ISSN-2277-9280

RESEARCH ARTICLE REEHANA SHAIK, *Int.J.A.PS.BMS , JUL-SEPT.2012, Vol.1(3), 240-247*

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

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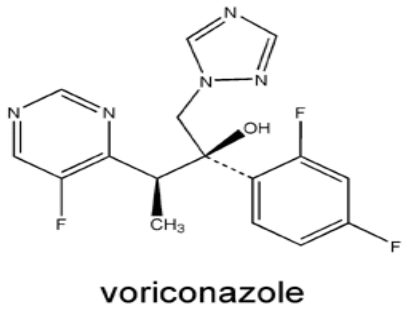
ABSTRACT

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 pharmaceutical 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 10-25µg/ml. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation y=0.026x-0.0036 with correlation coefficient of 0.99953 was obtained. Validation was performed as ICH guidelines for Specificity,linearity, accuracy, precision.

Keywords Voriconazole, uv spectrophotometric method

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.



voriconazole

Figure- 1. Chemical structure of voriconazole

OBJECTIVE

The aim of present work is to find out a simple, sensitive, specific, spectrophotometric method for the detection of Voriconazole in pharmaceutical dosage 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), ethanol ,water

OPTIMIZATION

Scanning and determination of maximum wavelength (λmax)

In order to ascertain the wavelength of maximum absorption (λmax) of the drug solution (10µg/ml) were scanned using UV-Visible spectrophotometer within the wavelength region of 200–380nm against reagent blank. The resulting spectrum was presented in Fig 2. and the absorption curve showed characteristic absorption maximum at 255 nm for Voriconazole.

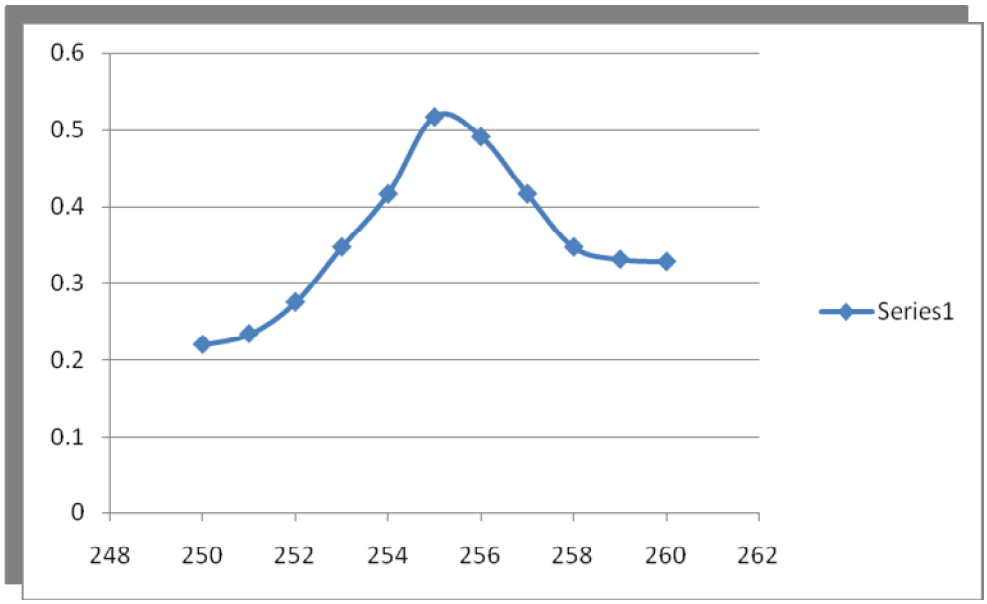


Fig.2. Absorbance spectrum of Voriconazole (10µg/ml).

Preparation of standard stock solutions:

Weigh accurately 10 mg of voriconazole in to a10 ml volumetric flasks. Then add small amount of ethanol to dissolve the drug and then volume was made up to 10 ml with the methanol (diluents). The concentration of standard stock solution is 1 mg/ml.

Preparation of working standard solution:

Transferred 5 ml from the above standard stock solutions in to 50 ml volumetric flasks and diluted up to the mark with diluent to get working standard solution of concentration 0.1 mg/ml. From this 2ml is diluted to 10 ml to get 20µg/ml.

Table 1. Linearity table of Voriconazole (pure drug) at 255nm

S.No	Concentration	Absorbance
1	0	0
2	10	0.264
3	12	0.305
4	14	0.352
5	16	0.411
6	18	0.459
7	20	0.52
8	22	0.575
9	24	0.627
	CC	0.99953
	Slp	0.026074
	Int	-0.00368

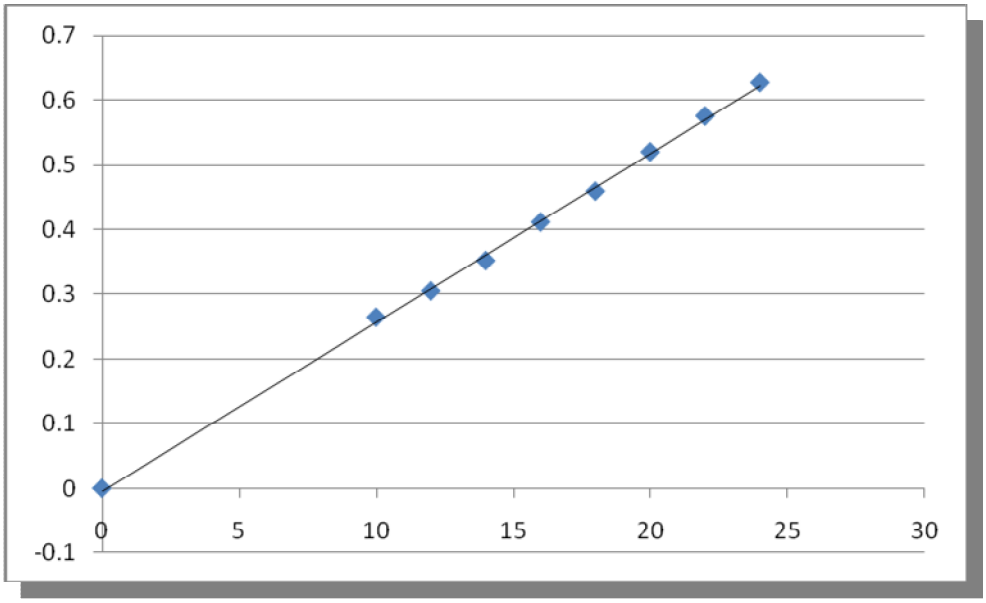


Fig.3. Linearity graph of Voriconazole (10 – 24 µg/ml).

VALIDATION OF METHOD PARAMETERS

LINEARITY

The aliquots of concentration ranging 10-24 µg/mL were prepared, linearity was found to be between 10-24µg/ml concentrations. The linearity results are tabulated in table 1.

Table: 1: Optical Characteristics.

Parameters	
λ (nm)	255 nm
Beer's Law limit (µg/ml)	10 – 24 µg/ml
Correlation coefficient	0.99953

ACCURACY

The accuracy, specificity, suitability and validity of the proposed methods were satisfied by conducting recovery studies. A known quantity of the drug was added to the pre analyzed sample formulation at 20%, 40% and 60% levels. The percentage recovery was calculated and given in Table no:2

S.NO	Recovery	Concentration	Absorbance	Amount found	% of Recovery
1	20%	12ppm	0.301	11.84	98.66
		12ppm	0.308	12.11	100.91
		12ppm	0.299	11.76	98
2	40%	14ppm	0.352	14	100
		14ppm	0.355	14.11	100.78
		14ppm	0.354	14.08	100.57
3	60%	16ppm	0.41	15.96	99.75
		16ppm	0.414	16.11	100.6
		16ppm	0.418	16.27	101.6

Table 2. Accuracy Data for Voriconazole.

PRECISION:

Repeatability:

The repeatability of the method was studied by measuring the absorbance at 255 nm of standard solutions of six replicate samples and measured the absorbance at 255 nm.

Repeatability of assay:

Interday precision: This was done by analyzing formulation by same analyst but for six days subsequently.

Intraday precision: This was done by analyzing formulation in same day for six times of individual preparation.

S.NO	Concentration(**µg/ml**)	Absorbance at 255nm	Statistical analysis (n=6)
1	20	0.745	Mean=0.748
2	20	0.748	
3	20	0.75	
4	20	0.751	%RSD=0.304
5	20	0.748	
6	20	0.746	

Table no 3. Intraday precision for Voriconazole

S.NO	Concentration(µg/ml)	day	Absorbance at 255nm	Statistical analysis at 255nm
1	20 µg/ml	1	0.738	Mean=0.7411
2		2	0.736	
3		3	0.741	
4		4	0.744	%RSD=0.58
5		5	0.74	
6		6	0.748	

Table no 4. Interday precision of Voriconazole

ESTIMATION OF VORICONAZOLE IN COMMERCIAL DOSAGE FORM

Take two vials which containing 10mg drug, equivalent to 1 vial weight was weighed accurately and transferred to a 10 ml volumetric flask. Then add small quantity of ethanol and sonicate it for 30 min to dissolve the drugs completely and then the volume was made up to the mark with the diluent and filtered through 0.45 µm membrane filters. From this, 2 ml was taken and diluted to100 ml with diluents to get 20 µg/ml concentration. The absorbance of this solution was measured at 255 nm against diluent as a blank.This procedure was repeated 3 times. Drug content in the vials was calculated. The results are summarized in table no:

Voriconazole (VFEND-10mg)	Concentration (ppm)	Absorbance	Average	% Assay
	20	0.522		
	20	0.52	0.516667	99.23
	20	0.508		

Table no 5. %Assay of Voriconazole

RESULTS AND DISCUSSION

From the optical characteristics of the proposed method, it was found that Voriconazole obeys linearity within the concentration range of 10 - 24 µg /ml. From the results shown in precision table-3 & 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-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.

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