Determination of Voriconazole and its Degradation products in Pharmaceutical formulations using High Performance Liquid Chromatography with Ultra-Violet Detection

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Abstract

Simple, sensitive and accurate stability indicating analytical method has been developed and validated for determination of voriconazole and its degradation products using RP-HPLC method with ultra-violet detection. The resulting method was applied for determination of voriconazole tablets (Vfend®). The procedure was developed and validated under acidic, basic, oxidative and photo-irradiated conditions. The compounds were separated on C_{18} column using 0.05 mol L^{-1} disodium hydrogen phosphate buffer (pH 5.5) : acetonitrile (1:1, v/v) as a mobile phase. at a flow rate of 1.0 mL per min and UV detection at 255 nm, the run time was less than 15 minutes The calibration curves were linear between 6.0 and 60 µg mL^{-1}. The proposed method was simple, highly sensitive, precise, accurate and, is useful for routine quality control analysis and stability testing. It was found that voriconazole was more sensitive to the basic conditions, photodegradation was observed only under severe conditions of light exposure and oxidation may also appear, however, it was stable in acidic medium.

Keywords:

Assay, Stability indicating method, Voriconazole, HPLC

1. Introduction

Voriconazole, ((2R, 3S) -2- (2,4-difluorophenyl) -3- (5-fluoro -4- pyrimidinyl) -1-(1H-1,2,4-triazol-1-yl)-2-butan-2-ol) (Fig. 1). Is the newest agent in the armamentarium antifungal. It is a triazole antifungal; structurally related to fluconazole, with a comparable spectrum of activity to itraconazole. Voriconazole inhibits the fungal ergosterol biosynthesis [1] This inhibition is more selective for fungal enzyme system than mammalian enzyme system[2]. The accumulation of 14-alphamethyl sterols results in a decrease in ergosterol, which is an essential component of fungal cell wall formation. The resulting cell wall abnormalities are thought to be responsible for voriconazole's antifungal activity. Voriconazole was approved by the Food and Drug Administration in May 2002 for the treatment of invasive aspergillosis and refractory infections of *Scedosporium apiospermum* and *Fusarium* spp.
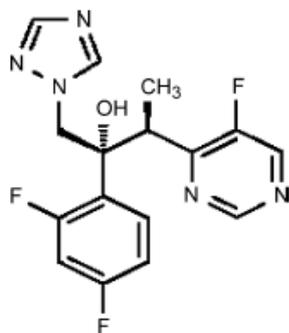
Fig.1. Chemical Structure of Voriconazole.

Studies have also shown it to be a promising agent for empiric treatment in febrile neutropenia [3]. Also it showed activity against *Candida* spp. Human pharmacokinetic data for voriconazole have been published by Purkins et al. [4]. A few methods were reported for the determination of voriconazole in human serum [5–7], and in aqueous humor [8], reports regarding the determination of impurities [9] and separation of stereoisomers [10] also appear in literature. So for no systematic HPLC method has been reported for determination of voriconazole in pharmaceutical injections. This paper reports a rapid and sensitive HPLC-UV method for routine quality control of voriconazole in pharmaceutical formulations and stability studies for the Voriconazole as a new pharmaceutical formulation.

2. Experimental

2.1 Materials and reagents

Voriconazole reference standard, 100.3% purity, was kindly supplied by SIGMA pharmaceuticals, Egypt. The tablets (Vfend®), containing 50 mg and 200mg of voriconazole, were obtained commercially. The excipients of the pharmaceutical formulation (hydroxypropyl cellulose, magnesium oxide, low-substituted hydroxypropyl cellulose, magnesium stearate, microcrystalline cellulose) were purchased from LUNA, local raw materials distributors, disodium hydrogen phosphate (AR grade) and acetonitrile (HPLC grade) were purchased from Merck, Darmstadt, Germany, all used reagents are of analytical grade.

2.2. Instrumentation

A liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a model LC-10Avp isocratic pump, SIL-10ADvp autosampler, CTO-10ACvp column oven, SPD-10Avp variable wavelength detector, SCL-10Avp system controller and Class-VP software were used to quantify the samples.

2.3. Chromatographic conditions

Compounds were separated on a Bondapack $^®$ C$_{18}$ column, (10 μm, 250 mm×4.6 mm.) at 30 ºC. The mobile phase was consisted of 0.05M disodium hydrogen phosphate buffer (pH 5.5) : acetonitrile (1:1, v/v) at a flow-rate of 1.0 mL min^{-1}. The UV detector was set at 255 nm, the total run time was less than 15 minutes and the sample size was 50 μL.

2.4. Preparation of stock solutions and standards

300 μg mL^{-1} stock solution of voriconazole was prepared in methanol, at room temperature and The calibration standards were prepared by diluting the appropriate amounts

of the stock solution with mobile phase.. Nominal concentrations were 6, 12, 18, 24, 30, 36 and 60 μg mL^{-1} of voriconazole,

2.5. Sample preparation

Randomly selected voriconazole tablets were powdered and diluted with the appropriate volumes of mobile phase for the assay test or used as it is for the content uniformity test. The samples were filtered using 0.45μ nylon sample filter (Whatman).

2.6. Stress treatment of voriconazole in acidic, basic, oxidative and photo-irradiation conditions

8 mL of the above stock solution (300 μg mL^{-1}) was diluted to 100 mL using 1N hydrochloric acid, 1N sodium hydroxide, 1.5% hydrogen peroxide solution or methanol. The acidic, basic and oxidative solutions were incubated at 50ºC for 2 hours; however, the neutral solution was irradiated under a CAMAG 200-W high-pressure mercury lamp for 40hrs. The distance between the light source and the sample was maintained at 25 cm. Samples in triplicates were analyzed using HPLC-UV analysis.

2.7. Quantification

20 μL of the standard solutions, voriconazole tablets solutions and forced degradation solutions were injected into the HPLC-UV system. The responses (peak area) for the major peaks were measured and the quantity of voriconazole was calculated from the equation Cs(Au/As) where Au is the area under the peak of the test solution and As the area under the peak of the standard solution and Cs is the concentration of voriconazole in the standard solution.

2.8. Linearity, lower limit of detection and lower limit of quantitation

Calibration curves were constructed form the working standard solutions of voriconazole at the concentrations range of 6.0 - 60 μg mL^{-1}. A linear regression was obtained by plotting the peak areas against concentrations. The correlation coefficient, intercept, slope and the confidence intervals for the slope and the intercept of the regression line were calculated the lower limit of detection (LLOD) and the lower limit of quantitation (LLOQ) were calculated as 3 S.D. and 10 S.D. of the blank, respectively, according to the treatment by Miller and Miller [11]. Before injecting solutions, the column was equilibrated for at least 20 minutes with the mobile phase.

3. Result and discussion

3.1. Method development

HPLC-UV method was developed to resolve voriconazole from its degradation products and to comply with the general requirements of system suitability. Effect of various variables as detection wavelength, types and quantites of the organic modifiers, column, column temperature, buffer concentration, and the pH of the mobile phase were studied.

Voriconazole and its degradation products showed maximum absorption at a wavelength of 255 nm. A reversed phase C$_{18}$ column (Hypersil ODS, 5 μm, 250×4.6 mm) with a mixture of acetonitrile and 0.01 mol L^{-1} disodium hydrogen phosphate (6:4, v/v), pH 5.5 was initially used, the retention times were short and unresolved peaks were obtained. Decreasing the percentage of acetonitrile from 60 to 50% resulted in longer retention time but the tailing factor was so high for the voriconazole peak, however, increasing of the

modifier ratio was associated with a shorter retention times for all compounds. Using of methanol as an organic modifier showed a high tailing factor and longer retention times. Hypersil C_{18} column of 5 μm was then replaced by Bondapack C_{18} column of 10 μm, the tailing factor was reduced and good peak shape was obtained. Maintaining column temperature at 30ºC showed good peak symmetry. Changing the concentration of disodium hydrogen phosphate from 0.01 to 0.05 M increased the peak symmetry reduced the tailing factor. pH 5.5 of the mobile phase was found to be optimum, decreasing the pH from 5.5 to 4 resulted in shorter retention times and unresolved peaks. The optimized chromatographic conditions as described above were applied for all measurements.

3.2. Specificity

Selectivity was demonstrated for voriconazole in a mixture of the drug excipients. No interferences were found at the retention time of voriconazole. The specificity was also demonstrated by induced degradation of voriconazole samples.

Stress treatment of voriconazole in acidic, basic, oxidative and photo-irradiation conditions). Under the studied conditions, It was found that voriconazole was degradated in aqueous solutions, especially under basic conditions. Photodegradation is observed only under severe conditions of light exposure. Oxidation may also appear.

The recovery of voriconazole was 99.3% with a total degradation of 0.7% in the case of 1 M HCl, however, the decomposition was significant when voriconazole was treated with 1N sodium hydroxide or 1.5% H_2O_2 where the voriconazole recovery was 63.0 and 87.0% and the total degradation was 37.0 and 13.0%, respectively. Voriconazole sample solutions showed a photodegradation of about 40 % (two photodegradation products appears) . Thus, the degradation products peaks were chromatographically resolved from voriconazole peak as shown in figures 2, 3, 4, 5, and 6 for voriconazole under neutral, acidic, basic, oxidative and photo-irradiation conditions, respectively. Also the peak purity check through the photodiode array detector was considered as a proof for the specificity of the proposed method as the spectrum taken for the peak of the voriconazole at different intervals were super imposed (figures 7) also the determined purity factor was lower than the calculated threshold (Fig.8).
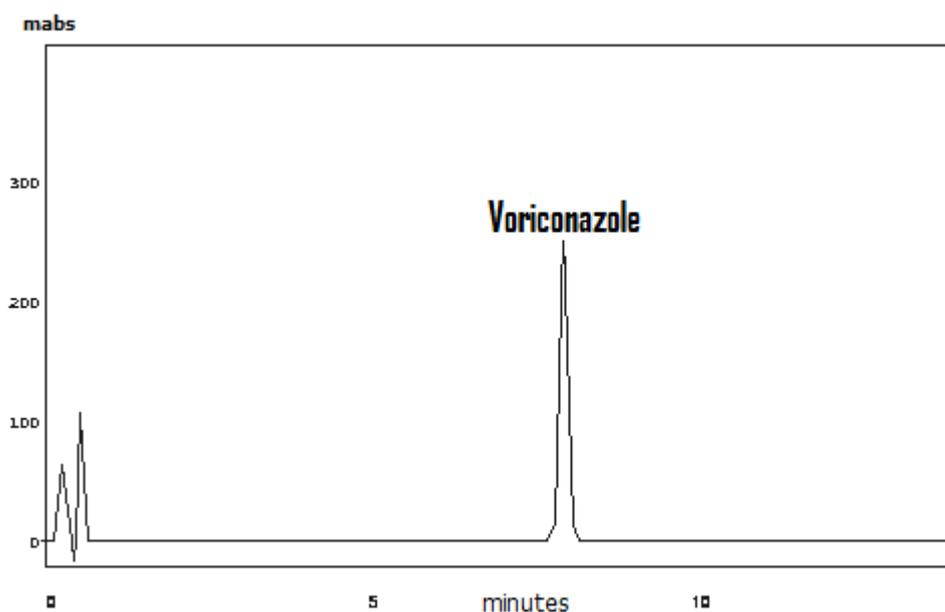
Fig.2 Voriconazole solution (24 mcg mL^{-1}) under neutral conditions.
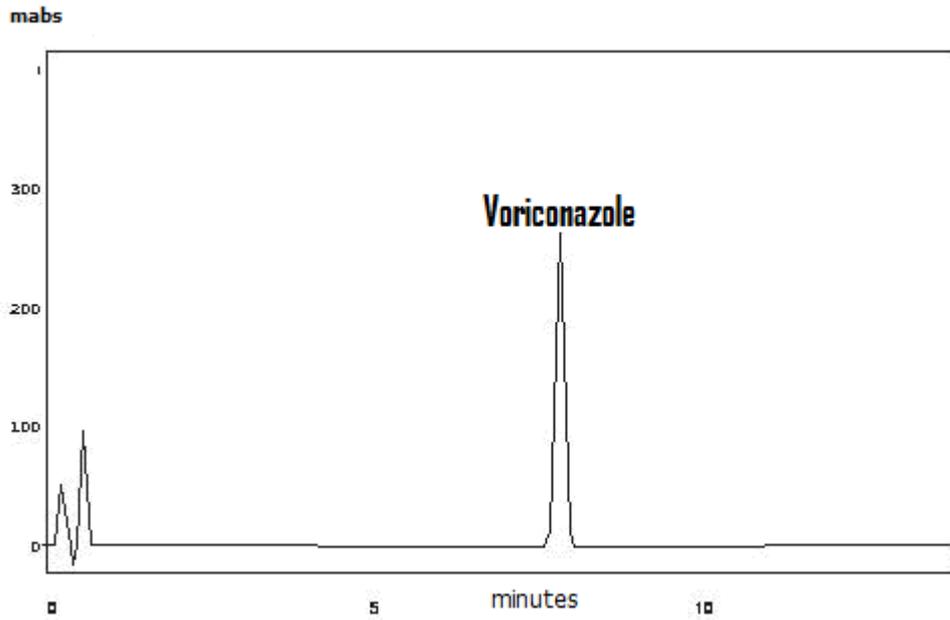
Fig.3 Voriconazole solution (24 mcg mL^{-1}) under acid degradation conditions.
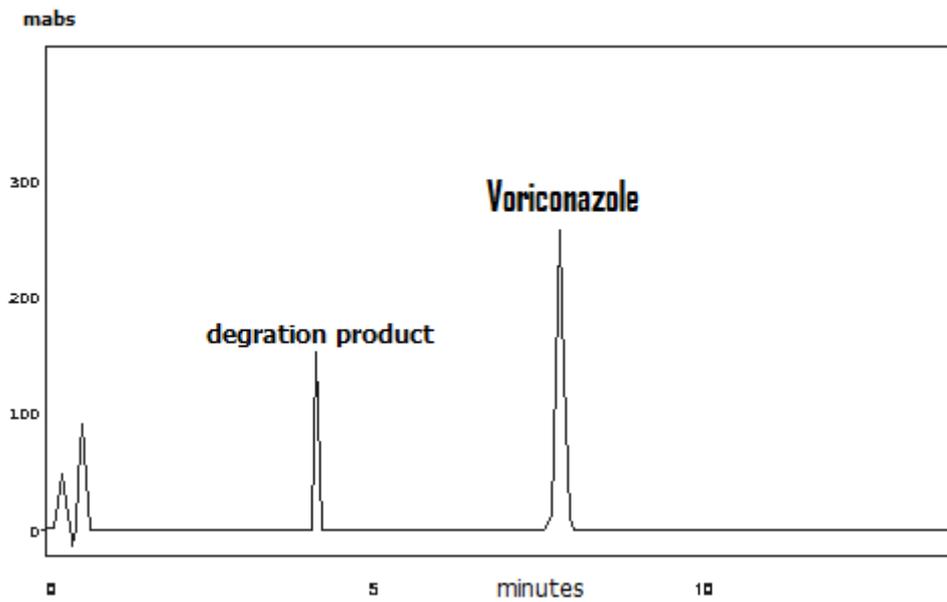
Fig.4 Voriconazole solution (24 mcg mL^{-1}) under basic degradation conditions.
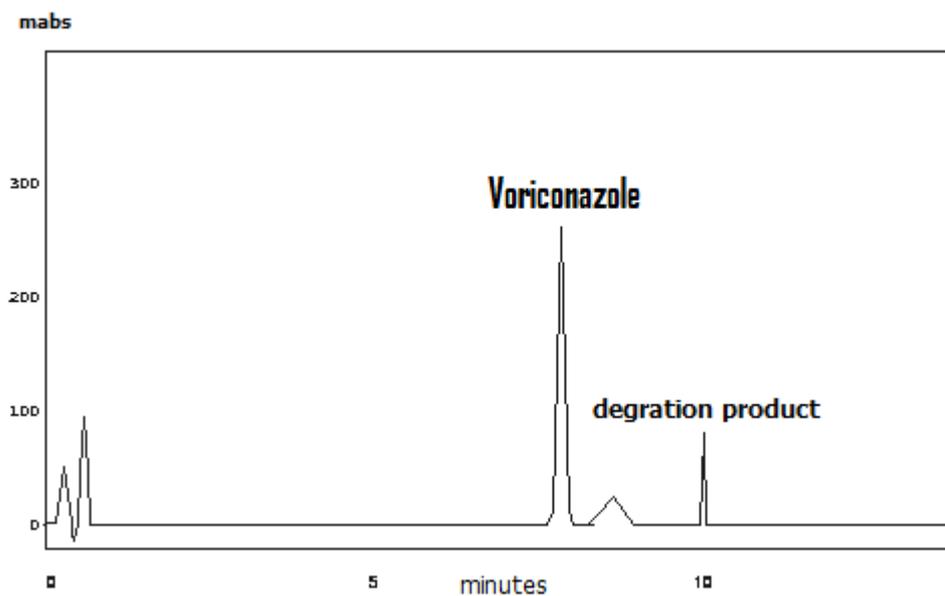
Fig.5 Voriconazole solution (24 mcg mL^{-1}) under oxidative degradation conditions.
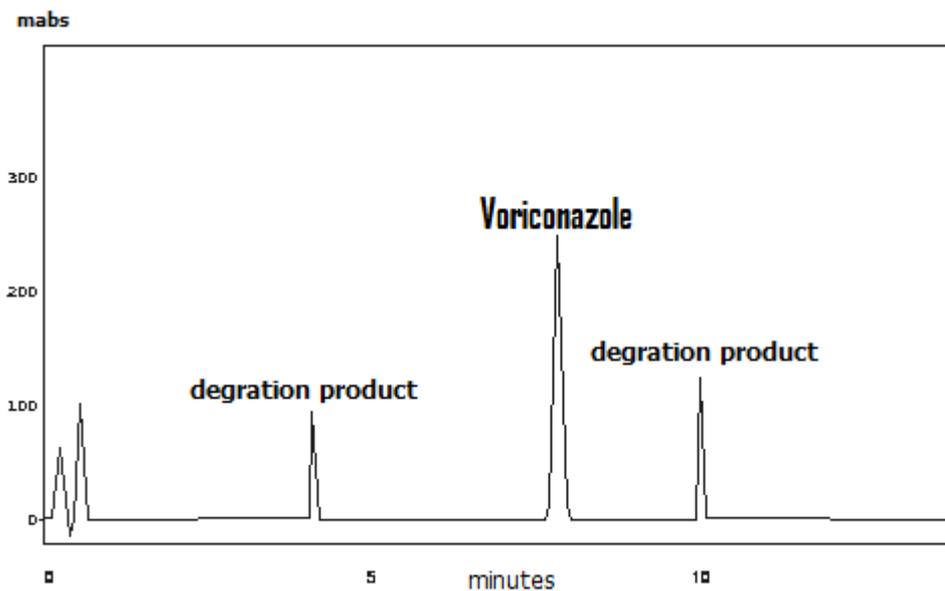
Fig.6 Voriconazole solution (24 mcg mL^{-1}) under photo-irradiation conditions.
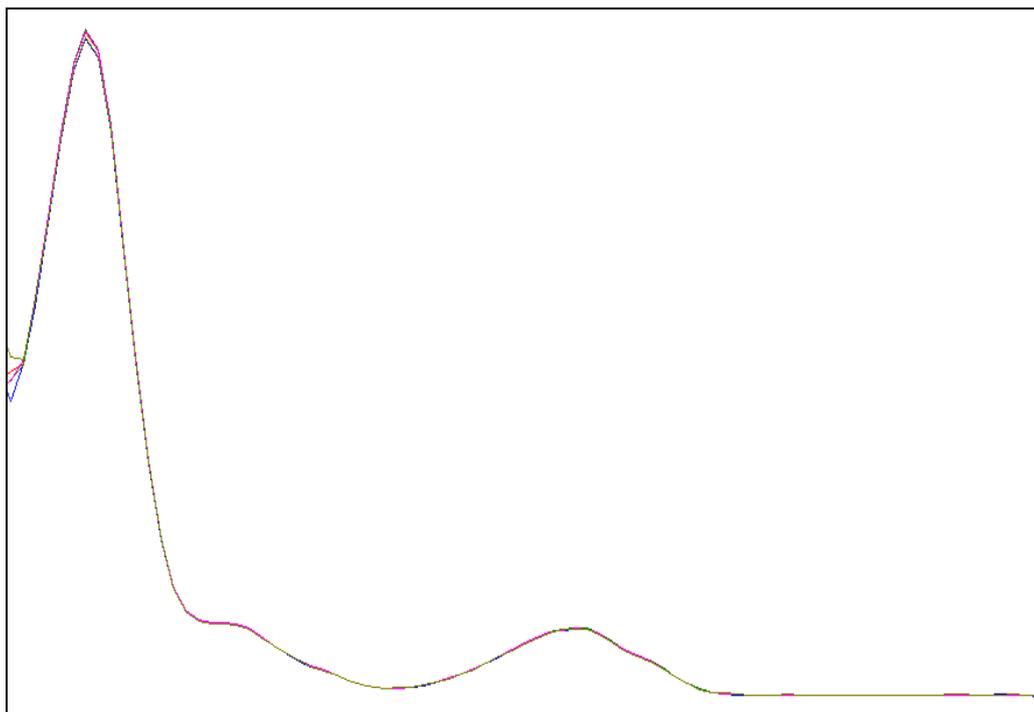
Fig.7 Voriconazole peak purity (the spectrum taken at different intervals for the peak are superimposed) .
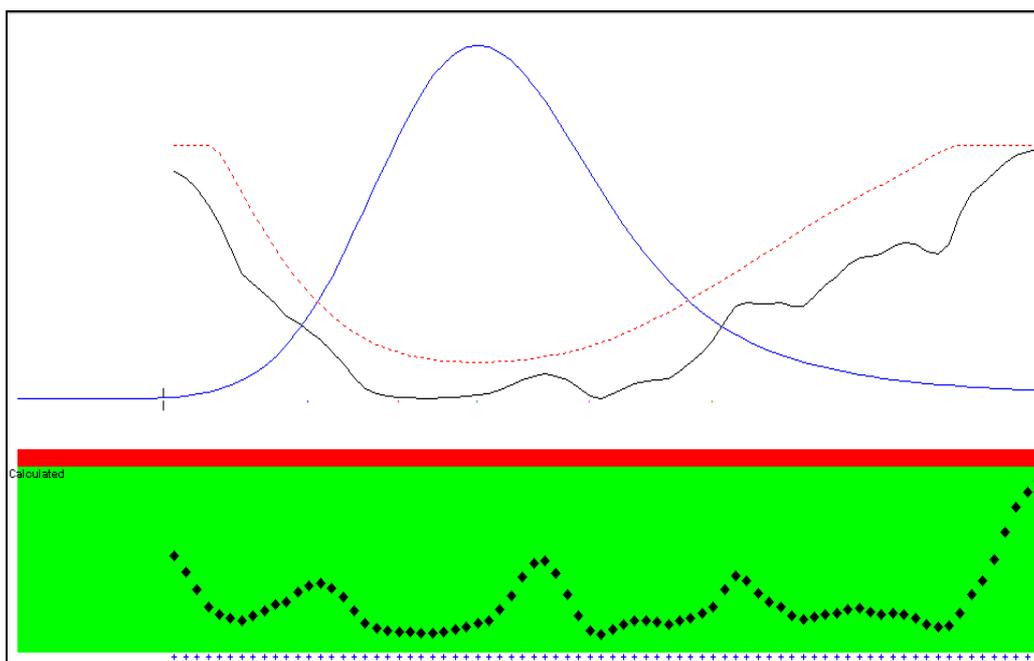
Fig.8 Voriconazole peak purity by PDA, the purity factor was lower than the calculated threshold

3.3. Linearity

The calibration curves (peak area vs. concentration) were constructed for voriconazole in pure solutions and in the drug-matrix solutions at concentrations of 6, 12, 18, 24, 30, 36 and 60 µg mL^{-1}, The mean of three replicate standards at each concentration level was used.

The resulting curves were found to be linear with correlation coefficients (r^2) of greater than 0.999 in both cases, correlation coefficients (r^2), slopes and intercepts are shown in (Table 1).

3.4. Sensitivity and accuracy

The limits of detection LOD and limits of quantitation, LOQ, were calculated for the calibration graphs of voriconazole as three and ten times of the noise level LOD and LOQ, respectively [12]. The values for LOD and LQD are given in Table 1. The accuracy of an analytical method is the closeness of test results obtained by that method to the true value[12]. The accuracy of the method was tested by analyzing different samples of voriconazole at various concentration levels ranged from 12 – 36 µg mL^{-1} in either pure solutions or in solutions comprising the drug-matrix used in tablet formulation, each concentration level was injected 3 times (*n=3*) and the average peak area was calculated. The results were expressed as percent recoveries of the voriconazole in the samples (Table 2). Table 2 shows that the overall percent recoveries of voriconazole in pure and drug-matrix solutions were 100.7% , relative standard deviation (R.S.D.)=1.41% and 99.9 (R.S.D.=1.19%), respectively.

Table1. Linearity of calibration curve for voriconazole in standard preparations and in drug-matrix preparation. Number of points in the regression line is 6 for each case.

Item	Calibration range (mcg mL^{-1})	Correlation coefficient	Slope	Slope 95% confidence interval for the slope[a]	Intercept	Slope 95% confidence interval for the intercept[a]	LOD (mcg mL^{-1})	LOD (mcg mL^{-1})
Voriconazole in standard preparation	6 - 60	0.9999	0.689	± 0.0118	-0.255	± 2.005	2.11	7.25
Voriconazole in drug-matrix preparation	6 – 60	0.9999	0.687	± 0.0190	1.455	± 3.122	3.45	12.10

[a]Confidence intervals of the slope and the intercept = (S.D of the slope or intercept x *t*), the value of *t* at 3 degree of freedom and 95% confidence level is 3.18

3.5. Stability of analytical solutions

Sample solutions of voriconazole in pure and drug-matrix solutions were tested for HPLC stability over 24 h at room temprature. The samples were analyzed in triplicates by the optimized HPLC method in fresh and stored solutions. The percent difference observed was in the range of -0.31 to -0.76 (Table 3), indicating the possibility of using standard solutions of voriconazole in pure or drug-matrix solutions over a period of 24 h without degradation.

3.6. Precision

As stated in the ICH [13]. and FDA[14]. guidelines, The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample under prescribed conditions.

This study precision was evaluated through repeatability and reproducibility. Various samples containing about 30 mcg mL^{-1} voriconazole in a synthetic matrix (drug-matrix) were analyzed by three independent analysts (six samples each) over 1 day and various days. The 1 day repeatability gave the overall percent recoveries of 100.1%, 101.2% and 99.9% with

%R.S.D. of 1.2, 0.62 and 0.26, respectively. The long-term reproducibility for all the analysis gave an over all recovery and R.S.D. of 100.4% and 0.96%, respectively.

Table 2. Estimation of the accuracy as an item for validation of the proposed HPLC method for the determination of voriconazole in standard or drug matrix solution.

Quantity added in mcg mL^{-1} of voriconazole	Standard solutions		Drug – matrix solutions	
	Quantity found in mcg mL^{-1}	Recovery (%)	Quantity found in mcg mL^{-1}	Recovery (%)
6.30	6.55	103.97	6.59	104.60
9.45	9.66	102.22	9.58	101.38
14.18	14.19	100.11	14.2	100.18
21.26	21.8	102.53	21.77	102.39
31.89	31.95	100.18	31.87	99.93
47.84	47.95	100.23	47.99	100.31
71.76	71.5	99.64	71.65	99.85
107.64	106.95	99.36	107.59	99.95
Calculations	Average	101.03	Average	101.07
	% R.S.D	1.64	% R.S.D	1.66

Table 3. Stability of voriconazole in standard and drug-matrix solutions over a period of 24h[a]

Sample	Quantity added in mcg mL^{-1} of voriconazole	Quantity Found		Difference (%)
		Fresh solution	Stored solution	
Standard solution of voriconazole.	14.18	14.19	14.21	-0.14
	31.89	31.95	31.9	0.16
	71.76	71.5	71.55	-0.07
Standard solution of voriconazole in the drug – matrix.	14.18	14.2	14.13	0.49
	31.89	31.87	31.53	1.07
	71.76	71.65	71.49	0.22

[a] Difference (%) = (Quantity Found in fresh solution - Quantity Found in stored solution)/ (Quantity Found in fresh solution) x 100.

3.7. Robustness

As defined by the ICH, the robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations in method parameters [15-16]. The optimum HPLC conditions set for this method have been slightly modified for samples of voriconazole (160 mg mL^{-1}) as a means to evaluate the method ruggedness. The small changes made included: the mobile phase ratio, the flow rate, the detection wavelength, the sonication time, the filtration system and the column (Table 4). Table 4 shows that the percent recoveries of voriconazole were good under most conditions and did not show a significant change when the critical parameters were modified. The tailing factor for voriconazole and the related compounds was always less than 2.6 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method would conclude that the method conditions are robust.

Table 4 Effect of experimental parameters on the percent recoveries of voriconazole[a]

Parameter	Modification	Recovery of voriconazole, %
Mobile phase	Buffer : ACN	
	50 : 50	100.1
	53 : 47	99.35 %
	47 : 53	101.1%
pH	5.5	100.1 %
	4.6	100.3 %
	5.4	100.2 %
Flow rate (mL min⁻¹)	1.0	100.1 %
	1.3	99.95 %
	0.90	99.87 %
Wavelength (nm)	255	100.1 %
	250	99.85 %
	245	99.90 %
Sonication time (minutes)	10	100.1 %
	15	100.4 %
	20	101.5 %
Filtration system	Nylon	100.1 %
	PTFE	100.7 %
	Centrifuge	99.4 %
Temperature	30ºC	100.1 %
	Room temperature	100.3 %

[a] ACN, acetonitrile; PTFE, polytetrafluoroethylene

3.8. Application

Samples of Vfend ® (n=6) were analyzed for voriconazole by this method and the results showed a percent recovery of 100.1% and a R.S.D. of 0.60% for 6 replicates. Also the method were used for analysis of raw materials samples which give results between 99.6% to 100.2%.

4. Conclusion

An HPLC method for the assay of voriconazole and its degradation products in the commercial drug products and in the tablet formulation was validated in this study. Voriconazole and the other related compounds which may coexist with it as impurities or as degradants gave chromatograms of very well resolved peaks which indicate the specificity of the method and the possibility of using it as an indicator of stability.

Slight changes in the experimental conditions did not affect significantly the resolution of the compounds of interest or their percent recoveries indicating the robustness of the method. All the statistical values (percent recovery, RSD, %D, confidence intervals of the slope and the intercept, LOD and LOQ) calculated were within the acceptable limits. The method can be used for the assay and chromatographic purity of the drug substance in its solid dosage form and for quality control purposes.

The proposed method was developed for estimation of voriconazole in its tablets dosage form. The method was successfully validated according to current ICH and FDA guidelines. This method could be used to optimize the formulation and in process control during manufacturing process and to assess the quality and performance of each tablet lot through the regular quality control testing of the pharmaceutical product.

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