Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

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

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Received: 04 Nov 2014 Revised and Accepted: 01 Dec 2014

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 KromasilC18 (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 quantitiation (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 $C_8H_{12}N_4O_5$ and molecular weight is 244.204 [6-8].

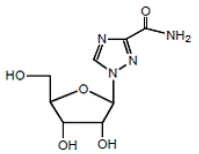

Fig. 1: Structure of Ribavirin

A detailed literature survey divulges bio anlytical 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 Kromasil C18 (250 X4.6 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.

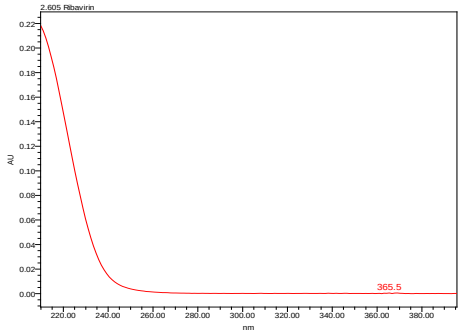

Fig. 2: UV spectrum of Ribavirin

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 chromatographies sample. 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.

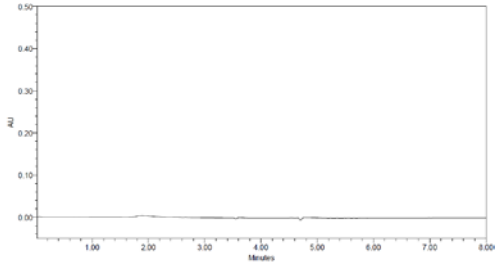

Fig. 3: Typical Chromatogram of Blank solution

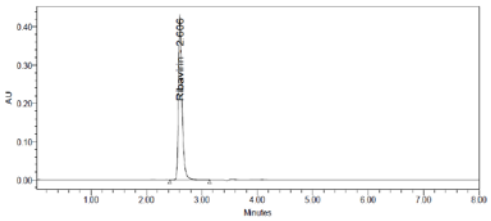

Fig. 4: Typical chromatogram of the standard solution

Table 1: System suitability studies results

Parameters*	Ribavirin
Retention time (min)	2.606
Number of Theoretical plates (N)	9096
Tailing factor(T)	1.32

* Mean of six injections

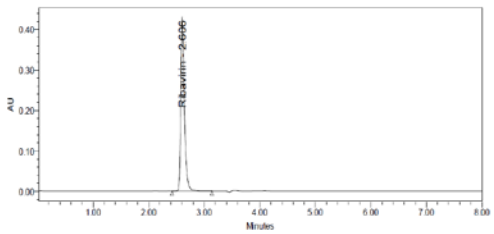

Fig. 5: Typical chromatogram for the tablet formulation

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 quantitiation (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 there by the precision of the system. System precision results are tabulated in table 2.

Table 2: System precision results

Injection number	Ribavirin
	Rt peak area
1	2.604 833684
2	2.606 838108
3	2.607 829483
4	2.607 836224
5	2.608 833188
6	2.608 823018
Average	832284
SD	5402.3
%RSD	0.6

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 3: Intra day precision results

N	Ribavirin %Assay
1	100.38
2	100.91
3	99.87
4	100.69
5	100.32
6	99.10
Average	100.21
SD	0.6505
%RSD	0.65

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.

Table 4: Linearity of the chromatography system

Drug	Linearity range (µg/ml)	R^2	Slope	intercept
Ribavirin	25-150	0.999	8300.4	1098.53

Table 5: Calibration data for Ribavirin

% Level	Concentration (µg/ml)	Peak Area 1	Peak Area 2	Peak Area 3
25	25	199277	197813	198585
50	50	432830	433171	432616
75	75	622389	628355	625398
100	100	827615	826828	827477
125	125	1036145	1033189	1036780
150	150	1249375	1248959	1243879
Regression equation		Y=8309.5+735.21	Y=8301.1+ 764.7	Y=8289.8+1795.6
Regression coefficient		0.999	0.999	0.999

Table 6: Results of Accuracy studies for Ribavirin

Concentration level (%)	*%Mean recovery
50	101.57
100	99.54
150	100.45

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 quantitiation (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.

ACKNOWLEDGEMENT

The authors would like to thank the management of A. K. R. G. College of Pharmacy, Nallajerla, for providing the necessary facilities to carry out of this research work. The authors are grateful to spectrum labs, Hyderabad for providing a gift sample of the drug.

CONFLICT OF INTERESTS

Declared None.

REFERENCES

1. Sidwell RW, Bailey KW, Wong MH, Barnard DL, Smee DF. *In vitro* and *in vivo* influenza virus-inhibitory effects of viramidine. Antiviral Res 2005;68(1):10-7.
2. Ascio GS, Leble LH, Vaha BH, Chan KA. Ribavirin for patients with crimean congo hemorrhagic fever a systematic review and meta-Analysis. J Antimicrob Chemother 2011;66(6):1215–22.
3. Bausch DG, Hadi CM, Khan SH, Lertora JJ. Review of the literature and proposed guidelines for the use of oral Ribavirin as post exposure prophylaxis for lassa fever. Clin Infect Dis 2010;51(12):1435–41.
4. Soares W, Thomas S, Thomson G, Garner P. Ribavirin for crimean congo hemorrhagic fever systematic review and meta-analysis. BMC Infec Dis 2010;10(207):1471-2334.
5. Sylvie LR, Francoise SL, Agnes PL, Jean PZ, Germain BS, Souvignet CL. Ribavirin quantification in combination treatment of chronic hepatitis c. AAC 2003;47(1):124-9.
6. Hema CT, Ugolini G, Wachara PS, Sung KW, Shuan GS, Thamatas LJ. Human rabies neuropathy genesis diagnosis and management. Lancet Neurol 2013;12(5):498–13.
7. Alvarez D, Dieterich DT, Brau N, Moorehead L, Ball L, Sulkowski MS. Zidovudine, use but not weight-based ribavirin dosing impacts anemia during hcv treatment in HIV-infected persons. J Viral Hepat 2006;13(10):683-9.
8. Bani SF, Carrat F, Pol S, Hor R, Rosenthal E, Goujard C, *et al*. Risk factors for symptomatic mitochondrial toxicity in HIV/hepatitis C virus coinfected patients during interferon plus ribavirin based therapy. J Acquir Immune Defic Syndr 2005;40(1):47-52.
9. Margarita M, Osvaldo R, Beatriz Z. Hplc–ms/ms method for the intracellular determination of ribavirin monophosphate and ribavirin triphosphate in cem_{ss} cells. J Pharm Biomed Anal 2009;49(5):1233–40.
10. Wilson ZS, Hai ZB, Thomas A, Xiangyu J, Weng N. Development and validation of a liquid chromatography tandem mass spectrometry method for the determination of ribavirin in human plasma and serum. J Pharm Biomed Anal 2002:29(2);83-94
11. Lou AM, Lori PL. Bioanalysis of ribavirin in rat plasma by high pressure liquid chromatography tandem mass spectrometry. J Med Chem Scien 1972:15;1150.
12. Zhi-LZ, Xi YY, Huai YP, Tie FC, Qiu XL. Rapid and sensitive hplc-ms/ms method for pharmacokinetic assessment of ribavirin in healthy chinese. BMC 2008:22(12);1409-15.
13. Li TY, Mai NY, Chin CL. A sensitive and specific method for the determination of total ribavirin in human red blood cells by liquid chromatography tandem mass spectrometry. J Chromatogr Sci 2003;41(5):255-60.
14. Manel A, Darius M, Matthias C, Thomas M. Multiplex liquid chromatography-tandem mass spectrometry assay for simultaneous therapeutic drug monitoring of ribavirin, boceprevir, and telaprevir. AAC 2013;57(7):3147-58.
15. Antonio D, Allende I, Marco S, Daniel G. Validation of liquid liquid extraction method coupled with hplc-uv for measurement of ribavirin plasma levels in hcv-positive patients. J Chromatogr B 2006:835(2):127-30.
16. Avolio A, Nicolò A, Simiele M, Turini S, Agnesod D, Boglione L. Development and validation of a useful hplc-uv method for quantification of total and phosphorylated-ribavirin in blood and erythrocytes of hcv positive patients. J Pharm Biomed Anal 2012:66(10);376-80.
17. Suman A, Naveen K, Ramana M. Validated rp-hplc method for the estimation of ribavirin in formulation. lirpbs 2011:2(2);704-9.
18. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1); 2005.