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Research Article

Quantification of Impurity-E in Voriconazole Powder for Solution for Infusion (200 mg/vial) by using High Performance Liquid Chromatography

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Abstract

A novel stability indicating liquid chromatographic method was developed and validated for the quantification of impurity-E((±)-β-Camphorsulfonic acid, (±)-Camphor-10-sulfonic acid)in Voriconazole powder for solution for infusion formulation. The separation was achieved on Novapak (150 x 3.9 mm, 4 µm) column using a movable segment consisting of pH 5.0 acetate buffer and acetonitrile gradient elution mode, at a flow rate of 1.0 ml/min. Column oven maintained at 35°C, inoculation quantity 50 µl, sample cooler temperature 5 °C and detection wavelength 286 nm. Chromatographic resolution between impurity-E and Voriconazole was found to be 22.1. Technique was extensively validated for the quantification of impurity-E in Voriconazole powder for solution for infusion formulation and established to be vigorous. Method was established extremely specific as all other related impurities were separated from the impurity-E. The Limit of quantitation (LOQ) and limit of detection (LOD) for impurity-E were 6.0µg/ml and 2.0 µg/ml respectively.

Keywords: Voriconazole; impurity-E; LOQ; LOD; Specification limit of Impurity-E 0.02%.

Introduction

Synthesis of drug substances often involves the use of reactive reagents and hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity and are to be controlled based on the maximum daily dose.

Voriconazole is chemically (2R,3S)-2-(2, 4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4triazol-1-yl)-2-butanol.[1] Voriconazole is a used to treat serious fungal or yeast infections[2], such as aspergillosis[3] (fungal infection in the lungs), candidemia (fungal infection into the blood) esophageal candidiasis[4] (candida esophagitis) or other fungal infections (infections in the skin, stomach, kidney, bladder or wounds). It inhibits the cytochrome P450[5-9] (CYP)-dependent enzyme 14-alpha-sterol demethylase, thereby disrupting the cell membrane and halting fungal growth[10]. Voriconazole has shown in-vitro activity against many yeasts and a variety of mold and dermatophyte isolates. It can be administered either orally or parenterally, exhibiting good oral bioavailability, wide tissue distribution including distribution into the central nervous system, and hepatic metabolism[11].

Molecular formula and molecular weight $C_{16}H_{14}F_3N_5O$ and 349.3 g/mol. Voriconazole drug substance monograph is available in the United States Pharmacopeia[12] and European Pharmacopeia, but Voriconazole for Injection monograph is not available in BP and USP.

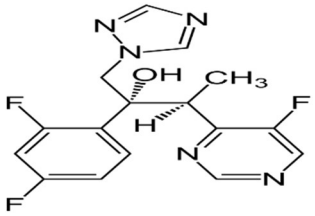

Figure 1: Chemical structure of Voriconazole

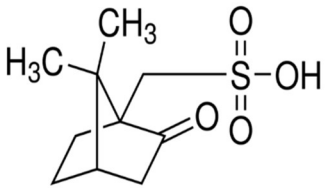

Figure 2: Chemical structure of Voriconazole Impurity-E

Impurity-E[(1R,4S)-7,7-Dimethyl-2-oxo bi cyclo [2.2.1] hept-1-yl] methane sulfonic acid. ((±)-β-Camphorsulfonic acid, (±)-Camphor-10-sulfonic acid) is the resolution reagent in voriconazole synthesis process. However, it is controlled in the final active substance specifications with a limit of not more than 0.10% under Ion chromatography method (as per EP monograph).

Impurity-E controlled in drug substance nevertheless this impurity also monitor in voriconazole drug product, due to drug product formulations using different types of excipients. These excipients may be reacts with voriconazole to form impurity-E. Hence impurity-E specifications limit not more than 0.20%, as per maximum daily dose of voriconazole (400 mg) and ICH In New Drug Products Q3b (R2)[13].

Literature survey reveals that several analytical methods reported in drug substance, drug product and biological formulations, liquid chromatographic methods RP-HPLC[14-26] have been used for the analysis of Voriconazole.

Ion chromatography (IC) method used during drug substance analysis, no extraction procedure needed for extraction of the impurities and main analyte while per USP and EP monograph (drug substance), but these API methods were not appropriate for formulations. For formulations, extraction process is appropriate for the extraction of the impurities as well as main analyte as drug product containing excipients and those excipients may bind with drug substance. Micro scale laboratories Ion chromatography instrument was not available because of IC instrument expense as well as analysis was also tricky.

Until now no RP-HPLC method was reported for quantification of impurity-E ((±)-β-Camphorsulfonic acid, (±)-Camphor-10-sulfonic acid) in Voriconazole powder for solution for infusion formulation.

In the present study, a new RP-HPLC method was developed and validated for the quantification of impurity-E in the Voriconazole powder for solution for infusion formulation.

Experimental

Chemicals and reagents

Ammonium acetate, Glacial acetic acid, Acetonitrile and milli-Q (AR Grade) was procured from Merck, India. Voriconazole impurity-E was obtained from Simson Life Sciences (SLS). The drug substance as well as Voriconazole formulation (Powder for solution) for do research obtained from Jodas Expoim Pvt. Ltd, Hyderabad, India.

Method development

Experiment-1

Initial trial was taken to check the feasibility of Ion chromatography method Voriconazole Drug substance (EP monograph) on finished product sample.

Preparation of solutions

Preparation of Sodium hydroxide solution

Accurately weighed and transferred 470 g Sodium hydroxide into 1000 ml volumetric flask. Dissolved and made up to the mark with water and mixed well.

Preparation of Movable segment

Prepared a combination of 1500 milliliter of water and 500 milliliter of methanol. To this added 175 µL of Sodium hydroxide solution and combined well. Filtered the solution through 0.45 µm membrane filter and sonicate to degas.

Preparation of 12 mM Sulfuric acid solution

Transferred 0.67 ml of Concentrated Sulfuric acid in to 1000 ml volumetric flask containing 700 ml of water and made up to mark with water and mixed well.

Chromatographic conditions

Column : Supelco Astec Cyclobond I 2000 RSP, 250 x 4.6 mm ID, 5 µm
Wavelength : 286 nm

Flow rate	: 1.0 ml/min
Injection volume	: 20 µL
Column Temperature	: 30°C
Auto sampler temperature	: 5°C
Elution Mode	: Isocratic
Runtime	: 30 minutes
Diluent	: Mobile phase

Impurity-E standard peak eluted at 6.05 minutes. Voriconazole analyte peak eluted at 6.39 minutes. Separation between Impurity-E and Voriconazole peaks found very less (less than 1.5). Based on the above observation, trials to be taken with different column.

Chromatographic conditions

Apparatus	: Ion chromatography is equipped with conductivity detector.
Column	: Omnipac PAX-100 250 x 4.0 mm, 8.5 µ (Part No. 42150)
Guard column	: Omnipac PAX-100 Guard column (Part No. 42151)
Suppressor solution	: 12 mM Sulfuric acid solution
Flow rate	: 1.0 ml/min
Injection volume	: 20 µl
Column Temperature	: 40°C
Elution	: Isocratic
Runtime	: 15 minutes

Impurity-E peak not detected in system suitability solution. It might be due to sample matrix. Based on the above observation, it is concluded that Voriconazole EP monograph method will not work for finished product.

Experiment-2

In this experiment-2, to check the feasibility of detection of Impurity-E in Voriconazole drug substance (EP Monograph) Enantiomer Impurity method.

Preparation of Buffer

Accurately weighed and transferred 0.77 g of ammonium acetate in 1000 ml of water and adjusted the solution pH to 5.0 ± 0.05 with glacial acetic acid.

Preparation of Mobile phase

Mixed 18 volumes of acetonitrile and 82 volumes of buffer. Filtered the solution through 0.45 µm membrane filter and sonicate to degas.

Experiment-3

To get the separation between Impurity-E and Voriconazole, trial has to be taken with a different column.

Preparation of solutions

Preparation of pH 5.0 acetate buffer

Accurately weighed and transferred 3.85 g of ammonium acetate in 1000 milliliter of irrigate and altered the solution pH to 5.02 with glacial acetic acid. Filtered the solution through 0.45 µm membrane filter and sonicated to degas.

Preparation of Mobile phase-A

pH 5.0 acetate buffer

Preparation of Mobile phase-B

Acetonitrile

Preparation of diluent

Water used as a diluent

Chromatographic conditions

Column	: Nova-pak C18, 150 x 3.9 mm ID, 4 µm
Wavelength	: 286 nm
Flow rate	: 1.0 ml/min
Injection volume	: 50 µl
Column Temperature	: 35°C
Auto sampler temperature	: 5°C
Elution Mode	: Gradient
Runtime	: 40 minutes
Diluent	: Water

Preparation of Voriconazole Impurity-E stock pile solution:

Accurately weighed and transferred 2.0 mg of Voriconazole Impurity-E, into 10 ml volumetric flask. Added 5 ml of diluent and liquefied the contents and made up to the mark with diluent and mixed well.

Preparation of System appropriateness solution:

Accurately weighed and transferred 10 mg of Voriconazole standard into 10 ml volumetric thermos. Added 5 ml of diluent and dissolved the contents. Inserted 1 ml of Voriconazole impurity-E stock solution**.** Diluted to the volume with diluent and mixed well.

Preparation of Standard solution

Transferred 2 ml of Voriconazole impurity-E stock solution into 20 ml volumetric flask and made up to the mark with the diluent and mixed well.

Preparation of Placebo solution

Taken 1 vial (Placebo) and reconstituted with 19 ml water for injection, the volume obtained solution is 20 ml and it contains 10mg/ml

voriconazole (as per pil Vfend) and injected into HPLC.

Preparation of Sample solution

Taken 1 vial (sample) and reconstituted with 19 ml water for injection, the volume obtained solution is 20 ml and it contains 10mg/ml voriconazole (as per pil Vfend) and injected into HPLC.

Impurity-E peak eluted at 7.856 minutes. Voriconazole peak eluted at 13.631 minutes. No interference was observed due to blank and placebo at impurity-E peak. Resolution between impurity-E peak and Voriconazole analyte found to be satisfactory. Therefore, the elution order was viewed from the chromatograms (Figure: 3.0-7.0).

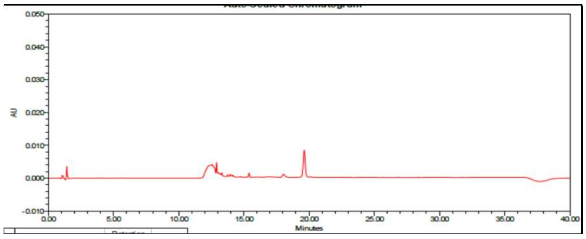

Figure 3: Typical chromatogram of Blank

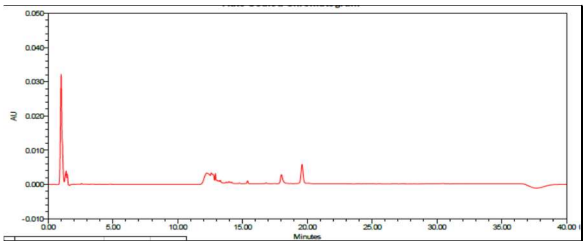

Figure 4. Typical chromatogram of Placebo

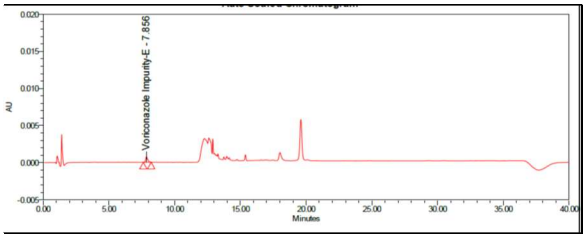

Figure 5. Typical chromatogram of Standard

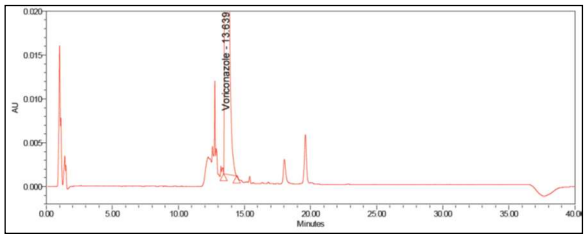

Figure 6. Typical chromatogram of Sample

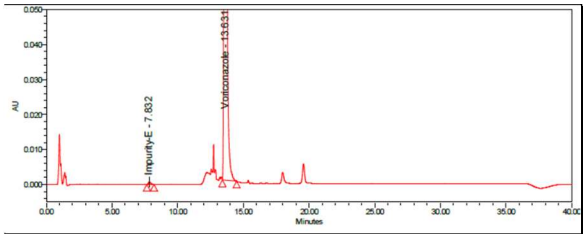

Figure 7. Typical chromatogram of Spiked sample

Analytical Method Validation:

Analytical method validation is the process of demonstrating that analytical procedures are suitable for their intended use. More specifically analytical method validation is matter of establishing documented evidence that provides a high degree of assurance that a facility or operation will consistently produce product meeting a predetermined specification. The following parameters were considered:

1. Specificity & System Suitability
2. Precision
3. LOD & LOQ
4. Accuracy (Recovery)
5. Solution stability
6. Force Degradation

System appropriateness

Equilibrated the chromatographic system with mobile phase until stable baseline is observed and solutions were injected as per sequence and system suitability parameters were recorded. System suitability test was performed each day before starting the parameter. Results obtained are tabulated in Table 1.0.

Preparation of Voriconazole Impurity-E stockpile solution:

Precisely weighed 2.0 mg of Voriconazole Impurity-E, interested in 10 ml volumetric thermos. Inserted 5 ml of diluent sonicated to dissolved and made up to the mark with diluent and mixed well.

Preparation of System appropriateness solution

Precisely weighed 10 mg of Voriconazole standard interested in 10 ml volumetric thermos. Inserted 5 ml of diluent and sonicated to dissolved. Inserted 1 ml of Voriconazole impurity-E stockpile solution**.** Diluted to the volume with diluent and mixed well.

Specificity and System Suitability

Blank, Placebo and Voriconazole other related impurities meddling

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded.

Chromatogram of blank solution (Figure: 8.0) showed no peak at the retention time of impurity-Eand Voriconazole analyte peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of impurity-Ein Voriconazole powder for solution for infusion formulation. Similarly chromatogram of panacea ((Figure: 9.0) demonstrated no crests at the retention time of impurity-Eand Voriconazole analyte crest. This indicates that the panacea utilized in sample preparation do not interfere in assessment of impurity-Ein Voriconazole powder for solution for infusion formulation. Method was established to be highly specific as all other related impurities (A, B, C, and D) were separated from the impurity-E.

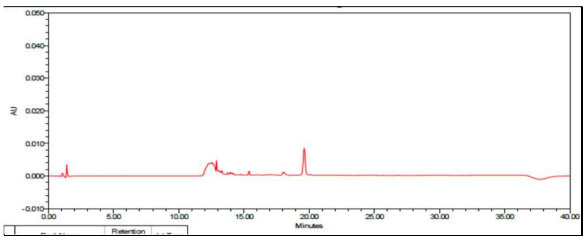

Figure 8: Typical chromatogram of Blank

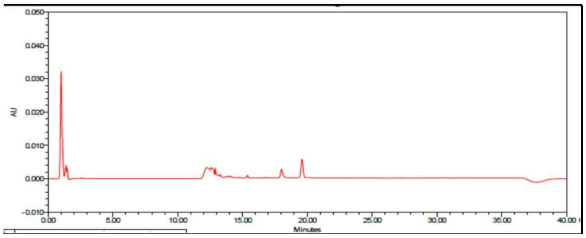

Figure 9: Typical chromatogram of Placebo

Table 1: Specificity Results

Sample	Retention time (min)	Purity Angle	Purity Threshold	Peak Purity
Standard solution				
Impurity-E	7.856	2.316	3.541	Pass
Sample solution				
Voriconazole	13.639	NA	NA	NA
Impurity-E	ND	NA	NA	NA
Spiked Sample solution				
Voriconazole	13.631	NA	NA	NA
Impurity-E	7.832	2.923	3.749	Pass
Blank and Placebo interference				
Blank	ND	NA	NA	NA
Placebo	ND	NA	NA	NA

Table 2: System appropriateness results

Name	Retention time (min)	Relative Retention time (RRT)	RS	Tailing factor	Plates count
Impurity -E	7.832	0.57	22.1	1.5	9608
VRC	13.631	1.00		1.3	83876

VRC –Voriconazole; RS - Resolution

Precision

System precision

System exactitude was demonstrated by systematized blank, Sensitivity solution, System suitability solution and Standard solution as per test technique and chromatographed the same into HPLC system. The peak areas and retention time of analyte were recorded for these system suitability injections. The System precision was evaluated by computing the % RSD for the peak area and retention time of these system suitability injections. The observations are tabulated Table: 3.0.

Table 3: System exactitude Results

S.No	Retention time	Area response
1	7.856	8295
2	7.850	7962
3	7.841	8234
4	7.834	8387
5	7.854	8275
6	7.85	8343
Avg.	**7.848**	**8249**
SD	**0.008**	**150.51**
%RSD	**0.11**	**1.82**

Method precision

Precision of the impurity was determined by prepared and injecting three control samples and three sample solutions spiked with impurity-E at specification echelon (0.2%). The tasters were arranged as per the technique and the consequence for

exactitude study is tabulated in Table: 4.0 and 5.0. Individual and mean %recovery should be in between 80-120. % RSD for % impurities found for unspiked sample should be not more than 10.0. % RSD for % recovery of known impurities for impaled sample should be not more than 10.0. The results were well within the limits. It is concluded that method is precise.

Table 4: Results of Method exactitude (Control sample)

No.of Preparations	Impurity-E (%)
1	ND
2	ND
3	ND
Average	**NA**
Std. Dev	**NA**
% RSD	**NA**

Table 5: Results of Method Precision (Spiked sample)

No.of Preparations	Impurity-E (%recovery)
1	96.7
2	98.1
3	100.0
Average	98.27
Std. Dev	1.66
% RSD	1.69

Limit of detection (LOD) & Limit of Quantitation (LOQ)

A solution containing 2.0 µg/ml of impurity-E standard was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all the three injections. A solution containing 6.0 µg/ml of impurity-E standard was injected six times. %RSD for the area response of analyte and impurity crest from six preparations of LOQ level should be not more than 15.0. The results for LOQ precision study is tabulated in Table: 6.0.The obtained results were well within the limit. It was concluded that method is precise at LOQ level.

Table 6: Results of LOQ Precision

S.No	Area Response
1	2310
2	2594
3	2487
4	2340
5	2593
6	2286
Average	**2435**
Std. Dev	**141.2799**
%RSD	**5.8**

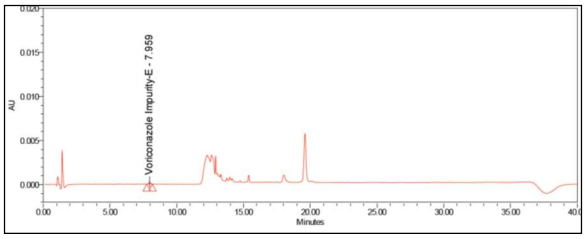

Figure 10. Typical chromatogram of LOQ precision sample

Accuracy

Recovery of impurity-E in Voriconazole was executed. The sample was taken and differing quantities of impurity-E symbolizes LOQ to 150 % of requirement echelon (0.2%) were inserted to the thermos. The impaled tasters were arranged as per the technique and the consequences are tabulated in Table: 7.0. LOQ level the %Recovery should be in between 75.0 to 125.0, above LOQ level the %Recovery should be in between 80.0 to 120.0. Accuracy at LOQ level to 150% level for Impurity-E is meeting the acceptance criteria. It is concluded that method is accurate.

Table 7: Recovery results of impurity-E

Preparation	Amount Added (%)	Amount Found (%)	% Recovery	% RSD
LOQ % level				
Prep-1	0.064	0.057	89.1	3.49
Prep-2	0.064	0.060	93.8	
Prep-3	0.064	0.061	95.3	
50% level				
Prep-1	0.107	0.101	94.4	4.21
Prep-2	0.107	0.102	95.3	
Prep-3	0.107	0.109	101.9	
100% level				
Prep-1	0.215	0.208	96.7	1.69
Prep-2	0.215	0.211	98.1	
Prep-3	0.215	0.215	100.0	
150% level				
Prep-1	0.322	0.315	97.8	1.35
Prep-2	0.322	0.308	95.7	
Prep-3	0.322	0.316	98.1	

Solution steadiness

The standard, sample and spiked sample solutions were injected into HPLC initial and after 24 hrs at every hiatus the % vicinity of impurity-E in impaled solution was evidenced and the dissimilarity in % vicinity with admiration to % vicinity obtained at first day intermission was estimated. The consequences are put into a table in Table: 8.0-10.0. Solution steadiness stricture was established and standard, sample and spiked sample solutions were stable for 24 Hours on bench top and in refrigerator (2-8°C) condition.

Table 8: Results of standard solution stability

Time interval	% Recovery or standard solution	
	Bench top	Refrigerator condition (2-8°C)
0 Hrs	NA	NA
24 Hrs	100.0	98.8

Table 9: Results of test sample solution stability

Time interval	% Recovery for impurity-E	
	Bench top	Refrigerator condition (2-8°C)
Initial	ND	
24 Hrs	ND	ND

Table 10: Results of spiked test sample solution stability

Time Interval	% Recovery for impurity-E	
	Bench top	Refrigerator condition (2-8°C)
Initial	96.7	
24 Hrs	97.65	96.30

Force Degradation

The forced degradation study was conducted to ensure that the analytical method is stability indicating and capable of separating degradants from the main analyte peak. The experiment involves evaluated the consequence of acid (5.0N HCl, 24 hours at 60°C heat), alkali (0.01N sodium hydroxide, 3hours on Bench top), hydrogen peroxide (30%H_2O_2, 24 hours on bench top) and Water (60°C for 24 hours) on Voriconazole tasters. The chromatograms acquired as of an assortment of stress circumstances are revealed in Figure:11.0.

The %impurity, %degradation and peak purity of impurity-E created in each and every one stress circumstances are established and recapitulated in Table:11.0. Voriconazole impurity-E was established to be more steady in applied acid, alkali, peroxide and hydrolytic stress circumstances.

Table 11: Degradation results of Voriconazole

Condition	Impurity E (%)	% Assay	Total Impurities (%)#	Mass Balance (%)
1	ND	100.5	0.03	NA
2	ND	73.7	27.50	100.7
3	ND	91.0	9.13	99.6
4	ND	80.7	21.59	101.8
5	ND	89.5	11.63	99.9

1 - Control sample; 2 - Acid degradation(5.0N HCl/5ml/60°C/24hrs); 3 - Base Degradation(0.01N NaOH/5ml/RT/3hrs); 4 - Peroxide Degradation(30%H_2O_2/5.0 ml/BT/24hrs); 5- Water degradation(Water/5ml/60°C/24hrs)

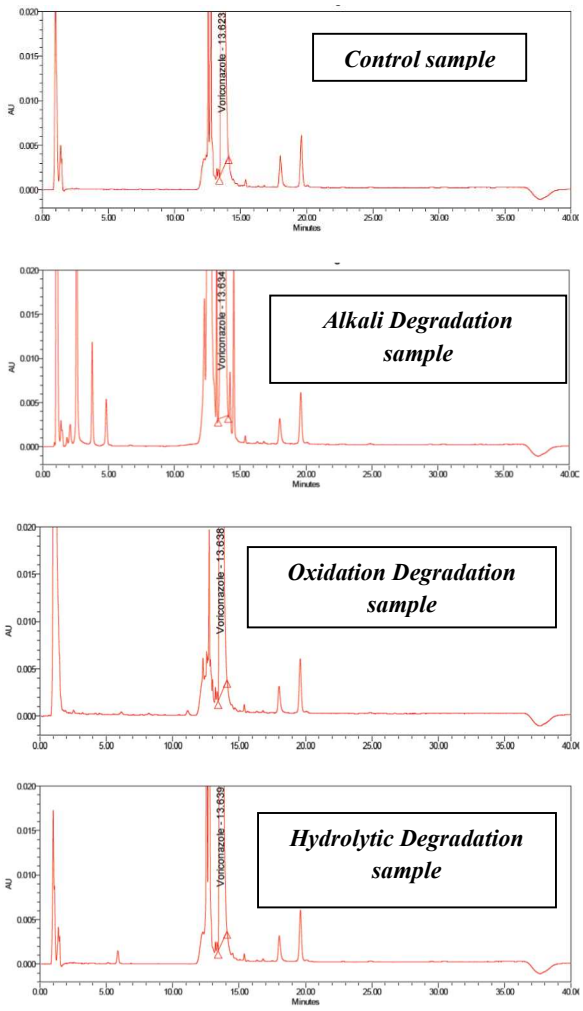

Figure 11: Typical chromatogram of degradation samples

Results & Discussion

A simple, fiscal, accurate and precise reverse phase HPLC technique was productively urbanized. The separation was attained on Novapak (150x3.9 mm, 4 µm) column using a mobile phase consisting of pH 5.0 acetate buffer and acetonitrile gradient elution mode at a flow rate of 1.0 ml/min. Column temperature maintained at 35°C, Injection volume 50µL, sample cooler temperature 5°C and Detection wavelength 286nm. The consequences gained were correct and reproducible. The technique urbanized was statistically authenticated in conditions of Selectivity, LOD & LOQ, correctness, exactitude and steadiness of solution.

For Selectivity, the chromatograms were evidenced for standard and sample solutions of impurity-E and Voriconazole. Selectivity studies reveal that the crest is well alienated from each other.

Therefore the technique is selective for the fortitude of impurity-E in Voriconazole powder for solution for infusion. There is no meddling of diluent and placebo at impurity-E and voriconazole crests.

The limit of detection and limit of quantitation for impurity-E 2.0&6.0 µg/ml respectively.

The accuracy studies were shown as % recovery for impurity-E at specification level. The results obtained were found to be within the limits. The relative standard deviation values of recoveries obtained for impurity-E are in the range of 1.35%-4.21%.

For exactitude studies six (6) repeat inoculations were executed. %RSD was concluded from the crest vicinities of impurity-E established to be 1.69% respectively. Solution stability of the Standard and sample solutions are stable for 24 hours when stored at room temperature (RT) and 2-8°C in refrigerator.

Hence, the chromatographic method developed for impurity-E in Voriconazole powder for solution for infusion formulation are fast, uncomplicated, responsive, exact, and precise. Therefore, the suggested technique can be productively useful for the custom analysis of the drug substance for assertion of its excellence all through its formulation.

Conclusion

The prospective Reverse Phase-HPLC method that can be determination of impurity-E in Voriconazole powder for solution for infusion at trace echelon concentration have been developed and validated as indicated by ICH strategy. The effectiveness of the method was ensured by the specificity, precision, accuracy and solution stability. Hence, the technique well suits for their future reasons and can be productively useful for routine analysis in laboratories and is appropriate for the excellence organize.

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Supporting Information

All data and material are available upon request.

Conflict of interests

The authors claim that there is no conflict of interest.

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