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METHOD DEVELOPMENT AND VALIDATION OF VORICONAZOLE BY USING UV SPECTROSCOPY

D.Snigdha*, T. Ramya Krishna, D. Snidgha, Afzal Nazneen, Nerella Mounika
Department of Pharmaceutical Chemistry, Talla Padmavathi Pharmacy College, Orus, Kareemabad, Warangal, Telangana, India

ABSTRACT
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.
Key Words: Uv Spectroscopy, Voriconazole

***Corresponding Author:**
D.Snigdha,
Talla Padmavathi Pharmacy College,
Orus, Kareemabad,
Warangal,Telangana,India.

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INTRODUCTION

Analytical chemistry may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition(atomic, molecular, phase) of substances and materials and their chemical structure. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture or determination of structure of chemical compounds. Drug analysis and assays play important role in the development, manufacture and therapeutic usage of drugs. This includes identification, characterization and determination of drugs in mixtures such as dosage forms. The drug analysis procedures play essential roles in pharmacokinetics of drugs in human and animals[1]. Bulk drugs are obtained by chemical synthesis, biosynthesis, isolation from plants or animals or biotechnological source. Dosage form is the bulk drugs manufactured into dosage form with help of additives prior to their use in patients. Drugs in different matrix are assayed by different methods such as chromatographic and spectroscopic technique, separation techniques, radio immuno assays etc. The modern trends in drug discovery and development with the aid of computer aided drug-designing promises the introduction of many newer drugs and multi component for various therapeutic activity.

EXPERIMENTATION MATERIALS AND METHODS

Chemicals and reagents

Chloroform was used throughout UV spectrophotometric method development and validation.

Instrumentation

UV spectrophotometric method was performed on double beam UV-visible spectrophotometer (Shimadzu, model 1700) having two matched quartz cells with 1 cm light path.

Selection of solvent

Chloroform was selected as ideal solvent for spectrophotometric analysis of Voriconazole

PREPARATION OF STANDARD STOCK SOLUTIONS

Accurately weighed quantity of 20 mg Voriconazole reference standard was transferred into 20 ml volumetric flask and dissolved and diluted up to the mark with chloroform to give a stock solution having strength 1000µg/ml. 100 µg/ml working standard solution was prepared by diluting 1 ml of stock solution to

10 ml with chloroform.

Preparation of Sample stock solution

For analysis of drug in capsule dosage form, 10 capsules were weighed accurately and the powder triturated in the mortar to get a fine powder. The capsule powder equivalent to 10mg was weighed and transferred to 10ml volumetric flask and dissolved with chloroform. The capsule solution was diluted to get a final concentration of 10µg/ml. The absorbance of these solutions measured at 267 nm. The amount of Voriconazole per capsule was calculated using the calibration curve.

Formula: %Purity=Sample absorbance / Standard absorbance X 100.

METHOD VALIDATION

The method was validated according to International conference on Harmonization (ICH) Q2B guidelines 1996 for validation of analytical procedure in order to determine the linearity, limit of detection, accuracy and precision.

LINEARITY & RANGE

Under the experimental conditions, the calibration graphs of the absorbance versus concentration were found to be linear over the range of 0.2-1.0µg/ml for proposed method. The statistical analysis of data obtained for estimation of Voriconazole is indicated chloroform of accuracy for the proposed methods evidenced by the low values of standard deviation and coefficient of variation.

ACCURACY

To determine the accuracy of proposed method, recovery studies were carried out by adding different amounts (50%, 100%, 150%) of standard bulk sample of Voriconazole within the linearity range were taken and add to pre analyzed formulation of concentration 10µg and percentage recovery values are calculated . Test should be prepared in triplets at each spike level and assay should be done as per the test method.

PRECISION

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. The precision of the ascertained by determination of six replicates of same concentration of sample and standard for method precision and system precision.

SYSTEM PRECISION

The system precision of the proposed method was ascertained by determination of six replicates of same concentration of standard drug within the Beer's range and finding out the absorbance. The absorbance, standard deviation, and %RSD were calculated.

METHOD PRECISION

The method Precision of the proposed method was ascertained by determination of six replicates of same concentration of sample drug within the Beer's range and finding out the absorbance. The absorbance, standard deviation and % RSD were calculated.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The detection limit of individual analytic procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantification limit of an individual analytic procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were calculated by using the relation 3.3S and 10 S respectively, where is the standard error of estimate and S is the slope. Calculated values of LOD and LOQ for Voriconazole were found to be 0.14 µg/ml and 0.43 µg/ml respectively.

RUGGEDNESS

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 10µg/ml of Voriconazole using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to reproducible, since there was no significant difference between analysts. Thus, the proposed method could be considered rugged

ROBUSTNESS

The robustness of the method was determined by introducing small changes in UV parameters, such as changing in the wavelength ± 4.

ANALYSIS OF PHARMACEUTICAL FORMULATIONS

The optimized spectrophotometric method applied to the direct determination of Voriconazole in tablet using calibration curve method without any sample extraction or filtration. From the absorbance value, the drug content per tablet was calculated.

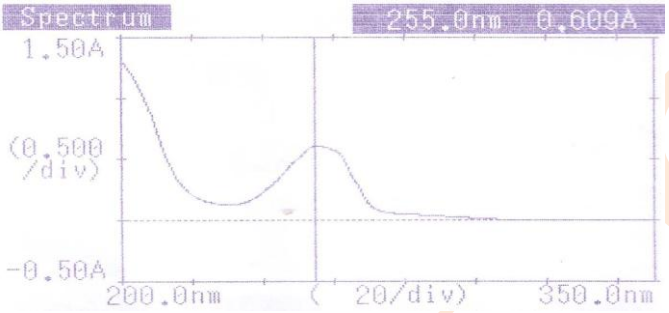
RESULTS AND DISCUSSION

OPTIMIZATION

Scanning and determination of maximum wavelength (λmax)

In order to ascertain the wavelength of maximum absorption (**λmax**) 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

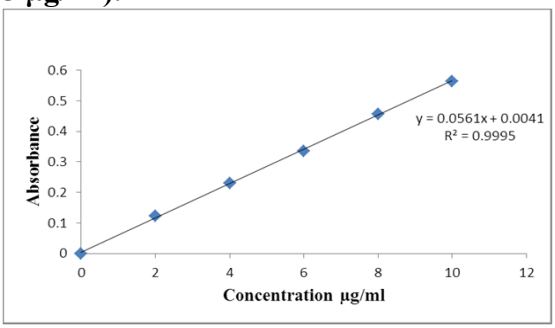
Working standard solution was prepared by taking 1 ml of the stock solution and diluting it to 10 ml with Milli Q Pore Water. So the final concentration of the standard Voriconazole is 25µg/ml.

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 **λmax** 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).



Linearity Data of Voriconazole.

S. No.	Concentration µg/ml	Absorbance
1	0	0
2	2	0.1316
3	4	0.2419
4	6	0.3516
5	8	0.4615
6	10	0.5612
Slope:0.056 Regression:0.999		

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.

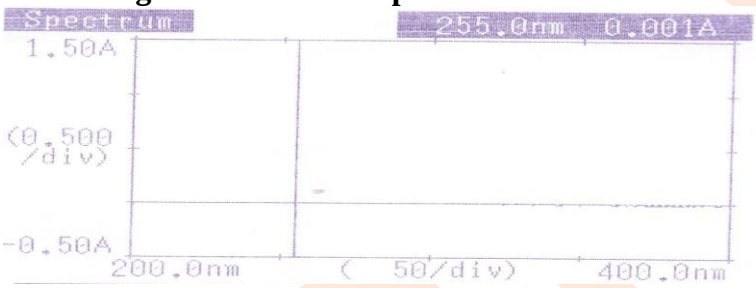
Table: 1.2: Optical Characteristics.

Parameters	
λ (nm)	**255 nm**
Beer's Law limit (µg/ml)	**5 – 35 µg/ml**
Sandell's sensitivity (µg/cm2/0.001 absorbance unit)	**0.041323**
Molar extinction coefficient (liter mole-1cm-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			
S1 : 80 %	20	25	0.488	101.96	Mean= 101.63
S2 : 80 %	20	25	0.486	100.55	S.D= 0.959
S3 : 80 %	20	25	0.490	102.38	%RSD= 0.943
S4 : 100 %	25	25	0.612	101.09	Mean= 100.70
S5 : 100 %	25	25	0.608	100.43	S.D= 0.343
S6 : 100 %	25	25	0.609	100.60	%RSD= 0.340
S7 : 120 %	30	25	0.734	99.57	Mean= 99.61
S8 : 120 %	30	25	0.733	99.43	S.D= 0.208
S9 : 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.

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.

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