ISSN: 0975-766X
Research Article
Available Online Through
www.ijptonline.com

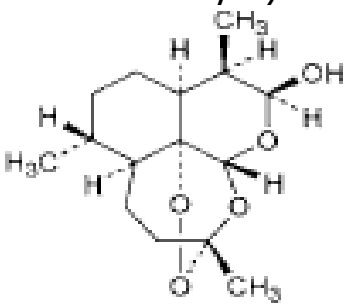
DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR VORICONAZOLE BY USING UV-SPECTROPHOTOMETER

Subhadip Roy*[1], BVV Ravi Kumar[1], Saswati Tarafdar[2]
[1]Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India.
[2]Mepro Pharmaceuticals Pvt Ltd, Surndranagar, Gujarat, India.
E-mail: subhadipr@indiatimes.com

Received on 23-02-2011 *Accepted on 05-03-2011*

ABSTRACT: The present research work discusses the development of a UV spectrophotometric method for Voriconazole. Simple, accurate and cost efficient spectrophotometric method has been developed for the estimation of Voriconazole in bulk and tablet dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ max) was found to be 255nm. The percentage recovery of Voriconazole was in the 99.61-101.63%. Beers law was obeyed in the concentration range of 5-35µg/ml. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation y=0.024x-0.000 with correlation coefficient of 0.9999 was obtained. Validation was performed as ICH guidelines for Specificity, linearity, accuracy, precision,

INTRODUCTION: Voriconazole (2R, 3S)-2-(2,4-diflurophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yl)-2-butanol isa triazole anti-fungal agent. The primary mode of action of voriconazole is the inhibition of fungal cytochrome P-450-mediated 14 alpha-lanosterol demethylation, an essential step in fungal ergosterol biosynthesis. The accumulation of 14 alpha-methyl sterols correlates with the subsequent loss of ergosterol in the fungal cell wall and may be responsible for the antifungal activity of voriconazole. Voriconazole has been shown to be more selective for fungal cytochrome P-450 enzymes than for various mammalian cytochrome P-450 enzyme systems.



OBJECTIVE

The aim of present work is to find out a simple, sensitive, specific, spectrophotometric method for the detection of Voriconazole in pharmaceutical tablet formulation.

INSTRUMENTS

UV-Visible double beam spectrophotometer (UV-1700, Pharmaspec, SHIMADZU Limited, Japan) with 1cm matched quartz cells and Digital balance (Citizen Co.)

CHEMICALS AND REAGENTS

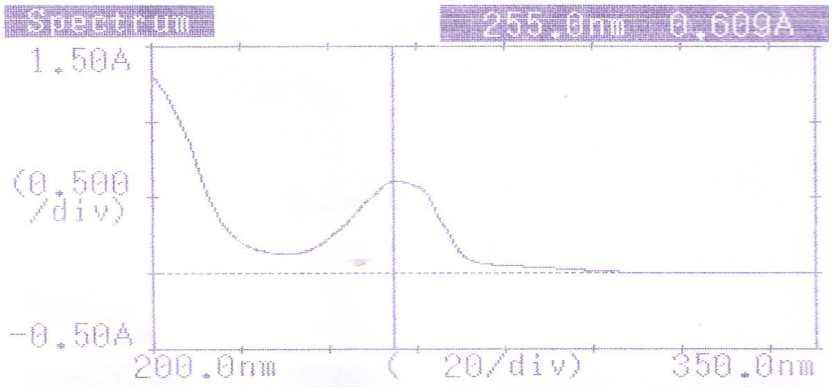
Methanol (Rankem) and Milli Q Pore Water

OPTIMIZATION

Scanning and determination of maximum wavelength (λ_{max})

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug solution (25µg/ml) in Milli Q Pore Water were scanned using UV-Visible spectrophotometer within the wavelength region of 200–380nm against reagent blank. The resulting spectrum was presented in Fig 1.1. and the absorption curve showed characteristic absorption maximum at 255 nm for Voriconazole.

Fig.1.1 Absorbance spectrum of Voriconazole (25µg/ml).



Preparation of Stock Solution

Stock solution was prepared by dissolving 12.5 mg of Voriconazole in 50 ml volumetric flask, add 5 ml of methanol to dissolve the drug, sonicate for 3 mins, cool it at room temperature and make up the volume 50 ml, with Milli Q Pore Water.

Preparation of Working Standard Solutions and construction of standard graph

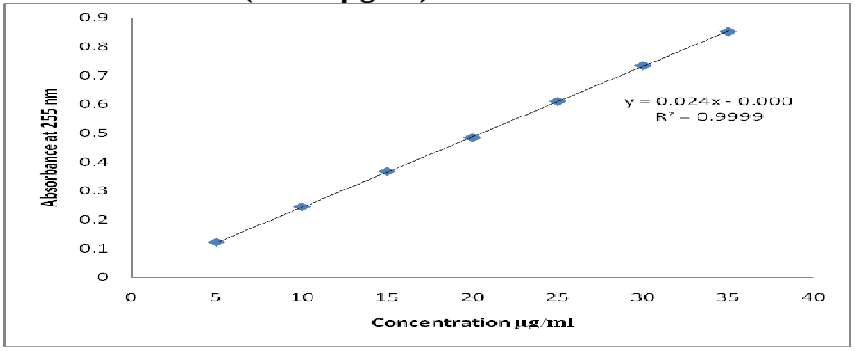
Working standard solution was prepared by taking 1 ml of the stock solution and diluting it to 10 ml with Milli Q Pore Water. So the final concentration of the standard Voriconazole is 25µg/ml.

To construct Beer's law plot for Voriconazole, different aliquots of Voriconazole were taken and diluted to 10 ml with Milli Q Pore Water to get the working standard solutions.The absorbances of each solution were measured at λ_{max} 255 nm against water blank. The results were shown in Table1.1. The standard graph for Voriconazole was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Fig1.2. The drug has obeyed Beer's law in the concentration range of 5 - 35 µg /ml.

Table 1.1. Linearity table of Voriconazole (pure drug) in Milli Q Pore Water at 255 nm.

Concentration (µg/ml)	Absorbance at 255 nm
5	0.121
10	0.244
15	0.367
20	0.484
25	0.610
30	0.734
35	0.852

Fig.1.2: Linearity graph of Voriconazole (5 – 35 µg/ml).



VALIDATION OF METHOD PARAMETERS

LINEARITY

The aliquots of concentration ranging 1-50 µg/mL were prepared in triplicate, but linearity was found to be between 5-35µg/ml concentrations. The linearity results are tabulated in table 1.2.

Table: 1.2: Optical Characteristics.

Parameters	
λ (nm)	**255 nm**
Beer's Law limit (µg/ml)	**5 – 35 µg/ml**
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	**0.041323**
Molar extinction coefficient (liter mole^{-1}cm^{-1})	**8453.06**
Correlation coefficient	**0.9999**

SPECIFICITY

Specificity is performed to determine the presence of exipients (fig 1.3).

Fig 1.3 Absorbance spectrum of Placebo.

SYSTEM PRECISION

The precision of the proposed method was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbances by the proposed method. From these absorbances Mean, Standard deviation %R.S.D was calculated. The readings were shown in table 1.3.

Table 1.3 System Precision Data.

Voriconazole	Absorbance at 255 nm
Std.1	0.608
Std.2	0.607
Std.3	0.607
Std.4	0.606
Std.5	0.608
Std.6	0.608
AVG.	**0.607**
Std. Deviation	0.001
% RSD	**0.134**

METHOD PRECISION

Six replicate samples from homogeneous powdered blend of Voriconazole tablets were analysed as per the method. (The percentage of label claim calculated) the results are tabulated in table 1.4.

Table 1.4 Method Precision Data.

NAME	Absorbance at 255 nm	Assay (%)
Std.1	0.609	95.78
Std.2	0.607	95.61
Std.3	0.607	95.44
Std.4	0.608	95.61

Std.5	0.606	95.44
Std.6	0.607	95.44
AVG.	**0.607**	**95.55**
STDEV	0.001	0.139
% RSD	**0.170**	**0.145**

ACCURACY

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of standard samples of Voriconazole to the Placebo of concentration 25 µg/ml. From that percentage recovery values were calculated. The results were shown in table 1.5.

Table 1.5 Accuracy Data.

Sample ID	Concentration(µg/ml)		Absorbance at 255 nm	%of Recovery	Statistical Analysis
	Pure drug	Placebo			
S_1 : 80 %	**20**	**25**	**0.488**	**101.96**	**Mean= 101.63**
S_2 : 80 %	**20**	**25**	**0.486**	**100.55**	**S.D= 0.959**
S_3 : 80 %	**20**	**25**	**0.490**	**102.38**	**%RSD= 0.943**
S_4 : 100 %	**25**	**25**	**0.612**	**101.09**	**Mean= 100.70**
S_5 : 100 %	**25**	**25**	**0.608**	**100.43**	**S.D= 0.343**
S_6 : 100 %	**25**	**25**	**0.609**	**100.60**	**%RSD= 0.340**
S_7 : 120 %	**30**	**25**	**0.734**	**99.57**	**Mean= 99.61**
S_8 : 120 %	**30**	**25**	**0.733**	**99.43**	**S.D= 0.208**
S_9 : 120 %	**30**	**25**	**0.736**	**99.84**	**%RSD= 0.209**

ESTIMATION OF VORICONAZOLE IN COMMERCIAL DOSAGE FORM

For analysis of commercial formulations, 20 tablets containing Voriconazole (50mg) were taken and powdered. The powder equivalent to 12.5mg (65 mg) of Voriconazole was taken in a 50 ml volumetric flask, add 5 ml of methanol to dissolve the drug, sonicate it for 15 mins. Cool it at room temperature and make up the volume with Milli Q Pore water. Filter the above solution with 0.45µ filter paper. From the above filtrate take 1 ml of the filtrate in a 10 ml volumetric flask and make up the volume with Milli Q Pore Water.

So the final concentration of the sample solution is 25µg/ml.

The absorbance of the solution was measured at 255 nm against reagent blank (table 1.6).

Table.1.6: Recovery from the formulation.

Sl.No.	Formulation	Labeled Amount (mg)	Amount Recovered (mg)	% Drug Recovered	% R.S.D
1	VORIPRO Mepro Pharmaceuticals	50	49.83 ± 0.116	99.66 ± 0.232	0.230

Each value is average of three determinations ±standard deviation.

RESULTS AND DISCUSSION

From the optical characteristics of the proposed method, it was found that Voriconazole obeys linearity within the concentration range of **5 - 35 µg /ml**. From the results shown in precision table-1.3 & 1.4, it was found that the % RSD is less than 2%, which indicates that the method has good reproducibility. From the results shown in accuracy table-.1.5, it was found that the percentage recovery values of pure drug to the Placebo were in between **99.61 – 101.63 %**, which indicates that the proposed method is accurate and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method.

CONCLUSION

The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulations without interference of excipients and other additives. Hence, this method can be used for the routine determination of Voriconazole in bulk samples and Pharmaceutical formulations.

REFERENCES

1. G. Srinubabu, Ch. A.I. Raju, N.Sarath, P.Kiran Kumar, J.V.L.N. Seshagiri Rao, Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design, Talanta 71 (2007) 1424-29.
2. Loralie J.Langman, Felix Boakye-Agyeman, Measurement of voriconazole in serum and plasma, Clinical Biochemistry 40 (2007) 1378-85.
3. Richard Gage, David A. Stopher, A rapid HPLC assay for voriconazole in human plasma, Journal of Pharmaceutical and Biomedical Analysis 17 (1998) 1449-53.
4. Claudia Michael, Jens Teichert, Rainer Preiss, Determination of voriconazole in human plasma and saliva using HPLC with fluorescence detection, Journal of Chromatography B, 865 (2008) 74-80.
5. Jeans-Baptiste Gordien, Arnaud Pigneux, Stephane Vigouroux, Simultaneous determination of five systemic azoles in plasma by HPLC with Ultra violet detection, Journal of Pharmaceutical and Biomedical Analysis 50 (2009), 932-38.
6. Ping Gu, Yuru Li, Development and validation of a stability indicating HPLC method for determination of voriconazole and its related substances, Journal of Chromatography.
7. A I H Adams and AM Bergold, Development and validation of HPLC method for the determination of voriconazole content in the tablet, Chromatographia, volume 62, number 7-8/ oct. 2005.
8. 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, Journal of Chromatographic Science,2007,45 (7), 409-414.

9. F Pehourcq, C Jarry, 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.
10. Stephanie Chhun, Elisabeth Rey, Aynes Tran, Simultaneous Quantification of voriconazole and posaconazole in human plasma by HPLC with ultra violet detection, Journal of Chromatography B, 2007, 852 (1-2): 223-228.
11. 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. Journal of AOAC INTERNATIONAL, 91(5); 1070-4.
12. International Conference on Harmonization, Draft Guideline on Validation Procedure, Definition and Terminology Federal Register, 1995, 60, 11260.
13. ICH, Q2A validation of analytical procedure, Methodology International Conference on Harmonization, Geneva, October 1994.

Corresponding Author:
Subhadip Roy*,
E-mail:*subhadipr@indiatimes.com*