Journal of Applied Pharmaceutical Science Vol. 10(1), pp 096-100, January, 2020
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2020.101013
ISSN 2231-3354

A novel analytical liquid chromatography–tandem mass spectrometry method for the estimation of Ribavirin in bulk and pharmaceutical formulation

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ARTICLE INFO

Received on: 26/09/2019
Accepted on: 09/11/2019
Available online: 03/01/2020

Key words:
Ribavirin, formulation, LC-MS/MS, validation, ICH.

ABSTRACT

The aim of this study is to develop and validate a method that is simple, precise, sensitive, and rapid compared to using the liquid chromatography–tandem mass spectrometry method for the quantitative determination of Ribavirin (antiviral drug) in its tablet formulation. The development and validation of the method were achieved using a column (Zorbax 50 mm × 4.6 mm × 5 µm) with mobile phase ammonium formate (pH: 7.50): acetonitrile in the ratio (30:70, v/v) with the flow rate of 0.5 ml/min. The retention time for Ribavirin was 1.1 minutes with the total run time of 2.5 minutes. The linearity range for Ribavirin was from 2 to 100 ng/ml with a correlation coefficient of 0.9956. The detection and quantitation limits of Ribavirin are 0.7 and 2 ng/ml, respectively. The percentage recovery of Ribavirin ranged from 94.00% to 98.33%. The percentage relative standard deviation for intraday and interday precision results was found to be 0.67%–2.11% and 1.92%–3.11%, respectively. The new method developed for Ribavirin drug was found to be rapid, sensitive, selective, and economical. The established method was the evaluation of Ribavirin in its marketed formulation (tablet). The values obtained from the analysis were found out to be within the acceptable limits as per the International Council for Harmonisation (ICH) guidelines.

INTRODUCTION

Ribavirin that is also known as virazole is having the chemical formula of 1-[(2R, 3R, 4S, 5R)-3, 4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-1, 2, 4-triazole-3-carboxamide. The molecular formula of Ribavirin is $C_8H_{12}N_4O_5$ with the molecular weight of 244.206 g/mol (Drug information, 2018).

From the literature review, it was observed that there are some methods used for the estimation of Ribavirin in biological matrices, such as serum (Danso *et al*., 2011), human plasma (Ferreiros *et al*., 2014; Loregian *et al*., 2007), rat plasma, and brain (Shi *et al*., 2015; Zironi *et al*., 2011), but no analytical method has been developed for the estimation of Ribavirin in its formulation. Furthermore, the reported methods on biological matrices were less sensitive to the quantification limit of more than 5 ng/ml and the run time of more than 5 minutes. Therefore, the aim of this present study was to develop a highly sensitive, simple, and economical method with less retention time for the estimation of Ribavirin drug than using the liquid chromatography–tandem mass spectrometry (LC-MS-MS) instrument.

EXPERIMENTS

Materials

The Ribavirin working standard was provided by the Indian Pharmacopeia Commission (IPC), New Delhi, India, as a gift sample. Ammonium formate that was purchased from Rankem Fine Chemical Ltd., LC-MS grade acetonitrile from Sigma Aldrich and water of LC-MS grade by Milli-Q Reverse osmosis (RO) System (Millipore, Bedford, MA, USA) were used.

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Instrumentation and conditions

The liquid chromatography system has been coupled with a tandem quadrupole mass spectrometer (Shimadzu 8030. Tokyo, Japan) having an LC-20AD pump, electrospray ionization interface, photodiode array (PDA) detector (SPD-M20), column oven (CTO-20AC),CEM-20Alite controller, and SIL-20AC autosampler. Lab solution data station software was used. Initially, different chromatographic conditions with different buffers were used. However, the use of acidic buffers (formic acid and acetic acid) affected the separation of the analyte. The use of ammonium acetate buffer gave a good separation of analyte, but the mass detection was less; after a number of trial and errors, the separation and quantification of Ribavirin separation (isocratic) was achieved by Zorbax C_{18} column (4.6 × 50 mm, 5 µm) as a stationary phase for rapid determination with ammonium formate (pH 7.5): acetonitrile at the ratio of 30:70, v/v at a flow rate of 0.5 ml/min, and an injection volume of 10 µl.

Selection of mass range

The Ribavirin drug solution (1,000 ng/ml) was directly injected into the LC-MS/MS and scanned over the range of 150–350 to determine the precursor ion (see Fig. 1). Furthermore, the multiple reaction monitoring (MRM) chromatograms were obtained for transitions 244.90→113.15 (+) collision energy (CE): −11.0 (quantification) and 244.90→96.05 (+) CE: −31.0 (detection), as shown in Fig. 2.

Preparation of mobile phase

For mobile phase preparation, 0.63 g of ammonium formate was dissolved in 1,000 ml of Millipore water and filtered through 0.45 µm using a Millipore filtration unit.

Preparation of Ribavirin standard stock and working solution

Ribavirin (100 mg) standard was taken and transferred into a 100-ml standard flask into which 50-ml methanol (diluent) was added and dissolved. Furthermore, the content was made up to the volume using the diluent. From the stock solution, dilutions were made to prepare the linearity range solutions of 2, 10, 20, 40, 60, 80, and 100 ng/ml. The quality control samples of low quality (LQC) 6 ng/ml, sample of medium quality (MQC) 50 ng/ml, and sample of high quality (HQC) 90 ng/ml were also prepared.

Validation study

As per ICH guidelines, parameters, such as specificity, accuracy, linearity, precision studies, detection limit (LOD), and quantitation limit (LOQ), were studied for the developed method (ICH, 2005).

Specificity

The excipients that interfere were determined by injecting standards into the system to determine the method specificity.

Linearity

Linear for Ribavirin was analyzed at six concentration levels ranging from 2 to 100 ng/ml. The linearity graph was used to calculate the standard deviation and regression coefficient.

Accuracy and precision

Recovery studies performed to determine the method accuracy at three concentration levels (LQC, MQC, and HQC) at replicates for each concentration were evaluated. For determining the precision of the method, three different quality control (QC) concentrations were taken and analyzed on the same day and on different days and the results were represented as percentage relative standard deviation (% RSD) for each concentration.

LOD and LOQ

LOD and LOQ for Ribavirin were calculated by the signal-to-noise ratio. The signal-to-noise ratio of 3:1 was used to determine the LOD, and the 10:1 signal-to-noise ratio was used to determine the LOQ.

Robustness

The robustness of the method depends on the experimental conditions (operators, mobile phase used, and types of columns). The parameters, such as mobile phase (acetonitrile) concentration, buffer (ammonium formate) concentration, and flow rate, were studied for robustness.

System suitability

The system suitability is an important parameter for method development, and various factors were studied, such as tailing factor and linearity range.

RESULTS AND DISCUSSIONS

Specificity

The excipients that interfere were determined by injecting standards and elution of no peaks along with the retention time of Ribavirin. Therefore, the developed method signifies the selectiveness for Ribavirin determination in its formulation (see Fig. 2).

Linearity

The linearity curve was determined for the Ribavirin drug at six concentration levels ranging from 2 to 100 ng/ml. The linearity curve shows a correlation coefficient of 0.9956 and with a regression equation of $y = 949.63x + 2168.6$ (see Fig. 3).

Accuracy and precision

The standard addition method was used to determine the method accuracy at three QC levels. The percentage recovery of Ribavirin ranged from 94.00% to 98.33%. The percentage RSD of intraday and interday precision results was found to be 0.67%–2.11% and 1.92%–3.11%, respectively (see Table 1). Application of the method intended to study Ribavirin in a commercial tablet formulation.

Determination of Ribavirin in its tablet formulation

Twenty tablets were accurately weighed and powdered; 10 mg powdered sample was transferred to a 10-ml volumetric flask, 5-ml acetonitrile was added and kept in a sonicator, and then the volume was made up to 10 ml using acetonitrile. After this, the resultant solution was shaken thoroughly and filtered through a syringe filter. The quality control samples of Ribavirin in different concentrations were prepared as follows: 6, 50, and 90 ng/ml (see Table 2 and Fig. 4).

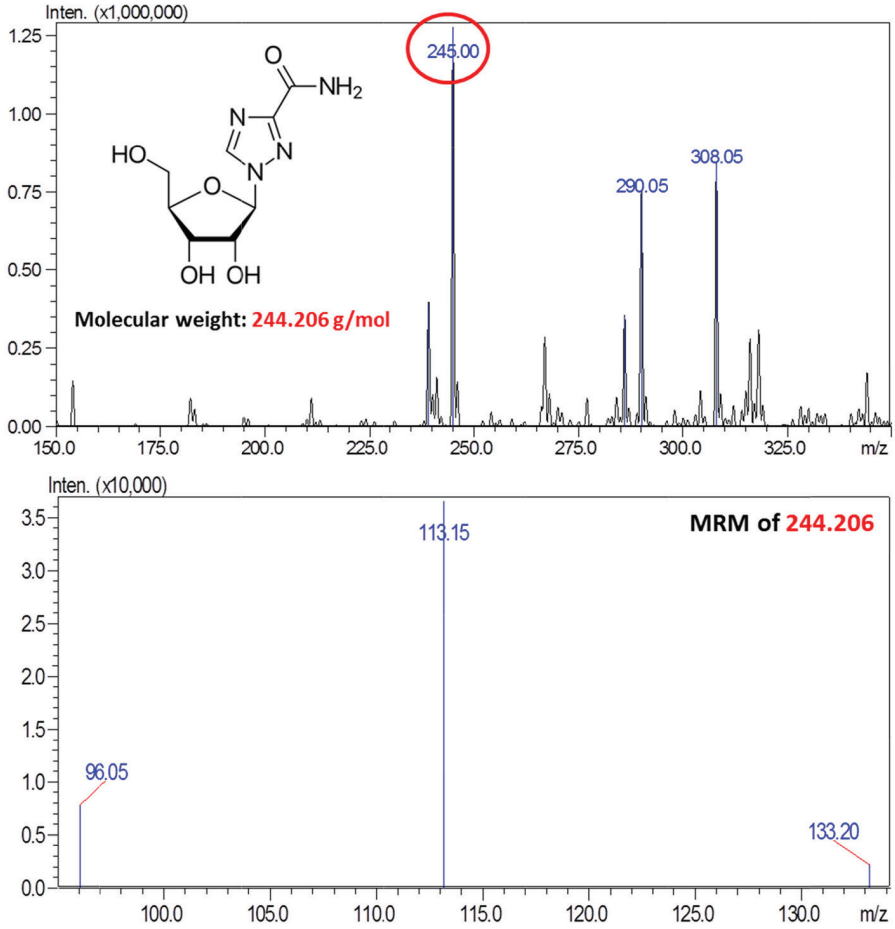

Figure 1. (a) Mass scan spectra and (b) MRM spectra for Ribavirin in positive mode.

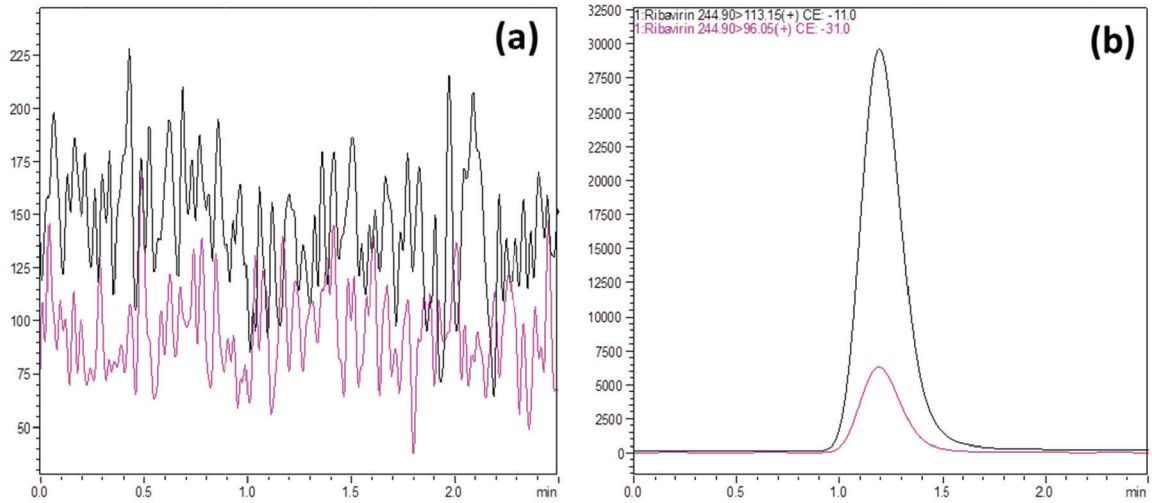

Figure 2. (a) Blank chromatogram of Ribavirin. (b) Standard chromatogram of Ribavirin.

LOD and quantification limit (LOQ)

LOD and LOQ for Ribavirin were calculated based on the signal-to-noise ratio. The LOD for Ribavirin was found to be 0.7 ng/ml, and due to its low LOD, the quantification limit was 2 ng/ml.

Robustness

The robustness of the method was determined by changing the operating conditions (operators, mobile phase used, and types of columns). The parameters, such as mobile phase concentration, were checked by changing the organic solvent

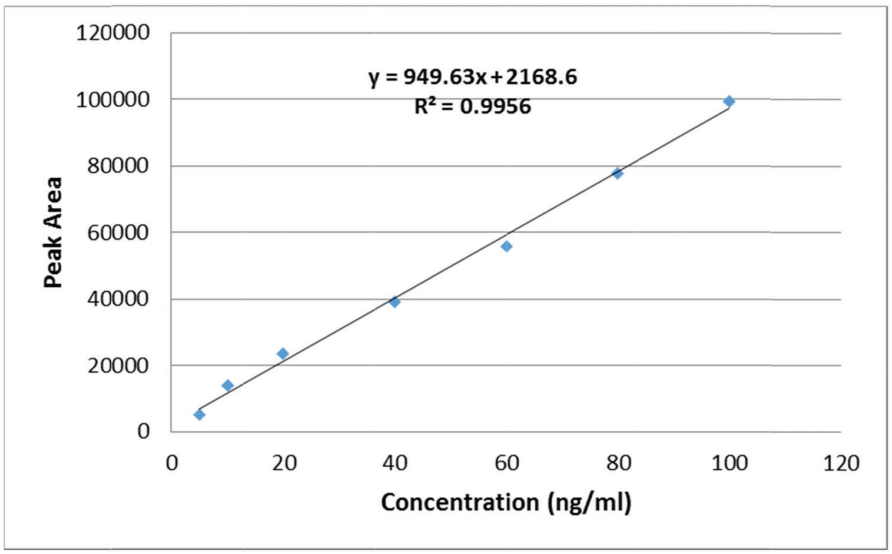

Figure 3. Linearity of Ribavirin.

Table 1. Accuracy and precision results of Ribavirin.

QC Samples (ng/ml)	Mean Conc. Found (ng/ml)	Intra-day		Inter-day	
		Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)
6	5.68 ± 0.30	94.66	2.11	92.34	3.11
50	48.40 ± 0.50	96.88	1.03	94.44	2.70
90	88.60 ± 0.60	98.44	0.67	96.27	1.92

Table 2. Recovery results for Ribavirin in its formulation.

Formulation	Label Claim	Assay level for QC sample (ng/ml)	Amount Found (ng/ml)	% Recovery
Ribavirin*	200 mg	6	5.64 ± 0.05	94.00
		50	47.90 ± 0.20	95.80
		90	88.50 ± 0.03	98.33

*Formulation 200 mg Rebetol.

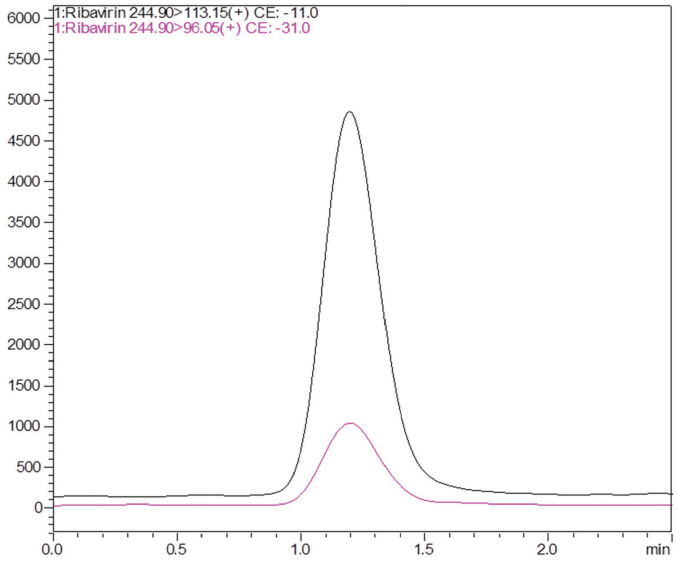

Figure 4. MRM chromatogram of Ribavirin in commercial formulation.

Table 3. System suitability parameter.

S.No	Parameters	Ribavirin
1	Limit of detection	0.7 ng/ml
2	Limit of quantitation	2 ng/ml
3	Theoretical plates	8,800
4	Tailing factors	1.1
5	Linearity range	2–100 ng/ml
6	Correlation coefficient (r^2)	0.9956

(acetonitrile) percentage (60%, 70%, and 80%) and buffer pH (6.0, 6.5, and 7.0) along with the mobile phase flow rate (0.2, 0.5, and 0.7 ml). The results were represented in terms of RSD %, and the obtained results were very much within the 3.0% limit.

System suitability

The system suitability of the method was determined against parameters such as tailing factor, linearity range, and validation parameters. The obtained results were found to be within the limit, indicating the suitability of the developed method (see Table 3).

CONCLUSION

The new method developed for the estimation of Ribavirin was found to be sensitive, simple, rapid, and economical with low retention time compared to the previously reported methods. The established method can be further used for the evaluation of Ribavirin in other marketed formulations. The values obtained from the analysis were found out to be within the acceptable limits as per the given ICH guidelines.

ACKNOWLEDGMENT

The authors are grateful to the Indian Pharmacopeia Commission (IPC), New Delhi, India, for providing Ribavirin as a gift sample.

CONFLICT OF INTEREST

All the authors declare that there are no conflicts of interest.

FINANCIAL SUPPORT AND SPONSORSHIP

None.

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How to cite this article:

Sharma P, Narenderan ST, Meyyanathan SN, Sangamithra R, Sanjay MS, Babu B, Kalaivani M. A novel analytical liquid chromatography tandem mass spectrometry method for the estimation of Ribavirin in bulk and pharmaceutical formulation. J Appl Pharm Sci, 2020; 10(1):096–100.