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Research Article

A NEW RP-HPLC METHOD FOR ESTIMATION OF VERICIGUAT IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validation of Vericiguat in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS C18 (4.6×250mm, 5µm) column with Methanol:Phosphate Buffer (55:45) v/v as mobile phase at a flow rate of 1.0 mL min^{-1} with UV detection at 225 nm; the constant column temperature was ambient. The run time under these chromatographic conditions was less than 8 min. The retention time of Vericiguat was found to be 2.252. The calibration plot was linear over the concentration range of 6 - 14 µg mL^{-1} with limits of detection and quantification values of 1.2 and 3.6 µg mL^{-1} respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%. The developed method was simple, precise, specific, accurate and rapid, making it suitable for estimation of Vericiguat in bulk and marketed pharmaceutical dosage form dosage form.

Keywords: Vericiguat, RP-HPLC, Validation, ICH Guidelines.

1. INTRODUCTION

HPLC is able to separate macromolecules and ionic species labile natural products, polymeric materials, and a wide variety of other high-molecular weight poly functional group. HPLC is the fastest growing analytical technique for the analysis of the drugs. Its simplicity, high specificity, and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids. In this, the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3-10µm [1, 2]. Modern LC uses very small particles for packing. The small particle size results in more rapid approach to the distribution equilibrium and consequently smaller plate height, so that a given length of column includes large number of plates which makes the column efficient and the peak narrow [3]. But close packing of these small particles reduces the flow rate of the mobile phase through the packed bed (the packings aid to develop high back pressure) and in order to achieve a reasonable flow rate it is necessary to apply pressure to the mobilephase. So, the designation, put forth as High-Pressure Liquid Chromatography [4, 5].

1.1. Drug profile

Table 1: Drug Profile [6]

Drug	Vericiguat
Synonym	Vericiguat, Véricigu at, Vericiguatum
Category	BCRP/ABCG2 Substrates, Cardiac Therapy, Guanylate Cyclase Stimulators
IUPAC name	Methyl N-[4,6-diamino-2-[5-fluoro-1-[(2-fluorophenyl) methyl] pyrazolo [3,4-b] pyridin-3-yl] pyrimidin-5-yl] carbamate
Molecular weight	426.328 g /mol
Molecular formula	$C_{19}H_{16}F_2N_8O_2$
Melting point	149-158°C
pKa	11.84
Log P	2.99

A search through the literature revealed that the drug has been examined with a variety of analytical approaches, notably HPLC, RP-UPLC, UPLC-MS-MS etc. The development of a basic, precise, reliable, and repetitive HPLC method for the quantification of Vericiguat tablet dosage form is described in the current method with low retention time as compared to other available methods along with economical mobile phase.

1.2. Structure

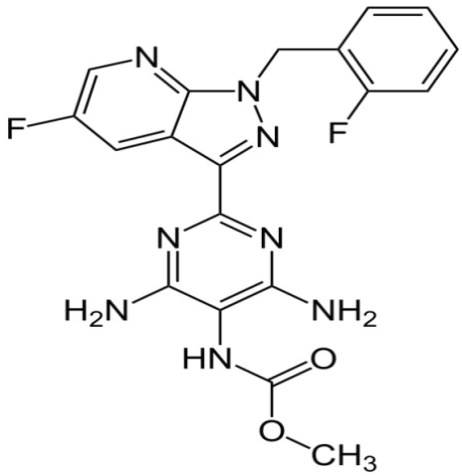

Fig. 1: Structure of Vericiguat

2. MATERIAL AND METHODS

2.1. Instruments used

For the experiments WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector HPLC was used. Vericiguat (Pure) was obtained from Sura labs, Water and Methanol for HPLC was procured from Lichrosolv (Merck) and Acetonitrile for HPLC was procured from Merck

2.2. Preparation of standard solution

2.2.1. Diluent Preparation

The Mobile phase was used as the diluent. Accurately weigh and transfer 10 mg of Vericiguat working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicated to dissolve and removal of air completely and make volume up to the mark with the Methanol.(Stock solution)

2.2.2. Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 55:45% v/v.

2.2.3. Preparation of Buffer and Mobile Phase

2.2.3.1. Preparation of Potassium dihydrogen Phosphate (KH_2PO_4) buffer (pH-3.6):

Potassium dihydrogen phosphate (6.8043g) was dissolved of in 1000 ml HPLC water and the pH was adjusted to 3.6 with diluted orthophosphoric acid. Filtered and sonicated the solution by vacuum filtration and ultra sonication.

2.2.3.2. Preparation of mobile phase

Accurately measured 550 ml (55%) of Methanol, 450 ml of Phosphate buffer (45%) were mixed and degassed in digital ultra sonicated for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

2.3. Procedure

Injected the standard by changing the chromatographic conditions and recorded the chromatogram and as shown in fig. 2 the conditions of proper peak elution for performing validation parameters as per ICH guidelines were noted [7].

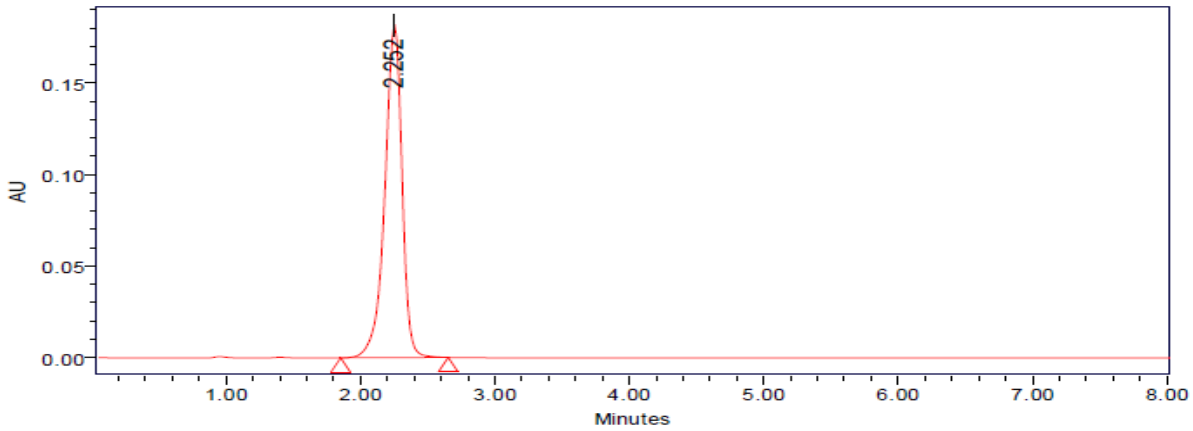

Fig. 2: Optimised Chromatogram for standard

2.4. Validation parameters [8-10]

2.4.1. System Suitability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.4.2. Preparation of Sample Solution

20 tablets were powdered and 10 mg equivalent weight of Vericiguat sample was taken into a 10mL clean dry volumetric flask and about 7mL of Diluent was added. The mixture was sonicated to dissolve it completely and volume was made up to the mark with the same solvent. Further 0.1ml of Vericiguat was pipette from above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent and injected and chromatogram was recorded and shown in fig. 3.

Three replicate injections of standard and sample solutions were injected and the results were calculated by using formula:

%Assay = {(Sample area × Weight of standard × Dilution of sample × Purity × Weight of tablet)/Standard area × Dilution of standard × Weight of sample × 100 × Label claim)} ×100

2.4.3. Preparation of Drug Solutions for Linearity

Ten mg of Vericiguat working standard was taken into a 10ml of clean dry volumetric flasks and about 7ml of diluents was added and sonicated to dissolve it completely and volume was made up to the mark with the same solvent (Stock solution).

2.4.4. Preparation of Level - I to Level V (6-14 ppm respectively of Vericiguat)

Varied amount (0.6ml to 0.14 ml) of stock solution was taken in to 10ml of volumetric flask and the volume was made up to mark with diluents and sonicated the solution for bubble entrapment using ultrasonicater.

Each level was injected into the chromatographic system and measured the peak area. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and correlation coefficient was calculated.

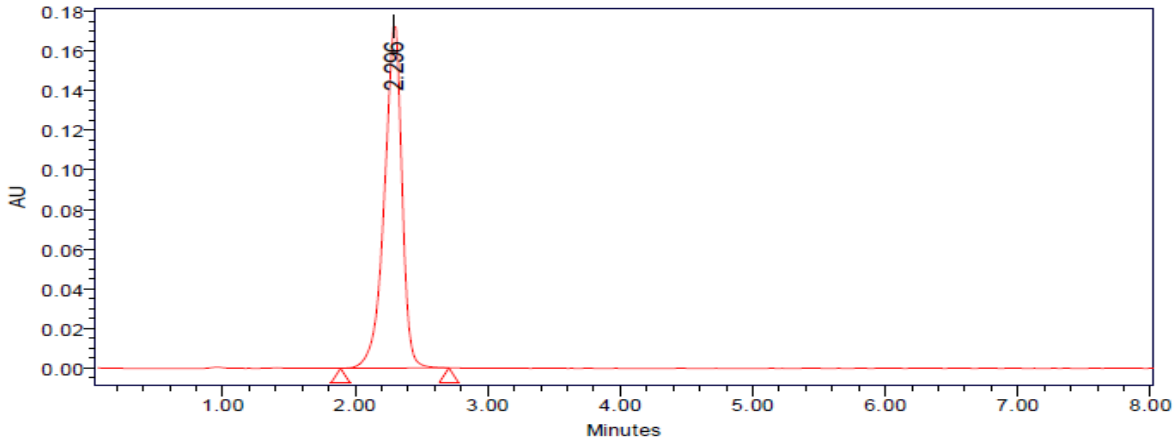

Fig. 3: Optimised Chromatogram for sample

2.5. Precision

2.5.1. Repeatability

2.5.1.1. Preparation of Vericiguat Product Solution for Precision

Vericiguat working standard (10 mg) was transferred into a 10ml of clean dry volumetric flasks, about 7ml of diluents was added and sonicated to dissolve completely and made the volume up to the mark with the same solvent (Stock solution).

Further, 0.1ml of the above Vericiguat stock solutions was pipetted into a 10ml volumetric flask and diluted up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.5.2. Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, precision was performed on different days by maintaining same conditions.

Analyst 1:

The standard solution of 10ppm of Vericiguat was injected for six times and measured the area for all six

injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution of 10ppm of Vericiguat was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

2.6. Accuracy

2.6.1. For preparation of 50% Standard stock solution

Vericiguat working standard (10 mg) was transferred of into a 10ml of clean dry volumetric flasks, 7mL of diluents was added and sonicated to dissolve it completely and made the volume up to the mark with the same solvent (Stock solution).

Further, 0.05ml of the above Vericiguat stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluents.

2.6.2. For preparation of 100% Standard stock solution

Further, 0.1ml of the above Vericiguat stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluents.

2.6.3. For preparation of 150% Standard stock solution

Further, 0.15ml of the above Vericiguat stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluents.

2.6.4. Procedure

Three replicate injections of individual concentrations (50%, 100%, 150%) were injected under the optimized conditions. The chromatograms were recorded and measured the peak responses. The amount found and amount added for Vericiguat was recorded and calculated the individual recovery and mean recovery values.

2.7. Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

2.7.1. For preparation of Standard solution

Ten mg of Vericiguat working standard was transferred into a 10ml of clean dry volumetric flask, 7mL of diluents was added and sonicated to dissolve it completely and volume up was made upto the mark with the same solvent (Stock solution).

Further, 0.1ml of the above Vericiguat stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluents.

In this trial it shows proper separation of peak and more plate count in the chromatogram and the tailing factor is within the limit. So, it is an optimized chromatogram.

3. RESULTS AND DISCUSSION

3.1. Method validation [11-15]

Table 2: Results of system suitability for vericiguat

S. No	PeakName	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vericiguat	2.277	1652847	185647	6589	1.24
2	Vericiguat	2.277	1653658	186254	6587	1.26
3	Vericiguat	2.267	1654521	185475	6584	1.28
4	Vericiguat	2.265	1653564	186594	6582	1.29
5	Vericiguat	2.277	1658745	185684	6895	1.24
Mean			1654667			
Std.Dev.			2355.764			
%RSD			0.142371			

3.2. Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

There was no interference from blank. So the method was specific.

3.3. Assay (Standard)

The % purity of Vericiguat in pharmaceutical dosage form was found to be 99.86%.

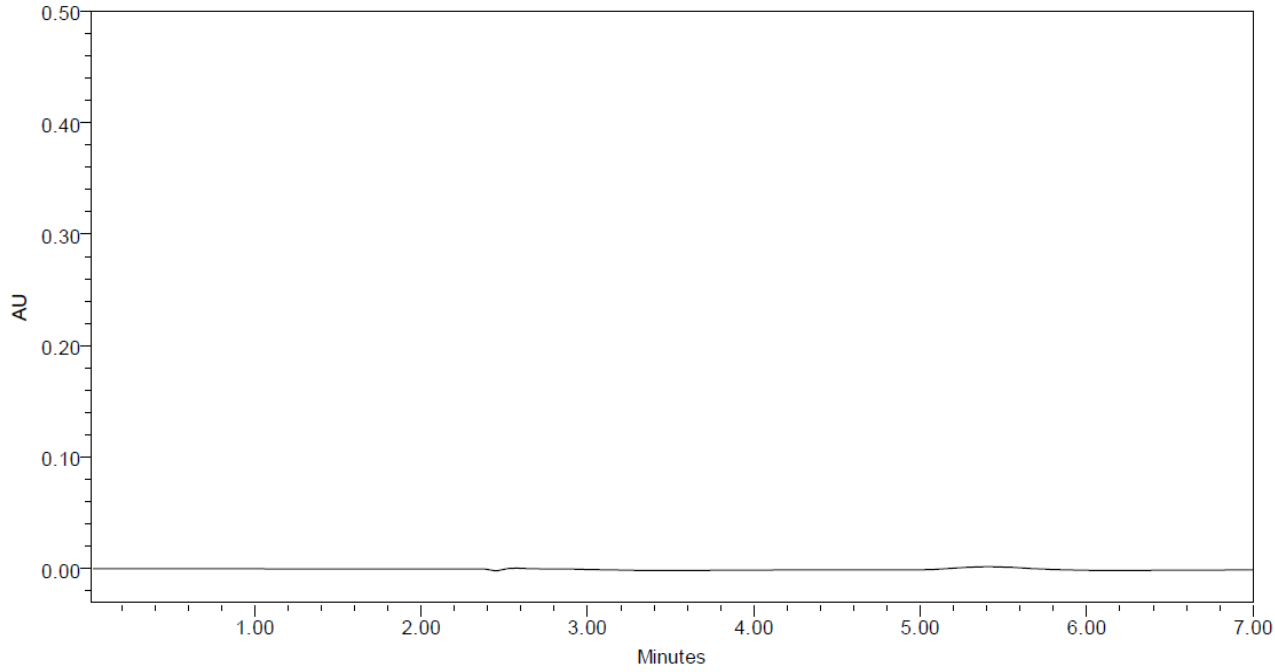

Fig. 4: Chromatogram for blank

Table 3: Assay data for Vericiguat

S. No	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	2.265	1658254	185468	1.24	6391	1
2	2.267	1658475	184524	1.23	6549	2
3	2.267	1658471	186598	1.25	6682	3
4	2.265	1658254	185468	1.24	6391	4
5	2.267	1658475	184524	1.23	6549	5
6	2.267	1658471	186598	1.25	6682	6

Table 4: Assay Results of Vericiguat

S. No.	Name of Compound	Label Claim	Amount found (mg)	% Purity
1	Vericiguat	10 mg	9.98	99.86

3.4. Linearity

Table 5: Data for linearity

Concentration µg/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778

3.5. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

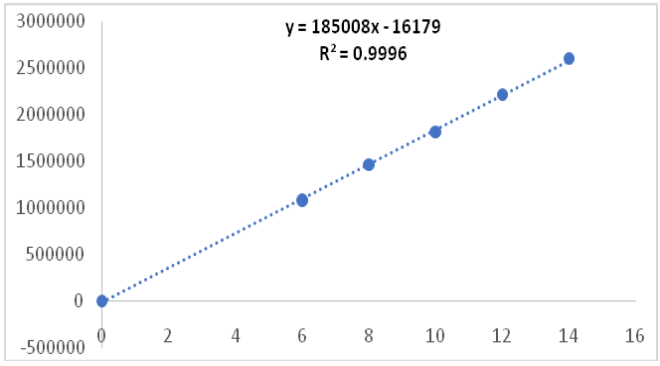

Fig. 5: Linearity curve of Vericiguat

3.5.1. Intermediate precision

Analyst 1

Analyst 2

3.6. Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 6: Results of repeatability for Vericiguat

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vericiguat	2.293	1658954	186958	1.26	6785
2	Vericiguat	2.276	1658745	187548	1.27	6854
3	Vericiguat	2.286	1659865	189854	1.26	6852
4	Vericiguat	2.277	1653254	186985	1.25	6784
5	Vericiguat	2.280	1654781	189542	1.24	6895
Mean			1657120			
Std.dev			2913.592			
%RSD			0.175823			

Table 7: Results of intermediate precision for Vericiguat

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vericiguat	2.274	1678541	186589	6587	1.26
2	Vericiguat	2.258	1685985	186598	6321	1.26
3	Vericiguat	2.267	1685745	186985	6385	1.25
4	Vericiguat	2.270	1685987	187854	6580	1.26
5	Vericiguat	2.264	1698526	187549	6721	1.27
6	Vericiguat	2.265	1685943	186598	6637	1.26
Mean			1686788			
Std.Dev.			6463.466			
%RSD			0.383182			

Table 8: Results of intermediate precision analyst 2 for Vericiguat

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Vericiguat	2.277	1665847	167481	6854	1.25
2	Vericiguat	2.255	1658989	167854	6785	1.26
3	Vericiguat	2.265	1659845	167895	6854	1.24
4	Vericiguat	2.255	1665964	167854	6895	1.26
5	Vericiguat	2.253	1659863	168585	6459	1.25
6	Vericiguat	2.252	1665986	167859	6456	1.26
Mean			1662749			
Std. Dev.			3501.766			
%RSD			0.210601			

Table 9: Accuracy results for Vericiguat (n=3)

% Concentration	Area	Amount added (ppm)	Amount found (ppm)	% Recovery
50%	109068.3	5	5.021	100.42
100%	202187.0	10	10.54	100.54
150%	297032.3	15	15.181	101.20

3.7. Limit of detection for vericiguat

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where σ = Standard deviation of the response, S = Slope of the calibration curve

Result obtained was 1.2µg/ml

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample

which can be quantitatively determined.

LOQ=10×σ/S

Where σ = Standard deviation of the response, S = Slope of the calibration curve

Result obtained was 3.6µg/ml

3.8. Robustness

The robustness was observed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Vericiguat. The method is robust only in less flow condition. The standard of Vericiguat was injected by changing the conditions of chromatography.

There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 10: Results for Robustness Vericiguat

Parameters	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 mL/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 mL/min	1758468	2.032	6254	1.32

4. CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Vericiguat in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Vericiguat was freely soluble in methanol, ethanol, and chloroform, soluble in ether, sparingly soluble in acetonitrile and octanol, and practically insoluble in water. Methanol was chosen as the mobile phase. The solvent system used in this method was economical. The % RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Vericiguat in bulk drug and in pharmaceutical dosage forms.

Conflict of interest

None declared

Source of funding

None declared

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