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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE ESTIMATION OF VERICIGUAT IN TABLET DOSAGE FORM

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ABSTRACT

A new, selective, precise and accurate RP-HPLC method was developed and validated for the estimation of Vericiguat in tablet dosage form. Riociguat was used as an Internal Standard(I.S). The chromatographic analysis was performed on Hemochrom C18 (250 mm × 4.6 mm, 5 µm) column with the mobile phase consisting of 0.1% Trifluoroacetic acid and Acetonitrile in the ratio of 65:35% v/v at the flow rate of 1 mL/min. The analyte was detected at the wavelength of 327 nm. The retention time of vericiguat was found to be 8.3 min. The method showed linear responses over the concentration range of 5-150 µg/mL of vericiguat with a regression coefficient of 0.999. LOD and LOQ values were 6.6 µg/mL and 19.9 µg/mL respectively. The accuracy of the proposed method as determined by recovery studies was found to be 99.57%. The Percentage RSD of each parameter was found to be within the limit as per the ICH guidelines.

KEYWORDS: RP-HPLC, Method development, Validation, Vericiguat, Riociguat, Internal Standard.

INTRODUCTION

Heart Failure(HF) is a growing medical and economic problem, affecting an average of 64.3 million people worldwide.[1-2] Among these, approximately 50% cases are of Heart failure with reduced Ejection Fraction(HFrEF).[3] Ejection Fraction(EF) is the percentage of blood pumped out by the heart with each contraction. An EF of 40% or less is an indication of HFrEF. Despite the recent advancement in therapies, the mortality rate in patients with HF has remained unacceptably high and the prognosis for these patients remains poor. Moreover, repeated hospitalizations and the need for additional parenteral therapy indicate an impaired quality of life.[4] For such patients, an urgent need for new treatment options is required. Vericiguat was approved by the U.S. Food and Drug Administration(FDA) in January 2021 for its use in reducing cardiovascular mortality and hospitalization in patients with HFrEF based on the Vericiguat Global Study in Subjects with Heart Failure with Reduced Ejection Fraction (VICTORIA) trial. The trial showed that in patients with high-risk heart failure, the incidence of death from cardiovascular causes or hospitalization for heart failure was lower among those who received vericiguat than among those who received placebo.[5]

Chemically, vericiguat is methyl {4,6-diamino-2-[5-fluoro-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b] pyridin-3-yl] pyrimidin-5-yl} carbamate. The molecular formula is C19H16F2N8O2 and the molecular weight is 426.39 g/mol.[6] The chemical structure of Vericiguat is shown in Fig.1.

Vericiguat is an oral soluble Guanylate Cyclase(sGC) stimulator. sGC is an enzyme that is activated by Nitric Oxide(NO) in the cells. The activation results in synthesis of the second messenger cyclic guanosine monophosphate (cGMP). cGMP decreases fibrosis of the heart and improves endothelial function. Increase in cGMP levels results in vasorelaxation, inhibition of smooth muscle cell proliferation, leukocyte recruitment, and platelet aggregation through a number of downstream processes.[7-9] Thus, the Nitric Oxide (NO)-sGC-cyclic guanosine monophosphate (cGMP) pathway plays a significant role in the regulation of cardiovascular system. In patients with cardiovascular disease, the oxidative stress may affect the binding of NO to sGC and consequently producing a relatively NO-resistant state.[10-12] Vericiguat, as a direct sGC stimulator, activates the cGMP pathway independent of nitric oxide involvement. It increases the enzymatic activity of sGC to generate cGMP independently of NO and thereby helps to prevent the myocardial and vascular dysfunction associated with decreased sGC activity in heart failure.[13-14] Vericiguat is unique as

compared to the first FDA approved sGC drug that is Riociguat as modifications to its structure have significantly decreased its susceptibility to oxidative metabolism, which results in a relatively long half-life and thus allowing for once-daily dosing.[7] The chemical structure of vericiguat and riociguat is shown in figure 1 and 2 respectively.

During Literature survey, one HPLC method was found for the estimation of vericiguat.[16] Several instrumental methods were found for the analysis of Riociguat.[17-22] However no method was found for vericiguat which used an Internal Standard (I.S). Hence this study was done to develop a reliable, accurate and precise method for the analysis of vericiguat in tablet dosage form. Riociguat was used as I.S as it is structurally very similar to vericiguat.

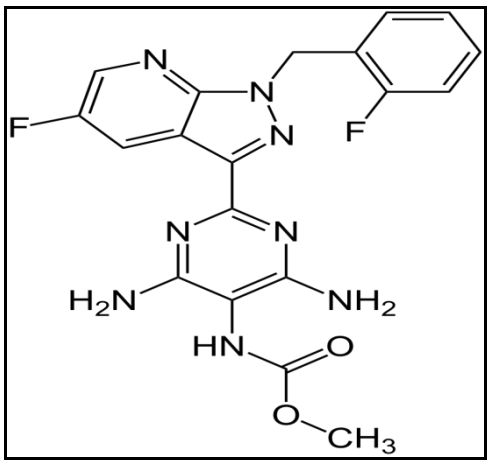

Fig.1: Structure of Vericiguat

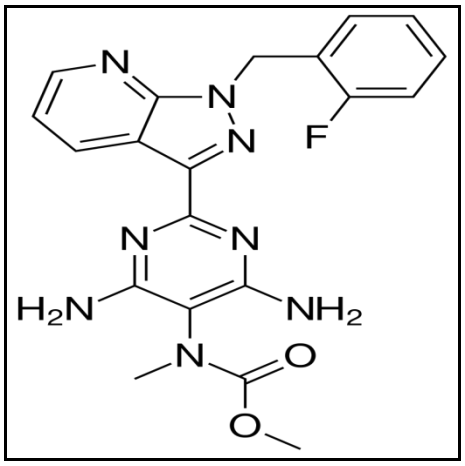

Fig.2. Structure of Riociguat(I.S)

MATERIALS AND METHODS

Instrumentation

Chromatographic analysis was performed on WATERS 2695 Alliance HPLC system equipped with WATERS 2996 Photo Diode Array Detector. Empower 3 software was used for data processing and evaluation. For all the spectrophotometric measurements PerkinElmer UV/VIS Spectrophotometer having PerkinElmer UV WinLab ES software version 6.0.4.0738 was used. Sartorius Analytical Balance was used for all the weighing.

Chemicals and Reagents

Vericiguat and riociguat Reference Standards with potency of 99.8% and 99% respectively were obtained from Central Drug Testing Laboratory, Mumbai. Vericiguat tablets with the brand name Verquvo® containing 10 mg Vericiguat were received as gift sample from Assistant Drugs Controller Office, Air Cargo, Mumbai. Acetonitrile (HPLC grade) from

Merck life science Pvt. Ltd, Trifluoroacetic acid 98% (AR grade) from Finer chemicals were used.

Determination of wavelength of maximum absorbance

20 mg of vericiguat standard was weighed accurately and transferred to 100 mL volumetric flask and volume was made up to the mark with methanol (200 µg/mL). The aliquot portion of the standard stock solution of vericiguat was diluted appropriately with methanol so as to obtain a solution of 10 µg/mL concentration. The above solution was scanned in the range of 400.0 nm to 200.0 nm using UV/Visible spectrophotometer using methanol as a blank. vericiguat showed maximum absorbance at 327 nm. So, the same wavelength was selected for the analysis of vericiguat.

Preparation of Mobile phase

Mixture of 0.1% Trifluoroacetic acid and Acetonitrile in the ratio of 65:35 v/v was used as mobile phase for the present study. Trifluoroacetic acid (0.1% v/v) was prepared by dissolving 1ml of Trifluoroacetic acid(98%) in 1000mL of milli-Q water. The solution was sonicated for 10 minutes and filtered through 0.45 µm nylon filter.

Preparation of Standard Solution

A stock solution of 500 µg/mL vericiguat was prepared. Similarly, 800 µg/mL stock solution of riociguat(I.S) was prepared separately. To prepare the working standard solution 10 mL of vericiguat stock solution and 5 mL of riociguat stock solution was added to 50 mL volumetric flask and the volume was made up using mobile phase so as to prepare working standard solution consisting of 100 µg/mL vericiguat and 80 µg/mL riociguat. This combined standard solution was used as the standard solution for all the analysis.

Preparation of Sample Solution

10 tablets of vericiguat were weighed and their average weight was determined. The tablets were crushed. An accurately weighed amount of powder equivalent to 10 mg of vericiguat was transferred to six different 100 mL volumetric flask. This was followed by the addition of 50 mL mobile phase to all the flasks. The mixture was then sonicated for 15 minutes. The solution was cooled to room temperature and to this 10 mL of stock solution of riociguat standard(800 µg/mL) was added. Further volume was made up to the mark using mobile phase. All solutions were filtered through a 0.45 membrane filter.

Method development and optimization

Various literature was obtained on the physical and chemical properties of Vericiguat.[14-15] Molecular, structural and solubility data revealed that vericiguat is a non polar drug, Initial trials on HPLC were carried out on Inert Sustain C18 (4.6 mm × 125 mm, 5 µm) column by considering the chemical nature of the molecule. Riociguat is structurally similar to vericiguat. Hence, it was selected as Internal Standard.

Several trials were done by using different mobile phase systems in different ratios to obtain the separation of vericiguat and I.S. Initial trials with Acetonitrile: Water(pH3)(50:50% v/v) gave poor peak shape. Also, long retention times and less resolution between the peak of vericiguat and internal standard was observed. Variations were made in mobile phase proportion, injection volume, and concentration of the standard solution. But none of the conditions gave acceptable SST parameters. Hence further trials were done by using 0.1% Trifluoroacetic acid: Acetonitrile (50:50% v/v). This trial was done on Hemochrom C18 (4.6 mm × 125 mm, 5 µm) column. Various trials were done with this mobile phase in different ratios, injection volume and concentration of the standard solutions of the analyte and I.S. Chromatograms with acceptable peak shape and SST parameters were obtained on this column. Finally, good peak shape, resolution and acceptable system suitability parameters were found with the mobile phase composition of 0.1% Trifluoroacetic acid:Acetonitrile in the ratio of 65:35% v/v on Hemochrom C18 (250 mm × 4.6 mm, 5 µm)column.

The best peak shape, size and resolution were obtained with the following chromatographic conditions:

Column: Hemochrom C18 (4.6 mm × 250 mm, 5 µm)

Flow rate: 1 mL/min.

Diluent: Mobile phase.

Programming: Isocratic.

Wavelength: 327 nm.

Run time: 12 min.

Injection volume: 10 µL

Method Validation Studies

The developed RP-HPLC method was validated for parameters such as specificity, linearity, precision, accuracy, LOD, LOQ, and robustness according to ICH guidelines.[22]

Specificity

Specificity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of other components expected to be present in the sample. Specificity for a given analyte is commonly measured and documented by resolution, plate number(efficiency) and tailing factor. For specificity, 10 µL solution of blank, standard and sample solution were injected into the HPLC system separately and the chromatograms are shown in Figures 3-5. There are no co-eluting peaks observed at the retention time of vericiguat. This indicates that the peak of the analyte was pure and this confirmed the specificity of the method.

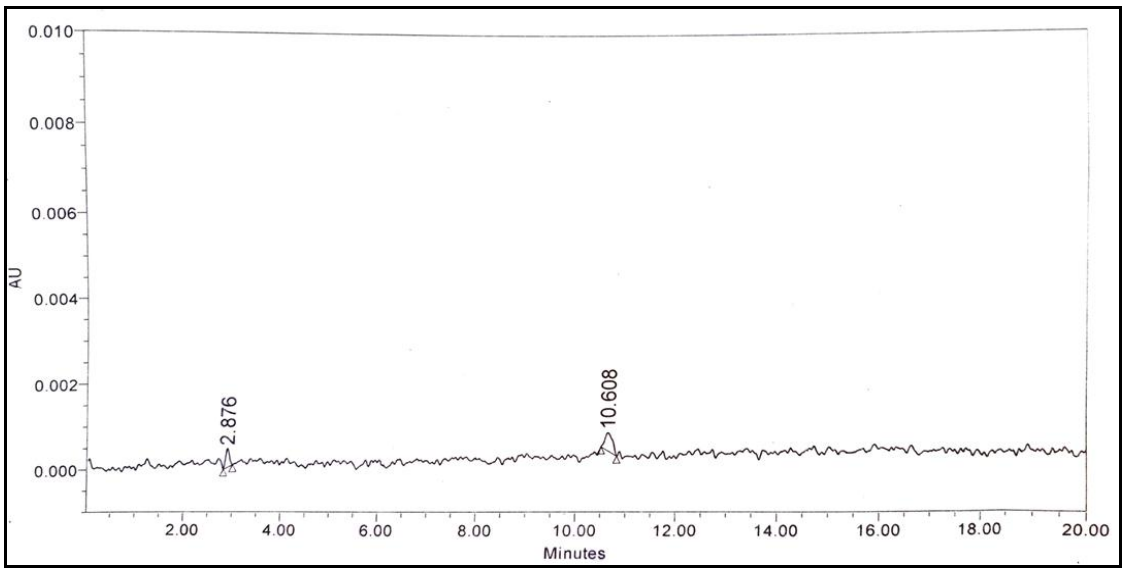

Fig. 3: Chromatogram of Blank.

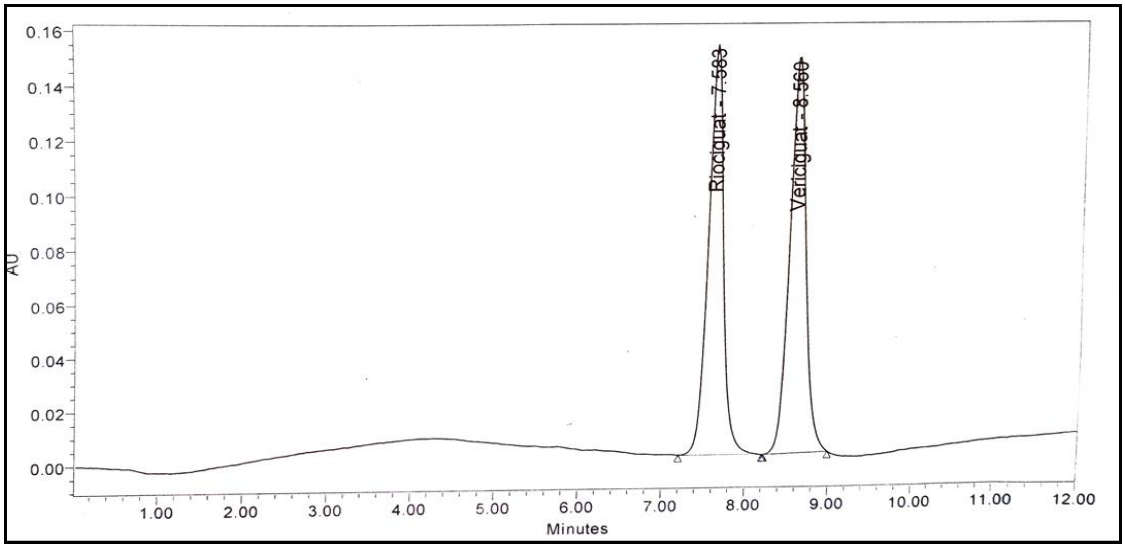

Fig. 4: Chromatogram of Standard.

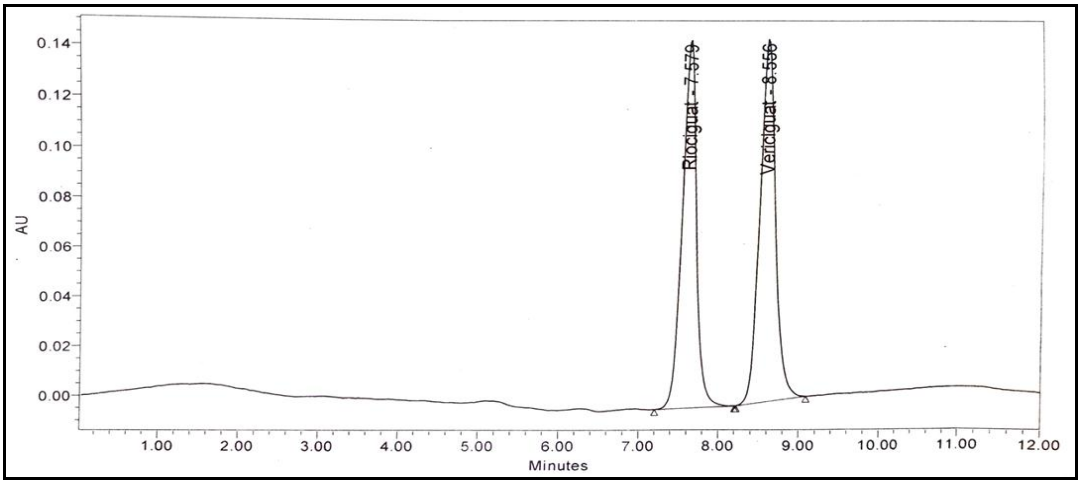

Fig. 5: Chromatogram of Sample.

System Suitability

System suitability parameters were analyzed to check the system performance by injecting mixed standard preparation (six replicates) into the HPLC. The chromatograms were recorded to evaluate SST parameters like %RSD of RT, resolution, tailing factor and Theoretical plates.

Table 1: System suitability parameters for Vericiguat.

Injection no.	Area of drug	Area of I.S	Peak Area Ratio(Drug/I.S)	RT of drug	Resolution	Tailing Factor	Theoretical Plates
1	1899780	1835768	1.034	8.339	2.824	1.23	9870
2	1915618	1848708	1.036	8.337	2.814	1.22	9884
3	1912929	1819743	1.051	8.337	2.812	1.24	9923
4	1903505	1823475	1.044	8.336	2.821	1.24	9945
5	1907332	1828200	1.043	8.339	2.814	1.25	9823
6	1933756	1816132	1.065	8.334	2.817	1.24	9861
Mean	1912153	1828671	1.045	8.366	2.817	1.236	9884.3
SD	12086.50	11966.65	0.011	0.002	0.005	0.010	44.016
%RSD	0.63	0.65	1.08	0.023	0.16	0.83	0.44
Limit	NMT 2.0%	NMT 1.0%	NMT 2.0%	NMT 1.0%	NMT 1%	NMT 2.0%	NMT 2.0%

Linearity

The Linearity range for this method was determined by preparing standard solutions of vericiguat in different concentrations. Equal amount of I.S was added. A standard stock solution of vericiguat was prepared which was diluted with mobile phase so as to prepare concentrations of vericiguat within the range of 5-150 µg/mL. Three injections from each concentration were analysed under the same conditions. The mean peak area ratio of vericiguat and I.S was plotted against corresponding concentrations to obtain the calibration

graph. The results of linearity studies showed linear relationship over the concentration range of 5-150 µg/mL for vericiguat. From the regression analysis, the linear equation obtained was: y = 0.0112x - 0.0096, and the correlation coefficient(r^2) value was found to be 0.999 indicating a linear relationship between the concentration of analyte and area under the peak.

Table 2: Linearity data for Vericiguat.

Linearity level	Concentration	Peak area ratio (Analyte/I.S)
1	5	0.05152
2	10	0.11847
3	20	0.21081
4	50	0.54836
5	75	0.81084
6	100	1.09546
7	125	1.36396
8	150	1.70346

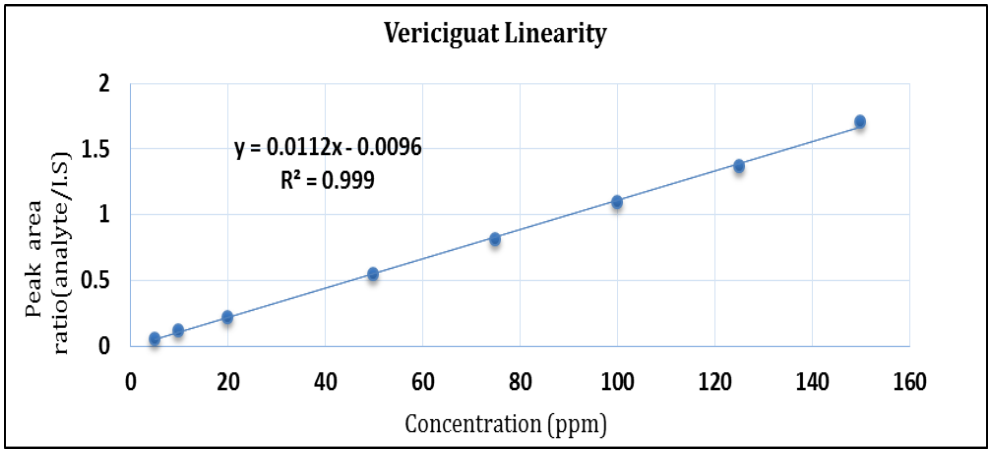

Fig. 6. Calibration curve for Vericiguat.

Sensitivity

Calculation of LOD based on the standard deviation of the response and the slope of a calibration curve is done using the following formula:

$$LOD = 3.3* \propto /S.$$

Where $\propto$ is the standard deviation of the response and S is the slope of the calibration curve.

The Limit Of Quantification (LOQ) is calculated similarly as the LOD using the formula:

$$LOQ = 10* \propto /S.$$

The LOD and LOQ value of Vericiguat was calculated to be 6.6 µg/mL and 19.9 µg/mL respectively.

Table 3: Sensitivity data of Vericiguat.

Molecule	LOD	LOQ
Vericiguat	6.6 µg/mL	19.9 µg/mL

Accuracy

The accuracy for this method was determined by recovery studies at three concentration levels (110%, 120%, and 130%) by standard addition method. Three samples from each concentration were injected. The percentage recovery of added Vericiguat and RSD were calculated for each of the replicate samples. The mean % recovery was 99.57%.

Table 4: Accuracy studies of Vericiguat.

% Level	Amount spiked(mL)	Amount recovered (mg)	% Amount recovered	% recovery	Mean recovery
100	0	10.22	102.20	102.20	99.57%
110	1	10.81	108.13	96.37	
120	2	12.03	120.37	98.504	
130	3	13.38	133.81	101.22	

Precision

The system precision and method precision (repeatability) of the proposed methods were determined by several measurements of standard solution and sample solution, respectively. For System precision, six measurements of the standard solution at the 100% concentration levels was determined on the same day. Method precision was established by six assay determinations of the sample solution at the 100% concentration levels on two different days. The %RSD and results obtained was calculated to evaluate repeatability of the results and all the values were found to be within limit.

Table 5: Precision data as Repeatability.

Sample no.	Day 1	Day 2
1	103.34	102.80
2	103.67	103.20
3	104.23	102.78
4	101.69	101.74
5	101.03	102.88
6	100.93	100.90
Mean	102.48	102.38
SD	1.44	0.88
%RSD	1.40	0.86
Limit	NMT 2.0%	NMT 2.0%

Robustness

Robustness of an analytical procedure is its capacity to obtain comparable and acceptable results on introducing small but deliberate variations in procedural parameters. Robustness of the proposed method was analysed by changing flow rate (± 2 mL), Mobile Phase composition (± 2%) and wavelength (± 2 nm). Under such different chromatographic conditions, three sample solutions of vericiguat were prepared and injected in triplicates along with six replicate injections of the working standard solution. Mean, SD, and % RSD of % estimation were calculated and reported. The results of robustness studies showed that a minor change in the method condition, such as phase ratio, flow rate and wavelength did not show any significant deviation in the results. The recovery and %RSD were within the acceptable limits.

Table 6: Robustness studies of Vericiguat.

Parameter	Change in parameter	% Estimation	Mean	SD	%RSD	Limit
Wavelength (± 2 nm)	325	101.01	101.13	1.29	1.27	NMT 2.0%
	327	102.48				
	329	99.92				
Flow rate (± 0.2ml)	0.9	100.72	102	1.12	1.10	
	1	102.48				
	1.1	102.81				
Mobile Phase composition (± 2 %)	63:37	102.04	101.72	0.51	0.49	
	65:35	102.48				
	67:37	103.05				

Assay

The assay results obtained shows high percentage recoveries and low SD values which confirms that the method is suitable for routine analysis of vericiguat in its tablet dosage form.

Table 7: Assay results for Vericiguat.

S. No.	Weight of standard (mg)	Sample weight (equivalent to 10mg)	Mean area ratio of analyte/IS	Sample/I.S area ratio	Assay %
1	10mg	250.64	1.0475	1.07991	103.34
2		250.54		1.08160	103.67
3		250.56		1.08762	104.23
4		250.39		1.06040	101.69
5		250.53		1.05402	101.03
6		250.41		1.05248	100.93
Mean					102.48
SD					1.44
%RSD					1.40

RESULTS AND DISCUSSION

A novel, precise, accurate RP HPLC method has been developed for the determination of vericiguat in its tablet dosage form. Chromatographic conditions were optimized by considering the chemical nature of vericiguat. Riociguat was used as I.S.

Analysis and evaluation of System Suitability parameters was done by injecting six replicates of the standard solution containing a working concentration of 100 µg/mL vericiguat and 80 µg/mL internal standard. The % RSD of parameters such as Area, retention time, tailing factor, resolution were evaluated. For this method, all parameters were found to be within the acceptance limits. The results of system suitability studies are summarized in Table 1. The chromatograms of blank, standard and sample are shown in Fig. 3, 4 and 5 respectively.

The results of Linearity studies over the concentration range of 5-150 µg/mL showed linear detector response with correlation coefficient of 0.999. The regression equation obtained was y=0.0112x - 0.0096 as shown in Fig. 6. The linearity data is shown in Table 2.

The LOD and LOQ values were found to be 6.6 µg/mL and 19.9 µg/mL respectively for vericiguat as mentioned in Table 3. Thus the method is found to be sensitive over a broad range.

The method was found to be accurate as the mean percent recovery of vericiguat sample solutions was found to be 99.57% which was within limit as shown in Table 4.

For precision studies vericiguat sample solutions were found to be within limits hence the method was found to be precise. The intermediate precision for the method was done by analyzing vericiguat tablet by the proposed method. The % RSD values of assay were found to be within the acceptable limits as shown in Table 5.

The method was sufficiently robust for normally accepted variation in parameters such as mobile phase ratio, flow rate and wavelength. % RSD of % assay during changes in method parameters was less than 2.0% and the results were not adversely affected by these changes. The results are tabulated in Table 6.

The assay results obtained showed high percentage recoveries and low SD values, which confirms that the method is suitable for routine analysis of vericiguat in its tablet dosage form. The results are summarized in Table 7.

Abbreviations

RP-HPLC: Reversed Phase High Performance Liquid Chromatography; I.S: Internal Standard; LOD: Limit of Detection; LOQ: Limit of Quantification; ICH: International Council for Harmonization; AR: Analytical reagent; UV-VIS: Ultraviolet-visible spectrophotometry; RT: Retention Time; NMT: Not More Than; SD: Standard deviation; %RSD: Percentage Relative Standard Deviation.

CONCLUSION

Thus, the RP-HPLC method developed for vericiguat is linear, accurate, precise, reproducible and specific as evident from the validation results. All the verification parameters were within the range according to ICH guidelines. In addition, the main features of this method is the use of Internal Standard Riociguat which makes the method more precise and accurate. Hence the proposed RP-HPLC technique can be used for the routine analysis of vericiguat drug in its tablet dosage form.

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