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VALIDATED AND A SENSITIVE UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF VORICONAZOLE IN BULK AND COMMERCIAL FORM

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ABSTRACT

A simple, sensitive and reliable UV spectrophotometer method has been developed for the estimation of voriconazole in the bulk and pharmaceutical dosage form. Estimation was carried out at wavelength of 223nm using methanol as solvent. The beer lambert's law range was observed in the range of 5-30µg/ml with correlation coefficient (R^2=0.995). The percentage recovery was found to be in the range of 98.62 to 101.25%. The proposed method was found to be simple, accurate, and reproducible. All the parameters of the analysis were chosen according to ICH [Q2 (R1)] guidelines and validated statistically using SD and %RSD

INTRODUCTION

Voriconazole is chemically (2R, 3S)-2-(2, 4-Difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1H-1, 2, 4- triazole-1-yl) butan-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 spectrophotometric method for the estimation of voriconazole with complete validation as per ICH guidelines.

MATERIALS AND METHODS:

A sample of voriconazole with a purity of 99.8% w/w was obtained from spectrum labs, Hyderabad. LABINDIA double beam UV/visible spectrophotometer and Elite analytical balance instruments were used. Chemicals and reagents are analytical grade. Voriconazole of 200mg with a brand name vorier® was purchased from a local Pharmacy.

Preparation of standard stock solution:

A standard drug solution of voriconazole was prepared by transfering 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 100ml volumetric flask and made up to the mark with methanol to get a concentration of 100µg/ml.

Absorption maxima: It was scanned by a UV spectrophotometer in the range of 200-400nm using methanol as blank. The maximum absorbance was found at 223nm and represented spectrum is shown in Figure 2.

Preparation of sample solution

10 tablets were weighed and powdered, an amount of powder equivalent to 100mg of voriconazole was weighed and dissolved in 100ml methanol and then filtered through Whattman filter paper (no.41) to get a clear solution of 1000µg/ml. From the above solution, 10ml was pipetted out and made up to 100ml with diluents to get a final concentration of 100µg/ml.

METHOD VALIDATION[13]

The objective of method validation is to demonstrate that the method is suitable for its intended purpose. The method was validated for linearity, precision, accuracy, robustness, ruggedness, LOD & LOQ.

Linearity: Different aliquots of voriconazole in the range of 0.5-3ml transferred into 10ml volumetric flask and made up to the mark with methanol. The solutions were analyzed at 223nm. A Calibration curve was plotted by taking concentration on the x-axis and absorbance on the y- axis.

Precision-The closeness of agreement between the obtained values by analyzing the sample multiple times under prescribed conditions. There are 3 levels: repeatability, intermediate precision, and reproducibility. Repeatability is a measure of the exactness under the same working conditions more than a short interior of time, i.e, under ordinary working states of the scientific technique with the same hardware. It is also known as intraday precision. Reproducibility is also known as inter-day

Precision.

Precision is expressed in terms of %Relative Standard Deviation.

$$\%RSD = \frac{\text{Standard Deviation}}{\text{Mean}} x100$$

Accuracy- Accuracy means the expression of closeness of agreement between the values which is accepted either as a conventional true value or an accepted reference true value and the value found, accuracy is assessed by using 9 determinations covering a minimum of 3 concentrations.

Robustness- The Robustness of an analytical procedure is the capacity to remain unchanged by small but deliberate changes in parameters.

Ruggedness: The Ruggedness of an analytical procedure is the degree of reproducibility of results by analyzing the same sample under a variety of conditions like laboratories, instrument, analysts, reagents etc.

Sensitivity: Limit of detection (LOD) and Limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines.

Limit of Detection-It is the lowest amount of the drug in the sample that can be detected, but not necessarily quantified.

Limit of Quantification- It is an amount of analyte that can be quantified with a specified limit of accuracy and precision**.**

Linearity: From the standard stock solution, the various dilutions in the concentration 5-30 µg/ml were prepared. The solutions were scanned at 223nm and the absorbance was recorded and shown in [Table1]. From this, calibration curve was obtained by plotting absorbance versus concentration of voriconazole and the linearity was represented in figure 3. The correlation coefficient was found to be 0.995.

Precision- The Repeatability of the method was checked by scanning 20µg/ml solution for 6 times represented in [Table 2]. Intraday precision was determined by checking the absorbance of (20µg/ml) on the same day (morning, afternoon, evening) and the results were represented in [Table 3]. Inter-day precision was determined by checking the absorbance of (20µg/ml) on three different days and the obtained results were represented in [Table 4].

Accuracy: An Accuracy study was conducted by spiking at three concentration levels (80%, 100%, and 120%). At each level, triplicate samples were scanned and the percentage recovery was determined and presented in the [Table 5].

Robustness: To determine the robustness of the method one parameter wavelength variation was made slightly different from the selected wavelength. No significant difference was found in the absorbance and hence proposed method was considered as robust and reports were reported in the [Table 6].

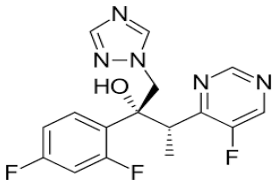

Figure 1: Chemical structure of voriconazole

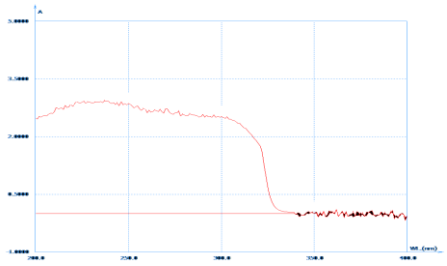

Figure 2: Absorbance spectra of Voriconazole

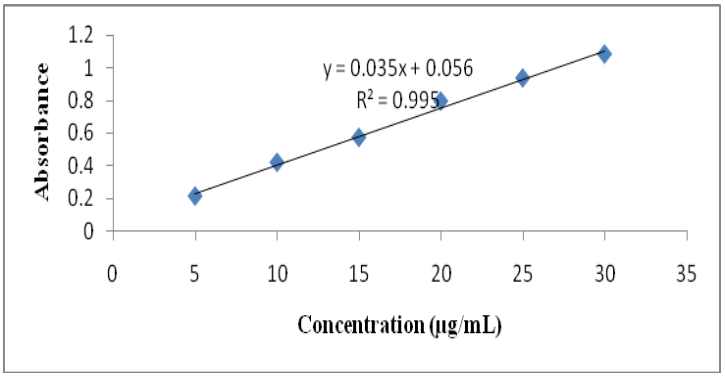

Figure 3: Calibration graph of voriconazole

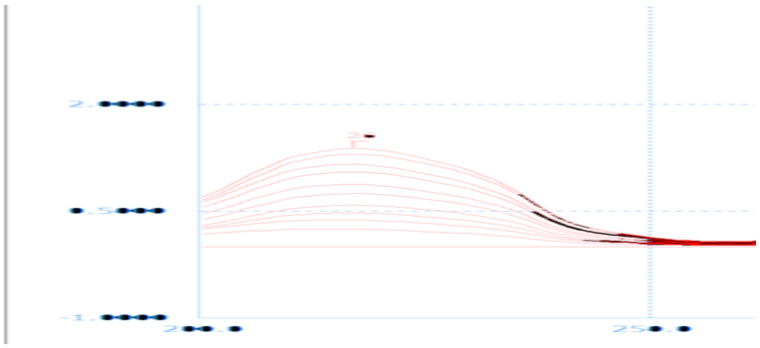

Figure 4: overlay spectra of voriconazole

Table 1: Linearity data

Concentration (µg/mL)	Absorbance
5	0.2118
10	0.4180
15	0.570
20	0.7934
25	0.9348
30	1.0815

Table 2: Repeatability data

Concentration (µg/mL)	Absorbance
20	0.7506
20	0.7473
20	0.7436
20	0.7439
20	0.7379
20	0.7390
%RSD	0.64%

Table 3: Intraday precision

Concentration (µg/mL)	%RSD			Mean %RSD
	Morning	Afternoon	Evening	
20	0.64	0.78	0.56	0.66

Table 4: Inter-day precision

Concentration (µg/mL)	%RSD			Mean %RSD
	Day 1	Day 2	Day 3	
20	0.64	0.78	0.56	0.66

Table 5: Accuracy data

% Level of Addition	Amount added (µg/mL)	Amount Found (µg/mL)	%Recovery	%Mean Recovery
80	16	15.81	98.83	99.21
100	20	19.80	99.01	
120	24	23.61	99.79	

Table 6: Robustness Results

Concentration (µg/mL)	Absorbance		
	λ1	λ2	λ3
20	0.7333	0.7506	0.7423
20	0.7333	0.7473	0.7421
20	0.7284	0.7436	0.7377
20	0.7294	0.7439	0.7340
20	0.7257	0.7379	0.7347
20	0.7245	0.7390	0.7335
%RSD	0.57%	0.64%	0.54%

Table 7: Ruggedness Results

Concentration (µg/mL)	Absorbance	
	Analyst 1	Analyst 2
20	0.7506	0.7398
20	0.7473	0.7392
20	0.7436	0.7548
20	0.7439	0.7571
20	0.7379	0.7427
20	0.7390	0.7410
%RSD	0.64%	1.07%

Table 8

LOD (µg/mL)	LOQ (µg/mL)
0.0282	0.0854

Ruggedness: The ruggedness of the developed method was checked by an analyzing the samples by different analysts at different days at similar operational conditions. The statistical analysis showed no significant differences between results obtained by employing different analysts and results were shown in the [Table 7].

Limit of detection and limit of quantification

Limit of detection is the lowest amount of an analyte in a sample that can be detected but not necessarily quantified, under the stated experimental conditions.Limit of quantification is the lowest amount of an analyte in a sample that can be quantified, under the stated experimental conditions. The LOD and LOQ for voriconazole were found to be 0.0282µg/ml and 0.0854µg/ml and results were shown in the [Table 8].

Assay of voriconazole Tablets: For the analysis, 10 tablets of voriconazole were weighed and finely powdered. An accurately weighed quantity of powder equivalent to 100mg quantity of voriconazole was taken in a 100ml volumetric flask. Few ml of methanol was added and sonicated for 15 minutes and then filtered through Whattman filter paper (No.41) and volume was adjusted to solvent. From the further dilution was made to get a final concentration of 20µg/ml and the % 5 assay was found to be 99.8%.

RESULTS AND DISCUSSION

The method was developed and validated as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, robustness, ruggedness, LOD, LOQ and specificity. Beer's law is obeyed of over the concentration range 5 to 30µg/ml, using regression analysis. The linear equation Y=0.035x+0.056 with a correlation coefficient of r^2=0.995. The precision results show %RSD <2 at each level, which indicates that the method is precise. The accuracy of the method was checked by recovery studies was found to be accurate within the range of 98.62 to 101.25%. The robustness and ruggedness results reveal that the method is more sensitive. There was no interference observed from the excipients present in the formulation, indicated that the method is specific. Determination of voriconazole in tablet formulation vorier® showed the content of voriconazole was very close to the label amount. The %RSD values in all the parameters were within the acceptable limit (<2%).

CONCLUSION

A validated UV spectrophotometric method has been developed for the estimation of voriconazole in bulk as well as the Pharmaceutical dosage form. The developed method was found to be simple, precise, specific, reproducible and linear over the concentration range studied. The proposed method can be used for routine analysis of voriconazole in bulk as well as pharmaceutical formulations.

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