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Original Research Article

A stability indicating RP-HPLC method development and validation for simultaneous quantification for assay of rifampicin in pharmaceutical solid dosage form

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ABSTRACT

A cost effective stability indicating reverse phase high performance liquid chromatography (RP-HPLC) for simultaneous quantification for assay of Rifampicin in pharmaceutical solid dosage form (tablets) was developed and validated. Chromatographic procedure was conducted using stationary phase packing c18 column (Luna), having 5 μm particle size with inner diameter (250 x 4.6) mm, mobile phase consists of 60% acetate buffer (pH 4.5) and 40% acetonitrile (HPLC grade of sharlau manufacturer). The detection wavelength was uv 254 nm with flow rate of 1.0 ml/min and injection volume was 10 μL with run time 10 min. Based on proposed method validation was conducted following system precision, specificity or selectivity, method precision, accuracy, linearity, robustness, solution stability, limit of quantitation (LOQ), limit of detection. It was observed the system (Waters, HPLC) was precise enough to quantify Rifampicin, no interference of blank, placebo or other known impurity at the elution zone of Rifampicin; % RSD for six sample solutions of Rifampicin was 0.9% (% average assay: 98.7%); accuracy observed 101.1% for 80% recovery, 100.4% for 100% recovery and 100.5% for 120% recovery; co-relation coefficient of linearity (r =1.000); limit of quantification (LOQ) found 0.18 ppm.

1. Introduction

Rifampicin is an antibiotic used for the treatment of tuberculosis and other infectious disease derived from *streptomyces mediterranei.* [1] Tuberculosis is nothing but a deadly infectious disease that is responsible for two million people death each year. Rifampicin is treated as the key to the current treatment of tuberculosis due to its active sterilizing ability. [2] Rifampicin is an antimicrobial drug for *Mycobacterium tuberculosis.* [3] In order to ensure its quality, International Pharmacopoeia (IP), British Pharmacopoeia (BP), United State of Pharmacopoeia (USP) narrated analytical procedure. [4–6] According to the outline of literature survey, high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), visible spectrophotometry method available for the quantification of Rifampicin (Figure 1: molecular structure of Rifampicin) in pharmaceutical solid dosage form. [7]

The purpose of this study is to develop and validate a stability indicating reverse phase high performance liquid chromatography (RP-HPLC) for assay quantification of Rifampicin in pharmaceutical solid dosage form.

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2. Materials and Methods

2.1. Instrument and chromatographic condition

The chromatographic condition was performed into waters Alliance HPLC 2695 system with PDA detector (USA) connected with empower 3 software; column: 4.6 x 250 mm inner diameter (ID), 5μm with particle size (Phenomenex Luna); flow rate: 1.0 ml/min; injection volume: 10μL, detector: uv 254 nm with run time 15 minutes.

2.2. Chemicals, reagent and standard

Rifampicin working standard (potency: 100.0%) used of Olon Active Pharmaceuticals manufacturer, acetonitrile and methanol used for HPLC grade of Scharlau manufacturer, sodium hydroxide and sodium acetate trihydrate used for analytical grade of scharlau manufacturer, glacial acetic acid used for analytical grade of Merck manufacturer.

2.3. Method development

Based on solubility of Rifampicin (at pH 7.06 solubility of Rifampicin 0.85 mg/ml),[8] maximum absorbance, pKa value of Rifampicin (Zwitterions with pKa 1.7 related to the 4-hydroxy and pKa 7.9 related to the 3-piperazine nitrogen),[9] molecular weight of Rifampicin and polarity of Rifampicin; detection and quantification reverse phase high performance liquid chromatography (RP-HPLC) method was optimized. The proposed detection and quantification technique was; mobile phase (60% acetate buffer pH 4.5 with 40% acetonitrile), diluent (60% phosphate buffer pH 7.0 with 40% methanol), wavelength 254 nm.

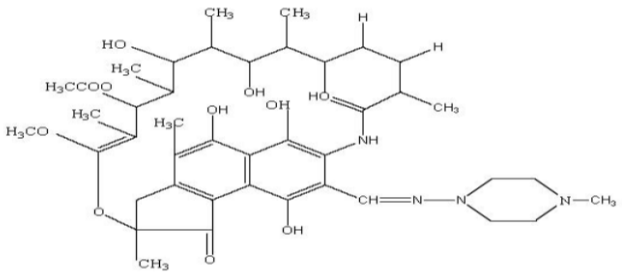

Fig. 1: Molecular structure ofRifampicin[9]

3. Preparation of Standard Solution

Accurately taken 20 mg of Rifampicin working standard into 100 mL volumetric flask. Added 60 ml of diluent with sonication for 10 minutes. Cooled to room temperature. Diluted up to the mark with diluent. Concentration 0.20 mg/ml

3.1. Preparation of sample solution

Taken 20 tablets and crushed them uniform. Accurately taken 200 mg equivalent of Rifampicin into 1000 ml volumetric flask. Added 200 ml of diluent and mechanically shaked at 200 rpm for 10 minutes. Added 600 ml of diluent to the same volumetric flask and sonicated for 15 minutes with intermittent shaking. Cooled to room temperature and diluted up to the mark with diluent. Centrifuged the solution at 7000 rpm for 3 minutes. Taken the supernatant solution for HPLC vial. Concentration: 0.20 mg/ml

3.2. Method validation

According to ICH Q2R1 analytical method validation guideline, this method was validated considering system precision, specificity, linearity, accuracy, method precision and intermediate precision.[10]

3.3. System precision

System precision of reverse phase high performance liquid chromatography (RP-HPLC) was confirmed by five replicate injection of standard solution for Rifampicin. The main objective of system precision was to identify whether the system for analysis was ready or not. Figure 2 for representative chromatogram of standard solution for Rifampicin.

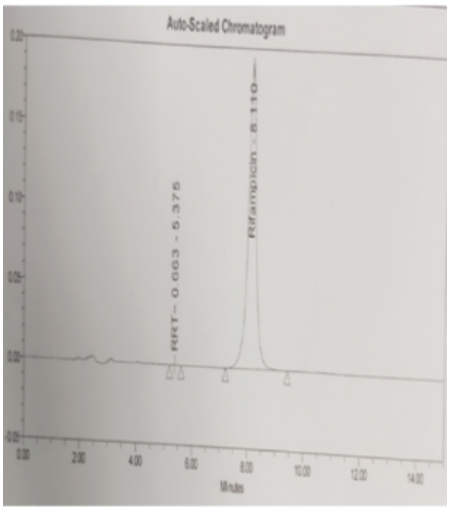

Fig. 2: Chromatogram of standard solution

3.4. Specificity

In order to conduct specificity study, Rifampicin quinone impurity 0.005 mg/ml, placebo, blank and test solution was run into the HPLC system. The aim of this study was to detect any interference available or not for impurity, blank and placebo at the elution zone of Rifampicin and also to confirm the peak purity of Rifampicin in test solution.

Figures 3 and 4 for impurity and peak purity chromatogram.

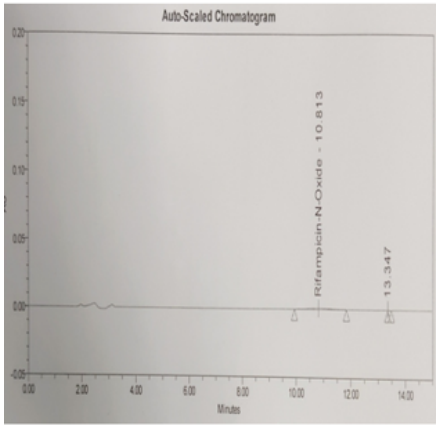

Fig. 3: Chromatogram of impurity

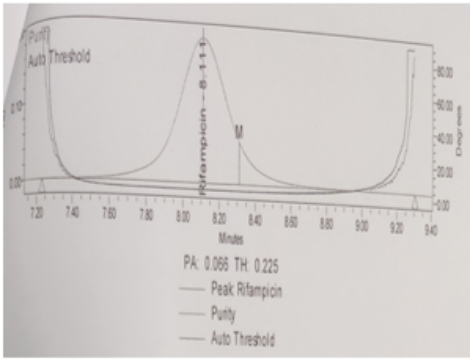

Fig. 4: Peakpurity of Rifampicin in test solution

3.5. *Linearity*

Linearity was estimated by injecting five standard solution ranges from concentration 0.15 mg/ml to 0.30 mg/ml covering 50% to 150% with respect to 100% standard concentration 0.20 mg/ml. Figure 5 for calibration curve of linearity study.

3.6. *Method precision*

Six independent sample solutions were prepared for method precision study using waters (USA) PDA reverse phase high performance liquid chromatography (RP-HPLC). Table 3 tabulated the method precision study results.

3.7. *Intermediate precision*

Intermediate precision study was executed in different day using six different sample solutions in Agilent (USA) PDA reverse phase high performance liquid chromatography. Table 3 for intermediate precision result.

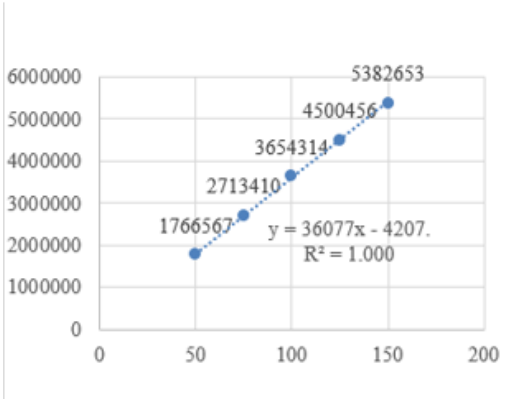

Fig. 5: Calibrationcurve of linearity

3.8. *Accuracy*

80%, 100% and 120% of Rifampicin spiked solution was prepared for 9 determinations to calculate the accuracy of the analytical method. Table 3 showed the results for accuracy results.

4. Limit of quantification

Diluent solution of standard solution was prepared to calculate the sensitivity of the method based on signal to noise ration. On 0.18 ppm concentration of Rifampicin standard solution the signal to noise ration observed 47. So, the sensitivity of the method claimed for 0.18 ppm.

5. Results and Discussion

5.1. *System precision*

Table 1: % RSD for replication five injection of standard solution

Standard solution	
No of injection	**Area/Response**
1	3803514
2	3758007
3	3833797
4	3763527
5	3814269
Average	3794623
standard deviation	32815.1
% RSD	0.86

Comment: The system was precise enough to detect and quantify of Rifampicin.

5.2. *Specificity*

The method was specific and sensitive for Rifampicin peak detection and peak purity of Rifampicin passed. No interference observed at the elution zone of placebo, blank and impurity solution. Purity angle of Rifampicin was 0.066

and purity threshold was 0.225 for test solution.

5.3. Linearity

Table 2: Correlation coefficient and regression analysis

Linearity range (mg/ml)	0.10 mg/ml to 0.30 mg/ml
Regression equation (y=mx +c)	36077x - 4207
Slope (m)	36077
Intercept (c)	-4207
Correlation coefficient, R^2	1.000

Comment: This method showed linear result within executed concentration of Rifampicin standard solution. So, the range of this method should be 0.10 mg/ml to 0.30 mg/ml concentration.

5.4. Method precision and intermediate precision

Table 3: Method precision & intermediate precision results

Sample Number	Method precision (Water PDA System)	Intermediate precision (Agilent PDA System)
	% Assay	% Assay
1	98.5	97.7
2	97.3	99.5
3	99.5	100.6
4	99.4	99.7
5	99.1	99.8
6	98.1	99.8
Average	98.7	99.5
% RSD	0.9	1.0

Comment: Both method precision and intermediate precision found satisfactory results. So, this method is precise and suitable for Rifampicin assay quantification in pharmaceutical solid dosage form.

5.5. Accuracy

Table 4: Nine determinations of accuracy results

Sample Name	% Recovery	Average Recovery
80%_accuracy sample_1	99.7	
80%_accuracy sample_2	102.1	101.1
80%_accuracy sample_3	101.5	
100%_accuracy sample_1	100.2	
100%_accuracy sample_2	100.3	100.4
100%_accuracy sample_3	100.6	
120%_accuracy sample_1	99.84	
120%_accuracy sample_2	100.91	100.5
120%_accuracy sample_3	100.65	

Comment: The recovery results of 80%, 100% and 120% concentration of Rifampicin found satisfactory.

6. Conclusion

Based on above analytical data, it can be concluded that this reverse phase high performance liquid chromatography (RP-HPLC) method is stability indicating, sensitive, specific for Rifampicin peak detection and quantification, precise and accurate for assay estimation in pharmaceutical solid dosage form.

7. Source of Funding

None.

8. Conflict of Interest

None.

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