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VALIDATED CHROMATOGRAPHIC METHOD FOR DETERMINATION OF VORICONAZOLE IN TABLETS THROUGH RP- HPLC

K. G. Suryawanshi, N. A. Porwar, K. A. Patil, K. K. Khadke, Y. R. Patil, Dr. K. R. Patil*, S. N. Patil, Dr. S. D. Barhate

Department of Pharmaceutical Chemistry, SES Arunamai College of Pharmacy, Mamurabad, Jalgaon Maharashtra India.

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***Corresponding Author: Dr. K. R. Patil**
Department of Pharmaceutical Chemistry, SES Arunamai College of Pharmacy, Mamurabad, Jalgaon Maharashtra India.
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ABSTRACT

Voriconazole is a broad-spectrum triazole antifungal agent widely used in the treatment of invasive fungal infections. The present study focuses on the development and validation of a simple, accurate, and cost-effective analytical method for the quantitative estimation of voriconazole using RP-HPLC and UV spectrophotometry. Chromatographic separation was achieved using a C18 column with a mobile phase consisting of methanol and 0.1% orthophosphoric acid in water (80:20 v/v), at a flow rate of 1.0 mL/min and detection wavelength of 256 nm. The method was validated according to ICH guidelines for parameters including linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method exhibited excellent linearity in the concentration range of 5–25 µg/mL with a correlation coefficient (R^2) of 0.999. Recovery studies confirmed the accuracy of the method with values between 98–101%, while precision studies showed %RSD less than 2%, indicating high reproducibility. The LOD and LOQ were found to be 0.0917 µg/mL and 0.2779 µg/mL, respectively. The developed method was successfully applied to the analysis of pharmaceutical formulations, demonstrating its suitability for routine quality control. Overall, the method is simple, sensitive, precise, and economical, making it ideal for the estimation of voriconazole in bulk and dosage forms.

KEYWORDS: Voriconazole, Quality Control, Antifungal, Chromatography.

INTRODUCTION

The most important antifungal is voriconazole (VOR), a derivative of triazoles. Its chemical formula is (2R, 3S)-2-(2,4-difluorophenyl)-3-(5-fluoro pyrimidin-4-yl)-1-(1,2,4-triazol-1-yl)-2-butanol (Figure 1).[1] Its mechanism of action, like

those of other azole antifungals, entails blocking the 14-sterol demethylase enzyme, which is necessary for the production of ergosterol in the fungal cell membrane. This inhibition favors fungal enzyme systems over human enzyme systems. In May 2002, the FDA approved VOR to treat invasive aspergillosis, Scedosporium apiospermum, and refractory infections caused by Fusarium species.[2] Several analytical methods for measuring VOR in different pharmacological dose forms, including as pure medicines, pharmaceutical formulations, and biological samples, were found in the literature review.These techniques mostly use chromatography, while electrochemistry has also been used.[3,4] These methods usually require a great deal of sample pre-treatment, cleanup procedures prior to analysis, and the use of expensive equipment that most quality control labs cannot afford. Visible spectrophotometry is a frequently used analytical method in clinical laboratories, hospitals, the pharmaceutical industry, and quality control. This approach is used because it is easy to use, inexpensive, quick, sensitive, selective, accurate, precise, widely available, and appropriate for pharmaceutical analysis. It is widely used in the analysis of several drug classes in their pure form, pharmaceutical formulations, and biological materials. According to what is currently known, very few spectrophotometric techniques have been documented for the quantitative measurement of VOR in pharmaceutical formulation.[5-12]

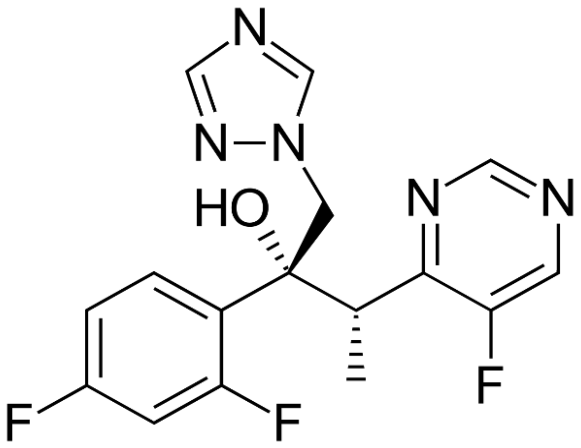

Fig No. 1: Structure of Voriconazole.

MATERIALS AND METHOD

Instruments

The analysis of the drug was carried out on Agilent Gradient System with auto sampler having an UV Spectrophotometric Detector. Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 100mm;5µm), a 20µl injection loop and UV730D Absorbance detector and running chemstation.

Selection of stationary phase

The column used in this method C_{18} Agilent The configuration of the column is 4.6 x 100 mm, particle size 5 µm. C_{18} column gives high non polar retentively, symmetric peak shape, highly reproducible and stable ideal for HPLC method

Solubility Studies

This study was carried out to find an ideal solvent in which drugs are completely soluble. Various solvents were tried for checking solubility of Voriconazole. From solubility studies it was concluded that of Voriconazole is poorly soluble in water however it is soluble in methanol PH adjusted 0.1%Orthophosphoric Acid, Buffer pH 3.0.

Chromatographic conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table No. 1: HPLC chromatographic conditions.

Sr. No.	Parameters	Description
1	HPLC	Agilent Gradient
2	Software	Chemstation 10.1
3	Column	(Agilent) C18
4	Particle Size	5 µm
5	Stationary	C_{18} (Agilent)
6	Mobile	Methanol: water
7	Detection	256nm
8	Flow Rate	1.0 ml/min
9	Temperature	Ambient
10	Sample Size	20 µl
11	PH	3.2
12	Run Time	15 min
13	Filter Paper	0.45 µm

Preparation of Stock Standard Solution

Standard Solution Stock I: (Voriconazole)

Accurately weight and transfer 10 mg Voriconazole working standard into 10 ml volumetric flask as about diluents Methanol completely and make volume up to the mark with the same solvent to get 1000µg/ml standard (stock solution) and 15 min sonicate to dissolve it and the resulting stock solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol : Water (0.1%OPA), prepared in (80 ml MEOH : 20 ml WATER v/v) solvent.

METHOD VALIDATION

In method validation for current method was carried as per International Conference on Harmonization (ICH) Q2R1 guidelines. Validation was done through linearity, accuracy, precision, repeatability and robustness.

1. Preliminary studies on Voriconazole

Melting point

The procured reference standard of Voriconazole were found to melt in the range of 155^{o}C respectively.

Solubility

Voriconazole nitrate, a topical broad-spectrum antifungal, is developed to supply an additional agent for the treatment of superficial cutaneous and mucosal infections. The drug was found to be Crystalline solid powder, solubility available in DMSO (20 mg/ml) and methanol.

Table No. 2: Solubility Data of Voriconazole.

MEDIA	RESULTS
Water	5.4 ± 0.298
pH 1.2 acidic buffer	5.2 ± 0.118
pH 2 phosphate buffer	9.96 ± 0.827
pH 4 phosphate buffer	14.96 ± 1.065
pH 6.8 phosphate buffer	16.22 ± 0.640

pH 6.8 phosphate buffer with 0.15 % SLS	24.74 ± 0.857
pH 7.4 phosphate buffer	22.84 ± 0.331

UV Spectroscopy

UV absorption of 20 mcg solution of Voriconazole in MEOH was generated and absorbance was taken in the range of 200-400 nm. λmax of Voriconazole was found to be 256 nm respectively.

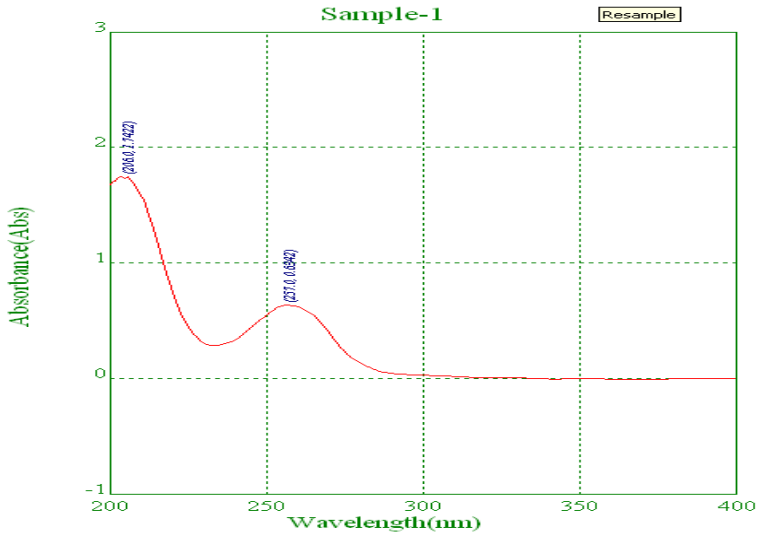

Fig No. 2: UV Spectrum of Voriconazole.

Analytical Method of Validation

In method validation for current Method was carried as per International Conference on Harmonization (ICH) Q2R1 guidelines. Validation was done through linearity, accuracy, precision, repeatability and robustness.

Linearity

The absorption spectra was recorded in the wavelength region of 200 - 400 nm in UV-Spectrophotometric methods.

Beer-Lambert's law was followed in the conc. range of **5-25** µg/ml for Voriconazole. Linearity was observed with correlation co-efficient (r2) values **0.998 with linear equation for y = 20.87x - 10.09** Voriconazole which is shown in Table The RP-HPLC Method for respective linear equation for Voriconazole was y = 20.87x - 10.09 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Voriconazole is depicted in **Fig No.3**

Table No. 3: Linearity data for Voriconazole.

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
HPLC Method	5	93.97	93.75	93.86	0.16	0.17
	10	203.11	201.36	202.24	1.24	0.61
	15	299.31	300.69	300.00	0.98	0.33
	20	403.69	404.12	403.91	0.30	0.08
	25	514.63	514.95	514.79	0.23	0.04
	Equation		y = 20.87x - 10.09			
	R^2		0.999			

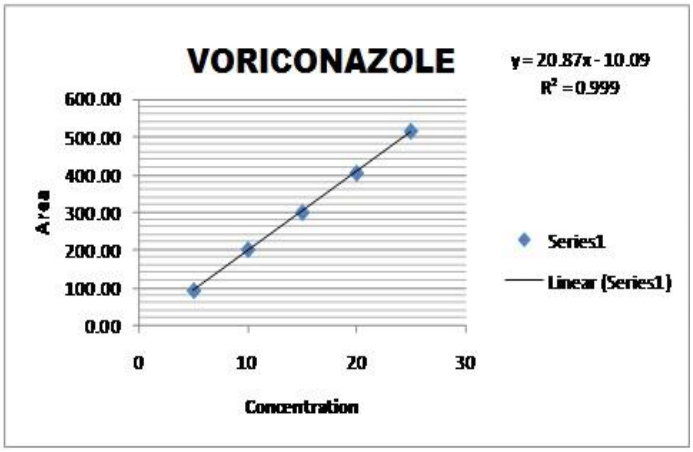

Fig No. 3: Calibration curve of Voriconazole.

2. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed **(Table No.4)** Statistical validation of recovery studies shown in **(Table No.5)**

Table No. 4: Recovery Studies Voriconazole for RP-HPLC method.

Method	Level (%)	Amt. taken (ug/ml	Amt. Added (ug/ml	Amount found Mean*±S.D.	Amt. recovered Mean*±S.D.	%Recovery Mean*± S.D.
HPLC	80%	10	8	17.95±0.06	7.95±0.06	99.34±0.76
	100%	10	10	20.02±0.08	10.02±0.08	100.18±0.74
	120%	10	12	21.99±0.01	11.99±0.01	100.07±0.29

Table No. 5: Statistical Validation of Recovery Studies Voriconazole.

Method	Level of Recovery (%)	% RSD	Standard Deviation*	Mean % Recovery
HPLC	80 %	0.06	0.06	99.34
	100 %	0.08	0.08	100.18
	120 %	0.01	0.01	100.07

***Denotes average of three determinations for RP-HPLC method**

Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-101% **(Table No.5)**

3. System suitability parameters :(Repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Voriconazole system suitability parameters were studied. The result shown in below **(Table No.6)**

Table No. 6: Repeatability studies on RP-HPLC for Voriconazole.

METHOD	Concentration of Voriconazole (mg/ml)	Peak area	Amount found (mg)	% Amount found
HPLC	15	299.51	14.80	98.71
METHOD	15	298.36		
	Mean	298.94		
	SD	0.96		
	%RSD	0.32		

Repeatability studies on RP-HPLC method for Voriconazole found to be ,The %RSD was less than 2%, which shows high percentage amount found in between 14.80 and percent amount 98.71 % indicates the analytical method that concluded .**(Table No.6)**

4. Precision

The method was established by analyzing various replicates standards of Voriconazole. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in **(Table No.7)** respectively.

Table No. 7: Result of Intraday and Inter day Precision studies on RP-HPLC method for Voriconazole.

Drug	Conc (µg/ml)	Interday Precision		Intraday Precision	
		Mean± SD	% Amt Found	Mean± SD	% Amt Found
Voriconazole	10	10.17 ± 0.66	101.76	10.07 ±5.68	100.71
	15	14.80± 0.91	98.67	30.33± 9.62	101.09
	20	19.96 ± 0.71	99.80	49.90 ±3.71	99.79

Intraday and Inter day Precision studies on RP-HPLC method for Voriconazole which shows the high precision %amount in between 101.76 % to 99.80 % indicates to analytical method that concluded.

5. Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done.

The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

Table No. 8: Result of Robustness Study of Voriconazole.

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	%RSD
		For Voriconazole	
Chromatogram of flow change 0.9 ml	20	399.32 ± 0.19	0.05
Chromatogram of flow change 1.1 ml	20	362.65 ±0.08	0.02
Chromatogram of comp change wavelength change 255 nm	20	397.2 ±0.39	0.10
Chromatogram of comp change wavelength change 257 nm	20	429.38 ±0.19	0.04
Chromatogram of mobile phase change 81+19 ml	20	414.6 ±0.40	0.10
Chromatogram of mobile phase change 79+21 ml	20	414.62 ±0.35	0.08

LOD and LOQ

LOD and LOQ were calculated from the linearity curve by using the formula

LOD = 3.3 X Avd. SD / Slope and

LOQ = 10 X Avd. SD / Slope

Table No. 9: LOD and LOQ Data of Voriconazole.

Parameters	Value
Slope	20.87
Intercept	0.58
Correlation coefficient R^2	$R^2 = 0.999$
LOD	0.0917
LOQ	0.2779

7.3 Analysis of tablet formulation

Procedure

Weigh 10 mg Voriconazole cream and calculated the average weight, accurately weigh and transfer the sample equivalent to 20 mg Voriconazole into 10 ml volumetric flask. Add about 10ml MEOH of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (10µg/ml). The simple chromatogram of test Voriconazole Shown in **(Fig No.4)** The amounts of Voriconazole per cream were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with cream formulation. Tablet Assay for %Label claim for %RSD Calculated, Result was shown in **(Table No. 9).**

Brand Name: Take 1.00 g sample in 100 ml v.f and make up the volume 100 ml with methanol means 20 mg in 100 ml methanol= 200 µg/ml voriconazole stock-ii

Table No. 10: Analysis of marketed formulation.

CON	20 µg/mL	20 µg/mL
AMT found	19.83	19.89
% L.C	99.18	99.45
S.D	0.06	0.04
% RSD	0.20	0.21

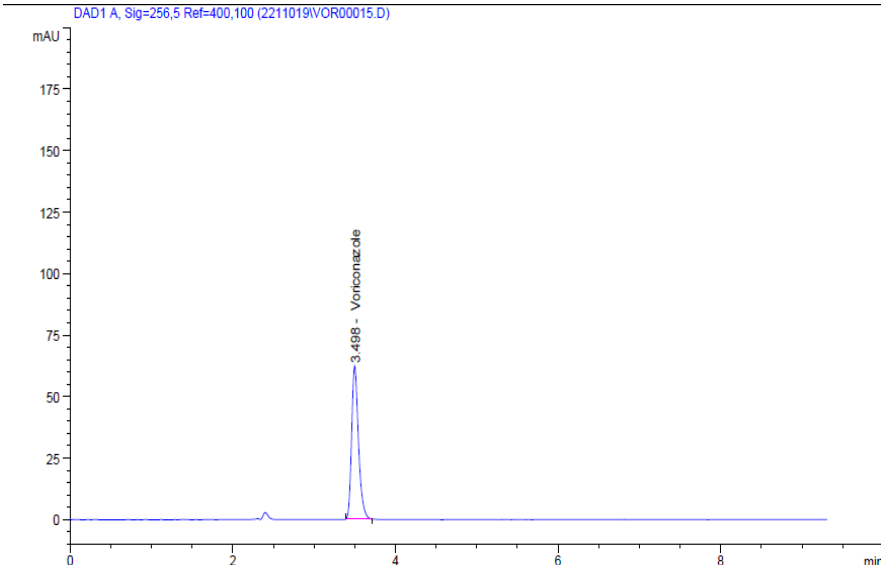

Fig No. 4: Chromatogram of marketed formulation.

CONCLUSION

In this method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, run time etc). The system with Methanol: (0.1% OPA) water (80: 20% V/V) with 1.0 ml/min flow rate is quite robust.The optimum wavelength for detection was 256 nm at which better detector response for the drug was obtained. The average retention time was found to be 3.4.In specificity it is found that there is no interference of any placebo and blank peaks with the drug of the analysis concern.The calibration was linear in concentration range of 5-25 µg/ml. The sensitivity for the voriconazole has been calculated and

the LOD and LOQ of the voriconazole was found to be 0.0917µg/mL and 0.2779 µg/mL The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 99.51 – 100.75 % Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % R.S.D. reported was found to be less than 2 %.

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