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Development and Validation of RP-HPLC Method for the Estimation of Rifapentine in Bulk and Pharmaceutical Formulation

Ansari Tahir*[1], Kalkotwar R. S[1], Patil Sachin[1], Momin Haseeb[2] and Jameel Asghar[3]

[1]Jagdamba Education Society's S.N.D College of Pharmacy Babhulgaon, Yeola-423401
[2]Nashik Rayat Education Society's S.S.G.M College Kopargaon.
[3]Mahatma Gandhi Viddyamandir's JantaVidyalay College of Art's And Science, Yeola-423401

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Address for Correspondence

Dept, of QAT, S.N.D College of pharmacy Babhulgaon, Yeola-423401
E-mail: tahiransari04@gmail.com

ABSTRACT

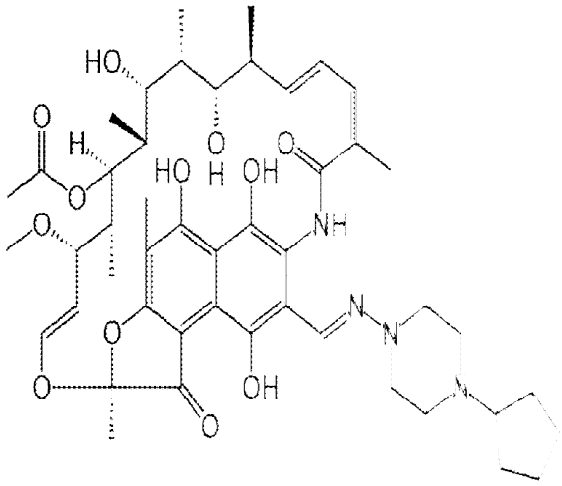
A simple, accurate, precise and sensitive reverse phase high performance liquid chromatography method was developed for estimation of Rifapentine in bulk and pharmaceutical dosage form. For HPLC method, Column: C18 (4.6ID x 250mm) in isocratic mode with mobile phase containing Acetonitrile: 0.01M KH_2PO_4 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. The method was validated for several parameters like accuracy, linearity, precision etc, 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, RP-HPLC, Dosage forms & Method validation.

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 leprae*in 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[5]. Literature survey reveals that only bio-analytical method has been developed for the estimation of Rifapentine in blood, plasma, serum etc[6-8]. The objective of the present work was to develop and validate a simple, sensitive, precise and accurate RP-HPLC method for the estimation of Rifapentine in bulk and pharmaceutical formulations.



Chemical Structure of Rifapentine

Chemical (IUPAC) name

Rifapentine is chemically (7S, 9E, 11S,12R,13S,14R,15R,16R,17S,18S,19E,21Z)-26-[(E)-N-(4-cyclopentylpiperazin-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}]triacont a-1,3,5(28),9,19,21,25(29),26-octaen-13-yl acetateor3-[N-(4-Cyclopentyl-1-piperazinyl) formimidoyl] Rifamycinis a piperazinyl-hydrazone derivative of 3-formyl Rifamycin SV[9,10].

MATERIALS AND METHODS

Instrumentation

A) 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.

B) HPLC Binary Gradient system 3000series, equipped with Serial dual plunger, UV/Visible detector and Inert-silC18, (4.6 ID×250mm) column with HPLC work station software was employed for the analysis.

Chemicals and reagents

All chemicals and solvents of HPLC grade were purchased from fine Chemicals Ltd, Mumbai, India. HPLC grade water was obtained from Milli-QRO water purification system. The pure drug Rifapentine and marketed formulation were obtained as gift sample from Lupin pharmaceutical LTD, Aurangabad.

Preparation of 0.01M Potassium phosphate buffer

For the preparation of 0.01M Potassium dihydrogen phosphate buffer, Dissolve1.36gm 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.

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 0.45 µm membrane filter (Millipore).

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.

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).

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 (pH-6) 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).

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).

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.

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.[11,12]

RESULTS AND DISCUSSION

i) 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.

ii) Precision Studies

Precision studies were carried out to ascertain the reproduciblity 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.

iii) 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.

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

Where,
σ = the standard deviation of the response
S = the slope of the calibration curve

iv) 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.

$$LOQ = 10\ \sigma/S$$

Where,
σ = the standard deviation of the response
S = the slope of the calibration curve

v) 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).

vi) 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.

vii) 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.

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 proposed HPLC 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 RP-HPLC 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: Optimized Chromatographic Condition

Mobile phase	Acetonitrile: 0.01M KH_2PO_4 buffer pH (6.0)
Ratio	80:20
Flow rate	0.8ml/min
Wavelength	478nm
Pressure	6-7Mpa
Injection Volume	20µl

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

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

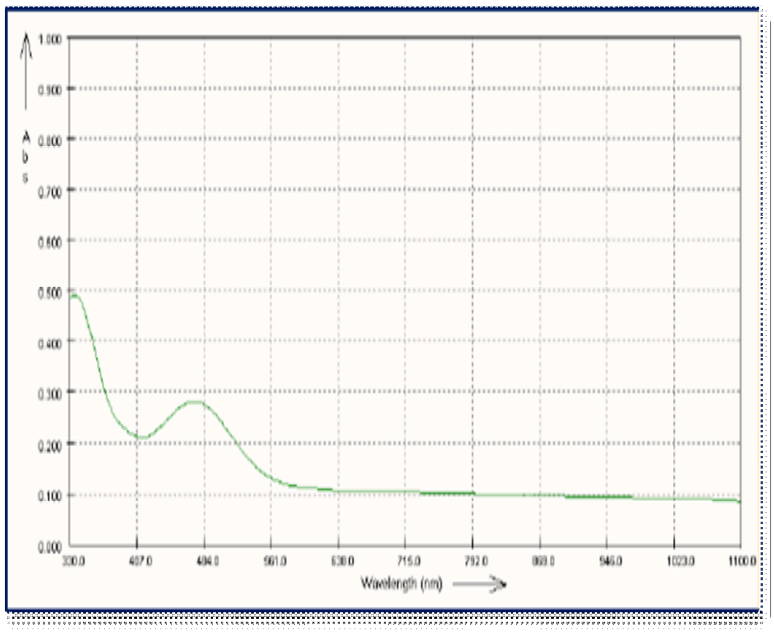

Figure 1. Absorbance Spectrum of drug

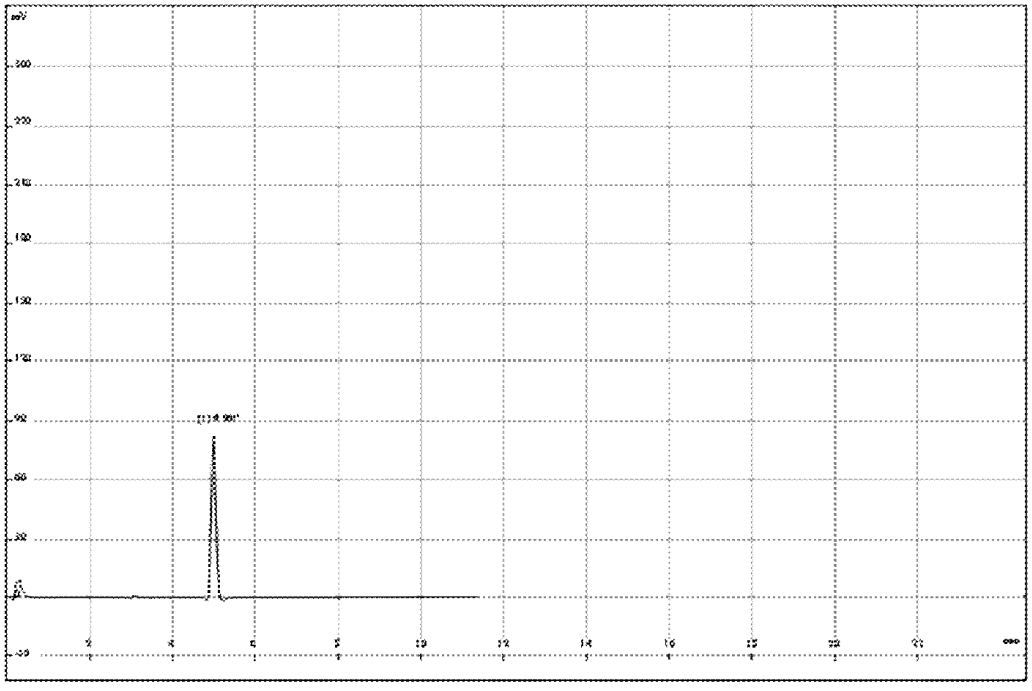

Figure 2. Chromatogram of RIFA (20µl/ml)

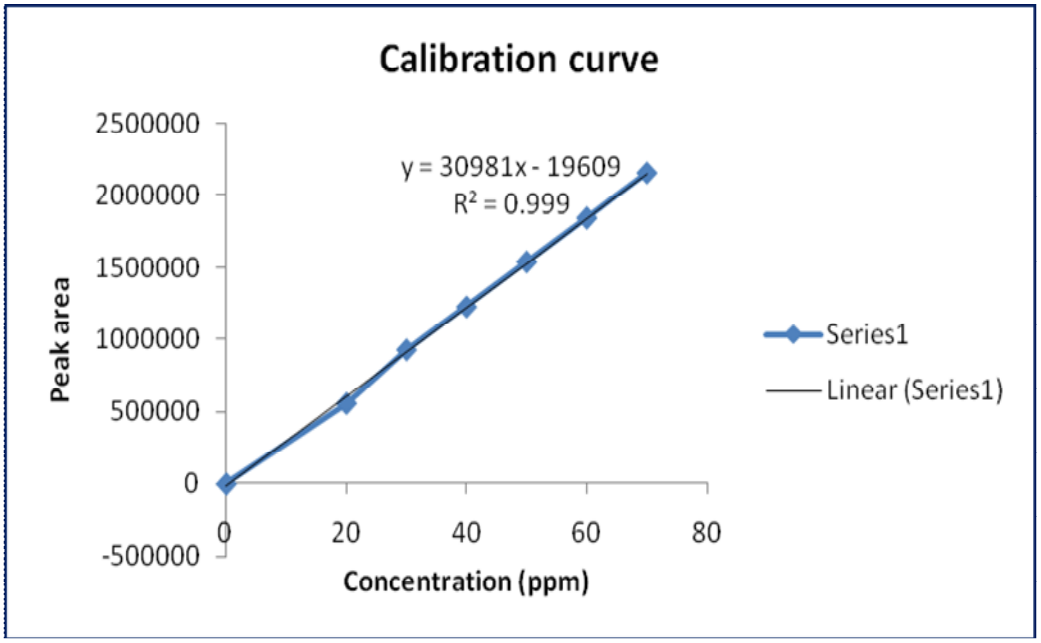

Figure 3. Calibration curves