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METHOD DEVELOPMENT AND VALIDATION OF VORICONAZOLE FOR INJECTION BY RP-HPLC

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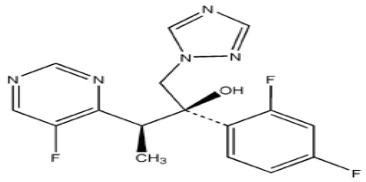
ABSTRACT

A new simple**,** precise, accurate and selective RP-HPLC method has been developed and validated for voriconazole in parental dosage form. The method was carried out on an Intersil ODS-C_{18} (150X4.6X5µ) column with a mobile phase consisting of mixed phosphate buffer, ACN and Methanol (65:30:5) and flow rate of 2.0 mL min-1. Detection was carried out at 257 nm. The retention time for VCZ was found to be 6.413 min. The VCZ % recovery was within the range between 99.55-99.63%. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per ICH guidelines. The developed method was validated for precision, accuracy, sensitivity and robustness. The developed method can be used for routine analysis of titled drug in formulation.

Keywords: Voriconazole; RP-HPLC, Intersil ODS, Validation.

INTRODUCTION

Voriconazole is prepared by Pfizer Pharma as a formulation and is used a Triazole antifungal medication that is generally used to treat serious, invasive fungal infections as under brand name Vfend [1-3]**.**



voriconazole

MATERIALS AND METHODS

Chemicals & Instrumentation

Voriconazole was obtained from Pfizer pharmaceuticals, India as a gift sample respectively. Acetonitrile (HPLC Grade), ortho-phosphoric acid, Methanol (HPLC Grade), Water for injection, were purchased from Merck (India) Ltd. India. Formulation Voriconazole injection was obtained from Natco containing VCZ (2mg/vial).

Analysis was performed on chromatographic system of Equipment High performance liquid chromatography ALLIANCE, PDA DETECTOR 2998 equipped with auto sampler. HPLC is controlled by Empower 2 software. Column Intersil ODS-C_{18} (150X4.6X5µ), Mobile phase having Buffer, ACN and Methanol (65:30:05) where Flow rate is 2mL/min, Wavelength is 257 nm, Injection volume 20 µL, Column oven at Ambient and Run time 11 minutes .Mobile phase and sample solutions were filtered through a 0.45µm membrane filter and degassed.

Preparation of Standard Solution

50mg of Voriconazole standard was accurately weighed and transfer into 100mL volumetric flask and 50mL of the diluent was added to it and shaken to dissolve and volume was adjusted to 100mL with diluent and sonicated for 15min to remove dissolve gases [4-7].

Preparation of Sample Solution

Take two vial which containing drug, to each vial add 18.8ml of water for injection, shake well to get a clear

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solution this solution was pooled in to 200ml volumetric flask, rinse the vial twice with water for injection. Made up the final volume. From the pooled sample take 5ml into 100ml volumetric flask then made up to final volume with diluents and mix. Filter the solution through membrane filter having the pore size 0.45µ [8-11].

Validation of Method

The HPLC method was validated in accordance with ICH guidelines [5,6,12].

Precision

The precision of the method was performed in the intra-day studies five repeated injections of standard solution were made and the response factor of drug peak and % RSD were calculated. From the data obtained the developed method was found to be precise.

Accuracy

The accuracy of measurement is defined as the closeness of the measured value to the true value. Typically, accuracy is represented and determined by recovery studies. This study was performed by spiking analyte matrices. For assay methods, spiked placebo samples are prepared at three concentrations of 80%, 100% & 120%.

Robustness

Robustness was evaluated by making deliberate variations in few method parameters such as variation of the temperature; flow rate, change in mobile phase composition, wavelength.

Limit of detection and Limit of quantitation

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, where 'ASD' is the average standard deviation and 'S' is the slope of the line.

RESULTS AND DISCUSSION

Selection of Chromatographic Conditions and Optimization of Mobile Phase:

Preparation of Phosphate buffer

Mix 1mL of orthophosphoric acid to 100mL of water filter the solution through 0.45 micron or finer porosity membrane filter and degas by sonication.

Preparation of mobile phase

Prepare a suitable quantity of a mixture of Buffer, ACN, and Methanol in the ratio 65:30:05 and degas by sonication.

Linearity

The linearity of the method was demonstrated over the concentration ranges of 7.94, 8.93, 9.92, 10.92, 11.91ppm for VCZ. preparation of standard stock solution 50mg working standard drug is transferred into 25mL volumetric flask which contains 2000 µg/mL further pipette out Aliquots of 4mL, 4.5mL, 5ml, 5.5mL, 6mL, and for Voriconazole was prepared from above prepared standard stock solution. Different concentrations of the pure drugs were injected into the chromatographic system. Calibration curve of Voriconazole were constructed by plotting peak area vs applied concentrations and shown in Fig. 2. The obtained results have shown an excellent correlation between peak area and concentration of pure drug within the concentration range. The correlation coefficient for the average area at each level vs concentration of analyte was calculated and presented in Table 1.

Precision

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or relative standard deviation. In the above Precision study was assessed by injection repeatability tests. For injection repeatability mixed standard solution of VCZ were injected in replicate. In this method precision was confirmed by low % RSD values of peak area for all components and reported in Table 2. The % RSD values was 0.09 value should be within 2 and the method was found to be precise

Accuracy

Preparation of Standard Solution

Accurately weighed about, 50 mg of voriconazole working standards are transferred separately into 25mL clean dry volumetric flask, added about 5mL of diluent and sonicated to dissolve it completely and make the volume up to the mark with diluent. Further pipette out 4 mL of the Voriconazole above stock solutions into a 50mL volumetric flask and diluted up to the mark with diluent to get the concentrations of 160µg/mL respectively. These stock solutions were filtered through 0.45µm membrane filter paper by using vacuum filter.

Sensitivity

The LOD VCZ was found to be 0.041271µg/ml respectively. The LOQ for VCZ was found to be 0.1250636µg/ml respectively. The low values of LOD and LOQ indicates high sensitivity of the method.

Robustness

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. the low values of % RSD (less than 2 %) indicating robustness of the method.

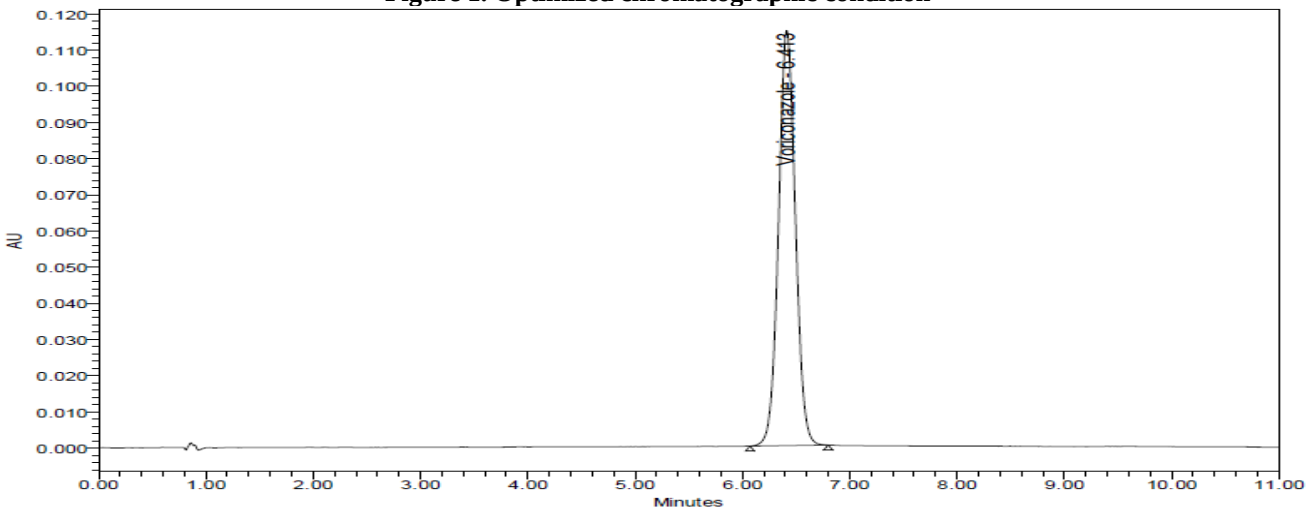
System Suitability Test

According to USP, system suitability test are integral part of liquid chromatographic methods. The

resolution, number of theoretical plates, peak asymmetry and capacity factor were calculated for standard solutions.

The results obtained from validation of the methods and system suitability studies are summarized in Table 5.

Figure 1. Optimized chromatographic condition



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Voriconazole	6.413	1251518	100.00	8022	1.04

Figure 2. Linearity calibration graph

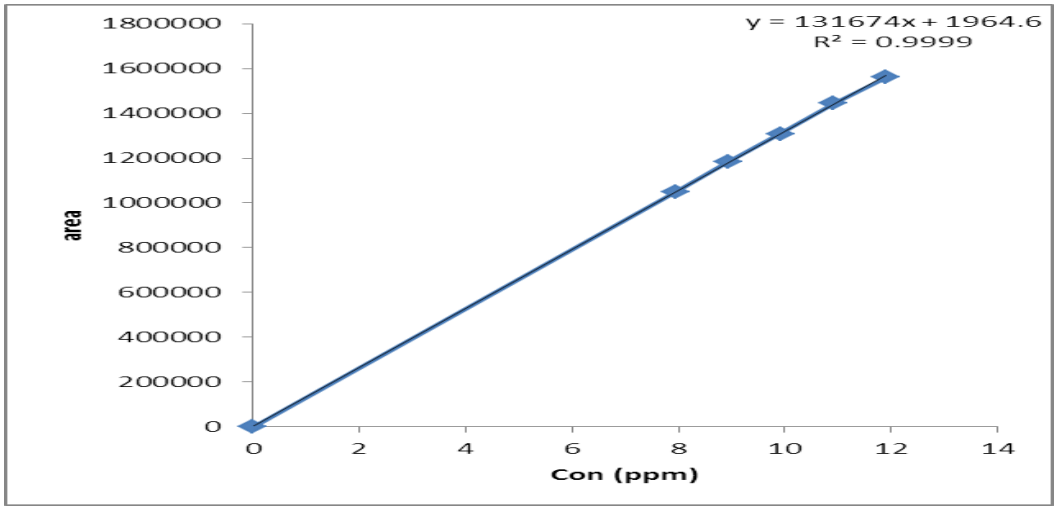


Table 1. Linearity data

S . No	Conc.(ppm)	Standard concentration (ppm)	Area
1	80%	7.94	1048997.3
2	90%	8.93	1182457.3
3	100%	9.92	1307767.6
4	110%	10.92	1444131.3
5	120%	11.91	1562098.3
Correlation coefficient			0.999965
Slope (m)			129774
Intercept (c)			21214
Bias for 100% response			0.91

Table 2. Precision

SNO	INJECTIONS	AREA
1.	**INJECTION 1**	1275625
2.	**INJECTION 2**	1273217
3.	**INJECTION 3**	1275606
4.	**INJECTION 4**	1273630
5.	**INJECTION 5**	1275597
6.	**INJECTION 6**	1275543

Table 3. Accuracy

drug	% Level	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean % recovery
VCZ	80	7.92	7.90	99.63	99.76
	100	9.91	9.90	99.96	
	120	11.89	11.85	99.71	

Table 4. Assay Results

S.No.	DRUG NAME	LABLE CLAIM (mg/vial)	AMOUNT FOUND (mg/vial)	% ASSAY FOUND
1	VORICONAZOLE	200	1.919461	202

Table 5. System suitability parameters

S.No.	Parameters	VORICONAZOLE
1	% R.S.D	0.12
2	Tailing factor (T)	1.04
3	No. of theoretical plates (N)	8022

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of voriconizole in formulation. The method was validated as per ICH guidelines.

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