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A NOVEL, SENSITIVE AND VALIDATED UV-SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF RIBAVIRIN IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Ribavirin is a nucleoside analogue used as an antiviral, anti-metabolite, anti-infective drug mostly used to treat Chronic Hepatitis-C.A specific and economic UV spectrophotometric method has been developed using Distilled Water as solvent at a predetermined λmax of 224nm. Beer limit was found in the range of 5-40 µg/mL and exhibited good correlation coefficient (R^2 = 0.999) and the regression equation was found to be y=0.021x+0.077.This method was successfully applied in the determination of Ribavirin content in a marketed formulation from the local market and the results were in good agreement with the label claim.The method was validated according to the ICH guidelines for Range, linearity, Accuracy, Precision, Robustness, Ruggedness, LOD, LOQ and Sensitivity. The obtained results proved that the method can be employed for the routine analysis of Ribavirin in bulk as well as for formulation.

KEYWORDS: Ribavirin, Method development, Validation, UV spectrophotometer, Precision, Accuracy.

INTRODUCTION

Ribavirin (Fig-1)chemically is(1-β-D-ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide or 1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1H-1,2,4-triazole-3-carboxamide.It is a nucleoside analogue, used as an antiviral, antimetabolite, anti-infective drug mostly used to treat Chronic Hepatitis-C.It is a white powder having molecular formula of $C_8H_{12}N_4O_5$ and a molecular weight of 244.2 g/mol. It is freely soluble in Distilled Water. The mechanism action of Ribavirin is by incorporating into viral RNA synthesis including

viral genome mutations and inhibiting normal viral replication. It also inhibits HCV by inhibiting polymerase enzyme and possesses a broad spectrum of activity against DNA and RNA viruses.

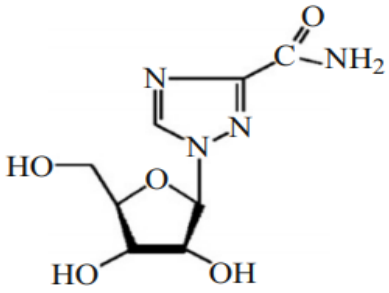

Figure 1: Chemical structure of Ribavirin.

A detailed literature survey for Ribavirin revealed that a very fewMass-Spectroscopic, HPLC and LC-MS[1-5] methods have been reported for the quantification of Ribavirin. No author reported the UV Spectrophotometric method for the determination of Ribavirin in pharmaceutical formulations. Hence an attempt was made to develop a simple, precise, accurate, and validated spectrophotometric method for the determination of Ribavirin in Bulk and Formulation.The developed method was validated as per ICH Guidelines.[6-8]

MATERIALS AND METHODS

Instruments and materials

A gift sample of Ribavirin with purity of 99.5% w/w was obtained from Spectrum labs, Hyderabad. LAB INDIA (T60) double beam UV / Visible Spectrophotometer and ELITE analytical balance were the instruments used. Chemicals and reagents used are of analytical grade. Ribavirin of 200mg with a brand name Ribasure® was purchased from the local pharmacy.

Preparation of standard stock solution

A standard drug solution of Ribavirin was prepared by adding 100mg of the drug into a 100mL volumetric flask and made up to mark with methanol to get a concentration of 1000µg/mL.

Preparation of working standard solution

From the above standard stock solution, 10mL of the sample was transferred to a 100 mL volumetric flask and made up to mark with Distilled water to get a concentration of 100

µg/mL. It was then scanned by a UV Spectrophotometer in the range of 200-400nm using Distilled water as a blank. The absorbance was found to be maximum at 224 nm.

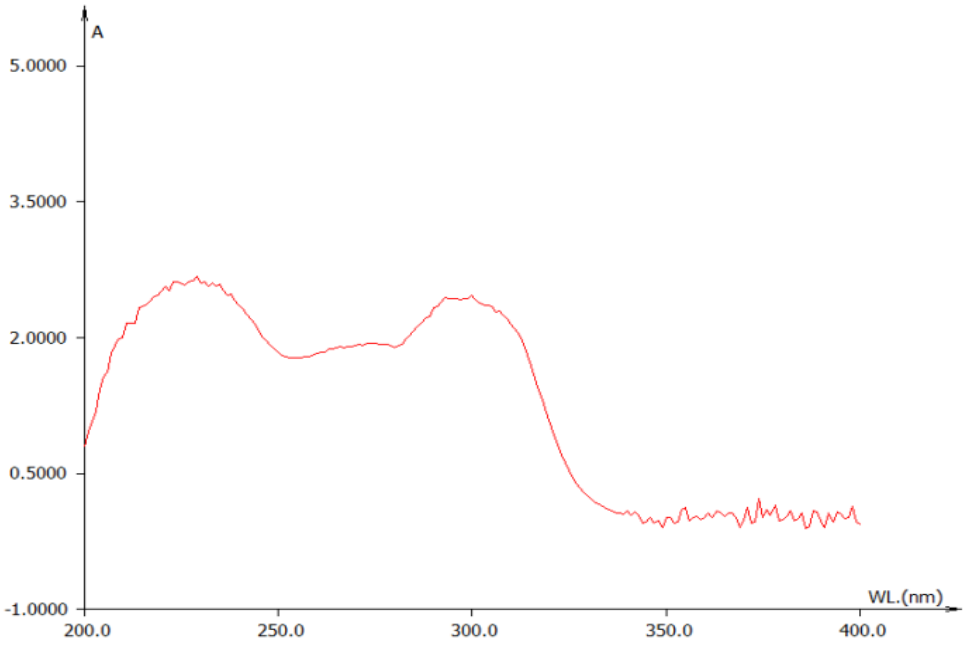

Figure 2: Determination of λ_{max} of Ribavirin.

Construction of calibration curve

Aliquots ranging from 5-40 µg/mL solutions were prepared by using Distilled water as solvent. The samples were then analyzed at a λmax of 224nm to get respective absorbance. The values [Table 1] are then plotted to get a calibration curve.

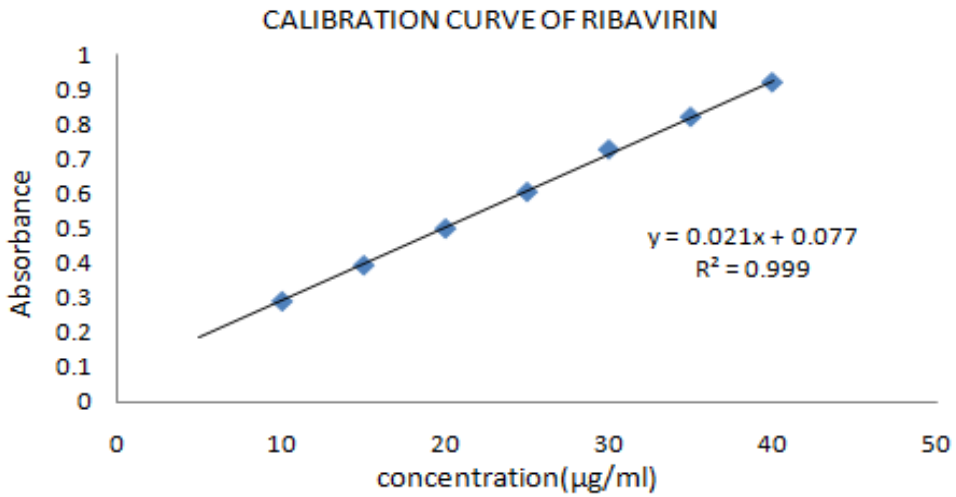

Figure 3: Standard calibration curve of Ribavirin.

Preparation of the assay solution

The proposed method was applied to analyze the commercially availableRibavirin capsules Ribasure® (200mg). 5 capsules are weighed and powdered the amount powder is equivalent to 100mgof Ribavirin was weighed accurately and transferred into 100 mL volumetric flask containing Distilled water which was further sonicated for 15 min with vigorous shaking the volume was brought up to 100mL with distilled. The solution was subjected to filtration through Whatman filter paper #44. The filtrate was diluted suitably with distilled water to get a final solution of 25 µg/ml concentration. This was subsequently analyzed using a Double beam UV-VIS spectrophotometer and taking distilled water as blank in the UV range 200-400nm. The spectrum was recorded as 224nm. The concentrations of the drug were calculated from the linear regression equation.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that is a specific activity will consistently produce the desired result, or a product meeting its predetermined specifications and quality characteristics. The method was validated according to ICH guidelines for various parameters like Range, Linearity, Precision, Accuracy, Robustness, Ruggedness, LOD, LOQ, and Sensitivity.[10-13]

Range

The range is an intervalbetween the upper and lower concentration analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Linearity

The ability of an analytical procedure is to produce test results that are directly proportional to the concentration of an analyte. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration. For estimation of linearity at least 5 concentrations are required.

Accuracy

Accuracy means the expression of closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference true value and the value found. Accuracy is assessed by using 9 determinations covering a minimum of 3 concentrations.

Precision

The closeness of agreement between the obtained values by analyzing the same sample multiple times under prescribed conditions. There are 3 levels of repeatability, intermediate precision, and reproducibility.

Repeatability is a measure of the exactness under the same working conditions more than a short interim of time, that is, under ordinary working states of the scientific technique with the same hardware it is also known as intraday precision

Reproducibility also is known as inter-day precision.

Precision is expressed in terms of % Relative Standard Deviation

$$\% \text{ RSD} = (\text{Standard deviation})/\text{Mean} \times 100$$

Standard deviation (SD)

$$SD = \sqrt{((\sum [\![(x-\bar{x})]\!]^2)/(n-1))}$$

Where n = no of entries

Ruggedness

The ruggedness of an analytical procedure is the degree of reproducibility of results by analyzing the same sample under a variety of conditions like laboratories, instruments, analysts, reagents, etc.

Robustness

Robustness of an analytical procedure is the capacity to remain unchanged by small but deliberate changes in parameters.

Sensitivity

Limit of detection (LOD) and Limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines.

Limit of Detection: It is the lowest amount of the drug in a sample that can be detected, but not necessarily quantitated.

$$LOD = (3.3 \times \sigma)/S$$

Where S= standard deviation

Limit of Quantification: It is an amount of analyte that can be quantitated with a specified limit of accuracy and precision.

$$LOQ = (10\times\sigma)/S$$

Linearity

Different aliquots of Ribavirin were prepared from the working standard solution (100µg/mL) in the range of 5-40 µg/mL. The solutions were scanned on a Double beam UV-VIS spectrophotometerin the UV range of 200-400nm using Distilled water as the blank. The spectrum was recorded at 224nm. The calibration plot was constructed as concentration Vs absorbance and can be shown in [Table1].

Table 1: Linearity of working standard solutions.

Concentration (µg/mL)	Absorbance (nm)
5	0.1635
10	0.2895
15	0.3927
20	0.4984
25	0.6034
30	0.7249
35	0.8184
40	0.9174

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inter-day variation study, the solutions of the same concentration 25µg/mL were prepared and analyzed six times, for five consecutive days, and the absorbance was recorded[Table 4]. In the intra-day variation study, six different solutions of the same concentration 25µg/mL were prepared and analyzed thrice a day (Morning, Afternoon, and Evening). And the % RSD was calculated and reported [Table3].

Table 2: Repeatability data.

Concentration (µg/mL)	Absorbance (nm)	Statistical analysis
25	0.5534	Mean: 0.54081 % RSD: 1.82%
25	0.5233	
25	0.5384	
25	0.5415	
25	0.5423	
25	0.5460	

Table 3: Intra-day study.

Concentration (µg/mL)	%RSD			Average % RSD
	1	2	3	
25	1.82	1.3	1.4	1.506

Table 4: Inter-day study.

Concentration (µg/mL)	% RSD					Average % RSD
	Day1	Day 2	Day3	Day4	Day5	
25	1.82	1.3	1.46	1.37	0.94	1.378

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations, i.e., 80, 100, and 120%, in which the amount of marketed formulation Ribasure® was kept constant and the amount of pure drug was varied. The solutions were prepared in triplicate and the accuracy was indicated by % recovery was calculated and reported in the [Table 5].

Table 5: Accuracy data.

Level of Addition (%)	%Recovery	% Mean Recovery
80	100.1	99.70
100	99.38	
120	99.64	

Robustness

Therobustnessof the method was carried out by analyzing the sample using two different wavelengths (±1 of λmax) that were and respective absorbance were recorded. The results are indicated in [Table 6].

Table 6: Robustness data.

Concentration (µg/mL)	Absorbance		
	223nm	224nm	225nm
25	0.6537	0.5534	0.5734
25	0.6597	0.5233	0.5769
25	0.6594	0.5384	0.5794
25	0.6618	0.5415	0.5776
25	0.6629	0.5423	0.5790
25	0.6631	0.5460	0.5831
%RSD	**0.45%**	**1.82%**	**0.5%**

Ruggedness

The ruggedness of the method was carried out by analyzing the sample using two different analysts and respective absorbance was recorded. The results are indicated in [Table 7].

Table 7: Ruggedness.

Concentration (µg/mL)	Absorbance	
	Analyst 1	Analyst 2
25	0.5534	0.6348
25	0.5233	0.6241
25	0.5384	0.6200
25	0.5415	0.6103
25	0.5123	0.6163
25	0.5460	0.6135
%RSD	**1.82%**	**1.3%**

Sensitivity

Limit of detection (LOD) and Limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines. They are calculated by checking absorbance using solvent and calculate using formulae and the results are shown in [Table 8].

Table 8: LOD & LOQ.

Limit of Detection	Limit of Quantification
0.026 µg/mL	0.08 µg/mL

RESULTS AND DISCUSSION

The method was developed and validated as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, robustness, ruggedness, LOD, and LOQ. Beers law obeyed over the concentration range of 5-40 µg/mL, using regression analysis the linear equation y=0.021x+0.077with a correlation coefficient of r^2 0.999.The precision results show % RSD less than 2 at each level which indicates clearly that the method is precise enough for the analysis of Ribavirin. The accuracy of the method was checked by recovery studies. The high recovery with values indicates the accuracy of the developed method. The robustness and ruggedness studies reveal that the method is enough robust and rugged. The LOD, LOQ values indicate that the method is more sensitive. There was no interference observed from the excipients present in the formulation, indicated that the method is specific. Determination of Ribavirin in capsule formulation Ribasure® showed the content of Ribavirin was very close to the label amount.The% RSD values in all the parameters were within the acceptable limit (<2%) All the characteristics of the method are represented in the [Table 9].

Table 9: Results of validated parameters.

Parameters	Results
Absorption maxima (nm)	224
Linearity range(µg/mL)	5-40
Regression equation	y= 0.021x+0.077
Correlation coefficient (R^2)	0.999
LOD(µg/mL)	0.026
LOQ(µg/mL)	0.085
Accuracy (% Recovery)	98.38-100.10
Robustness (%RSD)	0.92
Ruggedness (%RSD)	1.56
Precision Intraday precision (%RSD)	1.506
Inter-day precision (%RSD)	1.378

CONCLUSION

A UV spectrophotometric method has been validated for the estimation of Ribavirin in bulk as well as the pharmaceutical dosage form. The developed method was found to be simple, accurate, precise, specific, reproducible, and linear over the concentration range studied. The proposed method can be used for routine analysis of Ribavirinin bulk as well as Pharmaceutical formulations.

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