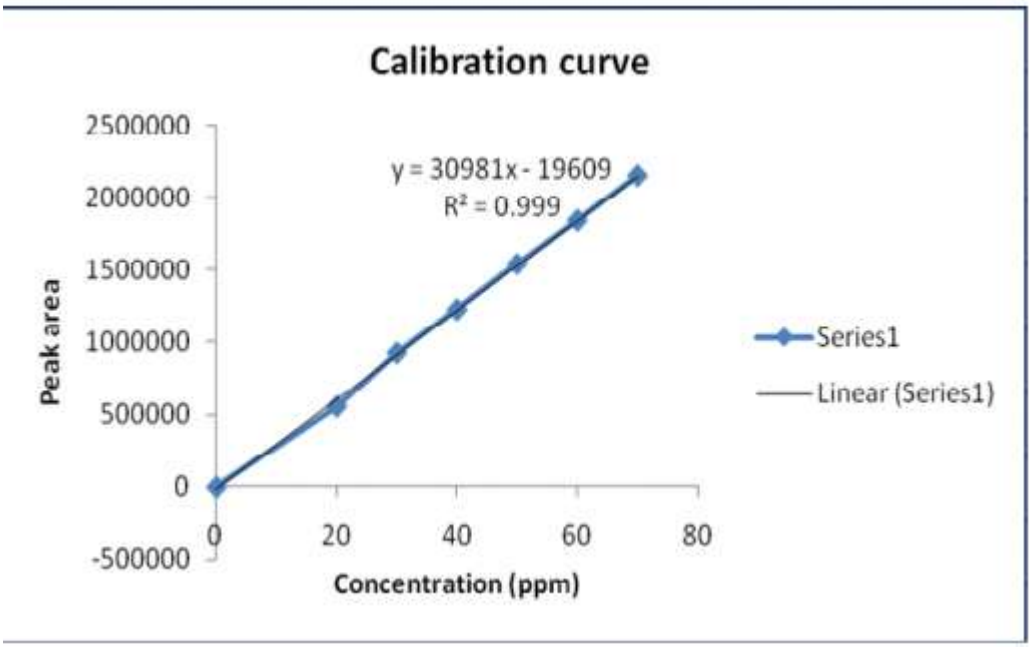
**[6]**

### 4.1.6. Preparation of Calibration curve:

The standard stock solutions of 1000µg/ml appropriate aliquots were transferred in different 10 ml volumetric flasks to make solution in range of 20-70µg/ml. A 20µl volume of each concentration was injected (n=3) three times into HPLC system, under optimized chromatographic conditions. The calibration curve was plotted using peak areas against concentration and regression data was collected. Calibration data of drug at optimized chromatographic condition is given in (Table 2), whereas the calibration curve is shown in (Figure 3)

**Table 2:** Calibration Data of Drug

| Sr.No. | Concentration (µg/ml) | Mean Area (n=3) | SD | %RSD |
|---|---|---|---|---|
| 1 | 20 | 554966 | 875.38 | 0.15 |
| 2 | 30 | 925018 | 991.46 | 0.10 |
| 3 | 40 | 1223584 | 2143.95 | 0.17 |
| 4 | 50 | 1537940 | 2150.18 | 0.13 |
| 5 | 60 | 1837444 | 3722.12 | 0.20 |
| 6 | 70 | 2148587 | 2846.08 | 0.13 |




**[6]**

## 5. UV:

### 5.1 Study of linearity curves:

An aliquot portions 0.4-2.4 Ml were transferred into series of six separate 10mL volumetric flasks and volume was adjusted to mark with methanol to obtain 24 µg/mL. The AUC between the–concentration of 4 two selected wavelengths(307- 350) were determined and calibration curves were constructed by plotting concentration versus AUC between the selected wavelengths. **[7]**

## 6. RP-HPLC:

### 6.1 Analysis of Pharmaceutical Formulations:

Rifapentine tablets (Rifapex) containing 150 mg of Rifapentine was selected for the analysis. 20 tablets were weighed accurately and average weight of single tablet was calculated. The powder equivalent to 10mg of Rifapentine was transferred to a 10ml volumetric flask and 2ml of above mentioned mobile phase was added to dissolve the sample. The final volume was adjusted to 10ml by mobile phase to get solution of 1000µg/ml. The above solution was filtered using Whatmann filter paper (No. 41). From these solutions 1ml of solution was pipette out and transferred to 10ml volumetric flasks and volume was made up to the mark using mobile phase so as to get the concentration 100µg/ml. This resulting solution was filtered from 0.22µm syringe filter before injection. A 20µl volume of sample was injected three times under optimized chromatographic condition and responses were recorded. The percentage drug content was calculated by measuring the peak areas and comparing it with the peak area of pure Rifapentine of respective concentration. The result of analysis of marketed formulation is shown in table no.3.

**Table 3:** Analysis of Marketed Formulation

| Marketed Formulation | Label Claim. (mg/Tablet) | Percentage Purity (%) | S.D | %R.S.D |
|---|---|---|---|---|
| Rifapex | 150 | 99.60 | 0.095 | 0.095 |

[6]

## 7. UV:

### 7.1 Preparation of in-house tablet formulation:

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

**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

### 7.2 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 prepared 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.

**[7]**

## 8. METHOD VALIDATION:

### 8.1 RP-HPLC:

### 8.1.1 RESULT AND DISCUSSION:

#### 8.1.2 Linearity and Range:

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 analyzed. The drug showed linearity in the range of 20-70µg/ml with correlation coefficient 0.9992. **[6,9,10]**

#### 8.1.3 Precision Studies:

Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatablity was determined by preparing six replicates of same concentration of the sample were prepared and analyzed under the optimized chromatographic condition for Intraday and Interday precision. 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 SD and %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 2. **[6,9,10]**

#### 8.1.4 Limit of Detection:

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. LOD was determined using the following equation designated by ICH guidelines. LOD =3.3 σ/S Where, σ = the standard deviation of the response S = the slope of the calibration curve. **[6,9,10]**

#### 8.1.5 Limit of Quantification:

Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOQ was determined using the following equation designated by ICH guidelines. LOQ =10 σ/S Where, σ = the standard deviation of the response S = the slope of the calibration curve. **[6,9,10]**

### 8.1.6 Robustness:

Robustness tests examine the effect operational parameters have on the analysis results. One factor changed at a time to estimate the effect. Thus replicate injections (n=3) were performed under small changes of chromatographic parameters. For the determination of method robustness a number of chromatographic parameters such as flow rate, detection wavelength, and mobile phase composition etc. were varied within a realistic range and the quantitative influence of the variables is determined. For the developed method the robustness study was carried out at concentration of 40µg/ml of Rifapentine. Each factor was changed at three levels (+1, 0, -1). **[6,9,10]**

### 8.1.7 Ruggedness:

The ruggedness of an analytical method is the degree of reproducibility of test results under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days, etc. Ruggedness of proposed method was determined by carrying out analysis by two different analysts and the result of analysis was expressed as SD and %RSD. **[6,9,10]**

### 8.1.8 Accuracy (recovery study):

Recovery studies was carried out by applying the standard addition method, to drug sample to which the known amount of pure Rifapentine was added corresponding to 50%, 100% and 150% of the label claim (standard addition method). At each level three injections were given and were compared with the corresponding peak areas of standard for the determination of the % drug recovery. The results obtained from the validation of developed method are summarized in table 3.

**Table 3:** Analysis of Marketed Formulation

| Marketed Formulation | Label Claim. (mg/Tablet) | Percentage Purity (%) | S.D | %R.S.D |
|---|---|---|---|---|
| Rifapex | 150 | 99.60 | 0.095 | 0.095 |

**Table 4:** Regression analysis data and summary of validation parameter for the Proposed RP-HPLC method

| Parameters | Results |
|---|---|
| Linearity Range (µg/ml) | 20-70 µg/ml |
| Slope (m) | 3098 |
| Intercept (c) | 1960 |
| Correlation Coefficient | 0.9992 |
| Limit of Detection (µg/ml) | 0.225 µg/ml |
| Limit of Quantitation (µg/ml) | 0.684 µg/ml |
| **Precision (%RSD)** | |
| Intra-day precision | 0.14 |
| Inter-day precision | 0.13 |
| Ruggedness | Rugged |

**Table 5:** Recovery data for the proposed method

| Drug | Label Claim | Amount of drug taken (µg/ml) | Amount added (%) | % mean recovery ±S.D (n=3) |
|---|---|---|---|---|
| Rifapentine | 150mg | 30 | 50 | 99.57±0.221 |
| | | 30 | 100 | 99.92±0.086 |
| | | 30 | 150 | 99.85±0.692 |

**[6,9,10]**

## 9. UV- SPECTROPHOTOMETRY:

### 9.1 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 cal- culation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axisis 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) versus AUC.

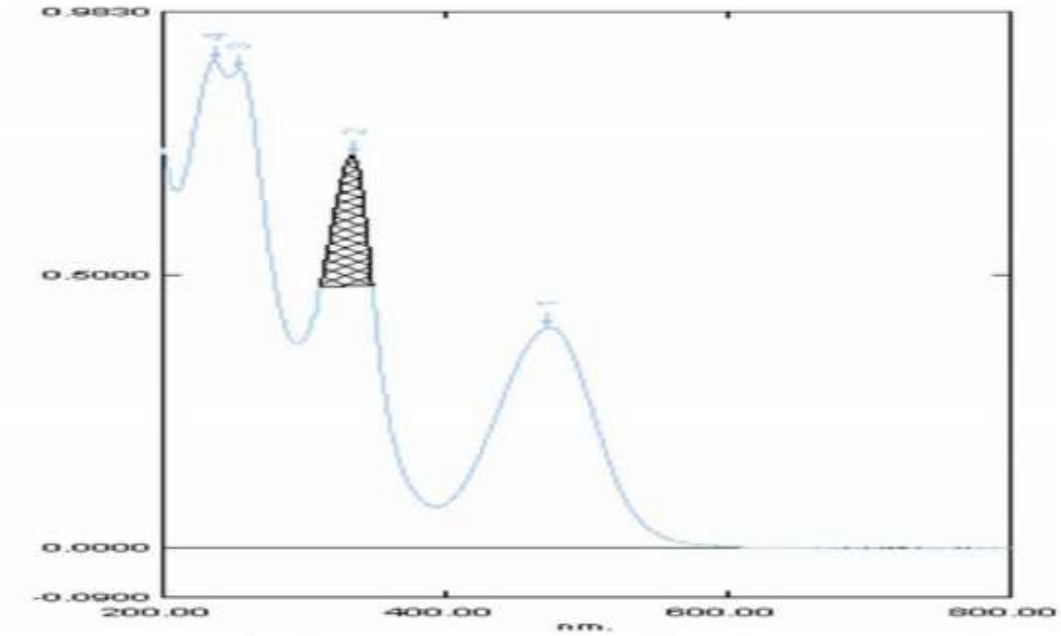



**Figure 2 : Area under curve spectrum of rifapentine**

**[7]**

## 10. VALIDATION OF METHOD:

The proposed method was validated as per ICH guidelines.

### 10.1 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 co- efficient; the value must be > 0.999 **[6,7,8]**

## 10.2 Precision:

The intra day 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.

**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 |

**[6,7,8]**

## 10.3 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 (8µg/mL). Three samples were prepared at each of these concentrations. The recovery of added drug was estimated by measuring there response and by fitting these values to the straight line equation of calibration curve.

**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 |

**[6,7,8]**

## 10.4 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.

**[6,7,8]**

### 10.5 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 percentrelative standard deviation.

TABLE 5: Ruggedness

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

n= no. of estimations

**[6,7,8]**

## 11. RESULT AND DISCUSSION OF UV METHOD:

The molecular structure of the rifapentine is presented in Figure1. In methanol ,Rifapentine showed maximum absorbance at 334 nm. It shows the absorption spectrum of rifapentine in Methanol for the method. Optical characteristics of rifapentine were calculated by the methods and presented . The intra-day and inter-day precision values (%RSD) were calculated and lying in the acceptable limits (dî2%)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 (dî2%). 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 .

**[7]**

## 12. ACKNOWLEDGEMENT:

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## 13. CONCLUSION:

The linear calibration curve was obtained at concentration range 20-70µg/ml with Correlation Coefficient (0.9992), Slope (3098) and Intercept (1960). The Limit of detection (LOD) and Limit of quantification (LOQ) found to be 0.225µg/ml and 0.684µg/ml for Rifapentine respectively by the HPLC method. The 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 RP-HPLC method is precise and accurate and hence can be used for the routine analysis of Rifapentine in bulk and pharmaceutical formulation. 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 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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