ISSN: 2321-3132

Research Article

International Journal of Chemistry and Pharmaceutical Sciences

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Method Development Validation and Degradation studies of Voriconazole Drug by RP-HPLC Method

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Received: 24 July 2014, Accepted: 30 August 2014, Published Online: 27 September 2014

Abstract

A rapid and sensitive RP–HPLC method with UV detection (260 nm) for routine analysis of voriconazole was developed. Chromatography was performed with mobile phase containing a mixture of acetonitrile and water (50:50, v/v) with flow rate of 1.0 ml min−1. Quantitation was accomplished with internal standard method. The procedure was validated for linearity (correlation coefficient = 0.9999), accuracy, robustness and intermediate precision. Experimental design was used for validation of robustness and intermediate precision. To test robustness, three factors were considered namely Percentage of acetonitrile in mobile phase, Flow rate and pH. An increase in the flow rate results in decrease of the drug found concentration, while the percentage of organic modifier and pH has no important effect on the response. For intermediate precision measure the variables considered were: analyst, equipment and number of days. The R.S.D. value (0.45%, n = 24) indicated a good precision of the analytical method. The proposed method was simple, highly sensitive, precise and accurate and retention time less than 4 min indicating that the method is useful for routine quality control.

Keywords: Voriconazole, HPLC, Degradation studies

Contents

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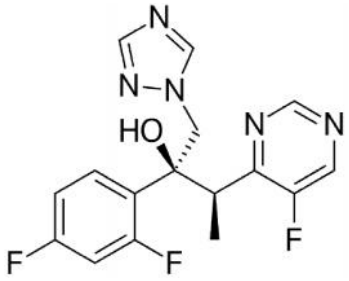
Manuscript ID: IJCPS2252

PAPER-QR CODE

1. Introduction

Voriconazole is a triazole antifungal medication that is generally used to treat serious, invasive fungal infections. These are generally seen in patients who are immunocompromised, and include invasive candidiasis, invasive aspergillosis, and certain emerging fungal infections. Voriconazole is chemically called as (2R,3S)-2-(2,4-Difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol. Formula is $C_{16}H_{14}F_3N_5O$ molecular weight 349.311 g/mol. Mechanism of action of the Voriconazole is a triazole antifungal 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.



2. Materials and Methods

Voriconazole was collected from MSN Laboratories, India.

Buffer preparation:

Accurately weighed 1.41gm of Disodium hydrogen Ortho phosphate was transferred into a 1000ml of Volumetric flask and about 900ml of Milli-Q water was added and sonicated to degas, finally the volume was made up with Milli-Q water and the pH was adjusted to 4.0 with dil. OPA.

Standard Preparation: (Voriconazole 1000µg/ml, Impurity-A 5µg/ml)

Accurately Weighed 1mg Impurity-A of Voriconazole was transferred into 10ml volumetric flask diluted to 10ml with diluents and labeled as Impurity stock. 10mg Voriconazole drug was weighed and taken into a 10 ml clean dry volumetric flask, 5ml diluent was added and, sonicated for 30 minutes and labeled as working solution. 0.5ml from the impurity stock solution was pipette out and transferred into flask labeled as working solution and made up the final volume with diluents.

Method development & Optimization:

Using Mobile phase consisting of different buffers and methanol at different concentrations and different mobile phase's pH values are attempted .A peak was observed in which the shape and retention time of Voriconazole was found to be broad compared to the buffer and acetonitrile composition of mobile phase. After selecting the best conditions based on peak performance, buffer solution and acetonitrile in the ratio 50:50 and using HPLC column is Agilent Zorbax C_{18} 250mmx4.65µm, the run times of the proposed method was 25mins with isocratic solution. Column temperature is 30°C,flow rate is 1ml/min, PDA Detector is mainly used this purpose. After injecting the standard solution volume was found to be 10µL. Retention times found were about 4.5 minutes for Voriconazole.

Table 1

Name	**Manufacture**
HPLC	Water e2695 Alliance HPLC system connected with PDA Detector 2998 and Empower2software
Ultra Sonicator	Fastclean Ultrasonic cleaner
pH meter	Lab India
Electronic Balance	Sartorius
HPLC column	Agilent Zorbax C_{18}

3. Result and Discussion

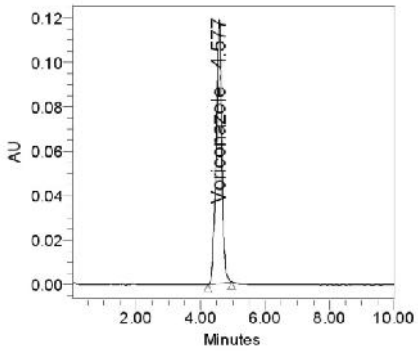


Figure 1

Table 2

Peak Name	RT	Area	USP Plate Count	USP Tailing
Voriconazole	4.550	1466295	4735	1.25

Method Validation:
Precision:

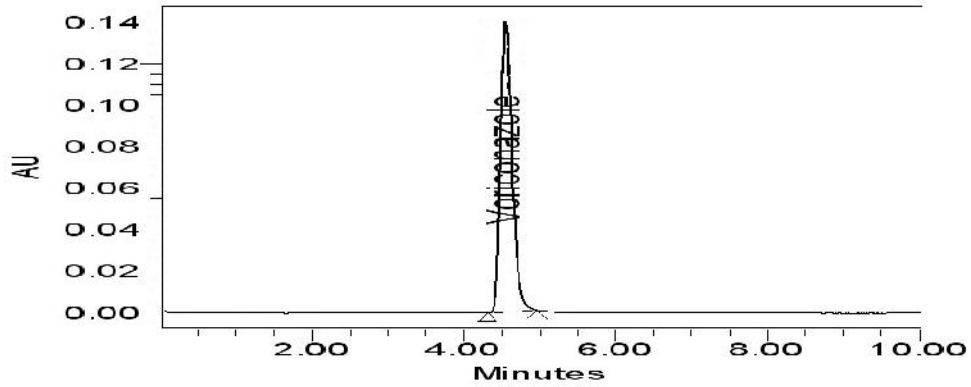


Figure 2

Table 2

Sample name	Sample weight	RT	Area	%Assay
Precision 1	25	4.564	12692820	98
Precision 2	25	4.573	12389747	97
Precision 3	25	4.575	12684412	96
Precision 4	25	4.575	12696633	96
Precision 5	25	4.578	12622426	95
Precision 6	25	4.578	12807765	96
%RSD				1.1

Accuracy: The spiked level was found to be at 50,100,150 and the % recovery was found to be 100%.
Linearity:

Table 3

Voriconazole		
%Level	Conc (mg/mL)	Area
25	0.002	27314
50	0.003	40432
100	0.005	69536
150	0.008	108581
200	0.01	136171

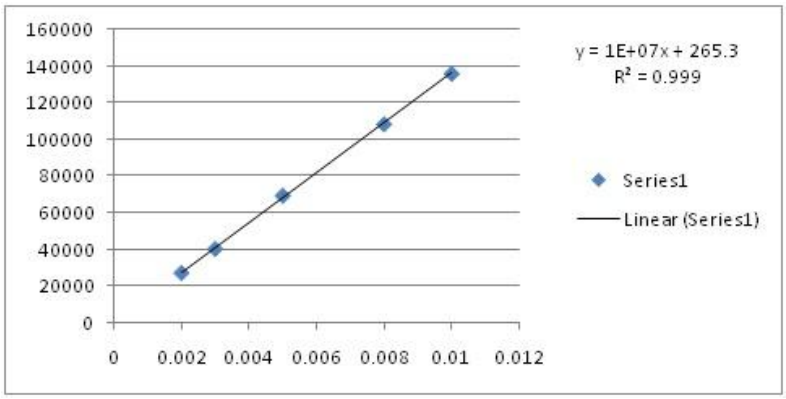


Figure 3

LOD:

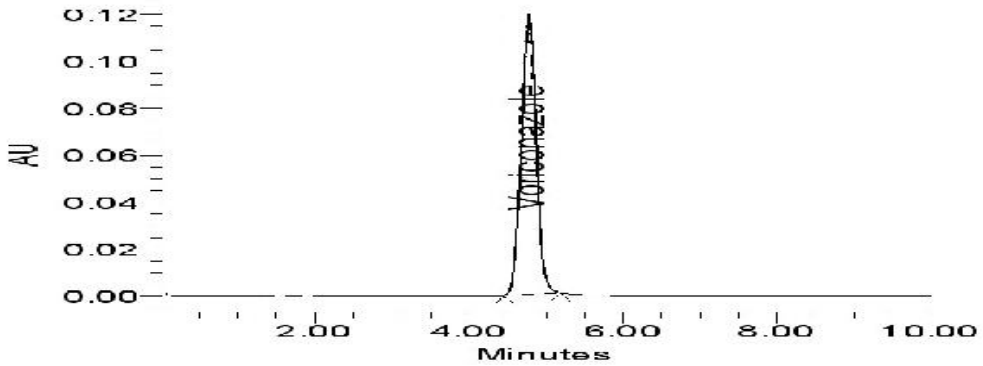


Figure 4

LOQ:

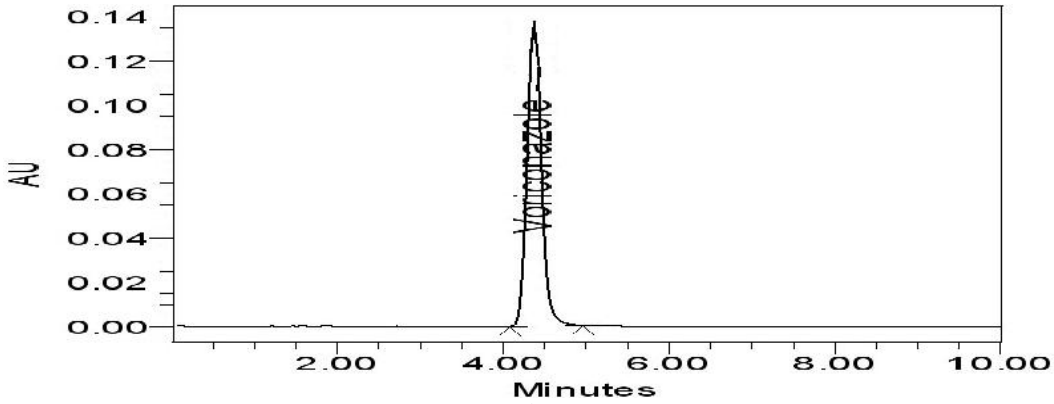
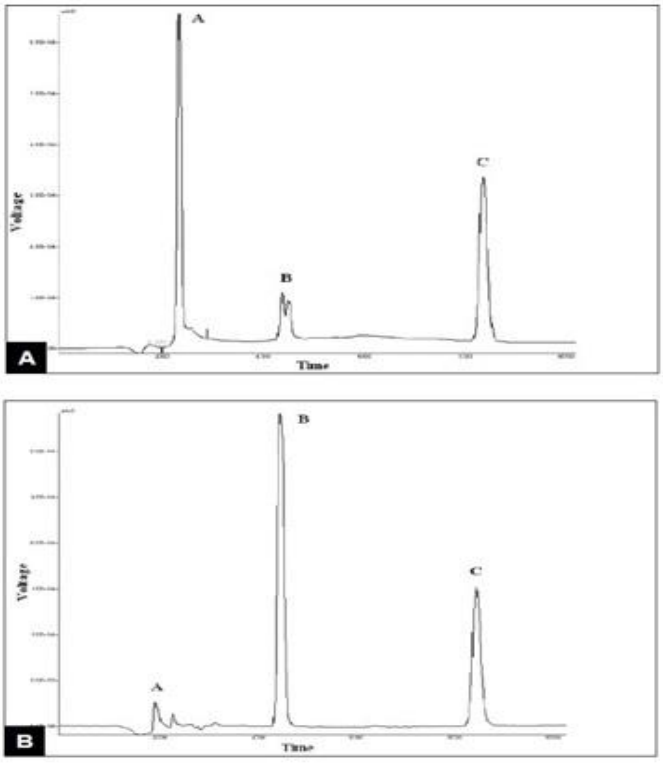


Figure 5

Discussion: LOD for Voriconazole was 9.9 µg/ml respectively, while LOQ was 10.2µg/ml.
Degradation Studies: Result of Stress Degradation Studies of Voriconazole



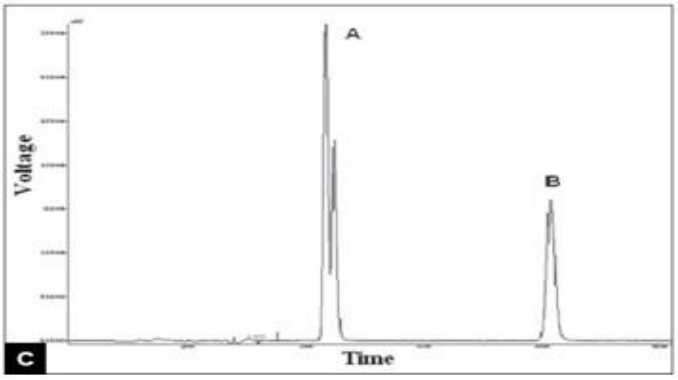


Figure 6: Chromatogram of voriconazole subjected to hydrolytic conditions

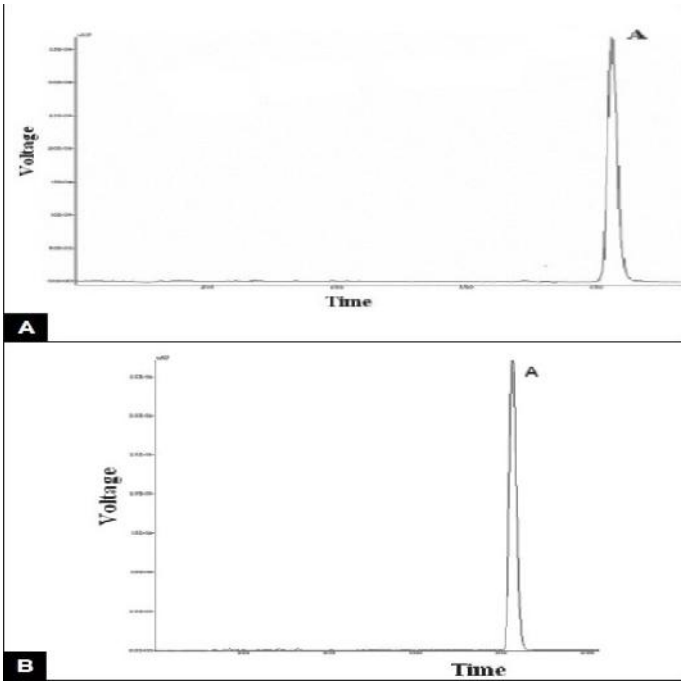


Figure 7: Chromatogram of voriconazole subjected to heat and light A: upon exposure to heat and B: upon exposure to light.

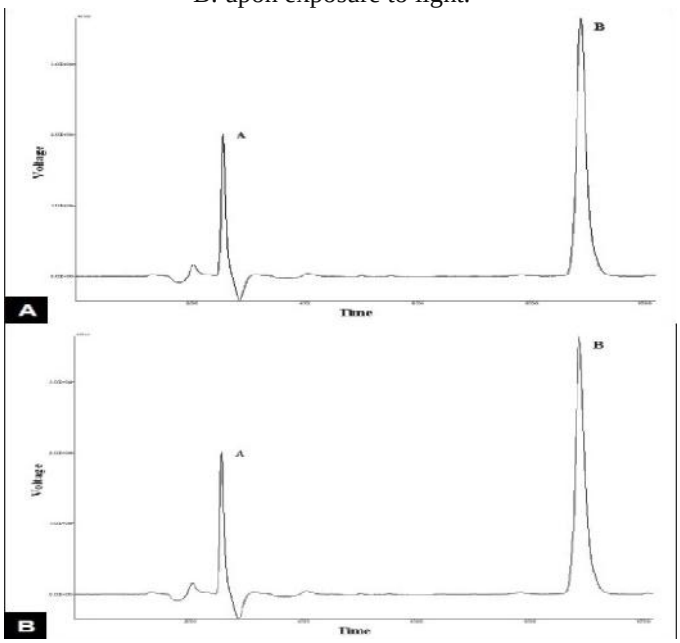


Figure 8: Chromatogram of voriconazole subjected to oxidative conditions

Table 4

Condition	Time(Min.)	%Degradation	Retention time of degradation products(min.)
0.1N NaOH	30	55.58	4.45
	60	78.75	4.44
	90	100	4.44
3N HCl	30	11.60	4.41
	60	16.92	4.41
	90	25.11	4.42
	120	29.99	4.45
	150	35.06	4.44
30% H_2O_2 (1ml)	15	1.17	None Detected
30% H_2O_2 (5ml)	20h	5.40	None Detected
Dry heat 70^0C	48h	0.24	None Detected
Photolytic	--	4.68	None Detected
Neutral(Reflux)	1h	56.92	4.3

4. Conclusion

The HPLC method described in this study is used for determination and validation of Voriconazole. LOD was found to be 9.9 µg/ml and LOQ was 10.2 µg/ml, the developed method may be recommended for routine and QC analysis of the investigated drugs to provide simple, accurate and reproducible quantitative analysis for the determination. The study shows that Voriconazole undergoes degradation in acidic, alkaline and neutral hydrolytic conditions whereas it is relatively stable when exposed to dry heat, oxidation and photolytic conditions. A stability-indicating method was developed, which resolved all the degradation products formed under variety of conditions. The degradation products formed under acid, alkali and neutral hydrolysis condition are same which is confirmed from UV spectrum of the degraded product. The method is proved to be simple, accurate, precise, specific and selective. Hence the method may be used for assay of the product during stability studies.

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