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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF VERICIGUAT IN MARKETED FORMULATION

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ABSTRACT

For the estimation of Vericiguat in the API and Marketed formulation, Following ICH criteria, an accurate, precise, specific, and robust stability indicating RP-HPLC technique was designed and validated. The development of method utilized by Zorbax eclipse plus C18 (250 mm × 4.6mm × 5µm) column with mobile phase of 10mM Potassium dihydrogen phosphate: Methanol (60:40 v/v) in isocratic mode. The flow rate kept at 1.0 mL/min at 256 nm wavelength. Vericiguat has retention time 6.9 min. Developed method to be validated according to ICH guideline in term of linearity, Accuracy, Precision, Robustness, and Forced degradation studies was performed. Linearity was achieved with a correlation coefficient was 0.9995 between the concentration 50 µg/mL to 150 µg/mL. Percentage recovery was discovered within the limits of 98% to 102%. %RSD was found to be less than 2% which is the specified in range. Studies of forced degradation reveal that Acid and Alkali have the highest rates of degradation. Impurity peak eluted at 3.1 and 3.0 mins in Acid and Alkali degradation respectively. Vericiguat did not degrade in Thermal and Photolytic degradation.

Key Words: RP-HPLC, Vericiguat, Validation, Eclipse plus C18 column, ICH guideline

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INTRODUCTION

Heart failure indicates that the heart is unable to pump enough blood for the body's metabolism, with the main symptoms being fatigue, fluid retention, and dyspnea. Systolic heart failure is defined by the heart's inability to adequately evacuate enough blood during the contraction phase.[1] Reduced ejection fraction with less than 40 percent of the blood move from the heart with each beat. Because of decreased blood flow, your entire body may get weary and cause issues. Heart failure (HF) has arisen as a global pandemic with 26 million individuals affected and the costing of global health system an estimated US$31 billion worldwide.[2] According to data from Register General of India (RGI) and Indian Council of Medical Research (ICMR) heart failure become the leading cause of death among the Indians. The nitric oxide (NO)-sGC-cyclic guanosine monophosphate (cGMP) has a essential effect on regulation of cardiovascular system. Vericiguat directly activates sGC, independently of and in synergy with NO, to create more cGMP, resulting in smooth muscle relaxation and vasodilation, which may improve cardiac function. [3,4].

In order to reduce the risk of cardiovascular death, heart failure (HF) hospitalization after an HF hospitalization, or the demand for outpatient IV diuretics, vericiguat is advised in patients with symptomatic chronic HF and an ejection fraction less than 45%. [5]. Vericiguat was approved by USFDA in January 2021 and CDSCO approval in 25th February 2022. It is available as Verquvo® by Bayer Zydus Healthcare pharmaceuticals. The dose of Vericiguat is starting from 2.5 mg once daily. The chemical name of Vericiguat is Methyl (4,6-diamino-2 (5-fluoro-1- (2-fluorobenzyl) -1H-pyrazolo [3,4-b] pyridine-3-yl) pyrimidin-5-yl) carbamate. vericiguat is white powder with a molecular weight of 426.39g/mole and a molecular formula of C19H16F2N2 O2. Vericiguat is slightly soluble in water and acetone acetone, very slightly soluble in acetone, methanol, ethanol, ethyl acetate freely soluble in dimethyl sulphoxide (DMSO). Survey of literature search there are only one RP-HPLC method for the quantification of vericiguat in marketed formulation at public domain [6]. The process of developing new drugs includes essential stress testing. Guidelines from the International Conference on Harmonization (ICH) Q3A(R2) and Q3B(R2) emphasize the need for studies to be done on drugs to determine their inherent stability characteristics [7].

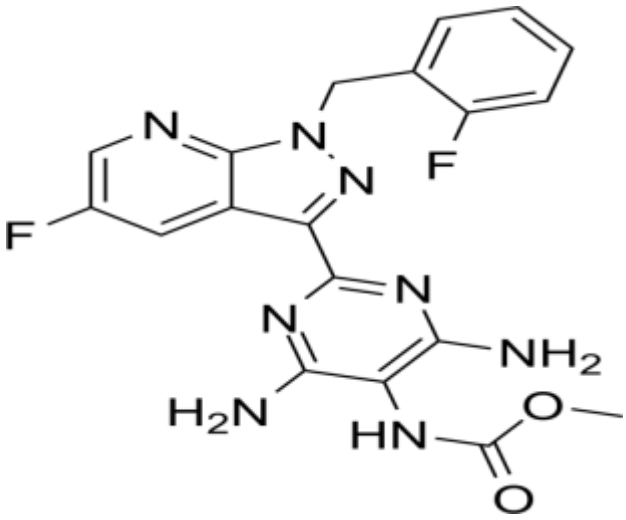

Figure: 1 chemical Structure of Vericiguat MATERIALS AND METHODS

Reagents and chemicals

Vericiguat tablets (marketed formulation) was purchased from the local market. HPLC grade chemicals are used for the development of method. HPLC grade Methanol and Hydrochloric acid were procured from Rankem lab. Potassium dihydrogen phosphate procured from Avantor Pvt.ltd. Other chemicals such as Sodium hydroxide, Hydrogen peroxide was procured from central drug house(p) ltd.

Instrumentation

The estimation of Vericiguat carried out by using Agilent 1260 infinity II HPLC system with photodiode array detector using Open lab CDS software as an integrator. pH meter (lab line), Analytical Balance (Mettler Toledo), Sonicator and centrifuge (Remi). The column used for separation of vericiguat was Zorbax Eclipse plus C18 (250 mm x 4.6 mm x 5 µm).

Preparation of Mobile phase

Mobile phase consists of 10mM Potassium dihydrogen phosphate buffer and Methanol in ratio of (60:40 v/v). The buffer solution was sonicated for 10 min and then filtered through 0.45 µm membrane filter and degassed before use.

Preparation of Diluent

The diluent made up of 10mM Potassium dihydrogen phosphate: Methanol (60:40 v/v).

Preparation of standard stock solution

Standard stock solution of vericiguat was prepared by dissolving accurately weighted 50 mg of vericiguat was taken into 50 mL volumetric flask and added 30 mL of diluent 10mM Potassium Dihydrogen Phosphate: Methanol (60:40 v/v). Sonicate

for 5 mins to properly dissolve. Volume was made up to the mark with the same diluent to get 1000 µg/mL standard stock solution.

Preparation of Standard solution (WSS)

From standard stock solution 1mL of solution was placed in a 10 mL volumetric flask and then diluted with 10 mL of diluent 10mM Potassium dihydrogen phosphate: methanol (60:40 v/v). The resulting solution has a 100 µg/mL concentration.

Sample Preparation for Formulation Analysis

10 tablets of Vericiguat were weighted individually and an average weight of 10 tablets was calculated. Taken equivalent weight 50 mg transferred to a 50 ml volumetric flask and dissolved in 30 ml of diluent. The mixture was vortex and sonicated for 30 min with intermittent shaking. Cooled the flask at room temperature then make up volume with diluent 10mM Potassium dihydrogen phosphate: methanol (60:40 v/v). Centrifuge at 5000 rpm for 10 mins to get clear supernant. Solution was then filtered through a 0.45 µm nylon syringe filter. Filtered solution was collected and 1mL of filtered solution was diluted up to 10 mL to achieve 100 µg/mL concentration.

Method development and Optimized Chromatographic condition

A RP-HPLC method was developed with the purpose of achieving a sufficient resolution of vericiguat and its degradation product. In this study zorbax eclipse plus C18 (250 mm x 4.6 mm x 5 µm) chosen for the achievement of good separation and resolution between drug and impurities. Then various mobile phase compositions and ratios were tried for better separation. Preliminary trials taken at isocratic mode using different ratio of water and methanol,0.1% formic acid in water and methanol,10mM potassium dihydrogen phosphate and methanol was used but obtained results show that Peak broadening effect observed. The estimation and separation of drugs in the presence of its degradants was done using isocratic mode on zorbax eclipse plus C18 (250 mm x 4.6 mm x 5 µm) reverse phase column with 1.0 mL/min flow rate, mobile phase having composition of 10mM potassium dihydrogen phosphate and methanol in ratio of 60:40 v/v was the optimized and resulted in well resolved and sharp peak in presence of degradation products. The 10 µl sample solution was injected using autosampler. The retention time of vericiguat was 6.9 min. The various system suitability parameters were observed and found within specified limits as per ICH guidelines.

Table: 1 Optimized chromatographic condition for method development

Parameters	Specification
Column	Zorbax eclipse plus C18 (250 mm x 4.6 mm x 5 µm)
Mobile phase	10mM Potassium dihydrogen phosphate: Methanol (60:40v/v)
Flow rate	1.0 mL/min
Detection wavelength	256 nm
Injection volume	10 µL
detector	Photodiode array detector

Sample Preparation

Vericiguat tablets available from local market with brand name of Verquvo (2.5 mg,5 mg and 10 mg, Bayer AG, Germany) taken tablet that contain label claim is 5 mg. Marketed formulation assay was performed under the optimized chromatographic condition. 10 tablets of vericiguat triturate and taken equivalent weight of 50 mg powder into 50 mL volumetric flask. All sample and standard solution prepared in 10mM Potassium dihydrogen phosphate: methanol (60:40 v/v).1 mL of sample solution taken into 10 mL volumetric flask to 100 µg/mL of sample solution was prepared and injected into HPLC system and calculate % Assay using noted peak area of sample.

Table: 2 Assay of formulation

Drug	Label claim	Amount found	% Label claim ±SD
Verquvo Tablet (Vericiguat)	5 mg	4.94 mg	98.8 ± 0.79

RESULTS

The developed method validated in terms of linearity, System suitability, Accuracy, Precision, Robustness in accordance with specification of ICH Q2(R2) guideline.

Specificity

Ability of method that assess analyte peak from other interference peak. Determine by recording chromatogram of 100 µg/mL of vericiguat standard and diluent. Diluent contain buffer solution: Methanol (60:40 v/v). Peaks were not detected in retention time of analyte peak that indicate there is no any interference in analyte peak. Analyte peak is separated from impurity peak or interference peak that indicates this method has specific.

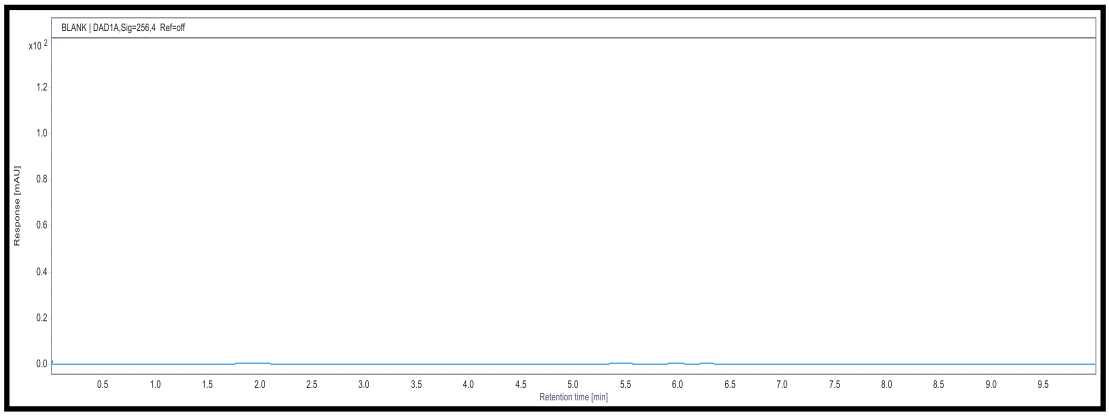

Figure 2: Chromatogram of Blank

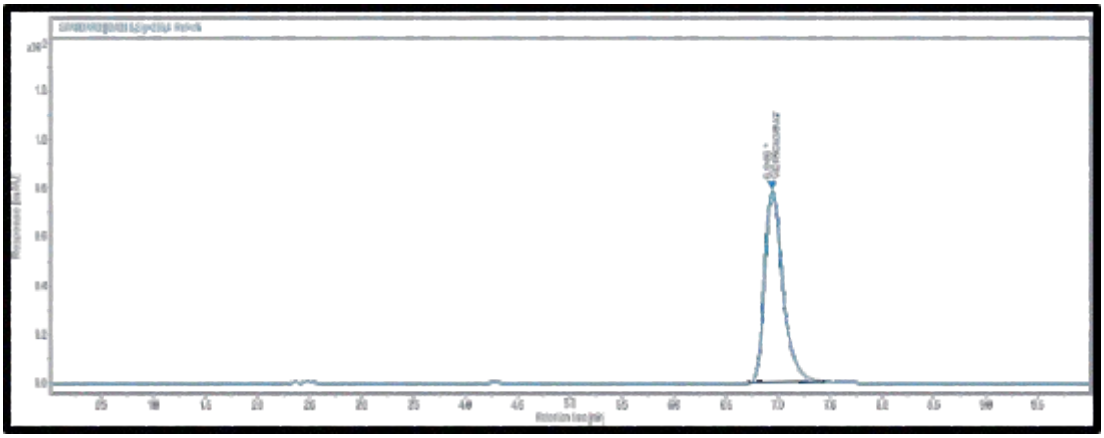

Figure: 3 Chromatogram of Vericiguat standard 100 µg/mL

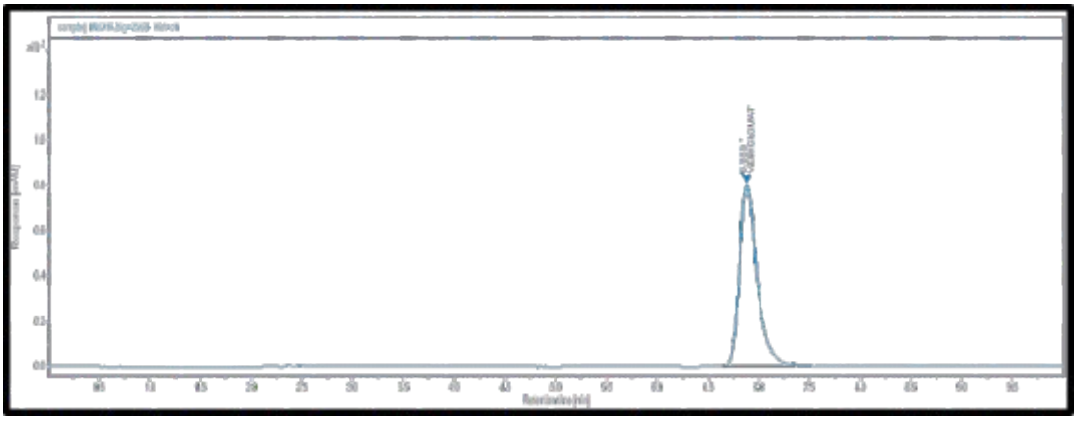

Figure: 4 Chromatogram of vericiguat sample 100µg/mL

System Suitability Testing

System suitability testing is performed to determine the column efficiency, resolution, and repeatability of a chromatographic system in order to confirm its ability to conduct a certain analysis. SST was done by injecting six replicates of standard solution 100 µg/mL. Table 3 summarizes the estimated peak area, tailing factor, theoretical plates and their average, SD, and %RSD.

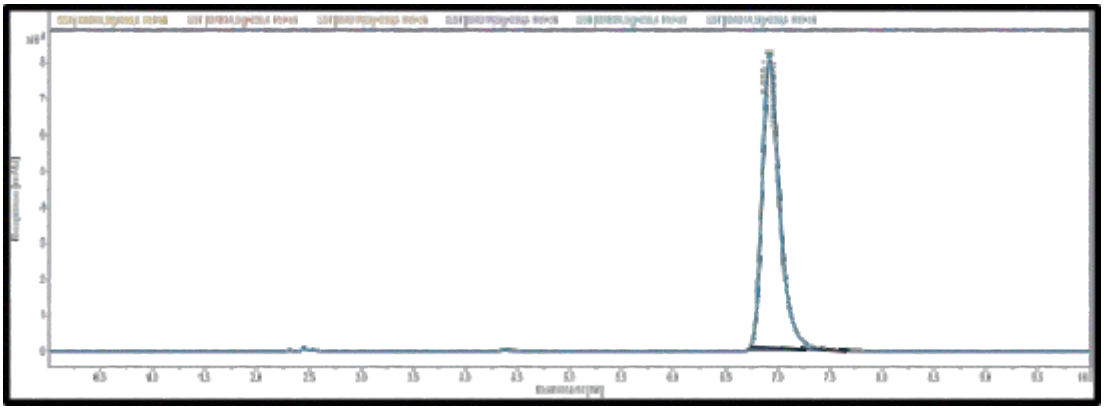

Figure: 5 Chromatogram for system suitability of vericiguat

Table: 3 System suitability testing data for vericiguat

Parameter	Results of Vericiguat
%RSD of Peak Area	0.52%
%RSD of Retention time	0.06%
Mean of Theoretical plates	8646
%RSD of Theoretical plates	0.35%
%RSD of Tailing factor	0.86%

Linearity

Linearity describe as the response is directly proportional to concentration of standard solution. From the standard solution series of the concentration in the range is 50 µg/ml to 150 µg/mL was prepared and injected each standard concentration. On the basis of peak area, the calibration curve was plotted by taking concentration on the x axis and peak area on the y axis. The results of linearity R^2 =0.9995 as shown in Figure 6.

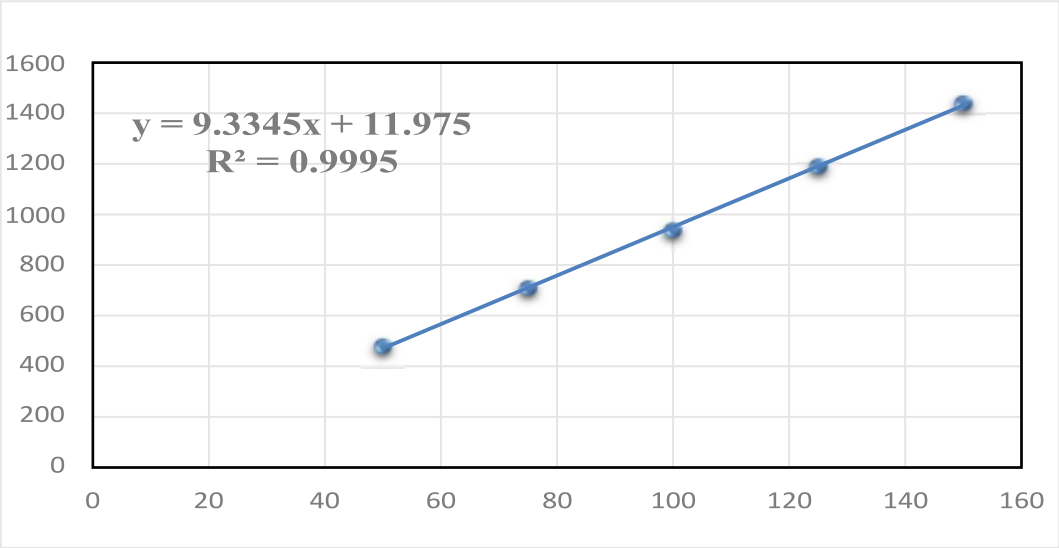

Figure : 6 Calibration curve of vericiguat

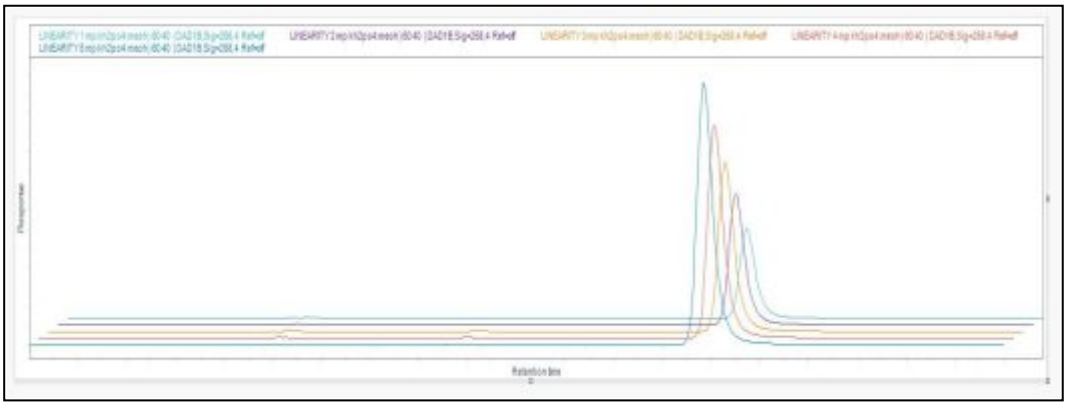

Figure :7 linearity overlay Chromatogram of vericiguat

Table: 4 Precision Data for Vericiguat

Concentration (µg/mL)	Peak Area
50	477.30
75	709.52
100	936.59
125	1187.75
150	1437.73
Correlation Coefficient(R^2)	0.9995

Table: 5 Precision Data for Vericiguat

Intraday Precision		Intraday Precision	
Sample No.	**% Assay**	**Sample No.**	**%Assay**
1	99.19	1	99.73
2	98.35	2	100.19
3	99.05	3	99.89
4	99.38	4	98.96
5	100.07	5	99.59
6	99.73	6	99.38
Mean	99.29	Mean	99.62
±SD	0.59	±SD	0.42
%RSD	0.60	%RSD	0.43

Accuracy

For an accuracy Study, performed by Placebo taken individually into various volumetric flasks and spiked in replicate with known amounts of Vericiguat at three different levels. 50% (Level 1), 100% (Level 2), and 150% (Level 3) of the standard concentration have been used to determine accuracy. The concentration was examined using the suggested procedure, and the amount of recovered Vericiguat was estimated (acceptance criteria: between 98 and 102%). As % Recovery is found within the acceptance criteria, the method is accurate. Results are summarized in Table 7.

Table :6 Accuracy results of vericiguat

Level	Amount added	Amount recovered	% Recovery	Average % Recovery(n=3)	±SD	%RSD
50%	50.3	50.6	101.2			
	50.6	50.3	100.6	100.2	0.69	0.69
	50.4	49.7	99.4			
100%	100.3	99.5	99.5			
	100.6	101.2	101.2	99.7	0.72	0.72
	100.5	99.8	99.3			
150%	150.4	149.9	99.7			
	150.3	150.7	100.2	99.8	0.36	0.36
	150.5	149.8	99.5			
Mean			99.9			
±SD			0.58			
%RSD			0.58			

Limit of Detection (LOD) and limit of Quantitation (LOQ)

The term "limit of detection" refers to the lowest amount of analyte present in a sample that can be detected but not measured.

LOD =3.3 x Standard/Slope

The lowest concentration of analyte in the sample that can be identified and quantified utilizing chromatographic conditions of an analytical technique with sufficient precision and accuracy is referred to as the limit of quantitation.

LOQ =10 × Standard/Slope

Limit of detection and limit of quantification performed by preparing a series of standard preparation of different concentration 10 µg/mL, 5 µg/mL, 2 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.3 µg/mL of vericiguat standard then injected into HPLC system to determine LOD and LOQ was

Table: 7 LOD and LOQ Data

Injection (n=3)	10 µg/mL	5 µg/mL	2 µg/mL	1 µg/mL	0.5 µg/mL	0.3 µg/mL
1	75.05	37.15	15.89	7.24	4.09	ND
2	74.98	37.28	16.75	7.14	4.23	ND
3	74.87	36.96	15.96	7.30	4.17	ND
Mean	74.96	37.13	15.96	7.22	4.15	-
±SD	0.09	0.16	0.08	0.08	0.06	-
%RSD	0.12	0.43	0.50	1.12	1.56	-

Robustness

The robustness of an analytical technique is a measure of its ability to be unaffected by small but deliberate variations in method parameter and provides an indication of its reliability during use. Robustness defines as change in small parameter that should not affect to method for estimation of vericiguat. Robustness performed by changes in parameter like Flow rate (±

0.2 mL/min), Wavelength (± 2 nm), Column temperature 25°C and 35°C.

Change in flow rate ± 0.2 mL/min: This procedure performed by changing flow rate proportion ± 0.2 mL (0.8 mL/min and 1.2 mL/min) for 100 µg/mL concentration of standard solution was injected three times into HPLC system by varying the flow rate conditions.

Change in column temperature: This procedure performed by changing column temperature 25°C and 35°C.Sample solution was injected in three times by varying conditions. Calculated SD and %RSD of peak area of the response.

Change wavelength ± 2 nm: This procedure performed by changing wavelength 100% concentration of sample solution was injected three times by varying wavelength. Noted and calculated peak area, Average, SD and %RSD.

Table 8: Robustness data of vericiguat

Method parameter	Theoretical plates	Tailing factor	%RSD of peak Area	%Assay of Vericiguat
Variation in Wavelength (254 nm)	8140	1.40	0.45	97.53
Variation in Wavelength (258 nm)	8165	1.40	0.54	96.45
Variation in Column oven temperature (25°C)	8817	1.38	0.39	97.34
Variation in column oven temperature (35°C)	8207	1.39	0.46	97.67
Variation in Flow rate (0.8 mL/min)	9488	1.40	0.34	96.79
Variation in Flow rate(1.2 mL/min)	7947	1.36	0.36	97.52

Forced degradation studies

Forced degradation studies include degradation of drug substance and drug product at the condition more severe than accelerated condition. This studies further facilitate the development of stable formulation with suitable storage condition. ICH guidelines demonstrate forced degradation condition such as acidic, alkali, photolytic, thermal and oxidative degradation. Stability data to understand the how the quality of drug substance and drug product change under the influence of various environmental factors. Performed acid degradation (5N HCL), Alkali degradation (5N NaOH), Oxidative degradation (3% H2 O2) Thermal degradation and Photolytic degradation. Representative chromatograms of these evaluations are shown in the following Figures 8,9,11,12,13.

Solution preparation

5N HCL preparation: 42.5mL of HCL taken into 100 mL volumetric flask and volume make up with milli-Q water.

5N NaOH preparation: 20gm of sodium hydroxide pellets are taken into 100 mL volumetric flask and volume make up to mark with milli-Q water.

3% H2 O2 preparation: 3mL of H2 O2 is pipetted out into 100ml volumetric flask and volume make up to mark using milli-Q water.

Procedure

Acid degradation studies: Accurately equivalent weight 5 mg vericiguat tablet

powder taken into 50 mL volumetric flask. Add 30 of diluent that contain 10mM Potassium dihydrogen phosphate: Methanol (60:40 v/v) for the dissolve sample and add 1mL of 5 N HCL kept Room temperature for 30 hrs after the completion of 30 hrs then add 1N NaOH for neutralized the solution and make up to the mark with diluent. Filter the solution and inject into HPLC system at optimized chromatographic condition. Observe the result that how much % of drug degradation.

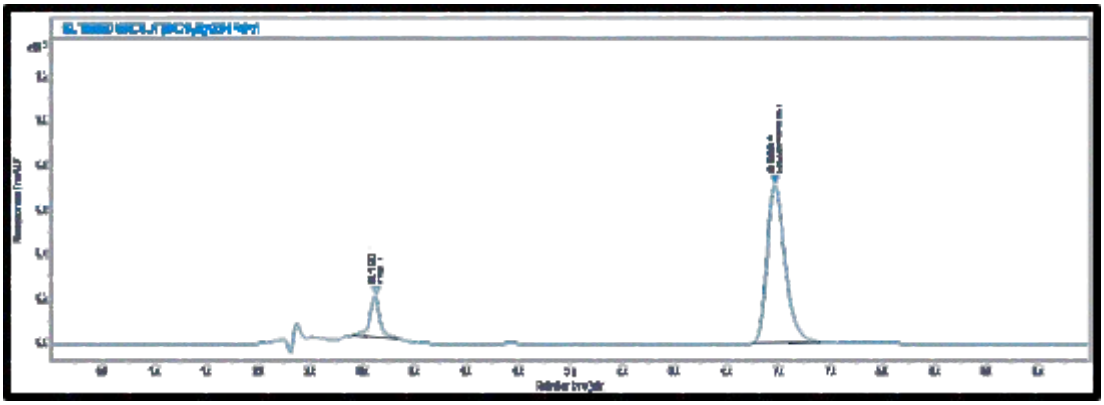

Figure: 8 Chromatogram of sample under 1mL of 5N HCL at Room Temperature for 30 hrs

Alkali degradation studies: Accurately equivalent weight 5 mg of vericiguat tablet powder taken into 50 mL volumetric flask. Add 30mL of diluent that contain 10mM Potassium Dihydrogen phosphate: Methanol (60:40v/v) for dissolve sample. Add 1mL of 1N NaOH kept at Room Temperature for 30 hrs. After completion of 30 hrs. add 1N of HCl for neutralized the solution and make up to mark with diluent. Filter the solution and inject into HPLC system at optimized chromatographic condition. Observe result peak area that how much % of drug degradation.

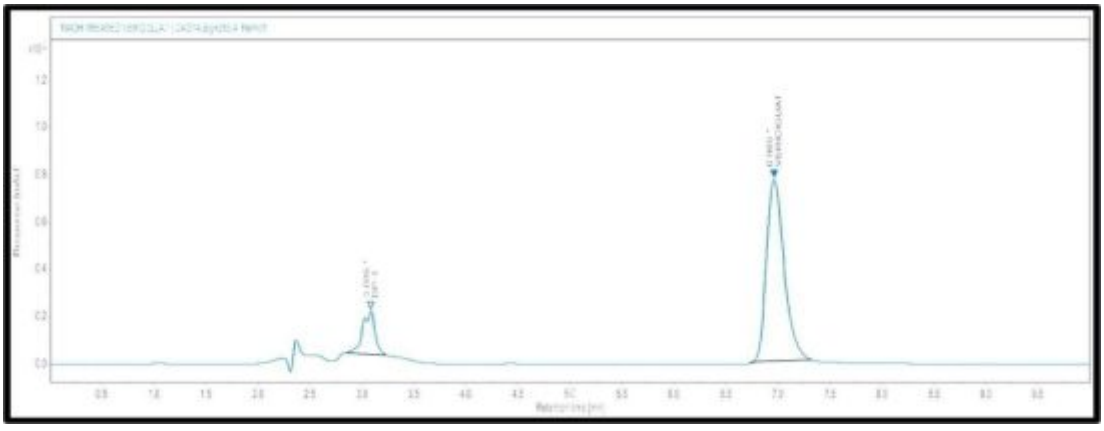

Figure: 9 Chromatogram of sample under 1ml of 5N NaOH at Room Temperature for 30 hrs

Oxidative degradation: Accurately equivalent weight 5 mg of vericiguat tablet powder taken into 50 mL volumetric flask. Add 30 mL of diluent for dissolve the sample. Add 1mL of prepared 3%H_2O_2 and then kept at Room Temperature for 48 hrs. Make up the volume to the mark with diluent. Check peak area under the optimized condition.

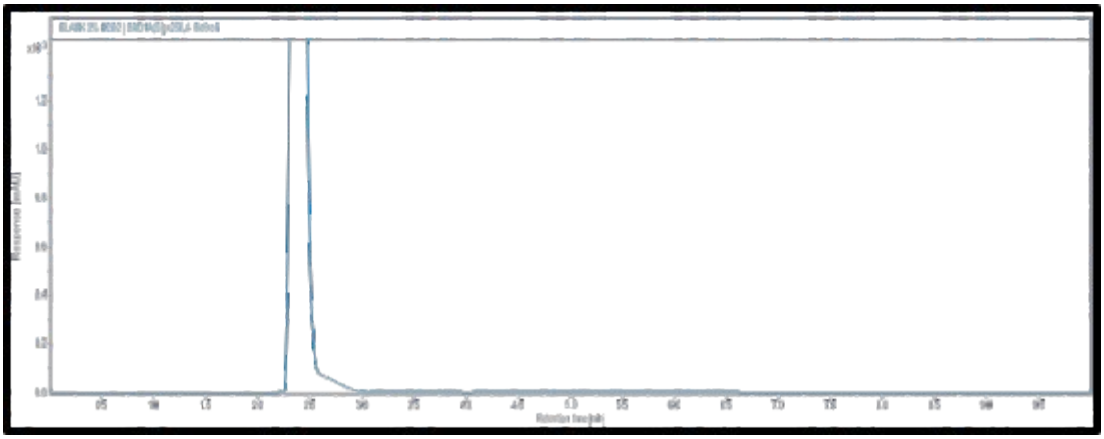

Figure: 10 Chromatogram of blank 3% H_2O_2

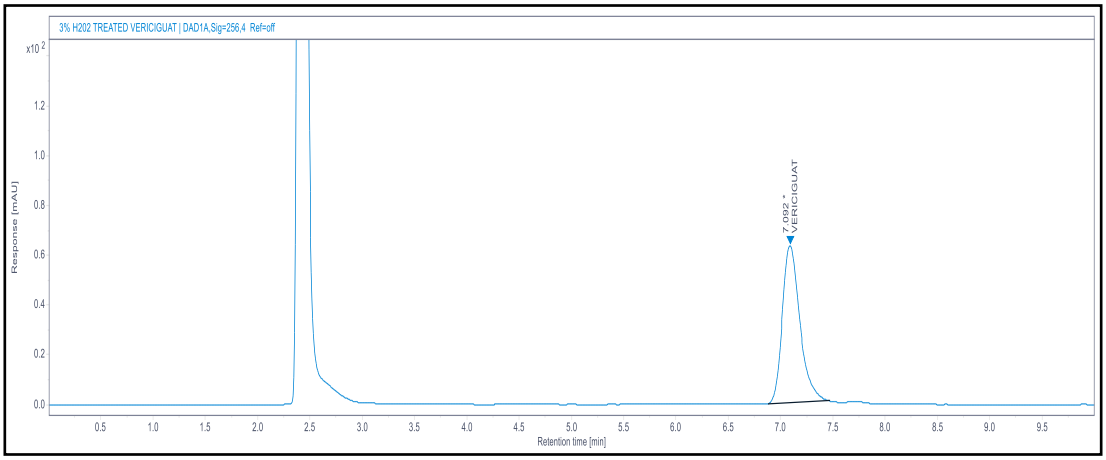

Figure: 11 Chromatogram of sample under 1mL of 3% H_2O_2 at Room Temperature for 48 hrs

Thermal degradation: Accurately weight the 10 tablets of vericiguat and triturated by using mortar and pestle to form fine powder. Taken 5 mg equivalent weight of vericiguat into 50 mL volumetric flask. Make up to volume with diluent. Flask placed on 80 °c heated water bath for 2 hrs. and after 2 hrs. inject the sample solution injected into HPLC system and observe the results.

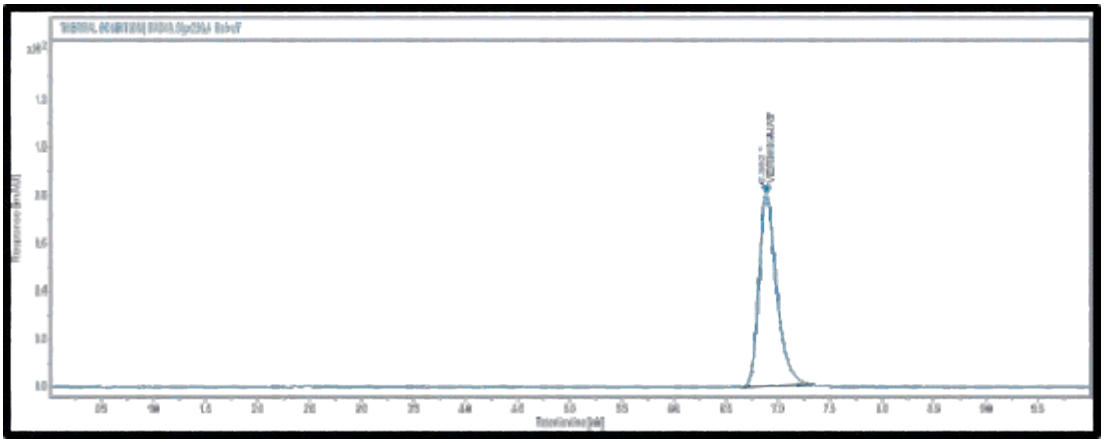

Figure: 12 Chromatogram of sample under at 80°C for 2hr

Photolytic Degradation: Taken 5 mg equivalent powder of sample put it directly in sunlight for 6 hrs. Make up the 50 mL volume with diluent and injected into HPLC system. Check the peak area at optimized condition show in Figure 13.

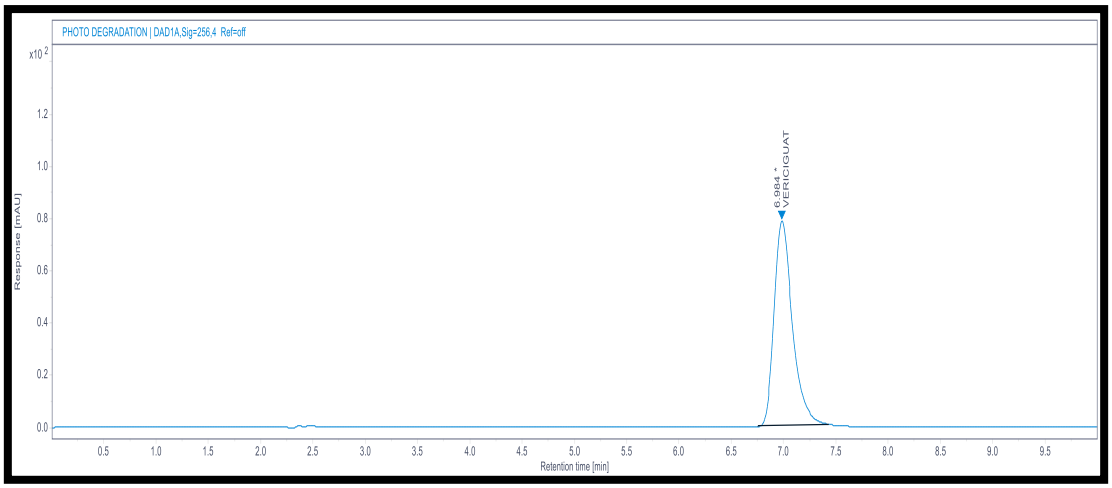

Figure: 13 Chromatogram of sample under directly in sunlight for 6 hrs

Table: 9 Results for forced degradation studies of vericiguat

Mode of Degradation	Condition	Retention time of Impurity	% Assay	% Degradation	Peak Purity
Sample as such	No Treatment	-	99.83	-	998.90
Acid Degradation	1mL of 5N HCL at Room Temperature for30 hrs	3.12	81.60	18.20	998.94
Alkali degradation	1mL of 5N NaOH at Room Temperature for 30 hrs	3.08	79.42	20.57	999.96
Peroxide Degradation	1mL of 3% H2O2 At Room Temperature for 30 hrs	-	97.59	2.41	998.93
Thermal degradation	80°C for 2 hrs	-	99.80	2.37	998.96
Photolytic degradation	Direct sunlight placefor 6 hrs	-	99.39	-	999.91

DISCUSSION

In this developed method the drug substance with various physicochemical characteristics is used in this created approach with the use of a photodiode array detector, the ideal analytical wavelength was found for vericiguat estimation. For effective drug separation in the presence of degradants, various mobile phases were tried. So that the chromatographic conditions could be adjusted, numerous chromatographic factors including wavelength detection, stationary phase, pH of the mobile phase and its composition, and mobile phase

flow rate were assessed. There were several different mobile phases tested in this study, including water, methanol, and 0.1% formic acid in water, but peak broadening was seen. As a result, the mobile phase, which consisted of 10mM potassium dihydrogen phosphate: methanol (60:40 v/v), demonstrated effective and superior separation of the drug and the degradation products produced during the forced degradation study. Several system suitability parameters were used to evaluate the method's system appropriateness. Zorbax Eclipse Plus C18 (250 mm x 4.6 mm x 5 µm) column was used for the project work, which has greater repeatability. According to ICH criteria, the findings of the system suitability parameter were determined to be within acceptable limit values. The determination of the detection and quantification limit values verified that the approach was sensitive enough to identify the drug even when degradants were present. Also, it was noted that the drug's recovery percentage was within the intended range. By injecting a blank, a standard, and a sample, along with completing a forced degradation study, the method's specificity was assessed. The statistical results from the robustness parameter demonstrate the resilience of the presented approach. It was discovered during the stress degradation investigation that vericiguat degrades under acidic, alkaline, and oxidative conditions. There were no degradation products seen under thermal and photolytic conditions. It was determined from the above that the degradants peak does not interfere with the Drug peak. LOD and LOQ was found to be 0.5 µg/ml.

CONCLUSION

For the purpose of determining the amount of vericiguat in commercial formulations, a Specific, Accurate, Robust, and Reliable RP-HPLC technique was established. The validation of the developed method indicates that the Drug and its degradation products were successfully separated. Active ingredient was profitably resolved with good resolution and quantified. Hence, the suggested validated stability-indicating method was successfully employed to work out Vericiguat in the bulk and pharmaceutical dosage form. Analysis of the pharmaceutical dosage form using developed method produced highly repeatable, reliable and high-quality agreement with the label claim of the drug. This study's findings support the assertion that the RP-HPLC technique for determining vericiguat in a tablet formulation is practical and efficient for use in research investigations, quality control, and routine vericiguat analysis in tablet dosage forms.

REFERENCES

(1) Puggia I, Rowland TJ, Miyamoto SD, Sinagra G, Mestroni L. Molecular and Cellular Mechanisms in Heart Failure. Heart Failure in the Child and Young Adult: From Bench to Bedside. Published online January, 2018:3-19.

(2) Murphy SP, Ibrahim NE, Januzzi JL. Heart Failure with Reduced Ejection Fraction: A Review. JAMA. 2020;324(5):488–504.

(3) Xia J, Hui N, Tian L, et al. Development of vericiguat: The first soluble guanylate cyclase (sGC) stimulator launched for heart failure with reduced ejection fraction (HFrEF). Biomedicine and Pharmacotherapy. 2022;149.

(4) Ambrosy A, Fonarow G, Butler J, et al. The Global Health and Economic Burden of Hospitalizations for Heart Failure. J Am Coll Cardiol. 2014; 63 (12): 1123–1133.

(5) Mechanism of action. Https://www.merckconnect.com/verquvo/mechanism-of-action. (Accessed on sept. 3, 2022).

(6) The Mustafa, Doaa Mustafa and Magdy, Nancy and El Azab, Noha F., First Validated Stability-indicating HPLC/DAD Method for Determination of Vericiguat in the Pharmaceutical Formulation; Application to Degradation Kinetic study, 2023; 259; 124498.

(7) Vericiguat drug profile. Https://go.Drugbank.Com/drugs/DB15456. (Accessed on sept. 3, 2022).