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Method Development and Validation for the Quantitative Determination of Vericiguat in Bulk Form and Marketed Pharmaceutical Dosage Forms by using RP-HPLC

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Abstract

The aim of the present study is to develop simple, precise, and accurate methods for quantitative estimation of Vericiguat in bulk form and marketed pharmaceutical tablet dosage form. The method was achieved on Symmetry C18 ODS (4.6mm×250mm) 5µm particle size column with mobile phase containing composition of Acetonitrile and Phosphate buffer (0.01M, pH-3.2) in the ratio of 30:70v/v at a flow rate 1.0ml/min with detection wavelength at 246nm. The linearity was obtained in the concentration range of 6-14 µg/ml for Vericiguat. The suitability of this method was proved by validation in accordance with ICH Q2 (R1) guidelines. The method was found to be accurate with percent recovery was found to be 100.130%. The %RSD for method repeatability and for intermediate precision were found be within the limits i.e. 0.441, 0.258 and 0.373 respectively. The proposed method was found to be simple and sensitive for routine quality control application of Vericiguat used in bulk form and pharmaceutical tablet dosage form.

Keywords

Vericiguat, RP-HPLC, Method Development, Validation, Accuracy, Precision.

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INTRODUCTION:

Vericiguat is a direct stimulator of soluble guanylate cyclase (sGC) used in the management of systolic heart failure to reduce mortality and hospitalizations. A key component of the NO-sGC-cGMP signaling pathway that helps to regulate the cardiovascular system, sGC enzymes are intracellular enzymes found in vascular smooth muscle cells (amongst other cell types) that catalyze the synthesis of cyclic guanosine monophosphate (cGMP) in response to activation by nitric oxide (NO)[1]. Cyclic GMP acts as a second messenger, activating a number of downstream signaling cascades that elicit a broad variety of effects, and these diverse cellular effects have implicated deficiencies in its production (primarily due to insufficient NO bioavailability) in the pathogenesis of various cardiovascular diseases. As a direct stimulator of sGC, Vericiguat mitigates the need for a functional NO-sGC-cGMP axis and thereby helps to prevent the myocardial and vascular dysfunction associated with decreased sGC activity in heart failure. Vericiguat was approved by the FDA in

January 2021 - developed by Merck under the brand name Verquvo-for use in certain patients with systolic heart failure[2]. Although not the first sGC stimulator to be granted FDA approval [riociguat] was approved in 2013 for use in pulmonary hypertension), Vericiguat is unique amongst its peers in that modifications to its structure have dramatically decreased its susceptibility to oxidative metabolism, resulting in a relatively long half-life and allowing for once-daily dosing[3]. The IUPAC name of Vericiguat is methyl N-[4,6-diamino-2-[5-fluoro-1-[(2-fluorophenyl)methyl]pyrazolo[3,4-b]pyridin-3-yl]pyrimidin-5-yl]carbamate. The Chemical Structure of Vericiguat is shown in following

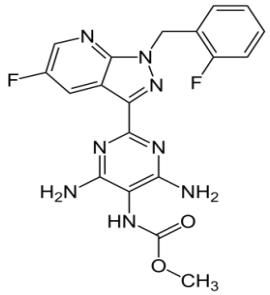

Fig-1: Chemical Structure of Vericiguat

MATERIALS AND METHODS:

Materials and Instruments:

The following are the list of instruments/equipment's /chemicals/reagents and standards to perform the HPLC Analysis[4] of the drug Vericiguat.

Equipment:

Table-1: List of Equipment

S.No.	Instruments/Equipment/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	T60-LABINDIA UV – Vis spectrophotometer
3.	High Precision Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry C_{18} Column, 250 mm x 4.6 mm and 5µm particle size
7.	P^H Analyser (ELICO)
8.	Vaccum Filtration Kit (Labindia)

Chemicals and Reagents:

Table-2: List of Chemicals used

S.No.	Name	Grade	Manufacturer/Supplier
1.	HPLC grade Water	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	HPLC	Loba Chem; Mumbai.
3.	Ethanol	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	HPLC	Loba Chem; Mumbai.
5.	DMSO	A.R.	Sd fine-Chem ltd; Mumbai
6.	DMF	A.R.	Sd fine-Chem ltd; Mumbai

Method Development:

HPLC Instrumentation & Conditions: The HPLC system[5] employed was **HPLC WATERS** with Empower2 Software with Isocratic with UV-Visible Detector.

Standard Preparation for UV-Spectrophotometer Analysis:

The Standard Stock Solutions – 10 mg of Vericiguat standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration.

It's canned in the UV spectrum[6] in the range of 200 to 400nm. This has been performed to know the maxima of Vericiguat, so that the same wave number can be utilized in HPLC UV detector for estimating the Vericiguat.

Selection of Wavelength:

The detection wave length was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The UV spectrum of Vericiguat was obtained and the Vericiguat showed absorbance's maxima at 246nm.The UV spectra of drug are follows:

Selection of Chromatographic Methods:

The proper selection depends upon the nature of the sample, (ionic or ion stable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and hence reversed phase chromatography[7] was selected.

Optimization of Column:

The method was performed with various columns like Hypersil C_{18} column, X- bridge column and X-terra (4.6 ×150mm, 5µm particle size), Symmetry C18 ODS (4.6mm×250mm) 5µm particle size Column[8] was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Mobile Phase Optimization:

Initially the mobile phase tried was Water: Methanol and Water: Acetonitrile and Methanol with TEA Buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate buffer (0.01M, pH-3.2) in the ratio of 30:70respectively.

Estimation of Vericiguat in bulk and pharmaceutical dosage form:

Preparation of Mobile Phase:

Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter[9].

Preparation of 0.01M Potassium dihydrogen orthophosphate Buffer Solution:

About 1.36086grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 3.20 with diluted orthophosphoric acid.

Diluent Preparation:

Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Assay

Preparation of the Vericiguat standard solution:

Accurately weigh and transfer 10 mg of Vericiguat, working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Further pipette 0.1ml of Vericiguat from stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent[10].

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[11-15].

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and taken weight 10 mg equivalent weight of Vericiguat sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Procedure:

Further pipette 0.1ml of Vericiguat from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Inject the three replicate injections of standard and sample solutions and calculate the assay[16] by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Analytical Method Validation

Validation[17]is a process of establishing documented evidence which provide a high degree of assurance that specific activity will consistently produce a desired result or product meeting its predetermined specification and quality characteristics.

System Suitability

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation[18] usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor time and theoretical plates.

Accuracy:

For preparation of 50% Standard stock solution:

Further pipette 0.05ml of Vericiguat from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% Standard stock solution:
Further pipette 0.1ml of Vericiguat from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
For preparation of 150% Standard stock solution:
Further pipette 0.15 ml of Vericiguat from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Vericiguat and calculate the individual recovery and mean recovery values[19].
Acceptance criteria:
The %RSD for each level should not be more than 2.
Precision:
Repeatability
Preparation of Vericiguat for Precision:
Further pipette 0.1 ml of Vericiguat from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.
Ruggedness
To evaluate the intermediate precision of the method, Precision was performed on different days by maintaining same conditions.
Procedure:
Day 1:
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
Day 2:
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
The % RSD for the area of five standard injections results should be no more than 2%.
Linearity:
Preparation of Level – I (6µg/ml of Vericiguat):
Further pipette 0.06 ml of Vericiguat from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of Level – II (8µg/ml of Vericiguat):
Further pipette 0.08 ml of Vericiguat from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of Level – III (10µg/ml of Vericiguat):
Further pipette 0.1ml of Vericiguat from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of Level – IV (12µg/ml of Vericiguat):
Further pipette 0.12ml of Vericiguat from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of Level – V (14µg/ml of Vericiguat):
Further pipette 0.14ml of Vericiguat from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Procedure:
Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.
Acceptance Criteria: Correlation coefficient should be not less than 0.999.
Limit of Detection:
The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.
Limit of Quantitation
The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.
Robustness:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .
Effect of Variation of flow Rate:
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.
Effect of Variation of mobile phase organic composition:
The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 70:30, 75:25 instead of 65:35, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.
Forced Degradation Studies:
The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the

degradation products from the pure active ingredient.

Acid Degradation Studies: To 1 ml of Vericiguat stock, 1 ml of 2N HCl was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N NaOH and makeup to final volume to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation Studies: To 1 ml of stock solution of Vericiguat 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N HCl and makeup to final volume to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. The sample of 20µl was injected into the system, and the chromatograms were recorded to an assessment of sample stability.

Oxidation Degradation Studies: To 1 ml of stock solution of Vericiguat 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solution was kept for 30 min at 60ºC.For HPLC study, the resultant solution was diluted to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Dry Heat Degradation Studies: The 1 ml of standard drug solution was placed in the oven at 60ºC for 6h to study dry heat degradation. For HPLC study, the resultant solution was makeup to final volume to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered through a 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

Photo Degradation Studies: The photo stability of the drug was studied by exposing the stock solution to UV light for 1day or 200Watt-hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (10µg/ml) solution and filtered with 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

Water Degradation Studies: To 1 ml of stock solution of Vericiguat, 1 ml of distilled water was added. The solution was kept aside for 30 min at 60 ºC. For HPLC study, the resultant solution was diluted to obtain (10µg/ml) cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl was injected into the HPLC system, and the chromatograms were recorded for the assessment of sample stability.

RESULTS AND DISCUSSION:

Development of Analytical Method (Optimization):

Wavelength Detection:

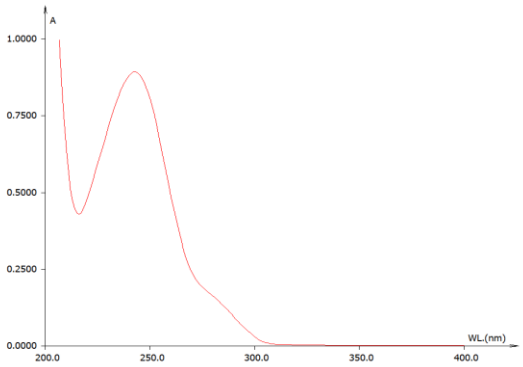

Fig-2: UV Spectrum of Vericiguat (246nm)

Observation: While scanning the Vericiguat solution we observed the maxima at 246nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Optimized Chromatographic Conditions:

Mobile phase: Acetonitrile: Phosphate buffer (0.01M, pH-3.2) (30:70v/v)
Column: Symmetry C18 ODS (4.6mm×250mm) 5µm particle size
Flow rate: 1 ml/min
Wavelength: 246 nm
Column temp: Ambient
Injection Volume: 20 µl
Run time: 10 minutes

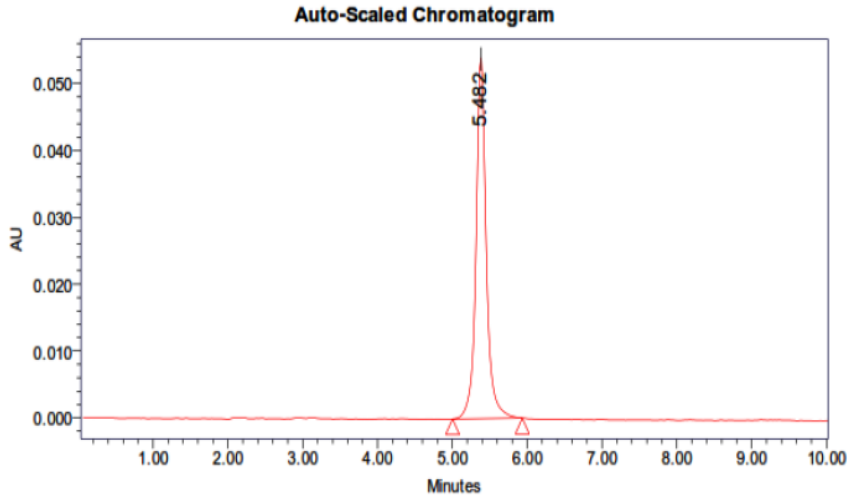

Fig-3: Optimized Chromatographic Condition

Analytical Method Validation

In this method, system suitability, linearity, precision, accuracy, robustness, LOD, LOQ, forced degradation are validated[20] for the selected Vericiguat drug.

System Suitability:

As per the test method, the standard solutions were prepared and injected into HPLC system from which the evaluated system suitability parameters[21] are found to be within the limits.

Table-3: Observation of System Suitability Parameters

S. No.	Parameter	Vericiguat
1	Retention Time (min)	5.453
2	Theoretical Plates	6967
3	Tailing factor	1.12
4	Peak Area (AUC)	647856

The system suitability parameters were found to be within the specified limits for the proposed method.

Specificity

The ICH documents[22] define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitates Vericiguat in drug product.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Vericiguat in present in the marketed pharmaceutical dosage form was found to be 99.85%.

Linearity

The ability of the method to produce results those are directly or indirectly proportional to the concentration of the analyst in samples within the limits[23].

Table-4: Chromatographic Data for Linearity Study of Vericiguat

Concentration µg/ml	Average Peak Area
6	468784
8	615798
10	768759
12	925748
14	1078765

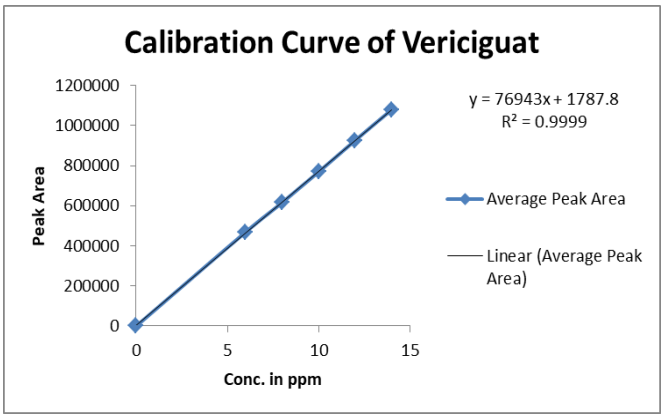

Fig-4: Calibration Curve of Vericiguat

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Vericiguat is a straight line.

Y = mx + c

Slope (m) = 76943

Intercept (c) = 1787

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity[24] is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 76943. These values meet the validation criteria.

Precision:

The precision[25] of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability: Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-5: Results of Repeatability for Vericiguat:

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vericiguat	5.419	645784	83685	6825	1.05
2	Vericiguat	5.405	642589	84932	6849	1.09
3	Vericiguat	5.478	643658	85847	6845	1.08
4	Vericiguat	5.466	648759	86295	6839	1.09
5	Vericiguat	5.493	649657	86587	6895	1.07
6	Vericiguat	5.466	647854	87853	6874	1.10
Mean			**646383.5**			
Std. Dev			**2853.319**			
%RSD			**0.441428**			

Intermediate Precision/Ruggedness:

Analyst 1:

Table-6: Results of Intermediate Precision for Vericiguat

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vericiguat	5.484	636854	84863	6758	1.09
2	Vericiguat	5.493	637489	84759	6726	1.08
3	Vericiguat	5.406	635762	84685	6749	1.09
4	Vericiguat	5.419	636984	84697	6698	1.07
5	Vericiguat	5.446	634856	84258	6728	1.08
6	Vericiguat	5.452	639689	84753	6699	1.08
Mean			**636939**			
Std. Dev.			**1649.149**			
%RSD			**0.258918**			

Analyst2:

Table-7: Results of Intermediate Precision Analyst 2 for Vericiguat

S. No.	Peak Name		Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vericiguat	5.491	628985	85698	6985	1.09
2	Vericiguat	5.482	624879	85479	6899	1.07
3	Vericiguat	5.416	625846	85748	6928	1.06
4	Vericiguat	5.482	623568	85647	6874	1.09
5	Vericiguat	5.495	628985	85246	6984	1.07
6	Vericiguat	5.427	628473	85924	6872	1.08
Mean			**626789.3**			
Std.Dev.			**2340.636**			
%RSD			**0.373433**			

Accuracy:

Accuracy[26] was determined by recovery studies which were carried out in three different concentrations levels (50%, 100%, and 150%). APIs with concentration of 5, 10, and 15µg/ml of Vericiguat were prepared. As per the test method, the test solution was injected three preparations each spike level and the assay was performed. The percentage of recovery values was found to be in the range of 100.134% for Vericiguat. %RSD values were found to be <2%. The results are given in Table 8.

Table-8: The Accuracy Results for Vericiguat

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	386559	5	5.00	100.000%	
100%	768536	10	9.965	99.650%	100.130%
150%	1164522	15	15.111	100.740%	

Limit of Detection for Vericiguat

The detection limit[27] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where, σ = Standard deviation of the response

S = Slope of the calibration curve

Result: 0.487µg/ml

Quantitation Limit for Vericiguat

The quantitation limit[28] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where, σ = Standard deviation of the response

S = Slope of the calibration curve

Result: 1.477µg/ml

Robustness

The robustness[29] was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Vericiguat. The method is robust only in less flow condition. The standard of Vericiguat was injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table-9: Results for Robustness

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	648759	5.484	6845	1.08
Less Flow rate of 0.9 mL/min	635248	5.599	6786	1.09
More Flow rate of 1.1 mL/min	659865	4.576	6528	1.05
Less organic phase	625986	7.415	6689	1.03
More organic phase	615869	3.827	6354	1.01

Stability Studies

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products[30] from the pure active ingredient.

Fig-10: Results of Forced Degradation Studies for Vericiguat

S. No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	648759	0	100%	100%
2	Acidic	539378.232	16.86	83.14	100%
3	Basic	603540.497	6.97	93.03	100%
4	Oxidative	545217.063	15.96	84.04	100%
5	Thermal	616450.801	4.98	95.02	100%
6	Photolytic	533344.773	17.79	82.21	100%
7	Water	625079.296	3.65	96.35	100%

SUMMARY AND CONCLUSION:

The proposed RP-HPLC system was developed and validated for the estimation of Vericiguat within the Bulk form and marketed pharmaceutical dosage form. The proposed method was validated following ICH Q2 (R1) guidelines by testing its parameters, including linearity, precision, accuracy, robustness, LOD, and LOQ. The forced degradation study proved the effectiveness of the proposed validated stability-indicating method. The newly developed method was simple, sensitive, accurate, economical, and sparing, which can be espoused in regular internal control tests in pharmaceutical industries.

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