Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 12
ISSN: 2321–3647(online)

# Validated UV-Spectrophotometric Method for the Assay of Voriconazole in Pure and Formulations

**B.V.Sreenivasulu[1*], C.Rambabu[2]**
*1. Department of Biochemistry, Acharya Nagarjuna University,Guntur,AP,India.*
*2. Department of Chemistry, Acharya Nagarjuna University,Guntur,AP,India.*

## ABSTRACT

A simple, accurate, cost effective and reproducible UV-Spectrophotometric method has been developed and validated for the estimation of voriconazole in pure and dosage forms. The present UV-spectrophotometric method is based on measurement of absorption at maximum wavelength of 256nm. The Beer's law of this method was obeyed in the concentration range of 5-30µg/ml with the linear equation of Y = 0.0218x - 0.0029 and correlation coefficient of 0.9996. The percentage recovery of voriconazole ranged from (99.96) in pharmaceutical dosage forms.

**Keywords:** Voriconazole, UV-Spectrophotometry, Dosage forms.

*Corresponding Author Email sreenubv@gmail.com
Received 22 November 2014, Accepted 29 November 2014

Please cite this article as: Sreenivasulu BV *et al.*, Validated UV-Spectrophotometric Method for the Assay of Voriconazole in Pure and Formulations. American Journal of Pharmacy & Health Research 2014.

## INTRODUCTION

Voriconazole[1,2] (Figure.1) is a second-generation triazole antifungal drug extensively used for the treatment of invasive aspergilliosis and other serious fungal infections. It is designated chemically as (2R,3S)-2-(2, 4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4triazol-1-yl)-2-butanol with an empirical formula of $C_{16}H_{14}F_3N_5O$ and a molecular weight of 349.3. It is available in local pharmacy in the trade name of VFEND® (voriconazole) as a lyophilized powder for solution for intravenous infusion, film-coated tablets for oral administration, and as a powder for oral suspension. Literature Survey revealed that the many HPLC [3-13] and very few spectrophotometric [14-16] methods in biological fluids and in pharmaceutical formulations has been reported. The purpose of the present study was to develop a simple, sensitive, accurate and precise UV-Spectrophotometric method for the determination of voriconazole in pharmaceutical formulations. The validated method was applied to the commercially available pharmaceutical formulations containing voriconazole.

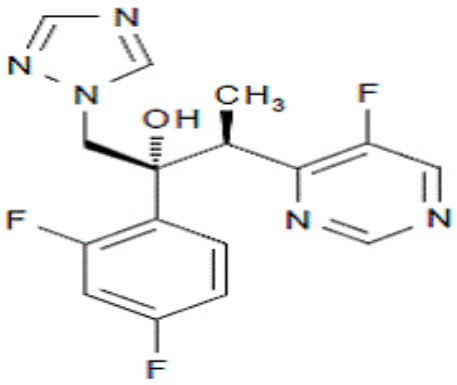


**Figure 1: Structure of voriconazole**

## MATERIALS AND METHOD

### Apparatus

The determination of voriconazole in pure and in different market brands was made by using UV-visible spectrophotometer (Shimadzu Model UV2400 PC series, Kyoto, Japan) in the range of λ 200-400nm, using DMSO as blank. The output signal was monitored and integrated using Dell computer equipped with Empower software and the same software was used to compute the standard regression curve analysis, and to calculate the mean, SD, %RSD and %recovery respectively. Analytical electronic balance was used.

### Materials, reagents and chemicals

Pure sample of voriconazole was procured as gifted sample by Dr.Reddys, Ltd., Hyd, India. Formulation samples used in the present study were commercially available in tablets forms

VFEND® containing 50mg of voriconazole were purchased from the local pharmacy. All other chemicals and reagents used in the present study were of analytical grade. Dimethyl sulfoxide (DMSO), Double distilled water was used as diluent in this present assay.

**Preparation of stock solution and calibration standards**

Accurate amount of pure voriconazole was weighed and transferred to 100ml volumetric flask. 50ml of Dimethyl sulfoxide was added into the flask and the flask was shaken manually till complete dissolution occurred and the final volume was made upto mark with the distilled water to obtain the final concentration 100µg/ml of voriconazole. An appropriate aliquot portion of voriconazole solutions (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0ml) from the above standard stock solution was carefully transferred to 10ml volumetric flasks and diluted with same diluent to obtain the final concentration of 5, 10, 15, 20, 25 and 30µg/ml of voriconazole.

**Analysis of voriconazole in market formulations**

Contents of 10 tablets of one brand [VFEND® containing 50mg of voriconazole] purchased from the local pharmacy were weighed and crushed to make fine powder using porcelain mortar and pestle. Briefly, a quantity of powder having an equivalent amount of 100mg of Voriconazole was weighed and dissolved in in 100ml volumetric flask containing 30 ml of dimethyl sulfoxide and mixed well and finally to dilute to the volume with distilled water to get final concentration of voriconazole 1000µg/ml (stock solution). Further different concentrations of voriconazole that obey within the linearity limits was prepared by transferring of different aliquots of stock solution of voriconazole into 10ml volumetric flask and diluting the mark with the same diluents and concentration of voriconazole in tablets was determined accordingly as described in previous section.

## RESULTS AND DISCUSSION

Scan the standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluent as a blank (Figure.2). At this fixed wavelength (256nm) a calibration curve constructed with the absorbance is so obtained versus the concentration ranges and the regression analysis for linearity plot (Figure.3) was made for the developed method using statistical approach respectively. The developed method has been validated by evaluation of the linearity, sensitivity, precision, accuracy and recovery as per ICH guidelines[17]. The Linearity of the method was evaluated by analyzing six concentration of each drug and each concentration was repeated three times. Linear regression equations with correlation coefficient ($R^2$) were obtained in the concentration range of 5.0-30.0µg/mL (R2: 0.9996) (Table.1). The LOD of the developed

UV-visible spectrophotometric method was made using the slope of the calibration curve and standard deviation of the response and the LOD for voriconazole was found to be 0.0274µg/ml respectively indicating the high sensitivity of the developed method. The precision of the developed UV-Spectrophotometric method was determined by repeatability studies which were carried out by repeating the analysis of voriconazole samples for 6 times. The %RSD value for precision was found to be less than <2% (Table.2) revealing that the developed method was precise. Accuracy of the present method was evaluated by subjecting the drug solution, at three different concentrations equivalent to 50, 100 and 150% of the active ingredient, by adding a known amount of voriconazole standard to a sample of known concentration for three times and calculating the recovery of voriconazole with RSD (%), and % recovery for each concentration. The mean % recoveries were in between 98.70-99.60% and were given in Table.3 revealing the good accuracy of the developed method. This method was applied to determine the content of voriconazole in market samples (tablet-forms). The content and percentage of voriconazole in market sample was found to be 49.89mg with an % recovery of 99.96%, respectively (n=3) (Table.4).

**Table: 1. Linearity study of voriconazole**

| Parameter | Results |
|---|---|
| $\lambda_{max}$ (nm) | 256 |
| Beer's law limits (µg/ml) | 5.0-30.0 |
| Molar absorptivity (1 $mol^{-1}.cm^{-1}$) | $2.998 \times 10^3$ |
| Sandell's sensitivity (µg.$cm^{-2}$/0.001 A.U) | 0.06234 |
| Regression equation (Y=a+bc);Slope (b) | 0.0218 |
| Intercept (a) | 0.0029 |
| Correlation coefficient (r) | 0.9996 |
| Relative standard deviation (%)* | 1.01 |
| LOD | 0.0274 |

**Table: 2. Results of precision study of voriconazole**

| S No | Name | ABS |
|---|---|---|
| 1 | Solution-1 | 0.425 |
| 2 | Solution-2 | 0.429 |
| 3 | Solution-3 | 0.432 |
| 4 | Solution-4 | 0.437 |
| 5 | Solution-5 | 0.434 |
| 6 | Solution-6 | 0.435 |
| Avg | 0.432 | |
| Std Dev | 0.00438 | |
| % RSD* | 1.01 | |

***** Average of six determinations

**Table: 3. Results of accuracy study of voriconazole**

| Accuracy | 50% | 100% | 150% |
|---|---|---|---|
| S No | Area | Area | Area |
| Injection-1 | 0.216 | 0.427 | 0.648 |
| Injection-2 | 0.214 | 0.429 | 0.651 |
| Injection-3 | 0.213 | 0.429 | 0.645 |
| Avg | 0.214 | 0.428 | 0.648 |
| Amt Recovered | 49.35 | 98.77 | 149.19 |
| %Recovery | 98.70 | 98.77 | 99.46 |

**Table:4. Application of proposed method for analysis of tablet Dosage Form**

| Pharmaceutical Formulation | Amount of voriconazole* | | % Recovery |
|---|---|---|---|
| | Labeled | Found | |
| VFEND® | 50 mg | 49.89 | 99.96 % |

***** Average of three determinations

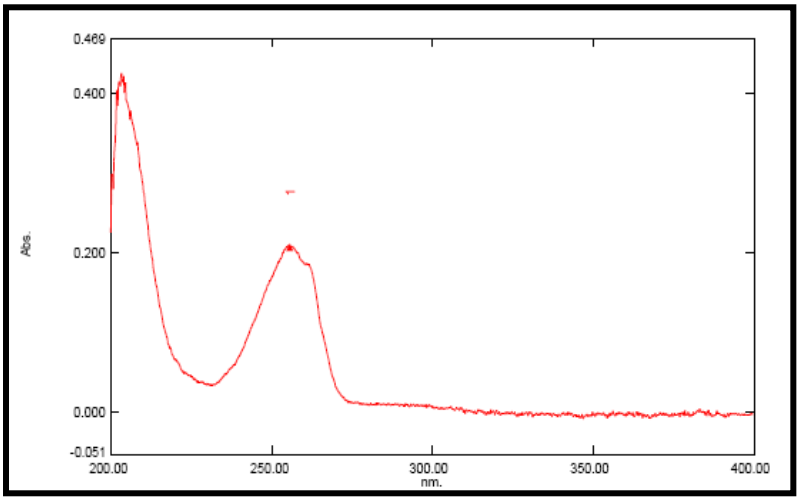


**Figure 2: UV Spectrum of voriconazole in DMSO**

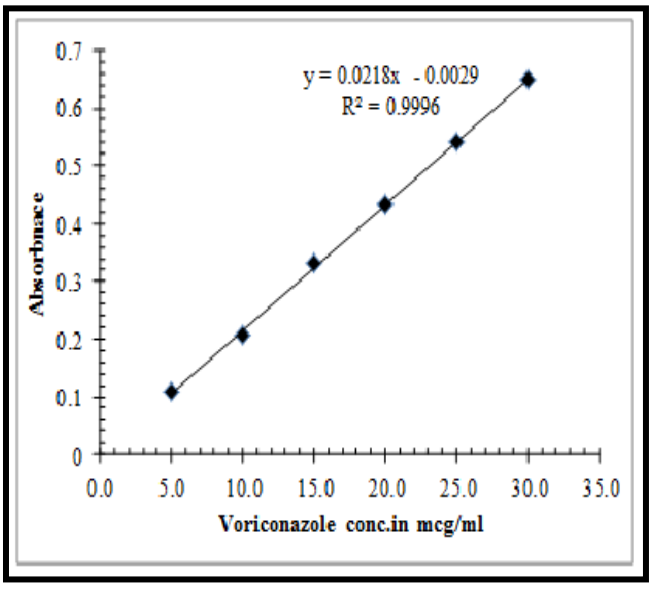


**Figure 3: Calibration Curve of voriconazole at 256nm**

## CONCLUSION

The developed UV-Spectrophotometric method was found to be rapid, simple, sensitive, accurate, precise and reproducible and can be used for routine quality control analysis of voriconazole in pure and formulations.

## ACKNOWLEDGEMENT

The authors are thankful to Bio Lee.Labs-Hyderabad, Andhra Pradesh and Dept. of Chemistry, Acharya Nagarjuna University, Guntur, AP, India for providing necessary for providing laboratory facilities to carry out this research and the authors greatly acknowledge Dr.Reddy's Labs .ltd, for providing the gifted sample of voriconazole.

## REFERENCES

1. Sabo J, Abdel-Rahman S. Voriconazole: A New Triazole Antifungal. The Annals of Pharmacotherapy 2000; 34:1032-1043.
2. Butters M, Ebbs J, Green S P, MacRae J, Morland M C, Murtiashaw CW, Pettman A J. Process Development of Voriconazole: A Novel Broad-Spectrum Triazole Antifungal Agent. Organic Process Research & Development 2001; 5:28–36.
3. Srinubabu G, Raju AI,Sarath N, Kiran Kumar P, Seshagiri Rao JVLN, Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design. Talanta 2007; 71:1424-29.
4. Loralie J. Langman, Felix Boakye-Agyeman. Measurement of voriconazole in serum and plasma. Clinical Biochemistry2007; 40:1378-85.
5. Richard Gage, David A. Stopher. A rapid HPLC assay for voriconazole in human plasma, Journal of Pharmaceutical and Biomedical Analysis 1998; 17:1449-53.
6. Claudia Michael, Jens Teichert, Rainer Preiss. Determination of voriconazole in human plasma and saliva using HPLC with fluorescence detection, Journal of Chromatography B 2008;865:74-80.
7. Jeans-Baptiste Gordien, Arnaud Pigneux, Stephane Vigouroux. Simultaneous determination of five systemic azoles in plasma by HPLC with Ultra violet detection. J Pharma Biomedical Analysis 2009; 50: 932-38.
8. Ping Gu, Yuru Li. Development and validation of a stability indicating HPLC method for determination of voriconazole and its related substances. J Chromatography.
9. Adams AIH, Bergold AM. Development and validation of HPLC method for the determination of voriconazole content in the tablet. Chromatographia 2005; 62: 7-8.

10. Shan Cheng, Feng Qiu, Jai Huang. Development and validation of a simple and rapid HPLC method for the Quantitative determination of voriconazole in rat and beagle dog plasma. J Chromatographic Science, 2007;45 (7): 409-414.
11. Pehourcq F, Jarry C. Direct injection HPLC micro method for the determination of voriconazole in plasma using an internal Surface reversed-phase column. Biomedical Chromatography 2004;18(9): 719-22.
12. Stephanie Chhun, Elisabeth Rey, Aynes Tran. Simultaneous Quantification of voriconazole and posaconazole in human plasma by HPLC with ultra violet detection. J Chromatography B 2007; 852 (1-2): 223-228.
13. Arun M Prajapati, Satish A Patel, Natvarlal J Patel. Development and validation reversed column HPLC and first-derivative UV spectrophotometric methods for estimation of voriconazole in oral suspension powder. Int J Chem and Pharma Sci 2012; 3(2):154-157.
14. Reehana.Shaik, Dhana Sree.M, G.Aravind. Development and validation of new analytical method for voriconazole for Injection by using UV-Spectrophotometer. Int. J. A.PS.BMS 2012;1(3): 240-247.
15. Subhadip Roy, Ravi Kumar BVV, Saswati Tarafda. Development and Validation of New Analytical method for voriconazole by using UV-Spectrophotometer. Int J Pharma Tech 2011;3(2): 1904-1912.
16. Deepan T, Alekhya V, Senniappan P and Dhanaraju MD. Method development and validation for the estimation of voriconazole by UV Spectroscopy. Int J Chem Pharma Sci 2014; 5 (2):164-167.
17. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedures. Text and Methodology ICH Q2 (R1): 2005.