VALIDATED HPTLC METHOD FOR DETERMINATION OF VORICONAZOLE IN BULK AND PHARMACEUTICAL DOSAGE FORM.

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ABSTRACT

A simple, accurate, precise and rapid high-performance thin-layer chromatographic method for determination of voriconazole as a bulk drug and in pharmaceutical formulations was developed and validated. The method employed TLC aluminum plates precoated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of methanol : toluene (3: 7 v/v) as mobile phase. Densitometric analysis of voriconazole was carried out at 255 nm. The system was found to give compact spots for voriconazole at R_f of 0.58 ± 0.02. The linear regression analysis data showed good linear relationship in the concentration range 200–1000 ng per spot. The limits of detection and quantitation were 12.05 and 36.55 ng/spot. The method was validated for precision, accuracy, specificity and robustness. The method has been successfully applied in the analysis of marketed formulation.

Keywords: V*oriconazole; Validation, HPTLC.*

INTRODUCTION

Voriconazole is designated chemically as (αR,βS)-α-(2,4-Difluorophenyl)-5-fluoro-β-methyl-α-(1H-1,2,4-triazol-1-yl-methyl)-4-pyrimideethanol[1]. It is used as an antifungal agent. Its primary mode of action is by inhibition of the fungal cytochrome P450-dependent 14α-sterol demethylase, an essential enzyme in ergosterol biosynthesis.

Literature survey reveals many analytical methods for its estimation. Voriconazole has been quantitatively assayed in biological fluids by HPLC[2-8]. Determination of drug in pharmaceutical dosage form has been reported by methods that includes spectrophotometric and chromatographic (HPLC) techniques as well[9-10]. No useful method for the analysis of voriconazole by HPTLC is yet reported. In the present investigation simple, accurate, sensitive and precise HPTLC method has been developed for determination of Voriconazole in the bulk and marketed formulation.

EXPERIMENTAL

Drugs, Reagents and Chemicals used:

Voriconazole was kindly provided by Alkem Laboratories Ltd. (Mumbai, India). Methanol (AR grade), Toluene (AR grade) were purchased from Sisco Research Laboratories Ltd; Mumbai.

Instrumentation:

Chromatographic separation was performed on a Merck TLC plates precoated with silica gel 60 F_{254} (10 cm ×10 cm with 250 μm thickness, E. Merck, Darmstadt, Germany, purchased by Anchrom Technologies, Mumbai, India). The samples were applied onto the plates using Camag

100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe as a band with 6 mm width using a Camag Linomat 5 applicator (Camag, Muttenz, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm). Densitometric scanning was performed on Camag TLC scanner 3 at 255 nm for all measurements and operated by winCATS software (V 1.4.2, Camag).

Preparation of Standard Stock Solution:

An accurately weighed sample (10 mg) of voriconazole was transferred to a 10 ml volumetric flask and dissolved in methanol to obtain a solution of strength 1000 µg/ml.

Preparation of calibration curves:

The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration 100 µg/ml. From standard solution, 2- 10 µl was spotted on the TLC plate to obtain final concentration 200-1000 ng/spot. The plate was developed in ascending vertical manner using solvent system methanol : toluene (3: 7 v/v) after 15 min of chamber saturation. Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm). The length of chromatogram run was 85 mm. The developed plates were dried in the current of air with the help of a drier. Densitometric scanning was performed in the absorbance mode at 255 nm. The slit dimension was kept at 5 x 0.45 mm. After completion of chromatographic analysis, peak areas of voriconazole were noted. Peak areas of voriconazole were plotted against corresponding concentrations and least square regression analysis was performed to generate the calibration equation.

The equations of the regression line is

$$y = 2.4905x - 1053.7 \quad (r = 0.9952)$$

Analysis of tablet formulation:

Twenty Tablets, each containing 200 mg voriconazole were weighed and finely powdered. A quantity of powder equivalent to 10 mg was weighed and transferred to 10 ml volumetric flask containing about 5 ml methanol, ultrasonicated for 10 min and then the volume was made up to 10 ml with methanol. The solution was filtered using whatmann filter paper No.41. From the filtrate appropriate dilutions were made in mobile phase to obtain concentration in the range of 200 to 1000 ng/spot. The amount of each drug present per tablet was estimated from the respective calibration curves.

Method Validation:

As per the ICH guidelines[11] the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation and robustness and specificity.

Linearity:

Linearity of the method was studied by spotting five concentrations of the drug prepared in the mobile phase in the range of 200-1000 ng/spot and noting the peak areas.

Accuracy:

For accuracy of method, recovery study was carried out by applying the method to drug sample to which known amount of voriconazole was added at level of 80, 100 and 120% of label claim (standard addition method). At each level of the amount, three determinations were performed and the results obtained were compared with expected results.

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra day studies, 3 repeated measurements of standard and sample solutions were made in a day and percentage RSD were calculated. In the inter day variation studies, 3 repeated

measurements of standard and sample solutions were made on 3 consecutive days and percentage RSD were calculated.

Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula[11]

$$LOD = \frac{3.3 \times \text{Standard Deviation of the y-intercept}}{\text{Slope of calibration curve}}$$

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula[11]

$$LOQ = \frac{10 \times \text{Standard Deviation of the y-intercept}}{\text{Slope of calibration curve.}}$$

Robustness:

Robustness is checked by making slight deliberate change in the experimental procedures. Mobile phases having different composition like methanol : toluene (3.2: 6.8 v/v) and methanol : toluene (2.8: 7.2 v/v) were tried and chromatograms were run. Robustness of the method was checked at three different concentration levels 200, 400, 600 ng/spot.

Specificity:

The specificity of the method was ascertained by overlaying UV spectra of spots for standard drug and sample at R_f 0.58 ± 0.02.

RESULT AND DISCUSSION

Optimization of Solvent System and Chromatographic Conditions:

Chromatographic separation studies were carried out on the stock solution of voriconazole. Initially the plates were spotted with 10 µL of stock solution and developed by linear ascending development method using neat solvents like toluene, hexane, methanol, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, etc. without chamber saturation. Based on the results of these initial chromatograms, binary and ternary mixtures of solvents were tried to achieve optimum peak parameter. The final mobile phase consisting of methanol : toluene in the ratio of (3: 7 v/v)was optimized since good R_f value of 0.58 ± 0.02 for voriconazole was obtained as shown in Fig. 1. The samples were applied in form of bands of width 6 mm on precoated aluminum sheets of silica gel 60 $F_{254.}$ The application position (X) and (Y) were kept at 10 mm and 10 mm respectively to avoid edge effect. Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm), using 15 mins of chamber saturation. The length of chromatogram run was 85 mm. The developed plates were dried in the current of dry air with the help of a drier. Densitometric scanning was performed in the absorbance mode at 255 nm. The slit dimension was kept at 5 x 0.45 mm.

Linearity:

When peak area was plotted Vs Concentration (ng/spot) voriconazole showed good correlation coefficient in concentration range of 200–1000 ng/spot. Linearity was evaluated by determining five standard working solutions.

Table. 1 summarizes Beer's law limit, linear regression equation and correlation coefficient for the method.

Analysis of tablet formulation:

The proposed method was also evaluated in terms of assay of commercially available tablets containing voriconazole. Three replicate determinations were performed on the accurately weighed amounts of tablets. The results obtained are shown in Table. 2.

Precision:

The proposed method was found to be precise as indicated by percent RSD (Relative Standard Deviation) not more than 1.5.

Accuracy:

The proposed method when used for estimation of voriconazole from pharmaceutical dosage form after spiking with working standard afforded recovery of 98–102% and result of recovery for voriconazole from the marketed formulation are listed in Table. 3.

Limit of Detection and Limit of Quantification:

The limit of detection was found to be 12.05 ng/spot, while the limit of quantitation was found to be 36.55 ng/spot for Voriconazole.

Robustness:

Robustness is checked by making slight deliberate change in the experimental procedures. The result obtained is shown in Table. 4.

Specificity:

The method was found to be specific since no interfering spots were seen when R_f values of standard and sample were compared. There is no difference in the spectra of sample and standard solution which indicate the specificity of the method (Fig. 2).

CONCLUSION

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and sensitive, thus can be used for routine analysis of Voriconazole in tablet dosage form.

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Table. 1: **Regression analysis of calibration curves for HPTLC**

Parameters	Results
Wavelength (nm)	255
Beer's Law Limit (ng/spot)	200-1000
Correlation coefficient (r)	0.9975
Linear regression Equation[2] (y = mx + c)	
Slope (m)	2.4905
Intercept (c)	1053.7
Limit of detection (ng/spot)	12.05
Limit of quantitation (ng/spot)	36.55
Precision indicated by %RSD	< 1.5%

[1]is the Detection Wavelength for HPTLC method.
[2]With respect to y = mx + c, where y is the peak area and x is the concentration (ng/spot).

Table. 2: Results of analysis of commercial formulation

Sr.no	Amount present in (mg/tablet)	Amount found in (mg/tablet)	% of Label claim*
1	200	200.10	100.05
2	200	199.55	99.75
3	200	199.35	99.67

*Average of three determinations.

Table. 3: Recovery studies of Voriconazole

Level	% Recovery	Mean	% R.S.D
80	100.54	99.86	0.84
	100.12		
	98.92		
100	99.85	100.99	0.99
	101.75		
	101.37		
120	99.50	99.74	0.88
	99.00		
	100.72		

Table. 4: Robustness studies of Voriconazole

Mobile Phase Composition	Peak Areas (AU)		
	200 ng/spot	400 ng/spot	600 ng/spot
Methanol : toluene (2.8: 7.2 v/v)	1540.3	2030.2	2634.5
Methanol : toluene (3.0: 7.0 v/v)	1515.4	2012.5	2620.1
Methanol : toluene (3.2: 6.8 v/v)	1565.1	2050.3	2660.3

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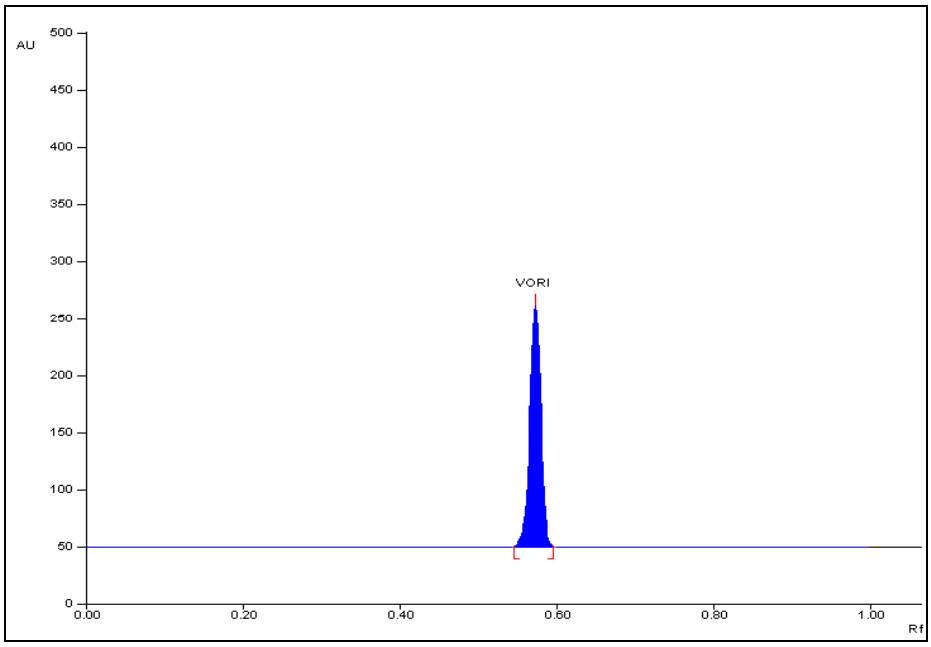

Fig. 1: HPTLC densitogram of Voriconazole (R_f 0. 58).

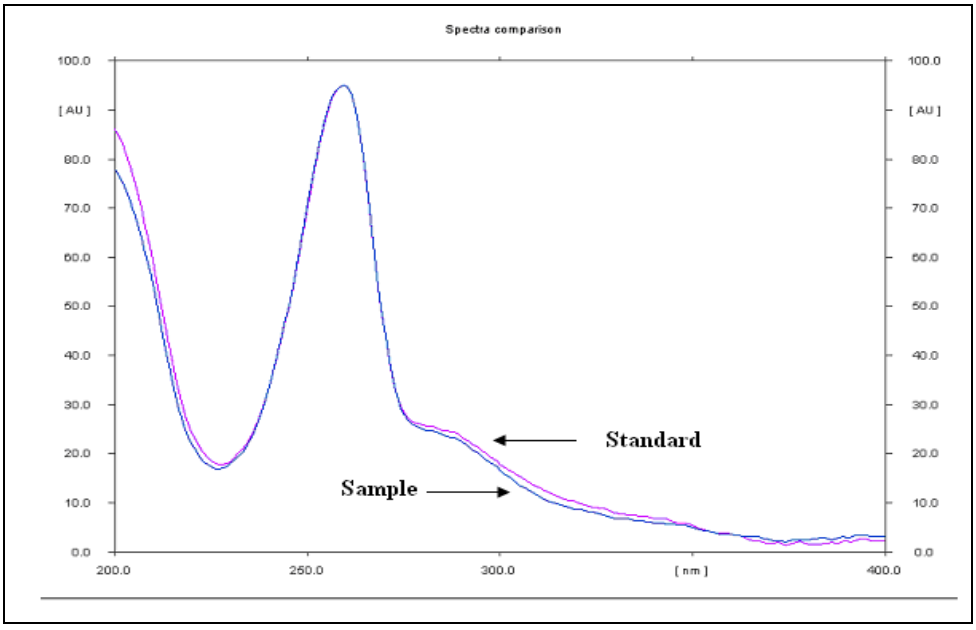

Fig. 2 : Overlain spectra of standard and sample Voriconazole (R_f 0.58 ± 02),taken on the CAMAG TLC scanner 3.

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