International Journal of Theoretical & Applied Sciences, 9(2): 99-105(2017)

ISSN No. (Print): 0975-1718
ISSN No. (Online): 2249-3247

Analytical Method Development and Validation of Impurity Profile in Rifapentine

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(Received 19 August, 2017, Accepted 10 September, 2017)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: A simple analytical method for determination of impurity profile in Rifapentine developed and validated. The proposed method was developed by using Thermo BDS-Hypersil C18 (250mm × 4.6mm, 5µm) column at 25˚C with a mobile phase containing a gradient mixture of sodium di-hydrogen orthophosphate and acetonitrile with a run time of 55 min at monitored wavelength of 254nm. The method is developed for identification and quantification of related impurities in the API. The Stability-indicating capability of method is demonstrated by forced degradation of Rifapentine. The developed method is validated as per the guidelines of International Conference on Harmonization (ICH). The developed method is precise and is capable of detecting and quantifying all the known and unknown impurities. The proposed method is linear, sensitivity; accuracy and simple mobile phase imply that the method is suitable for quantification of related impurities in Rifapentine.

Key words: Impurity profile, HPLC, Stability Indicating, ICH guidelines.

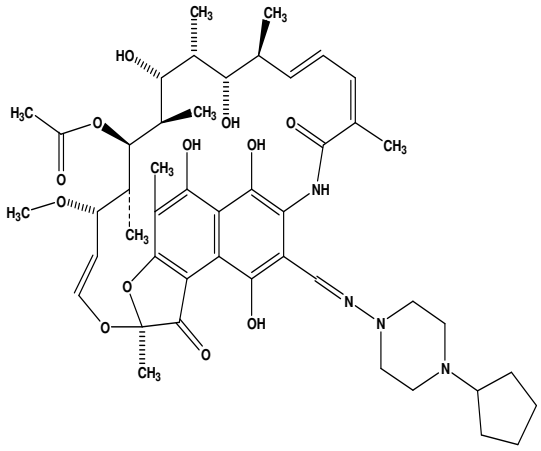
I. INTRODUCTION

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 [1-6].

It inhibits DNA-dependent RNA polymerase activity in susceptible cells. Specifically, it interacts with bacterial RNA polymerase but does not inhibit with a remarkably greater therapeutic efficacy against *Mycobacterium tuberculosis* and *Mycobacterium lepraeine* experimental infection. The drug has an advantage of five time's longer half-life than rifampicin and it is recommended for use in intermittent therapy [7].

Impurity profile is very crucial and critical during the synthesis of drug substances, as it can provide crucial data regarding the quality, safety, efficacy, toxicity of drugs, varies limit of detection(LODs) and limit of quantification(LOQs), structures of several organic and inorganic impurities, usually associated with bulk drugs and finished products.

High performance liquid chromatography is a relatively new technique that offers separation capabilities when compared to other classical analytical methods. The literature review showed that no single stability indicating High performance liquid chromatography method for all Rifapentine related impurities. Thus the paper describes the development and validation of a stability-indicating RP-HPLC method to monitor Rifapentine and Rifapentine related compounds.



Rifapentine

IUPAC Name:

(7S,9E,11S,12R,13S,14R,15R,16R,17S,18S,19E,21Z,26E)-26-{[(4-cyclopentylpiperazin-1-yl)amino]methylidene}-2,15,17,29-tetrahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-6,23,27-trioxo-8,30-dioxa-24-azatetracyclo[23.3.1.1^{4,7}.0^{5,28}]triaconta-1(28),2,4,9,19,21,25(29)-heptaen-13-yl acetate [8].

II. MATERIALS AND METHODS

A. Chemicals and reagents

Active Pharmaceuical ingredient standards, Impurity standards (Rifapentine N-Oxide, 3-Formyl Rifamycin and Rifapentine quinone) and test samples were supplied by Suven Life Sciences Ltd, R&D centre, Jeedimetla, Hyderabad, India, HPLC grade acetonitrile obtained from Rankem, ACS grade Sodium di-hydrogen orthophosphate procured from Merck, AR grade Sodium Hydroxide pellets procured from Rankem. Milli Q water used was obtained by using Millipore Integral water purification system.

B. Equipment

Analytical trials were carried out in Waters Alliance 2695 with 2996 PDA detector (Waters, Milford, MA,USA) consisting of a quaternary gradient solvent manager, sample manager and photo diode array(PDA) detector. The output signal was monitored through Empower3 software. The photo stability studies were carried out by using UV chamber (Advanced Instruments), Thermal stability studies were performed by using hot air oven (Hextec Instruments).

C. Chromatoraphic conditions

The chromatographic column of Thermo BDS-Hypersil C18 (250 X 4.6mm, 5µm) and the separation was achieved by gradient elution. Mobile phase A contains a mixture of 0.025M sodium dihydrogen orthophosphate buffer with pH adjusted to 7.7 with diluted sodium hydroxide solution and acetonitrile in the ratio of 90:10(v/v); and Mobile phase B contains a mixture of 0.025M sodium dihydrogen orthophosphate buffer with pH adjusted to 7.7 with diluted sodium hydroxide solution and acetonitrile in the ratio of 30:70(v/v). The gradient program (time (min)/ %B) was set as 0.00/20, 40.00/80, 45.00/80,47.00/20 and 55.00/20 with flow rate 1.0mL/min and injection volume of 20µL. The column temperature was maintained at 25˚C and the peaks were monitored at 254nm. Diluent is acetonitrile used for sample preparation [9].

D. Preparation of solutions

Preparation of standard solution

A stock solution containing 50µg/mL of Rifapentine,100µg/mL of Rifapentine N-Oxide, 80µg/mL of 3-Formyl Rifamycin and 100µg/mL of Rifapentine quinone with diluent. From the above stock solution 5µg/mL of Rifapentine,10µg/mL of Rifapentine N-Oxide, 8µg/mL of 3-Formayl Rifamycin and 10µg/mL of Rifapentine quinone were prepared with diluent and used for validation.

Preparation of sample solution

Fifty milli grams of sample was weighed and transferred in to a 50mL volumetric flask, dissolved and diluted to the volume with diluent.

E. Stress studies

Specificity is the capability of the method to measure the analyte response in the presence of potential impurities. Stress studies performed at the initial concentration of 1000µg/mL of Rifapentine solution to prove the stability indicating nature of the proposed method. Intentional degradation was attempted by stress conditions of photolytic for 1.2 million lux and ultra violet(UV) light, both at shorter and longer wavelengths for ~ 200Wh/m^3, heat (90˚C for 12h), acid(0.1M HCl at 90˚C for 1h), base(0.1 M NaOH at room temperature for 15min), hydrolysis (for 48h) and oxidation (3% H_2O_2 at 90˚C for 45min) to evaluate the capability of the proposed method to separate Rifapentine from its degradation products[10].

The peak purity values obtained from the stress samples was verified by using PDA detector. The peak purity angle values are less than peak purity threshold values in all the stress samples and demonstrate the analyte peak homogeneity. The results are reported in Table 2.

Table 1: Relative Retention Time and Relative Response Factor values of Rifapentine and Rifapentine related impurities.

Name of the Compound	Relative Retention Time(RRT)	Relative Response Factor(RRF)
Rifapentine N-Oxide	0.51	0.38
3-formyl rifamycin	0.75	0.82
Rifapentine Quinone	0.95	0.39
Rifapentine	1.00	1.00

Table 2: Peak purity values obtained for Rifapentine peak in stress studies.

Stress condition	Peak purity angle	Peak purity threshold
Acid degradation	0.04	1.00
Base degradation	0.05	1.00
Oxidation	0.05	1.00
Hydrolysis	0.05	1.00
Light degradation	0.07	1.00
Thermal Degradation	0.06	1.00

F. Method validation

The proposed method has been validated by HPLC as per ICH guidelines [11].

Precision. The repeatability of the test method was proved by injecting six individual test preparations of Rifapentine spiked with Rifapentine related impurities (Rifapentine N-Oxide at 1.0% level, 3-Formyl Rifamycin at 0.8% level and Rifapentine quinone at 1.0% level, with respect to the 1000µg/mL of Rifapentine). The intermediate precision of the method was evaluated using different analyst, different instrument and different lot of the same column. The %RSD (relative standard deviation) of peak area of each impurity was calculated for both precision and intermediate precision. The results are reported in Table 3.

Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were established for all the three impurities and Rifapentine by using calibration curve.

R =STEYX(Y values, X values), S is Slope of the calibration curve and finally determine LOD and LOQ by using the following formulae.

LOD = 3*R/S and LOQ = 10*R/S.

Precision was carried out for all the components at LOQ level by calculation % RSD of peak areas. The results are reported in Table 3.

Accuracy. The accuracy of an analytical procedure expresses the closeness and exactness of agreement between the true values and experimental values. The recovery was determined for all the impurities and Rifapentine at LOQ, 100, and 120% of the analyte concentration. The percentage recoveries are between 85% and 115%. The results are reported in Table 3.

Linearity. To establish the linearity of proposed method, Rifapentine and its related impurities, stock solutions prepared and diluted to obtain the required concentrations at different levels ranging from LOQ to 120%(i.e. LOQ, 80%, 90%, 100%, 110% and 120 %) to the specification level. The correlation coefficient, slope and y-intercept of the calibration curve were calculated. The results are reported in Table 3.

Robustness. The robustness study was carried out to evaluate the influences of small but deliberate variations in the chromatographic conditions, which have been described in the Chromatographic conditions section. The factors chosen for this study, which were critical sources of variability in the operating procedures such as mobile phase pH (±0.1) and flow rate (±0.1mL/min) were identified.

Table 3: Validation data for Rifapentine and Rifapentine related impurities.

Name of the Compound	Limit of Detection (Lod, %)	Limit of Quantitation (Loq, %)	Accuracy (%Recovery)		Linearity	System Precision (%Rsd)	Method Precision (%Rsd)
Rifapentine	0.01	0.02	Not applicable		Correlation coefficient:1.00, Slope:2146 , y-intercept: -3029	4.13	Not applicable
Rifapentine N-Oxide	0.07	0.20	At LOQ :103 , At 100%:110 At 120%:108		Correlation coefficient:1.00, Slope:1807, y-intercept:-21054	1.27	1.26
3-formyl rifamycin	0.02	0.05	At LOQ:102, At 100%:99, At 120%:100		Correlation coefficient:1.00, Slope:3480, y-intercept: -8570	0.93	0.75
Rifapentine Quinone	0.09	0.30	At LOQ:98, At100%:111, At120%:113		Correlation coefficient:1.00 Slope:1775, y-intercept: 9137	3.77	1.29
Name of the Compound	**Robustness**				**Ruggedness**		
	Flow at 0.9mL/min (%RSD)	Flow at 1.1mL/min (%RSD)	pH at 7.6 (%RSD)	pH at 7.8 (%RSD)	Second analyst (%RSD)	Second column (%RSD)	Second system (%RSD)
Rifapentine N-Oxide	0.33	1.23	2.20	1.81	1.81	1.54	2.33
3-formyl rifamycin	1.25	0	1.67	1.25	0.62	2.01	0.50
Rifapentine Quinone	0.86	1.01	0.85	2.33	1.23	3.54	1.44

The % RSD results for Rifapenrine related impurities were evaluated in the deliberately altered experimental conditions. The results are reported in Table 3.

Solution stability. Solution stability was established by injecting the same test sample solution at time intervals of 4hrs, 8 hrs, 12 hrs and 24 hrs. Test sample solutions were placed on bench top during the study and the results with those obtained from freshly prepared test sample solutions were compared. No significant changes were experienced up to 4 hours and slowly impurity levels have been increased over a period up to 24hrs. Hence, freshly prepared standard solutions and sample solutions are recommended.

III. RESULTS AND DISCUSSION

Relative Retention Times (RRTs) and Relative Response Factor values (RRFs) for Rifapentine and Rifapetine related impurities were established (Table 1).

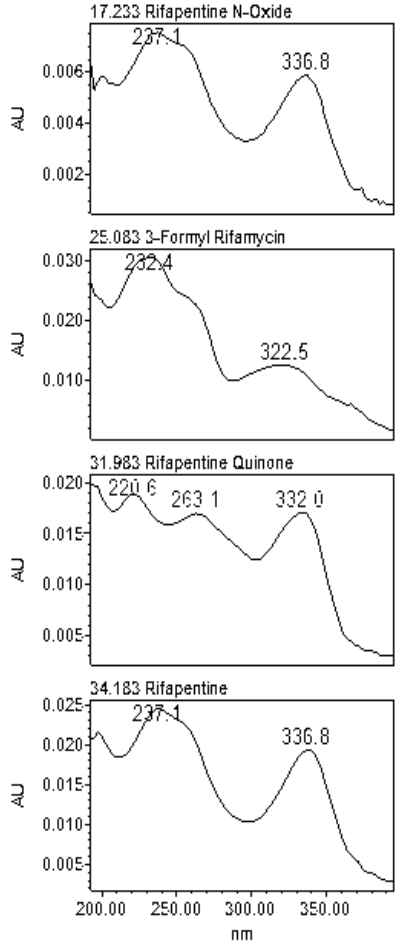


Fig. 1. UV spectra for Rifapentine and its impurities.

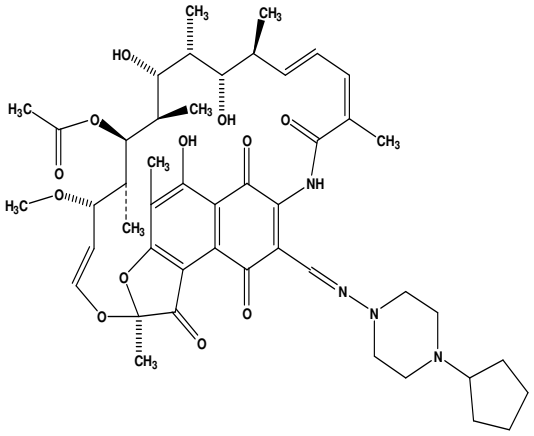
The impurities present in Rifapentine will be calculated by using the following formula.

$$\text{Impurity, \%w/w} = Rt/Rs \times Cs/Ct \times 1/RRF \times 100$$

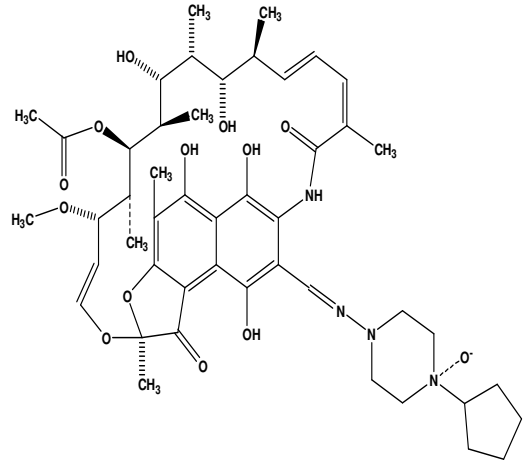
Where, Rt = Peak response of test sample, Rs=Peak response of impurity standard, Cs = Concentration of impurity standard and Ct =Concentration of test sample.

Analytical Method Development and Optimization Discussion

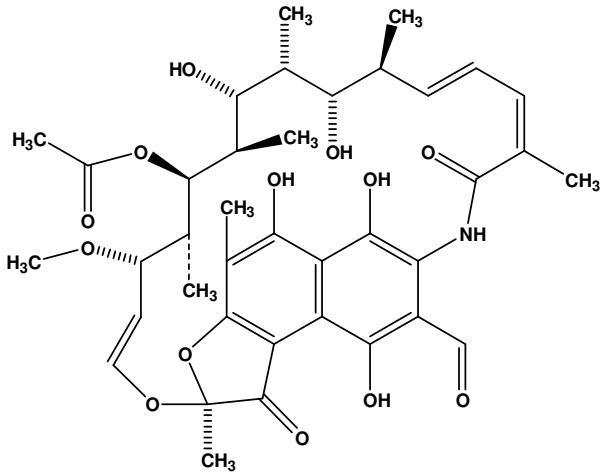
Chemical structure of Rifapentine related impurities are shown in Fig. 2. The impurities are labeled as Rifapentine N-Oxide, 3-Formyl Rifamycin and Rifapentine quinone.



Rifapentine quinone



Rifapentine N-Oxide



3-formyl rifamycin

Fig. 2. Structures of Rifapentine related impurities.

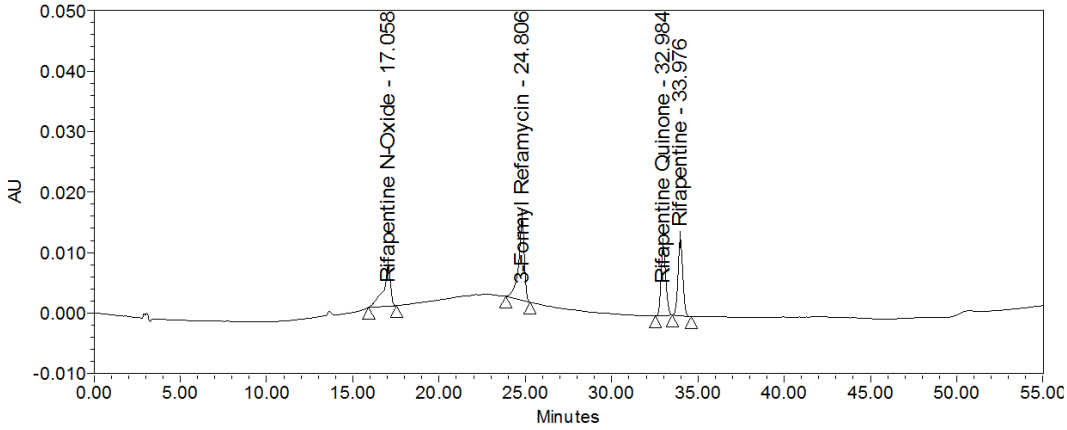


Fig. 3. Rifiapentine and it's related impurities at buffer pH at 7.5.

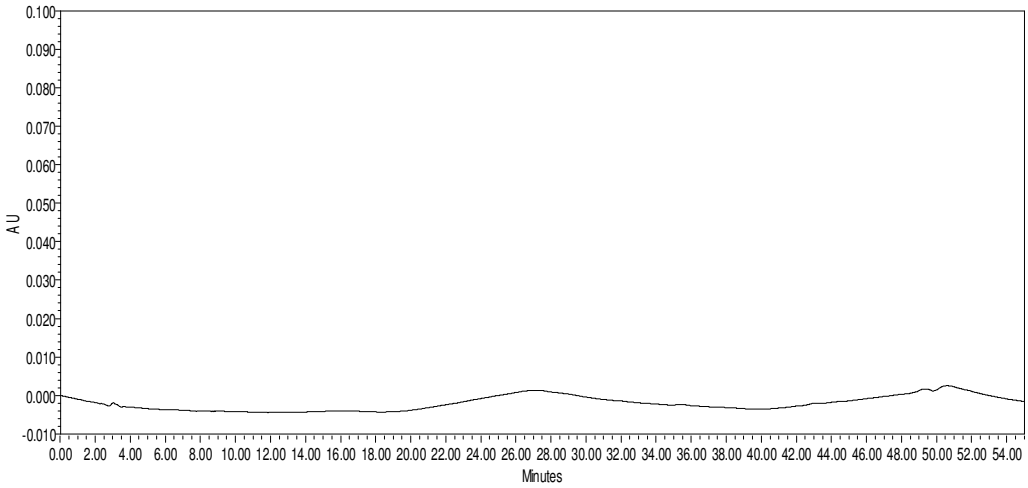


Fig. 4. Diluent.

After thorough screening of different compositions of organic modifiers, buffer pH and column oven temperatures, the separation was achieved using gradient program of different ratios of mixture of Buffer (a mixture of 0.025M sodium di-hydrogen orthophosphate, pH adjusted to 7.7 with diluted sodium hydroxide solution) and acetonitrile [12–15]. Initially poor resolution between Rifapentine and Refapentine quinone observed with the buffer pH of 7.5 (Fig. 3). Finally the method optimized with the buffer pH at 7.7 with an improved resolution. The method was optimized based on the peak shapes and resolution of Rifapentine and Rifapentine related impurities (Fig. 7). UV spectra of Rifapentine and Rifapentine related impurities showed in Fig. 1.

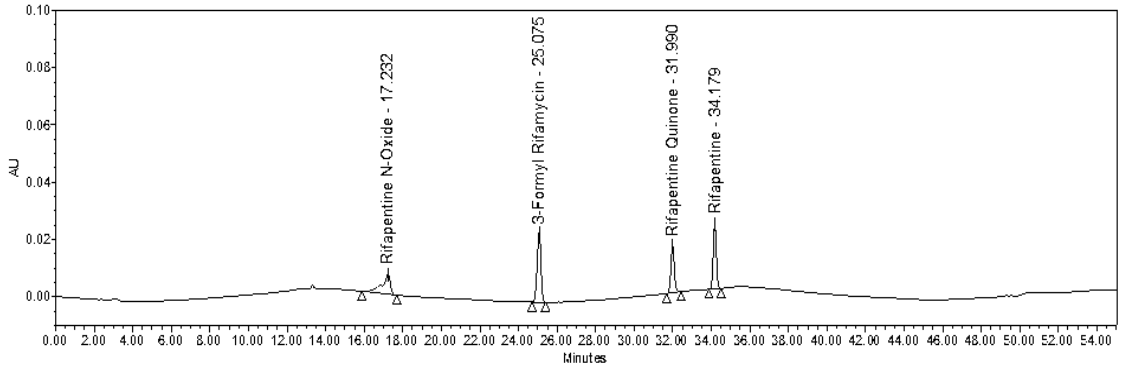


Fig. 5. Rifapentine at 5ug/ml, Rifapentine N-Oxide at 10ug/ml, 3-formyl Rifamycin at 8ug/ml and Rifapentine Quinone at 10ug/ml solution.

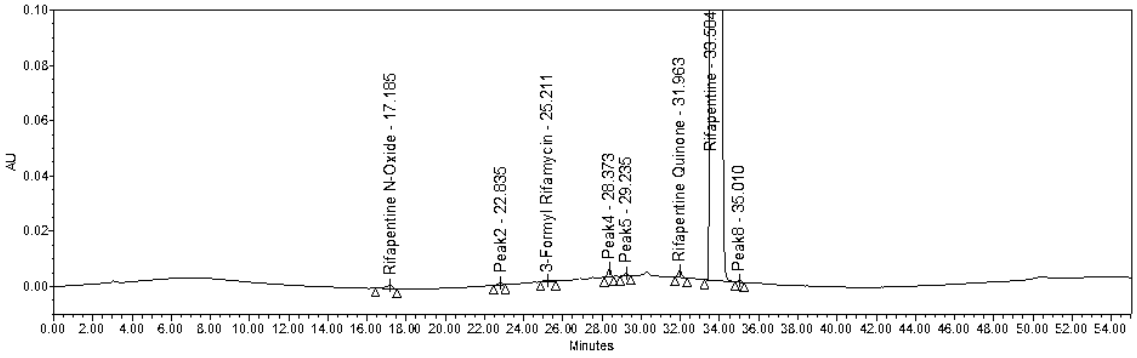


Fig. 6. Rifapentine test sample.

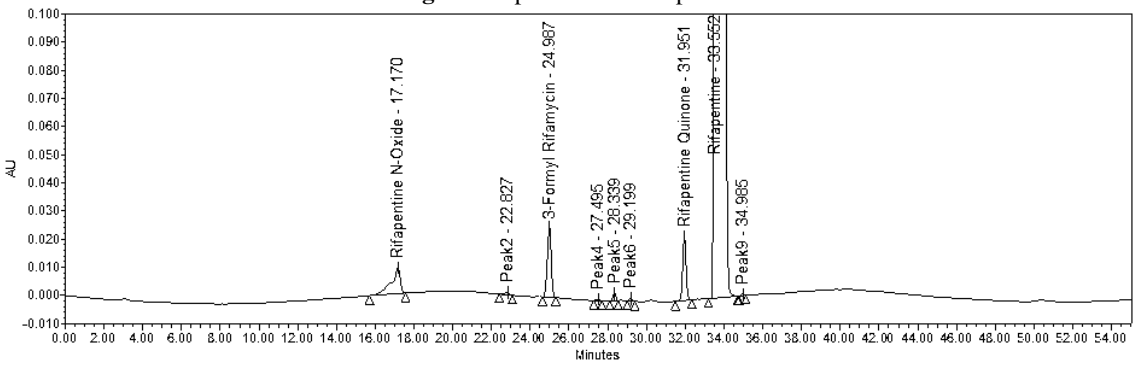


Fig. 7. Rifapentine spiked sample (spiked with impurities).

IV. CONCLUSION

The Proposed HPLC method is selective for the quantification of Rifapentine related impurities (Rifapentine N-oxide, 3-formyl rifamycin and Rifapentine quinone) present in Rifapentine. The linear curves obtained for Rifapentine and Rifapentine related impurities. The Limit of Detection (LOD) and Limit of Quantification (LOQ) values are found to be satisfactory. The results of %recovery were found to be satisfactory. The results obtained by the proposed method were reproducible. Hence, this method is suitable for detection and quantification of impurities present in Rifapentine.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Venkat Jasthi, CEO, Suven Life Sciences Ltd, Hyderabad for providing excellent facilities for carrying out this work.
The authors are grateful to Mr.C. Kameswara Rao, Dr.A.Veera Reddy and Dr.S.Udaya Bhaskar for their encouragement and support to carry out this work.

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