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Stability Indicating RP-HPLC Method for Rifampicin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Rifampicin is a first line medication used as an anti-tubercular agent. Rifampicin acts by binding and inhibiting *DNA dependent RNA polymerase.* It is active against gram-positive and negative both types of bacteria. The present work was undertaken with the aim to develop and validate a rapid and consistent RP-HPLC method in which the peaks will appear with the short period as per ICH Guidelines the clinical and pharmaceutical analysis of this drug requires effective analytical procedures for quality control and pharmacodynamic and pharmacokinetic studies as well as stability study. The chromatographic separation of Rifampicin was achieved on a C18 (4.6 x 250mm) analytical column with Acetonitrile & Water [HPLC Grade] in the ratio of 80:20 (v/v), as mobile phase at ambient temperature. The flow rate was 0.8 ml/min and UV detection was by absorption at 237 nm. The number of theoretical plates and tailing factor for Rifampicin were 5712 and 1.21 respectively. The linearity of the method was excellent over the range 10–100 ppm for the drug Rifampicin. The correlation coefficient was 0.9999. The percentage relative standard deviations of peak areas from five measurements were always less than 2%. The LOD for rifampicin was found to be 0.429 µg/ml. The LOQ for Rifampicin was found to be 4.125µg/ml. The drug content formulations were quantified by using the proposed analytical method. The developed HPLC method provides simple, accurate, Precise, reproducible and stability indicating for quantitative analysis for determination of rifampicin in bulk drug and pharmaceutical dosage form, without any interference from the excipients and in the presence of its acidic, alkaline, oxidative, dry and photolytic degradation products. Statistical tests indicate that the proposed HPLC method appear to be equally suitable for routine determination of rifampicin in the pharmaceutical dosage form in quality control laboratories, where economy and time are essential. This study is a typical example of the development of a stability-indicating assay, it is one of the rare studies where forced decomposition was done under all different suggested conditions and the degradation products were resolved. Hence, it is proposed for the analysis of the drug and degradation products in stability samples in industry. The method, however, is not suggested to establish the material balance between the extent of drug decomposed and formation of degradation products. As the method separates the drug from its degradation products. All these methods are simple, fast, accurate, precise, and economic and can be applied for routine analysis of drugs in the formulation and in bulk drug. So the proposed methods can be used for the routine quality control analysis of the bulk drugs as well as formulations.

INTRODUCTION

Tuberculosis is a chronic granulomatous disease and a major health problem in developing countries. It was first isolated in 1882 by a German physician named Robert Koch who received the Nobel Prize for this discovery. About one-third of world population is infected with *Mycobacterium tuberculosis.* In India, annually 2 million people develop the active disease every year. The therapeutic potential of Rifampicin (RIF) in tuberculosis is well recognized due to its unique ability to kill tubercle bacilli (*Mycobacterium tuberculosis*) when they undergo sporadic bursts of metabolism and growth. It can be used alone or in combination with other drugs, such as isoniazid (INH) and pyrazinamide, in the treatment of tuberculosis, leprosy and other infectious diseases especially those resulting from AIDS. Rifampicin acts by inhibiting bacterial *DNA –dependent RNA polymerase*, thus stopping the expression of bacterial genes. *Human RNA polymerase* is not inhibited. The drug is; Bactericidal and acts against both intro and extra-cellular organisms. Effective against tubercle bacilli resistant to other standard drugs and against some of the atypical mycobacterium; and the only drug, which acts on the persister's rifampin is readily absorbed from the gastrointestinal tract. Peak serum concentrations in healthy adults and pediatric populations vary widely from individual to individual. Following a single 600 mg oral dose of rifampin in healthy adults, the peak serum concentration averages 7 µg/ml but may vary from 4 to 32 µg/ml. Absorption of Rifampin is reduced by about 30% when the drug is ingested with food. Rifampin is widely distributed throughout the body. It is present in effective concentrations in many organs and body fluids, including cerebrospinal fluid. Rifampin is about 80% protein bound. Most of the unbound fraction is not ionized and, therefore, diffuses freely into tissues.

In healthy adults, the mean biological half-life of Rifampin in serum averages 3.35 hours after a 600 mg oral dose, with increases up to 5.08 hours reported after a 900 mg dose. With repeated administration, the half-life decreases and reaches average values of approximately 2 to 3 hours. The half-life does not differ in patients with renal failure at doses not exceeding 600 mg daily, and consequently, no dosage adjustment is required. Following a single 900 mg oral dose of rifampin in patients with varying degrees of renal insufficiency, the mean half-life increases from 3.6 hours in healthy adults to 5.0, 7.3, and 11.0 hours in patients with glomerular filtration rates of 30 to 50 ml/min, less than 30 ml/min, and in anuric patients, respectively

Currently, plasma levels of RIF are not monitored routinely in TB patients but it is clear that this would be advantageous if a simple and effective quantitative test were available. The high occurrence of tuberculosis in HIV infected subjects makes the management of HIV treatment complex. RIF is a very active antituberculosis drug that accelerates the metabolism of protease inhibitors. Due to the increasing necessity to monitor plasma concentrations in HIV patients with tuberculosis, different methods such as UV spectroscopy, Fluorimetry, Gas Chromatography, Polarography Amperometry and High-Performance Liquid Chromatography have been developed to measure RIF alone or in the presence of INH. These methods are expensive and need more expertise in experimentation. However, many of these methods suffer from limitations such as lengthy and tedious procedures, high sample volumes required, large quantities of solvents involved etc. The present study was undertaken with the objective to develop and validate a simple, sensitive HPLC assay procedure for the determination of Rifampicin in bulk as well as in pharmaceutical dosage form by modifying certain experimental conditions of the existing methods to enable good resolution of Rifampicin peak with shorter run time. After a number of trials using different combinations, we arrived at the present mobile phase and wavelength. In this paper, an accurate, reproducible and sensitive RP-HPLC method is described for the assay of rifampicin in bulk as well as in pharmaceutical dosage form. The assay involves the application of a C18 (4.6 x 250mm) analytical column with Acetonitrile and water [HPLC Grade] in the ratio of 80:20 (v/v), as mobile phase at ambient temperature, for the chromatographic separation and the use of UV detection.

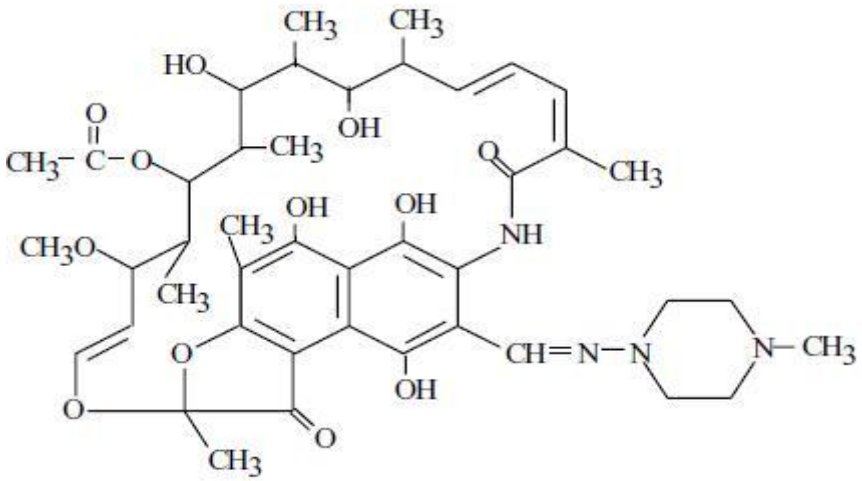

Fig. No. 1: The chemical structure of Rifampicin.

MATERIAL AND METHODS

Chemicals and Reagents Used:

The following chemicals were procured for the process: Water [HPLC Grade], Acetonitrile [HPLC Grade], and Rifampicin [Working standards] all the chemicals were procured from STANDARD SOLUTIONS and the tablets were collected from the Local market.

Apparatus and Chromatographic Conditions:

Equipment: High-performance liquid chromatography equipped with Auto Sampler and UV detector

Column: C18 (4.6 x 250mm, 3.5µm) or equivalent

Flow rate: 0.8 mL per min

Wavelength: 237 nm

Injection volume: 20 µl

Column temperature: Ambient

Runtime: 7min

Software: JASCO LC-4000Series HPLC

Model No: PU-4280.

MFD by: JASCO

Preparation of mobile phase:

The Mobile phase was prepared by mixing the Acetonitrile and Water (80:20) [HPLC Grade] and degassed in an ultrasonic water bath for 10 minutes. The resultant solution was filtered through 0.45 µ filter under vacuum filtration.

Diluents Preparation: The Mobile phase was used as Diluents.

Preparation of the Rifampicin Standard & Sample Solution:

Preparation of Stock solution:

The Stock solution was prepared by weighing accurately 10 mg of rifampicin [working standard] and transferred into a 10ml clean dry volumetric flask. About 7ml of the diluent was added to the volumetric flask and sonicated to dissolve it completely and the final volume was made up to the mark with the same solvent. From the above prepared Stock Solution pipette out 0.3 ml into a 10ml volumetric flask and the volume was made up to the mark with the diluent.

Sample Solution Preparation:

The Sample solution was prepared by weighing 5 tablets and calculated the average weight. Powdered the tablets and accurately weight, equivalent to 10 mg of Rifampicin [Sample] and transferred into a 10 ml clean dry volumetric flask. About 7ml of Diluent was added to the volumetric flask and sonicated to dissolve it completely and the final volume was made up to the mark with the same solvent. From the above prepared Stock Solution pipette out 0.3 ml into a 10ml volumetric flask and the volume was made up to the mark with the diluent. 20 µL of the standard, the sample was injected into the chromatographic system and measured the areas for the Rifampicin peaks and calculate the % assay by using suitable formulae.

System Suitability

The Tailing factor for the peaks due to Rifampicin in Standard solution should not be more than 1.5. The Theoretical plates for the Rifampicin peaks in Standard solution should not be less than 2000.

Assay

20µl of the sample solution was injected into HPLC under the conditioned described above.

The percent assay was calculated from the following formula;

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt.}{Label\ Claim} \times 100$$

Where

AT= Average area counts of Sample preparation.

AS= Average area counts of standard preparation

WS = Weight of working standard taken in mg.

WT =Weight of test taken in mg.

DS =Dilution of standard solution

DT =Dilution of sample solution

P = Percentage purity of working standard

System Suitability Results for Rifampicin:

1) The Tailing factor obtained from the standard injection was **1.46.**

2) The Theoretical Plates obtained from the standard injection was **5712.**

Assay Result for Rifampicin:

$$\frac{699483}{694667} \times \frac{10}{10} \times \frac{0.3}{10} \times \frac{10}{26} \times \frac{10}{0.3} \times \frac{99.8}{100} \times \frac{769}{300} \times 100 = 99.19\ \%$$

Validation development

1. Precision: It is a measure of the degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. (Table no.1)

Table no.1: The Precision result was summarized for the drug Rifampicin

Injection	Area
Injection-1	694877
Injection-2	695531
Injection-3	694977
Injection-4	694278
Injection-5	698676
Average Standard	695568
Deviation	1652.7
% RSD	0.27

Acceptance Criteria: The % RSD for the area of all the five standard injections should not be more than 2%.

2. Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on a different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. (Table no 2)

Table no.2: The Ruggedness result was summarized for the drug Rifampicin

Injection	Area
Injection-1	693012
Injection-2	692238
Injection-3	694880
Injection-4	694643
Injection-5	695446
Average Standard	694445
Deviation	1077.5
% RSD	0.17

Acceptance Criteria: The % RSD for all the five standard injections results should not be more than 2%

3. Accuracy: Proposed method when used for extraction and subsequent estimation of Rifampicin from the pharmaceutical dosage form after spiking with additional drug afforded recovery of 98-102% and mean recovery for Rifampicin from marketed formulation are listed in table No.3.

Table No. 03: Recovery results for Rifampicin

Excess drug added to the analyte (%)	**Theoretical content(µg)**	**Recovery (%)**
0	100	100.03
80	80	99.72
100	200	100.02
120	120	100.04

Acceptance Criteria: The % Recovery for each level should be in between 98.0 to 102.0

4. Linearity: It is the ability of the method to elicit test result that is directly proportional to an analytic concentration within a given range Rifampicin showed linearity in the concentration range of 10 µg/ml to 100 µg/ml (r^2 =0.999) for HPLC. For HPLC method, the linearity of calibration graphs and adherence of the system to Beer's law was validated by the higher value of correlation coefficient.

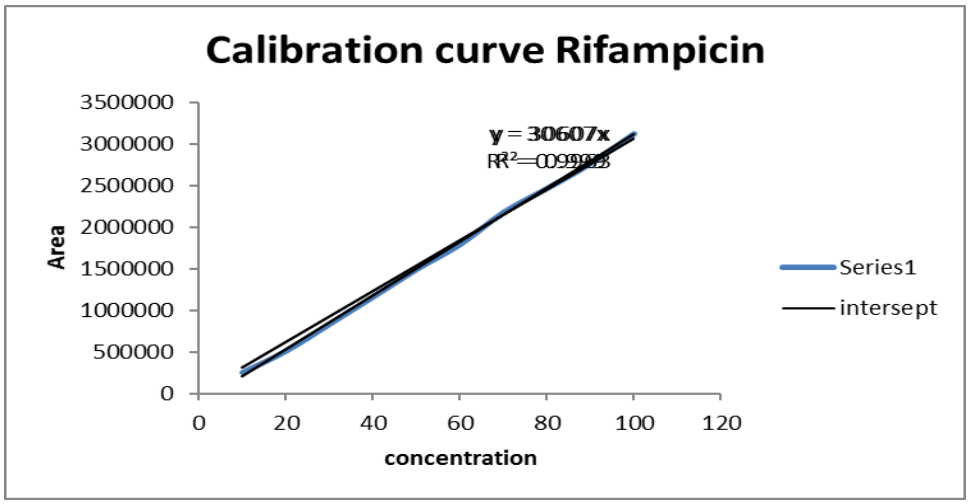

Fig 1: calibration curve for Rifampicin standard

Acceptance Criteria: The Correlation coefficient should not be less than 0.999.

Table No.04 Linear regression data for calibration curve

Parameters	**Rifampicin**
Linearity range	10-100 µg/ml
R^2	0.999
Slope	30607
S.E. of estimation	0.221

5. Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample**,** which can be detected but not necessarily quantities as an exact value.

Limit of Detection for Rifampicin:
Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank :45 µV
Signal Obtained from LOD solution :128 µV

S/N= 128/45=2.84 µg/ml

Acceptance Criteria: The S/N Ratio value should be 3 for LOD solution

6. Limit of Quantification: It is defined as the lowest concentration of the analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio

Limit of Quantification for Rifampicin:
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank : 45 µV
Signal Obtained from LOQ solution : 425 µV
S/N=425/45=9.44

Acceptance Criteria: The S/N Ratio value should be 10 for LOQ solution.

7. Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact of the method. Each factor selected to examine were changed at three levels (-1,0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n=5) of mixed standard solution were performed under small changes in chromatographic conditions. Results presented in table No.6 indicate that the selected factors remain unaffected by small variation of these parameters.

Table No.06: Robustness Parameters

Robustness Parameters	% RSD (NMT 2.0)	Theoretical Plates	Tailing (0.8-2.0)	Remarks
Flow rate +10%	1.65	8720	1.65	Robust
Flow rate -10%	1.80	9365	1.82	Robust
Wavelength +5nm	1.85	10800	1.75	Robust
Wavelength -5nm	1.68	11550	1.81	Robust
P^H of mobile phase +0.2 units	1.88	13450	1.65	Robust
P^H of mobile phase -0.2 units	0.90	11570	1.65	Robust
Mobile phase composition +2%	1.79	12365	1.83	Robust
Mobile phase composition -2%	0.95	11452	1.65	Robust

RESULT & DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of the drug in pharmaceutical dosage forms. In order to develop a method under isocratic conditions, mixtures Acetonitrile and Water [HPLC grade] in different combinations were tested as mobile phase on a C18 (4.6 x 250mm) column. A binary mixture of Acetonitrile and Water [HPLC grade] in 80:20v/v proportion was proved the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention times obtained for the drug Rifampicin was 2.400. (Fig. no. 2)

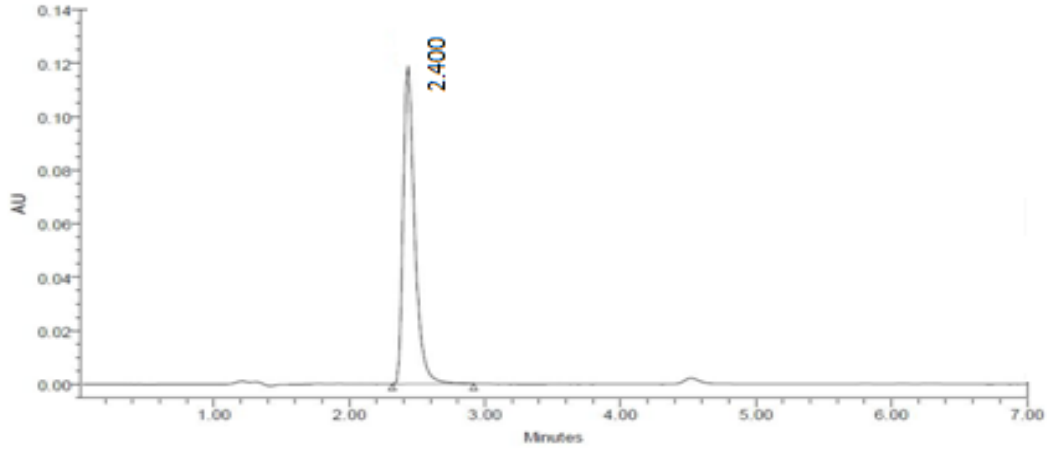

Fig. No. 2: Typical chromatogram of Rifampicin (RT=2.400 min).

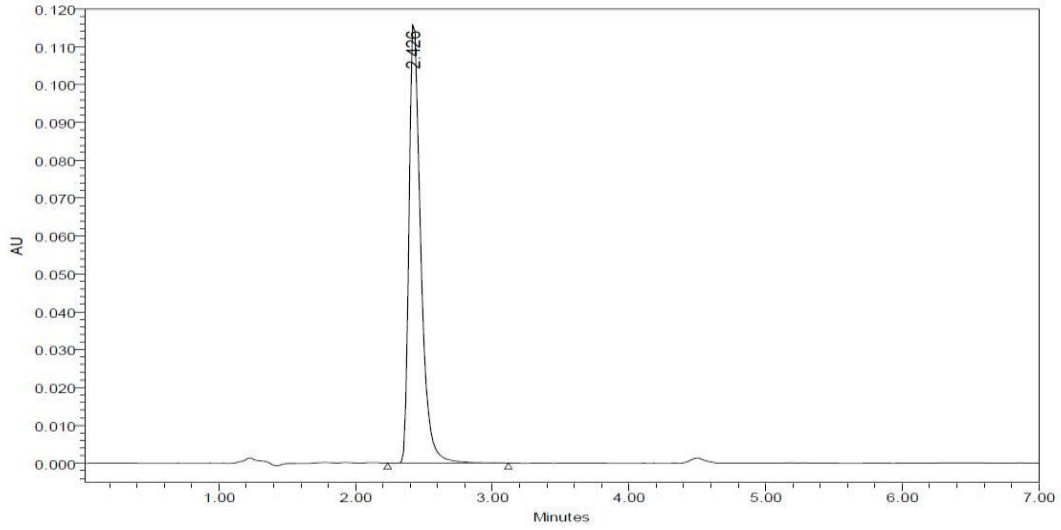

Fig. no. 3 A Typical Chromatogram for Rifampicin (Standard)

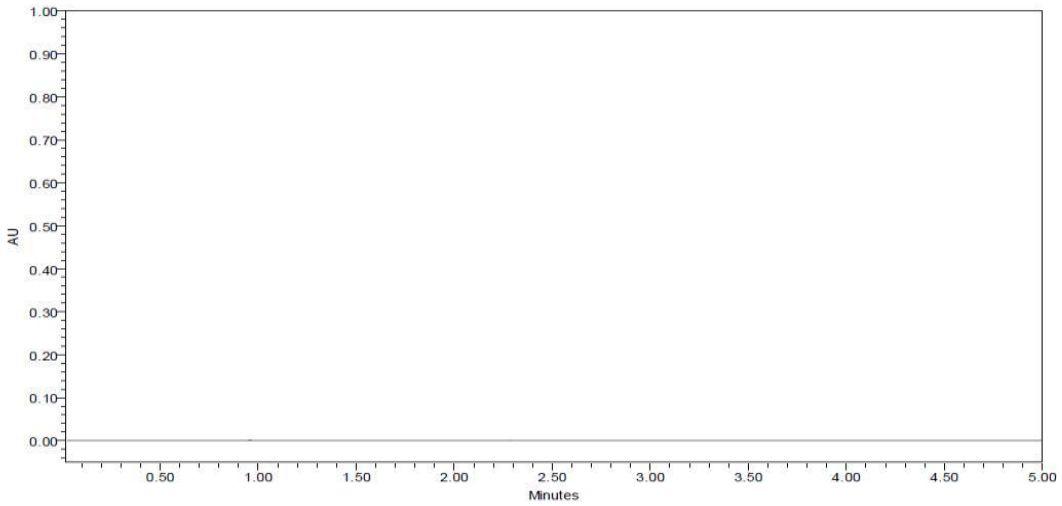

Fig. no. 4 A Typical Chromatogram for the Blank

The Precision data was represented in Table no. 1. The %RSD was found to be 0.70 and the chromatograph was represented in Fig. no. 5.

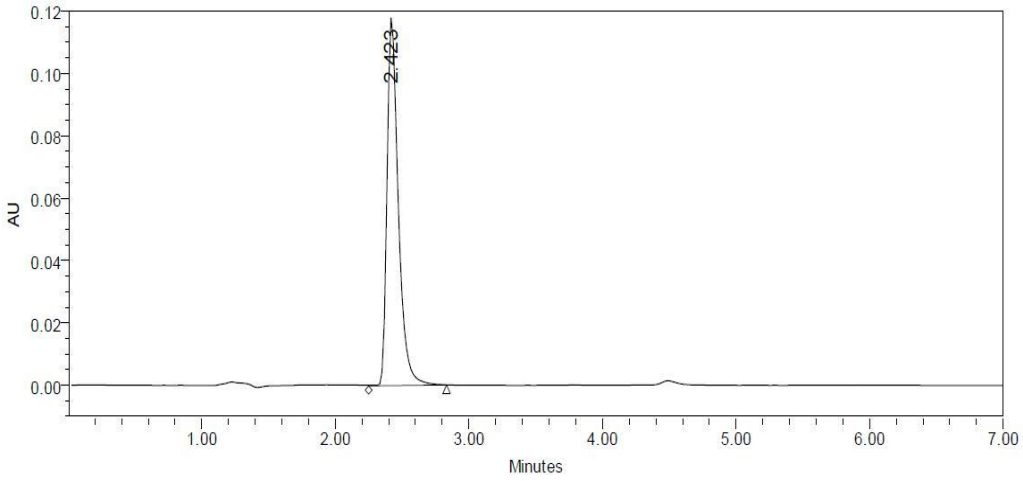

Fig. no. 5 A Typical chromatogram of Rifampicin (Precision)

When the drug Rifampicin was analyzed by the proposed method in the intra and inter-day (Ruggedness) variation, a low coefficient of variation was observed it was represented in Table no. 2. The %RSD was found to be 0.15 and the chromatogram was represented in Fig. no.6 which shows that the developed RP-HPLC method was highly precise.

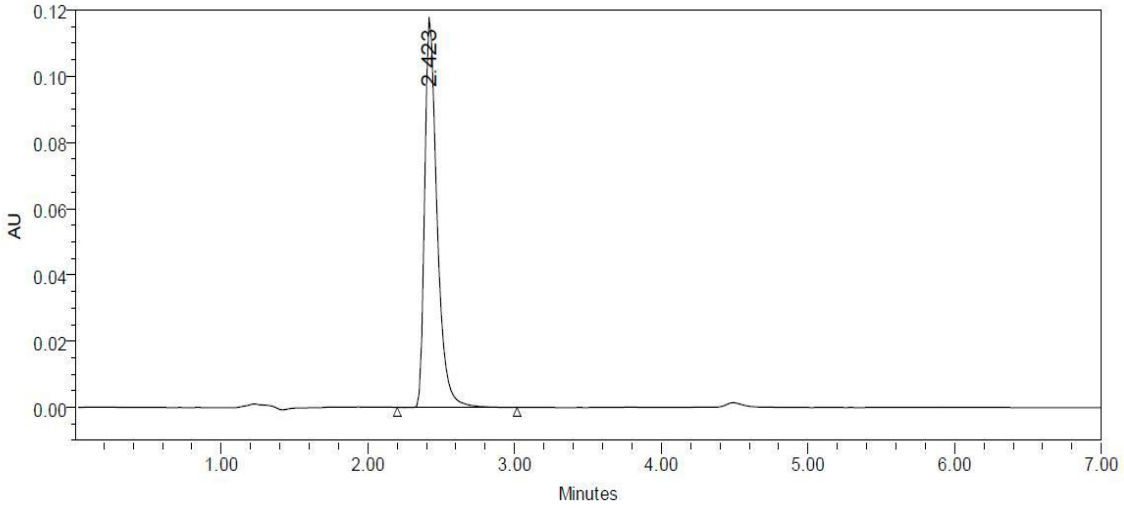

Fig. no. 6 A Typical chromatogram of Rifampicin (Intermediate Precision)

The Accuracy data were summarized in Table no. 3. The % Recovery was found to be 100.02% and the chromatograms for Accuracy 100% was represented in Fig. no. 7

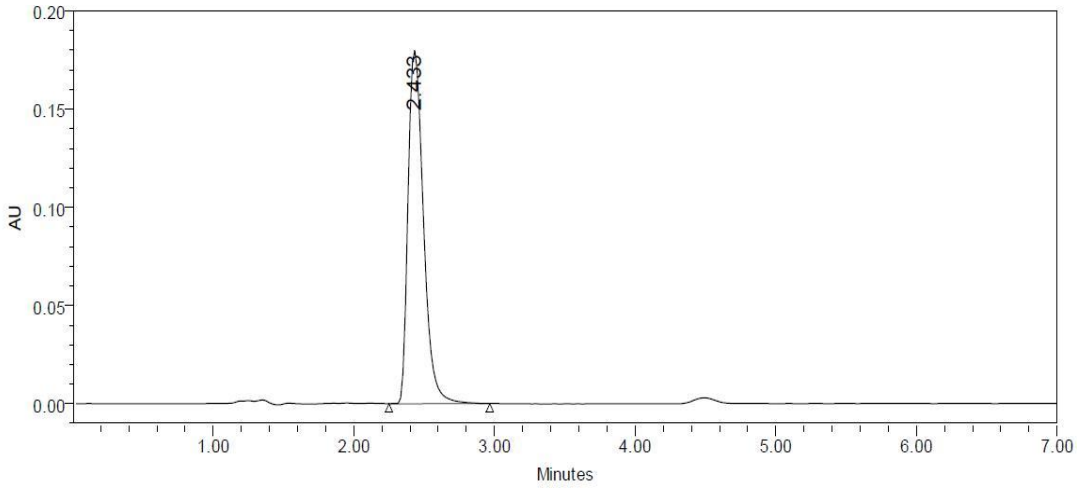

Fig. no. 7 A Typical chromatogram of Rifampicin (Accuracy 100%)

Linearity:

In order to test the linearity of the method, five dilutions of the working standard solutions of Rifampicin in the range of 20 to 100 ppm was prepared. The data were represented in Table no. 4. The Correlation Coefficient was found to be 0.996. Each of the dilutions was injected into the column and the Linearity Curve was represented in Fig. no.08.

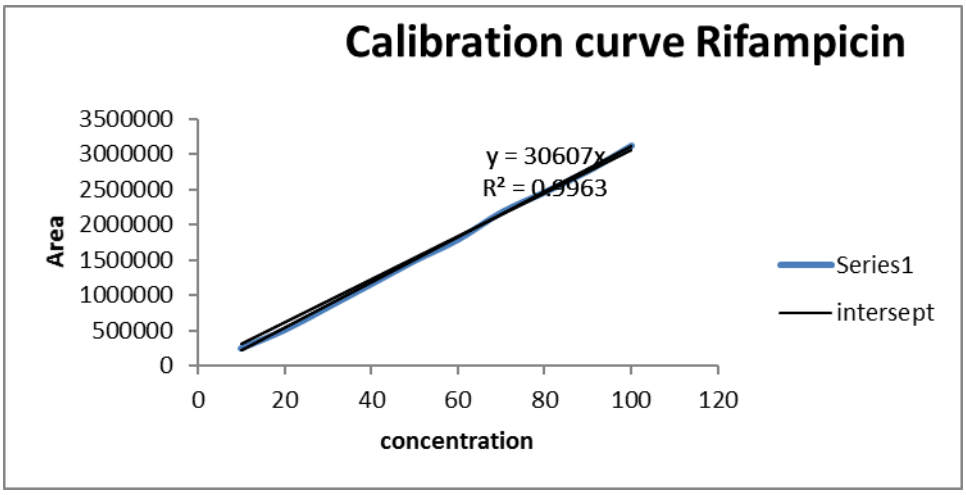

Fig. no. 08 Calibration Curve for the drug Rifampicin (Linearity)

Limit of detection and limit of quantification of the method were calculated basing on the standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD for Rifampicin was found to be 0.429µg/ml. The LOQ for rifampicin was found to be 0.085µg/ml. The drug content formulations were quantified by using the proposed analytical method. The chromatogram was represented in Fig. no. 09 & 10.

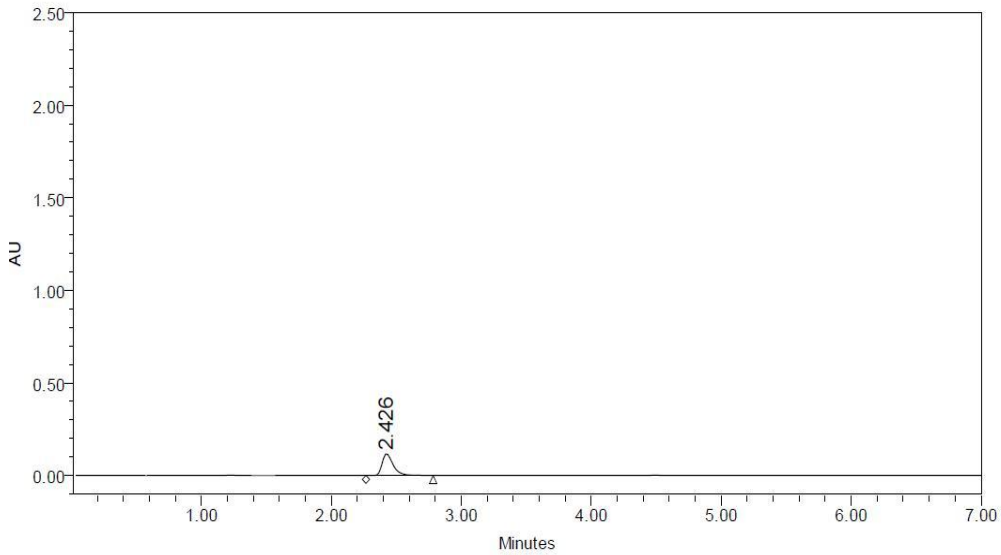

Fig. no. 09 Typical chromatogram of Rifampicin (LOD)

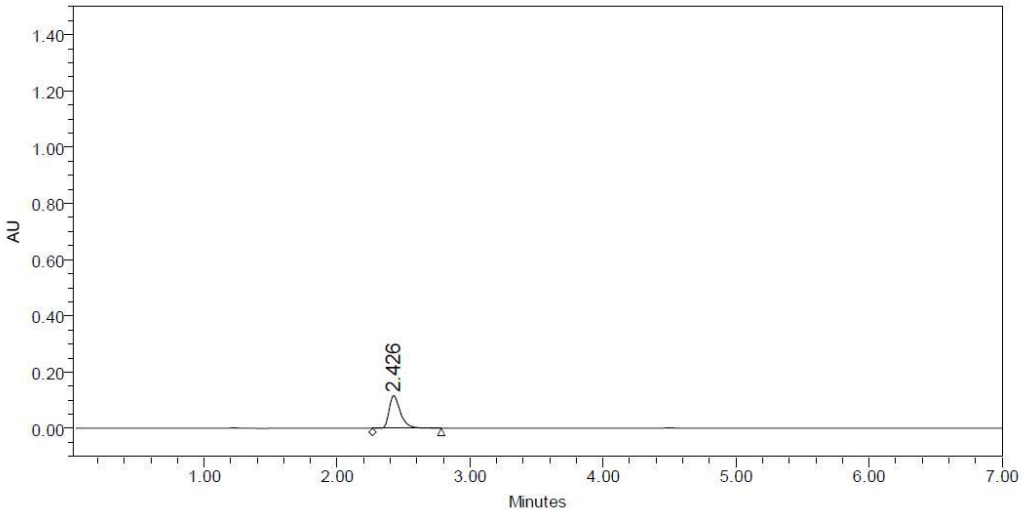

Fig. no. 10 Typical chromatograms of Rifampicin (LOQ)

Robustness:

Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Acetonitrile and Water in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in Table No. 5 & 6 and the chromatograms were represented in Fig. no. 11, 12, 13 &14.

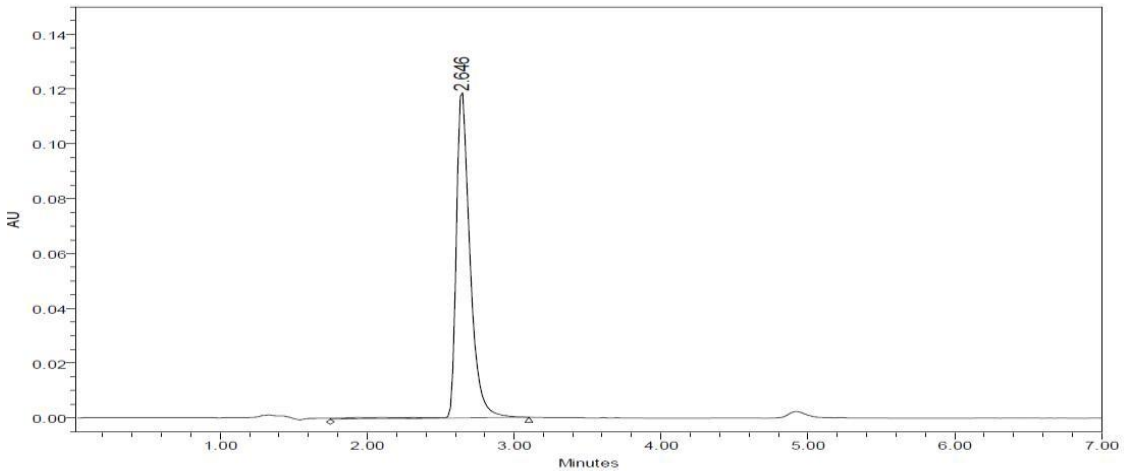

Fig. no. 11 A Typical chromatogram of Rifampicin for Robustness (Less flow rate)

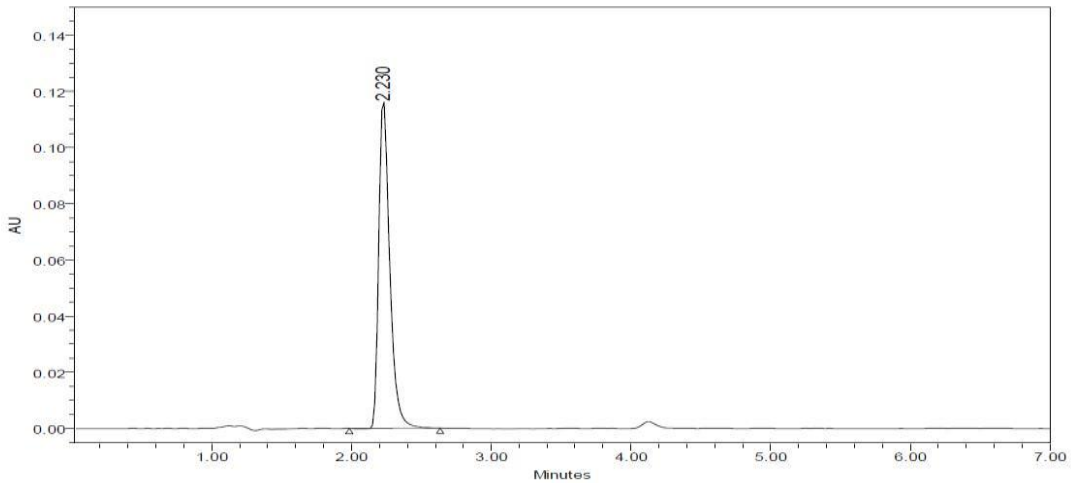

Fig. no. 12 A Typical chromatogram of Rifampicin for Robustness (More flow rate)

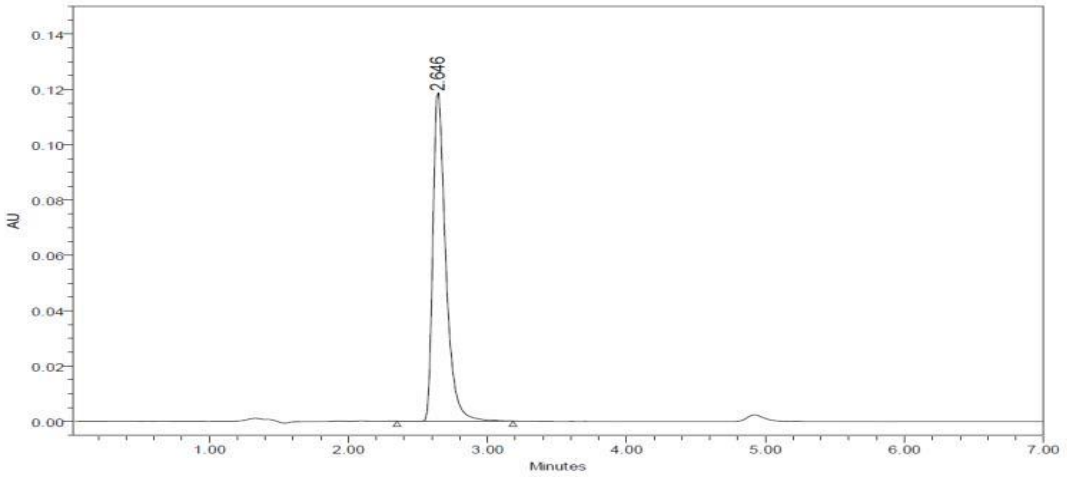

Fig. no. 13 A Typical chromatogram of Rifampicin for Robustness (Less organic Phase)

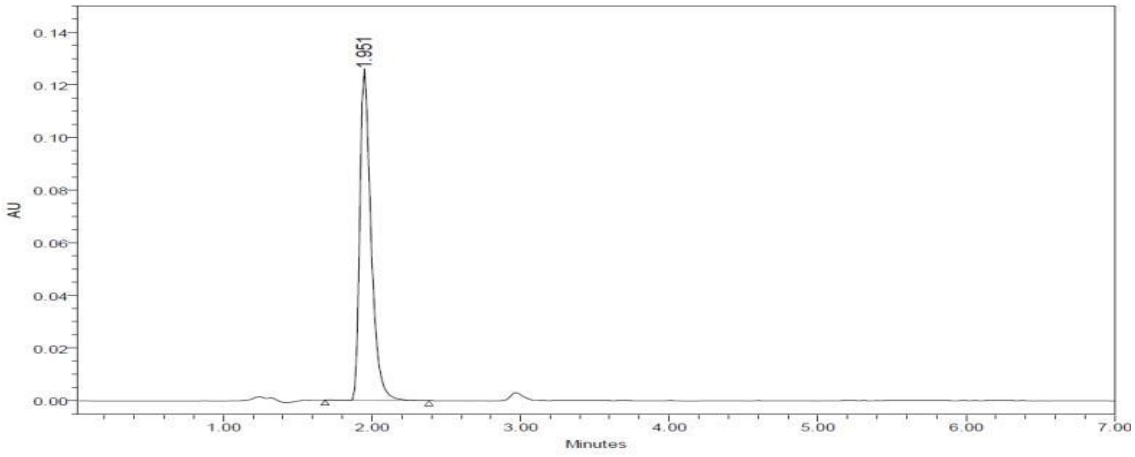

Fig. no. 14. A Typical chromatogram of Rifampicin for Robustness (More Organic Phase)

CONCLUSION

The above results clearly indicate that RP-HPLC can be applied for estimation for Rifampicin in Bulk and Pharmaceutical dosage form. The results can be calculated for the peak height and peak area but the precise and accurate results can be obtained by using peak area as compared to peak height. This HPLC method can be satisfactorily adopted wherever this instrument and facilities are available.

The developed HPLC method provides simple, accurate, Precise, reproducible and stability indicating for quantitative analysis for determination of Rifampicin in bulk drug and pharmaceutical dosage form, without any interference from the excipients and in the presence of its acidic, alkaline, oxidative, dry and photolytic degradation products. Statistical tests indicate that the proposed HPLC method appear to be equally suitable for routine determination of Rifampicin in the pharmaceutical dosage form in quality control laboratories, where economy and time are essential. This study is a typical example of the development of a stability-indicating assay, it is one of the rare studies where forced decomposition was done under all different suggested conditions and the degradation products were resolved. Hence, it is proposed for the analysis of the drug and degradation products in stability samples in industry. The method, however, is not suggested to establish the material balance between the extent of drug decomposed and formation of degradation products. As the method separates the drug from its degradation products

All these methods are simple, fast, accurate, precise, and economic and can be applied for routine analysis of drugs in the formulation and in bulk drug. So the proposed methods can be used for the routine quality control analysis of the bulk drugs as well as formulations.

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