WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.632
Volume 12, Issue 4, 1619-1627 Research Article ISSN 2278 – 4357

DEVELOPMENT AND VALIDATION OF NOVEL DRUG-VERICIGUAT QUANTIFIED USING RP-HPLC-PDA METHOD

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Article Received on
03 February 2023,

Revised on 24 Feb. 2023,
Accepted on 14 March 2023

DOI: 10.20959/wjpps20234-24509

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ABSTRACT

A novel, rapid, and economical RP-HPLC method was developed for the determination of Vericiguat in API (Active Pharmaceutical Ingredient). The developed method was validated as per the ICH guidelines. An Inertsil - ODS C18 (250 x 4.6 mm, 5µ) column at ambient temperature and an eluent system with a Methanol: Acetonitrile (60:40) ratio were used for chromatographic separation. This separation condition showed symmetrical peaks with high resolution at a wavelength of 225 nm using PDA detection at a 1.0 ml/min flow rate and injection volume at 20 µL. Vericiguat showed linearity for the range of 20-70µg/mL with a correlation of determination R^2=0.9999 and curve fitting (y=52296x + 6339). The accuracy was determined by recovery studies and was found in the range of 99.92-100.53% the results were within the acceptable limits for the method and the intermediate precision. RSD% of the determination of precision was ≤ 2%. The results for LOD and LOQ were 0.0047µg/mL and 0.01429 µg/mL respectively. The proposed method showed the results of system suitability, robustness, and ruggedness were within acceptable limits. It is noteworthy to state that so far, the literature survey revealed no analytical report on vericiguat. Hence reporting the first method for the development and validation of vericiguat using the RP-HPLC method.

KEYWORDS: Acetonitrile, PDA detection, RP-HPLC, Vericiguat.

1. INTRODUCTION

Vericiguat is a novel oral soluble guanylate cyclase (sGC) stimulant developed by Merck & Co., Bayer AG to treat chronic heart failure, and lowers hospitalization rate and ejection fraction.[1-2] Vericiguat is chemically known as methyl {4,6-diamino-2-[5-fluoro-1-(2-fluorobenzyl)-1Hpyrazolo[3,4-b]pyridin-3-yl]pyrimidin-5yl}carbamate (figure-1), its molecular formula is $C_{19}H_{16}F_2N_8O_2$ with a molecular mass of 426.39 g/mol.[3]

Riociguat, the first sGC stimulator, was licensed for treating pulmonary hypertension in 2013. Later, the structure was changed to considerably lessen its oxidative metabolic susceptibility, leading to the development of the drug vericiguat. The FDA approved the commercial version of Verquvo® in January 2021 for use in a subpopulation of patients with systolic heart failure. The European Medicines Agency concluded that Verquvo demonstrated better benefits than its risk and is approved for use within the EU.[4-5]

Vericiguat stimulates the production of sGC and cGMP without the need for NO, which enhances the effects of nitric oxide (NO) by stabilizing the NO-sGC connection. Therefore, cause vasodilation, which improves heart function by causing smooth muscle relaxation.[5-7]

It is spurring to learn that to a thorough assessment of the literature, there isn't any pharmacopeial record for vericiguat and not a single analytical method published for its quantification and validation. Hence, necessary to develop an RP-HPLC method for the rapid, precise, and accurate quantification of vericiguat.

Therefore, the current work aims to develop a new vericiguat method using the RP-HPLC methodology. The conformity with ICH recommendations serves as simultaneous validation of the current procedure.

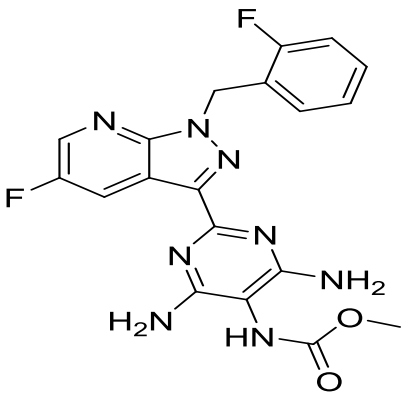

Fig No 1: Structure of Vericiguat.

2. MATERIALS AND METHOD

2.1 Material and reagent

Vericiguat (API) working standard was procured as a gift sample from Metrochem API Pvt Ltd. And the other chemicals used are Methanol (HPLC grade) and Buffer (KH_2PO_4), and all other chemical used.

2.2 Chromatographic Analysis

The analysis of the drug was done by using HPLC–Waters Model NO.2690/5 series Equipped with Reverse Phase (Inertsil) C18 ODS column (250x4.6mm; 5µm), a 20µL injection loop, PDA (Photodiode Array) detector and running Empower.2 software. Electronic balance (Sartorious) and sonicator (Fast Clean) were used. The wavelength (λ max) with the PDA detector selected for vericiguat was 225nm. The mobile phase of methanol: acetonitrile (60:40) V/V was filtered through 0.45-micron filter paper were used.

2.3 Stock Solution Preparation: The standard stock solution (1000 µg/mL. solution) was prepared by sonicating dissolving 100 mg of vericiguat in 100 ml of methanol for 30 minutes. From the above solution, taken 100ml and diluted with methanol and make up the volume to 100ml (100 µg/mL) and sonicate it for 10 minutes.

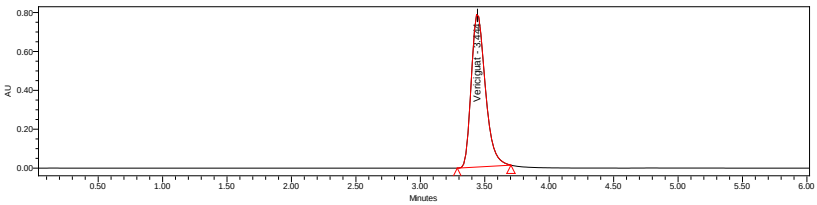

Fig No.2: Standard chromatogram.

2.8 Method validation

According to ICH criteria, the optimal method for vericiguat estimate has been validated. Key analytical parameters were validated more consistently by using chromatographic procedures in analytical applications.

(a) System suitability (b) Recovery (c) Linearity (d) Sensitivity (e) Accuracy (f) Precision (g) limits of detection (h) Limit of quantitation (i) Ruggedness (j) Robustness

2.8.1 System Suitability: By injecting the mobile phase as a blank followed by six duplicates with a range of the theoretical plates of vericiguat, system appropriateness parameters including retention time, tailing factor, and concentration (20-70µg/mL) were performed.

2.8.2 Linearity: Vericiguat standard stock solution (20–70µg/mL) was prepared, and a calibration curve between concentration and peak area was plotted to obtain a linear graph.

2.8.3 Accuracy

To ensure precision, the vericiguat sample was combined with a known quantity of the vericiguat standard drug. Injecting 50%, 100%, and 150% of the working concentration level under ideal chromatographic conditions estimates the quantity of drug recovered.

Three concentration levels 50%, 100%, and 150% were used to evaluate vericiguat recovery. The results of the % recovery indicate that the findings were accurate and consistent.

2.8.4 Precision: A system, method, and intermediate precision of the current approaches were assessed using standard and sample solutions, respectively.

Five measurements of the standard solution at a concentration of 40 ppm and 100% assay levels were employed to test the system's precision. Six readings of the standard solution at a concentration of 40µg/mL, with 100% assay level, were used to evaluate the method precision (Analyst 1) and intermediate precision (Analyst 2).

Six observations of the standard solution at a concentration of 40 µg/mL, with 100% assay level, were used to evaluate the technique precision (Analyst 1) and intermediate precision (Analyst 2). The peak area, RSD, and concentration were all noted, and the system's repeatability was calculated.

2.8.5 Ruggedness: Several analysts made six injections of the standard solution, and the results were recorded. For each peak area, the RSD %was determined and calculated.

2.8.6 Robustness: The robustness was assessed by making minute, intentional adjustments to the experimental conditions, such as wavelength, flow rate (0.8mL/min, 1 mL/min, 1.2 mL/min), and the composition of the mobile phase.

2.8.7 LOD and LOQ: By examining several concentrations of solutions and calculating the signal-to-noise ratio, the limit of detection and the limit of quantification of vericiguat were assessed. The signal-to-noise ratio for the concentration (LOD) is around 3:1, whereas the signal-to-noise ratio for the concentration (LOQ) is approximately 10:1.

3. RESULTS AND DISCUSSION

3.1 HPLC method development

The following parameters were taken for the method development.

Table No 1: Optimized Chromatographic Conditions.

Parameters	Method
Stationary phase (column)	Inertsil-ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: Acetonitrile (60:40)
Run time (minutes)	6 min
Volume: The volume ejection loop (ml)	20µL
Column temperature (°C)	Ambient
Flow rate (ml/min)	1.0 ml/min
Drug RT (min)	3.444min
Detection wavelength (nm)	225nm

The current work aimed to develop a rapid, simple, and economical assay of Vericiguat testing for API powder. Based on a review of the Vericiguat literature about their physical and chemical properties as well as multiple analytical methods carried out for Vericiguat, the innovative analytical RP-HPLC system Vericiguat was developed.

Different parameters were studied to create the analytical approach. Firstly, the solvent selection was based on drug solubility and stability. The mobile phase was selected based on the dissociation constant of aqueous and organic solvents (K-capacity factor). Different mobile phase ratios were investigated, however the mobile phase with methanol: acetonitrile (60:40) ratio was chosen because of its symmetrical peaks and high resolution.

Secondly, the maximum absorbance of Vericiguat was discovered to be 225nm. The injection volume was set at 20 µL, which resulted in a nice peak area. The Inertsil C18 column was employed in this work, and ODS picked a nice peak shape. The temperature of the ambient environment was determined to be adequate for the type of medication solution. Because of the good peak area, adequate retention duration, and good resolution, the flow rate was set at 1.0mL/min.

3.2 METHOD VALIDATION

3.2.1 Specificity: By contrasting mobile phase blank and standard solution chromatograms, specificity was assessed (Vericiguat 20 µg/mL). For this, 20 µL of each of the mobile phase blank and standard solutions were individually injected into the HPLC apparatus. Figures 3

and 4 display the findings of the chromatogram analysis. At the retention time of Vericiguat interference, as can be seen, there are no coeluting peaks. This finding demonstrates carrying out the peak purity using a PDA detector.

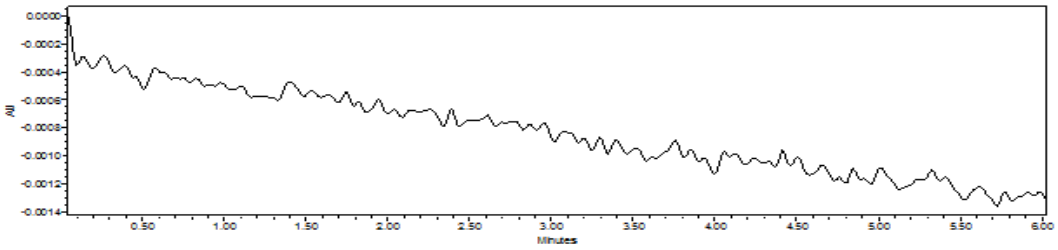

Fig No 3: Blank Chromatograph.

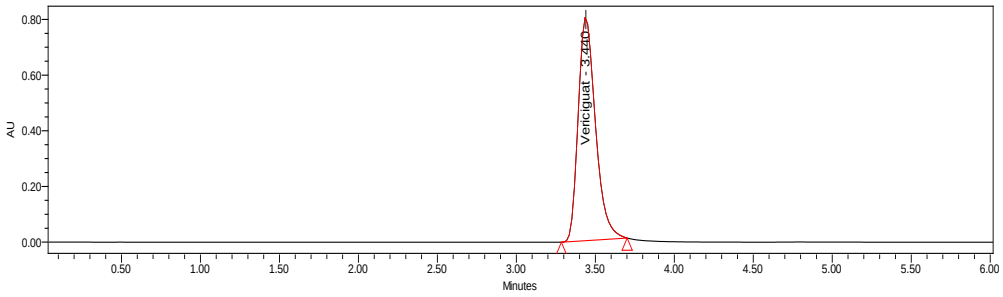

Fig No 4: Chromatogram Standard.

3.2.2 Linearity and Range: The calibration graph was produced by plotting the average peak area obtained from the RP-HPLC against the appropriate concentrations. The findings of the linearity investigation (Figure 5) revealed a linear relationship between vericiguat concentrations of 20 -70µg/mL. A linear equation, y = 52296x + 6339, was derived from the regression analysis. The area under the peak and analyte concentration were found to be linearly related, as indicated by the goodness-of-fit (R^2) value, which was found to be 0.999.

3.2.3 Accuracy: The degree to which an analytical process' results agree with the actual value is expressed by its accuracy. The accuracy findings showed a percentage recovery and RSD % values were within an acceptable range.

Table No 2: Data of Accuracy.

Concentration % of the spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	20	19.98	99.92	MEAN	100.12
50% Sample 2	20	19.98	99.92		
50% Sample 3	20	20.10	100.52	%RSD	0.3493
100% Sample 1	40	40.09	100.22	MEAN	100.33
100% Sample 2	40	40.09	100.23		
100% Sample 3	40	40.21	100.53	%RSD	0.17665
150% Sample 1	60	60.19	100.33	MEAN	100.39
150% Sample 2	60	60.19	100.32	%RSD	0.1163
150% Sample 3	60	60.31	100.53		

3.2.4 Precision: The system, method, and intermediate findings indicated that the precision is within acceptable parameters. The number of theoretical plates, the RSD, and the tailing factor were all computed; the findings are all within acceptable ranges and RSD was ≤.2%, and the tailing factor was ≤ 1 for a number of plates ≤1000.

3.2.5 Robustness: By examining the impact of slight changes in the RP-HPLC settings on the previously described system suitability parameters of the proposed method, the robustness of the analytical method was examined. A little change in the parameters of the approach, such as the mobile phase's composition, temperature, flow rate, and wavelength, is resilient within accepted limits, according to the results of robustness testing. Table 3 provides a summary of the results of flux rate variability showed an impact and it was discovered that the % RSD was less than 2.0%.

Table No 3: Data of Robustness.

Flow rate	Average Peak Area	Standard Deviation	%RSD
0.8mL	1432615.88	70.3826	0.00491
1.0 mL	2102984.66	48.4957	0.00230
1.2 mL	2843634.92	30.7947	0.0010

3.2.6 System suitability: The RSD% was less than 2.0%, showing that the standard solutions were stable for 24 hours under both conditions. The peak area, theoretical plate count, and tailing factor were within acceptable limits. Table 4 presents the outcomes.

Table No-4: Data of System Suitability.

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.446	2102936.24	11003	1.126
2	3.442	2102846.85	11040	1.126
3	3.441	2103011.54	11020	1.130
4	3.442	2102942.85	11038	1.127
5	3.440	2103038.45	11060	1.128
Mean	3.4422	2102955.18	11032	1.1274
SD	0.00228	74.7604	-------	-------
% RSD	0.066247	0.00355	-------	-------

3.2.7 Ruggedness: The assay percentage was within the acceptable range, and the RSD% was not more than 2.0%, demonstrating the system's robustness in terms of variability.

Table No 5: variability of the System.

S.NO:	Peak area	Assay % of Vericiguat
1	2102837.37	100.22
2	2102956.99	100.22
3	2102890.92	100.23
4	2103072.69	100.23
5	2103127.56	100.23
6	2102954.32	100.22
Mean	2102973.30	100.22
%RSD	0.00519	0.00520

3.2.8 Limit of Detection and Limit of Quantification (LOD and LOQ): In contrast to the limit of detection (LOD), which is the lowest amount of analyte in a sample that can be detected but not always quantitated, the limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be quantitatively identified with sufficient accuracy. The data show that Vericiguat has a LOD and LOQ of 0.0047 µg/mL and 0.01429 µg/mL respectively.

4. CONCLUSION

A simple, rapid, isocratic RP-HPLC Method was developed, validated, and assessed for Vericiguat in API. The proposed method uses the mobile phase composition of methanol: acetonitrile (60:40) as results showed symmetrical peaks and high resolution. Furthermore, the

method was validated for linearity, sensitivity, accuracy, and precision. The methodology successfully met the requirements for ICH guidelines and all parameters were within acceptable limits. The results indicate that the established method is reliable and reproducible. The method was first reported for analysis of Vericiguat (API) with a short retention time. Therefore, this method is applicable for routine quality control analysis in the pharmaceutical industry.

5. CONFLICT OF INTEREST

The authors are having no conflict of interest.

6. ACKNOWLEDGMENT

The authors are thankful to the management of GCP for providing the facilities for this study. They are also thankful to Metrochem API Pvt Ltd. (Hyderabad, India) and Active Pharma lab for providing the drug as a free sample for this study.

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