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# Method development and validation of RP-HPLC method determination of vericiguat in bulk and formulation

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## Abstract

**Abstrac**t: Analytical Chemistry is a measurement of science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine Attempts were made to develop RP-HPLC method for simultaneous estimation of Vericiguat from Tablet. For the RP - Agilent Tech. Gradient System with Auto injector, DAD Detector Reverse Phase (Agilent) C18 column (4.6mm x 100 mm;2.5µm), a 20µl injection loop and UV730D Absorbance detector and running chemstation 10.1 software.

**Methanol**: water (0.1% acetic acid), (50:50) v/v, pH 3.was used as the mobile phase for the method. The detection wavelength was 258nm and flow rate was 0.8 ml/min. In the developed method, the retention time of Vericiguat was found to be being 5.268min. The method has been found to be better than previously reported method, because of its less retention time, isocratic mode and use of an economical and readily available mobile phase, readily available column, UV detection and better resolution of peaks.

**Keywords:** RP-HPLC; Vericiguat; Method Development; Validation; Mobile phase

## 1. Introduction

Analytical Chemistry is a measurement of science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine. It seeks ever improved means of measuring the chemical composition of natural and artificial materials

In recent years research in analytical chemistry has concerned mainly on the development and application of physical and physicochemical analytical methods, instrumental analysis, in which their speed and sensitivity have far surpassed the classical methods of gravimetric and volumetric analysis.

Chromatography may be defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases. It is based on the difference in the rate at which the components of mixture move through a porous medium (called stationary phase) under the influence of some solvent or gas (called moving phase).

The chromatographic method of separation, in general involve the following steps:

- Adsorption or retention of a substance or substances on the stationary phase.

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- Separation of the adsorbed substance by the mobile phase.
- Recovery of the separated substances by a continuous flow of the mobile phase; the method being called elution.
- Quantitative and qualitative analysis of the eluted substances.

HPLC is high resolution, high pressure and speed liquid chromatography. It has several times resolving power than open column liquid chromatography hence it is used for speedy resolution of complex mixture, separation and determination of species in a variety of organic, inorganic, and biological materials.

## 2. Advantages of HPLC

- It provides a specific, sensitive and precise method for analysis of different complicated samples.
- There is ease of sample preparation and sample introduction.
- There is speed of analysis
- The analysis by hplc is specific, accurate and precise.
- It offers advantage over gas chromatography in analysis of many polar, ionic substances, metabolic products and thermolabile as well as non volatile substances

## 3. Drug profile

### 3.1. Vericiguat

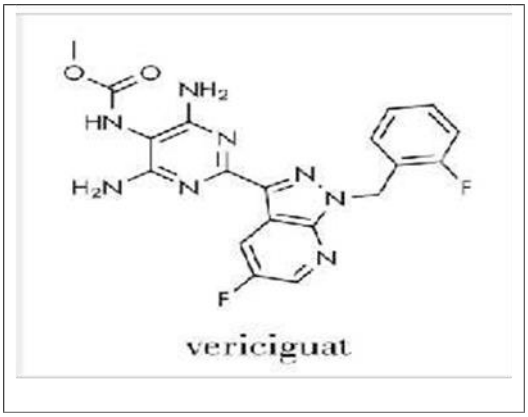


**Figure 1** Structure of Vericiguat

**Table 1** Profile of drug

| | |
|---|---|
| Molecular formula | $C_{19}H_{16}F_2N_8O_2$ |
| Molecular weight | 426.388 g·mol−1 |
| Chemical name | methyl *N*-[4,6-diamino-2-[5-fluoro-1-[(2 fluorophenyl)methyl]pyrazolo[3,4-b]pyridin-3-yl]pyrimidin-5-yl]carbamate |
| Description | White crystalline powder |
| Category | used to treat chronic heart failure |

## 4. Material and methods

### 4.1. Selection and Procurement of Drug

*4.1.1. Drug sample supplier*

**Table 2** Drug and Drug Supplier

| Name of Drug | Drug Supplier |
|---|---|
| Vericiguat | Swapnroop drugand pharmaceutical |

### 4.2. List of reagents & chemicals used

**Table 3** List of Reagents and Chemicals used

| Sr. No. | Name of chemicals | Manufacturer. |
|---|---|---|
| 1. | Acetonitrile (HPLC grade) | Merck Ltd., India |
| 2. | Methanol (HPLC) | Merck Ltd., India |
| 3. | water (HPLC grade) | Merck Ltd., India |
| 4. | 0.1% OPA (HPLC grade) | Merck Ltd., India |
| 5 | 0.1% Acetic Acid (HPLC grade) | Merck Ltd., India |

### 4.3. Selection of Analytical Technique

HPLC was selected as analytical technique for estimation of Vericiguat.

### 4.4. Instruments

The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector Equipped with Reverse Phase (Agilent) $C_{18}$ column (4.6mm x 100mm; 2.5µm), (*G1310A ISOPUMP*) pump, a 20µl injection loop and Diode Array Detector (DAD) and running Chemstation 10.1software.

### 4.5. Stock preparations

*4.5.1. Stock I : Standard Sample Preparation*

STD .Vericiguat10 mg in 10 ml Methanol = 1000 µgm/ml

*4.5.2. Stock II : formulation Preparation:-*

Take 15.78mgin 10 ml Methanol i.e. = 1000 µgm/ml

### 4.6. For Accuracy Solution Preparations

- Take 10 µgm/ml marketed formulation For Accuracy,
- 80 % = 0.1 ML marketed formulation and Add 8µgm/ml STD and make up vol 10 ml with mobile phase
- 100 % =0.1 ML marketed formulation and Add 10 µgm/ml STD and make up vol 10 ml with mobilephase 120 %= 0.1 ML marketed formulation and ADD12 µgm/ml STD and std makes up vol 10 ml with mobile phase

### 4.7. Instruments and Equipment's

**Table 4** Instrument (HPLC) Details used during Method Development

| | Name of Instrument | Company Name |
|---|---|---|
| 1 | HPLC Instrument | Agilent with auto sampler(DAD Detector) (chem-station) |
| 2 | UV-Spectrophotometer | Analytical Technologies Limited |
| 3 | Column($C_{18}$) | Agilent$C_{18}$ (100mmX 4.6mm,2.5µm) : |
| 4 | pH meter | VSI pH meter(VSI 1-B) |
| 5 | Balance | WENSAR™ High Resolution Balance. |
| 6 | Sonicator | Ultrasonic electronic instrument |

## 5. Experimental work

**Table 5** Chromatographic conditions (HPLC) details used during method Development

| 1. | HPLC | Agilent Tech. Gradient System With Auto injector, UV Detector |
|---|---|---|
| 2. | Software | chemstation 10.1 |
| 3. | Column | (Agilent) C18 column (4.6mm x 100mm |
| 4. | Particle size packing | 2.5 m |
| 5. | Stationary phase | C-18 ( Agilent) |
| 6. | Mobile Phase | Methanol : 0.1 % acetic acid 50 : 50 |
| 7. | Detection Wavelength | 258 nm |
| 8. | Flow rate | 0.8 ml/min |
| 9. | Temperature | Ambient |
| 10. | Sample size | 20 l |
| 11. | Ph | 3.2 |
| 12. | Run Time | 15 min |
| 13. | Filter paper | 0.45 m |

Analytical column : Agilent C18 Column (100mm x 4.6mm), 2.5µm particle size.

Injection volume : 20µl

Flow rate : 0.8 ml/min

Detection : 258 nm

Run Time : 15 min

Following Mobile phase were tried:

### 5.1. Method development of HPLC:

**Preparation of Stock Standard Solution:** Standard Solution Stock I : (Vericiguat)

Accurately weight and transfer 10mg Vericiguat working standard into 10 ml volumetric flask as about diluents Methanol completely and make volume up to the mark with the same solvent to get 1000µg/ml standard (stock solution) and 15 min Sonicate to dissolve it and the resulting stock solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol: (0.1%acetic acid)Water, prepared in 5.0ml MEOH: 5.0(0.1%acetic acid)ml Water v/v)solvent.

## 6. Result and discussion

Preliminary studies on Vericiguat.

### 6.1. Melting point

The procured reference standard of Vericiguat was found to melt in the range of 250-260 $^0$C respectively.

Solubility: The drug was found to be soluble in organic solvents such as Methanol, DMSO, DMF Insoluble in water.

### 6.2. UV Spectroscopy

UV absorption of 10 µg/mL solution of Vericiguat in methanol was generated and absorbance was taken in the range of 200-400 nm.258 nm λ max

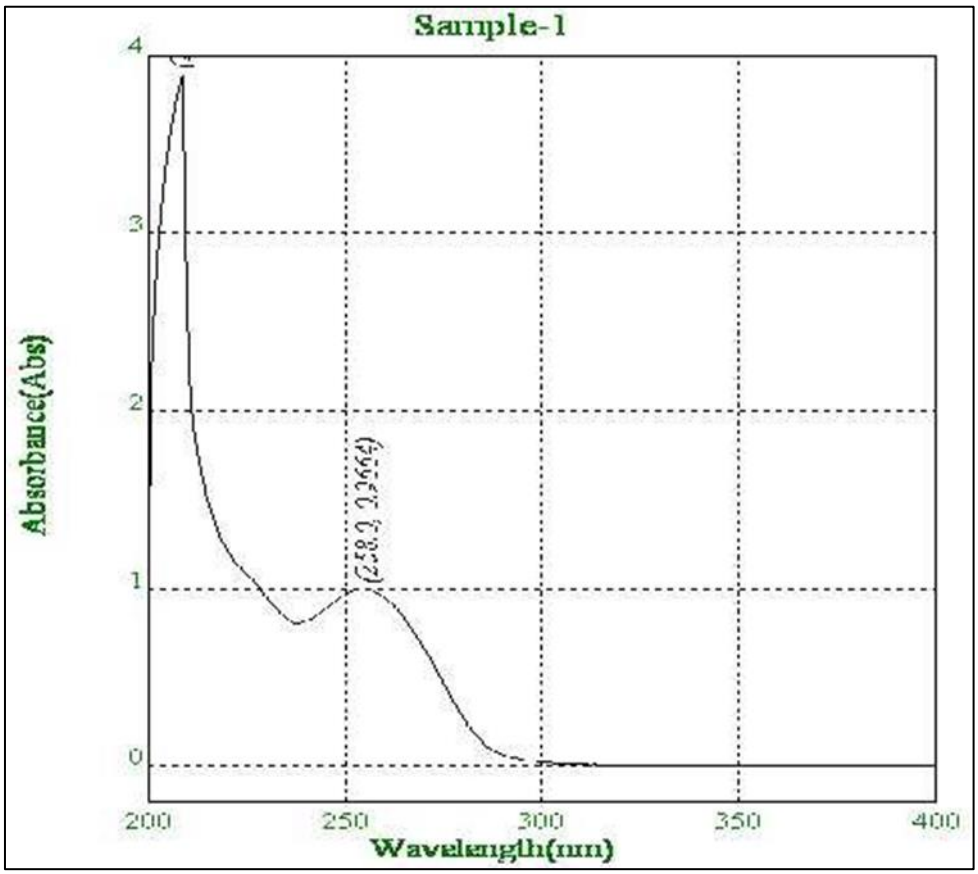


**Figure 2** UV spectrum of Vericiguat

Standard solutions were scanned in the range of 200-400nm, against 10 ml methanol and volume make with methanol solvent system as reference Vericiguat in methanol was found to be 258 nm, selected wavelength is 258 nm

### 6.3. Studies on the chromatographic behavior of Vericiguat.

After the selection of suitable mobile phase, it was then optimized for its reproducibility, sensitivity & accuracy. **The final chromatographic conditions selected were as follow:**

Analytical column: Agilent $C_{18}$ Column (100mm x 4.6 mm, 2.5µm particle size).

Injection volume: 20µl

Flow rate : 0.8 ml/min

Mobile phase : Methanol : water(0.1% acetic acid) (50: 50 % V/V)

Detection : 258 nm

Run Time : 15 min

### 6.4. Standard chromatogram of Vericiguat

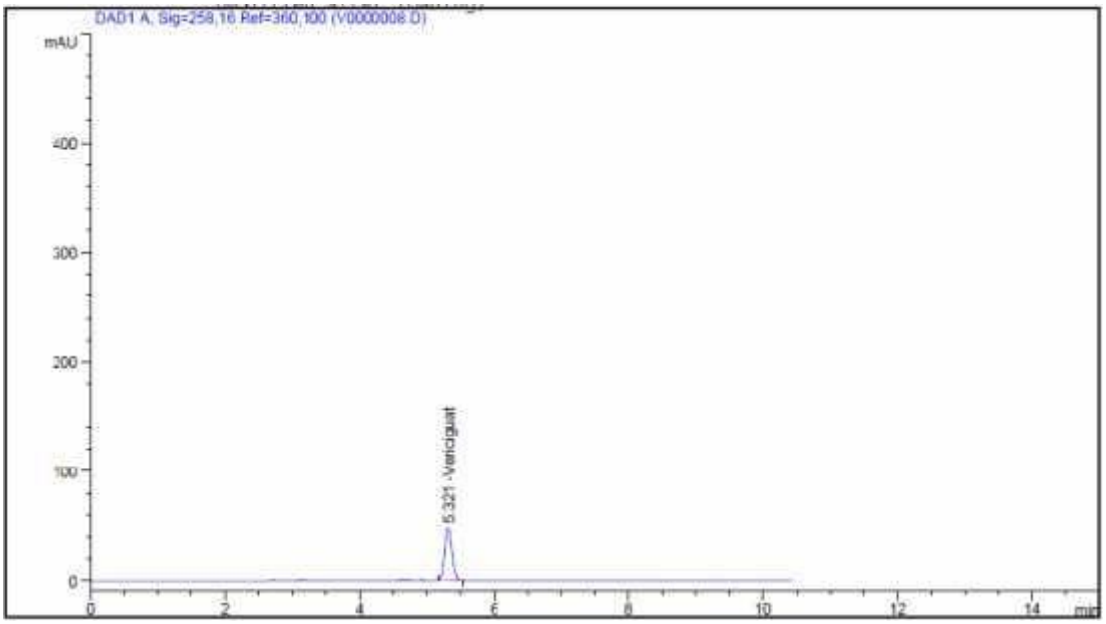


**Figure 3** Chromatogram of standard Vericiguat

**Table 6** Result for standard Chromatogram of Vericiguat

| No. | RT[min] | Area[mV*s] | TP | TF | Resolution |
|---|---|---|---|---|---|
| 1 | 5.321 | 358.03867 | 12504 | 0.86 | - |

### 6.5. Analytical of Method Validation:

*6.5.1. Linearity*

From Vericiguat standard stock solution, different working standard solution (10-50µg/ml) for HPLC were prepared in mobile phase 20µl of sample solution was injected into the chromatographic system using volume loop injector. Chromatograms were recorded.

**Table 7** Linearity study of Vericiguat

| Sr. No. | Concentrationµg/ml | Area Vericiguat |
|---|---|---|
| 1 | 10 | 358.86 |
| 2 | 20 | 667.03 |
| 3 | 30 | 995.09 |
| 4 | 40 | 1304.84 |
| 5 | 50 | 1606.57 |

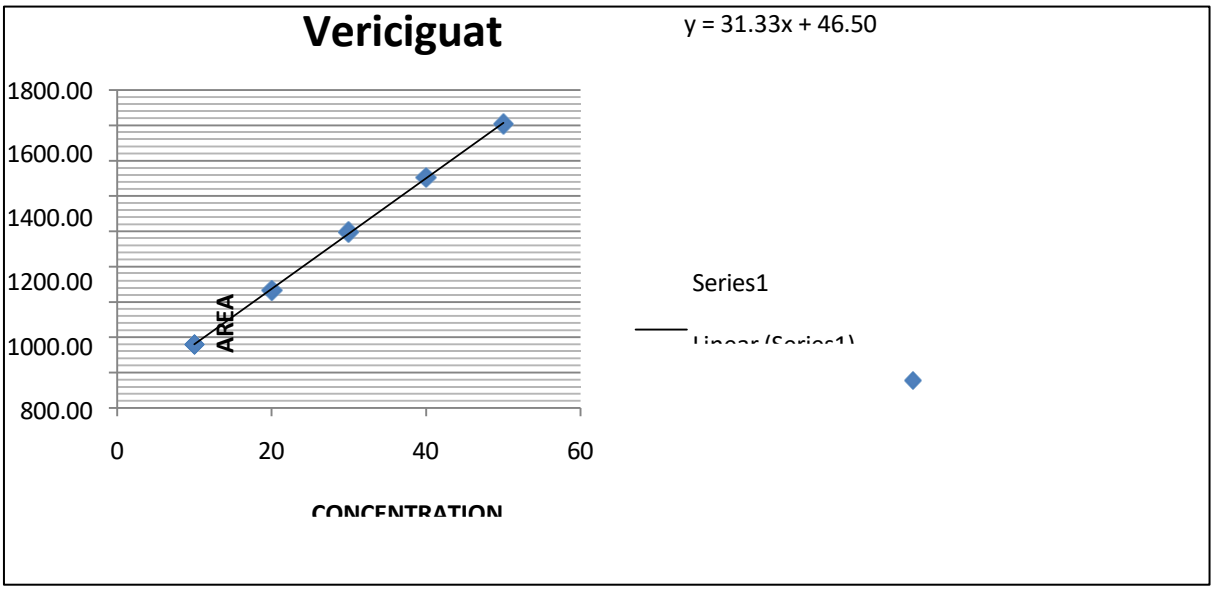


**Figure 4** Calibration curve of Vericiguat (HPLC)

**Table 8** Regression equation data for Vericiguat

| Regression Equation Data | Y=mx+c |
|---|---|
| Slope(m) | 31.33 |
| Intercept(c) | 46.50 |
| Correlation Coefficient | 0.999 |

Linearity of of Vericiguat was observed in the range of 10-50µg/ml. Detection wavelength used was 258 nm.

The calibration curve yielded correlation coefficient ($r^2$) 0.999 & 0.999 for Vericiguat respectively)

### 6.6. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed Tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed .Statistical validation of recovery studies shown

**Table 9** Result of Recovery data for Vericiguat

| Drug | Sr No. | Level (%) | Amt. taken (µg/ ml | Amt. Added (µg/ml | Area. Mean*± S.D. | Amt. recovered Mean*±S.D. | %Recovery Mean *± S.D. |
|---|---|---|---|---|---|---|---|
| | 1 | 80% | 10 | 8 | 17.95±0.01 | 7.96± 0.01 | 99.56±0.24 |
| HPLC | | | | | | | |
| method | | | | | | | |
| | 2 | 100 | 10 | 10 | 20.04±0.06 | 10.0±0.06 | 100.3± 0.64 |
| | | % | | | | | |
| | 3 | 120 | 10 | 12 | 21.84±0.03 | 11.84±0.03 | 98.67± 0.25 |
| | | % | | | | | |

*mean of each 2 reading.

**Table 10** Statistical Validation of Recovery Studies Vericiguat

| | Level of Recovery (%) | Mean % Recovery | Standard Deviation* | % RSD |
|---|---|---|---|---|
| HPLC | 80% | 99.56 | 0.24 | 0.24 |
| | 100% | 100.39 | 0.64 | 0.63 |
| | 120% | 98.67 | 0.25 | 0.26 |

*Denotes average of three determinations.

Accuracy of method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-102%

### 6.7. System suitability parameters :( Repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Vericiguat system suitability parameters were studied. The result shown in below

## 7. Result for Repeatability (SST):

**Table 11** Repeatability studies on Vericiguat (HPLC)

| Sr.No. | Concentration of Vericiguat(mg/ml) | Peak area | Amount found (mg) | % Amount found |
|---|---|---|---|---|
| 1 | 40 | 1313.9235 | 40.41 | 101.02 |
| 2 | 40 | 1310.9528 | 40.39 | 101.00 |
| | | Mean | 40.40 | 101.01 |
| | | SD | 2.10 | 2.10 |
| | | %RSD | 0.16 | 0.16 |

Repeatability studies Vericiguatwas found to be 101.01%, the %RSD was less than 2, which shows high percentage amount found in between 100% indicates the analytical method that concluded.

### 7.1. Precision

The method was established by analyzing various replicates standards of Vericiguat. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in respectively.

**Table 12** Result of Intraday and Inter day Precision for Vericiguat HPLC

| **Concⁿ (µg/ml) HPLC** | **Intraday Precision** | | | **Interday Precision** | | |
|---|---|---|---|---|---|---|
| | Mean± SD | %Amt Found | %RSD | Mean± SD | %Amt Found | %RSD |
| 10 | 354.01±1.46 | 98.15 | 0.41 | 354.31±0.17 | 98.25 | 0.05 |
| 30 | 991.50±9.19 | 100.54 | 0.93 | 995.87±7.72 | 101.01 | 0.78 |
| 50 | 1604.30±0.19 | 99.44 | 0.19 | 1630.51±14.42 | 101.12 | 0.88 |

*Mean of each 3 reading Intraday and Inter day Precision for Vericiguat which shows the high precision %amount in between 98% to 102% indicates to analytical method that concluded

### 7.2. Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

**Table 13** Result of Robustness Study of Vericiguat

| **Parameters** | **Conc.(µg/ ml)** | **Amount of detected(mean ±SD)** | **%RS D** |
|---|---|---|---|
| Mob-phase composition(51ml+49 ml)Methanol + 0.1% (acetic acid)water | 50 | 1612.0±3.08 | 0.19 |
| Mob-phase composition(49ml+51ml) Methanol + 0.1% (acetic acid)water | 50 | 1615.6±2.66 | 0.16 |
| Wavelength change 257nm | 50 | 1623.7±11.15 | 0.69 |
| Wavelength Change 259 nm | 50 | 1588.77±10.78 | 0.68 |
| Flow rate change(0.7ml) | 50 | 1842.38±2.24 | 0.12 |
| Flow rate change(0.9ml) | 50 | 1431.78±0.32 | 0.02 |

### 7.3. Robustness Study of Vericiguat

The changes were did flow rate (±1 ml/ $min^{-1}$), PH of mobile phase composition, andWavelength. %RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded. **(Table No.68)**

*7.3.1. Limit Detection*

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slopeThe limit of detection (LOD) may be expressed as:

**LOD = 3.3 (SD)/S**

= 3.3 X 1.25/ 31.33

**= 0.1320**

Where, SD = Standard deviation of Y intercept S = Slope

The LOD of Vericiguat was found to be 0.1320 (µg/mL) analytical methods that concluded.

*7.3.2. Limit Quantification*

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

**LOQ = 10 (SD)/ S**

=10 X 1.25/ 31.33

**= 0.40023**

Where, SD = Standard deviation Y intercept

S = Slope

The LOQ of Vericiguat was found to be **0.3949**(µg/mL) analytical methods that concluded.

**7.4. Analysis of formulation:-Procedure:**

Equivalent to 101.6mgVericiguat into 10 ml volumetric flask. Add about 10ml of diluents and Sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (40 µg/ml). The simple chromatogram of test Vericiguat Shown in **(Fig No: 459,60)** the amounts of Vericiguat per Tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with Tablet formulation. Tablet Assay for %Lable claim for %RSD Calculated, Results was shown in **(Table No.71,72).**

Brand Name : Verquvo (2.5mg)

Total weight of 20 tab Powder wt. = 5.08gm Avg. Powder Weight = 25.4mg

Eq.Wt for 10 mg= 10 x 25.4 / 2.5 = 101.6 MG

Take 101.6mgs in 10 ml Methanol i.e. = 1000 µg/ml tab solution ***-TAB STOCK –II***

Take 0.4 ml in 10 ml Mobile Phase i.e. = 40µg/ml tab solution

**Table 14** Analysis of marketed formulation. (HPLC)

| Sr.no | Amount present in mg | Area(I) | Amount found in mg | % Label claim |
|---|---|---|---|---|
| | VCG | VCG | VCG | VCG |
| 1 | 40 | 1305.08 | 40.17157 | 100.43 |
| 2 | 40 | 1309.5407 | 40.3141 | 100.79 |
| Mean | - | 1307.31 | 40.24 | 100.61 |
| SD | - | 3.158 | 0.101 | 0.252 |
| %RsD | - | 0.242 | 0.250 | 0.250 |

Analysis of marketed formulation were also % Lable Claim was found to be 98-101% Satisfactory are concluded.

Tablet Assay for % Lable Claim

**Table 15** Tablet for %Lable claim

| Sample | Label claimed | %Label claimed± SD | %RSD |
|---|---|---|---|
| Selepag | Vericiguat =2.5 mg | 100.61± 0.252 | 0.250 |

Tablet Assay for %Lable claim for were also was found to be 98% and 102%RSD are less than 2 satisfactory result that concluded

### 7.5. Ruggedness

The degree of reproducibility of test result obtains by the analysis of same sample under variety of Condition. Such as different analyst, laboratory Different instrument.

**Table 16** Analysis of Ruggedness.

| Assay | Drug | Conc | Amt.Found | %Amt Found | SD | %RSD |
|---|---|---|---|---|---|---|
| Vericiguat | Analyst1 | 66.00 | 66.926 | 101.40 | 0.06 | 0.31 |
| | Analyst2 | 66.00 | 66.899 | 101.36 | 0.96 | 0.97 |

### 7.6. Specificity

The analytes should have no interference from the extraneous components and be well resolved from them. Specificity is the procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

## 8. Summary and conclusion:

Vericiguat is guanylatecyclase (sGC) used to treat chronic heart failure, The present work deals with "Development and validation of RP-HPLC method for estimation of Vericiguat in Bulk Drug And it's Dosage form"

### 8.1. Summary for HPLC method

Attempts were made to develop RP-HPLC method for simultaneous estimation of Vericiguat from Tablet. For the RP - Agilent Tech. Gradient System with Auto injector, DAD Detector Reverse Phase (Agilent) C18 column (4.6mm x 100 mm;2.5µm), a 20µl injection loop and UV730D Absorbance detector and running chemstation 10.1 software.

Methanol: water (0.1% acetic acid), (50:50) v/v, pH 3.was used as the mobile phase for the method. The detection wavelength was 258nm and flow rate was 0.8 ml/min. In the developed method, the retention time of Vericiguat was found to be being 5.268min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible.

So the proposed methods can be used for the routine quality control analysis Vericiguat in bulk drug as well as in formulations.

### 8.2. Conclusions for HPLC method

The method provides selective quantification of Vericiguat This developed RP-HPLC method for estimation of Vericiguat is accurate, precise, robust and specific.

The method has been found to be better than previously reported method, because of its less retention time, isocratic mode and use of an economical and readily available mobile phase, readily available column, UV detection and better resolution of peaks.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

The authors declare no conflicts of interest, financial or otherwise.

*Availability of data and materials*

Supplementary material that is available on the publisher's website along with the published article contains spectroscopic data of all compounds.

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