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Original Article

A Validated Isocratic RP-HPLC Method for the Quantification of Vericiguat in Solid Dosage Forms

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ABSTRACT

An accurate, sensitive, precise, quick isocratic reverse phase HPLC (RP-HPLC) technique has been developed and validated for the quantification of Vericiguat in the solid dosage forms. The best separation was achieved on a 250 mm x 4.6 mm ID., 5µ-particle size Xterra®-Octadecyl-Silyl-3V-Reverse-Phase-C18-column with 0.03M KH2PO4 in water: acetonitrile (30:70 v/ v) with pH-3.2 in the isocratic mode of elution as mobile phase solvent at a speed of 0.5 ml/ min. UV detection was at 218 nm. Retention time of Vericiguat was found to be 5.8 minutes. With a correlation coefficient of about 0.998, peak-response was obtained as function of concentration over the range of 29.6 to 88.8 µg/ ml for Vericiguat. Vericiguat had shown to have a percentage assay of 109.73 %. Vericiguat had a limit of detection and a limit of quantification (LOQ) of 0.074 µg/ ml and 0.222 µg/ ml, respectively. The presence of excipients in the formulation had no effect on the assay method. The procedure is appropriate for use in QC- laboratories since it is quick and precise

Keywords: Vericiguat, Isocratic-RP-HPLC, solid dosage forms

INTRODUCTION

Vericiguat, (VERQUVO™; Merck & Co, Bayer AG), is the first Soluble Guanylate Cyclase (sGC) stimulator, approved to treat heart failure, to reduce the risk of cardiovascular death and heart failure hospitalization following a hospitalization for heart failure or need for outpatient intravenous (IV) diuretics in adults with symptomatic chronic heart failure and ejection fraction less than 45% [1]. Vericiguat directly stimulates sGC by binding to a target site on its beta-subunit, bypassing the need for NO-mediated activation, and enhances the effects of NO by stabilizing the NO-sGC binding [2] and in doing so causes an increase in the production of intracellular cGMP that results in vascular smooth muscle relaxation and vasodilation [3].

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Vericiguat restores the deficiency in this signaling pathway, through stimulation and activation of sGC, aiming to increase cGMP levels, with a reduction in HF-related oxidative stress and endothelial dysfunction [4].

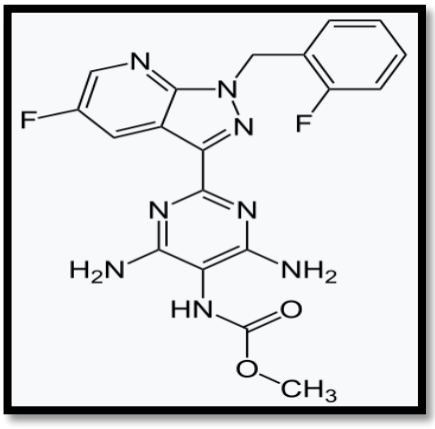

Figure 1: Structure of Vericiguat

It has a molecular formula and molecular weight is C19H16F2N8O2 and 426.4 g/mol respectively and its IUPAC name is Methyl (4,6-diamino-2-(5-fluoro-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b] pyridin-3-yl) pyrimidin-

5-yl) carbamate [5]. Very few methods for the determination of Vericiguat in oral fixed dosage form have been reported [6-12]. Furthermore, no official or preliminary monograph on this drug has been published in any of the compendial pharmacopoeias. The goal of this study was to develop an accurate and efficient RP-HPLC method to estimate Vericiguat in fixed dosage forms for oral administration. The validation of the devised approach is also addressed in this study, as per ICH standards [13].

Experimental:

Chemicals and Reagents:

- Vericiguat of 99% pure were acquired from Dr. Reddy's Laboratories Pvt Ltd, India.
- HPLC- Grade Acetonitrile - Rankem-Fine-Chemicals
- Potassium di hydrogen phosphate obtained from Qualigen-Fine chemicals.
- Chromatographic-Grade water was obtained from Rankem-Fine-Chemicals

Chromatographic-Instrument:

Quantitative HPLC was carried out on a waters 2996 high-performance liquid chromatograph with a PDA detector module, which included an automated injector with a 20 microliters injection volume and a quadra-pump. The column utilized was a Reverse_Phase_XTerra-Octa-Decyl-Silane-3V-C18 column (250mm x 4.6 mm internal diameter with particle size 5µm). Empower Software was installed on the HPLC equipment. The ambient column temperature was maintained and eluted over 14.0 minutes at a mobile solvent speed of 0.5 ml/ min under isocratic conditions. The organic modifier used is acetonitrile, while the mobile phase is 0.03M KH2PO4 in water: Acetonitrile (30:70 v/ v) with pH-3.2. It was degassed and filtered via 0.45-m Nylon membrane filters before use. For the analyte, UV detection at 218 nm was used as the wavelength of detection with a PDA detector. 0.03M KH2PO4 in water: Acetonitrile (50:50 v/ v) is used as diluent to make the standard dilutions. Vericiguat was eluted at 5.8 minutes.

Preparation of the Primary Standard Drug solutions: To make the primary standard stock solution, 37mg of Vericiguat was dissolved in a volumetric flask (100 ml) with 20ml of diluent ie., 0.03M KH2PO4 in water: Acetonitrile (50:50 v/ v), sonicated for 15 minutes, and then brought up to 50 ml with the diluent to get the primary standard stock solution containing 740µg/ ml of Vericiguat.

Preparation of Working Standard Drug Solution: After adding 5 ml of the primary working standard solution to the 50 ml volumetric flask, the flask was filled with 50 ml of the diluent. This resultant solution, which includes 74 ug/ ml of Vericiguat, is suitable for use as a working standard solution. The working stock solution was kept in a cool, dark place that was controlled to be at 4°C.

Sample Preparation:

After measuring the weight of each individual tablet, we were able to calculate the average weight of twenty Verquvo® tablets prepared in-house. The tablets were crushed into a powder form and a sample weight equivalent to containing 37-mg of pure Vericiguat was taken. This was shifted to a 50 ml pre-calibrated-measuring flask, and dissolved in a blend of 0.03M KH2PO4 in water: Acetonitrile (50:50 v/ v). After being sonicated in the diluent and strained via Whattman#41 filter paper, the resultant primary working sample solution contained 740 µg/ ml of Vericiguat. After quantitatively transferring 5 ml of the filtrate to a 50 ml pre-calibrated-measuring flask, the diluent was added to bring the volume of the solution to 50 ml. This solution served as the working testing solution having 74 µg/ ml of Vericiguat. The stock solutions were kept in a dark place at 4 degrees centigrade.

Discussion and Results:

The purpose of this research was to create a chromatographic technique for the quantifiable determination of fixed-dose of Vericiguat.

Optimized Chromatographic Conditions:

The chromatographic conditions were optimized finally using 0.03M KH2PO4 in water: Acetonitrile (30:70 v/v) with pH-3.2 as the eluting solvents in isocratic mode at a flow rate of 0.5 ml/ min with an injection volume of 20 µL. Run time was 14 minutes, at an ambient column oven temp with 0.03M KH2PO4 in water :Acetonitrile (50:50 v/ v), sonicated and degassed, as the diluent in a XTerra RP18 C18 column (250mm x 4.6 mm internal diameter with particle size 5µm). The detection was done by Photo diode array (PDA) detector at a wavelength of 218nm. The retention time was found to be 5.8 mins.

Linearity:

Aliquots of Vericiguat working stock solutions were placed in various 10 ml volumetric flasks and made the volume up to the 10 ml with the mobile phase, yielding in final strengths of 29.6- 88.8µg/ ml (Table 2). The peak areas and retention times of the drug solution (loaded at 20µL) were measured thrice in the column. Using a PDA-detector set at 218 nm, a linearity-graph was generated by plotting peak areas-vs- Vericiguat concentrations in µg/ ml

Accuracy:

The approach's accuracy was found by evaluating the drugs' recovery using the standard-spiking method. To assess if the analytes contained in the formulation caused positive or negative interventions, known amounts of each drug equivalent to 10 percent standard drug solution were added to 80 percent, 100 percent, and 120 percent of the target test concentrations a formulation mixture. Each set-of-addition was replicated thrice at each dilution level. The results are compared to a competent reference standard after extraction of sample preparation. The percentage of analytes recovered by the assay was used to assess the accuracy. Table 3 shows the results of accuracy investigations on standard solution and process-related impurity; recovery measurements suggest that the procedure was accurate.

Precision:

Quality-control samples in 100 % (w/ v) dilution were used to assess intraday and inter-day precision. On the same day, six replicates of the target concentrations were examined for intra-day variation, and six replicates were examined for inter-day variation on three different days. The method's repeatability is indicated by the low RSD value (1%). (Table 4)

Limits of Detection and Quantification:

The method's LOD was set at the lowest concentrations of active pharmaceutical components with a signal-to-noise (S/N) ratio of around 3. (LOD). The lowest active therapeutic medication concentrations that can be assessed with acceptable precision and accuracy while maintaining a signal-to-noise (S/N) ratio of roughly 10 (LOQ).

Method Applicability:

This study evaluated the newly created method by applying it to pharmaceutical tablets for the estimation of Vericiguat.

Results and Discussion:

Optimization of Chromatographic Conditions:

An isocratic RP- HPLC procedure for assaying the active ingredients was developed due to lack of an easy, reproducible, and quick-to-use method for the determination of Vericiguat concentrations in formulary matrices. The effect of various HPLC technique variables were examined on the result of the study to optimize the chromatographic parameters, various proportions of CH3CN-H2O, CH3CN: O-H3PO3 and CH3CN-KH2PO4 buffer were tested. After several early investigatory tests, 0.03M KH2PO4 in water: Acetonitrile with pH-3.2 binary system at the proportion of (30:70 v/ v) was chosen over other mobile phases because it resulted in improved peak shape of the active component. This procedure gives the good chromatographic detection of analyte after multiple exploratory & investigatory trail runs. The active pharmaceutical analyte had excellent UV sensitivity and was interference-free at 218 nm. The analyte peak was highly defined and without any incidence of tailing under these conditions. The set of conditions previously noted in this article were chosen for additional validation after considering the entire body of data acquired from this extensive study.

Method Validation Tests:

Method precision (RSD, percent), method accuracy (recovery percent & %RSD,), linear range (r2), and LOD & LOQ were explored as recommended method validation characteristics.

Linearity:

With a correlation coefficient of 0.998, the graph of chromatographic-peak areas of the analyte versus respective concentrations was shown to be linear in the band of 29.6-88.8 µg/ ml for Vericiguat (Table 2). The least square fit data of linear regression analysis was derived from the measurements and is given in Table 1. The regression line for Vericiguat is y = 172589x. Table 1 presents the regression parameters for this technique that include slope, intercept and % RSD. These findings suggest that there was a significant correlation.

Accuracy:

Individual recovery of analyte at 80 %-dilution level on w/v basis, 100 %-dilution level on w/v basis and 120 %-dilution level on w/v basis of prescribed concentrations was 82.81 % to 119 % for Vericiguat, demonstrating the method's accuracy. The RSD was usually less than 1% in these data, demonstrating that the technique seems to be very accurate and generates consistent results (Table 3)

Precision:

Table 4 summarizes the intraday and interday fluctuation in precision analysis. The method's repeatability is indicated by the low RSD value (less than-1%). These results show that the approach has a high level of precision and repeatability, both within a single analytical run and across multiple runs (Table 4).

Limit-of-Detection & Limit-of-Quantifications:

Vericiguat has a limit of detection (LOD) and limit of quantification (LOQ) of 0.074 µg/ ml and 0.222 µg/ ml respectively. These numbers illustrate the method's high sensitivity, which is essential in most investigations, as well as the fact that it can be used to detect and quantify analytes over a wide concentration range.

Specificity:

The retention time for Vericiguat was determined to be 5.8 minutes, according to the representative chromatogram given in Figure 2. When the pharmaceutical tablet matrices were evaluated, no indication of excipient interference signals was observed in the respective retention time of the chromatogram. It indicates that the analyte was not disturbed by any probable merging peaks. As a result, this technique can be employed with certainty.

Table 1: Regression analysis & Operating-System Suitability Results

Study-Parameter	Vericiguat
Retention Time (min)	**5.8**
Peak areas	**12169016**
Percentage of peak areas	**99.48**
USP-Tailing	**1.23**
Theoretical Plates	**9360.21**
Resolution	**11.4**
Linear range (µg/ ml)	**29.6-88.8**
Limit-of-Detection (µg/ ml)	**0.074**
Limit-of-Quantification (µg/ ml)	**0.222**
Correlation-Coefficient (r^2)	**0.998**
Assay-in-Percentage (%)	**108.77**

Table 2: Summary of the standard calibration Curve for Linearity experiment

Calibration Standard Dilution Level	Concentration of Vericiguat (µg/ ml)	Peak Area
40 %	29.6	5244364
60 %	44.4	7554246
80%	59.2	10442599
100 %	74	12835916
120 %	88.8	15131202

Table 3: Accuracy evaluation by Spike-analysis method

Accuracy study at 80% target level	Injection Number	Vericiguat (Verquvo)	
		Standard Soln	**Spiked Soln.**
Verquvo-® tablet dosage form solution at 80% level was spiked with 10% of standard solution of Vericiguat	1	10310814	11893026
	2	10441476	11928847
	3	10342150	11912587
	Mean area	10392895	11916385
	Std. Dev	66942	120615
	% RSD	0.62	0.99
	%Recovery		104.63
80% of the target concentration is equivalent to Vericiguat 400 µg/ ml in 0.03M KH2PO4 in water: Acetonitrile (50:50 v/ v) as the diluent.			
Accuracy study at 100% target level	**Injection Number**	**Vericiguat (Verquvo)**	
		Standard Soln.	**Spiked Soln.**
Verquvo-® tablet dosage form solution at 100% level was spiked with 10% of standard solution of Vericiguat	**1**	**13174252**	**14392305**
	2	**13165413**	**14386513**
	3	**13147851**	**14383425**
	Mean area	**13155779**	**14387638**
	Std. Dev	**25238**	**2141**
	% RSD	**0.22**	**0.09**
	% Recovery		**82.81**
100% of the target concentration is equivalent to Vericiguat 500 µg/ ml in 0.03M KH_2PO_4 in water: Acetonitrile (50:50 v/ v) as the diluent.			
Accuracy study at 120% target level	**Injection Number**	**Vericiguat (Verquvo)**	
		Standard Soln.	**Spiked Soln.**
Verquvo-® tablet dosage form solution at 120% level was spiked with 10% of standard solution of Vericiguat	**1**	**15489409**	**17374064**
	2	**15630346**	**17301206**
	3	**15812299**	**17343279**
	Mean area	**15600112**	**17330140**
	Std. Dev	**896049**	**55488**
	% RSD	**0.62**	**0.33**
	%Recovery		**119.0**
120% of the target concentration is equivalent to Vericiguat 600 µg/ ml in 0.03M KH2PO4 in water: Acetonitrile (50:50 v/ v) as the diluent.			

Table 4: Evaluation of precision with-in-day and day-to-day analysis

Intra-Day Precision study of 100% standard dilution containing 500µg/ ml of Vericiguat			Inter-Day Precision study of 100% standard dilution containing 500µg/ ml of Vericiguat	
S.no	**Vericiguat**		**Vericiguat**	
	Ret. time	**Peak area**	**Ret. time**	**Peak area**
1	**5.469**	**12118799**	**5.737**	**12423348**
2	**5.630**	**12498825**	**5.741**	**12342997**
3	**5.678**	**12238216**	**5.603**	**12345453**
4	**5.661**	**12355307**	**5.686**	**12397761**
5	**5.722**	**12330351**	**5.856**	**12337335**
6	**5.829**	**12401614**	**5.736**	**12385627**
Average	**5.665**	**12330912**	**5.726**	**12355211**
Std. Dev	**0.118**	**106110**	**0.082**	**75872**
% RSD	**2.09**	**0.88**	**1.44**	**0.62**

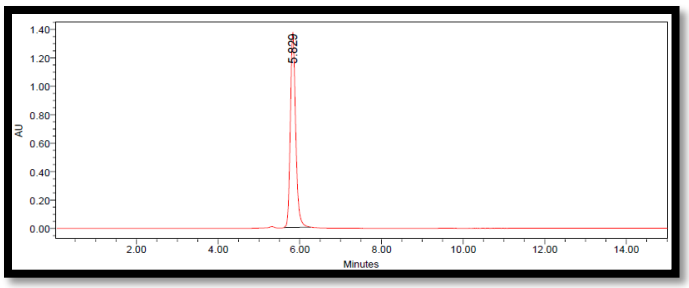

Figure 2: Chromatogram of Vericiguat-74µg/ ml analyzed by optimized Isocratic RP-HPLC method

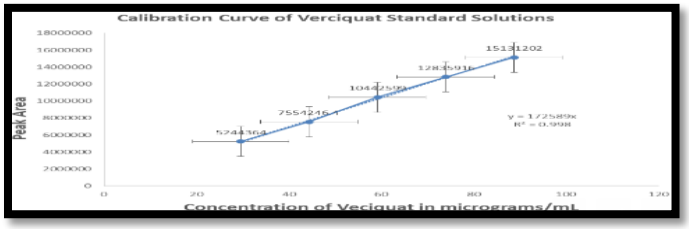

Figure-3: Linearity graph of Vericiguat dilution of standard solutions

Conclusion:

In this study, an efficient and commonly available HPLC method for the analysis of Vericiguat in pharmaceutical dosage form was devised. This method's key advantages are its significantly economical method and ease of operation. All these features are critical in operation, especially when analyzing a large number of samples. The validation experiments demonstrated that the procedural approach has a large calibration concentration range, adequate precision & accuracy and practically reliable sensitivity. The method can be used for regular analysis in formulation QC-studies and allows for a straightforward, selective, sensitive, and specific assessment of Vericiguat.

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