An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF VORICONAZOLE IN THE BULK AND PHARMACEUTICAL FORMULATION

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ARTICLE INFO

Key Words

Voriconazole, RP-HPLC, Method Development

Access this article online Website: https://www.jgtps.com/

Quick Response Code:

ABSTRACT

A simple, precise, specific, and accurate RP-HPLC method has been developed and validated for the estimation of Voriconazole in bulk and capsule dosage formulation. The separation was achieved by Enable C18 column (250 X 4.6mm, 5µm particle size) using a mobile phase consisting of Methanol and acetonitrile (60:40 v/v) at a flow rate of 1ml / min using detection wavelength at 256nm. The method was developed in isocratic mode. The retention time was around 2.841 minutes. The method showed linearity with correlation coefficient R^2=0.998 over the range of 10-60 µg/mL. The mean recoveries were found to be in the range of 98.5-98.9% for Voriconazole. The method was validated as per ICH guidelines for linearity, the limit of detection, the limit of quantification, accuracy, precision, and robustness, ruggedness. The method can be successfully applied for routine analysis of the quantitative determination of Voriconazole in the pharmaceutical dosage form.

INTRODUCTION

Voriconazole is chemically (2R, 3S)-2-(2, 4-Difluorophenyl)-3-(5-fluoropyrimidine-4-yl)-1-(1H-1, 2, 4- triazole-1-yl) butane-2-ol[1]. Voriconazole is a triazole antifungal medication[2] used to treat serious fungal infections. It is used to treat invasive fungal infections that are generally seen in patients who are immune comprised. A Literature survey reveals a very few spectrophotometric and HPLC methods were reported for the estimation of voriconazole[3-12], but there is still a need for the development of a sensitive HPLC method for the estimation of voriconazole with complete validation as per ICH guidelines.

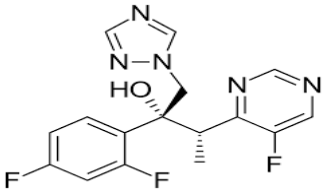

Figure 1: Chemical structure of voriconazole

MATERIALS AND METHODS

Chemicals and reagents

Voriconazole with a purity of 99.8% w/w was obtained as a gift sample and Market formulation of Voriconazole (Vorier®) was procured from the local market, chemicals and reagents used are of analytical grade. Chemicals like Acetonitrile and water are of

HPLC grade obtained from Merck Life sciences Pvt Limited, Mumbai.

Instruments and Apparatus

ELITE analytical balance and Shimadzu LC-20 AD HPLC with abinary pump, and a UV SPD-20A detector are used. Rheodyne injector fitted with a 20 µL loop was used and data were recorded and analyzed using LC solutions software. An enable C18 column (250 ×4.6mm, 5µm particle) was used.

Chromatographic conditions

Chromatographic separation was achieved in an isocratic mode. An Enable C18 Column (250 X 4.6mm, 5µm particle size). The mobile phase consisting of methanol: acetonitrile (60:40 v/v). The mobile phase was filtered through Nylon 0.45µm, 47mm membrane filter and was degassed before use. The flow rate was 1.0mL/min. The determination was carried out at 256nm and the injection volume was 20µl. The total run time was 20 min were optimized for the separation of Voriconazole. The samples were analyzed by a UV detector covering in the range of 200-400nm.

Diluent: Methanol and acetonitrile in the ratio 60:40 was used.

METHOD DEVELOPMENT

Accurately weighed 100mg of drug and was transferred into 100ml clean dry volumetric flask the contents were dissolved using diluents and sonicated for 15mins, later the volume was made up using diluents. Further 10ml from the above stock solution was transferred to a 100ml volumetric flask and made up to mark with diluents to get a concentration of 100µg/ml. In setting up the optimized conditions for development, the choice of detection wavelength was based on the scanned absorption spectrum obtained from the UV spectrum of Voriconazole, which was obtained by scanning the sample over the wavelength range of 200-400nm against blank as diluent. After a thorough examination of the spectra, the maximum absorbance was obtained at the wavelength 256nm as shown in Figure 2, which was selected for further analysis.

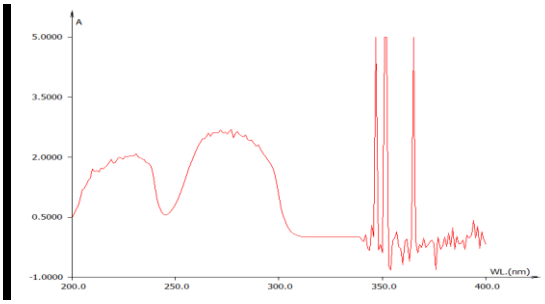

Figure 2: Absorbance spectra of Voriconazole

Preparation of mobile phase: Methanol and acetonitrile are separately taken into individual flasks and sonicated for 30 min and filtered using a 0.45 µ filter under vacuum filtration.

Preparation of standard stock solution: A standard drug solution of voriconazole was prepared by transferring 100mg of the drug into a 100ml of volumetric flask and made up to the mark with methanol to get a concentration of 1000µg/ml.

Preparation of working stock solution: From the above standard stock solution, 10ml of the sample solution was transferred to a 100ml volumetric flask and made up to the mark with diluents to get a concentration of 100µg/ml.

Preparation of sample solution: The proposed method was applied to analyze the commercially available Voriconazole - Vorier® (200mg). 10 tablets were weighed and powdered, the amount equivalent to 100mg of was weighed Voriconazole accurately and transferred into 100mL volumetric flask containing methanol which was further sonicated for 30 min with vigorous shaking, the volume was brought up to 100mL with methanol. The solution was subjected to filtration through Whattman filter paper #44. The filtrate was diluted suitably with diluents to get a final solution of 100µg/ml concentration. This was subsequently analyzed using a UV detector; the chromatogram obtained was recorded at 256 nm.

Method validation: Validation is a process of establishing documented evidence, which provides a high degree of assurance that is a specific activity, will consistently produce the desired result, meeting its predetermined

specifications and quality characteristics. The method was validated according to ICH guidelines for various parameters like Linearity, Precision, Accuracy, Robustness, Ruggedness, LOD, LOQ, Range, Sensitivity and Selectivity.[13-16]

System suitability: System suitability was determined from six replicate injections of the standard solution before the analysis and the chromatograms were recorded. Set up a chromatographic system, Allow the HPLC system to stabilize for 30mins, inject blank preparation (single injection) and standard preparation (six replicates), Relative standard deviation (RSD) of peak area for six replicates of the standard was calculated. System suitability parameters like symmetry, theoretical plate, and tailing factor were also recorded. The system suitability data is reported in [Table 2].

Specificity: Specificity is the ability to assess accurately the analyte in the presence of components which may be expected to be present in the sample matrix such as impurities or excipients. There should not be any interference of the diluents or placebo at the retention time of drug substances.

Observation: It is observed from the above data, diluents or excipients peaks are not interfering with the Voriconazole peak.

Linearity: Different aliquots of Voriconazole were prepared from the working standard solution (100µg/mL) in the range of 10-60 µg/mL respectively. Calibration curve showing concentration Vs peak area was plotted [Table 4].

Observation: The correlation coefficient for the linear curve obtained between concentrations Vs Peak area was found to be 0.9983.

Precision: The precision of the method was demonstrated by an inter-day variation study. In the inter-day variation study, the solutions of the same concentration 30µg/mL, 40µg/ml, 50µg/ml were prepared and analyzed six times, for two consecutive days, and the peak area was reported [Table 5,6, 7].

Observation: The % RSD for the area of 6 injections was found to be less than 2. Hence the results obtained were found to be satisfactory.

Accuracy: The accuracy of the method was determined by preparing solutions of three different levels, i.e., 80, 100, and 120%, in which the amount of marketed formulation Vorier® was kept constant (30µg/L) and the concentration of pure drug was varied, that is 24µg, 30µg, and 36µg for 80, 100, and 120% respectively. The solutions were prepared in triplicate and the accuracy was indicated by % recovery and was calculated and reported in the [Table 8].

Observation: The percentage mean recovery of Voriconazole was found to be 98.69%.

Limit of detection and limit of Quantification: The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on the standard deviation of the y-intercept and slope of the calibration curve by using the following formulas.

Limit of detection: 3.3* standard deviation / slope

Limit of quantification: 10* standard deviation / slope

Robustness: The robustness of an analytical method was a measurement of its capability to remain unaffected by small but deliberate variations in the method parameters. Robustness was done by changing the flow rate (±0.1ml/min) and wavelength (±1nm). The results had not much affected hence, the proposed method was found to be robust.

Observation: From the observation, it was found that the system suitability parameters were within the limit at all variable conditions

Ruggedness: Ruggedness is termed by the degree of reproducibility of results by analyzing the same sample under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, and materials.

Table 1: Optimized chromatographic conditions

HPLC Instrument	Shimadzu LC solutions Software UV detector SPD-**20A**
UV-Visible Spectrophotometer	Lab India (T60)
Column	Enable C18 column (250 X 4.6mm, 5µm).
Mobile phase	Methanol: Acetonitrile (60:40)
Flow rate	1mL/min
Detection wavelength	256nm
Run time	18 minutes
Retention time	2.841mins

Table 2: System Suitability Studies

Parameters	**Voriconazole**
Retention time	2.841
Theoretical plates	2850.171
Tailing factor	1.439

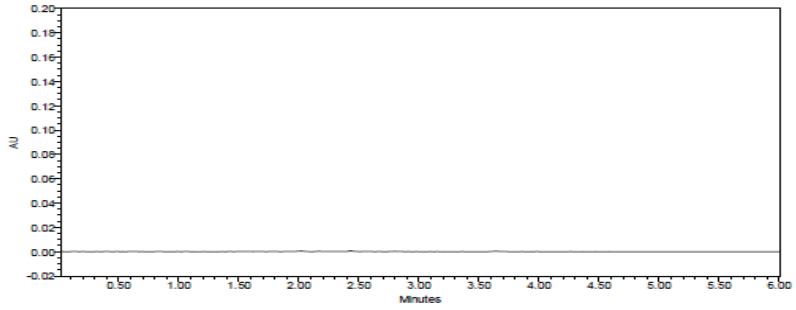

Figure 3: Chromatogram of Blank

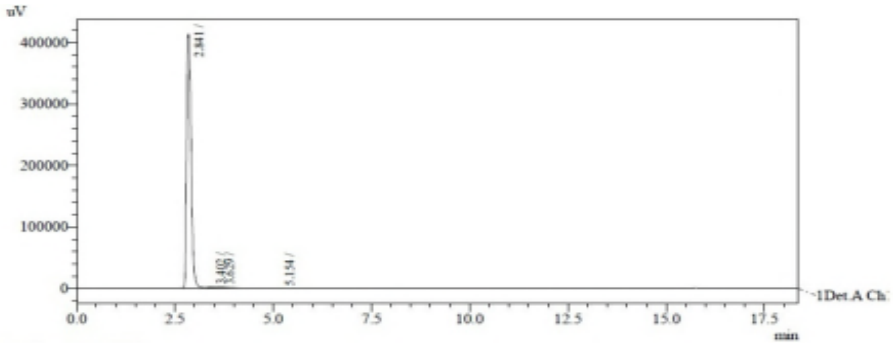

Figure 4: Chromatogram of standard

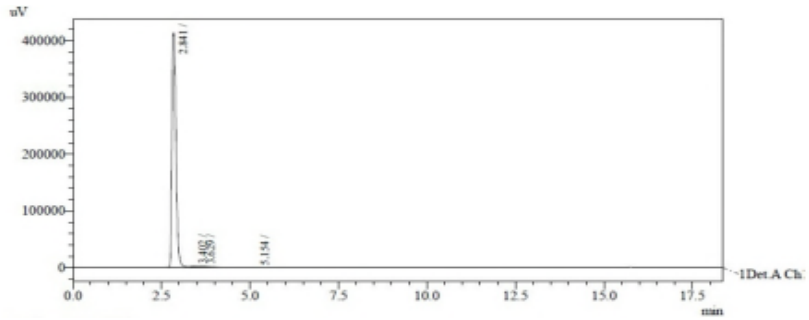

Figure 5: Chromatogram of sample

Table 3: Specificity results for standard and sample

Parameters	Voriconazole standard	Voriconazole sample
Retention time (min)	2.8	2.8
Number of Theoretical plates (N)	2850	3219
Tailing factor (T)	1.439	1.412

Table 4: Linearity data for Voriconazole

Concentration (µg/ml)	Peak area
10	482762
20	852862
30	1324627
40	1707897
50	2257286
60	2607185

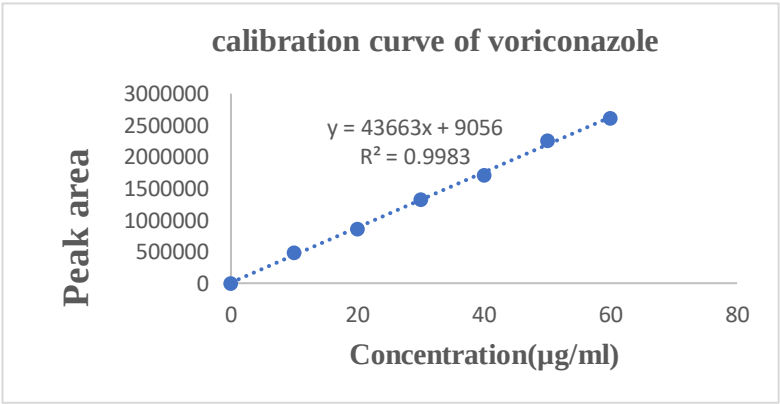

Figure 6: Calibration curve of Voriconazole

Table 5: Inter-day precision (30µg/ml)

Concentration (µg/mL)	Peak Area (Day-1)	Peak Area (Day-2)
30	1324547	1384638
30	1327654	1386586
30	1330427	1389734
30	1334443	1392674
30	1337485	1396523
30	1340534	1398734
	Avg: 1332515	**Avg:** 1391481
	%RSD: 0.45%	%**RSD:** 0.39%

Table 6: Inter-day precision (40µg/ml)

Concentration (µg/mL)	Peak Area (Day-1)	Peak Area (Day-2)
40	1707842	1707524
40	1727716	1723626
40	1737926	1736537
40	1747647	1746581
40	1757807	1752007
40	1727892	1728926
	Avg: 1734471	**Avg:** 1732533
	%RSD: 1.00%	%**RSD:** 0.93%

Table 7: Inter-day precision (50µg/ml)

Concentration (µg/mL)	Peak Area (Day-1)	Peak Area (Day-2)
50	2257206	2257282
50	2259338	2261291
50	2261141	2268130
50	2263382	2274229
50	2265247	2256073
50	2267291	2241923
	Avg: 2262267	**Avg:** 2259821
	%RSD: 0.16%	%**RSD:** 0.49%

Table 8: Accuracy data

Level of Addition	Tablet amount	Amount added	Drug found	% Recovery	%Mean Recovery
80%	30	24	29.5	98.46	98.69
100%	30	30	29.5	98.66	±0.700
120%	30	36	29.6	98.96	

Table 9: LOD and LOQ values

Parameters	µg/ml
Limit of detection	0.24
Limit of quantification	0.95

Table 10: Ruggedness data

Concentration (µg/mL)	Peak Area (Analyst-1)	Peak Area (Analyst-2)
30	1324547	1384638
30	1327654	1386586
30	1330427	1389734
30	1334443	1392674
30	1337485	1396523
30	1340534	1398734
	Avg: 1332515	**Avg:** 1391481
	%RSD: 0.45%	**%RSD:** 0.39%

Table 11: Summary of Validation Parameters for the proposed method

Parameters	Results
Absorption maxima (nm)	256nm
Linearity range (µg/mL)	10-60
Regression equation	y= 43663x+9056
Correlation coefficient (R^2)	0.998
LOD (µg/ml)	0.24
LOQ (µg/ml)	0.95
Accuracy (% Recovery)	98.5-98.9
Precision	
Interday precision (%RSD)	
Day-1	0.1039%
Day-2	0.1090%
Assay (%)	98.84

The method was studied by two different analysts. The results were reported [Table 10].

Observation: From the observation the %RSD values between two analysts, are not greater than 2%, hence the method was found to be rugged.

Assay: The percentage purity of Voriconazole (Vorier®, 200mg) was found to be 98.84%.

RESULTS AND DISCUSSION

The present study was aimed at developing a new Sensitive, Precise, Accurate HPLC method for the estimation of Voriconazole in the bulk and pharmaceutical dosage form. In order to achieve an optimum separation of the component peak, a mixture of Methanol and Acetonitrile in the ratio 60:40 as a mobile phase on an Enable C18 stationary phase. A binary mixture of Methanol: Acetonitrile in the ratio 60:40 v/v was selected as the chromatographic peaks were well defined and resolved with less tailing, less retention time. The retention time obtained for Voriconazole was 2.841min. Each of the samples was injected six times and the same retention times were observed in all the cases. The peak areas of Voriconazole were reproducible as indicated by a low coefficient of variation. A good linear relationship (r^2= 0.998) was observed between the concentration

of Voriconazole and the respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was y=43663x+9056 (where y gives peak area and x is the concentration of drug).In [Table 12]. The intraday and Inter-day precision was carried out and the proposed method was found to be precise as the %RSD value was less than 2. High recovery values obtained from three different levels, as the proposed method was checked by recovery studies. The high recovery values indicate the accuracy of the developed method. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and retention time. This indicates the robustness of the method. The lowest value of LOD and LOQ obtained by the proposed method indicates the sensitivity of the method. All the results obtained are represented in [Table 11].

CONCLUSION

Hence, it can be concluded that the proposed HPLC method is simple, precise, and accurate for the determination of Voriconazole in bulk as well as in the pharmaceutical dosage form. Moreover, The HPLC method enables faster quantification of Voriconazole with an analysis time of three minutes without the interference of excipients, the proposed method can be used for routine quality control of pharmaceutical formulation containing Voriconazole.

Acknowledgment: The authors are thankful to the Principal and the management, Vignan Institute of Pharmaceutical Technology, Visakhapatnam for providing necessary facilities to carry out this research work.

REFERENCES:

1. K.Richardson, 35th Interscience conference Antimicrobial agent's chemotherapy, 1995:125.
2. P.T.Troke, 35th Interscience conference antimicrobial agent's chemotherapy, 1995:125.
3. Anurekha Jain, UV spectrophotometric Estimation of voriconazole in bulk and tablet dosage form. *Asian Journal of chemistry*, 2009, 21(2): 1627-1629.
4. Beckette and stenlake, Practical pharmaceutical chemistry, 1986, 2, 2nd edition.
5. Pennick, Antimicrobial Agents chemotherapy, 2003, 44: 2348.
6. Srinubabu, *Talanta*, 2007, 71: 1424.
7. Stopher and R. Gage, *Journal of chromatography b*, 1997, 691:441.
8. Sridhar, Validated RP-HPLC method for the estimation of voriconazole in bulk and tablet dosage form. *Indian Journal of research in pharmaceutical and Biosciences*, 2010, 1(1): 14-18.
9. Randolph, Determination of voriconazole in aqueous humour by liquid chromatography ionization-mass spectroscopy, *Journal of chromatography* 2002, 776: 213-220.
10. Markus wenk, Fast and reliable determination of the anti-fungal drug voriconazole in plasma using monolithic silica rod liquid chromatography. *Journal of chromatography B*, 2006, 832: 313-316.
11. Richard Gage, HPLC assay for voriconazole in human plasma, *Journal of pharmaceutical and biomedical analysis*, 1998, 17: 1449-1453.
12. Teresa Araujo, Validation of LC-MS/MS method for determination of voriconazole in rat plasma, clinical pharmaceutics, *Journal of pharmaceutical and medical analysis*, 2007, 44: 985-900.
13. Tangri Pranshu, Validation: A Critical Parameter for Quality Control of Pharmaceuticals. *Journal of Drug Delivery & Therapeutics*, 2012, 2(3): 34-40.
14. Validation of Analytical procedure Methodology ICH "Harmonized Tripartite Guidelines," 1996:18.
15. ICH – Guidelines Q2 (R1), Validation of Analytical Procedure: Text and Methodology.
16. ICH- Guidelines Q2B, Technical Requirements for Registration of Pharmaceutical for Human Use Guideline on validation of Analytical Procedures-Methodology, Geneva, Switzerland.