Ind. J. Pharm. Edu. Res., 2026; Krupapharmacon:274-285.
https://www.ijper.org

Original Article

RP-HPLC Method Development and Stability-Indicating Assay of Vericiguat: An Analytical Quality by Design and Green Chemistry Approach

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ABSTRACT

Objectives: The present studies describe the systematic development and validation of a stability-indicating and environmentally sustainable RP-HPLC method for the quantification of Vericiguat in bulk form by applying AQbD and green chemistry principles. **Materials and Methods:** Method optimization was performed by employing custom design, where critical method parameters and critical analytical attributes were evaluated statistically. Optimal chromatographic separation was achieved on a Grace C18 column (250 × 4.6 mm, 5 µm) under isocratic conditions using potassium dihydrogen phosphate buffer and methanol (60:40, v/v, pH 4.5) at a flow rate of 0.75 mL/min, with detection at 254 nm. Method validation and degradation studies conducted according to ICH guidelines. Environmental sustainability was assessed using green analytical assessment tools. **Results and Discussion:** The optimized method showed a global desirability of 0.75. Vericiguat exhibited a retention time of 3.713 min, plate count of 8253, and tailing factor of 1.53. The method demonstrated excellent linearity over 50-150 µg/mL with an R^2 value of 0.999. The limit of detection and quantification were 0.082 and 0.20 µg/mL, respectively. Significant degradation was observed under acidic and thermal stress conditions, with degradation of 11.07% and 11.00% within 20% limit. AGREE and MoGAPI scores of 0.79 and 86 confirmed sustainability. The Custom Design approach enabled optimization through contour plots and desirability studies, while validation, stability-indicating, and greenness were established according to ICH guidelines. **Conclusion:** The developed method is novel, reliable, accurate, environmentally sustainable, and suitable for routine quality control analysis of Vericiguat in bulk drug.

Keywords: AQbD, Custom design, Green analytical chemistry, Method development, RP-HPLC.

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INTRODUCTION

Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) is a widely employed analytical technique for the separation, identification, and quantification of chemical entities in pharmaceutical, biological, and industrial samples. Method development involves the systematic optimization of chromatographic parameters to achieve reliable and accurate analysis. The AQbD approach enhances method development by incorporating predefined objectives, scientific understanding, and risk-based strategies. Unlike conventional trial-and-error methods, AQbD enables the prediction and control of variability through statistical evaluation and experimental design. This approach ensures method robustness, reliability, regulatory compliance, and consistent performance throughout the analytical lifecycle.[1]

Design of Experiments (DoE), a mathematical models and statistical tools, to study the effect of method variables in a structured and integrated manner. This approach improves method robustness, reliability, and reproducibility.

Vericiguat [VER] chemical structure (Figure 1) chemically as methylN-[4,6-diamino-2-[5-fluoro1-[(2-fluorophenyl) methyl]pyrazolo[3,4-b]pyridin-3-yl]pyrimidin-5-yl]carbamate, having the molecular formula of $C_{19}H_{16}F_2N_8O_2$ and molecular weight of 426.38 g/mol. Vericiguat is a medication used to treat systolic heart failure to lower hospitalization and death rates. Vericiguat directly stimulates Soluble Guanylate Cyclase (sGC) to enhance cGMP production through the NO-sGC-cGMP pathway involved in cardiovascular regulation. It improves myocardial and vascular function in heart failure independent of nitric oxide availability.

Most reported UV, HPLC, and HPTLC methods for vericiguat rely on conventional trial-and-error optimization without applying

DOI: 10.5530/ijper.krupapharmacon.44

AQbD principles. They lack structured risk assessment and do not define a robust design space and rarely assessed environmental sustainability using modern green analytical metrics leaving their ecological impact unaddressed. Therefore, the present study focuses on the development, optimization, and validation of a robust, stability-indicating, and environmentally sustainable reversed-phase high-performance liquid chromatographic method for the quantification of vericiguat in bulk drug.[2-4]

MATERIALS AND METHODS

Chemicals

Acetonitrile, NaH_2PO_4, Na_2HPO_4, K_2HPO_4, KH_2PO_4, HCl, NaOH, HPLC grade water, and methanol were procured from Merck India Pvt. Ltd., Mumbai, India and from SD Fine-Chem Limited, India. Vericiguat was obtained from Sunlight Sciences, Hyderabad, India.

Methods

The HPLC analysis was conducted using the Waters HPLC 2695 system in combination with the UV/Visible detector Model No. (2489). Validation and development of the HPLC techniques were made easier using the Empower 3 software. To select components and evaluate answers for optimal chromatographic conditions, Custom Design and JMP PRO 14 software were used to optimize the procedure.[5]

An Ishikawa bone diagram in (Figure 2) helps determine the underlying cause of problem by highlighting the possible causes and their sub-causes that influence the product's Critical Analytical Attributes (CAAs). Risk Estimation Matrix (REM) studies were used to identify CMPs, which are high-risk elements that have a strong impact on CAAs. The highest-risk factors identified by developing the REM and categorized them as low, medium, or high.

Risk assessment

Analytical Quality Risk Management (QRM) is a systematic method for identifying, monitoring, communicating, and analyzing threats to data integrity throughout the product lifecycle. A thorough effort to identify all pertinent variables that may affect the quality of the data can be ensured by using tools such as Ishikawa diagrams. These variables include standards, reagents, sampling, sample processing, and equipment operating conditions.

Preparation of solutions

Preparation of buffer (0.1M KH_2PO_4 buffer)

After weighing, 1.70 g of KH_2PO_4 were added into 250 mL volumetric flask. After dissolving the KH_2PO_4, water was added to the solution to dilute it, sonicated for 10 min and filtered through a 0.45 µm membrane filter.

Mobile Phase Preparation

After being combined and allowed to degas for 10 min in an ultrasonic water bath, 100 mL (10%) of KH_2PO4 buffer and 900 mL (90%) of methanol underwent filtration using a 0.45 µm filter using vacuum filtration.[6]

Standard stock solution preparation

The 10 mg working standard of vericiguat was weighed and transferred into a volumetric flask of 10 mL capacity. Then, diluent was added and sonicated for 30 min, before making up the volume with same diluent to obtain the desired stock solution, from which serial dilutions were prepared to achieve different concentrations

Methods development trials

Initial trials are necessary in order to optimize the final procedure. Using HPLC instrument method development trials were developed.

Methods validation

Ensuring that a certain technique is suitable for intended purpose and yields accurate and dependable results is an essential procedure in the pharmaceutical industry. It involves a series of tests and assessments to confirm the performance, robustness, and reliability of the analytical method.[7]

Linearity

Vericiguat solutions were injected at concentrations varying between 50 and 150 µg/mL. According to the graph, the Correlation Coefficient (R^2) value was found to be 0.999, indicating that Vericiguat passes linearity over a 50% to 150% range.[8]

Accuracy

We added a known dosage of the medication to the samples at three different concentrations 50%, 100%, and 150% to verify the accuracy. The samples were then examined using the optimized procedure. By comparing the observed amounts to the projected amounts, the percentage recovery was determined.[9]

Precision

Six vials were produced and injected each time, with 5 mL being pipetted out and diluted from the stock solution to yield 100 µg/mL. The range of 97-103% is the acceptable requirement % Relative Standard Deviation (%RSD) should not be more than 2% between six replicas.

Limit of detection and limit of quantification

Limit of detection is defined as the peak area times the baseline noise (three times the lowest concentration level), which also serves to identify the smallest amount of the substance that is

able to be identified with accuracy. LOQ is defined as the peak area times the signal-to-noise ratio (above ten), which indicates the smallest amount of the substance that can be consistently quantified or measured.[10]

Robustness

This test is performed by changing method parameters in the system such as Temperature (±2°C), Flow rate (±10°C), pH (±0.2) and Composition of mobile phase (±5%).

System suitability

The system suitability requirements are fulfilled. Plate count was determined to be more than 2500, tailing less than 2.0, appropriate peak area, Rt, and percentage RSD less than 2%. It was discovered that there were 4800 USP plates, with a USP tailing ratio of 1.20%. Retention time was determined to be 4.012 min, while RSD was found to be 0.2%.[11]

Forced degradation study

Forced degradation studies were carried out under acidic, alkaline, oxidative, thermal, hydrolytic, and photolytic conditions according to ICH guidelines to evaluate the stability-indicating capability of the developed RP-HPLC method. The degradation products formed during stress studies were adequately separated from the principal vericiguat peak without interference, confirming method specificity. Peak purity analysis demonstrated that the analyte peak remained spectrally homogeneous under all stress conditions.

Acid stress

To make 50 mL, 5 mL of Vericiguat were diluted by pipetting it out of the stock solution. After taking off 1 mL solution and adding it in volumetric flask of 10 mL, 5 mL of 0.1 N HCl that had been sufficiently diluted was added, and the combined mixture underwent 30 min of sonication. After being placed into an HPLC vial, the aforementioned solution was injected into the HPLC apparatus.[12,13]

Base stress

To make 50 mL, 5 mL of Vericiguat were diluted by pipetting it out of the stock solution. After taking off 1 mL solution and adding it in 10 mL volumetric flask of 10 mL, 5 mL of 0.1 N NaOH that had been sufficiently diluted was added, and the combined mixture underwent 30 min of sonication. After being placed into an HPLC vial, the aforementioned solution was injected into the HPLC apparatus.[14,15]

Hydrolysis

To make 50 mL, 5 mL of Vericiguat were diluted by pipetting it out of the stock solution. After taking off 1 mL solution and adding it in 10 mL volumetric flask, 5 mL of Water that had been sufficiently diluted was added, and the combined mixture underwent 30 min of sonication. After being placed into an HPLC vial, the aforementioned solution was injected into the HPLC apparatus.[16]

Peroxide

To make 50mL, 5 mL of Vericiguat was pipetted out from the stock solution and diluted to 50 mL. After taking off 1 mL solution and adding in to a 10 mL volumetric flask. 5 mL of 1% H_2O_2 was added, mixed well, and diluted to the correct volume with diluent. The resulting mixture was sonicated for 30 min, transferred into an HPLC vial, and injected into the HPLC system.[17,18]

Heat

To make 50 mL, 5 mL of Vericiguat were diluted by pipetting it out of the stock solution. 1 mL solution was withdrawn and transferred into 10 mL volumetric flask filled to the appropriate level with a diluent that has been heated at 60°C for 30 min, poured into HPLC vial and injected into the HPLC apparatus.[19,20]

Sunlight

To make 50 mL, 5 mL of Vericiguat were diluted by pipetting it out of the stock solution. 1 mL of solution was withdrawn and transferred to 10 mL volumetric flask that had been correctly diluted with a solution that had been exposed to sunlight for six days. Solution was poured into the HPLC vial and injected to the HPLC apparatus.[21]

Greenness Assessment

The environmental impact of the developed RP-HPLC method was evaluated using established analytical greenness assessment tools such as AGREE metric and MoGAPI. The method employed relatively less hazardous solvents, moderate solvent consumption, and minimal sample preparation steps. The overall greenness

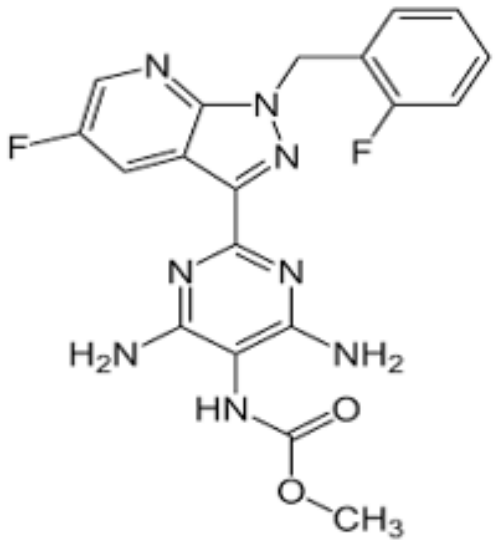

Figure 1: Chemical structure of Vericiguat.

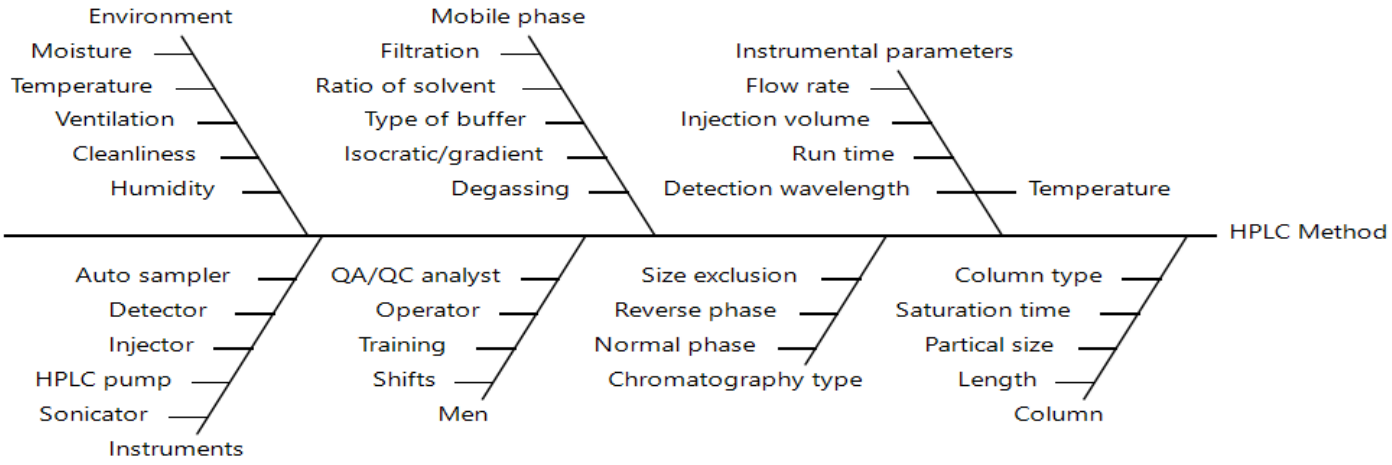

Figure 2: Ishikawa fishbone diagram.

Table 1: Method runs by custom design.

Sl. No.	Flow rate(A)	Column Length (B)	Solvent Composition (C)	Retention Time	Peak Area	Tailing Factor
1.	0.5	250	50	3.575	6436192	1.13
2.	0.75	250	60	3.458	4525634	1.66
3.	1	250	70	3.854	2574239	1.75
4.	1	150	50	2.657	1004760	1.35
5.	1	150	50	2.806	1029307	1.34
6.	1	250	70	4.269	4084072	1.92
7.	0.5	150	70	3.498	4589743	1.23
8.	0.5	150	70	3.541	3469749	1.18
9.	0.75	250	60	3.714	4589843	1.53
10.	0.5	250	50	3.352	3657235	1.51
11.	1	150	50	3.206	1381245	1.52
12.	0.5	150	70	5.326	2984625	0.98

Legend: This table presents the experimental runs generated using the custom design.

score indicated that the proposed method can be considered environmentally friendly and suitable for routine quality control analysis.[2]

RESULTS AND DISCUSSION

In fact, these analytical techniques serve as a reliable indicator of a high-quality product's robustness over the course of its lifetime. Any HPLC method's primary objective is to isolate, and quantify the target analyte or analytes from any contaminants and/or excipients. It is crucial to identify a system's Critical Quality Attributes (CQA) that might affect the analytical method's quality.[22] All statistical evaluations and optimization calculations for the present study were performed using Design-Expert® software (trial version). The software is developed by Stat-Ease Inc., Minneapolis, MN, USA.

Design of experiment

The goal of the Design of Experiment (DoE) method is to systematically monitor and identify changes in output responses through intentional alteration of process input variables (factors) in one or more trials. DoE is essentially a technique for methodically determining connections between variables. For the purpose of creating trustworthy methods in Quality Control (QC) situations, this framework is essential.[23]

Custom Design

A custom design was used to improve the process. Three parameters were selected and optimized: column length, flow rate, and buffer (KH_2PO_4) concentration. It displays the various ranges of three parameters that were measured: 50-70% KH_2PO_4, 0.5-1.5 mL/min flow rate, and 150 and 250 column lengths. The research design with 12 runs was created and analyzed using JMP PRO 14, as depicted in Table 1.

Risk assessment

Analytical Quality Risk Management is a systematic method for identifying, monitoring, communicating, and analyzing threats to data integrity throughout the product lifecycle. A thorough effort to identify all pertinent variables that may affect the quality of the data can be ensured by using tools such as Ishikawa diagram. These variables include standards, reagents, sampling, sample processing, and equipment operating conditions.

To assess risks associated with parameters affecting critical attributes such as resolution, accuracy, precision, tailing, etc., risk matrices are employed. Initially, basic matrices were developed based on scientific knowledge and methodological experience during the initial stages of development (Table 2).

Design of experiment by custom design

Effect Summary

In Figure 3, It is clear that variables like buffer concentration, column length, and flow rate affect the procedure. To show importance, a bar that represents the column length, flow rate, and buffer crosses the blue vertical line. To understand and optimize the process as a whole, it is essential to consider how these factors interact.

Model fit of specific HPLC technique responses

Data from all 12 experimental runs were included in the design to evaluate model fitness and optimize chromatographic responses. From (Figure 4) ANOVA analysis confirmed that R^2=0.81 and

Source	LogWorth		PValue
Flow rate(mL/min) (0.5,1)	3.385		0.00041
Column length (150,250)	2.800		0.00159
Buffer (KH2PO4) concentration in mobile phase (50,70)	2.017		0.00962

Figure 3: Effect summary.

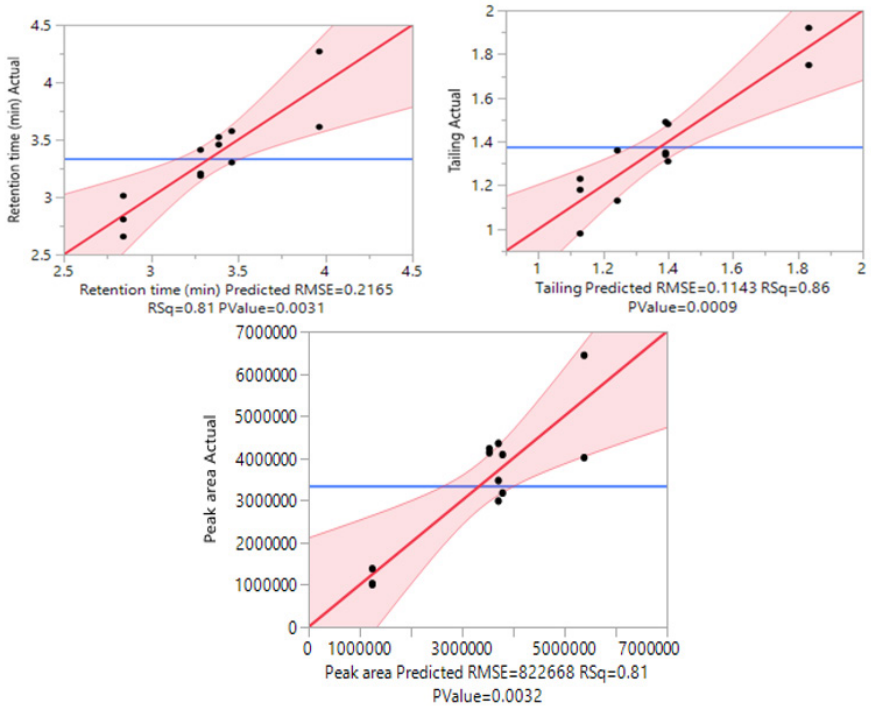

Figure 4: Actual vs Predicted plot for selected responses (retention time, peak area, tailing).

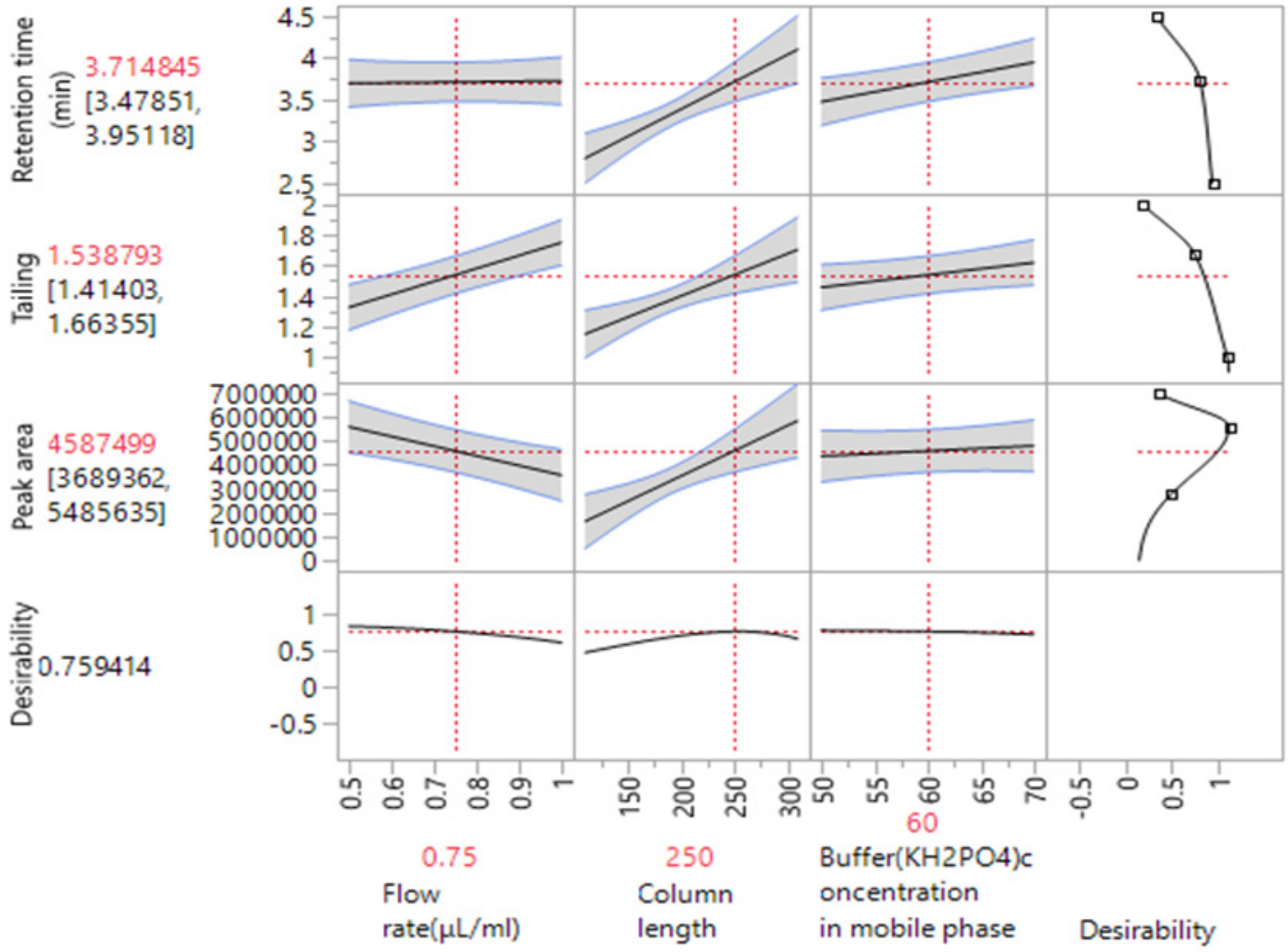

Figure 5: Prediction profiler with maximized desirability.

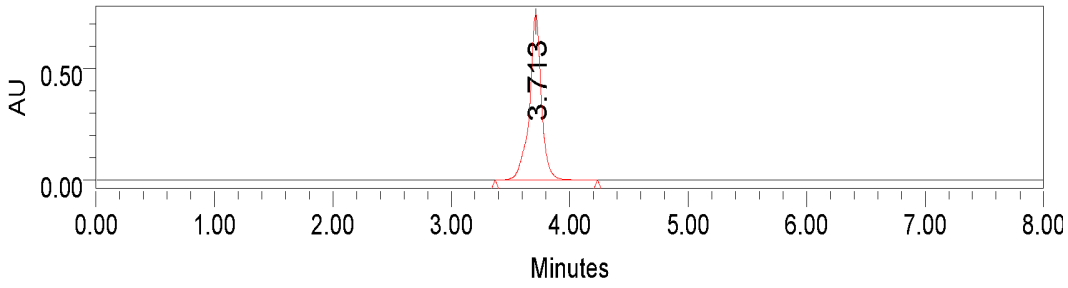

Figure 6: Chromatogram of vericiguat.

p=0.0031 for response retention time, R^2=0.81 and p=0.0032 for response peak area, and R^2=0.86 and p=0.0009 for response tailing are all statistically significant with p-values less than 0.05, indicating that the selected critical method parameters significantly influenced chromatographic responses. The obtained regression models demonstrated acceptable predictability and suitability for chromatographic optimization.

Prediction profiler of the optimized method and statistical optimization of selected response of HPLC method

Numerical optimization

The prediction profiler shows a continuous correlation between multiple responses and various parameters, providing a thorough graphic of the variability that can exist within the extreme results (Figure 5). Shows that the greatest global desirability value of

75.00% provides the chance of achieving the desired goal for all three responses, which do not vary too much and therefore considered to be the best method indicating the validity of the model.

Method Optimized chromatogram

A 60:40 (v/v) mixture consisting of potassium dihydrogen phosphate buffer (KH_2PO_4) and methanol makes up the mobile phase. The column flow rate is set at 0.75 mL/min. The method, developed to analyze Vericiguat, exhibits a retention time of 3.713 min, tailing factor of 1.53, and a plate count of 8253, as depicted in Figures 6, 7 of the chromatogram.

Method validation of analytical techniques

In order to create an appropriate RP-HPLC procedure that ensures precise and efficient separation of the components in the sample, multiple mobile phases such as methanol, acetonitrile, HPLC grade water, and phosphate buffer, at varied proportions with variable flow rates were used. A definite peak could not be observed after testing numerous combinations of mobile phases with variable ratios, and the retention duration was longer than expected. In the end, a well-defined peak (Figure 6) was obtained with RT time 3.713 min with a flow rate of 0.75 mL per minute using (KH_2PO_4) potassium dihydrogen phosphate buffer and, Methanol at a ratio of 60:40 v/v. This technique approach is effective throughout a range of concentrations from 50 to 150 mg per milliliter (Table 3).

System suitability

The system suitability parameters pass for tailing, which is less than 2.0, acceptable peak area, Retention time was found to be

Table 2: Risk Estimation Matrix (REM).

CAAs / CMAs	Organic phases	Mobile phase pH	Flow rate	Injection volume	Column oven temperature	Column type	Column length
Peak area	High	Medium	Low	High	High	High	Low
Retention time	Medium	High	High	Low	Low	Medium	High
Peak tailing	High	High	High	Medium	Low	Low	Medium
Theoretical plate	Medium	Low	Medium	Low	Medium	Low	Low

Legend: This table shows the qualitative risk ranking of method parameters.

Table 3: Method Development trials.

Sl. No.	KH2Po4: Methanol (v/v)	Column	Flow rate (mL / min)	Retention time	Area	USP Plate Count	Tailing	Inference
1.	50:50	X-Bridge, C18, 250X4.6mm, 5µm	0.8	5.066	5019746	2712	1.03	Less theoretical plates and distorted chromatographic shape
2.	60:40	X-Bridge, C18, 250X4.6mm, 5µm	0.8	4.970	3971115	2431	1.10	The plate counts outside the acceptable limit and unsatisfactory peak symmetry
3.	55:45	Grace, C18, 250X4.6mm, 5µm	0.8	5.769	3934430	2567	1.05	Gaussian Peak profile but higher retention time
4.	60:40	Thermo, C18, 250X4.6mm, 5µm	1.0	4.169	3782405	6570	0.80	Higher retention time
5.	60:40	GRACE, C18, 250X4.6mm, 5µm	0.8	3.713	5223762	8253	0.92	Theoretical plate count outside the acceptable limit
6.	70:30	SHIMPAC SOLAR, C18, 150X4.6mm, 5µm	1.0	2.538	2182040	5537	1.50	Acceptable peak symmetry and all outcomes are within range.

Legend: This table shows the trials performed during RP-HPLC method development.

3.713 min, plate count, which is more than 2500, and % RSD, which is less than 2 (Table 4).

Accuracy

We added a known dosage of the medication to the samples at three different concentrations-50%, 100%, and 150%-to verify the accuracy. As seen in (Table 5), the developed approach passes these criteria since accuracy at three distinct concentrations was within the range of 97-103%.

Precision

Six vials were produced and injected each time, with 5 mL being pipetted out and diluted from the stock solution to yield 100 µg/mL. The range of 97-103% is the acceptable requirement % Relative Standard Deviation (RSD) should not be more than 2% between 6 replicas. With a precision %RSD of 0.25%, the recommended method provided a high degree of accuracy.

Linearity and range

Vericiguat solutions were injected at concentrations varying between 50 and 150 µg/mL. According to the graph, the correlation coefficient (R^2) was found to be 0.999, indicating that Vericiguat passes linearity over a 50% to 150% range as seen in (Figure 8).

Robustness

This test is performed by changing method parameters in the system such as Temperature (±2°C), Flow rate (±10%), pH (±0.2) and Composition of mobile phase (±5%) (Table 6).

Detection and Quantification limit

The measured values are within the acceptable range, and there is low measurement variability. LOD and LOQ were determined using the S/N ratio, which was found to be 3.4 and 10.3, respectively. Each % RSD number is less than 2%. 0.082 µg/mL, and 0.2 µg/mL were determined to represent the LOD and LOQ, respectively. Small changes in the flow rate, temperature, etc. of the experiment. Overall, the %RSD values of these differences are less than 2%, which has no appreciable effect on the results.

Degradation studies

Vericiguat's stability was examined by purposefully exposing it to a variety of extreme environments, including heat, light, water, peroxide, acidic, and basic conditions. The drug degraded noticeably at all environmental conditions as shown in Table 7; Figures 9 and 10 summarises the specific results of these forced deterioration investigations. Significant degradation was observed under acidic and thermal stress conditions, with the

Table 4: System suitability studies.

Sl. No.	Test sample	Peak	Rt in (min)	Area (µV*sec)	USP Plate Count	USP Tailing
1.	STD-2	Vericiguat	4.000	472852	4694	1.20
2.	STD-2	Vericiguat	4.012	473568	4780	1.20
3.	STD-2	Vericiguat	4.029	472877	4800	1.20
4.	STD-2	Vericiguat	4.053	474575	4796	1.20
5.	STD-2	Vericiguat	4.061	476884	4689	1.20
Mean				474151.2		
%RSD				0.4		

Legend: This table summarizes system suitability results for the developed method.

Table 5: Accuracy studies or %Recovery studies.

Spiked level	Sample Weight	Sample Area	µg/mL added	µg/mL found	% Recovery
50%	22.50	231712	2.475	2.44	98
50%	22.50	232515	2.475	2.44	99
50%	22.50	232900	2.475	2.45	99
100%	45.00	469259	4.950	4.93	100
100%	45.00	470073	4.950	4.94	100
100%	45.00	469367	4.950	4.93	100
150%	67.50	711284	7.425	7.48	101
150%	67.50	710696	7.425	7.47	101
150%	67.50	711515	7.425	7.48	101

Legend: This table shows recovery results demonstrating method accuracy.

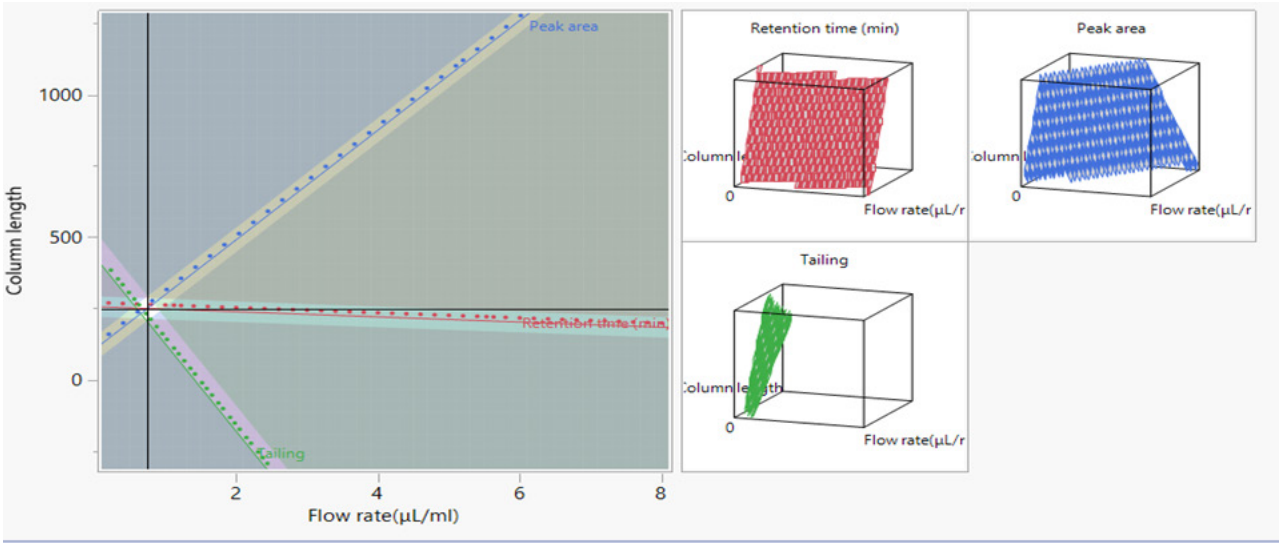

Figure 7: Profiler showing effect of method components on the responses.

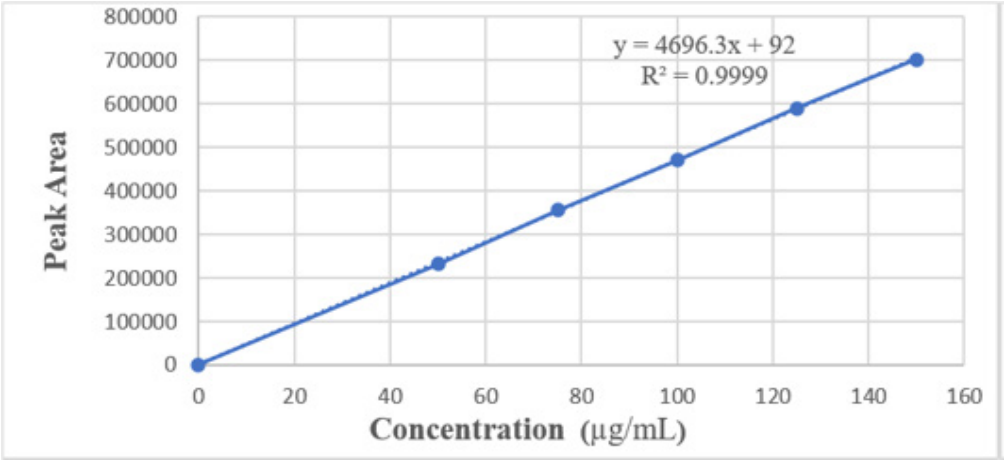

Figure 8: Linearity curve of Vericiguat.

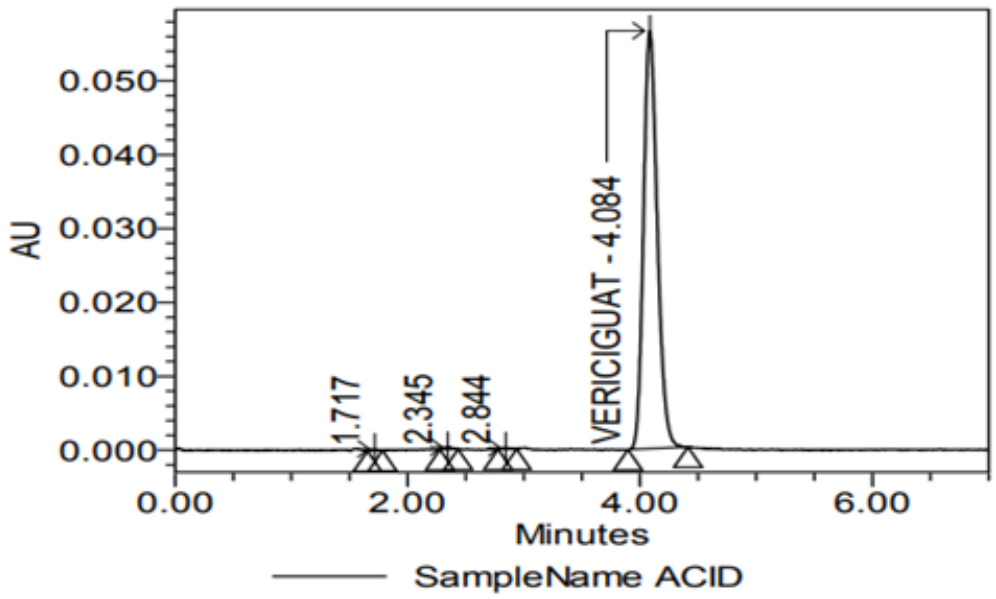

Figure 9: Acid chromatogram.

Table 6: Robustness assessment by altering flow, pH, and the composition of the mobile phase.

Sl. No.	Name of the sample	Peak	Retention time (min)	Area (µV*sec)	Plate Count (USP)	Tailing (USP)
1.	FLOW-A	Vericiguat	3.251	392471	4483	1.22
2.	FLOW-B	Vericiguat	4.786	584573	5419	1.23
3.	pH-1	Vericiguat	3.985	472546	4794	1.26
4.	pH-2	Vericiguat	3.974	473631	4792	1.26
5.	COMP-1	Vericiguat	4.786	584573	5419	1.23
6.	COMP-2	Vericiguat	4.636	446939	4531	1.22

Legend: This table presents robustness evaluation under deliberate variations.

Table 7: Results of forced degradation studies.

Stress Conditions	Sample weight (mg)	% Assay	%Degraded
Acid stress	45.00	88.93	11.07
Base stress	45.00	92.58	7.42
Peroxide stress	45.00	93.11	6.89
Heat stress	45.00	89.00	11
Photo stress	45.00	94.07	5.93
Hydrolysis stress	45.00	98.11	1.89

Legend: This table summarizes degradation behavior under various stress conditions.

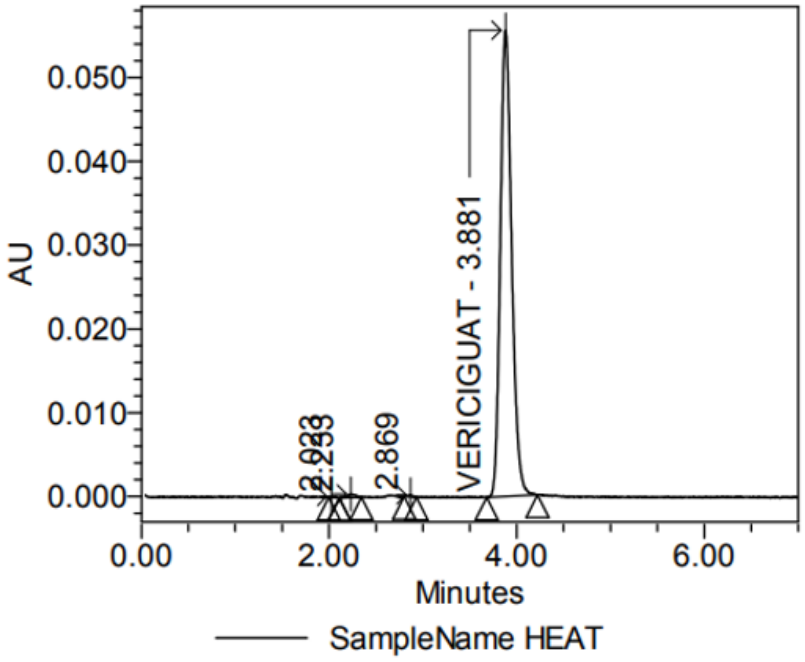

Figure 10: Heat chromatogram.

degradation percentage of 11.07% and 11.00% within the 20% limit according to ICH guidelines.

Greenness Evaluation

The Evaluation of Greenness

AQbD based RP-HPLC method was evaluated using both AGREE and MoGAPI tools. The GREEN assessment yielded an overall score of 0.79, indicating moderate compliance with green analytical chemistry principles supported by low sample consumption, minimal energy requirement, acceptable operator safety, and multi-analyte capability. However, the use of non-green organic solvents and offline sample preparation limited the overall greenness. The MoGAPI evaluation resulted in a score of 86, reflecting good greenness, attributed to operation, and controlled safety hazards. Although moderate solvent

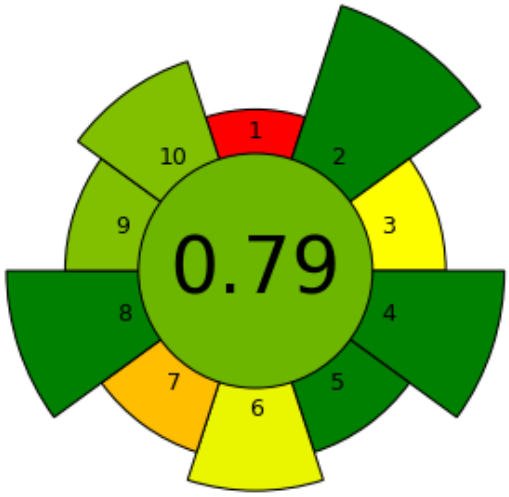

Figure 11: AGREE Tool.

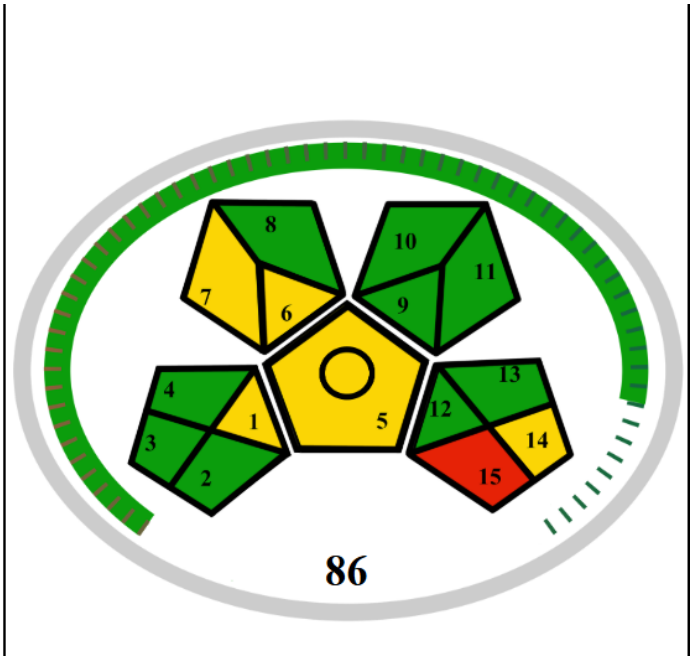

Figure 12: MoGAPI Tool.

usage and waste generation were observed, the overall method demonstrated an acceptable environmental profile. Together, AGREE and MoGAPI results confirm that the developed method achieves a balanced integration of analytical performance and environmental sustainability, making it suitable for routine quality control analysis (Figures 11, 12).

CONCLUSION

In this work, a reliable and environmentally conscious RP-HPLC method was developed for the estimation of vericiguat by incorporating Analytical Quality-by-Design principles. The use of a structured optimization approach allowed clear identification of factors that influence method performance, resulting in a procedure that is both efficient and resource-conservative. The method fulfilled all key validation criteria, showing strong linearity, good precision, excellent accuracy, and low detection limits. Its ability to withstand forced degradation conditions confirms its suitability as a stability-indicating tool. The green analysis was performed using AGREE and MoGAPI tools, demonstrating its environmental sustainability. Overall, this AQbD-guided and sustainability-focused chromatographic method offers a practical, robust, and greener alternative for routine analysis and quality control of vericiguat.

ACKNOWLEDGEMENT

The authors sincerely thank the faculty of Krupanidhi College of Pharmacy, Bangalore, for providing the required facilities and financial support.

ABBREVIATIONS

AQbD: Analytical Quality by Design; **RP-HPLC:** Reverse-Phase High-Performance Liquid Chromatography; **VER:** Vericiguat; **DoE:** Design of Experiments; **sGC:** Soluble Guanylate Cyclase; **cGMP:** Cyclic Guanosine Monophosphate; **NO:** Nitric Oxide; **CMP:** Critical Method Parameters; **CAA:** Critical Analytical Attributes; **REM:** Risk Estimation Matrix; **QRM:** Quality Risk Management; **ICH:** International Council for Harmonisation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **RSD:** Relative Standard Deviation; **ANOVA:** Analysis of Variance; **USP:** United States Pharmacopeia; **AGREE:** Analytical GREEnness; **MoGAPI:** Modified Green Analytical Procedure Index.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Vinod S, Nayak A, Prakash SR, Govindarajan S. RP-HPLC Method Development and Stability-Indicating Assay of Vericiguat: An Analytical Quality by Design and Green Chemistry Approach. Indian J of Pharmaceutical Education and Research. Krupapharmacon 2026:274-85.