ISSN : 0974-7419 Volume 16 Issue 10

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 16(10) 2016 [441-447]

Development and validation of stability indicating method for determination of related substance in ribavirin drug substance

Subbarao Eedara[1]*, Pradeep B.Kumar[2], B.M.Rao[3], M.B.V.Narayana[1]
[1]Analytical Research, Sai Life sciences Ltd., Turkapally, Hyderabad 500085, (INDIA)
[2]Department of chemistry, KL University, Guntur 500085, (INDIA)
[3]Corporate quality control, Dr Reddy's laboratories Ltd., Hyderabad 500085, (INDIA)
E-Mail: eedara81@gmail.com

ABSTRACT

A simple and accurate and precise reverse phase liquid chromatographic method was developed for the determination of the Assay and related substance in Ribavirindrug substance. Ribavirin is abroad spectrum Anti-Viral agentshows varying degrees of clinical efficacy in a variety of human viral infections including viral hepatitis, respiratory tract infections and haemorrhagic fevers. The Chromatographic separation between Ribavirin and its related substances was achieved using a YMC- Pack ODS-AQ; 150 x 4.6 mm, 3µm column, buffer contains 1.0g of anhydrous sodium sulfate and 2ml of 5%V/V Orthophosphoric acid and adjusted pH to 2.8 with 5% v/v orthophosphoric acid, mobile phase contains buffer as Mobile phase A and buffer: Acetonitrile (95:05 %v/v) as Mobile phase B using a binary gradient mode with flow rate of the mobile phase kept at 1.0 mL/min. The sample concentration was 0.5 mg/mL. The column temperature was maintained at 25°C and the detection wavelength was 220 nm. The injection volume was 5µL. The resolution between the critical pair of peaks (Impurity-A&Ribavarin)was found to be greater than 2.0. The limit of detection (LOD) and limit of quantification (LOQ) of Impurity-A is 0.1ng/mL, and analyte were 0.3 ng/mL, for 5µl injection volume. The test solution and mobile phase was observed to be stable up to 48 h after the preparation. The method was validated and found good results of precision, Selectivity and Solution Stability. The proposed method was found to be suitable and accurate for the quantitative determination of the assay and related substances during release and stability testing of Ribavirin active pharmaceutical ingredient.

KEYWORDS

High performance liquid chromatography;
Assay;
Related substances;
Validation;
Quantification and ribavirin.

INTRODUCTION

Ribavirin is described chemically as 1-β-D-ribofuranosyl-1H-1,2,4-trizole-3-carboxamide (Figure 1) and Impurity-A is described chemical name as Ribose triazole carboxylic acid(Figure 2). It is one of the Anti-Viral drug[1] used in treatment of patients with Chronic Hepatitis C. High-Performance Liquid Chromatographic Determination of Ribavirin in Whole Blood to Assess Disposition in Erythrocyteswas reported in the literature[2-3]. Determination of ribavirin in human serum and plasma

Full Paper

by capillary electrophoresis was also reported[4]. A rapid rp-hplc method development and validation for the quantitative estimation ribavirin in tablet[5], Stability-Indicating HPLC–DAD Determination of Ribavirin in Capsules and Plasma[6-7] were also reported. So far to our knowledge no stability indicating HPLC method for determination of the assay and related substances of Ribavirin drug substance has been reported.

Attempts were made to develop a suitable stability indicating LC method that can be used to determine the Assay and related substances bulk samples of Ribavirin. The present work describes a new, simple and accurate reverse phase liquid chromatographic method for the detection of the process-related impurities and degradation products generated from forced degradation studies which may be present in the bulk drug. The developed method was validated to ensure the compliance in accordance with ICH guidelines.

EXPERIMENTAL

Chemicals and reagents

Samples of Ribavirin and its related substances, Impurity-A (Imp. A), (Figure 2) were received from a Sai Life Science ltd., Hyderabad, India. The HPLC grade acetonitrile was purchased from Rankem fine chemicals, India, Analytical Reagent grade Sodium sulfate anhydrous, Ortho phosphoric Acid was purchased from Merck fine chemicals, India, HPLC grade water was produced internally by using Milli-Q, Millipore water purification system

Instrumentation

The LC system, used for method development and validation was from Waters alliance 2695 series with 2489/2998 Detectors (Waters corporation 34 Maple street., Milford, Massachusetts, 01757USA) consists of Quaternary gradient pump, auto sampler, Column oven and variable wavelength detector. The output signal was monitored and processed using Empower software on Pentium computer (Hewlett Packard).

Sample preparation

The stock solutions were prepared separately by dissolving the appropriate amounts of the related substances and compound in diluent, acetonitrile: water, 50:50 (v/v). The target analyte concentration was fixed as 500ng/mL.

RESULTS AND DISCUSSION

The objective of this work is to develop suitable stability indicating HPLC method for the assay and related substances and degradation products that were present in ribavirin drug substance. The mixture of related compounds (Imp. A,) and Ribavirin was used in the method development. Different Reverse phase stationary phases were employed during method development namely Zorbax SB-C8, 250x4.6 mm, 5µm, (Agilent Technologies, USA), Luna C-18 150x4.6 mm, 3 µm (Phenomenex, USA), Zorbax SB-C18, 150x4.6 mm, 3.5µm, YMC- Pack ODS-AQ; 150 x 4.6 mm, 3µm column Different trails were made during the method development and the details were mentioned in the TABLE 1.

Optimized chromatographic conditions

TABLE 1 : Results of different trails

Trial	HPLC Conditions	Remarks
1	Zorbax SB-C8, 250X4.6 mm, 5µ Mobile phase: 0.1% v/v TFA in water (MP-A) and 0.1% v/v TFA in Acetonitrile (MP-B) Flow rate: Flow rate: 1.0 mL/min, Column temperature: 25 °C In Volume: 10 ul	Poor resolution
2	Column: Luna C-18 150X4.6mm, 3 µm Mobile phase: 10 mm Ammonium Acetate and (MP-A) and Acetonitrile MP-B) Column temperature: 30 °C	Poor resolution Analyte peak shape is broad
3	Column: Zorbax SB-C18, 150X4.6 mm, 3.5µm Mobile phase: 10 mm Ammonium Acetate (MP-A) and Acetonitrile MP-B) Flow rate: 1.0 mL/min, Column temperature: ambient	Peak tailing were Observed

TABLE 2 : Forced degradation study results

S. No.	Stress condition	Stress Solution	Duration	%Degradation
1	Acidic		24 hours	9.8
2	Basic		24 hours	12.5
3	Thermal		24 hours	0.1
4	Peroxide		24 hours	4.8
5	Photolytic		24 hours	0.1

TABLE 3 : Validation results

Parameter		Results		
	Name	Resolution	Tailing Factor	Theoretical Plates
System Suitability	Ribavirin	NA	1.089	12578
	Impurity-A	5.83	1.23	8797
	Name	Retention Time	Purity Angle	Purity Threshold
Specificity	Ribavirin	4.108	0.07	23.14
	Impurity-A	3.290	15.24	23.14
	Name	System Precision (%RSD)		Method Precision (%RSD)
Precision Results	Ribavirin	1.3		2.32
	Impurity-A	1.1		2.81

Chromatographic separations were achieved only YMC- Pack ODS-AQ; 150 x 4.6 mm, 3µm column, buffer contains 1.0g of anhydrous sodium sulfate and 2 mL of 5%v/v Orthophosphoric acid and adjusted pH to 2.8 with 5% v/v orthophosphoric acid, mobile phase contains buffer as Mobile phase A and buffer: Acetonitrile (95:05 %v/v) as Mobile phase B using a binary gradient mode with flow rate of the mobile phase kept at 1.0 mL/min. The test sample concentration was 0.5 mg/mL in diluent, (Diluent is water). The column temperature was maintained at 25°C and the detection wavelength was 220 nm. The injection volume was 5 µL. The total analysis time for each run was 45 min. Good separations of all impurities and degradants within short run time were observed on YMC- Pack ODS-AQ; 150 x 4.6 mm, 3µm column.

Typical retention times of Imp. A, is 3.6 min., and analyte is4.36 min. The system suitability[8] results were given in TABLE: 2.

Method validation

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.[9] Specificity was tested by injecting the spiked sample of Ribavirin with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Moreover, identification of impurity-A was confirmed with retention time as compared with those of pure standards and also conform the peak purity of the analyte and impurity –A. Results were given in TABLE: 3

Forced degradation studies were performed for bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of Photolytic degradation as per ICH Q1B, Thermal degradation (at 60° C), acid hydrolysis (using 0.5 N HCl), base hydrolysis (using 0.5 N NaOH), and oxidative degradation (using 3.0% H_2O_2) to evaluate the ability of theproposed method to separate Ribavirin from its degradation products. For heat and light studies, study period was 10 days where as for acid, base, and oxidative degradation it was 48 hours. In all the stressed conditions peak purity of Ribavirin peak was passes by PDA detector. The chromatogram was presented in Figure: 4 and the results were presented in TABLE: 2.

Full Paper

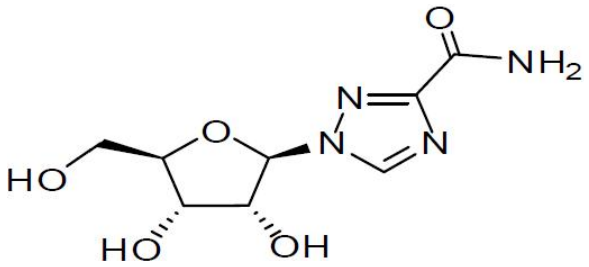


Figure 1 : Chemical structure of1-β-D-ribofuranosyl-1*H*-1, 2, 4-trizole-3-carboxamide (Ribavarin)

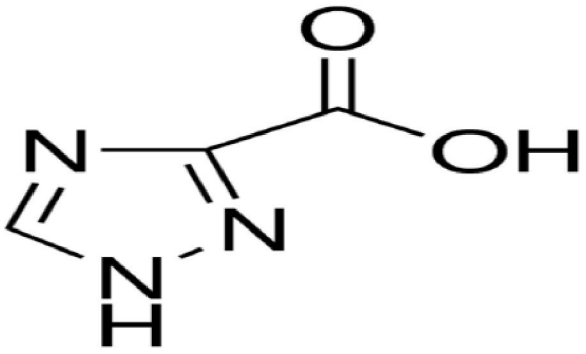


Figure 2 : Chemical structure of ribose triazole carboxylic acid (Imp. A)

Precision

Method precision

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions[9].

The system suitability was performed using 0.5 mg/mL ribavirin standard and impurity -A. Themethod precision for 0.5 mg/ mLRibavirinsample. spiked with 0.20% of Imp. A with respect to analyte concentration with six different preparations. the percentage relative standard deviation (%RSD) of method repeatability and system suitability for impurities was confirms good precision of the method. Results were given in TABLE:3

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample[9]. The linearity of the method was checked at six concentration levels of impurity-A i. e. from LOQ (0.03ng/mL) to 15ng/mL. The coefficient of regression of the calibration curvewas found to be greater than 0.99, andand%y –Intercept is getting is 3.5 % for impurity-A. Thus confirming the excellent correlation existed between the peak area and concentration of the impurities.

Limit of detection and limit of quantification

The limit of detection (LOD) represents the concentration of analyte that would yield a signal to noise ratio of 3[9]. The limit of detection (LOD) of Imp. A, 0.01ng/mL respectively for 5 µl injection volume. The limit of quantification (LOQ) represents the concentration of analyte that would yield a signal to noise ratio of 10[9]. The limit of quantification (LOQ) of Imp. A, were 0.3ng/mL, for 5µL injection volume. The precision for Imp-A at LOQ level was good, the relative standard deviation was found to be below 5.2%.

Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the present method, for the quantification Imp. A, The study was carried out at LOQ, 0.1, 0.2 0.3, and 0.5 % of target analyte concentration (0.5 mg/mL) of Imp. A, The percentage recoveries of impurities were ranged from 95.8 to 103.4 in samples of Ribavirindrugsubstance.

Robustness

The robustness of an analytical procedure[9] is measure of its capability to remain unaffected by small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage. In the varied chromatographic conditions viz. flow rate, mobile phase ratio and column temperature, the resolution between RibavirinImp. A, peaks was found to be >2.5 illustrating the robustness of the method.

Solution stability and mobile phase stability

Solution stability was studied by keeping the test solution spiked with impurities in tightly capped volumetric flask at temperature 25 ^{o} $\pm$ 2 ^{o} C on a laboratory bench for 24 h. Content of impurities was checked for every 12 h interval and compared with freshly prepared solution. No variation was observed in the content of impurities in sample solu-

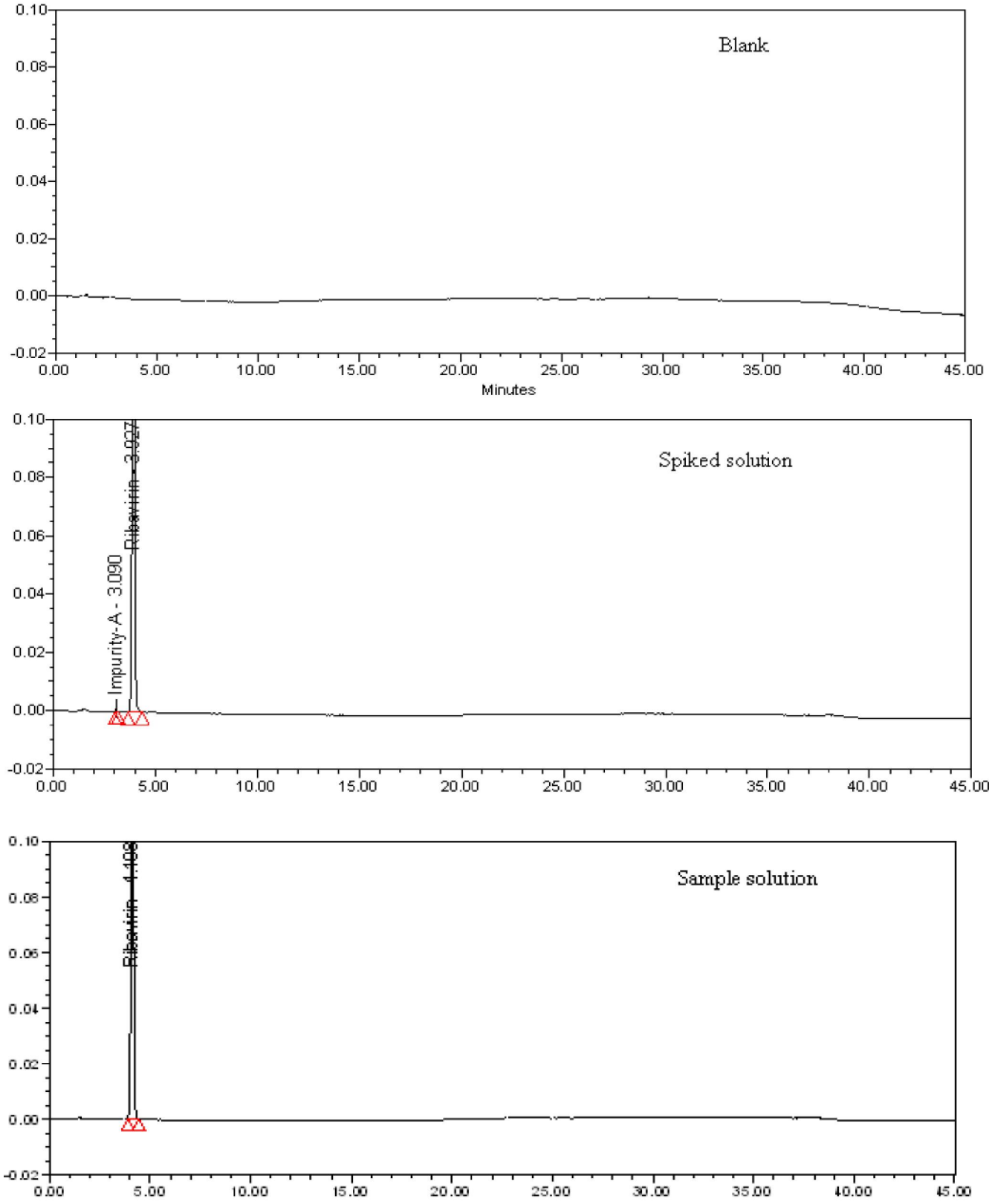
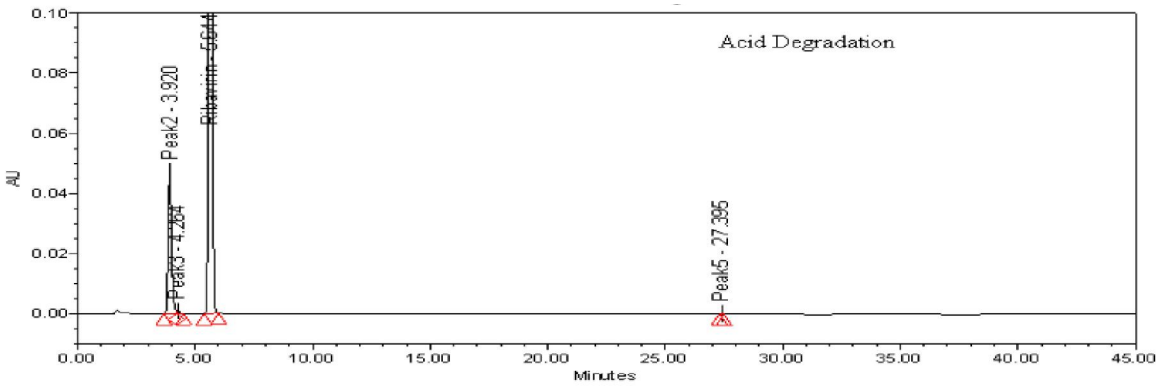


Figure 3 : HPLC chromatograms of blank, impurity-a spiked and ribavirin sample

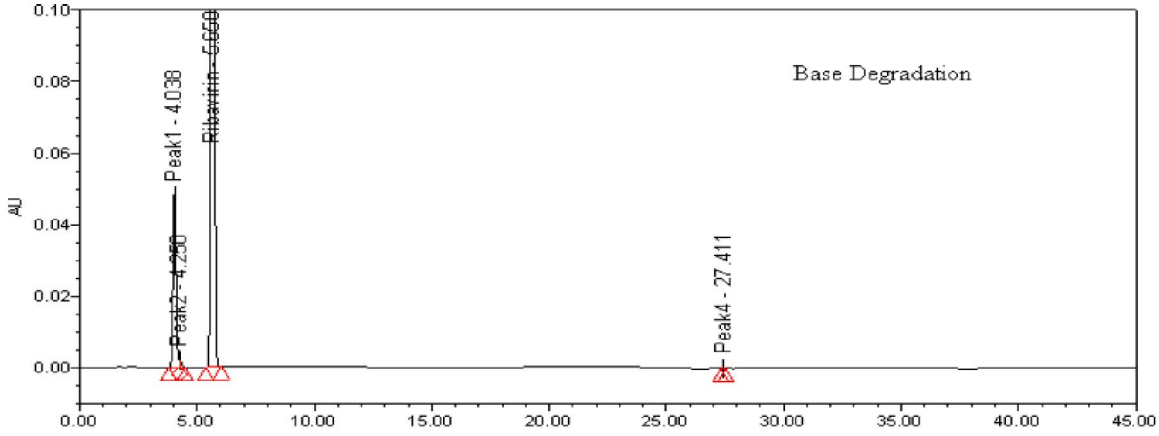
tions prepared in diluent were stable up to 48 h.

Mobile phase stability was carried out by evaluating the content of impurities in sample solution spiked with impurities, which were prepared freshly

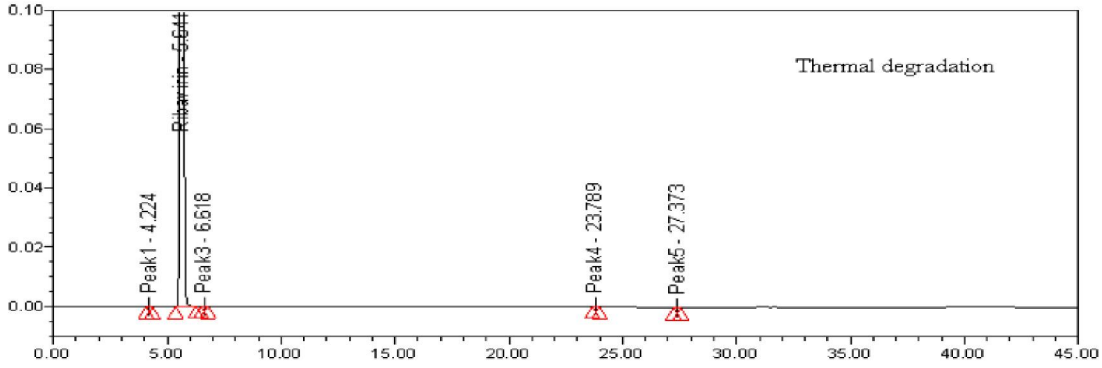
Full Paper



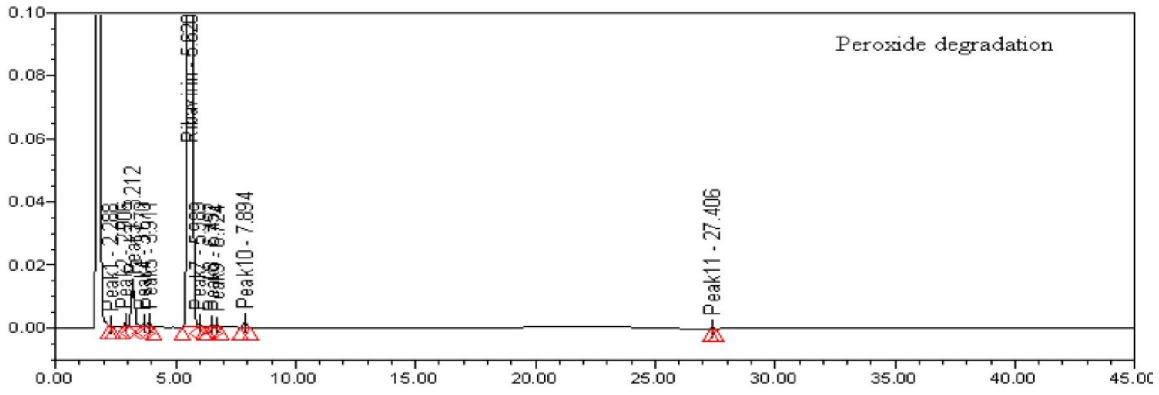
(a)



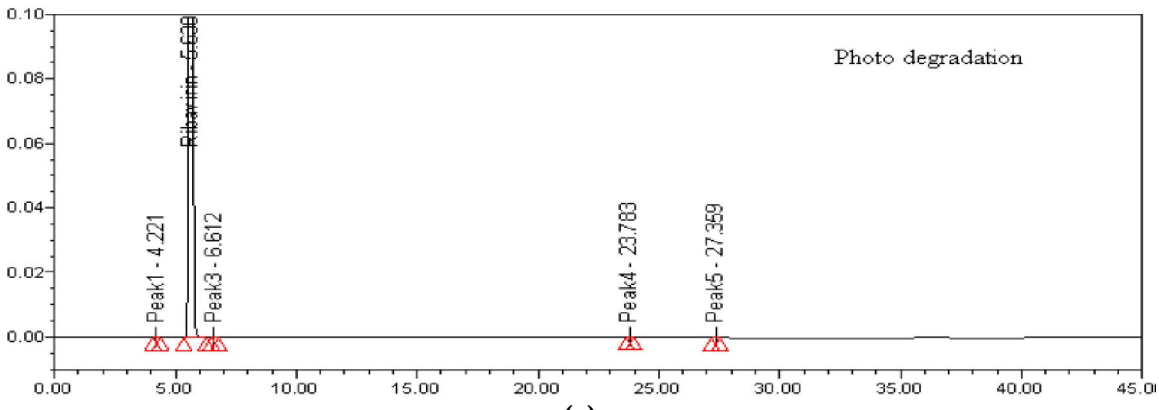
(b)



(c)



(d)



(e)

Figure 4 : Degradation study chromatograms a) acid, b) base, c) thermal, d) peroxide and e) photolytic

at every 12 h for 48 h. The same mobile phase was used during the study period. No variation was observed in the content of impurities for the study period and it indicates prepared mobile phase was found to be stable up to 48 h.

CONCLUSION

In this study, the simple, accurate and well-defined stability indicating HPLC method for the determination of the Assay and related substances and degradation products in Ribavirin was described. Ribavirin under various stress conditions were studied and presented. Based on the validation study results obtained, concluded that, the analytical method used for the related substances and Assay of Ribavirin drug substance by HPLC method is suitable for release and stability testing analysis during manufacturing.

ACKNOWEDGEMENTS

The authors wish to thank the management of Sai Life Sciences Ltd., and colleagues at analytical research department for supporting the research work.

REFERENCES

[1] H.Matsumoto, S.Hara, N.Nagata, K.Ikeda; Heterocyclic, **41**, 47 **(1995)**.
[2] Masato Homma, L.Anura; Jayewardene November, **43(11)**, 2716-2719, **(1999)**.
[3] Frank C.Bekkering1, Johannes T.Brouwer1; **33**, 319–486, February, **(2001)**.
[4] Michael C.Breadmore; Regula Theurillatand Wolfgang Thormann, **25**, 1397–1726 **(1997)**.
[5] G.Raveendra Babu, A.Lakshmana Rao, J.Venkateswara Rao; SCImago, SJR**7, (2014)**.
[6] Rim Said Haggag, Said Fathalla Belal; Ismail Ibrahim Hewala1 and Ola Ahmed El Rouby2, J Chromatogr.Sci., doi: 10.1093/chromsci/bmt067, **(2013)**.
[7] Zhi-Ling Zhou1, Xi-Yong Yu1, Min Yang1, Huai-Yan Peng2, Tie-Feng Chen; **22(12)**, 1321–1449 **(2001)**.
[8] United States Pharmacopeia, Asian Edition, **621**, 1225 **(2004)**.
[9] ICH Harmonised Tripartite Guideline on validation of Analytical Procedures: Q2(R1), November 2005 and Analytical procedures and method validation for the drug and Biologics Guidance for industry, July, **(2015)**.