



# RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF VERICIGUAT IN BULK AND TABLET DOSAGE FORM

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**Abstract:** For the quantitative determination of Vericiguat in bulk and tablet dosage form, a new validated HPLC method was validated in the present work. The mobile phase composed of Acetonitrile: Methanol: Water in 0.1% Triethylamine (50:10:40, v/v/v) with a flow rate of 1 ml/min. The column was a Phenomenex Luna C<sub>18</sub> column (150 mm × 4.6 mm id; 5 μm particle size). UV detectors at 258 nm were used to monitored. The calibration curve (R<sup>2</sup> value: 0.9999) was linear in the concentration range of 20 to 100 μg/ml. The suggested method was validated in accordance with ICH guidelines and successfully applied for the assay of Vericiguat in tablet dose form.

**Index Terms - Vericiguat, Raw material, HPLC method and Tablet dosage form**

## I. INTRODUCTION

Vericiguat is a novel new oral soluble guanylate cyclase (sGC) medication for the treatment of chronic heart failure that also reduces hospitalization and ejection fraction rates.<sup>[1-2]</sup> Therefore, inducing vasodilation relaxes smooth muscle, which enhances cardiac function.<sup>[3-5]</sup> A survey of the literature employing the RP-HPLC method revealed that no method has been developed for the analysis of Vericiguat by using this mobile phase. The purpose of this work is to develop a Vericiguat dosage form determination RP-HPLC method that is simple, accurate and reliable. The developed method was validated as per ICH norms<sup>[6-7]</sup>.

## II. MATERIALS AND METHODS

### Materials and Reagents

Verquvo tablet that were marked to contain 10 mg of Vericiguat was the tablet dose form used in this method. The mobile phase utilized was Acetonitrile: Methanol: Water in 0.1% Triethylamine (50:10:40, v/v/v).

### The Instrument and chromatographic conditions

A manual injecting facility with a 20 μL capacity per injection was used with the Shimadzu HPLC system (Shimadzu corporation Kyoto, Japan), which included a pump (LC - 20AD solvent deliver module, SPD-20A UV- Visible detector), both of which were operated by Lab solutions software. Phenomenex Luna C<sub>18</sub> (150 mm × 4.6 mm, 5.0 μm particle size) was the column that was used. Different mobile phases were tried in order to find the best condition for separation of Vericiguat. The mobile phase composed of Acetonitrile: Methanol: Water in 0.1% Triethylamine (50:10:40, v/v/v) which had a flow rate of 1.0 ml/min. At 258 nm, UV detection was determined. A 0.45 μm membrane filter was used to filter the mobile phase and samples. Before usage, mobile phase was degassed with a Sonica ultrasonic cleaner (model 2200 MH). The other instrument used are hot air oven. The Shimadzu AUX-220 electronic balance was used for all weighing.

## Preparation of standard and sample solutions

### Mobile phase

Acetonitrile: Methanol: Water in 0.1% Triethylamine (50:10:40, v/v/v) is programmed as RP-HPLC method.

### Preparation of standard stock solution

Accurately weighed quantity of 10 mg of Vericiguat API was transferred into 10 ml volumetric flask and dissolved in methanol and diluted up to mark to get concentration of 1000 µg/ml.

### Preparation of working standard solution

From above standard stock solution of Vericiguat 0.4ml of solution was taken into 10 ml volumetric flask and was made to the mark with the methanol to get 40 µg/ml of Vericiguat.

### Preparation of sample stock solution

The average weight of 20 tablets was determined. Sample stock solution was prepared by dissolving tablet powder equivalent to 10 mg of Vericiguat was transferred to 10 ml volumetric flask. Then 8 ml methanol was added and sonicated for 10 mins to ensure complete solubilization of drug. After sonication, volume was made up to the mark with methanol to get concentration of 1000 µg/ml.

### Preparation of sample solution

Filter the sample stock solution with whatman filter paper. Then, 0.4ml was withdrawn and diluted to 10 ml using methanol to get concentration of 40 µg/ml is a sample stock solution

### Validation

The proposed method was validated as per ICH guidelines.

### Linearity

Different aliquots of 0.2 - 1.0 ml of standard stock solution was transferred into series of 10 ml volumetric flasks, separately and the volume was made up to the mark with mobile phase to get concentrations 20, 40, 60, 80 and 100 µg/ml, respectively.

### Accuracy

To the preanalysed sample solution, a known amount of standard stock solution was added at different levels i.e. 50, 100 and 150%. The solutions were reanalyzed by proposed method.

### Precision

The reproducibility of this method was determined by analyzing tablets at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision).

## III. RESULTS AND DISCUSSION

Method development and optimization The HPLC procedure was optimized with a view to develop a suitable LC method for the determination of Vericiguat in tablet dosage form. Initially, methanol and water in different ratios were tried. But Vericiguat gave broad peak with tailing, so acetonitrile was added and mixtures of acetonitrile, methanol and water in different ratios were tried. It was found that Acetonitrile: Methanol: Water in 0.1% Triethylamine (50:10:40, v/v/v) gave acceptable retention times (4.063 min) with flow rate of 1.0 ml/min as shown in figure 1.

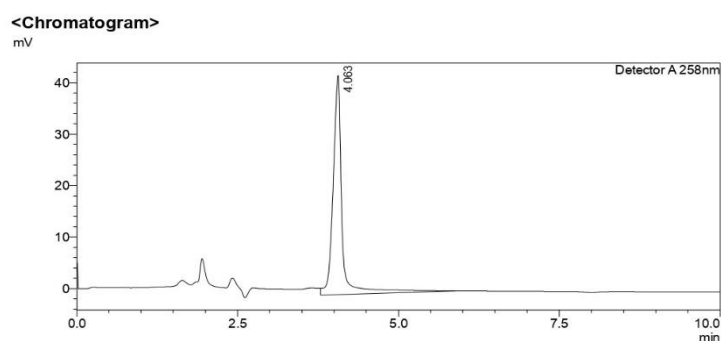


Figure 1: Chromatogram of Vericiguat obtained at optimum chromatographic conditions.

### Method Validation

The described method has been validated which include parameters like linearity, system suitability, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification)

### System suitability

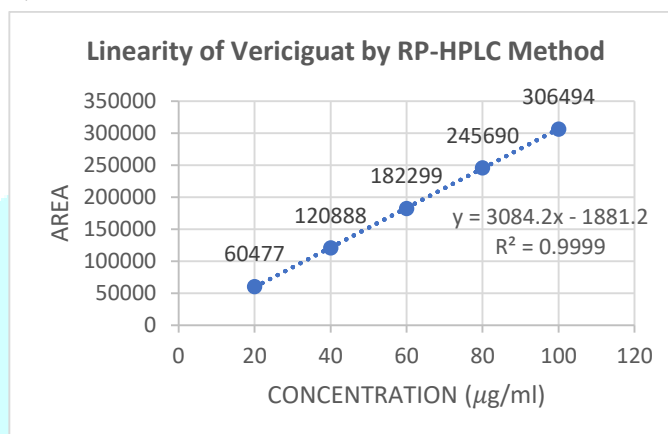
System suitability and chromatographic parameters were validated such as tailing factor and theoretical plates was calculated. The results are given in table 1

**Table I: Quantification of Verquvo Formulation**

Parameters	Vericiguat	Recommended limits
Retention time	4.063	RSD $\leq 2$
Peak area	120882	RSD $\leq 2$
Tailing factor (T)	1.436	T < 2
Theoretical plate (N)	4288	> 2000

### Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solution of Vericiguat at different concentration in the range of 20 -100  $\mu\text{g/ml}$  with correlation coefficient ( $r^2$ ) of 0.9999. Results are given in figure 2.



**Figure- 2: Linearity of Vericiguat.**

### Accuracy

Accuracy of the proposed method was determined by performing the recovery experiment. The recovery experiment was studied by adding known amount of standard Vericiguat to the pharmaceutical product and calculating the recovered standard amount. At 50%, 100% and 150% standard addition level mean recovery of Vericiguat found to be 99.88%, 100.28% and 100.16% respectively.

### Precision

Precision was evaluated at the repeatability and intermediate precision levels. For repeatability analysis, six independent portions of a tablet dosage form were processed through the full analytical method and results was evaluated obtaining a % RSD value of 0.8132 and average % purity of 100.12.

### Robustness

Robustness study was conducted by deliberate changes in mobile phase composition and flow rate, revealed that there was no significant variation in % assay.

### Limit of detection (LOD) and limit of quantification (LOQ)

The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration.

## IV. CONCLUSION

The Vericiguat in tablet formulation was determined using the HPLC method, which was developed and validated in accordance with ICH guidelines. According to the validation studies, the developed method was found to be quick, easy, accurate, precise, selective and economical. Therefore, this method is simple to use for regular analysis of Vericiguat in tablet dosage form.

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