International Journal of Research in Pharmacy and Life Sciences

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R E S E A R C H A R T I C L E

RP-HPLC Stability Indicating method for Separation of Impurities in Voriconazole

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A B S T R A C T

The author developed RP-HPLC method for the determination of the impurities of Voriconazole with adequate resolution in Voriconazole Injection. The current official USP monograph uses two methods for the determination of Voriconazole impurities in Voriconazole Injection and the resolution between impurities Retention time Impurity- RRT at Impurity-C (0.43), impurity–D (0.52) is not appropriate. The analytical method uses inertsil ODS-3V 250-mm x 4.6 mm, 5 µm, (or) Equivalent column and the mobile phase consists of Sodium Dihydrogen Phosphate, water and ACN adjusted the pH with diluted ortho phosphoric acid with a column temperature at the injection volume To finalize the above chromatographic conditions. pH condition of the mobile phase was optimized with different pH. To improve the retention time and resolution for impurity C and Impurity-D ion pairing reagent was introduced in the mobile phase by using the chemicals & reagents in AR Grade. (Sodium Dihydrogen Phosphate). By Using Filter Membrane 0.45 µm nylon membrane filter and degas to separate the Impurities By using Auto Sampler Equipment. And Calculate the Potency of Working Standard As is Basis, Relative Response Factor and add the Label claim.

Key words: Voriconazole, Impurity-C, Impurity- D, HPLC, pH, Membrane Filter

A R T I C L E I N F O

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PAPER QR-CODE

A R T I C L E H I S T O R Y: Received 15 March 2018, **Accepted** 19 April 2018, **Available Online** 24 May 2018

Citation: *Dr Srinivas Jagarlapudi, et al.* RP-HPLC Stability Indicating method for Separation of Impurities in Voriconazole.*Int. J. Res. Pharm, L. Sci.,* 2018, 6(1): 25-30.

C O N T E N T S

1. Introduction

Voriconazole (Vfend, Pfizer) is a triazole antifungal medication used to treat serious fungal infections. It is used to treat invasive fungal infections that are generally seen in patients who are immune compromised. These include invasive candidiasis, invasive aspergillosis, and emerging fungal infections.Voriconazole binds and inhibits ergosterol synthesis by inhibiting CYP450-dependent 14-alpha sterol demethylase. The inhibition of 14-alpha sterol demethylase results in a depletion of ergosterol in fungal cell membrane.

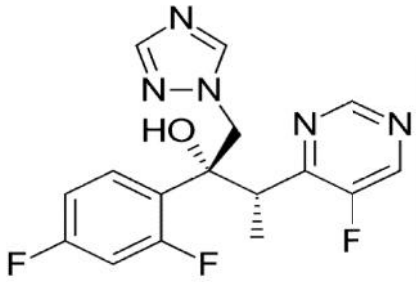

Fig 1: Chemical structure of Voriconazole

The main aim of present work to develop analytical method for determination of impurities(C & D) in Voriconazole. The method was validated as per ICH guidelines and is simple, rapid and reliable gradient RP-HPLC for the determination of Voricconazole impurities in Voricconazole injection.

2. Materials and Methods

Chemicals and Reagents:

API of voriconazole was procured from gift sample of Celon labs, Hyderabad. HPLC grade water and Acetonitrile were purchased from merck laboratories, Mumbai. AR grade Di sodium hydrogen phosphate also used.

Instrument:

A High performance liquid chromatography system with gradient elution capability, a spectro photometric UV detector and an auto sampler (Agilent 1260 HPLC or equivalent). Weighing Balance. Ultra Sonicator.

Chromatographic conditions:

Column	: ODS- 250-mm x 4.6 mm, 5 µm,
(or) Equivalent	
Make	: inertsil ODS-3V
Flow rate	: 1 mL per minute
Injection size	: 20 µL
Wave length	: 254 nm
Column Temperature	: 40˚c
Run time	: 50 minutes

Preparation of Buffer: Weigh accurately about 7.8 g (0.05M) of Sodium dihydrogen phosphate dihydrate in 1000 mL of water. Filter through membrane filter and degassed with ultra sonicator.

Preparation of Mobile phase:

Mobile phase A and B was prepared for mixing of Buffer and Acetonitrile in the ratio 800:200, filter through 0.45 µm nylon membrane filter and degas.

Preparation of Standard solutions

Diluted Standard [Concentration: 0.001 mg/mL]: Weight accurately 20mg of working standard in 100 ml volumetric flask, pipette out 1ml of solution in 200 ml of volumetric flask.

Sensitivity solution [Concentration: 0.0001 mg/mL]: Pippete 1 ml from above standard solution in 10 ml volumetric flask.

Sample Preparation [Concentration: 1 mg/mL]: Open one Sample vial, reconstitute with 19 ml of water in each vial and transfer this solution into 200 mL volumetric flask, mix and make up to the volume with diluent.

Placebo Preparation [concentration:1 mg/mL]: Open one Placebo vial, reconstitute with 19 ml of diluent in each vial and transfer this solution into 200 mL volumetric flask, mix and make up to the volume with diluent.

3. Results and Discussion

Method validation

Method validation parameters like specificity, linearity, accuracy, precision, LOD&LOQ and robustness, system suitability along with stability indicating studies are stastically validate to as per ICH guidelines Q2(R1).

Specificity:

Blank, Placebo, Standard, sample & Individual impurities were Injected in HPLC with PDA detector and observed the interference of blank and placebo.There is no significant interference of Blank and Placebo. The interference was observed in main peak, it having impurities C and D. Results are shown in fig 1,2 and 3.

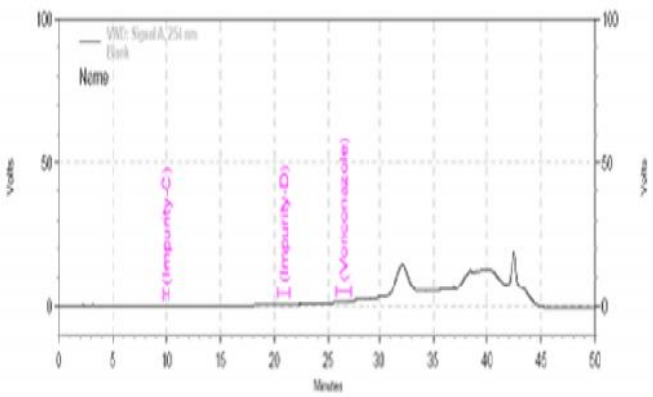

Fig 2: Chromatogram for Blank

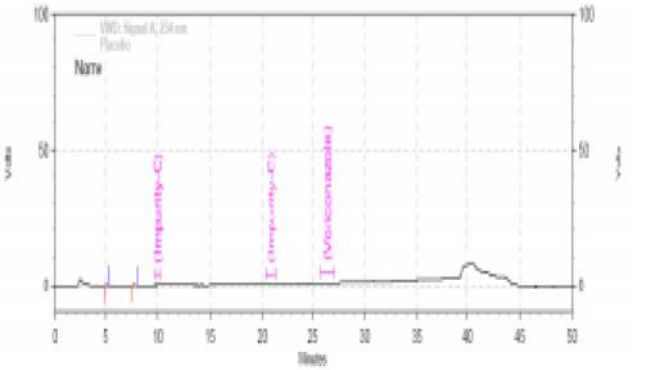

Fig 3: Chromatogram for Placebo

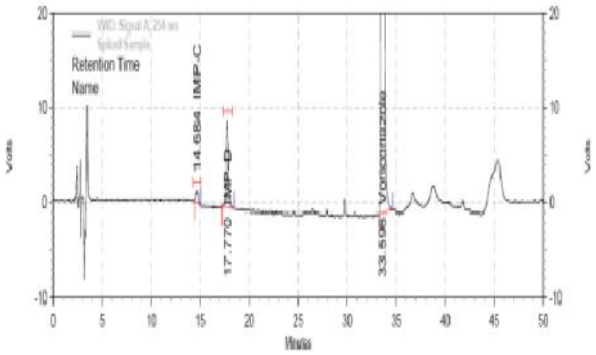

Fig 4: Chromatogram for Main peak

Linearity:

The linearity of response for Voriconazole & its impurities were determined at seven different concentration levels as shown in the following table and enclosed graphically. The results are calculated from linearity graph using the linearity equation. Regression analysis shows linear relationship between concentration and the response of analyte with in specified range. Results were shown in table 1.

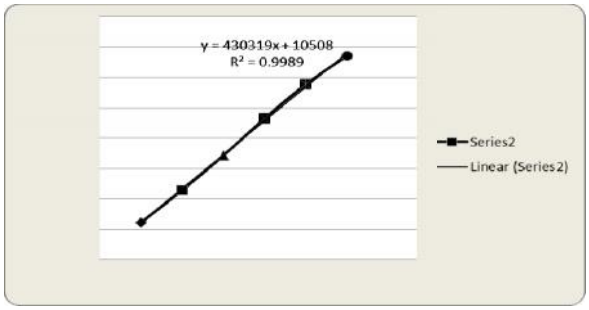

Fig 5: Calibration Curve of Voriconazole

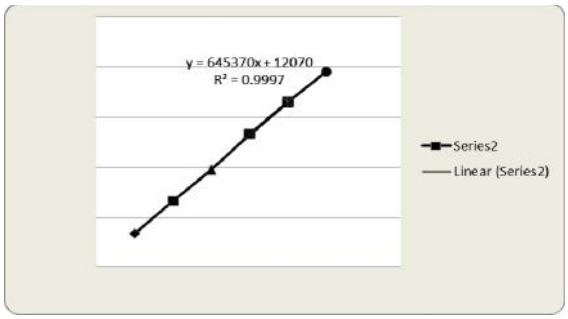

Fig 6: Calibration Curve of Impurity-C

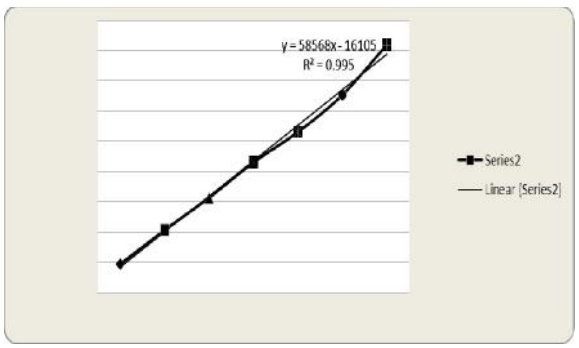

Fig 7: Calibration Curve of Impurity-D

Accuracy:

A known amount of both analyte, and Excipients in appropriate levels above and below the normal levels expected in the sample was prepared and analyzed by the proposed method and the results are shown in the following table 2.

Acceptance criteria: The % Recovery should be in between 85.0 to 115.0 & 75.0 to 125.0 for LOQ.

Observation: The results indicate that the recovery of analyte is accurate within the specified range.

Precision

Intraday and intermediate precision was carried and calculate the %RSD.

Repeatability (Intraday): Six preparations of sample were prepared and measured the absorbance; the results along mean value for the RS of Voriconazole for injection were shown in the table3.

Intermediate Precision: Six preparations of sample were prepared and measured the absorbance; the results along mean value for RS of Voriconazole for injection were shown in the table 4.

Acceptance criteria: % RSD should not be more than 10.0, For Unknown impurities NMT 15.0%

Observation: The % RSD value indicates a good degree of precision within the specified range.

LOD &LOQ:

The LOD/LOQ of Voriconazole & its impurities were established with proper trails. So that Signal to Noise ratio is about 10 for LOQ and about 3 for LOD. The Concentrations and S/N ratios are given below table 5.

Robustness:

To establish the robustness of the method employed for analysis of Related substances of Voriconazole for injection, the method was challenged for various parameters like Change in Flow and Change in Column temperature. The observations in different conditions are tabulated in 6 and 7.

Acceptance criteria: Analytical method should not be affected by changing the small variations in method parameters.

Observation: There is no significant effect on the result by doing small changes in the Column temperature and Flow rate.

Forced Degradation studies:

Forced degradation experiments were conducted to establish the stability indicating capability of the method. For each stress experiment, Drug product was stressed under conditions shown in bellow Table and analyzed as per the proposed analytical method using a HPLC equipped with a photodiode array detector.

Observation: Compound significant degradation was observed in Acid hydrolysis, Base Hydrolysis and Oxidation and thermal degradation conditions. Hence method is considered to be stability indicating.

Solution stability:

Spiked impurity sample solution and standard solutions (2 ppm) were prepared and stored at ambient temperature and refrigerated conditions. The analysis of each solution was repeated at periodic intervals covering a time period of 24 hrs. Standard solutions were stable for 24 hr when stored at

room temperature and at refrigerated conditions. Standard & Sample solutions are not stable up to 24 hrs at Room temperature & Refrigerator (2°-8°C) so inject freshly prepared samples and maintain sample temperature at 5°C.

Table 1: Linearity results for Voriconazole and imp C & D

S. No	Level	Voriconazole		Impurity C		Impurity D	
		PPM	Peak area	PPM	Peak area	PPM	Peak area
1	25	0.2597	122695	0.5039	336888	0.2636	47786
2	50	0.5195	229447	1.0079	662417	0.5273	103901
3	75	0.7792	343358	1.5118	975419	0.7909	157433
4	100	1.0390	465331	2.0157	1326952	1.0545	215713
5	125	1.2987	577701	2.5197	1649665	1.3182	265785
6	150	1.5585	671765	3.0236	1950809	1.5818	326945
7	200	2.0780	934946	4.0315	2730348	2.1091	439607
Correlation Coefficient			**0.9993**	**0.9992**		**0.9999**	
Slope			**443204.36**	**670946.25**		**211879.75**	
Y-Intercept			**962.3566**	**-2486.7213**		**-8959.6721**	

Table 2: Recovery results for Voriconazole and Imp C& D

Sample No.	Recovered (%)		
	Voriconazole	Imp-C	Imp-D
LOQ Prep-1	99.3	106.5	116.7
LOQ Prep-2	85.3	97.1	115.8
LOQ Prep-3	88.6	106.9	115.1
50% Prep-1	113.8	104.4	114.3
50% Prep-2	113.6	103.4	97.3
50% Prep-3	113.6	100.0	96.2
100% Prep-1	104.4	102.6	96.1
100% Prep-2	102.1	102.6	99.4
100% Prep-3	104.0	103.0	99.2
150% Prep-1	100.7	109.8	107.4
150% Prep-2	101.3	108.8	94.2
150% Prep-3	100.6	111.5	100.8
200% Prep-1	101.1	112.4	103.3
200% Prep-2	105.3	112.0	106.0
200% Prep-3	101.2	114.6	105.3
Mean	**102.3**	**106.4**	**104.5**
Standard deviation	**8.012**	**5.064**	**7.851**
% RSD	**7.83**	**4.76**	**7.51**

Table 3: Repeatability results for Voriconazole

Sample No.	Un Known-Max	IMP-C	IMP-D	Total Impurities
Sample-1	0.1314	0.4057	0.1273	0.6644
Sample-2	0.1348	0.4116	0.1263	0.6727
Sample-3	0.1312	0.4066	0.1287	0.6665
Sample-4	0.1351	0.4108	0.1270	0.6729
Sample-5	0.1344	0.4102	0.1265	0.6711
Sample-6	0.1321	0.4077	0.1252	0.6650
Avg.	**0.1322**	**0.4088**	**0.1268**	**0.6688**
Stdev.	**0.002**	**0.002**	**0.001**	**0.004**
%RSD	**1.3**	**0.6**	**0.9**	**0.6**

Table 4: Intermediate precision results for Voriconazole

Sample No.	Un Known-Max	IMP-C	IMP-D	Total Impurities
Sample-1	0.1419	0.4355	0.1386	0.7160
Sample-2	0.1276	0.4150	0.1365	0.6791
Sample-3	0.1421	0.4353	0.1391	0.7165

Sample-4	0.1273	0.4155	0.1377	0.6805
Sample-5	0.1411	0.4332	0.1383	0.7126
Sample-6	0.1279	0.4098	0.1353	0.6730
Avg.	0.1347	0.4241	0.1376	0.6963
Stdev.	0.008	0.012	0.001	0.021
%RSD	5.7	2.8	1.0	3.0

Table 5: Results for LOD & LOQ

Peak	Conc. of LOQ (PPM)	S/N Ratio (LOQ)	Conc. of LOD (PPM)	S/N Ratio (LOD)
Voriconazole	0.050	11.11	0.017	2.74
IMP-C	0.047	10.77	0.015	2.00
IMP-D	0.200	10.24	0.066	2.94

Table 6: Robustness results for Change in Flow rate

Parameter	Actual 1.0 ml/min	Change-1 0.8 ml/min	Change-2 1.2 ml/min
%RSD of Three replicate injections of reference solution	2.15	3.43	1.17
Recovery of Sensitivity Solution and Diluted Standard	112.19	103.39	114.73
Theoretical Plates of Voriconazole peak	121079	152915	112264

Table 7: Robustness results for Change in Column temperature

Parameter	Actual 40°C	Change-1 35°C	Change-2 45°C
%RSD of Three replicate injections of reference solution	2.15	0.85	2.87
Recovery of Sensitivity Solution and Diluted Standard	112.19	92.58	103.9
Theortical Plates of Voriconazole peak	121079	132510	134144

Table 8: Forced degradation results

Stress Type	Stress Conditions	% Assay	% Impurities	Mass Balance
Unstressed	Control Sample	100.0	0.31	NA
Acid Hydrolysis	1N HCL at RT for 1 Hour	98.3	1.41	99.7
Base Hydrolysis	1N NaOH at RT for Immediate	92.5	17.84	110.3
Base Hydrolysis	0.1N NaOH at RT for Immediate	100.5	0.320	100.8
Oxidation	1% H_2O_2 at RT for 1 Hour	98.2	0.52	98.8
Thermal Stress	60°C for 6 hours	102.1	1.63	103.7

4. Conclusion

The developed method was highly precise and acuurate to separate the impurities with adequate resolution. The method is superiror than USP in terms of resolution and chromatographic conditions.The results experimentally obtained meet the acceptance criterias of specificity, LOD/LOQ, linearity& range, precision, accuracy, Robustness, Ruggedness, solution suitability and Stability indicating method. The present method was can be adopted day to day in quality control department for releasing the final product and for the stability study analysis. Any significant change in the method, which may influence product quality, will have to be revalidated.

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