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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RIBAVIRIN IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for estimating stability of Ribavirin in tablet dosage form. Chemically, Ribavirin is 1-beta-D-Ribofuranosyl-1, 2, 4-triazole-3-carboxamide. The chromatographic separation of Ribavirin was achieved using C18 (250x4.6mm) column with a mobile phase containing a mixture of Acetonitrile: Water (60:40 v/v). The flow rate was 1ml/min and effluent was monitored at 218.0 nm. The retention time for Ribavirin was found to be 2.70 min. The relative standard deviation for intraday and interday precision in tablet was always less than 2%. The method was validated for linearity, range, precision, accuracy, specificity, selectivity, intermediate precision, ruggedness, robustness, stability and suitability.

KEYWORDS: Ribavirin, Reverse phase HPLC, Accuracy, Precision, Robustness, LOD, LOQ, Specificity.

INTRODUCTION

Ribavirin (RIB) is used as Antiviral[1]. Chemically it is 1-beta-D-Ribofuranosyl-1, 2, 4-triazole-3-carboxamide.[1] Its molecular formula and molecular weight are C8H12N4O5 and 244.2 respectively. Ribavarin is extremely water soluble. It is more stable in acidic medium. Literature survey reveals that many analytical methods such as UV spectrophotometric[2,3] and HPLC methods[3-7] are reported for determination of Ribavarin individually from pharmaceutical dosage form and HPLC[8] methods are reported for determination of RIB with other drugs in combined dosage form.

The aim of this work was to develop a simple, accurate, reproducible and sensitive method for determination of Ribavarin using rapid, convenient and simple reverse phase HPLC method.

MATERIALS AND METHODS

Chemicals and reagents

Pure sample of Ribavarin was procured from Emcure Pharmaceuticals Ltd., Pune. Methanol (HPLC grade), Ortho-phosphoric acid (AR grade) and Acetonitrile (HPLC grade) were obtained from Qualigen Laboratories Pvt. Ltd., Mumbai.

S. No.	Instrument	Make/Model
1	HPLC	Agilent 1120 Compact LC
2	UV Spectroscopy	Shimadzu-1700 UV/VIS
3	Balance	LC/GC
4	Ultrasonic bath	Life care

Instruments used

Optimization of Chromatographic conditions

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for Ribavirin. The combination of mobile phase methanol: water (50:50, 70:30 and 80:20 (v/v) was tried. The mobile phase Acetonitrile: water (50:50, 60:40, 80:20 v/v) was also tried. Acetonitrile: Water (60:40 v/v) at a flow rate of 1.0 ml/min was found to be satisfactory and gave symmetric and well resolved peaks for Ribavirin. The chromatogram was recorded at 218.0 nm as spectrum of Ribavirin showed maximum response at this wavelength.

Chromatogram showed symmetrical peaks with good shapes; tailing factor for Ribavirin was within range & the resolution of standard drug was satisfactory. Retention time for Ribavirin was found to be 2.70 min. The system suitability parameters observed by using this mobile phase are reported.

Preparation of mobile phase

HPLC grade Acetonitrile & HPLC grade Water (60:40v/v) was filtered through 0.45 µm membrane filter & sonicated on ultrasonic bath for 15 min

Preparation of standard stock solution

Ribavirin standard stock solution was prepared by transferring 10 mg of Ribavirin working standard into a 100 ml volumetric flask, approximately 30 ml of HPLC grade distilled water was added and sonicated for 20

min. The volume was made up to 100 ml with distilled water to get the concentration of 100 µg/ml. This solution was filtered through a 0.45µm pore size Nylon 66 membrane filter.

Selection of analytical wavelength

Here λ_{max} of Ribavirin was found to be 218.0 nm. Hence 218.0 nm was selected as wavelength of analysis.

Calibration curve

Appropriate aliquots of standard stock solutions of RIB were diluted with mobile phase to obtain concentrations in the range of 0.5, 1, 2, 4, 6, 8, 10 and 12µg/ml of RIB. Each solution was injected into HPLC system (Fig.1). The calibration curve of RIB was constructed by plotting peak area verses concentration separately. The linearity of RIB was found to be in the concentration ranges of 0.5-12 µg/ml. (Table 1), at their respective maximas. The coefficient of correlation was found to be 0.9990 for RIB (Table 1).

Analysis of Tablet formulation

The marketed tablet formulation containing 400 mg of Ribavirin was used. Twenty tablets were accurately weighed, transferred to a clean and dry mortar and ground into a fine powder. The quantity of powder equivalent to 10 mg of Ribavirin was weighed accurately and transferred to 100 ml volumetric flask and 30 ml of distilled water was added. The contents were sonicated for 20 min and made up to the mark with the distilled water. This solution was filtered through a 0.45µm pore size Nylon 66 membrane filter. From this stock solution, 0.4, 0.6, 0.8 and 1 ml solution was pipetted out and transferred to 10 ml volumetric flask separately and made volume up to the mark with mobile phase to get the concentration 4, 6, 8 and 10 µg/ml of RIB. The solution was injected into HPLC system (Fig.3). The results of the assay of tablet formulation and its statistical validation data is given in Table 3.

RESULTS AND DISCUSSION

Ribavirin was well resolved using mobile phase composition of Acetonitrile: Water (60:40 v/v) at flow rate of 1 ml/min, UV detection wavelength 272.0 nm and injection volume 20 µl. The HPLC system was found to best for analysis. The retention time for Ribavirin was found to be 2.70 min.

Table 1: Linearity of Ribavirin.

Sr. No	Concentration (µg/ml)	Peak Area* (mAU)
1	0.5	58260
2	1	105520
3	2	212040
4	4	436150
5	6	699120
6	8	932160
7	10	1165200
8	12	1398240

***Average of Three determinations.**

Table 2: System Suitability Parameters.

Area*	625836
Retention Time*	2.70 mins.
Theoretical Plates*	3654
Tailing Factor*	1.14
Regression equation	y = 11733x - 10829
Slope	11733
Intercept	10829
Correlation coefficient(r^2)	0.9996
Limit of Detection (µg/ml)	0.0035
Limit of Quantitation (µg/ml)	0.0102

***Average of three determination.**

Table 3: Analysis of Tablet Formulation.

Tablet sample	Label claim (mg/tablet)	Amount found* (mg/tablet)	% Label claim found*	± Standard deviation	Standard error
RIB	400	398.85	99.71	0.1264	0.0423

***Average of six determination Brand: Rebetol.**

Table 4: Intra-Day Precision.

Conc. (µg/ml)	Peak Area	Conc. Found (µg/ml)	% Purity	S.D	% R.S.D
10	1165202	9.93	99.30	0.0051	0.0052
10	1165212	9.93	99.30		
10	1165213	9.94	99.40		
10	1165208	9.94	99.40		
10	1165211	9.93	99.30		
10	1165200	9.93	99.30		

Table 5: Inter –Day Precision.

Day	Peak Area	Conc. (µg/ml)	% Purity*	S.D*	% R.S.D*
D-1	1165210	10	99.31	0.020736	0.020876
D-2	1165213	10	99.31	0.030111	0.030311
D-3	1165206	10	99.31	0.026833	0.027011

***Average of six determinations**

Table 6: Accuracy.

Level of recovery	Amount present (mg)	Amt of Std Added (mg)	Amt of drug recovered(mg)	%Recovery	Average of determinations
80%	400	320	319.87	99.95	
	400	320	319.75	99.92	99.93
	400	320	319.68	99.90	
100%	400	400	399.67	99.91	
	400	400	399.72	99.93	99.94
	400	400	399.83	99.95	
120%	400	480	479.87	99.97	
	400	480	479.76	99.95	99.95
	400	480	479.82	99.96	

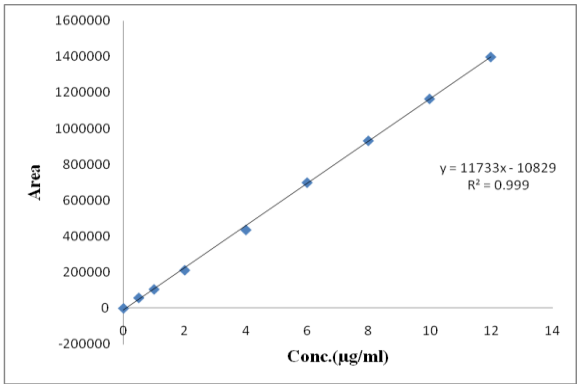

Fig. 1: Linearity of Ribavirin.

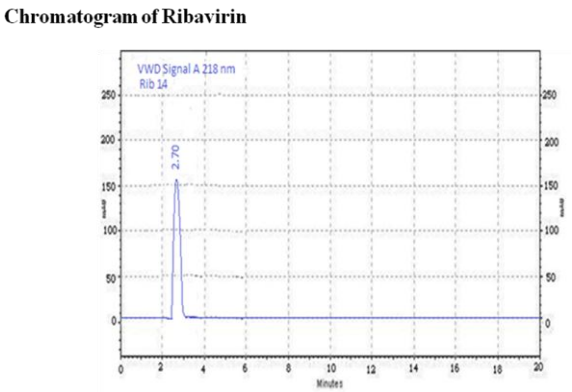

Fig. 2: Chromatogram of Ribavirin.

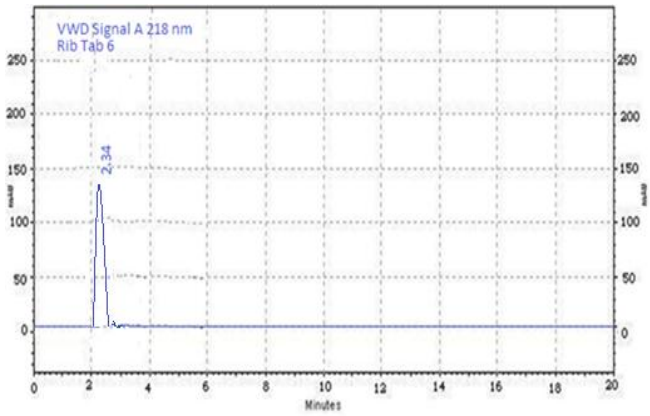

Fig. 3: Chromatogram of Ribavirin in Tablet Formulation.

METHOD VALIDATION[9,10,11]

1. **Specificity:** The specificity of HPLC method was ascertained by analyzing standard drug and sample solutions. The retention time of Ribavirin (RIB) the sample solution were confirmed by comparing with that of the respective standards.
2. **Linearity:** The linearity for RIB was selected at 0.5-12 µg/ml. The correlation coefficients were selected at 0.9990. The results are shown in Table 1.
3. **Limit of detection (LOD):** The limit of detection was determined by the analysis of sample with known concentrations of analyte and by establishing

the minimum level at which the analyte can be detected. The results are shown in Table 2.

4. **Limit of Quantitation (LOQ):** Limit of Quantitation was determined by the analysis of sample with known concentration of analyte and by establishing the minimum analyte can be reliably quantities.(Table 2).
5. **System suitability:** System suitability test are integral part of method development and are use to ensure adequate performance of chromatographic system. Retention Time (Rt), Theoretical plates (N), Tailing factor (T) were evaluated for six replicate injections of the drug at concentration of 10µg/ml. The results are shown in Table 2

Precision

Repeatability: In this study, six replicates injections of standard solution were injected for repeatability. The repeatability data was expressed in terms of % R.S.D. & was found to be less than 2%. The results are shown in Table 4.

Intermediate precision: The precision of the method was demonstrated by Intra-day and inter-day precision variation studies. In the intra-day studies, six repeated injection of standard solution was made and the response factor of drug peak and % R.S.D were calculated. In the inter day variation studies six repeated injection of standard stock solution was made for after 24 hours to 48 hours and response factor of drug peak and % R.S.D was calculated[7,8]. From the data obtained the developed method was found to be precise. The results are shown in Table 5.

6. **Accuracy:** Recovery studies were carried out by applying the method to drug content present in tablet dosage form to which known amount of Ribavirin was added at 80%, 100%, 120% levels. The recovery study was performed three times at each level. The results of recovery study along with its statistical validation are given in Table No**.** 6

7. **Robustness:** The robustness of study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors chosen for this study were the flow rate (±0.1ml/min), mobile phase composition Acetonitrile and water (60:40 %v/v) and temperature 25^0C.[12,13]

CONCLUSION

The reversed phase-HPLC method developed was fully validated showing satisfactory data for all the method validation parameters tested. A simple, rapid, reproducible, accurate and precise RP- HPLC method was developed for the quantitative estimation of Ribavirin in tablet dosage form. The proposed method can be conveniently used by quality control department to determine the assay of pharmaceutical preparations.

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