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STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF RIOCIGUAT IN BULK & PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The Paper involves Development of a simple, precise and stability-indicating Reverse Phase High Performance liquid chromatographic method for determination of Riociguat in Bulk as well as in Marketed formulation, using an Inertsil ODS-3 C_{18} column with a mobile phase composed of Buffer: ACN (50:50, v/v), pH 4.5, adjusted using glacial acetic acid with a run time of 15 mins and wavelength for Estimation at 323nm using PDA Detector. The Retention time for Riociguat was found to be at 5.6 mins. Linearity for Riociguat was established in the range of 1.00-3.00 µg/ml having correlation coefficient R^2 value as 0.9999. The Accuracy was found to be in the range of 98.00% - 102.00%. Forced Degradation studies revealed that the method developed to be stability-indicating. The results Obtained showed that the proposed method is suitable for the precise determination of Riociguat in bulk and its formulation.

KEYWORDS: Riociguat, HPLC, Development and Validation, Stability Indicating Method.

INTRODUCTION

Riociguat belongs to the new class of Drugs having therapeutic activity namely guanylate cyclase stimulators. It is used for treatment of two forms of pulmonary hypertension namely, chronic thromboembolic hypertension and chronic arterial hypertension.[1] It has a dual mode of action, directly stimulating soluble guanylate cyclase independent of nitric oxide, and also by increasing the sensitivity of soluble guanylate cyclase to nitric oxide, which increases the level of cyclic guanosine monophosphate, which ultimately results in vasorelaxation and antiproliferative and antifibrotic effects.[1] Chemically Riociguat is Methyl{4,6-diamino-2-[1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl]-5-pyrimidinyl}methylcarbamate.[1]

The Literature survey revealed that a HPLC method is available for the estimation of Riociguat from its bulk drug and dosage form[2], Therefore it was proposed to develop simple, precise and stability indicating method for estimation of Riociguat.

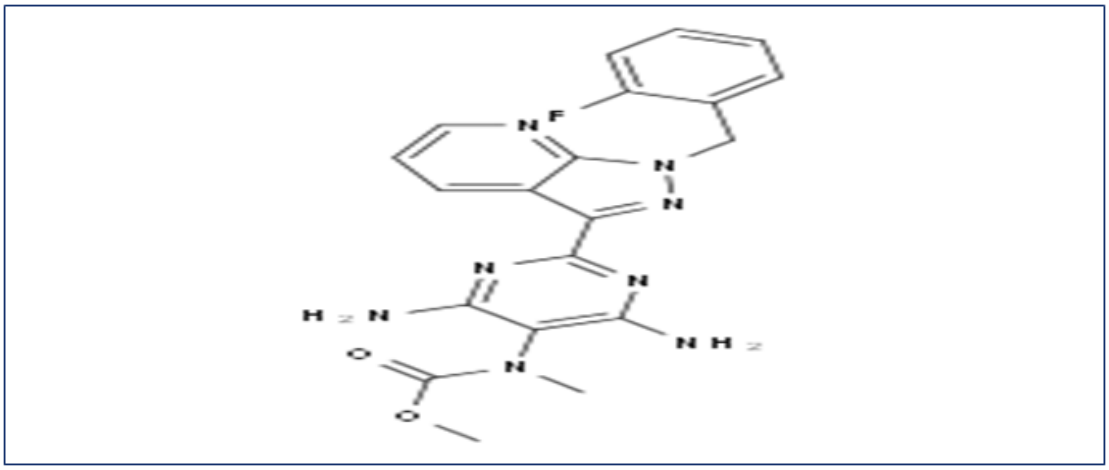

Fig. 1: Structure of Riociguat.[3]

MATERIALS AND METHOD

Materials: Riociguat standard was procured from MSN LABS, India as a gift sample and RIOCI Tablets (Marketed Formulation) was purchased from the local market. HPLC grade water was procured from J.K. Labs Mumbai and other reagents such as Acetonitrile, Methanol, and IPA was procured from Merck chemicals cooperation Ltd. Mumbai India.

Instrumentation: The Estimation of Riociguat was carried using JASCO HPLC system with Jasco MD-2018 plus Intelligent PDA Detector using ChromNAV software as an integrator, Analytical Balance (Mettler Toledo), pH meter (Lab India) and a sonicator (Spectralab) The column used for separation of Riociguat was INERTSIL ODS-3 (250 mm×4.6 mm×5 µ).

Method Development[4]

Various trials were conducted for the selection of mobile phase for the method development. The summary of these Trials is stated in Table. 1.

Table 1: Experimental Trials for choice of Mobile Phase.

Mobile Phase composition	Observation	Inference
Water : Methanol	No precision in retention time, very broad peak with tailing.	Use of Acetonitrile required for improving Peak shape
Water : Acetonitrile	No precision in retention time, but improvement in peak shape.	Use of buffer to improve precision in retention time.
Buffer : Acetonitrile	Precision in retention time with good peak shape & resolution.	Selected for optimization of the method.

Preparation of Mobile phase: The Mobile phase was Composed of Ammonium Acetate buffer and Acetonitrile in the ratio of 50:50 v/v; pH was adjusted to 4.5 using glacial acetic acid. This mixture was further sonicated for 15 mins with intermittent shaking, and used as a Mobile phase and diluent for the Analysis of the samples.

Preparation of standard solution: 10 mg of Riociguat was accurately weighed and transferred to 10 ml of volumetric flask; about 4 ml of methanol was added and sonicated. Volume was further made up using methanol to give a stock solution of 1000µg/ml (Solution A). This Solution A was further diluted using a diluent to get the final concentration of 2.00 µg/ml solution (working standard).

Preparation of sample solution (Marketed formulation): 10 RIOCI Tablets containing Riociguat (2.5mg) were accurately weighed and average weight was found out. Then these 10 Tablets were crushed and finely powdered and the powder equivalent to 10 mg of Riociguat was taken and transferred into a 10 ml volumetric flask. 7 ml of methanol was added and sonicated with intermittent shaking for a few minutes the volume was made up to the mark with Methanol (Solution A). Solution A was further diluted using the diluent to get the final concentration of 2.00µg/ml (working standard).

Method Validation[4,5]

The Developed Method was validated on the parameters such as system suitability, Specificity, Linearity, Precision, Accuracy and Sensitivity in accordance with the Specifications of ICH guidelines.[5]

System suitability: System suitability was evaluated by injecting six replicates of Riociguat standard (2.0 µg/ml), and the parameters such as % RSD of Peak area, retention time and number of theoretical plates were tested.

Specificity: Specificity of the method was determined by recording the chromatograms of a Standard solution of Riociguat (2.0 µg/ml) and blank (Diluent). Specificity signifies the identification of the analyte, interference from other peaks.

Linearity: The linearity of the method for Riociguat was studied by the injecting the solutions over the concentration range of 1 µg/ml -3 µg/ml respectively and the correlation coefficient was found. Drug levels of these concentrations were prepared and injected six times into the HPLC system keeping the injection volume constant. The calibration curve was

plotted as the mean peak area of the analyte against the concentration of the drug in µg/ml.

Precision: Precision was assessed at three different concentrations of Riociguat i.e. 1.00 µg/ml, 2.0µg/ml and 3.00 µg/ml (50%, 100% and 150% of the working level) at different time intervals on same day (Intraday Precision) and by repetition on next day (Interday precision).

Accuracy: The % Recovery study was performed by using minimum 3 concentration levels, each in triplicate determinations. It was carried out by spiking 50%, 100%, and 150 % of working concentrations to blank diluent of Riociguat standard in triplicate.

Sensitivity: The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the Standard solution of Riociguat using the developed HPLC method. This was done until a signal to noise ratio of NLT 3:1 and NLT 10:1 is maintained for LOD and LOQ respectively.

Forced Degradation Studies

Preparation of Riociguat Stock Solution

50 mg standard of Riociguat was accurately weighed and dissolved in 50 ml of Methanol to give a solution of 1000µg/ml (Stock solution). 2.00 ml of this solution was further diluted to 10 ml using diluent. This solution was the untreated solution. (200µg/ml). Forced degradation studies were then carried out by subjecting this stock solution to following stress conditions.

- Acid Hydrolysis
- Base Hydrolysis
- Oxidative Degradation
- Thermal Degradation
- Photolytic Degradation

Acid Hydrolysis: 2 ml of Stock solution (1000µg/ml) was taken in 10 ml volumetric flask and was subjected to 2 ml of 1N HCL, this mixture was kept in water bath for 1 hr. at 60°C, after 1 hr. the stressed solution was cooled to room temperature and neutralized with the corresponding base, the volume was made up with diluent, Finally this solution was filtered through 0.45µ filter paper and this solution was loaded into HPLC and the corresponding chromatogram was recorded.

Base Hydrolysis: 2 ml of Stock solution was taken in 10 ml volumetric flask and was subjected to 2 ml of 0.5 N NaOH this mixture was kept in water bath for 30 mins at 60°C, after 30 mins the stressed solution was cooled to room temperature and neutralized with the corresponding acid and volume was made up with diluent, Finally this solution was filtered through 0.45µ filter paper and this solution was loaded into HPLC and the corresponding chromatogram was recorded.

Oxidative Degradation: 2 ml of Stock solution was taken in 10 ml volumetric flask was subjected to 3 ml of 3% H_2O_2 and this mixture was kept for 30 mins at RT, after 30 mins volume was made up with diluent, Finally this solution was filtered through 0.45µ filter paper and this solution was loaded into HPLC and the corresponding chromatogram was recorded.

Thermal Degradation: 2 ml of Stock solution was taken in 10 ml volumetric flask; this Solution was heated on a water bath for 8 hrs. at 60°C. After heating the flask containing the stressed solution was cooled to room temperature, and volume was made up with diluent. Finally this solution was filtered through 0.45µ filter paper and this solution was loaded into HPLC and the corresponding chromatogram was recorded.

Photolytic Degradation: 2 ml of Stock solution was taken in 10 ml volumetric flask and was subjected to sunlight for 6hrs, after 6 hrs. This stressed solution was allowed to attain room temperature and volume was made up with diluent. Finally this solution was filtered through 0.45µ filter paper and, this solution was loaded into HPLC and the corresponding chromatogram was recorded.

RESULTS AND DISCUSSION

The Summary of Optimized Chromatographic Conditions is stated in Table 2.

Table 2: Optimized Chromatographic Conditions.

Parameter	Specification
HPLC Pump	Jasco PU-2089 Plus Quaternary Gradient HPLC Pump
HPLC Detector	Jasco MD-2018 Plus intelligent PDA Detector
Integrator	ChromNAV Chromatogram Software
Column	INERTSIL ODS-3 C18 column (250mm x 4.6mm x 5µ)
Mobile Phase	Ammonium Acetate Buffer : Acetonitrile (50:50 v/v, pH 4.5)
Wavelength	323 nm
Injection Loop	10µl
Flow Rate	1ml/min

System suitability: The standard solution of 2.0µg/mL was injected in six replicates and mean of system suitability parameters were estimated and summarized in Table 3.

Table 3: System Suitability Data of Riociguat.

Sr. No.	System Suitability Parameters	Observations	Acceptance Criteria
1.	Riociguat Standard Solution	2.0 µg/ml	--
2.	Area % RSD	0.4%	NMT 2%
3.	Retention Time	5.6 mins	--
4.	NTP	8256	NTP > 2000
5.	Symmetry Factor	1.26	0.8-2.0

Specificity: The method was quite selective for Riociguat as there was no other interfering peak seen around the retention time of Riociguat (Rt-5.6 mins). Also the baseline did not show any significant peak. Thus the method was found to be highly specific for Riociguat. Representative chromatogram for Riociguat standard is shown in Fig. 2 and blank in Fig.3.

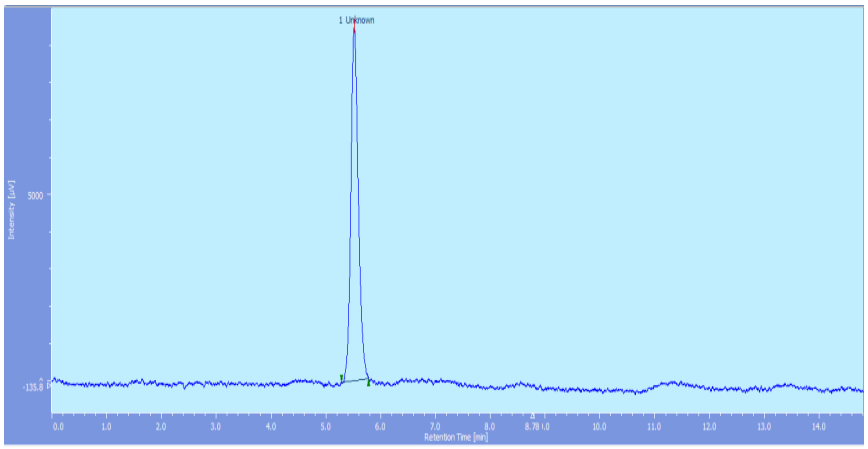

Fig. 2: Chromatogram of Riociguat standard 2.00µg/ml.

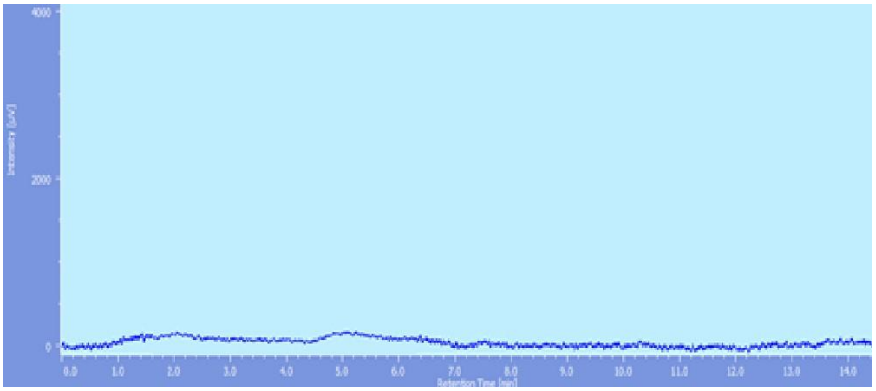

Fig. 3: Chromatogram for Blank run.

Linearity: The Linearity was confirmed in the range **1.00µg/ml – 3.00µg/ml**. The Correlation Coefficient (r^2) was found to be **0.9999** and the equation of the line was found to be **y = 41,138.7787x-4,815.4575** as evident from the calibration curve. Thus, the data showed that the response to be linear. This clearly indicates that an excellent correlation existed between the peak area and concentration of the analyte**.** The calibration curve is shown in Fig. 4 and the Linearity data is summarized in Table 4.

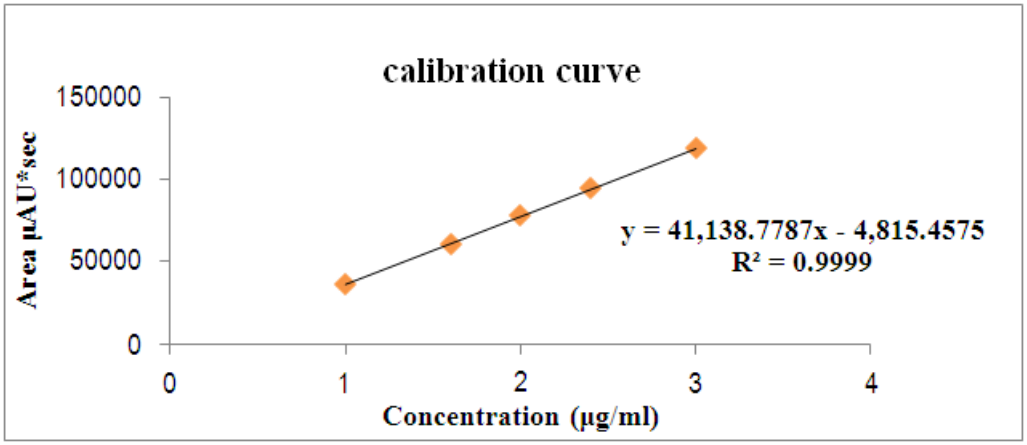

Fig. 4: Calibration Curve for Riociguat.

Table 4: Linearity Data for Riociguat.

Con. µg/ml	Peak Area (µAU*sec)								
	Lin 1	Lin 2	Lin 3	Lin 4	Lin 5	Lin 6	Mean	SD	%RSD
1.0	36778	36567	36456	36543	36456	36567	36561.17	117.9	0.32
1.6	60345	60456	60456	60334	60423	60345	60393.17	58.18	0.09
2.0	77865	77678	77678	77867	77678	77567	77722.17	119.4	0.15
2.4	94345	94567	94567	93456	93567	94234	94122.67	491.9	0.52
3.0	118234	118345	118341	118457	118789	118902	118511.3	270.6	0.22

Precision: Intraday Precision (Repeatability) of Riociguat was determined by taking six replicates of 50%, 100% and 150% of working level i.e. 1.00µg/ml, 2.0µg/ml and 3.00µg/ml concentration at different time intervals and Interday Precision by taking six replicates of same 3 concentration on two consecutive days, The % RSD values of the intra-day precision & Interday precision study were found to be 0.110% & 0.116% for Riociguat. This is confirmed that the method developed to be Precise.

Accuracy: % Recovery study was performed using a minimum of 3 concentration levels, each in triplicate determinations. It was carried out by spiking 50%, 100% and 150% of working level concentration to a blank diluent of Riociguat in triplicates.

Results for % Recovery summarized in Table 5.

Table 5: Accuracy data of Riociguat.

Sr. No.	Concentration (µg/ml)	% Recovery
1	1	100.58
2	2	99.93
3	3	99.90

Sensitivity: The LOD value for Riociguat was found to be 0.10 µg /mL & the LOQ value 0.50µg/mL respectively. This proved that the method developed to be sensitive.

Application of method for the Marketed Formulation of Riociguat

The Developed method was applied successfully for the determination of Riociguat in Marketed formulation. Representative chromatogram for the Marketed formulation of Riociguat is shown in Fig.5 and the Assay Result is stated in Table. 6.

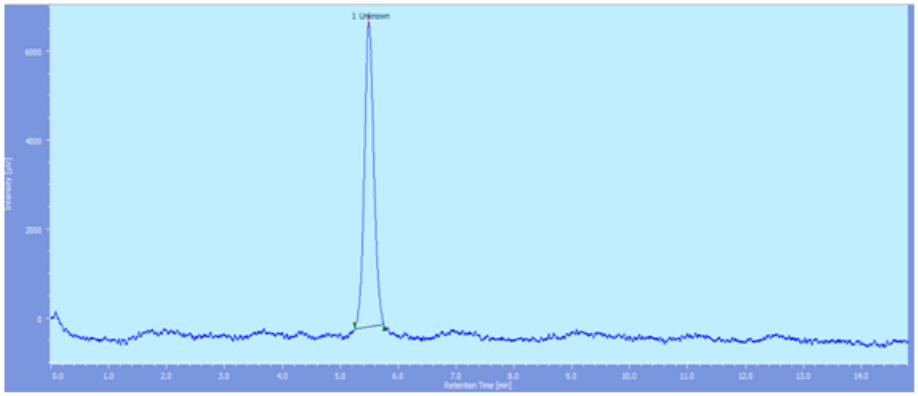

Fig. 5: Chromatogram of the Marketed formulation of Riociguat.

Table 6: Assay of the Marketed formulation.

Sample	Assay (%)
Marketed formulation	98.90

Stability Indicating Assay Method of Riociguat by HPLC

Forced Degradation studies were carried out to prove the method is stability-indicating.[6] Riociguat was degraded by subjecting it to different stress conditions such as Acid Hydrolysis, Base Hydrolysis, Oxidative Degradation, Thermal Degradation and Photolytic Degradation, to suggest the Drug's degradation behaviour. Representative chromatograms of these evaluations are shown in the following Figures.

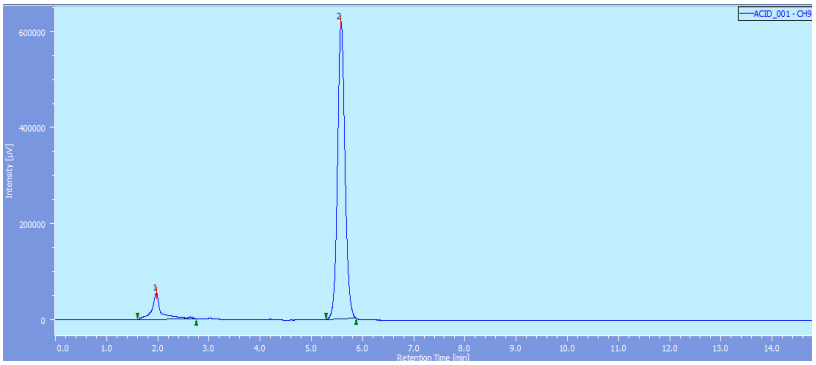

Fig. 6: Chromatogram of Riociguat solution subjected to Acid Hydrolysis.

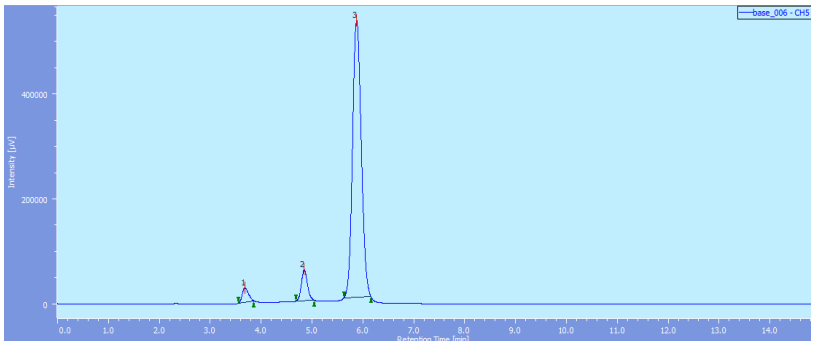

Fig. 7: Chromatogram of Riociguat solution subjected to Base Hydrolysis.

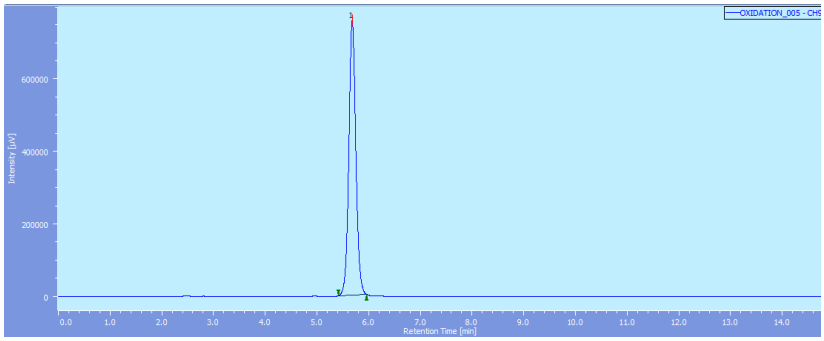

Fig. 8: Chromatogram of Riociguat solution subjected to Oxidative Degradation.

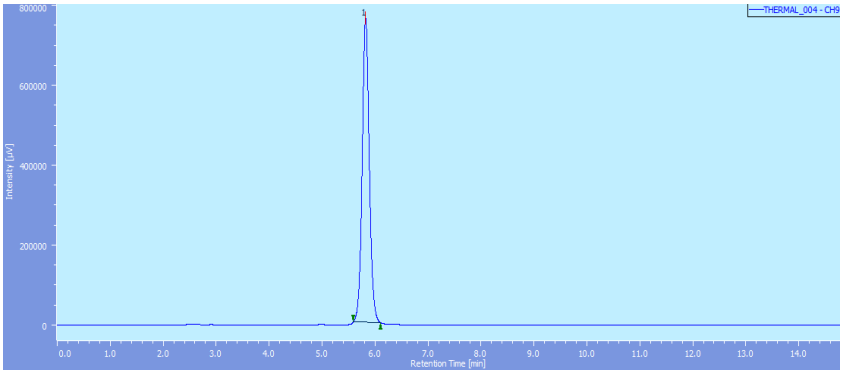

Fig. 9: Chromatogram of Riociguat solution subjected to Thermal Degradation.

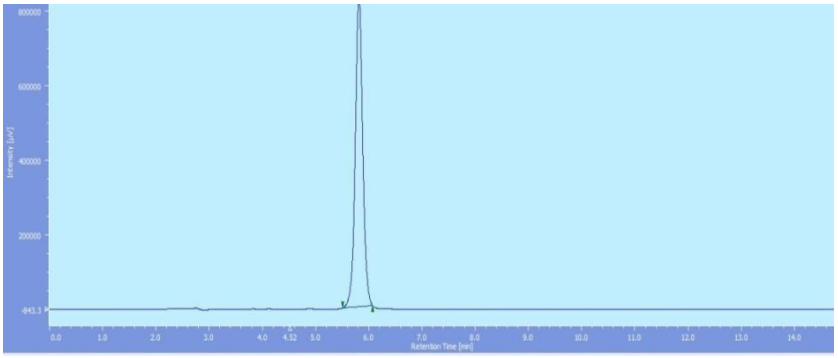

Fig. 10: Chromatogram of Riociguat solution subjected to Photolytic Degradation.

Table 7: Summary of Forced Degradation Evaluations for Riociguat.

Sr. No.	Degradation Condition	Retention time of Degradation products (Mins)	Riociguat Percent Degradation (%)
1	Acid Hydrolysis	2.2	3.3
2	Base Hydrolysis	3.65, 4.80	10.2
3	Oxidative Degradation	-	-
4	Thermal Degradation	-	-
5	Photolytic Degradation	-	-
6	Total Degradation	13.5 %	

The Forced Degradation studies indicated that Riociguat is susceptible to Acid and Base Hydrolysis, and the Method Developed to be Stability Indicating.

CONCLUSION

The RP-HPLC assay method developed for determination of Riociguat is linear, accurate, precise, rapid and specific as evident from the validation results thus can be used to provide a convenient and reproducible approach for estimation of Riociguat in bulk as well as in marketed formulation. The Developed method for the drug is also stability indicating and can be conveniently used for quality control to determine the assay in regular product development, production and stability samples for routine analysis of the samples.

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