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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR VORICANAZOLE IN BULK DRUG

[1]Eswara Rao Bammidi*, [1]Vaikuntarao Lakinani, [1]Subrahmanyam [1]Lanka, Pavitra. Paila
[1]Department of Chemistry, GITAM Institute of Sciences, GITAM University, Visakhapatnam, India.
Email:eswar.bammidi@gmail.com

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Abstract:

The objective of the current study was to develop a validated, sensitive, specific and stability-indicating reverse phase HPLC method for the quantitative determination of Voriconazole and its potential impurities. The determination was done for active pharmaceutical ingredient and its pharmaceutical dosage form in the presence of degradation products, and its process-related impurities. The drug was subjected to stress conditions of hydrolysis (acid and base), oxidation, photolysis and thermal degradation per International Conference on Harmonization (ICH) prescribed stress conditions to show the stability-indicating power of the method. Impurity profiling is to identity as well as to quantity of impurity in the pharmaceuticals, is now gaining critical attention from regulatory authorities. The different Pharmacopoeias, such as the British Pharmacopoeia (BP), United States Pharmacopeia (USP), and Indian Pharmacopoeia (IP) are slowly incorporating limits to allowable levels of impurities present in the API's or formulations. In the developed HPLC method, the resolution between Voricanazole and its process-related impurity was found to be 1.29 Regression analyses shows an "r" value (correlation coefficient) 0.999 for Voricanazole and its one impurity. The method employed a linear gradient elution and the detection wavelength was set at 260nm. Mobile phase consists of mixture of acetonitrile and water (50:5v/v) with flow rate of 1.0ml/min. The R.S.D. value (0.45%, n= 24) and retention time less than 4min indicating that the method is useful for routine quality control.

Keywords: Stability, Voriconazole, HPLC, Development and Validation.

Introduction:

In the pharmaceutical world, an impurity is considered as any other organic material, besides the drug substance, or

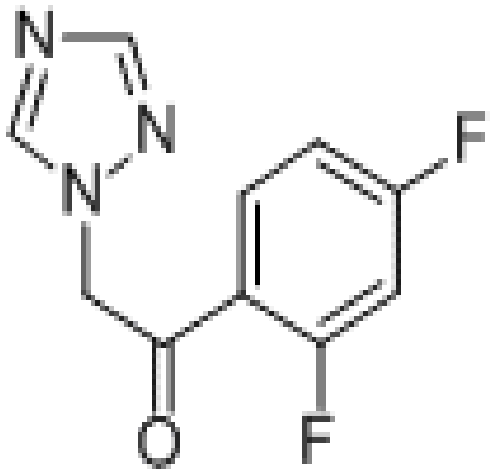
ingredients, arise out of synthesis or unwanted chemicals that remains with API's. The impurity may be developed either during formulation, or upon aging of both API's and formulated API's in medicines [1]. Voriconazole (is VFEND) is a second-generation antifungal agent belonging to azole class. Its chemical designation is (2R, 3S)-2- (2,4- difluorophenyl)-3- (5-fluoropyrimidin-4-yl)-1-(1H-1,2,4-triazol-1-yl) butan-2-ol (Figure 1). Azoles, in general work primarily through inhibition of cytochrome P-450-14-α- demethylase, thereby inhibiting the steps in the synthesis pathway for fungal membrane production and growth.Literature survey reveals that all of the above analytical methods are used only for the quantification of Voriconazole, not for the the quantification of known related compounds and degradation impurities of Voriconazole in Tablet dosage form. Voriconazole degrades significantly under base hydrolysis stress conditions as compared with acid hydrolysis [2, 3, and 4]. A RP- HPLC method was developed for the quantitative determination of Voriconazole, along with its degradation and diasteromeric impurities in tablet dosage form [5]. This present work describes an accurate, rapid, specific and reproducible method for the determination of Voriconazole and its impurity in bulk drug samples and pharmaceutical dosage forms along with method validation as per ICH norms.

2. Experimental

2.1. Chemicals:

Samples of Voriconazole was gift sample from MSN Laboratories, India.HPLC grade Acetonitrile, analytical reagent grade potassium dihydrogen phosphate, and phosphoric acid were purchased from Merk, Darmstadt, Germany. High purity water was prepared by using Millipore Milli-Q plus water purification system. All samples and impurity used in this study were of greater than 99.0% purity.

Ethanone, 1-(2, 4-difluorophenyl)-2-(1H-1,2,4-triazol-1-yl)-(CAS No. 86404-63-9)



2.2. Equipment:

The HPLC system, used for method development, forced degradation studies and method validation was waters e2695 binary pump plus auto sampler with PDA Detector 2998 with Empower2software (Waters Corporation, MA, USA). The output signal was monitored and processed using Empower 2 software on Pentium computer (Digital equipment Co). Water batch equipped with temperature controller was used to carry out degradation studies for all solution, photo stability studies were carried out in a photo stability chamber (Mack Pharmatech, Hyderabad, India). Thermal stability studies were performed in a dry air oven (Mack Pharmatech, Hyderabad, India).

2.3. Chromatographic conditions:

The chromatographic column used Agilent Zorbax C_{18} 250nmx4.65µm, the run times of proposed method was 25min with isocratic solution. Column temperature is 30^0C, flow rate is 1ml. After injecting the standard solution volume was found to be 10µL. Retention times found were about 4.5 minutes for Voricanazole. Buffer consists of 10mM Potassium dihydrogen phosphate, pH adjusted to 2.8 using phosphoric acid. Mobile phase consists of the mobile phase was 1.2mL min^{-1}. The HPLC gradient program was set as: time (min)/ % solution B:0/10, 20/80, 20.1/10 and 25/10. The column temperature was maintained at 35^0C and the detection was monitored at a wavelength of 260nm. The injection volume was 10µL. Water was used as diluents. The concentration is 300µg/mL for related substances method and 100µg/mL for Assay method.

2.4.1. Preparation of Standard Solutions:

A) **Impurity-A 5µg/ml:**

Accurately weighed 1mg Impurity-A Voricanazole was transferred into 10ml volumetric flask diluted to 10ml with diluents and labelled as Impurity stock.

B) Voricanazole 1000µg/ml:

10mg Voricanazole drug was weighed and taken into a 10ml clean dry volumetric flask, 5ml diluents was added and, sonicated for 30minutes and labelled as working solution. 0.5ml from the impurity stock solution was pipette out and transferred into labelled as working solution and made up to the final volume with diluents.

C) Preparation of Buffer solution:

Accurately weighed 1.41gm of Disodium hydrogen Ortho phosphate was transferred into 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 dilute OPA.

2.4.2 Specificity:

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The specificity of the Voricanazole in the presence of its impurity namely impurity–A and degradation products was determined by developed HPLC method. Forced degradation studies were also performed on Voricanazole to provide an indication of the stability indicating property and specificity of the proposed method [13-18]. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (105°C), acid hydrolysis (0.1N HCl), base hydrolysis (0.1 NaOH) and oxidation (3% H_2O_2). For heat study period was 2days and for light studies, study period was to illuminate the sample for 1.2 million Lux hours, where as for acid, base and peroxide hydrolysis the test period was 48 h. Peak purity of stressed samples of Voricanazole was checked by using 2998 Photo diode array detector of Waters Corporation, MA, USA.

2.5: Analytical Method Validation:

The developed chromatographic method was validated for linearity, precision, accuracy, sensitivity, robustness and system suitability.

2.5.1 Precision:

The Precision of the related substance method was checked by injecting the samples at different concentrations of Voricanazole spiked levels at 50,100 and 150. Precision study was also determined by performing the same procedures on a different day (interday precision). Assay method precision was evaluated by carrying out assays of test sample Voricanazole against qualified reference standard. The %RSD of assay values obtained was calculated.

2.5.2 Sensitivity:

Sensitivity was determined by establishing the Limit of detection (LOD) and Limit of quantitation (LOQ) for impurity by injecting a series of dilute solutions with known concentration. The precision study was also carried out at the LOQ level by individual preparations of impurity-A calculated the %RSD for the areas of impurity.

2.5.3 Linearity and Range:

Linearity test solutions for assay method were prepared from stock solution at five concentration levels from 50 to 200% of assay analyte concentration (50, 75, 100, 150 and 200 µg mL-1).

A linearity test solution for related substance method was prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at seven concentration levels. From LOQ to 200% of the permitted maximum level of the impurity (i.e. LOQ, 0.0375, 0.075, 0.1125, 0.15, 0.225 and 0.3%) was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the corresponding predicted responses. Linearity was checked for three consecutive days in the same concentration range for both assay and related substance method and calculated the %RSD Value of the slope and Y-intercept of the calibration curve. Upper and lower levels of range were also established.

2.5.4 Accuracy:

The accuracy of the assay method was evaluated in triplicate at five concentration levels, i.e., 50, 75, 100, 150 and 200µg/ml in bulk drugs. At each concentration, three sets were prepared and injected triplicate.

2.5.5. Robustness:

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (R_S) between Voricanazole and impurity-A were evaluated. Flow rate of mobile phase is 1ml/min.

3. Results and Discussion:

3.1. Method Development and Optimization:

Method validation parameters are indicated in table No: 2. The HPLC method carried out in this study aimed at developing chromatographic system capable of eluting and resolving Voricanazole from its process related impurities and degradation products that comply with the general requirements for system suitability. Initial trials were done on C18 column (100 mm × 3 mm i.d., particle size 3.5 µm) using Mobile phase consists of mixture of acetonitrile and water (50:5, v/v) with flow rate of 1.0ml/min. After many logical trials, chromatographic condition was established such that which could be suitable for separation of drug and seven known impurities along with the degradation products.

After selecting the best conditions based on peak performance, buffer solution and acetonitrile in the ratio 50:50 and using HPLC column is 250mmx4.65m, 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 Agilent Zorbax C_{18} standard solution volume was found to be 10L. Retention times found were about 4.5 minutes for Voriconazole. Standard solution volume was found to be 10L. Retention times found were about 4.5 minutes for Voriconazole.

Table-1: Results of Accuracy study for impurity-A.

Impurity-A		
%Level	**Conc (mg/mL)**	**Area**
25	0.002	28539
50	0.003	44239
100	0.005	73714
150	0.008	116398
200	0.01	145432

Related substances Method Validation					
Product Name	Voriconazole	Label claim	1	Date of Analysis	
Parameter	Method precision				
Working/Reference Standard Details:					
Working/Reference Standard name		**Potency(%)**	99.9		
Standard preparation:					
Standard wt. (mg)	10.7	Dilution-2	1	Dilution-4	1
Dilution-1	10	Dilution-3	10	Dilution-5	1
Sample preparation:					
Sample wt. (mg)/ No.of units	Dilution-2	1	Dilution-4	1	**Sample wt. (mg)/ No.of units**
Dilution-1	10	Dilution-3	1	Dilution-5	Dilution-1
Average wt.(mg)	1				

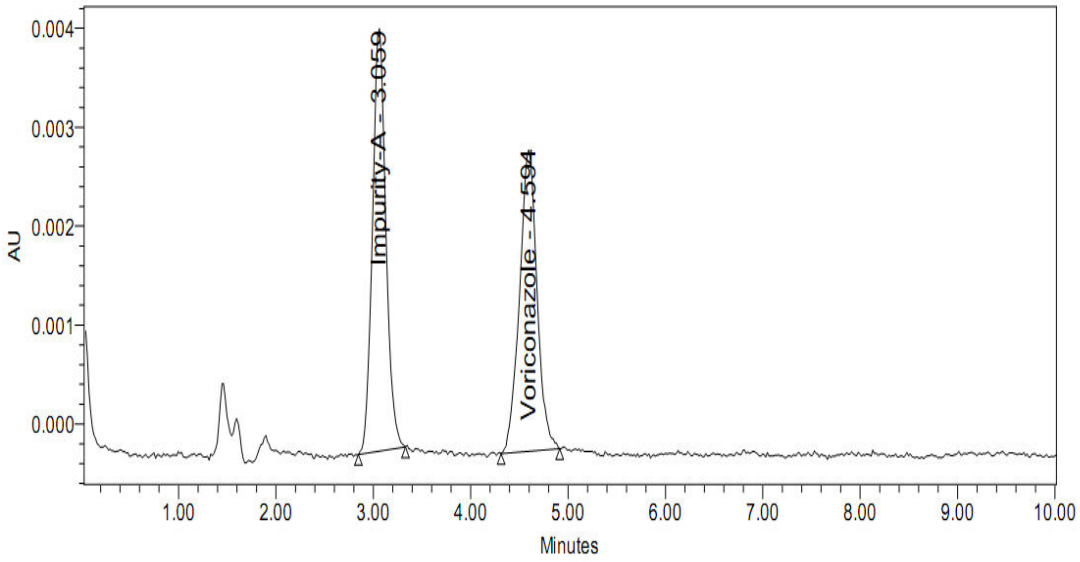
Calculations

Std Response No.	1	2	3	4	5	6	**Mean**	**% RSD**
std response	1469094	1466295	1451091	1454692	1472297	1481141	1465768	0.8

Sample No.	**Sample-1**	**Sample-2**	**Sample-3**	**Sample-4**
Sample wt	10	10	10	10

(mg)/No.of units									
Impurity name	**RRF**	**Area**	**% w/w**	**Area**	**% w/w**	**Area**	**% w/w**	**Area**	**% w/w**
Impurity-A	1	61526	0.449	62278	0.454	62900	0.459	63306	0.462
Total impurities		0.45		0.45		0.46		0.46	

Figure-1: Typical chromatogram of Voricanazole with impurity-A.



Peak Name: Impurity-A

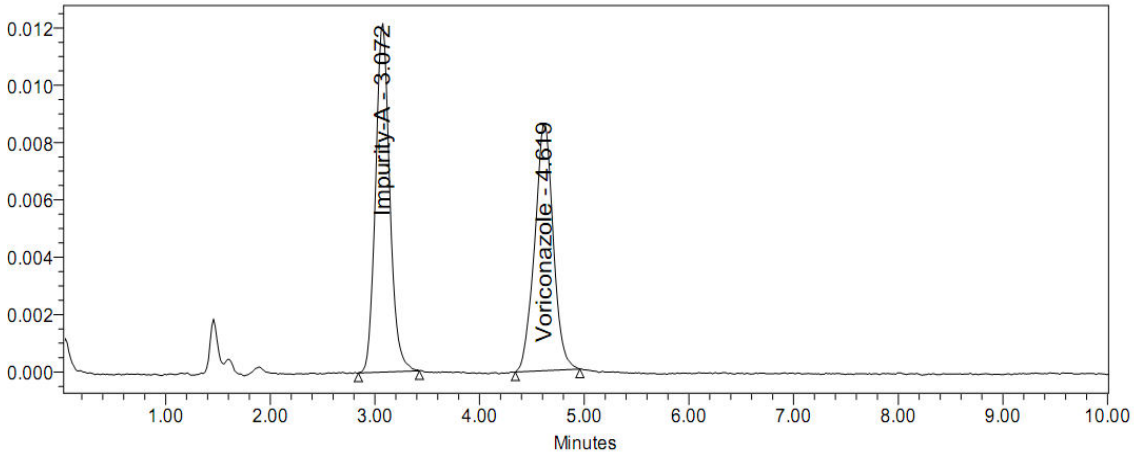
	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Impurity-A	3.072	115055	2511	1.12
2	Impurity-A	3.074	118084	2487	1.11
3	Impurity-A	3.078	116054	2419	1.12
Mean			116398		
% RSD			1.3		

3.2. Assay analysis:

Analysis was performed for different batches of Voricanazole in bulk drug samples (n=3) ranged from 99.88-99.96%.

4. Conclusion:

The HPLC method developed for Assay and related substance determination of Voricanazole in bulk drug is precise, accurate, sensitive and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of Voricanazole samples.



References:

1. Alsante K M, Boutres P, Couturier M A, Fridmann R C, Harwood J W, Horan G J, Jensen A J, Liu Q, Lohr L L, Morris R, Raggon J W, Reid G L, Santafianos D P, Sharp T R, Tucker J L and Wilcox G E (2004) Pharmaceutical Impurity Identification: A Case Study Using a Multidisciplinary Approach. Journal of Pharmaceutical Sciences 93 (9): 2296.
2. Eldin AB, Shalaby AA, Youssif M, Maamly. Development and validation of Stablility Indicating RP-HPLC Method for Estimation of Voriconazole in Bulk drug. Intnt Journal of Sci Nanao.52 , 229-238, (2010).
3. Kheter AB, Sinha PK, Damle MC, Mehendre R., Development and Validation of Staability Indicating R.P- HPLC mehthod for Voriconazole. Ind J Pharma Sci, 3, 509-514, (2009). http://dx.doi.org/10.4103/0250-474X.58178.
4. Gu P, Li Y., Development of Validation of a stability-indicating HPLC method for determination of Voriconazole and its related substances. J Chromatogr Sci, 47, 594-598, (2009).
5. http://dx.doi/10.1016/j.clinbiochem.2007.07.024.
6. Shaikh, Kabeer A, Ashish T. Patil, A Validated Stability–Indicating Liquid Chromatographic Method for Determination of Degradation Impurities and Diastereromers in Voriconazole Tablets, Scientia Pharmaceutica, 80, 879-888, (2012).

Corresponding Author:

[1]Eswara Rao Bammidi*,

Email: *eswar.bammidi@gmail.com*