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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD FORESTIMATION OF RIOCIGUAT IN BULK AND PHARMACEUTICAL DOSAGE FORM

Pamil Desai & Ankita Bhavsar

Research Scholar, Department of Pharmaceutical Quality Assurance, Sat Kaival College of Pharmacy, Sarsa, Gujarat, India

ABSTRACT

Stability indicating high performance liquid chromatography method for the analysis of Riociguat was developed and validated. The column used was Hypersil BDS C_{18} (250 X 4.6 mm 5.0 µ) with a flow rate of 1.0 ml/min using UV detector at 233 nm. The chromatograms were developed using (0.05M potassium dihydrogen orthophosphate, ph-5.0) Buffer: Methanol (10:90% V/V) as a mobile phase. The described method was linear over a concentration range of 25-75 µg/ml for the assay of Riociguat. The retention times of Riociguat was found to be 4.403 min. The % recovery of Riociguat was found to be 101.55%. The limit of detection and limit of quantification were found to be 1.95 µg/ml and 5.90 µg/ml respectively. The % RSD of Riociguat was found to be 0.73 %. The method developed is robust. The drug was exposed to acidic, basic, oxidative, photolytic and thermal degradation. The peaks of degradation products were well-resolved from the peak of the standard drug with significantly different values. Results showed that the developed method is simple, specific, accurate and robust for the determination of Riociguat in its formulation. The method can effectively separate the drug from its degradation products and it can be considered as a stability-indicating assay.

***KEYWORDS**: Riociguat, Methanol, Method Development, Validation, Stability Study*

Article History

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INTRODUCTION

Riociguat is a vasodilator. It works by widening the blood vessels that connect the heart to the lungs. This increases the blood supply to the lungs, which reduces the workload of the heart.[2]

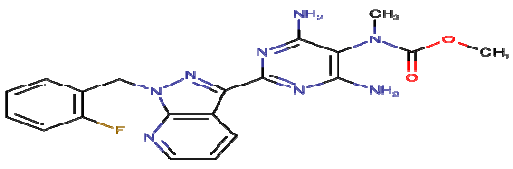

Figure 1: Chemical Structure of Riociguat

The drug was approved on 16.04.2018 by CDSCO and is not official in any pharmacopoeia.[3]

Literature survey reveals that very few analytical methods have been reported for estimation of Riociguat but no stability indicating method has been reported till date.

MATERIALS AND METHOD

Instrumentation

Weighing Balance used Scale Tec, Micro Balance - Mattler Toledo, pH meter - LAB INDIA, Melting point apparatus - LAB INDIA MR-VIS, IR AFFINITY-1 - SHIMADZU, UV Spectrophotometer - SHIMADZU, Sonicator - Sonorax, HPLC - SHIMADZU. Pump –LC-20 AT, Injector - I3000, Detector - SPD 20 A UV Detector, Column - Column, 250 X 4.6mm C_{18} 5.0 µ, Hypersil BDSwere used.

Materials and Reagents

Methanol - HPLC (Merck, India Limited), Potassium dihydrogen ortho phosphate – AR grade (Merck, India Limited), Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide (30%) Analytical Reagent (Merck, India Limited) was used.

Preparation of Solutions

Preparation of 0.1N HCl

A solution of 0.1N HCl was prepared by taking 0.85 ml concentrated HCl in 100 ml volumetric flask and diluted up to mark with water.

Preparation of 0.1 N NaOH

A solution of 0.1N NaOH was prepared by dissolving 0.4 gm NaOH pallets in 100 ml water.

Preparation of 3% H_2O_2

A solution of 3 % H_2O_2 was prepared by taking 10.0 ml of 30 % H_2O_2 in 100 ml volumetric flask and diluted up to mark with water.

Preparation of the Mobile Phase

The mobile phase was prepared by mixing 0.05M Potassium dihydrogen ortho phosphate buffer pH 5.0: Methanol (10:90 %v/v).

Standard Preparation (50 µg/ml Riociguat)

An accurately weighed 50 mg of Riociguat was then transferred in 100 ml of the volumetric flask, dissolve and volume make up with diluent. Further, dilute 1ml of this solution was transferred into a 10 ml volumetric flask and the volume was adjusted up to mark with diluent to get a concentration of Riociguat50 µg/ml.

Sample Preparation

(Marketed formulation preparation) An accurately weighed 25 mg of powder was transferred into 50 ml volumetric flask, added about 30ml of diluent into it, shake for 10 minutes and made up to volume with diluent and mixed well.

METHOD VALIDATION[4]

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, the limit of quantification, robustness and system suitability.

Linearity The developed method has been validated as per ICH guidelines (Zucman D, 2007). Working standard solutions of Riociguat in the mass concentration range of 25 µg/ml to 75 µg/ml was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Riociguat was obtained by plotting the peak area ratio versus the applied concentrations of Riociguat. The linear correlation coefficient was found to be 0.9993. Results are shown in table 1.

Table 1: Linearity of Riociguat

S. No	Conc(µg/ml)	Area
1	25	770.565
2	32.5	1186.479
3	50	1559.379
4	62.5	1924.313
5	75	2436.120

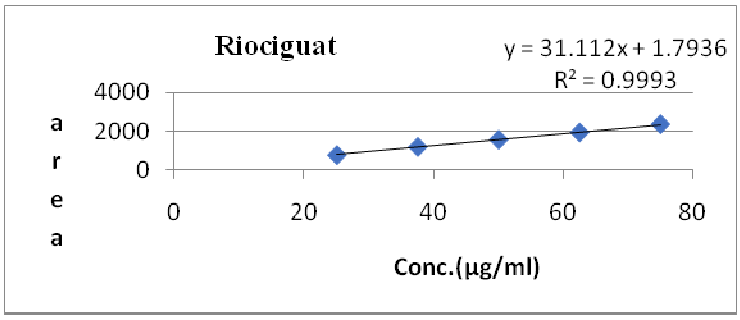

Figure 2: Calibration Curve of Riociguat

Table 2: Precision parameters of Riociguat

Injection	Concentration(µg/ml)	Intra Day	Inter Day
1	50	1542.313	1564.033
2	50	1553.106	1578.157
3	50	1542.256	1571.816
RSD		0.40	0.45

Precision

Repeatability of the method was checked by injecting replicate injections of 50 µg/ml of the solution for three times on the same day as the intraday precision study of Riociguat and the RSD was found to be 0.40 for intraday and 0.45 for interday. Results are shown in table 2.

Accuracy

The accuracy of the method was determined by calculating the recovery of Riociguat by the method of standard addition. A known amount of Riociguat (8 µg/ml, 10 µg/ml and 12 µg/ml) was added to a pre quantified sample solution

and the amount of Riociguat was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Riociguat was estimated by measuring the peak area ratios by fitting these values to the straight line equation of the calibration curve. From the above determination, percentage recovery was calculated and the average recovery was found to be 101.56%. Results are shown in table 3.

Table 3: Recovery Results

Recovery	Conc. of Sample	Recovery	% of Recovery
80%	8 µg/ml	8.14	101.80
100%	10 µg/ml	10.12	101.24
120 %	12 µg/ml	12.20	101.63

Specificity

The specificity of the method was determined by comparing test results obtained from the analysis of sample solution containing excipients with that of test results those obtained from standard drug.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 1.95 µg/ml and 5.90 µg/ml respectively as per ICH guide-lines. Results are shown in table 4.

Table 4: Results of LOD and LOQ

Parameter	Measured
LOD	1.95 µg/ml
LOQ	5.90 µg/ml

Table 5: Robustness Results

Parameter	Modification	Peak Area	% of Change
Standard	No change	1550.085	–
Mobile phase	buffer: Methanol (12:88% v/v)	1551.519	0.09
Flow Change	1.2ml/min	1533.880	1.05
pH	5.02	1568.729	1.20

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied. Results of Robustness are shown in Table 5.

System Suitability Parameter

System suitability tests were carried out on freshly prepared standard stock solutions of Riociguat and it was calculated by determining the standard deviation of Riociguat standards by injecting standards in six replicates and the values were recorded in Table 6.

Table 6: System Suitability Parameters of Riociguat

Parameters	Values
λ max (nm)	233nm
Beer's law limit (µg/ml)	25-75 µg/ml
Correlation coefficient	0.9993
Retention time	4.393min
Theoretical plates	7030
Tailing factor	1.345
Limit of detection	1.95 µg/ml
Limit of quantification	5.90 µg/ml

Standard chromatogram of Riociguat was shown in figure 3 and chromatographic condition in Table 7

Table 7: Chromatographic Conditions

S. NO	Test HPLC Conditions	Result
1	elution	Isocratic
2	API conc	50 µg/ml
3	Mobile Phase	Buffer : Methanol (10:90% v/v)
4	pH	5.0 With 0.1 N NaOH
5	Column	250 X 4.6mm C_{18} 5.0 µm, HypersilBDS
6	Wavelength	233nm
7	Flow	1 ml\min
8	Runtime	15mins
9	Retention time	4.393 min
10	Area	1539.297
11	Theoretical plates	7030
12	Tailing factor	1.345
13	Diluent	Mobile phase
14	Injection Volume	20 µl

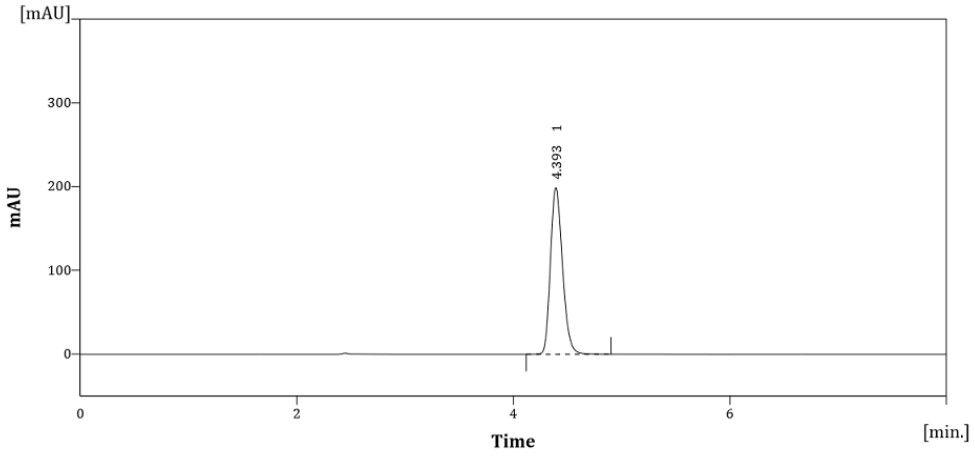

Figure 3: Standard Chromatogram of Riociguat

Name	RT Time	Height	Area	Conc.	Tailingfactor	Theoretical Plates
Riociguat	4.393	198.934	1539.297	100	1.345	7030

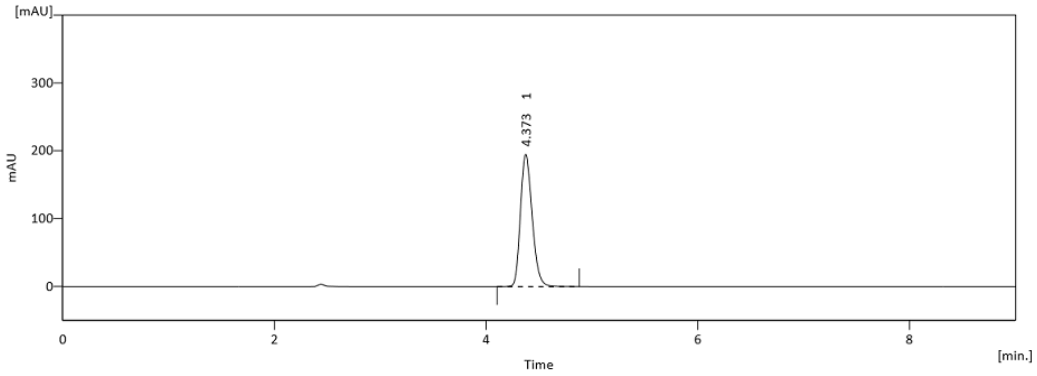

Figure 4: Formulation Chromatogram of Riociguat

Name	RT Time	Height	Area	Conc.	Tailing Factor	Theoretical Plates
Riociguat	4.373	195.797	1508.563	100	1.393	6966

Table 8: Formulation Results of Riociguat

Formulation	Dosage	Sample Concentration	Amount Found	% Estimation
Rioci(Tablet)	1.5 mg	50 µg/ml	48.95	97.90

Optimization of the Chromatographic Conditions

The nature of the sample, its molecular weight and solubility decide the proper selection of the stationary phase. The drug Riociguat being non-polar is preferably analyzed by reverse phase columns and accordingly, C_{18} column was selected. So the elution of the compound from the column was influenced by the polar mobile phase. The concentration of the water and Methanol were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase(0.05M Potassium dihydrogen phosphate Buffer: Methanol 10:90 v/v), pH 5.0 with 0.1NNaOH. The retention time of Riociguat was found to be 4.393min, which indicates a good base-line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that the developed method was accurate and precise. The system suitability and validation parameters are given in Table 6. The high percentage of recovery of Riociguat was found to be 101.56% indicating that the proposed method is highly accurate. The proposed liquid chromatographic method was applied for the determination of Riociguat in tablet formulation. The result for Riociguat was comparable with a corresponding labeled amount. The absence of additional peaks indicates no interference of the excipients used in the tablets.

Forced Degradation Studies [5]

Forced degradation studies like acid/base, thermal, oxidation, and Photolytic of the drugs were carried out. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the methods. The proposed stability – indicating RP-HPLC method was validated as per ICH Q1A (R2) guidelines[6].

For acid degradation 2 ml of 0.1N HCl for 30 min 12.83% Riociguat degradation were observed.

Base degradation with 2ml of 0.1N NaOH 30 min the 9.81 % Riociguat degradation was observed.

During oxidative degradation 2 ml of 3% H_2O_2 for 30 min the 15.46 % Riociguat degradation were observed.

Thermal degradation at 105°C for 30 min the 14.61 %. Riociguat degradation was observed in Thermal degradation.

Photolytic degradation at UV chamber for 30 min the 12.10 %. Riociguat degradation were observed in Photolytic degradation.

Acidic Degradation

1 ml sample stock solutions of Riociguattaken into 10ml of the volumetric flask, added 2 ml 0.1N HCl into it and it was kept at room temperature for 30 min. Then added 2ml of 0.1N NaOH to neutralize it and volume was made up to mark with diluent and mixed well and injected. A chromatogram is shown in (Figure -5,6)

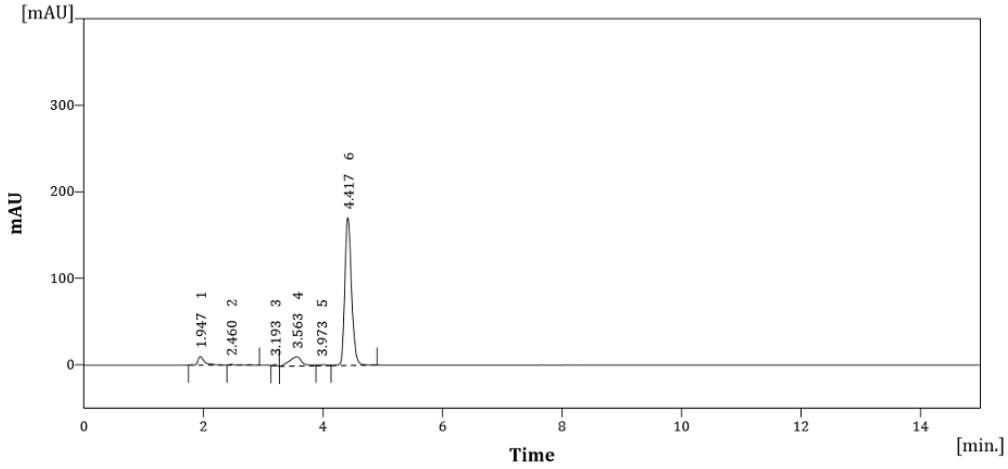

Figure 5: Chromatogram of Standard Riociguat acid Degradation (0.1N HCl, 2 ml for 30 min)

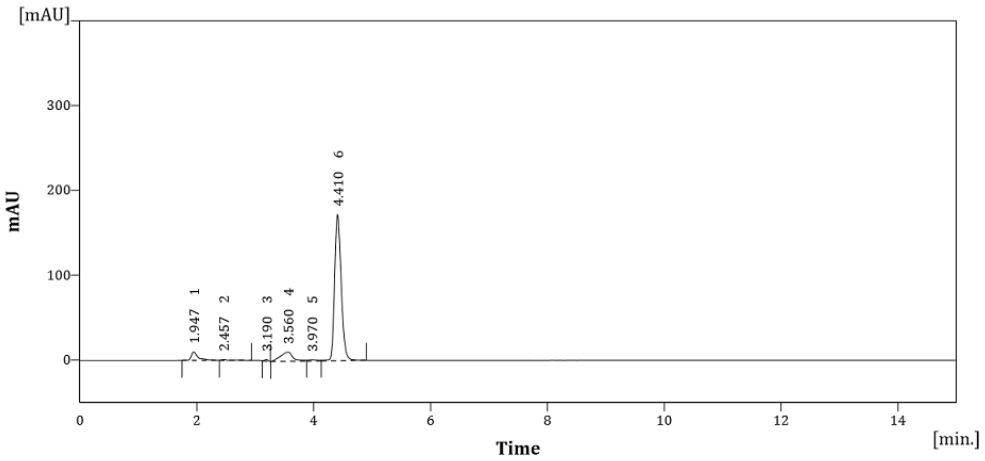

Figure 6: Chromatogram of Sample Riociguat Acid Degradation (0.1N HCl, 2 ml for 30 min)

Basic Degradation

1ml sample stock solutions of Riociguat taken into 10ml of the volumetric flask added 2ml 0.1N NaOH into it and it was kept at room temperature for 30 min. Then added 2ml of 0.1N HCl to neutralize it and volume was made up to mark with diluent and mixed well and injected. A chromatogram is shown in (Figure 7).

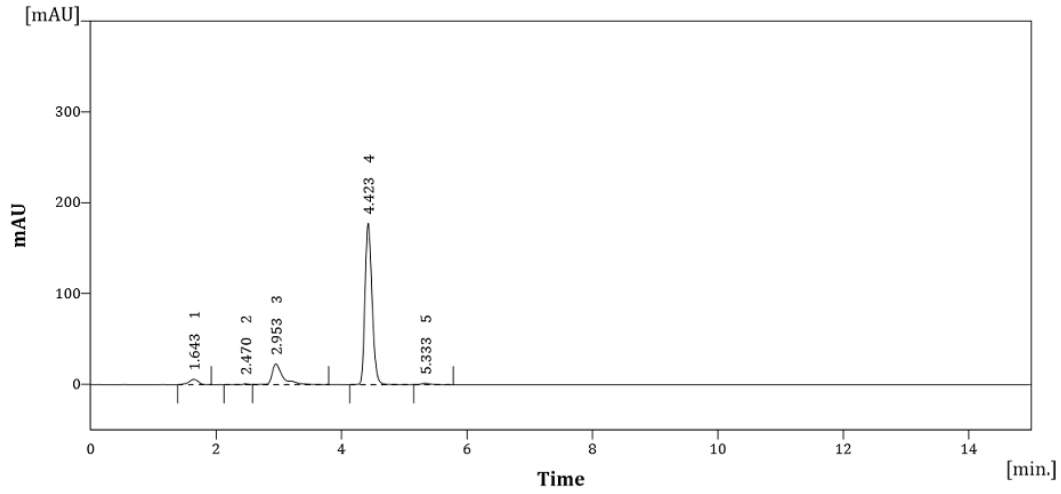

Figure 7: Chromatogram of Riociguat Base Degradation (0.1N NaOH, 2 ml for 30 min)

Oxidative Degradation

1ml sample stock solutions of Riociguat taken into 10ml of the volumetric flask added 2ml 3% H_2O_2 solution into it and it was kept at room temperature for 30min. Then volume was made up to mark with diluent and mixed well and injected. A chromatogram is shown in (Figure 8).

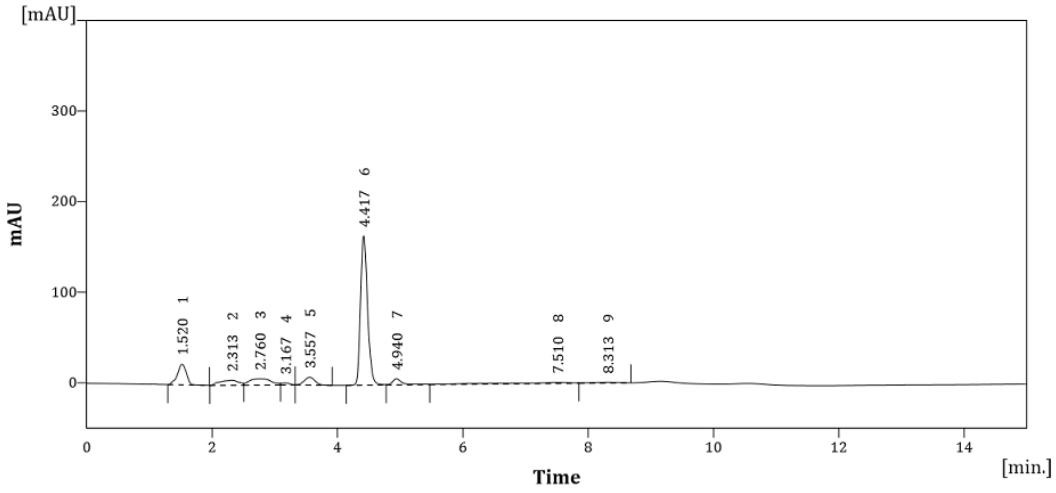

Figure 8: Chromatogram of Riociguat Oxidative Degradation (3% H_2O_2, 2 ml for 30 min)

Thermal Degradation

1ml sample stock solutions of Riociguat were taken into 10ml of the volumetric flask; it was kept for 30 min at 105°C temperature. Then volume was made up to mark with diluent and mixed well and injected. A chromatogram is shown in (Figure 9).

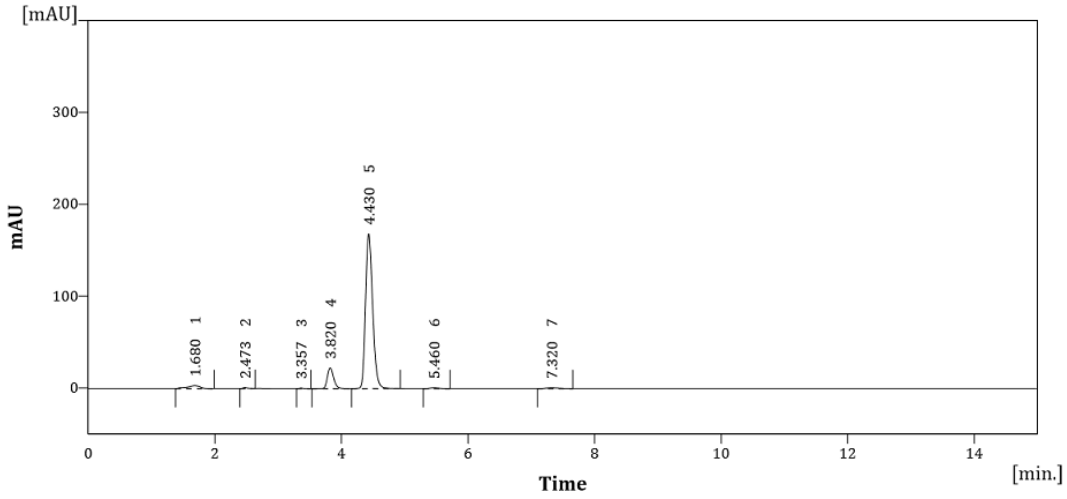

Figure 9: Chromatogram of Riociguat Thermal Degradation (105°C, for 30 min)

Photolytic Degradation

1ml sample stock solutions of Riociguat were taken into 10ml of the volumetric flask; it was kept for 30 min in sunlight. Then volume was made up to mark with diluent and mixed well and injected. A chromatogram is shown in (Figure 10).

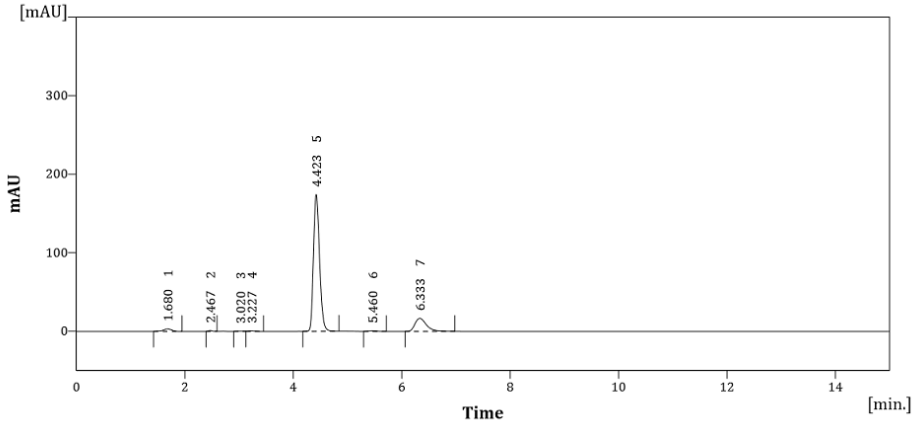

Figure 10: Chromatogram of Riociguat Sunlight Degradation (Sunlight 30 min)

CONCLUSIONS

An attempt has been made to develop simple, rapid, accurate methods for estimation of Riociguat by High-Performance Liquid Chromatography. This method was validated as per ICH guideline (Q2R1). For RP-HPLC linearity of Riociguat were found in the range 25.0-75.0 µg/ml. The result of the analysis of pharmaceutical formulation by the proposed method is reproducible and reliable. It is a good agreement with the label claim of the drug. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the methods. The proposed stability – indicating RP-HPLC method was validated as per ICH Q1A (R2) guidelines. Stability indicating method can be successfully applied to perform long – term and accelerated stability studies in Pharmaceutical dosage form. It can also be used to check the quality of the product after different storage condition and when stress degradation is carried out. The results indicated the suitability of the method to study the stability of Riociguat under various forced degradation condition

viz. acid, base, oxidative, and thermal degradation. It can be concluded that the developed method may be employed for analysis of stability samples of Riociguat.

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