A RAPID RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION RIBAVIRIN IN TABLETS

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ABSTRACT

Objective: To develop an accurate, precise and linear Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method and validate as per ICH guidelines for the quantitative estimation of Ribavirin (200mg) in tablets.

Methods: The optimized method uses a reverse phase column, Enable Make Kromasil C18 (250 X 4.6 mm; 5μ), a mobile phase of phosphate buffer (pH 4.2): acetonitrile in the proportion of 85:15 v/v, flow rate of 1.0 ml/min and a detection wavelength of 215 nm using a PDA detector.

Results: The developed method resulted in Ribavirin eluting at 2.606 min. Ribavirin exhibited linear in the range 25-150μg/ml. The precision is exemplified by the relative standard deviation of 0.4%. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.24ng/ml and 0.73ng/ml respectively.

Conclusion: An accurate, precise and linear RP-HPLC method was developed and validated for the quantitative estimation of Ribavirin in VIRAZIDE (200mg) tablets as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

Keywords: RP-HPLC, Ribavirin, Method development, Validation, Estimation.

INTRODUCTION

Ribavirin is (fig. 1 1-β-D-ribofuranosyl-1H-1, 2- 4triazole-3-carboxamide) a nucleoside anti metabolite antiviral agent that blocks nucleic acid synthesis and used against both RNA and DNA viruses [1]. Ribavirin is used for a variety of viral hemorrhagic fevers like Lassa Lassa fever, Crimean-Congo hemorrhagic fever Crimean-Congo hemorrhagic fever, Venezuelan hemorrhagic fever, hantavirus infection chronic hepatitis C respiratory syncytial virus [2-4].

Ribavirin has been commercially available in 40mg, 200mg, 400mg, 600mg of tablet strengths and 200mg capsule strength. Brand names include Copegus, Rebetol, Ribasphere, Vilona, and Virazole. Ribavirin is an odorless powder. It is soluble in water, ethanol and dimethyl sulfoxide, slightly soluble in alcohol. Its chemical formula is C₉H₁₂N₄O₅ and molecular weight is 244.204 [6-9].

Fig. 1: Structure of Ribavirin

A detailed literature survey divulges bio analytical methods for the analysis of Ribavirin individually and in various combinations in biological matrices [9-14] and few RP-HPLC methods for the determination of assay of Ribavirin in bulk and in tablet, capsule dosage forms [15-17]. In the present study, the authors report a rapid, sensitive, accurate and precise HPLC method for the quantitative estimation of Ribavirin in VIRAZIDE tablets.

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure sample of Ribavirin with purities greater than 99% was obtained as a gift sample from Spectrum labs, Hyderabad, India and tablet formulation [VIRAZIDE] was procured from local Pharmacy, Hyderabad, India with the labelled amount 200mg of Ribavirin. Acetonitrile (HPLC grade) water (HPLC grade), Potassium di-hydrogen ortho phosphate (AR Grade) and ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India). 0.45μm Nylon membrane filters were obtained from the Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu Prominence Liquid Chromatography comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable Make KromasilC18 (250 X 4.6 mm; 5μ). A manually operating Rheodyne injector with 10 μL sample loop was equipped with the HPLC system. The HPLC system was controlled with “Empower2” software. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (Sonica, model 2200 MH) and UV-Visible Spectrophotometer (Shimadzu UV-1800 series, software-UV probe version 2.42) were used in this study.

Method

Selection of wavelength

The suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Ribavirin. Suitable wavelength selected was 215 nm fig. 2.

Chromatographies conditions

The developed method uses a reverse phase C18 column, Enable make KromasilC18 (250 X 4.6 mm; 5μ), mobile phase consisting of Potassium di-hydrogen ortho phosphate buffer (adjusted using dilute with ortho phosphoric acid pH 4.2; acetonitrile in the proportion of 85:15 v/v). The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 10μl for every injection. The detection wavelength was set at 215 nm.

Buffer preparation

The buffer solution was prepared by adding 1.36 mg of Potassium di-hydrogen ortho phosphate to 1000 ml of HPLC grade water and
later pH was adjusted to 4.2 using 30% v/v of ortho phosphoric acid in water. The buffer was then filtered through 0.45 μm nylon membrane filter.

Fig. 2: UV spectrum of Ribavirin

Mobile phase preparation
The mobile phase was prepared by mixing phosphate buffer and acetonitrile in the ratio of 85:15 v/v and later it was sonicated for 10 minutes for the removal of air bubbles.

Preparation of working standard solution
10mg of Ribavirin was accurately weighed and taken in 10 ml clean and dry volumetric flask containing 7 ml of diluent (same as the mobile phase) and then sonicated for 30 minutes made up to 10 ml. This is considered as working standard solution (100μg/ml). 1 ml was pipetted out from above stock solution into a 10 ml volumetric flask and then make up to the final volume with diluent (100μg/ml).

Preparation of stock and working sample solution
Ten tablets were weighed separately and the average weight was determined. The average weight was weighed from the ten tablets grinded in a pestle and mortar, 1500mg transferred to a 100 ml volumetric flask containing 70 ml diluent and then sonicated for 25 minutes, followed by filtration through 0.45μ nylon membrane filter to get a sample stock solution of 0.0667mg/ml. 0.1 ml of the above stock solution was pipetted out and made up to 10 ml to get working sample solution equivalent to a concentration of working standard of 100μg/ml.

RESULTS AND DISCUSSION
Method development
A Reverse phase HPLC method was developing the system suitability parameters like tailing factor (T), the number of theoretical plates (N), the runtime and the cost effectiveness. The optimized method developed resulting in the election of Ribavirin at 2.606 min. Fig. 3 and 4 represent chromatograms of blank solution and the standard solution (100μg/ml) respectively. The total run time is 8 minutes. System suitability tests are an integral part of method development and used to ensure adequate performance of the chromatography system. Retention time (Rt), the number of theoretical plates (N) and peak Asymmetric factor was evaluated for six replicate injections of the standard at the working concentration. The results are given in Table 1.

In order to test the applicability of the developed method to a commercial formulation, VRAZIDE was chromatographed at working concentration (100μg/ml) and it is shown in fig. 5. The sample peak was identified by comparing the retention time with the standard drug fig. 4. System suitability parameters were within the acceptable limits, ideal for the chromatography system. Integration of the separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug Ribavirin by the proposed HPLC method.

Fig. 3: Typical Chromatogram of Blank solution

Fig. 4: Typical chromatogram of the standard solution

Table 1: System suitability studies results

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ribavirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>2.606</td>
</tr>
<tr>
<td>Number of Theoretical plates (N)</td>
<td>9096</td>
</tr>
<tr>
<td>Tailing factor(T)</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>* Mean of six injections</td>
</tr>
</tbody>
</table>

Fig. 5: Typical chromatogram for the tablet formulation

Method validation
Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines [18] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, and ruggedness, limit of detection (LOD) and limit of quantitation (LOQ).

Specificity
Fig. 3-5 for blank, standard drug solution and sample chromatogram reveal that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drugs as
blank have no peak at the retention time of Ribavirin. Accordingly, it can be concluded that, the method developed is said to be specific.

**Precision**

**System precision**

Six replicate injections of the standard solution at the working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning the peak area for the drug, which indicates the acceptable reproducibility and hence by the precision of the system. System precision results are tabulated in table 2.

<table>
<thead>
<tr>
<th>Injection number</th>
<th>Ribavirin</th>
<th>Rt peak area</th>
<th>peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>2.604 833684</td>
<td>833684</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>2.606 838108</td>
<td>838108</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2.607 829483</td>
<td>829483</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2.607 836224</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>2.608 831188</td>
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<td>6</td>
<td></td>
<td>2.608 823018</td>
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</tr>
<tr>
<td>Average</td>
<td></td>
<td>2.608</td>
<td>832284</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td>5402.3</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td></td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Repeatability (Intra day precision)**

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 3).

<table>
<thead>
<tr>
<th>N</th>
<th>Ribavirin</th>
<th>%Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100.38</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100.91</td>
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<td>3</td>
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</tr>
<tr>
<td>5</td>
<td>100.32</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>99.10</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>100.21</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.6055</td>
<td></td>
</tr>
<tr>
<td>%RSD</td>
<td>0.65</td>
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</tr>
</tbody>
</table>

**Linearity**

Standard solutions of Ribavirin at different concentration level (25%, 50%, 75%, 100%, 125% and 150%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding peak area. The results show an excellent correlation between peak areas and concentration level of the drug within the concentration range (25-150μg/ml) for the drug and the results are given in table 4-5. The correlation coefficient of Ribavirin is greater than 0.99, which meet the method validation, acceptance criteria and hence the method is said to be linear.

**Accuracy**

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as showed in Table 6. The accepted limits of recovery are 98% - 102% and all observed data are within the required range, which indicates good recovery values and hence the accuracy of the method developed.

**Sensitivity**

The sensitivity of measurement of Ribavirin by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 0.24ng/ml and 0.73 ng/ml.

**CONCLUSION**

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision, and linearity, limit of detection and limit of quantitation, for the quantitative estimation of Ribavirin in tablets.

The precision is exemplified by the relative standard deviation of 0.4 %. A good linear relationship was observed in the drug between concentration ranges of 25 and 150μg/ml. Accuracy studies revealed that mean recoveries were between 98 and 102%, an indicative of
accurate method. Accordingly, it can be concluded that the developed reverse phase isocratic HPLC method is accurate, precise and linear and therefore the method can be used for the routine analysis of Ribavirin in tablets.

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CONFLICT OF INTERESTS

Declared None.

REFERENCES