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STABILITY INDICATING HPLC METHOD FOR RIBAVIRIN AND ITS PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The present work describes a specific, stability indicating HPLC method for determination of Ribavirin (1) and its pharmaceutical dosage forms.

Ribavirin was chromatographed on a microbondapak C18 column utilizing a simple mixture of 0.01M dibasic potassium phosphate and methanol (95 : 5). The detection was done at 207 nm.

The available literature was scanned to locate the various methods(2,3) available along with the one reported in USP XXII.

A comparative study was made of the proposed method and USP method and the advantages over the USP method have been discussed.

The low value of Relative standard deviation and recovery of the drug in the range of 99.1% to 101.5% indicates a good precision and non-interference of the method.

INTRODUCTION

Ribavirin, chemically, 1-beta-d-ribofuranosyl - 1,2,4-triazole-3-Carboxamide, is a broad spectrum virustatic agent, indicated in the treatment of type A hepatitis, herpes goster, herpes simplex, viral respiratory infections and viral childhood diseases like measles (4-8).

Triazole Carboxylic Acid (TCA), Ribose triazole carboxylic acid (RTCA) and Triazole carboxamide (TCO) are the related impurities of Ribavirin which appear at different retention times when chromatographed with Ribavirin under proposed HPLC conditions. The functional UV absorbing group in TCA and RTCA being the same, it is difficult to separate the two.

The proposed method is specific with respect to the above impurities which can be easily identified in bulk drug or capsule formulations, if present.

EXPERIMENTAL

Reagents & Materials:

Ribavirin, TCA, RTCA, TCO were obtained from M/s Viratek, California. All the reagents were of "Guaranteed Reagent" grade and all the solvents were of HPLC grade. Distilled, deionized water (obtained from Milli-Q) filtered through 0.45 micron membrane filter was used throughout the experiment.

Chromatographic Instrumentation:

The HPLC System comprised of a dual piston reciprocating pump (Model 510), LC Spectrophotometer (Model 486), a computing integrator (Model 746) [All from WATERS] and a Rheyodyne injector (Model 7125) was used. The experiment was performed on a microbondapak C18 reverse phase column(3.9 mm x 30 cm). The flow rate of the mobile phase was 1.0 ml/min. The detector sensitivity was set at 1.0 a.u.f.s and the eluents were monitored at 207 nm.

The mobile phase consisted of 0.01M potassium dihydrogen orthophosphate and methanol (95 : 5). The pH of this mobile phase was found to be 4.6.

Standard and Sample Preparation:

Ribavirin stock standard solution of 1 mg/ml concentration was prepared. The stock was suitably diluted

Table - 1
Analytical Data of Ribavirin and its formulations

Product	Code *	Declared Amount	Estimated Amount	% Recovery
Ribavirin Bulk Drug		98.5 to 101.0%	99.26	99.26
Ribavirin Syrup	A	50.0 mg/5ml	49.8 mg/5ml	99.60
Ribavirin Capsules	B	100 mg/Cap	98.99 mg/Cap	98.99
	C	200 mg/Cap	201.02 mg/Cap	100.50

* Commercially available formulations.

to obtain a final concentration of 25 mcg/ml with mobile phase.

Impurities (TCA, RTCA and TCO) stock solutions were prepared of 0.1 mg/ml concentration in methanol.

The contents of twenty capsules were mixed and powder equivalent to 100 mg of Ribavirin was weighed in a 100 ml volumetric flask and diluted upto the mark with mobile phase. The preparation was stirred well and filtered. The filterate was suitably diluted to obtain a final concentration of 25 mcg/ml Ribavirin in mobile phase.

Ribavirin Syrup was also suitably diluted with mobile phase to obtain a final concentration of 25 mcg/ml of the drug.

All the preparations (standard, sample and impurities) were injected separately and the chromatograms were recorded. The results obtained of the commercially available formulations are tabulated in Table - 1.

Table - 2
Accuracy and Recovery study of Ribavirin

Added Amount (mg)	Recovered Amount (mg)		Percentage Recovery	
	Proposed Method	USP Method	Proposed Method	USP Method
2	1.987	1.972	99.35	98.60
4	3.932	3.982	98.30	99.55
6	6.002	5.899	100.03	98.32
8	8.012	8.102	100.15	101.27
10	9.983	10.151	99.83	101.51

RSD : Proposed Method : 0.757% ; USP Method 1.48%.

Precision, Linearity & Recovery Study

The method precision was evaluated by repeated assays of commercial formulations over separate periods of one day and one week. The within day precision was determined by performing five consecutive assays within a period of eight hours. The day today reproducibility of the method was determined by analysing the same sample (single operator) on seven consecutive days.

Under the proposed conditions Ribavirin showed a linear response over a range of 0 - 100 mcg/ml. The impurities also showed a linear response in the range of 0 - 50 mcg/ml.

The accuracy of the procedure was evaluated by proposed method and USP method. Known amount of the drug was added to the placeboes and were analysed by both the method. The recovery data obtained from the study was in the range of 98% to 101% by the proposed method and was in the range of 98.3 to 101.5% by the USP method, the relative standard deviation was 0.757% and 1.48% respectively. The data is presented in Table - 2. It is apparently clear from the data that the proposed method is very precise and accurate.

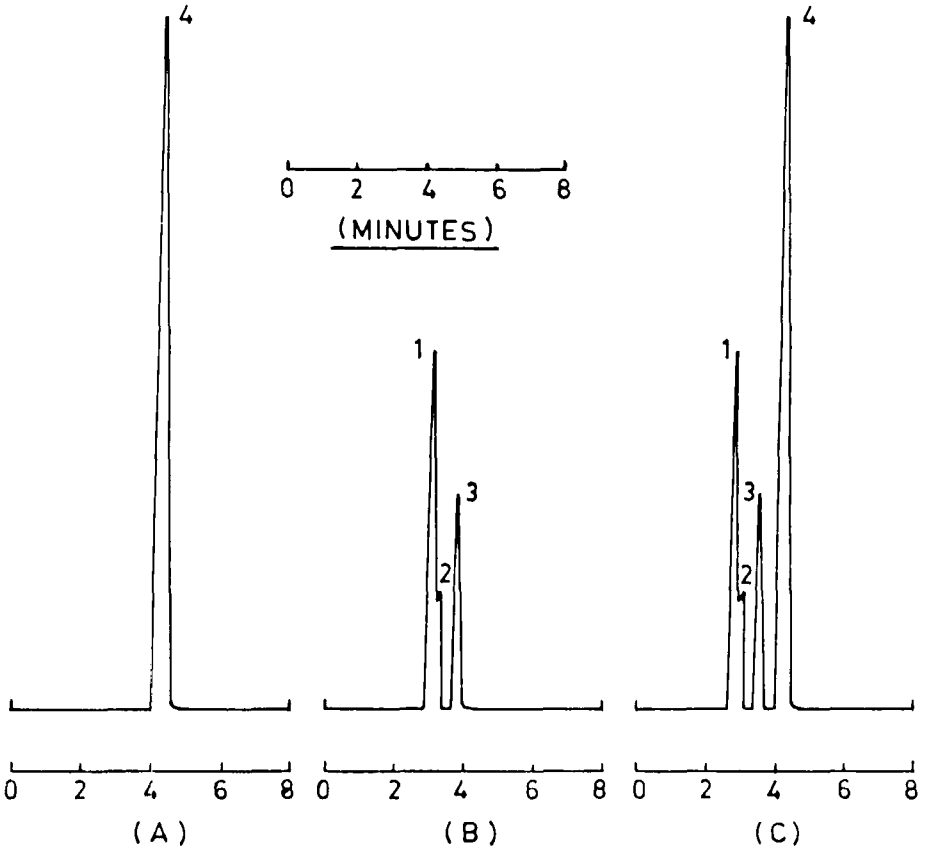

FIGURE-1

TYPICAL CHROMATOGRAMS OF RIBAVIRIN[A;4]: TCA, RTCA, TCO[B;1,2,3 respectively] AND THEIR COMBINATION[C].

Results & Discussion:

The proposed method is simple, accurate and specific with respect to the impurities mentioned. The typical chromatograms of Ribavirin (pure drug) and its impurities are shown in Figure-1.

The method was compared with the official method of USP. The results are presented in Table-3. The operating conditions of USP employ a ion-exchange column which is a rare column to procure. The proposed method employ a

Table - 3

Product	Declared Amount	Recovery by Proposed Method (%)	Recovery by USP method (%)
Ribavirin Bulk Drug	-	99.26	98.97
A	50 mg/5ml	99.60	99.39
B	100 mg/Cap	98.99	99.33
C	200 mg/Cap	100.50	99.67

easily available C18 column and the usability of C18 columns is much more than the ion-exchange column. More over the specified conditions in USP maintain the column at 65 C which requires an additional equipment set up to maintain the temperature. In the proposed method the column is maintained at ambient temperature.

Looking at the simplicity of the proposed method and the complexity of the USP method the authors feel that the proposed method has an edge over the USP method, and can be used in routine quality assurance analysis.

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