Int. J. Pharm. Investigation, 2024; 14(3):822-832.
https://www.jpionline.org

Original Article

Development of a Robust and Reliable RP-HPLC Method for the Estimation of Vericiguat in Tablet Dosage Form: Greenness Analysis using AGREE Penalties

Arram Madhavi[1,2], Medidi Srinivas[1,*], Niraj Gupta[2]

[1]Department of Pharmaceutical Chemistry, Geethanjali College of Pharmacy, Cheeryal, Keesara, Medchal, Hyderabad, Telangana, INDIA.
[2]Department of Pharmaceutical Chemistry, School of Pharmacy, OPJS University, Rawatsar Kunjla, Near Sankhu Fort, Rajgarh (Sadulpur), Jhunjhunu Road, Churu, Rajasthan, INDIA.

ABSTRACT

Objectives: A simple and sensitive analytical method was developed to quantify the vericiguat in tablet formulation. **Materials and Methods:** The analyte was separated on a Kromacil C_{18} column (250 mm×4.6 mm×5 µm particle size) using a mobile phase of potassium dihydrogen orthophosphate pH 2.5 adjusted with orthophosphoric acid and acetonitrile (50:50% v/v) pumped at 1.0 mL/min. Detection of the effluent was done using a UV detector at a wavelength of 252 nm. **Results:** The retention time for vericiguat was 4.42 min. The drug showed linearity within the concentration range of 30-70 µg/mL. The accuracy of the method was considered satisfactory and the mean recovery percentage in the acceptable range of 99.92-100.61%. **Conclusion:** The RP-HPLC method was successfully developed and validated according to ICH guidelines. The AGREE software was used to assess the environmental friendliness score of the proposed method, which was determined to be 0.79. The proposed method was simple, precise, sensitive, rapid, and robust for estimating vericiguat in tablets.

Keywords: RP-HPLC, Vericiguat, Agree score.

Correspondence:
Prof. Medidi Srinivas
Department of Pharmaceutical Chemistry, Geethanjali College of Pharmacy, Cheeryal, Keesara, Medchal, Hyderabad-501301, Telangana, INDIA.
Email: drmsr9@gmail.com

Received: 02-03-2024;
Revised: 17-04-2024;
Accepted: 05-05-2024.

INTRODUCTION

Heart failure is one of the leading causes of illness and death globally. Despite advancements in medical treatment, the prognosis remains grim. Heart Failure (HF) is a clinical syndrome caused by inadequate ventricular filling or the heart's inability to pump blood effectively. Symptoms like exercise intolerance, fluid retention, and dyspnea can indicate this condition.[1] The prevalence of heart failure is expected to rise significantly, with estimates projecting a 40% increase from 5.7 million in 2012 to 8 million in the next eight years, putting strain on healthcare systems.[2]

HF can have a wide range of underlying causes, many of which are multifactorial. These causes may include genetic abnormalities, hypertension, ischemic heart disease, arrhythmias, valvular heart disease, and exposure to infections, toxins, infiltrative diseases, radiation, and high-output situations.[2]

DOI: 10.5530/ijpi.14.3.92

Copyright Information :
Copyright Author (s) 2024 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : EManuscript Tech. [www.emanuscript.in]

HF exhibits diverse presentations, prompting classification based on Left Ventricular Ejection Fraction (LVEF) to guide diagnosis and management. Three distinct phenotypes emerge such as i) HF with reduced Ejection Fraction (HFrEF) with LVEF less than 40%, ii) HF with mid-range Ejection Fraction (HFmrEF) with LVEF between 41% and 49%, and iii) HF with preserved Ejection Fraction (HFpEF) with LVEF greater than 50%.[3] HFrEF is primarily treated medically with "angiotensin receptor-neprilysin inhibitors", "angiotensin-converting enzyme inhibitors", and "Sodium-Glucose cotransporter-2 (SGLT-2) inhibitors".[4]

Historically, researchers have not designed HFrEF therapies to address endothelial dysfunction or target the NO pathway.[5] The molecular processes involving Nitric Oxide (NO) signaling are diverse and play a crucial role in maintaining cardiovascular homeostasis. Nitric oxide synthase is a continuous process that produces Nitric Oxide (NO), which is an endogenous vasodilator released from endothelial cells. The primary mechanism through which nitric oxide acts intra-cellularly is by stimulating Guanylyl Cyclase (sGC) in its soluble form.

It is further activated by Nitric Oxide (NO) binding to its heme group, elevates cyclic 3', 5'-Guanosine Monophosphate (cGMP) levels in the cells of smooth muscle which subsequently leads to vascular relaxation.[6]

The NO/sGC/cGMP regulatory circuit is vital in maintaining the homeostasis of cardiovascular and pulmonary systems, as well as organs such as the brain, kidney, and liver.[7] In terms of pharmacology, sGC stimulators increase the activity of sGC without relying on NO. When combined with endogenous NO, they mutually enhance its effects. In contrast, sGC activators exhibit selective binding and activation of the oxidized, heme-free form of sGC, bypassing the requirement for NO and heme presence.[8]

Vericiguat (VCG) is a novel oral soluble guanylate cyclase stimulant that directly activates soluble guanylate cyclase. It achieves this by binding to a distinct site from nitric oxide, thereby enhancing the cyclic Guanosine Monophosphate (GMP) pathway.[9] Moreover, it aids in stabilizing the binding of nitric oxide to its target, thereby increasing the responsiveness of soluble guanylate cyclase to naturally occurring nitric oxide. As a result, it improves the heart and blood vessel function by both amplifying the body's natural nitric oxide and directly activating a key enzyme. VCG exhibits enhanced pharmacokinetic stability, excellent oral bioavailability, and a prolonged half-life, facilitating once-daily oral administration. These distinctive features, coupled with its unique pharmacodynamic profile, contribute to a more moderate impact on blood pressure compared to other sGC-inducing drugs.[10]

VCG is a light yellow to brown solid, chemically recognized as "methyl(4,6-diamino-2-(5-fluoro-1-(2-fluorobenzyl)-1H-py razolo[3,4-b] pyridin-3-yl) pyrimidin-5-yl) carbamate" (see Figure 1). In the pharmaceutical industry, having an efficient analytical method for analyzing individual drugs or drug combinations is crucial. After reviewing the literature, it was discovered that a stability-indicating HPLC/DAD method,[11] a spectrophotometric strategy based on the diazo coupling reaction with 8-hydroxyquinoline,[12] and an eco-friendly simultaneous estimation of vericiguat and its alkaline degradation products[13] were all revealed. Additionally, a recent study reported a method for detecting vericiguat in rat plasma using UPLC-MS/MS has been reported.[14] However; these methods lack sensitivity and accuracy when quantifying VCG at low concentrations. Additionally, these methods are expensive, time-consuming, and not suitable for routine analysis. Therefore, we have chosen to utilize a cost-effective UV-coupled HPLC technique, which is widely available in laboratories with limited financial resources and requires routine monitoring of VCG in its formulation. Considering these factors, the objective of this study is to develop and validate an RP-HPLC method for quantifying VCG in tablet formulation following ICH guidelines under Q specification.[15] The environmental friendliness score of the developed method was eventually established through greenness estimation of the developed conditions.

MATERIALS AND METHODS

Chemicals and reagents

In this study, high-grade HPLC reagents and solvents were utilized. The solvents used were CH_3CN (acetonitrile), KH_2PO_4 (potassium dihydrogen orthophosphate), CH_3OH (methanol), and H_3PO_4 (orthophosphoric acid), which were supplied by SD Fine Chem Ltd., located in Mumbai, India. Vericiguat with a certified purity of>99.11% was received as a gift sample from Ascentyo BioSciences, Hyderabad, India. Verquvo® (each tablet contains 10 mg of vericiguat), marketed by Bayer Zydus Pharma Ltd., India and was purchased from local pharmacy store.

Instruments

The study was conducted using the Shimadzu HPLC system (Model No. LC-20AD) coupled with a UV detector (Model No. SPD-M20A). Data acquisition was done using Empower software version 2. Chromatography was performed using a Kromacil C_{18} column (dimensions: 250 mm×4.6 mm, 5 μm). Weighing was done using an analytical balance from Mettler Toledo.

Chromatographic conditions

The chromatographic conditions for the analysis were as follows: A mobile phase consisting of a volumetric ratio of 50:50 (KH_2PO_4: CH_3CN) was used in isocratic mode. The analysis was conducted under ambient temperature with a mobile phase flow rate of 1.0 mL/min. Each run involved injecting 20 μL of the sample into the HPLC. The UV detector was set to a detection wavelength of 252 nm to detect VCG in the effluents from the column (Figure 2).

Buffer preparation

To prepare the diluent, 3.48 g of KH_2PO_4 was dissolved in 1000 mL of water. A solution of orthophosphoric acid was added

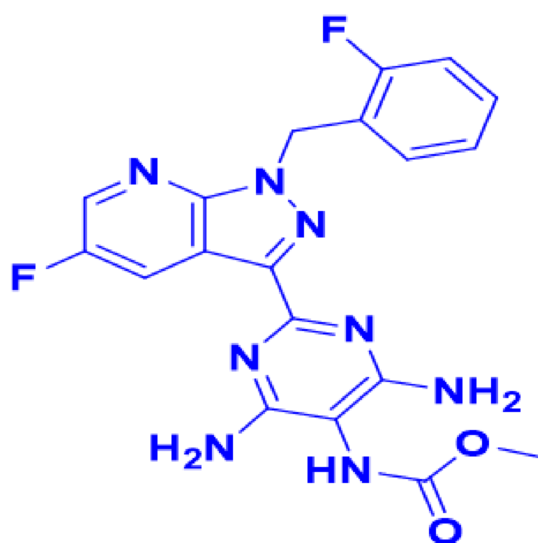

Figure 1: Chemical structure of Vericiguat.

to achieve a final pH of 2.5. It was then filtered using a nylon membrane filter (0.45 μm) and degassed before being used as the diluent in the HPLC analysis.

Preparation of standard stock solution

In a Volumetric Flask (VF) with a capacity of 100 mL, 50 mg of VCG powder was added. The flask was then filled with the diluent and sonicated for 20 min. The volume was adjusted to 100 mL using the diluent. Next, 1 mL of the solution was transferred to a 10 mL VF and filled with 10 mL of diluent to achieve a concentration of 50 μg/mL of VCG.

Selection of the detection wavelength

HPLC analysis was performed using an 50 μg/mL solution of VCG. To create the working solution, the primary standard stock solution was diluted using the mobile phase as the diluent. UV spectrophotometer was employed to scan the prepared solution in the 190-400 nm wavelength range. The mobile phase was used as a reference to obtain accurate absorbance measurements. The purpose of this scanning process was to identify the wavelength at which the VCG compound absorbs light the most, which would be optimal for its detection in subsequent HPLC analyses.

Preparation of sample solution from dosage form

Twenty film-coated tablets of Verquvo® (each tablet contains 10 mg of vericiguat) were weighed, and their average weight was determined. The tablets were crushed to ensure homogenous powder. An aliquot of the VCG powder equivalent to 50 mg was transferred into a 100 mL VF for further preparation and analysis. The flask was filled with the diluent and subjected to sonication for 20 min. A 1 mL aliquot of the resulting solution was transferred into a 10 mL VF and diluted with 10 mL of diluent, resulting in a final concentration of 50 μg/mL.

Analytical method development

The method development process for HPLC analysis of VCG involved optimizing various parameters while keeping specific constants. Mobile phase composition (solvent systems and ratios), column selection, and flow rates were systematically adjusted to achieve desirable chromatographic separation. However, to ensure consistency and facilitate validation, several parameters remained unchanged: detector type, injection volume (20 μL), oven temperature (25±2°C), and elution mode. For each set of chromatographic conditions, a spectrum was recorded at the chosen detection wavelength. During the method's development, other criteria that were taken into account were peak height, column pressure, accuracy, resolution, analysis time, and solvent efficiency per run.

Validation

The established chromatographic conditions were deemed suitable for further method validation. The validation process adhered to the ICH Q2 guidelines[15] and incorporated an assessment of reagent and solvent stability, ensuring the reliability and reproducibility of the analytical procedure.

Evaluation of system suitability

System suitability tests were conducted to ensure the reliability of the HPLC system. Six injections at a concentration of 50 μg/mL were made to measure column efficiency, plate count, and tailing factor. The results met the predetermined criteria, confirming the consistency of the system and its adherence to specified limits.[16]

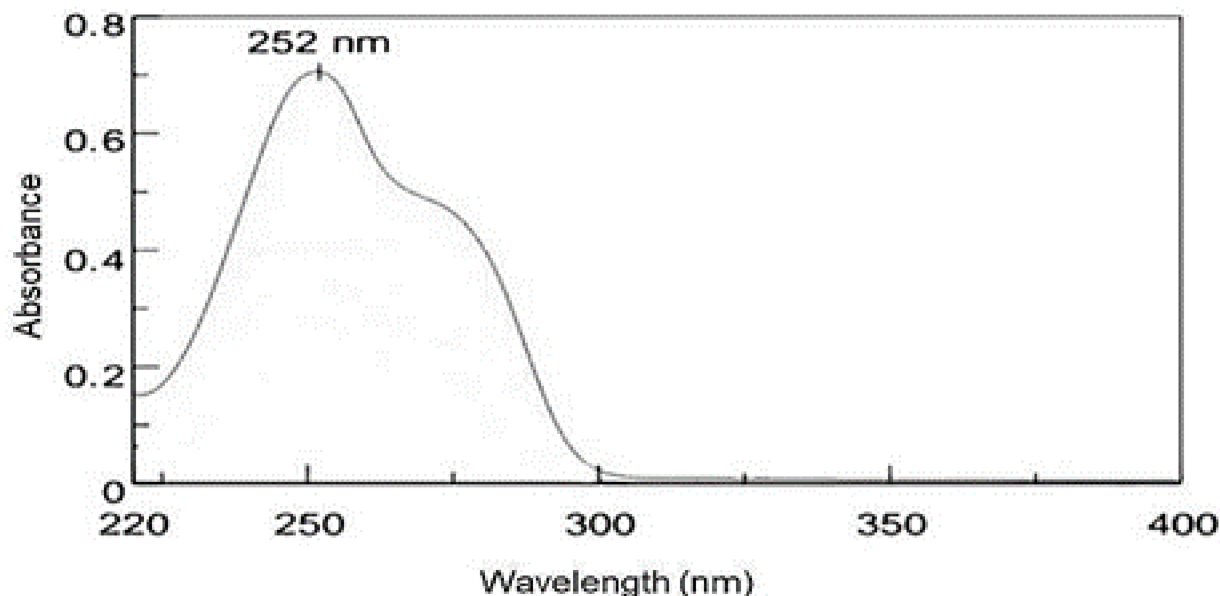

Figure 2: UV spectrum of Vericiguat.

Specificity and selectivity

The specificity and selectivity of the method were confirmed by the ability to detect VCG in the sample without interference. The chromatogram of the VCG reference standard showed a positive result, while the blank (containing only the diluent) showed no response or interference. Figures 3 and 4 show the chromatograms of the standard and blank injections, respectively.

Linearity

The linearity of VCG was evaluated by preparing dilutions ranging from 30-70 µg/mL from the standard stock solution. Peak area responses were measured for each concentration in the HPLC analysis.

Precision

Precision was assessed by conducting six repeated injections of the standard solution, which resulted in a low% RSD of 0.2, indicating high precision and consistent results. Similarly, six injections of the test solution showed a % RSD of 0.3, within the acceptable limit of 2.0% for precision, demonstrating the reliability of the method in sample analysis.

Intermediate precision (IP)

Intermediate precision was evaluated by having two analysts use separate HPLC instruments in different labs on different days to analyze the standard solution. Despite time-based variations, both obtained nearly identical assay results, with a negligible difference of 0.1% and an RSD below the acceptable limit of 2.0% on both days.

Accuracy or recovery studies

The accuracy of the HPLC method was verified through a triplicate recovery study. VCG at concentrations of 40, 50 and 60 µg/mL was injected into pre-analyzed samples. The mean recovery percentage was computed from these studies to validate accuracy.

Robustness

The robustness of the HPLC method was assessed by deliberately adjusting the flow rate and wavelength. These variations did not significantly alter the chromatogram, tailing factor, or plate count, indicating that the method remains robust and unaffected by fluctuations in flow rate and wavelength, ensuring accuracy and precision.

Forced degradation studies

The standard VCG sample was subjected to degradation under various stress conditions, including acidic, alkaline, oxidative, thermal, photostability, and neutral environments. For acidic and alkali degradation, the samples were refluxed with 2N hydrochloric acid and 2N sodium hydroxide at 60°C for 30 min each. Oxidative degradation was induced using 20% Hydrogen Peroxide (H_2O_2) and refluxed at 60°C for 30 min. Thermal degradation occurred at 105°C for 6 hr, photostability degradation by exposure to UV light for 7 days or 200 W h/m^2, and neutral degradation by refluxing in water for 6 hr at 60°C. The VCG samples were diluted to a final concentration of 50 µg/mL. 20 µL of each sample was injected into the system, and the chromatograms were recorded to evaluate the sample's stability.

Solution stability

Sample and standard solution stability assessments were conducted to ensure their integrity throughout the analysis. To assess sample stability, triplicate samples were kept at ambient temperature and refrigerated (2-8°C) for one and two days, respectively. The percent Relative Difference (%RD) between these stored samples and a freshly prepared sample was calculated. This confirmed that the samples remained stable during storage. Similarly, standard solution stability was tested. Duplicate standard solutions were stored at room temperature and under refrigeration for 1 and 2

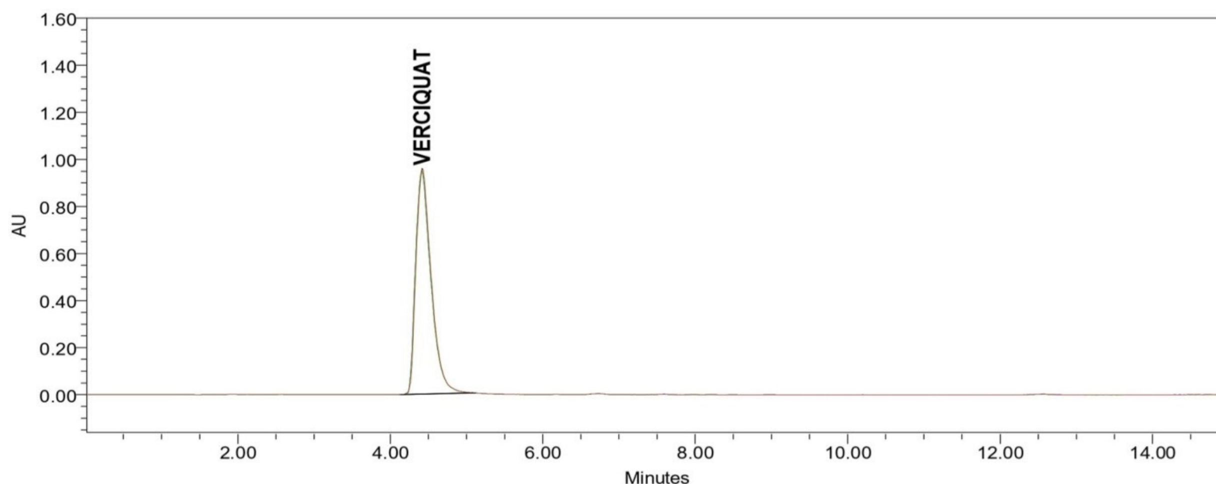

Figure 3: Optimized Chromatogram of Standard.

days. The percent assay and %RD were calculated by comparing the stored solutions with freshly prepared standards. This verified the stability of the standard solutions throughout the analysis period.

To ensure mobile phase stability, its appearance, system suitability parameters, and repeatability were evaluated after 1 and 2 days of storage at room temperature. This assessment confirmed the suitability of the mobile phase for consistent analysis throughout the planned duration.

Application of the developed method

The developed and validated HPLC method proved effective in determining and quantifying the precise amount of VCG present within commercially available tablets. As described in the materials and method section, sample solutions were prepared following the established protocol. Each sample was then injected into the HPLC system three times (triplicate injections) to ensure data accuracy and reliability.

Greenness of RP-HPLC method

The level of greenness was calculated using AGREE® software version 0.5 beta from the University of Vigo, Spain, which was accessible in an online repository.[17] Several criteria related to the approach were evaluated, including sample size, procedural steps, sample transformation level, derivatization, sample analysis rate per hour, organic phase ratio, quantity of hazardous compounds, energy consumption, and operator safety. The score and penalties are intended to assess any technique in relation to the human operating environment and geosafety.[18,19] Penalty points were deducted based on predetermined criteria and displayed as a pictogram. The central point of the spherical pictogram shows the final greenness score achieved by deducting penalty points. A pictogram with a core colour that is green or similar to green indicates a higher level of environmental safety, while colours further from green suggest lower environmental safety.

RESULTS

Method development and optimization

This research aimed to develop and validate a reliable method using UV-coupled RP-HPLC to quantify the amount of VCG in film-coated tablets. We combined theoretical knowledge with practical experimentation to successfully achieve this goal. The theoretical understanding of VCG's physico-chemical properties, derived from available literature, guided the selection of suitable chromatographic conditions.

The preliminary approach was developed using complete VCG information such as chemical structure, molecular mass, partition coefficient, solubility, and absorption spectra by UV. Solubility is critical in determining the best mobile phase compositions and columns for HPLC method development. VCG exhibits limited solubility in water but readily dissolves in organic solvents such as dimethyl sulfoxide and acetonitrile. This characteristic makes acetonitrile a suitable choice for preparing stock solutions of VCG. The compound is lipophilic (logP - 2.99), and has a molar mass of 426.388 g per mole.

VCG displayed notable Ultraviolet (UV) absorbance at 252 nanometers (nm) in a potassium dihydrogen orthophosphate: acetonitrile solution (50:50 v/v), as shown in Figure 2. This conveniently coincided with the optimal wavelength for quantifying VCG (Figure 2). Leveraging this initial observation, and through meticulous optimization of various parameters (see methods section), an accurate HPLC method was developed.

For an ideal analytical method, the detection wavelength should strike a balance between achieving the lowest Limit of Detection (LOD) and maximizing peak area. Fortunately, in this case, both criteria were met at 252 nm. This wavelength offered not only the minimum detectable concentration of VCG but also resulted in the largest peak area, ensuring accurate quantitation. Furthermore, 252 nm was free from potential interference from excipients co-eluting with VCG at its retention time of 4.42 min. The purity of the obtained peak at this wavelength further solidified the choice of 252 nm as the optimal detection wavelength. Consequently, considering both sensitivity and method conditions, 252 nm emerged as the optimal detection wavelength, fulfilling all necessary criteria.

Considering the physicochemical properties of VCG, both C_8 and C_{18} analytical columns were evaluated for their suitability in analysis. The column packed with C_{18} was surpassed the C_8 column in terms of important chromatographic characteristics such as Resolution (*Rs*), optimal plate Number (*N*), and Tailing factor (*Tf*). This indicates superior peak separation and sensitivity on the C_{18} column. Subsequently, to determine the optimal detection wavelength, the instrument's Limit of Detection (LOD) at 252 nm was measured using the UV detector.

Since VCG remains neutral during separation, the mobile phase can be a simple mixture of a buffer solution and an organic solvent. Among the various tested mobile phases, a mixture of KH_2PO_4 buffer (pH 2.5) and CH_3CN (50:50 v/v) yielded superior results. This combination produced symmetrical peaks for VCG with excellent resolution while also providing an appropriate retention time. Increasing the acetonitrile concentration significantly impacted peak resolution, widening band spacing. However, decreasing the concentration led to stronger retention but with varying selectivity, band spacing, and resolution. This highlights the importance of careful solvent selection and composition for optimal method development. Ultimately, a 50:50 (v/v) mixture of KH_2PO_4 buffer (pH 2.5) and CH_3CN proved to be the ideal mobile phase composition for quantifying VCG.

Flow rate also played a crucial role in separation, influencing VCG's retention time. While varying the rate from 0.5 to 1.5 mL/min, the optimal value for plate Number (*N*) and Resolution (*Rs*)

between components was found to be 1.0 mL/min. Notably, the quantification was performed at room temperature (25±2°C) due to VCG's neutral characteristics. It's important to remember that, in reversed-phase chromatography, higher temperatures often decrease selectivity for neutral compounds.[16]

Leveraging VCG's neutral nature for optimal separation, the temperature was maintained constant throughout the experiment. Following thorough analysis, the optimal set of parameters for the analytical method was determined to be a C_{18} column, a flow rate of 1 mL/min, a mobile phase consisting of potassium dihydrogen orthophosphate buffer (pH 2.5) and acetonitrile in a ratio of 50:50 v/v, and a detection wavelength of 252 nm. Under these optimized conditions, a symmetrical and well-defined peak of VCG was observed with an average retention time of 4.42 min, demonstrating the effectiveness of the chosen parameters.

The analysis showed a percent Relative Standard Deviation (%RSD) of less than 2%, indicating exceptional precision. Additionally, the column pressure remained significantly below the recommended limit. For most procedures, column pressures under 150 bar are ideal, while pressures under 200 bar are safe.[16]

System suitability parameters

The precision of the HPLC system, characterized by its repeatability, was assessed by calculating the %RSD from six consecutive injections of the standard solution. A requirement was set for the RSD not to exceed 2% to be considered acceptable. Since the RSD of the standard solution fell within this limit, indicating precision within the specified criterion, the system proved reliable for accurate VCG quantification in samples. These findings are detailed in Table 1.

Table 1: System suitability parameters for Vericiguat.

Sl. No.	Parameter	Vericiguat	Acceptance criteria
1.	Retention time (RT).	4.42	--
2.	Theoretical plates (N).	9835	NLT 2000
3.	Tailing factor (T).	1.75	NMT 2.0
4.	Linearity range (µg/mL).	30-70	--
5.	Detection Limit (µg/mL).	0.05	--
6.	Quantification limit (µg/mL).	0.15	--
7.	Regression data: Slope.	268092	--
8.	Regression data: Intercept.	308792	--
9.	Regression data: Correlation coefficient.	0.998	--

Table 2: Linearity of Vericiguat.

Sl. No.	Drug	Values of X and Y variables						Correlation co-efficient
1.	VCG	Variable	1	2	3	4	5	0.998
		X	30	40	50	60	70	
		Y	8399016	10992333	13559554	16632608	18983486	

Table 3: Precision Study.

Sl. No.	System Precision		Method Precision	
	Rt	AUC	Rt	AUC
1	4.41	13574321	4.42	13569342
2	4.42	13566234	4.43	13573127
3	4.42	13589987	4.42	13548291
4	4.41	13542013	4.42	13555678
5	4.43	13557789	4.44	13591023
6	4.44	13580456	4.42	13578901
Mean	4.4	13568466	4.4	13569393
SD	0.0	17087.6	0.0	15528.0
% RSD	0.2	0.2	0.2	0.3

Table 4: Intermediate Precision or Ruggedness Study.

Analyst Name	Analyst I			Analyst II		
Area of Std.	13583042			13563987		
Sl. No.	Concentration (µg/mL)	AUC	Assay (%)	Concentration	AUC	Assay (%)
1	50	13573246	99.93	50	13580321	99.98
2	50	13558976	99.82	50	13567489	99.89
3	50	13564123	99.86	50	13592034	100.07
4	50	13592784	100.07	50	13593546	100.08
5	50	13550678	99.76	50	13550679	99.76
	Mean	13567961.4	99.9	Mean	13576813.8	100.0
	SD	16111.1	0.1	SD	17985.4	0.1
	% RSD	0.2	0.2	% RSD	0.3	0.2
Difference between mean assay of two different analysts=0.1 %						

Table 5: Accuracy study.

Sl. No.	Level	Amount added (µg/mL)	Mean (*n*= 5)	% Recovery
1.	80%	40	10856431	99.92
2.	100%	50	13578321	99.96
3.	120%	60	16399650	100.61

Table 6: Robustness Study.

Parameters	Variation	Mean Peak area	%RSD	Tailing factor	No. of Theoretical Plates
Wavelength minus.	250 nm	13574628	0.95	1.70	7034
Wavelength plus.	254 nm	13575123	0.81	1.75	7265
Flow rate minus.	0.8 min/mL	13582976	0.68	1.26	6912
Flow rate plus.	1.2 min/mL	13583457	0.83	1.26	7811
Organic phase ratio change (less).	Buffer: Acetonitrile (80:20).	13559861	0.43	1.71	6924
Organic phase ratio change (more)	Buffer: Acetonitrile (60: 40)	13560131	0.51	1.71	8013
Column change.	Merck C_{18} column (250 mm×4.6 mm×5 µm).	13597324	0.29	1.70	7956
Temperature minus.	20ºC	13583463	0.31	1.16	6991
Temperature plus.	30ºC	13582916	0.69	1.32	7518

VCG exhibited a Retention Time (RT) of 4.42 min, confirming its effective separation and timely detection. Through meticulous selection of mobile phase composition, optimal wavelength, and parameter fine-tuning, the HPLC method for VCG analysis was tailored to meet the desired criteria: swift analysis and satisfactory resolution. This rigorous optimization ensures reliable and efficient VCG quantification, meeting stringent analytical demands.

Specificity and selectivity

The method demonstrated high specificity and selectivity, allowing for the accurate detection of VCG in the sample. The chromatogram of the VCG reference standard showed a distinct peak, while the blank containing only the diluent showed no response or interfering peaks. This confirms that there are no interferences from other sample components, as shown in Figures 3 and 4 (standard and blank injections, respectively). The chromatogram of the VCG solution displayed a sharp,

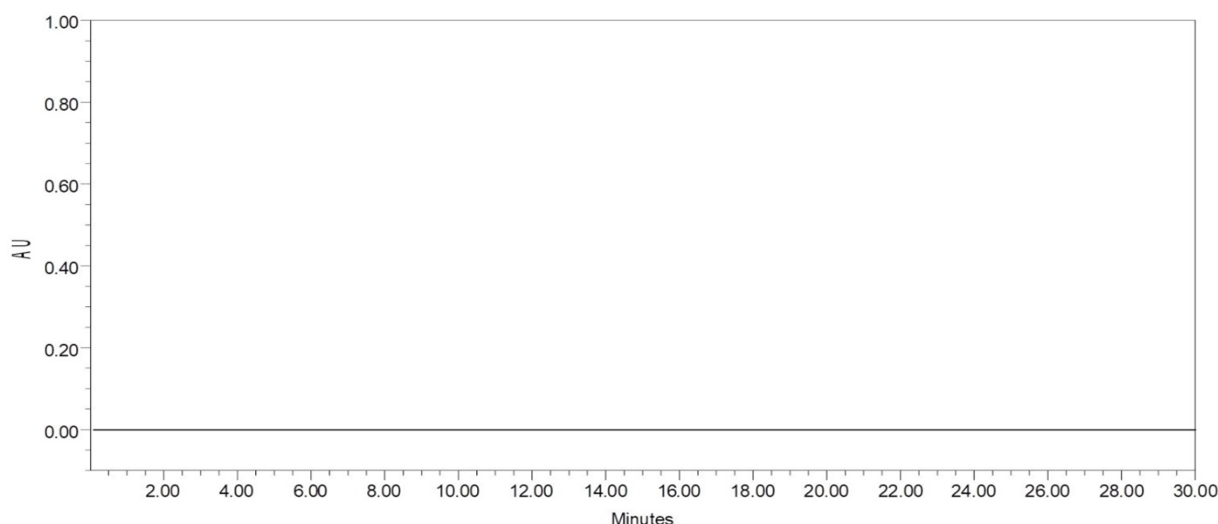

Figure 4: Blank Chromatogram.

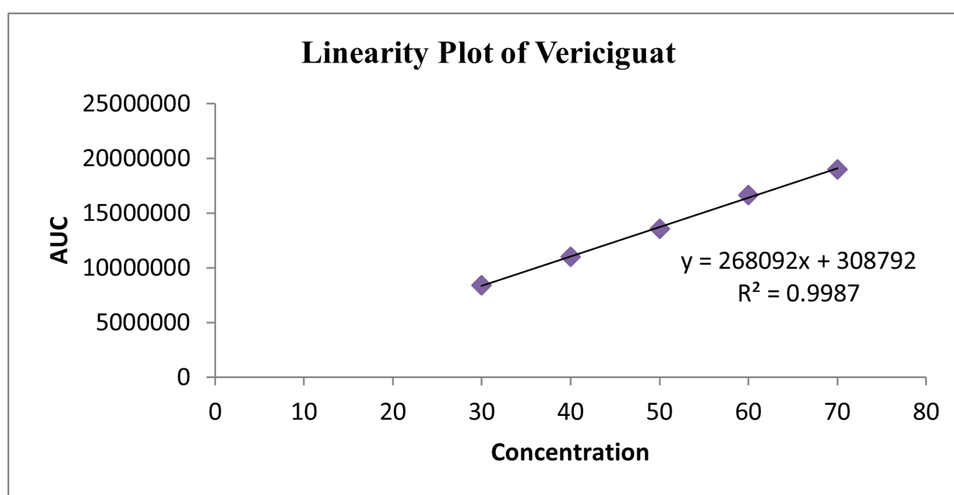

Figure 5: Linearity plot of Vericiguat.

well-separated, and symmetrical peak at a retention time of 4.42 min (Figure 3). Importantly, there were no interfering peaks from the placebo matrix at this retention time, indicating the excellent specificity of the method. This means that the method can accurately identify and quantify VCG even in the presence of other components from the sample.

Linearity

A linearity graph was generated for VCG, with concentration in µg/mL on the *x*-axis and Area Under the Curve (AUC) on the *y*-axis. Within the range of 30-70 µg/mL, a linear correlation was observed between drug concentrations and peak area responses. The results, depicted in Figure 5 and summarized in Table 2, highlight the importance of linearity in analytical methods. This characteristic ensures precise drug quantification across a wide range of concentrations, based on the measurement of peak areas. The HPLC method exhibits excellent linearity within the specified concentration range, establishing a strong relationship between concentration and peak area response, which facilitates accurate VCG analysis.

Precision

The precision of the method, including the repeatability of both sample and standard preparations, was found to be satisfactory. Table 3 confirms these findings, demonstrating the reproducibility and reliability of the HPLC method in VCG analysis. This validation supports the routine use of the method for accurate quantification across various types of samples.

Intermediate Precision (IP)

The consistent performance of the HPLC method across diverse laboratories, instruments, and analysts, even on different days, emphasizes its robustness. Its intermediate precision affirms its suitability for routine use, ensuring consistently accurate and reliable results under varying experimental conditions. Table 4 displays the percentage RSD values for intermediate precision,

and they are below 2.0%, confirming the precision of the method and instrument. This consistency guarantees dependable and minimally variable results, as evidenced by the acceptable recovery rate range of typically 99.9-100.01%.

Accuracy

The assay yielded a mean recovery percentage in the range of 99.92-100.61 signifying the accuracy of the HPLC method in quantifying the VCG content within expected values. Results from Table 5, which displays spiked concentrations and mean recovery percentages, validate the reliability of the method for quantitative analysis, showcasing successful VCG recovery from varied spiked samples. This accuracy, as evidenced by the recovery falling within the acceptable range, confirms the satisfactory performance of the method in accurately measuring the VCG content.

Robustness

Table 6 presents the results from robustness studies, detailing the variations tested and their impact on the performance of the method. The confirmed robustness of the HPLC method affirms its suitability for routine application, ensuring consistent and reliable results even under slightly altered operating conditions. Notably, insignificant changes in peak areas and retention times highlight the method's ability to deliver reliable outcomes across different conditions. In comparison to previously reported analytical methods in the literature, this HPLC method excels with shorter retention times, enhanced theoretical plates (indicating improved resolution), and a mobile phase that promotes better separation of VCG from other constituents. Consequently, its

Table 7: Forced degradation data (± SD) of the method*.

Stress Condition	% Amount Degraded	% Amount Recovered
Acidic	3.31±0.81	96.69±2.71
Alkali	1.98±0.68	98.02±2.28
Neutral	0.06±0.87	99.94±2.09
Oxidative	1.27±0.91	98.73±0.89
Thermal	0.52±0.09	99.48±1.85
Photostability	0.64±0.13	99.36±1.67

*The mean of the three results (for each condition); SD: Standard deviation.

Table 8: Analysis of Marketed Formulation.

Commercial Formulation	Ingredients	Labeled Amount (mg)	Amount Found (mg)	Found %
Verquvo™	Vericiguat	10 mg	9.93 mg	99.3

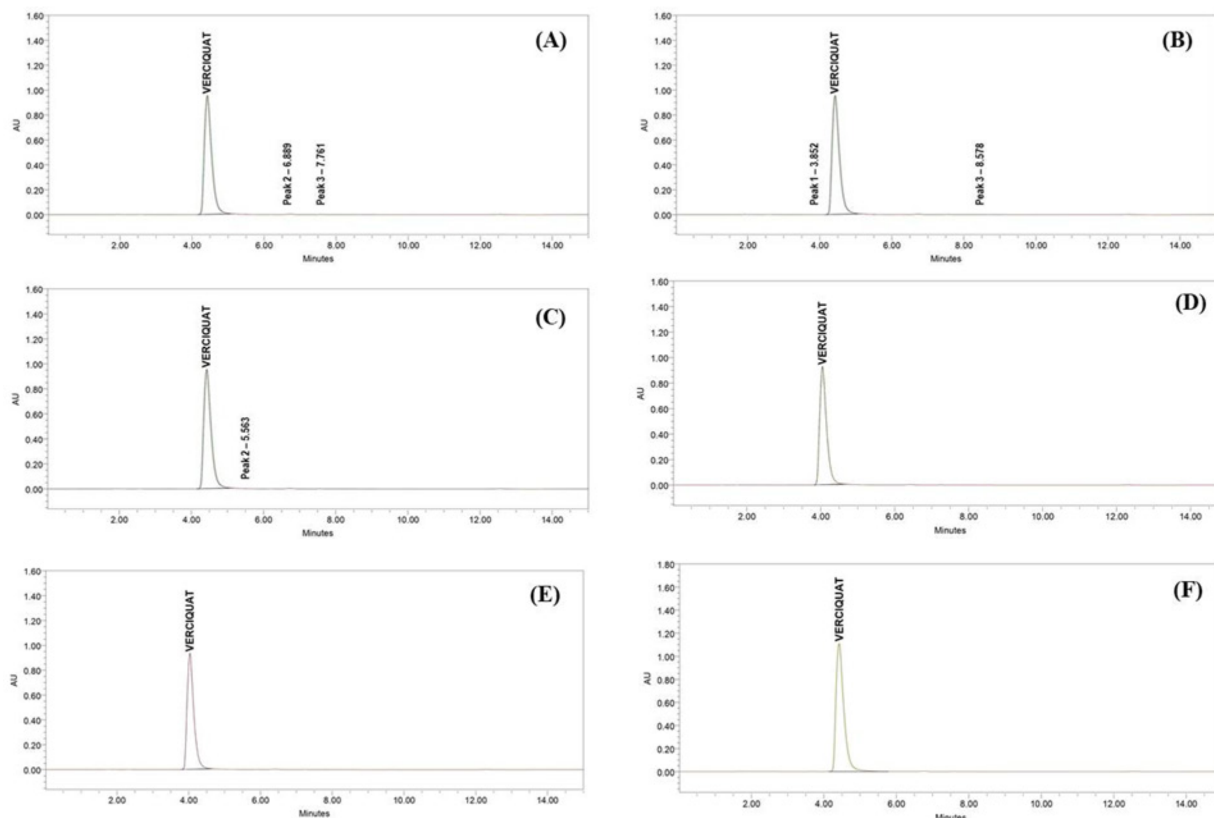

Figure 6: Chromatograms showing different degraded under different stress conditions.

Figure 7: Greenness of the developed method using AGREE software.

increased efficiency and precision make it more suitable for the routine quantification of VCG across several sample types.

Forced degradation studies

The standard solution was subjected to various stress conditions as outlined in the methods. The VCG showed approximately 3.31% degradation under acidic conditions, with approximately two degradation peaks observed (Figure 6 (A)). Under alkaline conditions, the VCG experienced approximately 1.98% degradation with two degradation peaks noted (Figure 6 (B)). In conditions of oxidation, the VCG exhibited around 1.27% degradation, with one degradation peak identified (Figure 6 (C)). Under specified conditions of temperature, photostability, and neutral environments, degradation was minimal, less than 1%, and no degradation peak was observed in Figures 6 (D to F). The forced degradation results are shown in Table 7.

Analysis of marketed formulation

To determine the content of vericiguat in Verquvo™ commercial tablets, each containing 10 mg of VCG, were selected. Percentage assay of the VCG tablets that were found as 99.3% indicates that the analyzed tablets have percentage purity within the acceptance limits as per ICH guidelines. Table 8 displays the results.

Greenness of RP-HPLC method

The greenness of the method guarantees its environmental safety. An increase in the number of substances, chemicals, reagents, procedures, and steps in the created method would have a more detrimental impact on the environment. Hence, it is crucial that the analysis is conducted using an environmentally friendly approach.[17] Various greenness assessment software programs provide a comprehensive analysis of the advantages and disadvantages of each method.[18,19] AGREE shows penalties using contour tones based on proximity to the desirable greenness, while also considering worker safety. The developed method's AGREE score was assessed to be 0.79, with penalty points of 0.21 deducted (Figure 7). This is because the mobile phase contains 50% v/v acetonitrile, which is known to have mild toxicity to aquatic life and is flammable. The retention time for VCG is less than 5 min in a single run, as shown in Figure 3, which minimizes wasting of the mobile phase. In the literature, a significant amount of VCG was wasted due to the time-consuming sample analysis time in a single run. VCG wastage was significantly higher in the literature due to the time-consuming sample analysis time in a single run.

DISCUSSION

The stability indicating RP-HPLC assay method is important in the measurement of intrinsic stability, as well as the qualitative and quantitative evaluation of active pharmaceutical ingredients. Only a few analytical techniques for VCG have been established so far. However, no RP-HPLC method based on the greenness approach has been developed for estimating VCG with high sensitivity. As a result, efforts were made to establish a reliable stability indicating the RP-HPLC method. The described method's retention time for VCG was 4.42 min, indicating that the approach has a good and effective retention time and can be considered affordable because it decreases solvent consumption and analyte run time. As a result, it is possible to conduct a quick analysis of a variety of samples. The calculated and statistical findings of the parameters of validation were within the acceptability limits specified by ICH.

CONCLUSION

Following the ICH guidelines, an HPLC method was established and validated. The method utilized a Shimadzu HPLC model (LC-20AD) with detection at 252 nm using ultraviolet (UV) light. The injection volume was 20 µL, and a Kromacil C_{18} column (250 x 4.6 mm, 5 µm) was used with isocratic elution. The resulting method is rapid, robust, straightforward, precise, sensitive, and cost-effective. It offers key benefits such as a brief run time (below 7 min) and excellent resolution. The % RSD values for all validation parameters met the criteria, confirming the method's suitability for routine analysis of vericiguat in laboratories and quality control. Furthermore, in terms of ecological safety, the validated technique was deemed environmentally friendly when assessed on a greenness scale. The pharmaceutical sector can utilise it for stability testing and evaluating dosage forms (bulk or finished) in a single run lasting less than 10 min.

ACKNOWLEDGEMENT

The authors are thankful to the Teja Educational Society for their support to carry out this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RP-HPLC: Reverse phase high-performance liquid chromatography; **RSD:** Relative standard deviation.

REFERENCES

1. Tomasoni D, Adamo M, Lombardi CM, Metra M. Highlights in heart failure. ESC Heart Fail. 2019;6(6):1105-27. doi: 10.1002/ehf2.12555.
2. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. Heart. 2007;93(9):1137-46. doi: 10.1136/hrt.2003.025270.
3. Julián MT, Pérez-Montes de Oca A, Julve J, Alonso N. The double burden: type 1 diabetes and heart failure-a comprehensive review. Cardiovasc Diabetol. 2024;23(1):65. doi: 10.1186/s12933-024-02136-y. PMID: 38347569; PMCID: PMC10863220.
4. Kansakar S, Guragain A, Verma D, Sharma P, Dhungana B, Bhattarai B, *et al.* Soluble Guanylate Cyclase Stimulators in Heart Failure. Cureus. 2021;13(9):e17781. doi: 10.7759/cureus.17781.
5. Grześk G, Witczyńska A, Węglarz M, Wołowiec Ł, Nowaczyk J, Grześk E, *et al* Soluble Guanylyl Cyclase Activators-Promising Therapeutic Option in the Pharmacotherapy of Heart Failure and Pulmonary Hypertension. Molecules. 2023;28(2):861. doi: 10.3390/molecules28020861.
6. Farah C, Michel LYM, Balligand JL. Nitric oxide signalling in cardiovascular health and disease. Nat Rev Cardiol. 2018;15(5):292-316. doi: 10.1038/nrcardio.2017.224.
7. Sandner P, Follmann M, Becker-Pelster E, Hahn MG, Meier C, Freitas C, *et al*. P. Soluble GC stimulators and activators: Past, present and future. Br J Pharmacol. 2021;1-22. doi: 10.1111/bph.15698.
8. Sandner P, Stasch JP. Anti-fibrotic effects of soluble guanylate cyclase stimulators and activators: A review of the preclinical evidence. Respir Med. 2017;122:S1-S9. doi: 10.1016/j.rmed.2016.08.022.
9. Follmann M, Ackerstaff J, Redlich G, Wunder F, Lang D, Kern A, *et al.* Discovery of the Soluble Guanylate Cyclase Stimulator Vericiguat (BAY 1021189) for the Treatment of Chronic Heart Failure. J Med Chem. 2017;60(12):5146-61. doi: 10.1021/acs.jmedchem.7b00449.
10. Armstrong PW, Pieske B, Anstrom KJ, Ezekowitz J, Hernandez AF, Butler J, *et al.* VICTORIA Study Group. Vericiguat in Patients with Heart Failure and Reduced Ejection Fraction. N Engl J Med. 2020;382(20):1883-93. doi: 10.1056/NEJMoa1915928.
11. Mustafa DM, Magdy N, El Azab NF. The first validated stability-indicating HPLC/DAD method for quantitation of Vericiguat in its pharmaceutical formulation; Application to degradation kinetic studies. Talanta. 2023;259:124498. doi: 10.1016/j.talanta.2023.124498.
12. Ainousah BE, Abdelazim AH, Almrasy AA, Abdel-Kareem RF, Ghoneim MM, Gamal M. Color based spectrophotometric determination of vericiguat using diazo coupling reaction; assisted computational calculations. Journal of Molecular Structure. 2023; 1294:136317.
13. Mustafa DM, Magdy N, El Azab NF. Different spectrophotometric methods for simultaneous quantitation of Vericiguat and its alkaline degradation product: a comparative study with greenness profile assessment. Sci Rep. 2023;13(1):23077. doi: 10.1038/s41598-023-50097-1.
14. Ding C, Guo C, Fang L, Li Y, Wang Z, Dong Z. Determination of vericiguat in rat plasma by UPLC-MS/MS and its application to drug interaction. J Chromatogr A. 2023; 1709:464401. doi: 10.1016/j.chroma.2023.464401.
15. Borman P, Elder D. Q2 (R1) validation of analytical procedures. ICH Qual. Guidel. 2017;5;127-66.
16. Snyder LR, Kirkland JJ, Glajch JL, Practical HPLC Method Development, John Wiley and Sons, 2012.
17. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE-Analytical GREEnness Metric Approach and Software. Anal Chem. 2020;92(14):10076-10082. doi: 10.1021/acs.analchem.0c01887.
18. Płotka-Wasylka J, Wojnowski W, Complementary green analytical procedure index (Complex GAPI) and software. Green Chem. 2021;23:8657-65.
19. Pena-Pereira F, Tobiszewski M, Wojnowski W, Psillakis E. A tutorial on AGREEprep an analytical greenness metric for sample preparation. Advances in Sample Preparation. 2022;3:100025. https://doi.org/10.1016/j.sampre.2022.100025

Cite this article: Madhavi A, Srinivas M, Gupta N. Development of a Robust and Reliable RP-HPLC Method for the Estimation of Vericiguat in Tablet Dosage Form: Greenness Analysis using AGREE Penalties. Int. J. Pharm. Investigation. 2024;14(3):822-32.