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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF VORICONAZOLE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A validated stability indicating HPTLC method for determination of Voriconazole in bulk and pharmaceutical dosage form has been developed. Chromatographic separation was performed on aluminum plates precoated with Silica Gel 60 F_{254} using Toluene: Methanol (8:2 v/v) as mobile phase followed by densitometric scanning at 256 nm. The chromatographic conditions gave compact spot for Voriconazole at Rf value of 0.45 ± 0.02 and specificity in accordance with international conference on harmonization (ICH) under prescribed stress conditions. The calibration curve was found to be linear in the range 400 - 1600 ng/band. The limit of detection and quantitation were found to be 20.22 and 61.30 ng/band, respectively. The proposed method can be applicable for the routine analysis of voriconazole in bulk and formulation.

Keywords: Voriconazole, HPTLC, Stability indicating method.

INTRODUCTION

Voriconazole chemically (2R, 3S)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol belongs to a class of antifungal medicines used to treat serious and invasive fungal infections[1]. Literature survey reveals following methods reported viz., UV-VIS spectrophotometric method[2,3], simple LC methods for determination of Voriconazole in bulk and formulation[4-6], stability indicating HPLC Method[7-9], stability indicating UPLC method[10], Determination of Voriconazole in human plasma, Rat and Beagle Dog Plasma methods[11-14].

There were no reports found for stability indicating High Performance Thin Layer Chromatographic (HPTLC) method. Thus new simple, accurate, precise stability-indicating HPTLC assay method has been developed and validated for the determination of Voriconazole in bulk and pharmaceutical dosage form as per ICH guidelines[15,16].

MATERIALS AND METHODS:

Voriconazole standard was kindly provided by Alkem Laboratories Ltd., Mumbai, India. Voriconazole tablets were procured from local pharmacy. Methanol, Acetone and all other reagents used in this study were of AR grade purchased from Merck Pvt. ltd, Mumbai.

Selection of analytical wavelength

The standard solution of Voriconazole in methanol was scanned over wavelength range 200 to 400 nm by using UV-Visible spectrophotometer. Wavelength 256 nm was

selected for analysis where Voriconazole showed higher absorbance (Figure 2).

Chromatographic conditions

Pre-coated silica gel 60 F_{254} TLC (E-Merck, Germany) plates (10x10 cm) were used as stationary phase. TLC plates were pre-washed with methanol and activated at 110°c for 10 min prior to application. The standard samples of Voriconazole were spotted on pre-coated TLC plates in the form of bands of length 4 mm using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat-5 applicator (Camag, Switzerland). The chromatographic development was carried using toluene: methanol (8:2 v/v) as mobile phase with chamber saturation time of 20 minutes and the migration distance of 80 mm. Densitometric scanning was performed using Camag TLC scanners 3 at 256 nm, operated by win CATS Software (Version 1.4.3, Camag). Deuterium lamp was used as a radiation source. All weighing was done on Shimadzu balance (Model AY-120).

Preparation of standard solution

A standard stock solution of Voriconazole was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. One ml of this solution was further diluted to 10 ml to get 100 µg/ml solution of Voriconazole.

VALIDATION

Linearity and Range

The calibration curve was obtained in the range of 400 - 1600 ng/band by applying different volumes (4-16 µl) of stock solution (100 µg/ml) on TLC plate. Each standard in six replicates was analyzed and peak areas were recorded. Standard calibration graph was plotted of peak area Vs concentration applied.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation study. In the intraday study 3 replicates of 3 standard concentrations (600, 800 and 1000 ng/band) were analyzed in a day and percentage RSD was calculated (Table 1). For the inter day study 3 standard concentrations (600, 800 and 1000 ng/band) were analyzed on 3 consecutive days and percentage RSD was calculated (Table 2).

Accuracy

To check the accuracy of the method, recovery studies were carried out by over-spotting standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %. Basic concentration of sample chosen was 600 ng/band. The areas were noted after development of plate. The drug concentration was calculated using regression equation. (Table 3).

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for drug in sample was confirmed by comparing the Rf and spectra of the spot with that of standard drug spot. The specificity of the method was also ascertained by peak purity profiling studies by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on Win CATS software (Version 1.4.3, Camag).

Robustness

The robustness of the method was studied, during method development by small but deliberate variations in method parameters like time from application to development (0, 30, 60, 120 min) and time from development to scanning (0, 30, 60, 120 min). One factor at a time was changed to study the effect on the peak area of the drug. Study was carried out at a concentration level of 1000 ng/band.

Stress degradation studies

Stress degradation studies were carried under condition of acid/ base as well as neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared. The blank subjected to stress in the same manner as the drug solution. Dry heat and photolytic degradation was carried out in solid state.

Degradation under alkali condition

1 ml working standard solution of Voriconazole (1000 µg/ml) was mixed with 1 ml of 0.1 N NaOH (methanolic) and 8 ml of methanol. Solution was kept for 24 Hrs. 10 µl of the resulting solution was spotted on TLC plate.

Degradation under acid condition

1 ml working standard solution of Voriconazole (1000 µg/ml) was mixed with 1 ml of 0.1 N HCl (methanolic) and 8 ml of methanol. Solution was kept for 24 Hrs. 10 µl of the resulting solution was spotted on TLC plate.

Degradation under neutral condition
1 ml working standard solution of Voriconazole (1000 µg/ml) was mixed with 1 ml of distilled water and 8 ml of methanol. Solution was kept for 24 Hrs. 10 µl of the resulting solution was spotted on TLC plate.

Degradation under oxidative condition
1 ml working standard solution of Voriconazole (1000 µg/ml) was mixed with 1 ml 3 % solution of H_2O_2 (methanolic) and 8 ml of methanol. Solution was kept for 2 Hrs. 10 µl of the resulting solution was spotted on TLC plate.

Degradation under dry heat
Dry heat studies were performed by keeping drug sample in oven (60^0 C) for a period of 24 Hrs. Sample was withdrawn, dissolved in methanol and diluted to get 1000 µg/ml. 1 ml was further diluted to get 100 µg/ml solution of which 10 µl volume was spotted on TLC plate.

Photo-degradation studies
Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool white fluorescent light to achieve an illumination of 1.2 Million Lux.Hr. Sample was weighed, dissolved and diluted to get 1000 µg/ml. 1 ml was further diluted to get 100 µg/ml solution of which 10 µl volume was spotted on TLC plate.

RESULTS AND DISCUSSION

Optimization of mobile phase
Method development for Voriconazole was started with the development of densitogram with neat solvents and combinations of Toluene, n-hexane, Ethyl acetate, and Methanol in different ratios. Toluene: Methanol in the ratio of (8:2 v/v) was selected as the mobile phase which resulted in good resolution and acceptable peak parameters. The Rf found to be 0.45 ± 0.02. (Figure 3). The results were found to be linear in the concentration range 400 – 1600 ng/band with correlation coefficient of 0.995. The results of validation are summarized in Table 4.
Drug was subjected to various forced degradation conditions. Although drug shown reduced percentage assay under all conditions; the peak for degradation product was observed under only base degradation (Figure 4). Summary of stress degradation results is given in Table 5. Peak purity results greater than 0.9990 indicate that peak is homogeneous in all stress conditions tested. The unaffected assay of drug in the tablet confirms the stability indicating power of the method.

CONCLUSION

Voriconazole degrades significantly under base hydrolysis as compared with acid hydrolysis. Proposed study describes that stability indicating HPTLC method for the estimation of Voriconazole in pharmaceutical dosage form is simple, specific, selective, robust, rugged and precise.

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Table 1: Intraday study for Voriconazole

Concentration(ng/band)	Intraday mean area*	% Recovery	SD	% RSD
600	2760.7	100.2	17.47	0.63
800	3665.7	100.9	29.93	0.81
1000	4530.9	100.4	26.76	0.59

*** Average of 03 determinations**

Table 2: Interday study for Voriconazole

Concentration(ng/band)	mean area*	% Recovery	SD	% RSD
600	2754.03	99.98	8.64	0.31
800	3672.4	101.1	34.50	0.93
1000	4561.3	101.3	30.22	0.66

*** Average of 03 determinations**

Table 3: Determination of accuracy for Voriconazole

Level	Conc.(ng/band)		Area	Average	% recovery	% RSD
50	600	300	3991.9	4001.7	101.2%	0.25
			4001.1			
			4012.2			
100	600	600	5316.5	5305.1	102.7	0.19
			5302.9			
			5295.1			
150	600	900	6539.9	6506.1	102.03	0.46
			6480.3			
			6499.1			

Table 4: Summary of validation study

Sr. No.	Validation Parameter	Results
1.	Linearity	y = 4.393x+118.6, R^2=0.998
2.	Range	400-1600 ng/band
3.	Precision	% RSD
	A) Intraday precision	0.67
	B) Interday precision	0.63
4.	Accuracy	% RSD
	50 %	0.25 %
	100 %	0.19 %
	150 %	0.46 %
5.	LOD	20.22 ng/band
6.	LOQ	61.30 ng/band`
7.	Specificity	Specific
8.	Robustness	Robust

Table 5: Summary of stress degradation of Voriconazole

Stress Degradation Condition	Percent Assay	Percent degraded (%)	R_f of degradation product	Peak purity	
				r(s,m)	r(m,e)
Base (0.1 N NaOH, kept for 24 Hrs)	76.25%	23.75	1) 0.27	0.9998	0.9992
			2) 0.49	0.9995	0.9996
Acid (0.1 N HCl, kept for 24 Hrs)	80.70%	19.3	-	0.9996	0.9998
Neutral (0.1 N HCl, kept for 24 Hrs)	71.36%	28.44	-	0.9997	0.9994
H_2O_2 3% (kept for 2 Hrs)	87.96%	12.04	-	0.9992	0.9994
Heat dry (60^0C, 24 Hrs)	73.97	26.03	-	0.9990	0.9991
Photo stability (UV, 200 watt hrs/square meter	86.23%	13.77	-	0.9997	0.9994
Florescence , 1.2 million Lux. Hrs)	82.67%	17.33	-	0.9998	0.9995

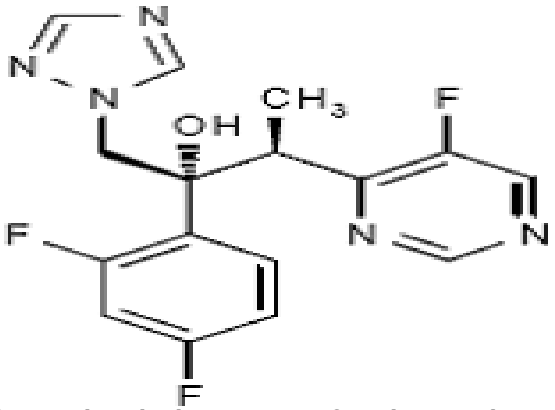

Fig. 1: Chemical structure of Voriconazole

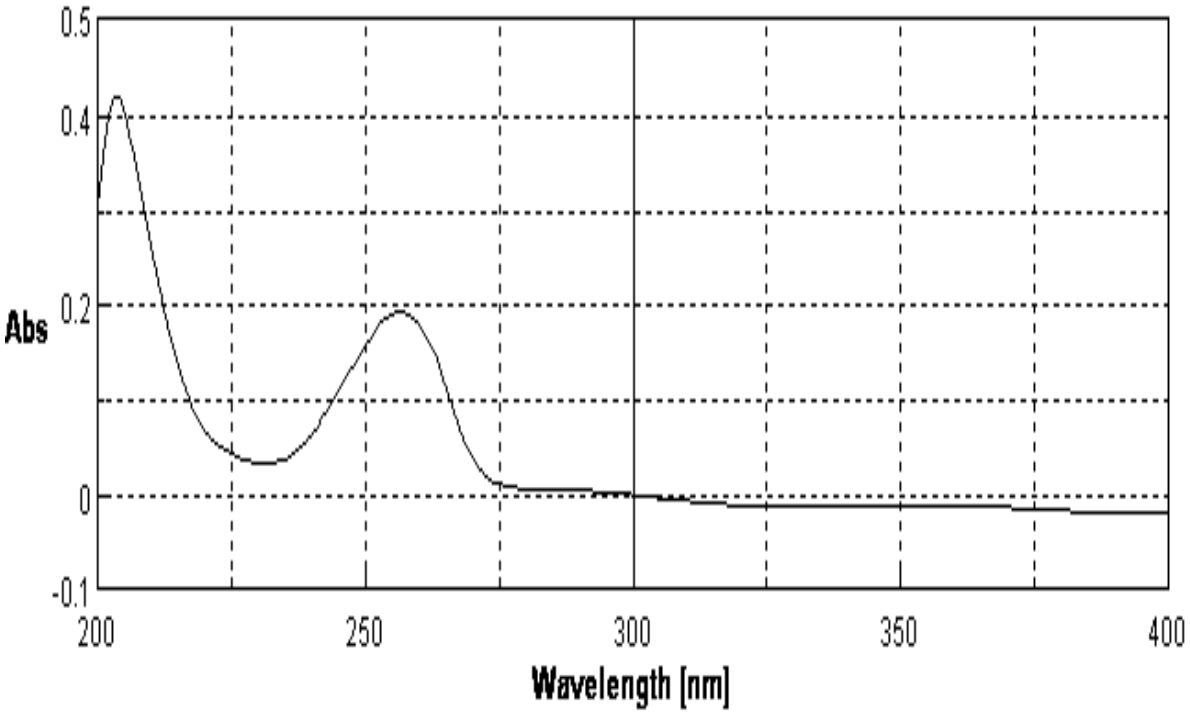

Fig. 2: UV spectra of Voriconazole between 200 and 400 nm

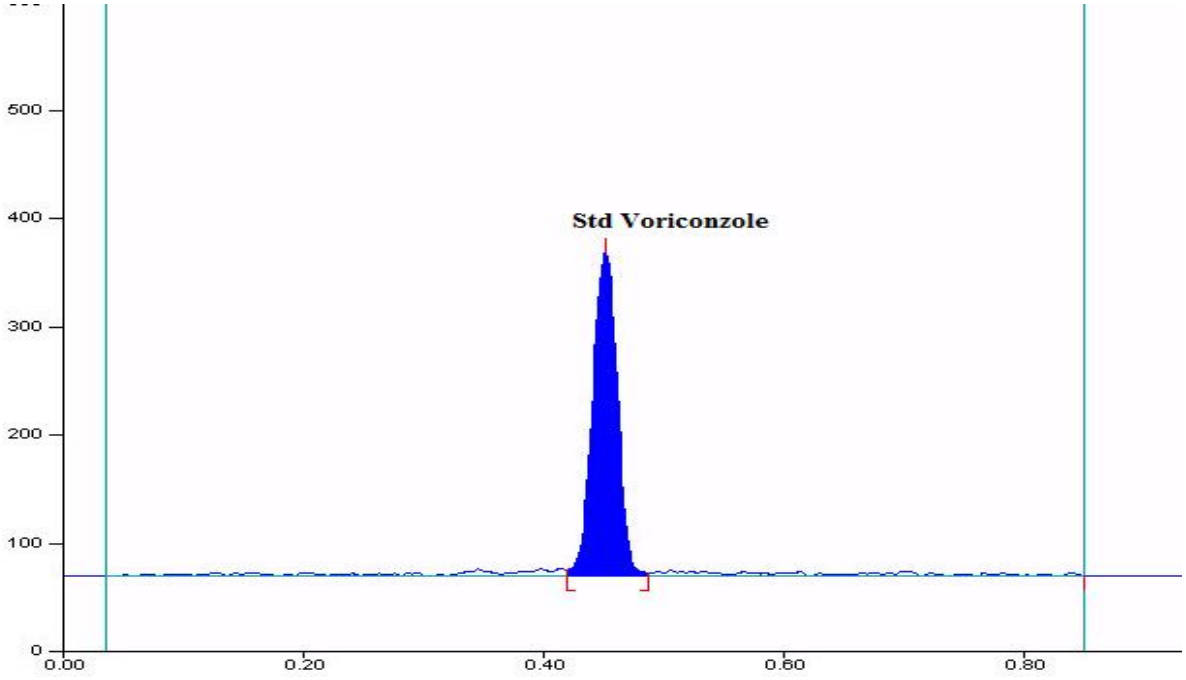

Fig. 3: Typical Densitogram of standard Voriconazole (100 ng/band)

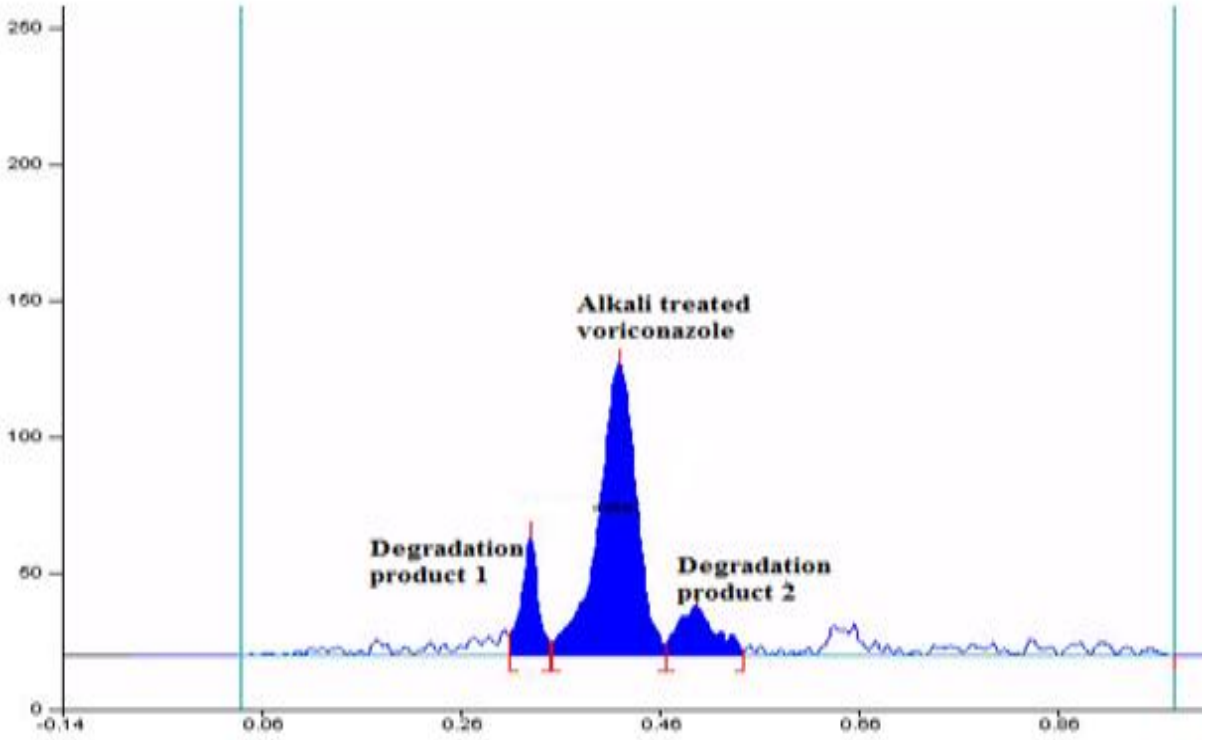

Fig. 4: Alkali treated Voriconazole

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