ORIGINAL ARTICLE

Development and Validation of Impurity Profiling and Stability Studies of Vericiguat using Ultra Performance Liquid Chromatography Method

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Abstract

Background: To develop a stability-indicating, simple, accurate, and rapid reverse phase ultra-performance liquid chromatography (RP-UPLC) method for the detection and quantification of organic impurities in Vericiguat bulk drug, ensuring the safety of drug therapy through a selective analytical approach. **Materials and Methods:** Vericiguat bulk drug, along with its known impurities (impurity-1 [Imp-1] and impurity-2 [Imp-2]) were used. Simple and economical solvents and mobile phase components were employed in the ultra-performance liquid chromatography method. Linearity of the method was established over the concentration ranges of 37.5–225 µg/mL for Vericiguat, and 2.5–15 µg/mL for both Imp-1 and Imp-2, with a correlation coefficient (r^2) of 0.999 for all analytes. Stress degradation studies were performed under various conditions to assess stability, particularly oxidative stress. Robustness was evaluated by deliberately varying method parameters. Recovery studies were conducted to assess accuracy and specificity in the presence of formulation excipients. **Results:** The developed method showed excellent resolution of impurities within a short run time. It was found to be robust and unaffected by small variations in method parameters. The stress testing revealed that Vericiguat is most sensitive to oxidative degradation, particularly in the presence of peroxide. Recovery results were consistent with label claims, with no interference from excipients. **Conclusion:** The developed RP-UPLC method is simple, fast, accurate, precise, robust, and economical. It is suitable for routine quality control analysis of Vericiguat and its impurities in bulk drug form.

Key words: Method validation, organic impurities, reverse phase ultra-performance liquid chromatography, stability-indicating method, stress degradation, Vericiguat

INTRODUCTION

The development and validation of analytical methods for the determination of impurities and the stability of pharmaceutical compounds is a critical aspect of drug development and quality control.[1,2] Vericiguat is a promising therapeutic agent used for the treatment of heart failure, and it is essential to ensure its purity and stability during manufacturing, storage, and distribution.[3,4]

One of the most commonly used techniques for the analysis of pharmaceutical compounds is ultra-performance liquid chromatography (UPLC). This method allows for the separation, identification, and quantification of individual components in complex mixtures, such as drug formulations and impurities. UPLC is a fast, sensitive, and efficient analytical technique that has become a cornerstone of modern pharmaceutical analysis.[5-7]

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In this context, the development and validation of an impurity profiling and stability study method for Vericiguat [Figure 1] using UPLC is of great significance.[8,9] This analytical method can provide critical information about the purity, degradation products, and stability of Vericiguat, which can be used to evaluate its quality, safety, and efficacy.[10,11] Moreover, the development and validation of this method can serve as a template for the analysis of other pharmaceutical compounds, contributing to the advancement of drug development and quality control.[12,13]

Literature review

A review of the literature reveals that pharmacokinetic and pharmacodynamic studies of Vericiguat in patients with heart failure and reduced ejection fraction have been reported.[14,15] To the best of our knowledge, no UPLC method has been documented for the impurity (Imp) profiling and stability analysis of Vericiguat. Furthermore, no such efficient analytical method that is both time- and cost-effective has been reported. As a result, we aimed to develop a rapid, accurate, reproducible, and economical UPLC-based method for Imp profiling and stability assessment of Vericiguat.[16,17] The developed method was validated in accordance with the International Conference on Harmonisation (ICH) guidelines.[18,19] This method is designed to determine Imp levels in the dosage form to ensure its safety and therapeutic efficacy.[20]

MATERIALS AND METHODS

Materials

The pure Vericiguat, which was a gift from Icon Laboratories in Vijayawada, was used to prepare respective standard solutions for method development and validation studies. Impurities A and B were obtained from Sree Icon Laboratories, Vijayawada. For the method development and validation study, Verquvo TM brand formulated samples and a placebo were used. HPLC grade acetonitrile and formic acid (Rankem, Mumbai, India), and orthophosphoric acid (Rankem) were used for diluent and mobile phase preparation. pH was measured using a calibrated pH metre.

Instruments

Waters Acquity UPLC bridged ethyl hybrid (BEH) Shield C8 (100 × 2.1 mm, 1.7 m) column was used to separate all impurities. Waters' Acquity UPLC system with photodiode array (PDA) detector was used for the entire development and validation study.

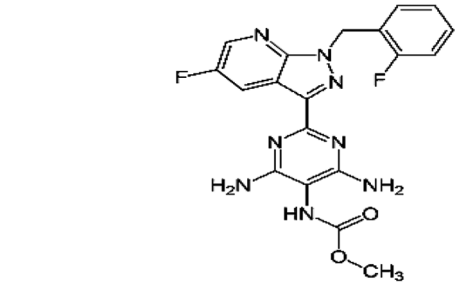
Mobile phase and diluent preparation

1 mL formic acid dissolved in 1 L of water; filter through a 0.45 nylon filter. A 20:80 mixture of 0.1% formic acid and acetonitrile was transferred into a glass mobile phase bottle. The bottle was sonicated for 5 min to thoroughly mix the reagents. Orthophosphoric acid was used to adjust the pH to 3.0. To remove the dissolved gases and contaminants, the mobile phase was filtered through a 0.45 µm filter. For solution preparation, the mobile phase was used as a diluent.

System suitability solution preparation

Preparation of standard solution

To make a standard stock solution, 15 mg of Vericiguat working standard was transferred into a 10 mL clean, dry volumetric flask, diluted to volume with diluent, and



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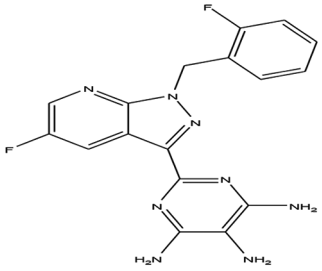

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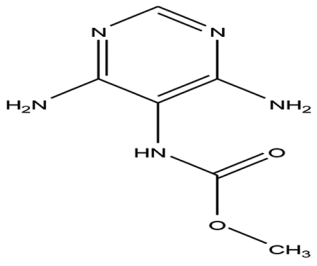

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Figure 1: Struture of Vericiguat (1), Impurity-1 (2), Impurity-2 (3)

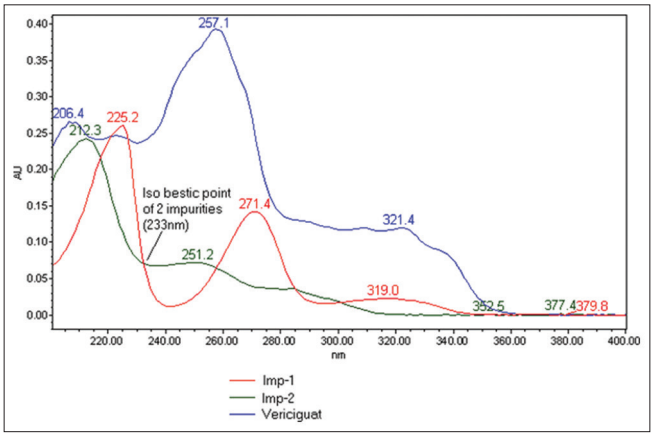

Figure 2: Photodiode array spectrum of Vericiguat and its impurities

sonicated to dissolve completely and make the volume up to the mark with the same solvent.

In a 10 mL volumetric flask, 1 mL of standard stock solution was transferred. The volume was increased with diluent to achieve 150 ppm of Vericiguat.

Preparation of Imp stock solution

Weigh 5 mg of Vericiguat Imp-1 and 5 mg of Vericiguat Imp-2 into a 10 mL volumetric flask, add 7 mL of diluent, sonicate for 30 min, and make the volume up to the mark with the same diluent. Pipette 2 mL of the above solution into a 10 mL volumetric flask and dilute to the desired concentration with diluents.

System suitability criteria

We defined system suitability parameters, such as resolution, capacity factor, signal-to-noise ratio, and theoretical plates to develop a robust analytical method.

Imp calculation

The corresponding principle analyte peak in the standard solution was compared to every known Imp that eluted in the sample chromatogram. The unknown degradant peaks were quantified by degradation of the individual active pharmaceutical ingredient (API) and by defining the relative retention time (RRT) with respect to the principle analyte peak. The unknown Imp was calculated using the analyte.

Method validation

ICH Q2 guidelines were followed in the validation of the devised approach. The new UPLC method was validated using tests on system appropriateness, specificity (selectivity and forced degradation [FD]), linearity, precision, accuracy, sensitivity (limit of detection [LOD] and limit of quantification [LOQ]), analytical solution stability, and robustness.

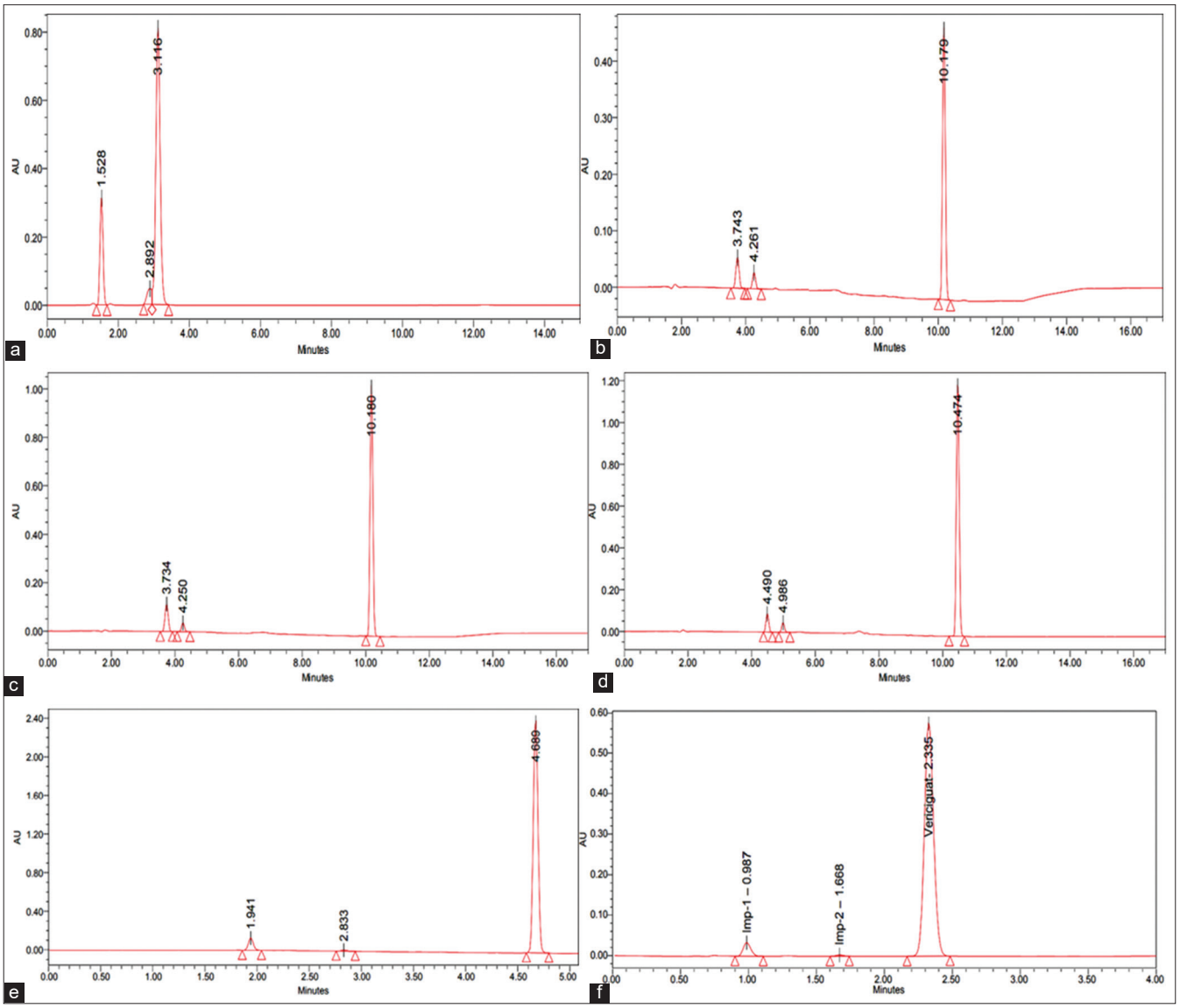

Figure 3: (a) Trail-1, (b) Trail-2, (c) Trail-3, (d) Trail-4, (e) Trail-5, (f) Optimized chromatograms

Accuracy

The accuracy study involved spiking all known impurities alongside the Vericiguat working standard at the LOQ level, as well as at 50%, 100%, and 150% of the Imp limit in the placebo. Six LOQ preparations and three 50%, 100%, and 150% preparations were injected into the system. The percentage recovered amount was calculated for each known Imp, and the principle analyte peak was compared to the spiked amount.

Precision

System precision

Six injections were made from the same standard preparations, and the relative standard deviation (RSD) for the replicate injections was calculated.

Method precision

Six sample solutions were prepared according to the procedure and injected into the UPLC system. The percentage RSD was calculated for all known impurities and analytes.

Specificity

To demonstrate the specificity of the developed method, the diluent was injected separately as a blank solution, placebo solution, Imp 1 and 2 solutions, and Vericiguat standard solution. The Imp spiked solutions and sample solution were prepared and injected into the UPLC system. The PDA detector was used to record the response of the individual analytes as well as the peak purity.

FD study

A FD study of the sample and placebo was performed to demonstrate the method's selectivity and to assess the method's stability in indicating the nature of the method. Heat, acid, base, and oxidation were used to stress both the sample and the placebo. UPLC was used to analyse the stressed samples. The acid degradation was performed using 1 N hydrochloric acid for 1 h at 60°C, and the alkali degradation was performed using 1 N sodium hydroxide for 1 h at 60°C. Thermal degradation was achieved by keeping the sample and placebo at 105°C for 24 h. For the oxidative stress study, 3% hydrogen peroxide was used for 12 h. The photolytic degradation was accomplished by exposing the drug sample to light for a period of time.

Linearity

Vericiguat working standard solutions and samples with all known impurities were made and injected in triplicate from 150% to LOQ (150%, 120%, 100%, 80%, 50%, 20%, and LOQ). The average area of each analyte was plotted against the concentration in µg/mL at each level of the linearity graph. We computed the intercept, slope, and correlation coefficient.

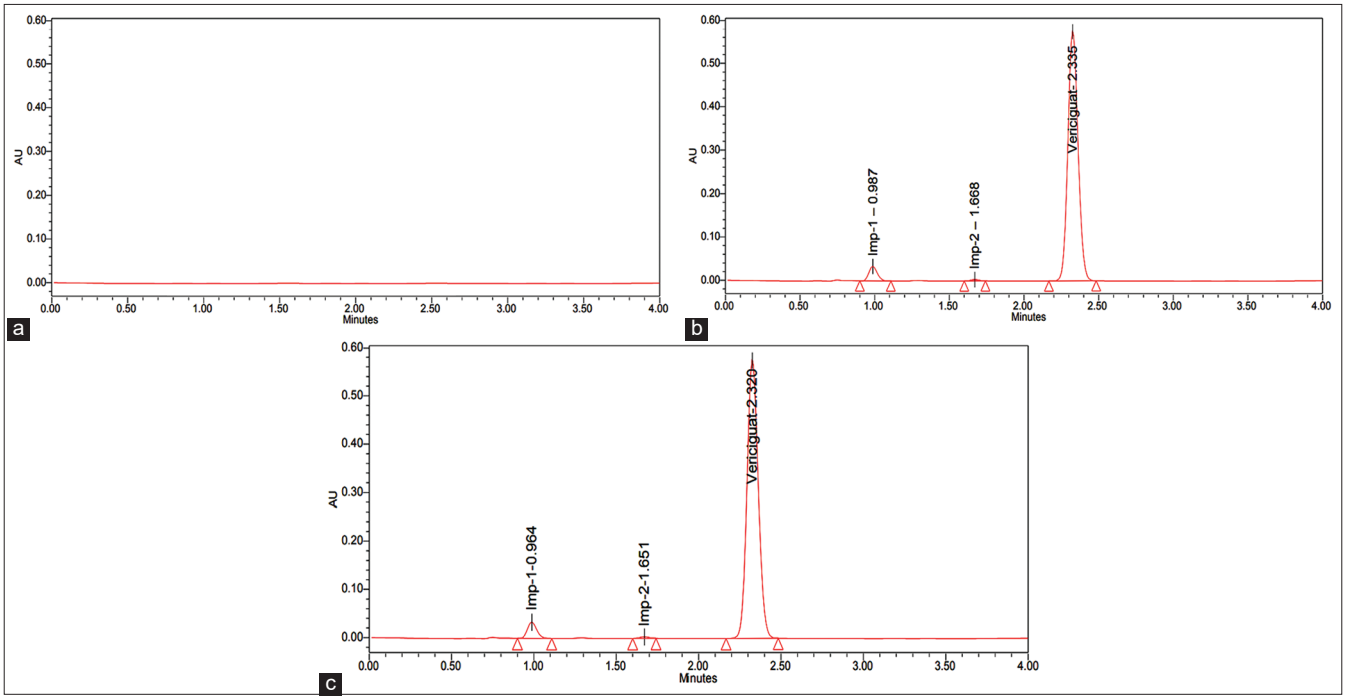

Figure 4: Chromatogram of (a) blank, (b) standard, (c) sample

Range

The area under the curve and concentration for linear response were used to determine the concentration range of analyte and Imp solutions.

Robustness

This study was carried out by purposefully changing critical method parameters, such as flow rate (0.1 mL) and mobile phase composition (0.2. To test the robustness of the analytical method, diluent, standard solution, sample solution, and Imp-spiked sample solution were injected.

RESULTS AND DISCUSSION

Method development

A reverse phase UPLC method was developed for the simultaneous estimation of Vericiguat and its related impurities. The method development process involved systematic optimization of chromatographic conditions, starting with the selection of an appropriate detection wavelength. Based on ultraviolet spectral analysis using a PDA detector, 233 nm [Figure 2] was selected as the detection wavelength due to optimal absorbance for Vericiguat and its impurities.

Multiple chromatographic trials (TRAIL-1 to TRAIL-5) [Figure 3] were conducted using different column types, mobile phase compositions, and buffer systems to achieve sharp peaks, acceptable resolution, and minimal tailing. Early trials with BEH Shield RP-18 and C8 columns using acetonitrile and 0.1% triethylamine or formic acid in various ratios showed issues, such as peak splitting, poor baseline separation, excessive tailing, or high peak response.

The optimized method was achieved using a Waters Acquity UPLC BEH Shield C8 column (100 × 2.1 mm, 1.7 µm) with a mobile phase consisting of acetonitrile and 0.1% formic acid in an 80:20 ratio. The flow rate was maintained at 0.2 mL/min with an injection volume of 5 µL. The retention times (RT) for Vericiguat Imp-1, Imp-2, and the main compound were 0.987 min, 1.668 min, and 2.335 min, respectively [Figure 4, Tables 1 and 2]. The method provided good resolution, acceptable tailing factors, and high plate counts, confirming its suitability for further validation.

The final method is simple, rapid, and robust, making it appropriate for the routine analysis of Vericiguat and its impurities in bulk drug form.

Validation of method

Linearity and range

The linearity of the method was evaluated over the concentration range of 37.5–225 µg/mL for Vericiguat, and 2.5–15 µg/mL for both Imp-1 and Imp-2. A series of solutions were prepared by diluting the stock solution to obtain six different concentrations, labeled as Solutions 1 through 6. Each solution was injected into the UPLC system in accordance with the established test procedure. Calibration curves for Vericiguat, Imp-1, and Imp-2 were constructed by plotting the peak area against the corresponding concentration. The resulting chromatograms and linear regression data confirmed the linear relationship for all analytes across the tested concentration range are shown in [Figures 5 and 6, Table 3].

Accuracy

The accuracy of the method was evaluated for Vericiguat, Imp-1, and Imp-2 by performing recovery studies at three concentration levels: 50%, 100%, and 150% of the target concentration. Each level was prepared in triplicate by spiking equivalent amounts of Vericiguat, Imp-1, and Imp-2 into separate volumetric flasks, in accordance with the test method. The spiked samples were analyzed, and the percentage recovery for each analyte was calculated. The average % recovery was determined for each level to assess the accuracy of the method results are shown in [Table 4].

Table 1: Optimized chromatographic conditions

Parameters	Observations
Column	Waters acquity UPLC BEH Shield C_8 (100×2.1 mm, 1.7 µm)
Mobilephase	Acetonitrile and 0.1% formic acid (80:20)
Detection wavelength	233 nm
Flowrate	0.2 mL/min
Injection volume	5 µL
Run time	4 min
Temperature	Ambient

Table 2: Vericiguat and its impurities system suitability parameters

S. No	Parameter	Vericiguat	Imp-1	Imp-2
1	Retention time	2.335	0.987	1.668
2	USP plate count	8786	6449	5913
3	USP tailing factor	0.98	1.14	1.02
4	USP resolution	5.78	6.37	6.37
5	Area	5452137	262991	25270

Imp-1: Impurity-1, Imp-2: Impurity-2

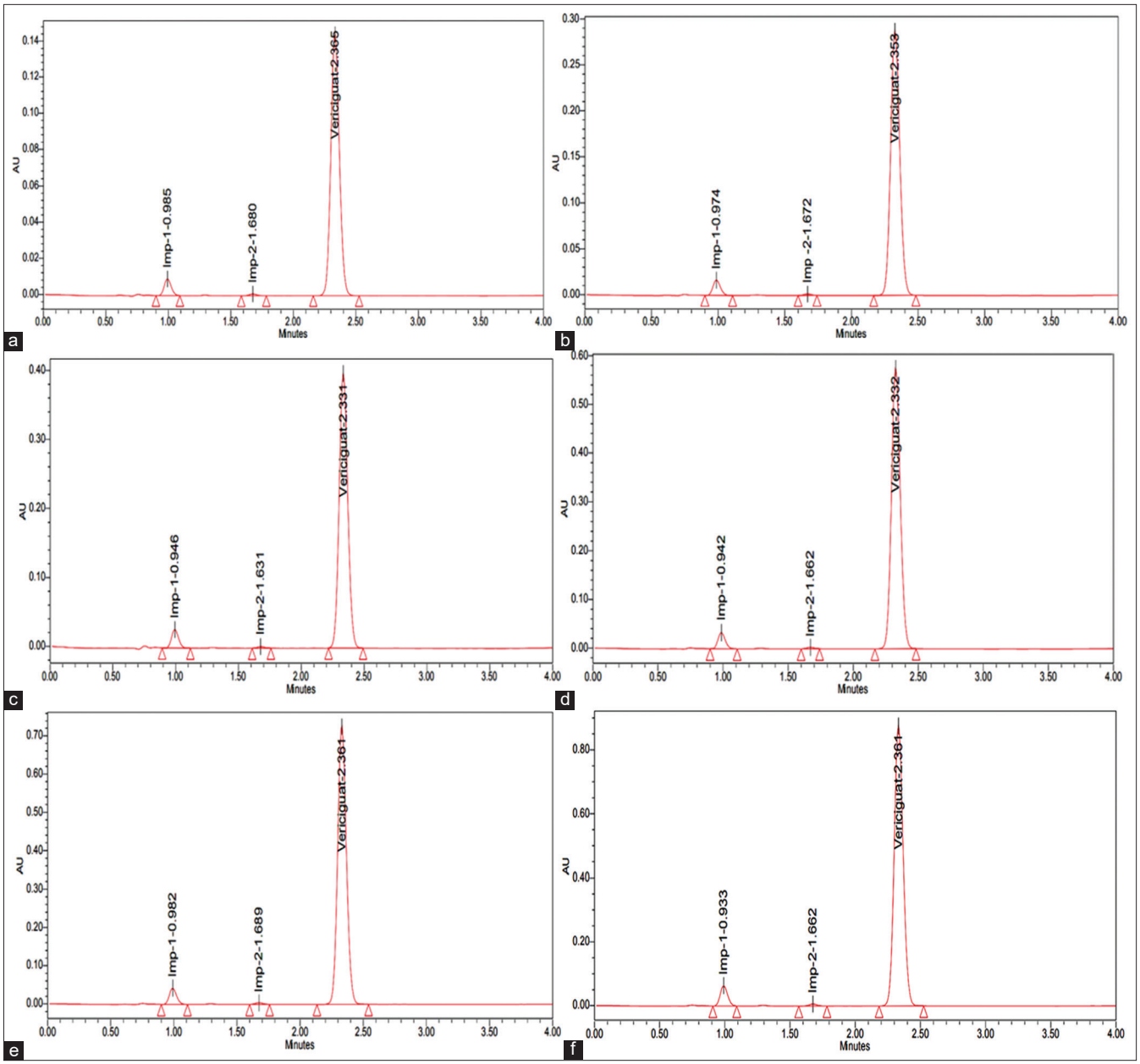

Figure 5: Linearity chromatograms of varicigut at (a) 37.50, (b) 75, (c) 112.50, (d) 150, (e) 187.50, and (f) 225 µg/mL

Precision

During the method and intermediate precision study, the % RSD of known impurities, single maximum, and total impurities was found to be <15% for six sample preparations [Tables 5 and 6].

Specificity and Selectivity

At the RT of known impurities and principle analyte peaks from blank and placebo solution, no interference was observed. All of the peaks were properly separated from one another.

LOD and LOQ determination

Vericagut, Imp-1, and Imp-2 had LOD and LOQ values of 0.45, 0.03, 0.03 µg/mL and 1.5, 0.1, and 0.1 µg/mL, respectively, results presented in [Table 7].

Robustness

We found during the robustness investigation that small changes in flow rate and organic phase composition had no effect on system appropriateness characteristics, including RT, RRT, and resolution. The developed method was only mildly sensitive to changes in isocratic composition results shown in [Tables 8 and 9].

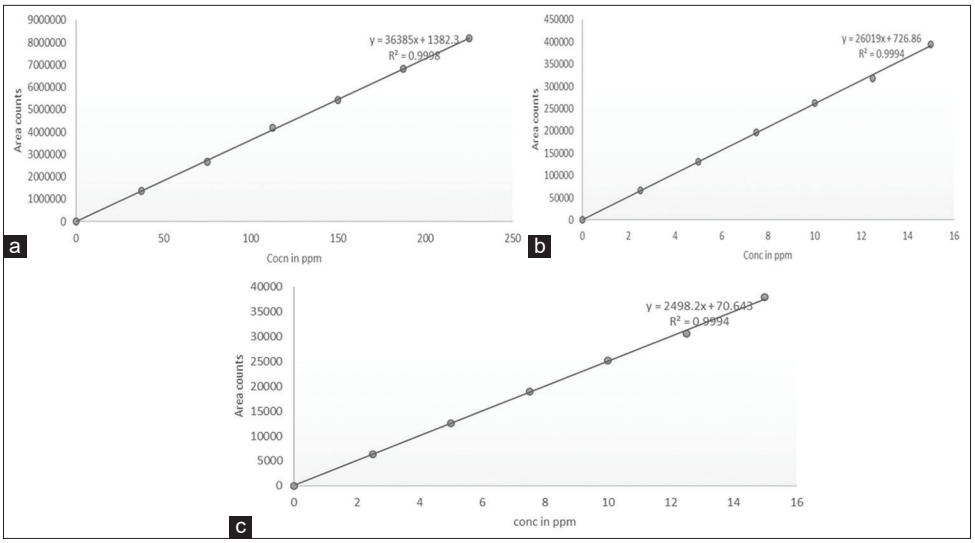

Figure 6: Calibration curve for (a) Vericiguat, (b) Impurity-1, and (c) Impurity-2

Table 3: Linearity parameters for Vericiguat, Imp-1 and Imp-2

Parameters	Vericiguat		Imp-1		Imp-2	
	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
	37.50	1363021	2.50	65747	2.50	6317
	75.00	2676055	5.00	131495	5.00	12635
	112.50	4189102	7.50	197243	7.50	18952
	150.00	5441068	10.00	263351	10.00	25254
	187.50	6815171	12.50	318738	12.50	30587
	225.00	8178205	15.00	394486	15.00	37905
Linearity range	37.5–225 µg/mL		2.5–15µg/mL		2.5–15 µg/mL	
Correlation coefficient	0.99988		0.99969		0.99968	
Regression equation	y=36384.69×+1382.29		y=26018.51×+726.86		y=2498.20×+70.64	
Slope	363849		26018.51		2498.20	
Intercept	1382.9		726.86		70.64	

Imp-1: Impurity-1, Imp-2: Impurity-2, Conc: Concentration

Table 4: Accuracy results for Vericiguat, Imp-1, and Imp-2

%Level	Analytes average area	% Recovery	% Relative standard deviation
50 Vericiguat	2688033	98.6	0.28
Imp-1	386978	100	0.50
Imp-2	323838	99.5.	1.55
100 Vericiguat	5451707	100	0.15
Imp-1	771463	99.7	0.02
Imp-2	652104	100.2	0.31
150 Vericiguat	8176832	100	0.15
Imp-1	1159786	99.9	1.32
Imp-2	967128	99.0	0.22

Imp-1: Impurity-1, Imp-2: Impurity-2

Table 5: System precision results for Vericiguat, Imp-1, and Imp-2

Parameters	Vericiguat	Imp-1	Imp-2
Mean	5452941	263175	25326
STD	5522.668	767.020	65.432
%RSD	0.10	0.29	0.26

RSD: Relative standard deviation, Imp-1: Impurity-1, Imp-2: Impurity-2

Table 6: Method precision results for Vericiguat, Imp-1, and Imp-2

Parameters	Vericiguat	Imp-1	Imp-2
Mean	5448818	263610	25355
STD	11371.644	887.381	55.791
%RSD	0.21	0.34	0.22

RSD: Relative standard deviation, Imp-1: Impurity-1, Imp-2: Impurity-2

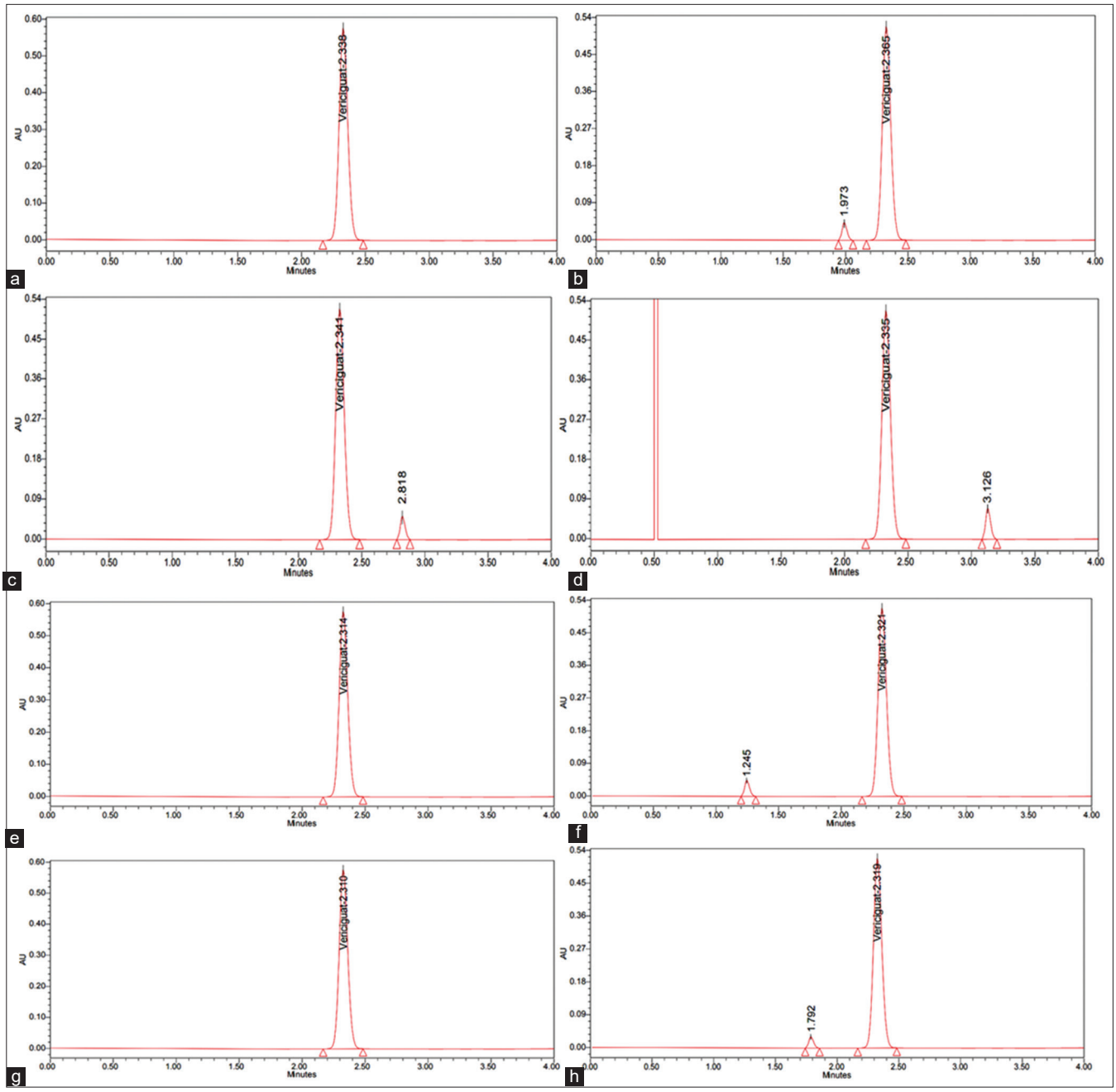

Figure 7: Degradation chromatograms of (a) control, (b) acid, (c) alkali, (d) peroxide, (e) hydrolysis, (f) reduction, (g) thermal, and (h) photolytic

Table 7: Limit of detection and limit of quantification

Sample	LOD (µg/mL)	LOQ (µg/mL)
Vericiguat	0.45	1.5
IMP-1	0.03	0.1
IMP-2	0.03	0.1

Imp-1: Impurity-1, Imp-2: Impurity-2, LOD: Limit of detection, LOQ: Limit of quantification

FD study

During the FD study, we found no significant degradation in acid (1%), alkali (3.5%), thermal (1.9%), or photolytic (1%). During oxidative stress, significant degradation (11.6%) was observed. We found no interfering peaks at the RT of the principle analyte peak and known impurities during the FD study. All of the peaks [Figures 7 and 8] were tested for peak purity index using a PDA detector and found to be pure. This demonstrated the stability of the developed method results are shown in [Table 10].

DISCUSSION

A simple, precise, accurate Imp profiling and stability studies are develop by the UPLC method. At present, only

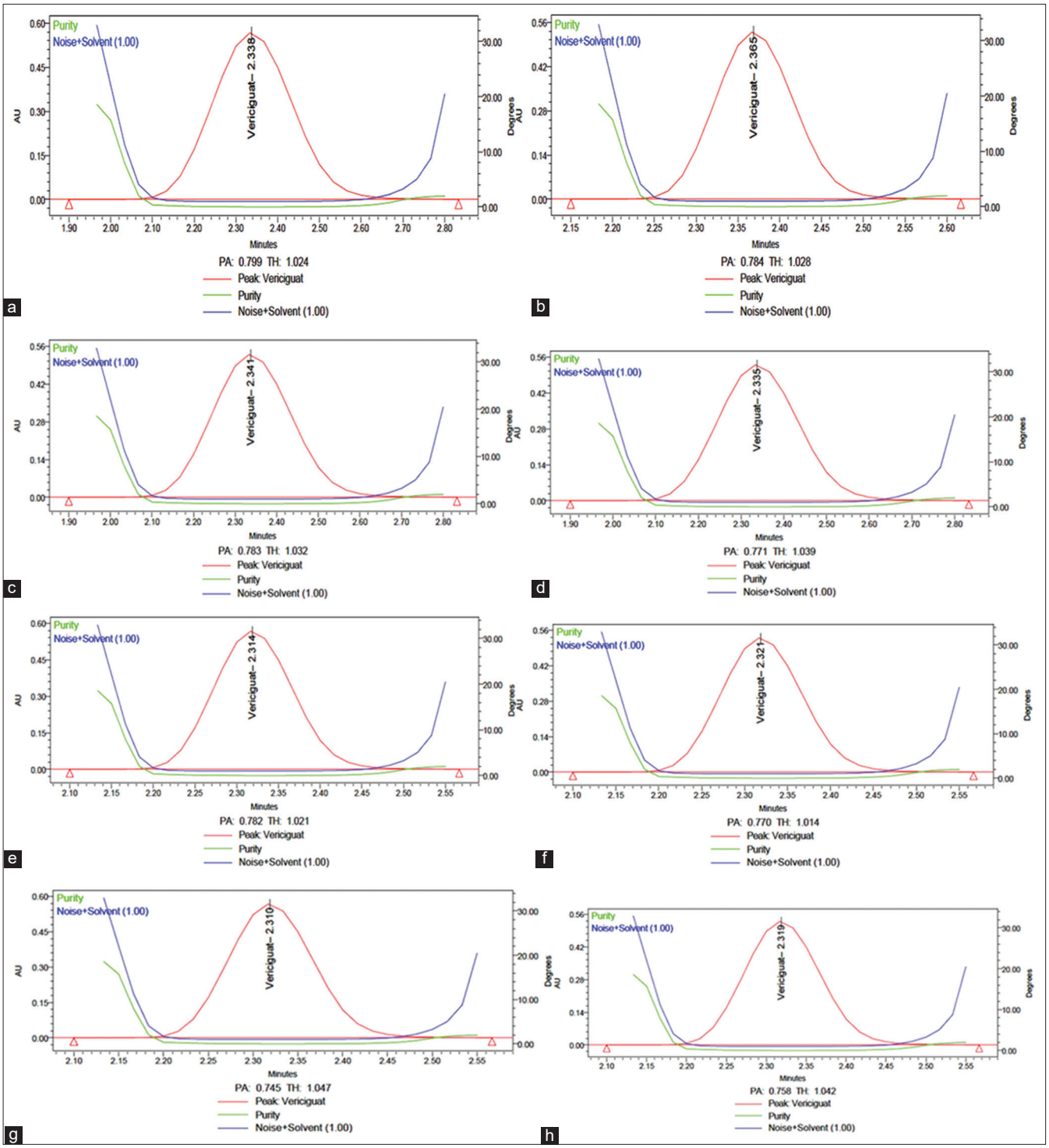

Figure 8: Purity plots of (a) control, (b) acid, (c) alkali, (d) peroxide, (e) hydrolysis, (f) reduction, (g) thermal, and (h) photolytic

pharmacokinetics and pharmacodynamics of Vericiguat in patients with heart failure and reduced ejection fraction was found but to the best of our knowledge, no reported stability-indicating UPLC method for estimating Vericiguat is available. Furthermore, there was no such effective analytical method reported that was effective in saving time and money. As analytical research, our primary goal was to create an analytical technique that is quick, precise, repeatable, and economical. The UPLC method was selected for the development of a stability-indicating technique for determining verification because GC and LC-MS are much more costly and fragile than UPLC. The developed method was validated as per the guidelines of the ICH.

Table 8: Robustness data of Vericiguat, Imp-1, Imp-2 (Effect of variation of flowrate)

S. No.	Flowrate (mL/min)	Peak area		
		Vericiguat	Imp-1	Imp-2
1	0.18	5641624	273641	27856
2	0.18	5629182	274056	27698
3	0.18	5653253	274480	27741
%RSD		0.21	0.15	0.29
1	0.22	5288749	241651	24512
2	0.22	5271365	242890	24620
3	0.22	5293431	242099	24698
%RSD		0.22	0.26	0.38

RSD: Relative standard deviation, Imp-1: Impurity-1, Imp-2: Impurity-2

Table 9: Robustness data of Vericiguat, Imp-1, Imp-2 (effect of variation in mobile phase composition)

S. No.	Composition	Peak area		
		Vericiguat	Imp-1	Imp-2
1	Organic Minus	5864122	295216	28869
2	Organic Minus	5841273	293120	28741
3	Organic Minus	5870314	293963	28652
%RSD		0.26	0.36	0.38
1	Organic Plus	5138693	232394	22216
2	Organic Plus	5122347	231627	22415
3	Organic Plus	5146820	233112	22507
%RSD		0.24	0.32	0.66

RSD: Relative standard deviation, Imp-1: Impurity-1, Imp-2: Impurity-2

Table 10: Results of forced degradation studies of Variciguat

Type of degradation	Purity angle	Purity threshold	Percentage degradation
Control	0.799	1.024	0
Acid	0.784	1.028	11.9
Alkali	0.783	1.032	13.1
Peroxide	0.771	1.039	15.2
Reduction	0.770	1.014	11.3
Hydrolysis	0.782	1.021	0.5
Photolytic	0.745	1.047	1.1
Thermal	0.758	1.042	10.8

CONCLUSION

A simple, sensitive, stability-indicating, and precise UPLC method for the estimation of Vericiguat and its impurities has been developed. The mobile phase and solvents are simple to prepare and economical. The proposed method is able to separate impurities with good resolution and with a short run time of 4 min. Stress studies concluded that Vericiguat undergoes degradation during the peroxide study. Validation was done using various validation parameters, such as system suitability, linearity, precision, specificity, accuracy, and robustness, which were found to be within the acceptance criteria. It is important to know the safety levels of impurities in a drug, by that the patient will get safe and effective medication. No hazardous chemicals were used in the analysis. Hence, the method is safe and eco-friendly. The present work concluded that the stability-indicating method by UPLC shows satisfactory and reproducible results without interference with the placebo and degradation products. Hence, these can be used for routine analysis of Vericiguat and its impurities.

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AUTHOR'S CONTRIBUTION

All the authors are equally contributed. All authors read and approved the manuscript.

AVAILABILITY OF DATA AND MATERIALS

All the data and materials are available on request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT OF PUBLICATION

Not applicable.

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