Journal of Applied Pharmaceutical Research
Volume 12 Issue 2, Year of Publication 2024, Page 57 – 67
DOI: 10.18231/j.joapr.2024.12.2.57.67

Check for updates

Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com *ISSN: 2348 – 0335*

DEVELOPMENT AND VALIDATION OF A QbD-BASED RP-HPLC METHOD FOR VERICIGUAT QUANTIFICATION

Shubham Mandhare, Rahul Godge*, Akshay Vikhe, Shubham Talole

Article Information
Received: 7th December 2023
Revised: 19th March 2024
Accepted: 8th April 2024
Published: 30th April 2024

Keywords
Quality by Design (QbD), RP High Performance Liquid Chromatography (RP-HPLC), Vericiguat, Central Composite Design (CCD)

ABSTRACT
Aim: An RP-HPLC method for Vericiguat using the QbD approach was developed and validated by ICH guidelines. **Method:** The ICH (Q2R1) guidelines have been followed in the development and validation of an RP-HPLC technique by considering several validation parameters like linearity, precision, LOD, LOQ, and accuracy. The study was performed on Agilent Tech using the C18 column (4.6x250 mm; 5 µm) and Chemstation 10.1 software with statistical data analysis, and the detector used was UV (DAD). **Results:** The mobile phase used for separation was Methanol: 0.1% OPA in the ratio of (76:24) at room temperature, the flow rate was 0.8ml/min, and the wavelength was 331nm. The results indicated that the quantification limit was 0.7209 µg/ml, and the detection limit was 0.2379 µg/ml. **Conclusion:** The validation studies confirmed that the developed method is fast, accurate, precise, cost-effective, selective, and useful for routine analysis of vericiguat in tablet dosage forms.

INTRODUCTION

Vericiguat is a new, orally soluble guanylate cyclase (sGC) drug used to treat heart failure while reducing hospitalization rates and improving ejection fraction [1-5]. Vericiguat relaxes smooth muscles by inducing vasodilation, thereby improving cardiac function.

More importantly, a comprehensive review of the available lite rature using the RP-HPLC method revealed a lack of specific methods for analyzing veriguat using this cell. This work aims to provide a reliable, accurate, and simple RP-HPLC technique for determining vericiguat dose forms. The procedure has been validated according to ICH guidelines [6-7]. Combining product specification, risk assessment, critical procedures (CPPs), and critical attributes (CQAs) to create a manufacturing environment is quality by design or QbD.

This comprehensive strategy aims to be well integrated into the drug development and review, leading to final drug approval & ongoing monitoring [8]. In the field of analysis this method is called Quality Analysis by Design (AQbD) [9-12]. Using QbD in the analysis process provides easy control by working in the design environment and following the rules of life. Therefore, QbD has attracted the attention of pharmaceutical companies and research centers [13-16]. High-performance liquid

*Department of Pharmaceutical Quality Assurance, Pravara Rural College of Pharmacy Pravaranagar, Tal-Rahata, District-Ahmednagar, Maharashtra, India

***For Correspondence:** **rahulgodge@gmail.com**

chromatography (HPLC) and reversed-phase high-performance liquid chromatography (RP-HPLC) are the pharmaceutical industry's most commonly used analytical methods. Quality has become critical, especially using the Quality by Design (QBD) approach.

Vericiguat is an oral soluble guanylate cyclase (sGC) stimulant jointly developed by Merck and Bayer. This medication is desi gned to treat heart failure, thereby reducing the cost of hospitali zation and improving ejection fraction.

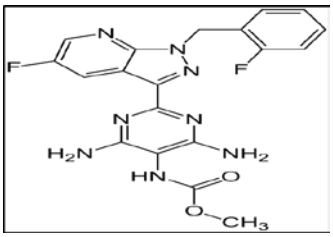


Figure 1 Structure of Vericiguat

MATERIAL AND METHODS

Materials

Chemicals and Reagents

Merck Ltd., Mumbai, supplied HPLC-grade methanol, and Thermo Fisher Scientific India Pvt Ltd, Mumbai, supplied HPLC water. Swapnaroop Drugs and Pharmaceuticals, Sambhajinagar, obtained Vericiguat's working standard.

Chromatographic conditions

The column used was C18 (250x ID 4.6 mm, particle size: 5µ), wavelength 331 nm, and ambient temperature. Methanol: 0.1% orthophosphoric acid (76:24) was used as the mobile phase with a 0.8 ml/min flow rate. The injection volume was 20 µl.

Method

Preparation of standard solutions

The 10 mg of vericiguat was accurately weighed and diluted to a 10 mL volumetric flask using methanol to produce 1000 µg/mL of standard solution of vericiguat. The stock solution was stored at room temperature until analysis. It was diluted with a suitable concentration to obtain a calibration curve.

Calibration curve

A working solution of vericiguat was prepared from the stock solution by an adequate dilution using Methanol: 0.1% OPA in the ratio of (76:24). Calibration standards were prepared by diluting this stock solution to obtain the concentration levels of 10,20,30,40, and 50µg/ml. Sonication was used to mix solids in the solution, which increases the dissolution rate and removes dissolved gasses from the liquid, resolving the peaks and showing moderate column back pressure [3].

Statistical Methods

Development of HPLC methods by using an analytical QbD Approach

Analytical Target Profile (ATP): This means creating a specific target or set of parameters that meet the needs of the analytical method. For instance, in the context of quantitative analysis of the Active Pharmaceutical Ingredient (API), these goals are defined to ensure accurate and precise determination. Therefore, the current research focused on the estimation of vericiguat in the given drug formulation [2,4].

Risk assessment

Critical process parameters (CPPs), regarded as independent factors, and critical quality attributes (CQAs), independent variables, were determined using a risk assessment. Critical quality attributes (CQAs) were determined for method characteristics such as retention time, peak asymmetry, resolution, theoretical plates, and area. Similarly, CPPs such as PH, wavelength, column, ratio of mobile phase, and flow rate wear were found to impact the method’s performance.

Experiment design and chromatographic conditions optimization

Vericiguat was scanned in a 200-400 nm UV range scan, and the highest absorption wavelength of Vericiguat was 331 nm. The elution was carried out at room temperature on a cromosil C18 column, exhibiting various or distinct peak shapes with various advantageous system suitability parameters. In HPLC, a UV detector was used to determine vericiguat. Three primary factors were considered in the optimization process. The mobile phase composition, the wavelength, and the flow rate. These were resolved using the quality by the Quality by Design (QBD) methodology or technique. The Design of Experiments (DOE) strategy was followed in the execution of five chromatographic runs, with a design expert selected based on the information provided by specialist software. The area, retention time, peak asymmetry, and theoretical plate categories were used to record the responses. After that, the software’s indication of attractiveness was used to choose the ideal chromatography condition [3].

Design software

The experiment's design was planned using Design Expert software 13 and the central composite design method.

Method validation

The ICH Q2 (R1) guideline was followed during validation to verify the methods' intended use. A number of criteria were evaluated, such as assay, robustness, accuracy, linearity, precision, percentage Recovery, limit of detection (LOD), and limit of quantification (LOQ) [8].

Linearity

The working solutions were prepared from a standard stock solution of 1000 µg/ml by diluting 1-5 ml of vertiguat in a 10 ml volumetric flask using mobile phase to give a concentration of 10 to 50 µg/ml. The graph was plotted by concentration against the relevant peak regions to create a calibration curve.

Precision

A working solution of concentrations 20, 30, and 40 µg/ml was prepared and tested for both intraday and interday precision. Intraday precision was performed in morning and evening sessions, duplicates of the solutions were injected and results were interpreted. The interday precision study was performed for the two distinct days. The resulting areas and percentage of relative standard deviation (RSD) were calculated for every series of experiments.

Accuracy

By ICH guidelines, the accuracy studies were performed at three different accuracy levels: 80%, 100%, and 120%. The resulting areas and percentage of relative standard deviation (RSD) were calculated for every series of experiments.

Robustness

The method robustness was evaluated by deliberately introducing minor variations in key parameters, specifically the wavelength, mobile phase and flow rate. The dependability of the method was verified through a thorough assessment of the robustness study.

LOD AND LOQ

The limit of detection (LOD) is defined as the smallest amount of the analyte in the sample that can be detected, even if it cannot be identified. When quantification is possible, this threshold is referred to as the limit of quantification (LOQ).

ASSAY

To determine concentration of vertiguat, 20 tablets were weighed, their mean weight was determined and finely powdered. The weight of triturated tablet equivalent to 50 mg of vertiguat was transferred into a 50 mL volumetric flask containing 30 mL methanol, sonicated for 30 min and diluted up to 50 mL with methanol to give 1000 µg/ml concentration. The sample solution was filtered using 0.45-µm filter (Millipore, Milford, MA). The solution was further diluted to give final concentration of 10 µg/ml. A 20 µl of sample solution was injected to HPLC under optimum conditions of temperature. The peak areas were measured at 331 nm. A placebo study found no interference of excipients like lubricants, glidant, or binders while performing analytical studies by RP-HPLC [3,8].

RESULTS AND DISCUSSION

Optimization of HPLC method by analytical QbD approach:

Experiments were conducted to optimize the composition of the mobile phase by adjusting the ratio of the methanol to 0.1% OPA at various proportions, including 80:20, 70:30, 60:40, and 75:25. In the initial chromatographic conditions, they applied the outlined in Table 1. The levels selected for the Faced Centered Central Composite Design (CCD) and the corresponding layout for 8 Quality by Design trials are presented in Tables 2 and 3, respectively.

Statistical Analysis of Method responces for peak asymmetry

The Study employed statistical analysis through the use of analysis of variance (ANOVA) to evaluate the impact of different variables and their interactions on the peak asymmetry response. Specific levels in CCD were chosen based on process factors and by determining faced centre values by selecting responses and conducting experiments to which ANOVA is applied. This optimization process involves adjusting the levels of the predictor variables in order to maximize or minimize the response variable, depending on the specific objective. To optimize the proposed method for retention time, the first step would be to define the desired target value for retention time. This could be a specific value that meets the requirements of the experiment or a range within which the retention time should fall. Next, the various experimental factors that can affect

retention time will be identified. These factors could include temperature, pressure, flow rate, column length, solvent composition, and other relevant variables. Optimized chromatographic conditions using the AQbD approach are shown in Table 5, and ANOVA for the quadratic model is shown in Table 6. The mobile phase's flow velocity and percentage composition are the independent variables selected from the Centered Composite Design (CCD). The chromatogram is Vericiguat optimized, is given in Figure 2

Table 1: Initial Chromatographic Method Development Conditions (HPLC)

Trial No.	**Wavelength (nm)**	**Mobile Phase Composition (%Methanol: 0.1%OPA)**	**pH of Mobile Phase**	**Sample Volume (µl)**	**Flow rate (ml/min)**	**Run time (min)**
1	331	80:20	3.0	20	0.8	8.22
2	331	70:30	3.0	20	0.8	8.99
3	331	60:40	3.0	20	0.8	8.10
4	331	75:25	3.0	20	0.8	7.99
5	256	75:25	3.0	20	0.8	8.33

Table 2 Faced Centered Central Composite Design (CCD)

	Name	**Units**	**Low**	**High**
A	Methanol	%	75	77
B	Flow rate	ml/min	0.7	0.9

Table 3 Design of experiment showing factors and responses for 8 QbD trials using CCD

	Factor 1	**Factor 2**	**Response 1**	**Response 2**	**Response 3**	**Response 4**
Run	**A: Composition of mobile phase (Methanol)**	**B: Flow Rate**	**Retention Time (RT)**	**Area**	**Theoretical Plates (N)**	**Telling Factor/ Asymmetry Factor**
	%	**ml/min**	**min**	**(Area Unit)**	**(Units)**	**(Units)**
1	76	0.9	3.163	1116.4763	6520	0.71
2	77	0.8	3.512	1146.1781	7761	0.73
3	75	0.7	4.223	1421.2661	7281	0.67
4	75	0.8	3.693	1288.7880	6430	0.70
5	77	0.7	4.033	1454.8691	7232	0.68
6	77	0.9	3.136	1158.0546	6406	0.71
7	75	0.9	3.298	1146.6871	5988	0.70
8	76	0.7	4.092	1469.6793	7032	0.67

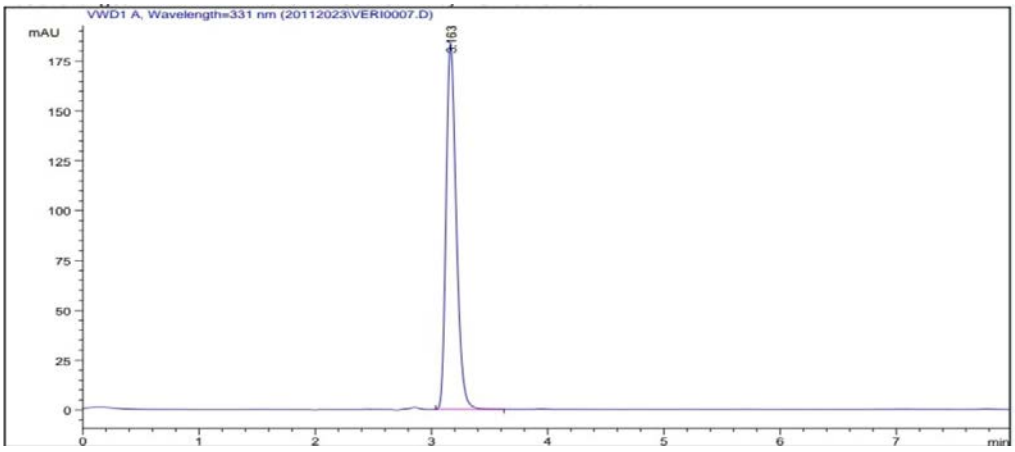


Figure 2 Chromatogram for optimized condition of Vericiguat

Table 4 Suggested optimized chromatographic condition by AQbD approach

Flow rate (ml/min)	% Composition (methanol:0.1% OPA)	Wavelength (nm)
0.8	76:24	331

Table 5 ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.32	5	0.2632	4525.32	0.0002	significant
A-Methanol	0.0473	1	0.0473	814.01	0.0012	
B-Flow rate	1.26	1	1.26	21684.82	< 0.0001	
AB	0.0002	1	0.0002	3.37	0.2078	
A^2	0.0027	1	0.0027	46.42	0.0209	
B^2	0.0065	1	0.0065	112.32	0.0088	
Residual	0.0001	2	0.0001			
Cor Total	1.32	7				

Results of an analysis of variance (ANOVA) are shown in Table 5, where the A Shows factor is significant (p = 0.0012), suggesting that the level of A-methanol significantly impacts the response variable. B- Flow This factor is highly significant (p < 0.0001), indicating that the flow rate of factor B significantly affects the response variable. Interaction between A and B- The interaction between factors A and B is insignificant (p = 0.2078), suggesting no significant interaction effect between these two factors. A^2- The quadratic effect of factor A is significant (p = 0.0209), indicating a non-linear relationship between factor A and the response variable. B^2- Similarly, the quadratic effect of factor B is significant (p = 0.0088), indicating a non-linear relationship between factor B and the response variable.

Factor coding is **coded.** Sum of squares is **Type III – Partial**

The **Model F-value** of 4525.32 implies the model is significant, **P-values** less than 0.0500 indicate model terms are significant.

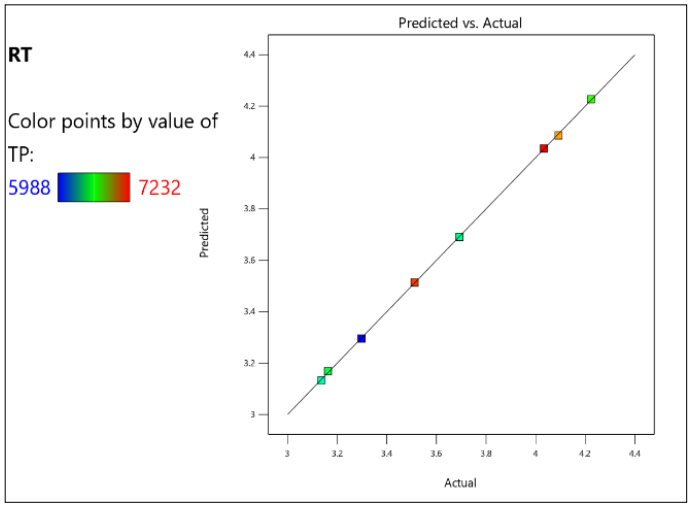


Figure 3(A): Plot of Predicted vs. Actual data retention time of Vericiguat

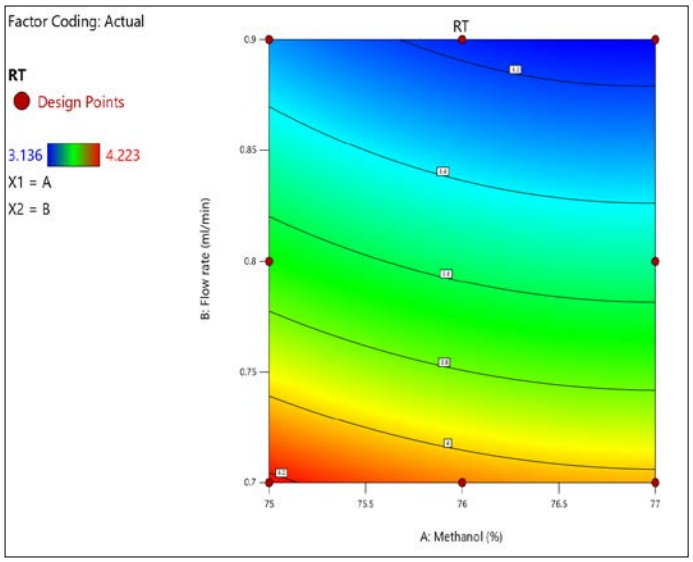


Figure 3(B): Counter plot depicting the impact of methanol and flow rate on retention time

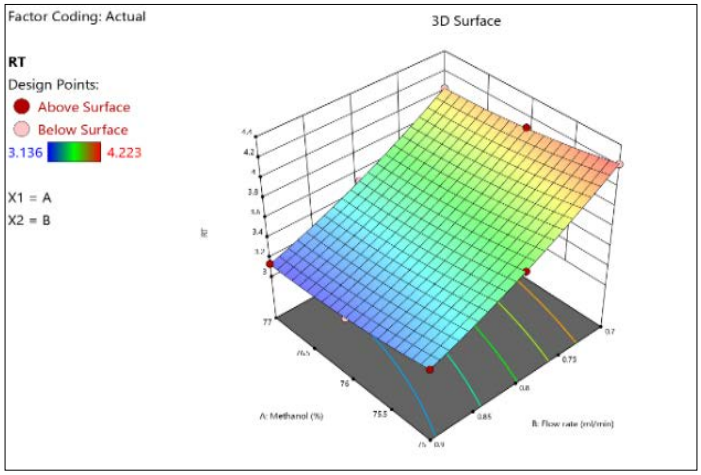


Figure 3(C): 3D Response Surface of the Retention time.

ANOVA for Linear model

Figure 3(A) shows the varying concentrations of methanol in percentage and the predicted versus actual results. 3(B) shows the impact of changing flow rate on methanol concentration, and 3 (C) is used to determine the influence of flow rate, Wavelength, and mobile phase composition on dependent variables. Table 6 shows ANOVA for Linear model Response 2: PA.

Table 6: ANOVA for Linear model Response 2: PA

Source	Sum of squares	df	Mean Square	F-Value	p-value	
Model	1.441E+05	2	72034.35	18.35	0.0050	Significant
A-Methanol	1588.91	1	1588.91	0.4047	0.5527	
B-Flow Rate	1.425E+05	1	1.425E+05	36.29	0.0018	
Residual	19632.89	5	3926.58			
Core Total	1.637E+05	7				

Factor coding is **coded**. Sum of squares is **Type III - Partial**

Figure 4A shows a Plot of Residual vs. run number peak area of vericiguat, which gives significant flow rate; Figure 4(B) shows a Plot of Predicted vs. Actual data peak area of vericiguat; Figure 4 (C) shows a Counter plot depicting the impact of Methanol and Flow rate on peak area; and Figure 4(D) shows the 3D Response Surface of the peak area.

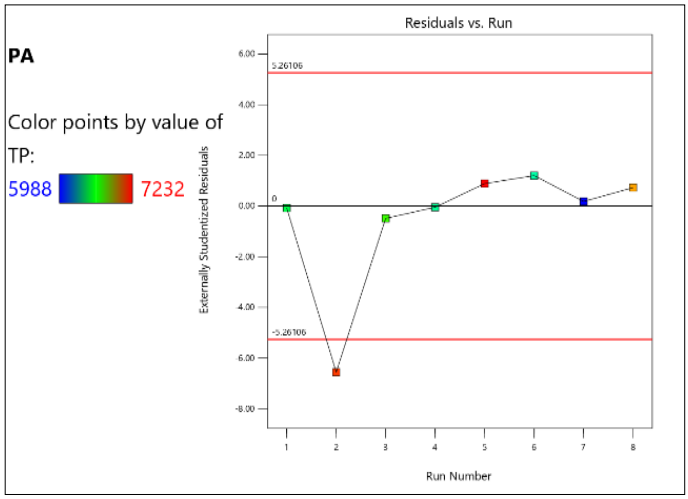
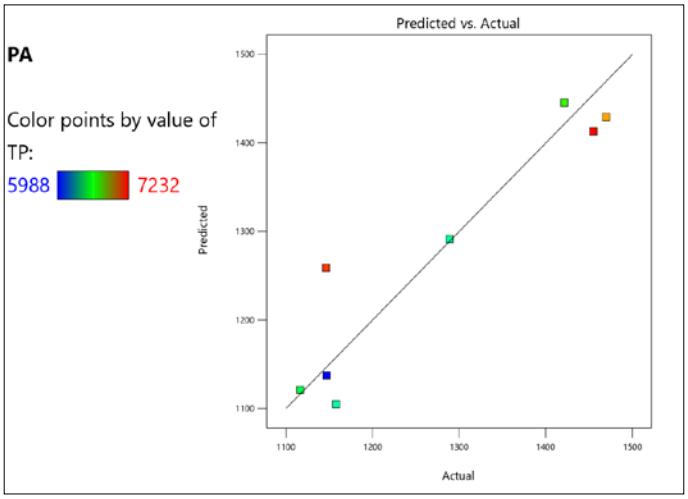
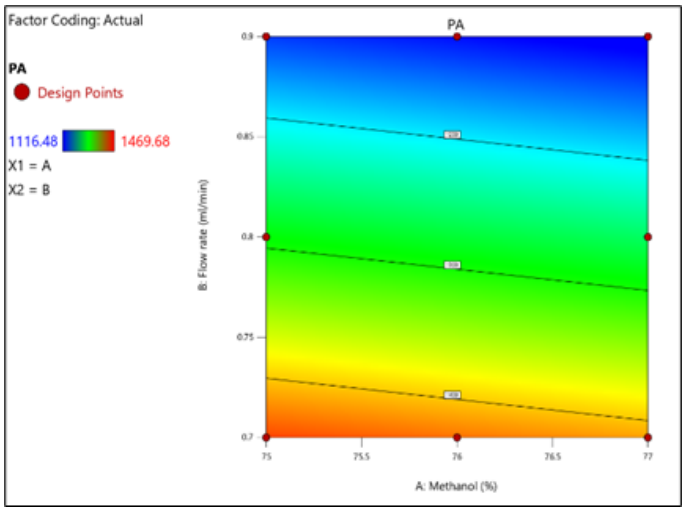


Figure 4(A): Plot of Residual vs run number peak area of vericiguat Figure



4(B): Plot of Predicted vs. Actual data peak area of vericiguat



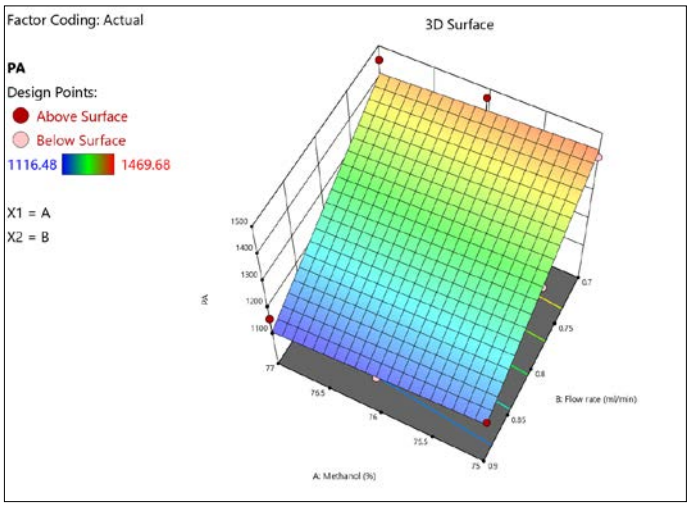


Figure 4 (C): Counter plot depicting the impact of methanol and flow rate on peak area

Figure 4(D): 3D Response Surface of the peak area

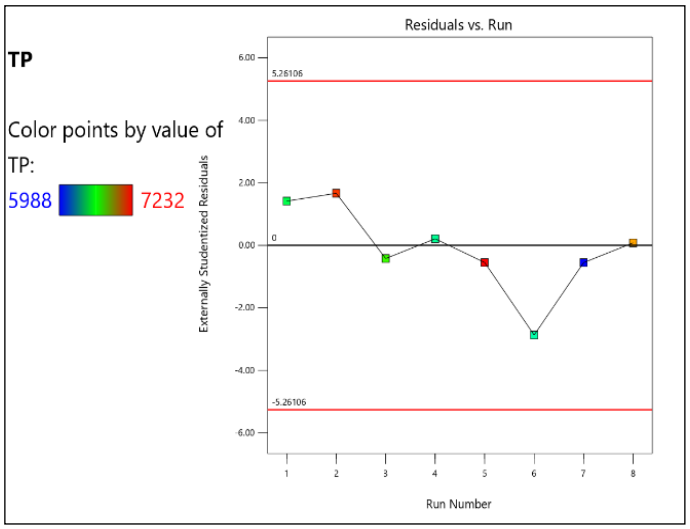
Table 7. ANOVA for Linear model Response 3: TP

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.169E+06	2	5.846E+05	22.00	0.0033	significant
A-Methanol	4.817E+05	1	4.817E+05	18.13	0.0080	
B-Flow rate	6.875E+05	1	6.875E+05	25.88	0.0038	
Residual	1.328E+05	5	26567.47			
Cor Total	1.302E+06	7				

Factor coding is **coded.**

Sum of squares is **Type III - Partial**

The sample F value of 22.00 shows that the model is significant. Table 6 shows ANOVA for the linear model with Response 3 TP. Figure 5(A) shows the Plot of Residual vs run number Theoretical plates of vericiguat, figure 5(B) shows the Plot of Predicted vs. Actual data theoretical plates of vericiguat, Figure 5(C) shows a Counter plot depicting the impact of Methanol and Flow rate on Theoretical plates and figure 5(D) 3D Response Surface of the Theoretical plates.



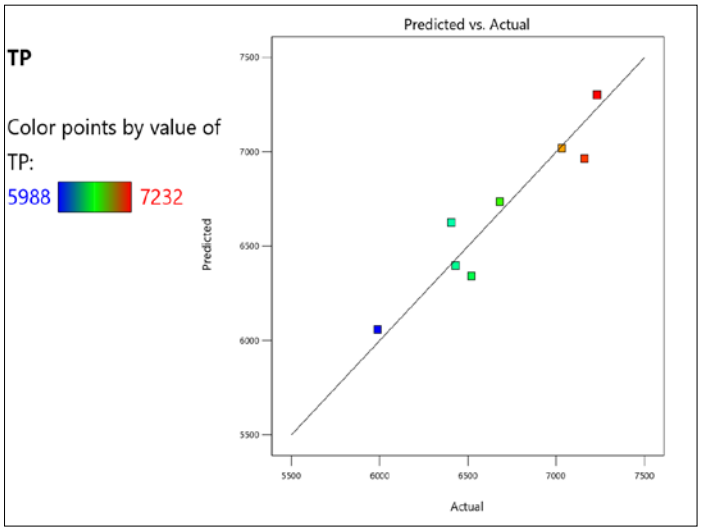
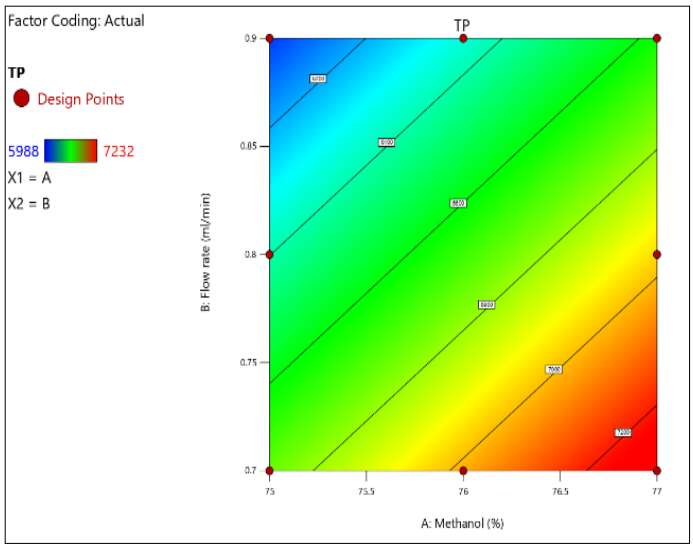


Figure 5(A): Plot of Residual vs run number Theoretical plates of vericiguat

Figure 5(B): Plot of Predicted vs. Actual data Theoretical plates of vericiguat



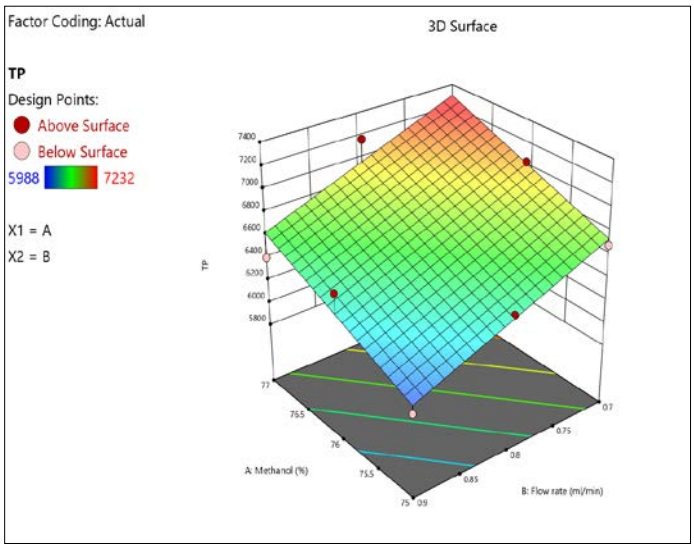


Figure 5(C): Counter plot depicting the impact of Methanol and Flow rate on Theoretical plates

Figure 5(D): 3D Response Surface of the Theoretical plates.

Table 8: ANOVA for Linear model

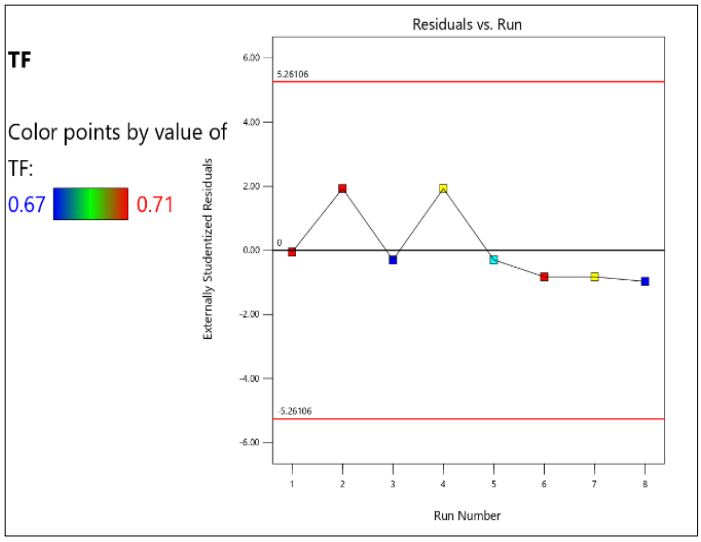
Response 4: TF

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0018	2	2.46E+05	12.25	0.0118	significant
A-Methanol	0.0001	1	1.32E+05	2.02	0.2143	
B-Flow rate	0.0017	1	6.123E+05	22.47	0.0051	
Residual	0.0004	5	12137.21			
Cor Total	0.0022	7				

Factor coding is **coded.**

Sum of squares is **Type III - Partial**

The **Model F-value** of 12.25 implies that the model is significant. Table 8 shows ANOVA of the linear model with 4T response, and figures 6A and 6B show a Plot of residual and predicted versus a tailing factor of vericiguat, and 6C shows a Counter plot depicting the impact of Methanol and Flow rate on tailing factor and 6 D shows 3D Response Surface of the tailing factor



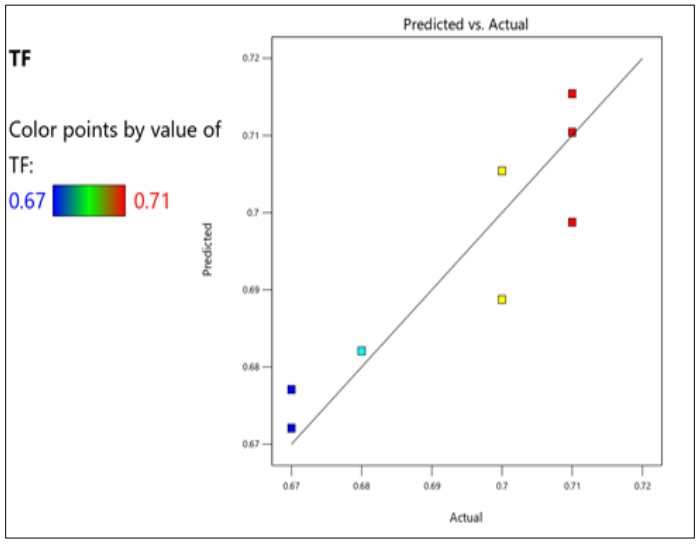
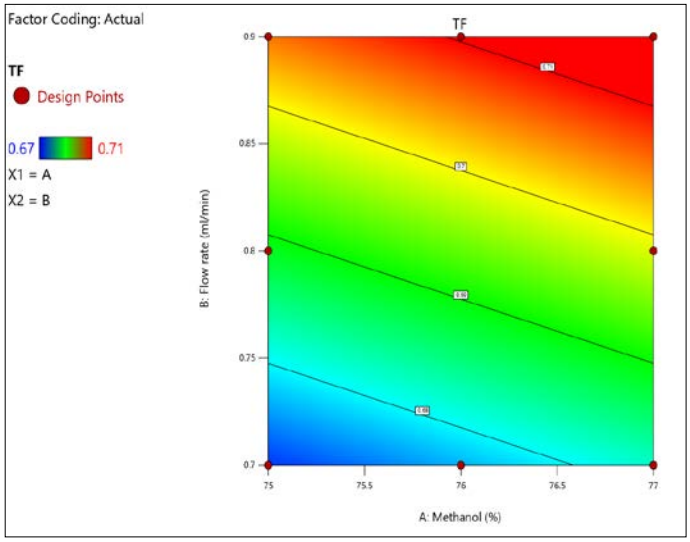


Figure 6(A): Plot of Residual vs run number tailing Factor of vericiguat

Figure 6(B): Plot of Predicted vs. Actual data tailing factor of vericiguat



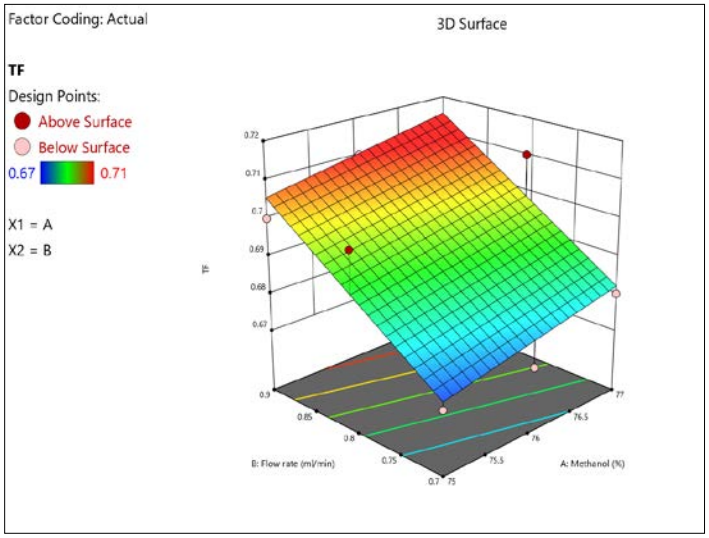


Figure 6(C): Counter plot depicting the impact of Methanol and Flow rate on tailing factor

Figure 6(D): 3D Response Surface of the tailing factor

In response surface plot is used to study dependent variable parameters like retention time, theoretical plates, peak area and tailing factor with independent variable parameters like flow rate, mobile phase and pH. In the present study response surface is above the planner which shows independent variables are producing significant results which optimized the method.

Method Validation

Linearity

Based on the findings from the linearity studies, Calibration curve is constructed by its plotting the drug concentration verses the corresponding area, as illustrated in Figure 7. The resulting line was determined to be a straight line, demonstrating high linearity with a correlation coefficient (r^2) of 0.9999 where there were no deviations. Equation of line is y = mx + c, was derived, with m representing the slope and c representing the y-intercept. The stated range of 10-50 µg/ml was founded to be consistent with the recognized linear association is considered appropriate for the quantitative determination of vericiguat Concentration.

Precision

The result of the intraday and interday precision tests are shown in the table 5. For the given readings, the mean area, standard deviation (SD), and relative standard deviation (%RSD) is to be calculated. The precision of the results was within acceptable bounds, with % RSD values continuously falling below 2%. This conformance proves the accuracy of the procedure. Table 9 shows Intraday and Interday precision studies of tablet formulation.

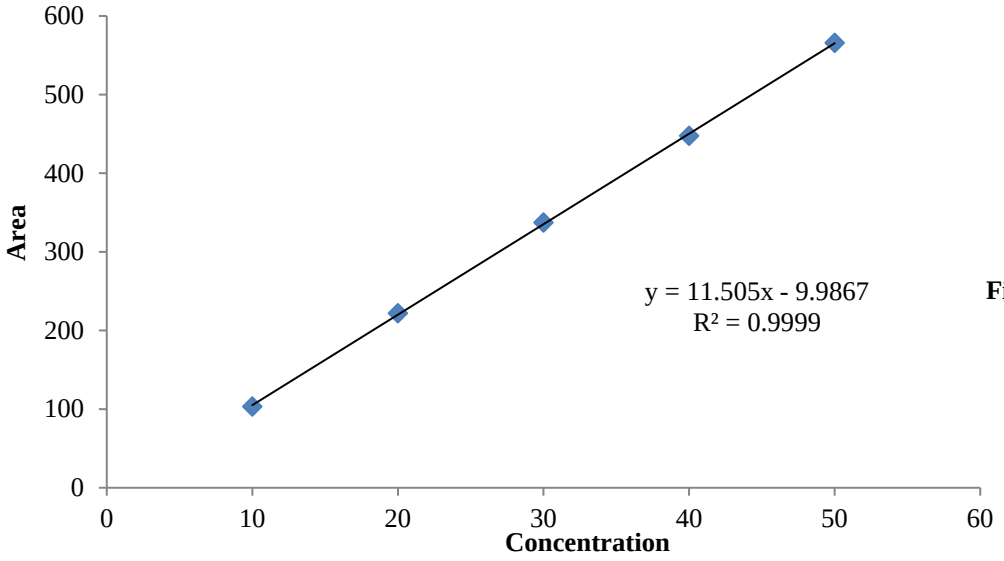


Figure 7: Linearity of Vericiguat

Table 9: Intraday and Interday precision

Concentration (µg/ml)	Intraday Precision		Interday Precision	
	Mean Area ± SD	% RSD	Mean Area ± SD	% RSD
20	223.91+0.87	0.39	221.72+1.06	0.48
30	335.47+1.81	0.54	335.96+0.22	0.07
40	448.35+2.17	0.48	448.37+1.33	0.30

Accuracy: Accuracy studies were conducted at three levels: 80,100 and 120%. The results of accuracy studies

Table 10: Accuracy studies. Test was passed with specification RSD < 2 %.

% Level	Concentration (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	Mean% recovery
80%	8	197.8268	8.07	100.88	101.24%
	8	198.4754	8.13	101.59	
100%	10	220.9915	10.09	100.85	100.31%
	10	219.7560	9.98	99.78	

Table 10: Results of robustness studies

Parameter Varied	Concentration (µg/ml)	Area	Mean	SD	%RSD
Wavelength (nm)					
330	40	443.3965	443.5	0.20	0.05
	40	443.6857			
332	40	433.0657	433.04	0.04	0.01
	40	433.0149			
Mobile Phase (mL)					
77+23	40	449.6707	449.9	0.38	0.09
	40	450.2132			
75+25	40	449.3774	449.76	0.55	0.12
	40	450.1497			

Robustness

Table 8 outlines the findings from the robustness studies. Despite minor differences in the experimental parameters, the observed % RSD values below the limit of 2% suggest that the method was not significantly affected. Table 11 shows the results of robustness studies by varying wavelength and mobile phase.

LOD and LOQ

The Limit of Detection (LOD) was calculated to be 0.2379 µg/ml, and the Limit of Quantitation (LOQ) was determined to be 0.7209 µg/ml.

CONCLUSION

In the present study, a simple, rapid, and sensitive QbD- based RP-HPLC Method for Vericiguat in tablet formulation was developed by using a Mobile phase was Methanol: 0.1% OPA in the ratio of (76:24), flow rate of 0.8ml/min and the retention time obtained was 3.163. Whereas per literature mobile phase used for method development is OPA: Acetonitrile in ratio of 40:60%v/v, the flow rate was set 1.0ml/min and Retention time obtained was 4.23 min. When compared with literature Methanol is cheaper solvent, flow rate was less than 1 ml/min and retention time is also less and Validation parameters as per ICH guidelines are more accurate in terms of linearity, precision, accuracy, LOD and LOQ compared to literature which confirms developed method is more accurate, precise and cost effective and it is suitable for routine analysis of vericiguat in tablet dosage forms.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

Rahul Godge and Shubham Mandhare contributed experimentally. Rahul Godge and Shubham Talole prepared and analyzed the sample results. Akshay Vikhe prepared the manuscript draft, on which all authors commented. All authors equally reviewed the final manuscript.

REFERENCES

[1] Alhakeem M, Ghica M, Pîrvu C. Analytical quality by design with the lifecycle approach: A modern epitome for analytical method development. *Acta Marisiensis – Seria*, **65(2)**, 37 – 44 (2019)

[2] Dewi MK, Pratama R, Arifka M, Chaerunisaa A. Quality by Design: Approach to Analytical Method Validation. *Sciences of Pharmacy*, **1(1)**, 33-40 (2022).

[3] Foram P, Yadav P. Stability Indicating HPLC Method Development and Validation of Vericiguat in tablet dosage form. *World Journal of Pharmacy and Pharmaceutical Sciences*, **12(7)**, 420-429 (2023).

[4] Joshi NA, Amrutkar SV, Upasani CD. Analytical Quality-by-Design Approach to Stability Indicating RP-HPLC Method Development and Validation for Estimation of Favipiravir in Bulk and Formulation. *European Chemical Bulletin*, **12(9)**, 118-133(2023).

[5] Kang C, Lamb Y. Vericiguat: A review in chronic heart failure with reduced ejection fraction. *American Journal of Cardiovascular Drugs*, **22(4)**, 451-459 (2022).

[6] Kumar L. Quality-by-design driven analytical method (AQbD) development and validation of HPLC-UV

technique to quantify rivastigmine hydrogen tartrate in lipidic nanocarriers: Forced degradation, and assessment of drug content and in vitro release studies. *Microchemical Journal*, **193**, 108944 (2023).

[7] Mahapatra A, Meyyanathan S. Approach of analytical quality by design and regulatory need. *International Journal of Health Sciences*, **6(S5)**, 2572–2592 (2022).

[8] Pravalika P, Jephthah G. A new RP-HPLC method for estimation of vericiguat in bulk drug and pharmaceutical dosage form. *Journal of Advanced Scientific Research*, **14(09)**, 37-43 (2023).

[9] Mandpe SR, Parate VR. Method optimization and analysis of flurbiprofen loaded Eudragit L100 nanoparticles using RP-HPLC technique: A central composite design approach. *Materials Today: Proceedings*, **45(6)**, 4777-4786 (2021).

[10] Mandpe SR, Parate VR. Experimental design approach, screening and optimization of system variables, analytical method development of flurbiprofen in nanoparticles and stability- indicating methods for HPLC. *Futur J Pharm Sci.*, **8**, 38 (2022).

[11] Nagar P, Garg M, Chauhan C. Analytical Quality by Design (AQBD) Approach for HPLC method development, method optimization and validation. *Quality Assurance*, **13(2)**, 103-110 (2022).

[12] Ramalingam P. QbD considerations for analytical development. *In Pharmaceutical Quality by Design*, 77-108 (2019).

[13] Sawarkar KT, Asnani AJ. Quality by Design (QbD): Building and Enhancing Quality of Pharmaceuticals. *International Journal of Pharmaceutical Research and Applications*, **7(3)**, 1738-1748 (2022).

[14] Urmi KF, Nawaz M, Islam SM. Analytical quality by design approach to RP-HPLC method development and validation for simultaneous estimation of esomeprazole and naproxen in modified-release dosage form. *Future Journal of Pharmaceutical Sciences*, **8(1)**, 1-16 (2022).

[15] Volta e Sousa L, Gonçalves R. Analytical method lifecycle management in pharmaceutical industry: A review. *AAPS Pharmscitech*, **22**, 1-14 (2021).

[16] Varnekar DS, Sanjay NT. Quality by Design Approach-To Analytical Method Validation. *International Journal for Multidisciplinary Research*, **5(2)**, 1-8 (2023).