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RP-HPLC Determination of Voriconazole in Pure and Pharmaceutical Dosage Forms

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A reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the estimation of voriconazole in bulk and pharmaceutical formulations. The quantification was carried out using a RP-C-18 Hypersil BDS column (250 mm × 4.6 mm i.d., 5 µ particle size) in isocratic mode with mobile phase comprising water, acetonitrile and methanol in the ratio of 50:25:25 v/v. The mobile phase was pumped at a rate of 1.5 mL/min and detection was carried out at 256 nm. The linearity was found to be in the range of 20-400 µg/mL. The proposed method was statistically evaluated and can be applied for routine quality control analysis of voriconazole in tablets.

Key Words: Voriconazole, RP-HPLC, Tablets.

INTRODUCTION

Voriconazole[1] is chemically (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol. It is a new[2] antifungal agent mainly used in immunocompromised patients for treatment of invasive aspergillosis, candidaemia in non-neutropenic patients, Flucanozole resistant serious invasive candidiasis, esophageal candidiasis and serious fungal infections[3] due to *Scedosporium* and *Fusarium* species. Literature survey reveals that few UV[4], HPLC[5-7] and LC-MS[8] methods have been reported for estimation of voriconazole in pharmaceutical formulations and biological fluids. The proposed RP-HPLC method was simple, accurate and precise for estimation of voriconazole in tablet dosage forms.

EXPERIMENTAL

An isocratic high performance liquid chromatograph (Waters Alliance 2695 separations module) equipped with 600e controller pump, 776 autosampler, 2487 dual λ absorbance detector was used. The chromatographic column used was a 250 mm × 4.6 mm i.d., Hypersil BDS RP-C-18 column with 5 µm particles.The system was equipped with Empower soft ware on Pentium computer.

HPLC grade acetonitrile, methanol, from E. Merck (India) Ltd., Mumbai and milli-Q water were used for preparing the mobile phase.

Chromatographic conditions: The mobile phase consists of milli-Q water, acetonitrile and methanol in the ratio of 50:25:25 v/v. The mobile phase was mixed thoroughly and filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1.5 mL/min. The column was maintained at 25 ± 2 °C and the volume of each injection was 20 µL. The eluent was monitored at 256 nm.

Procedure: About 40 mg of pure sample of voriconazole was weighed accurately and transferred to 100 mL volumetric flask and dissolved in 40 mL of mobile phase. The solution was sonicated for 5 min and then the volume made up with further quantity of mobile phase to get 400 µg/mL solution. Subsequent dilutions of this solution ranging from 20-400 µg/mL were made in 10 mL volumetric flasks. The solutions were filtered through 0.45 µm membrane filter and then 20 µL of filtrate was injected each time into the column at flow rate of 1.5 mL/min. Evaluation of the drug was performed with at 256 nm. Peak area was recorded for all peaks. A plot of peak area *versus* the respective concentration gives the calibration curve. The regression of drug concentration over the peak area was computed. The regression equation was used to estimate the amount of voriconazole in tablets.

Estimation of voriconazole in tablet dosage forms: Tablet powder equivalent to 80 mg was taken in 200 mL volumetric flask and 100 mL of mobile phase was added. The solution was sonicated for complete solubility of the drug, made up to the mark with the mobile phase and filtered through a 0.45 µm membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. The contents of the flask were made up to volume with mobile phase and mixed well. Each of the solutions (20 µL) was then injected five times into the column. From the peak areas, the drug content in tablets was quantified using the regression equation obtained from pure sample.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, accurate and precise HPLC method for the analysis of voriconazole in tablet dosage forms. The retention time for voriconazole was 12.986 min. The peak areas from such different concentrations set up above were calculated and are shown in Table-1. A good linear relationship ($r = 0.9999$) was observed between the concentration of voriconazole and the respective peak area. The regression curve was constructed by linear regression fitting and its mathematical expression was $y = 17414x + 916.79$ (where y is peak area and x is the concentration of voriconazole). The intra-day and inter-day variations of the method were determined using three replicate injections of 4 different concentrations, which were prepared and analyzed on the same day and 3 different days over a period of 2 weeks, a low coefficient of variation was observed (Table-2). This shows that the present HPLC method is highly precise.

To ensure reliability and accuracy of the method, recovery studies were carried out mixing a known quantity of drug with preanalyzed sample and the contents were reanalyzed by the proposed method. The values are shown in Table-3. About

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Drug concentration (µg/mL)	Peak area	Drug concentration (µg/mL)	Peak area
20	350328	240	4210360
40	692711	320	5605252
80	1375724	400	6927119
60	2788086		

Regression Equation from 20-400 µg/mL; Y = 17414X + 916.79 (r = 0.9999).

TABLE-2
PRECISION OF THE PROPOSED METHOD

Concentration of voriconazole (µg/mL)	Observed concentration of voriconazole (µg/mL)			
	Intra-day		Inter-day	
	Mean (n = 3)	CV (%)	Mean (n = 3)	CV (%)
20	19.98	0.301	19.96	0.150
40	39.98	0.104	39.95	0.076
80	79.97	0.047	79.96	0.052
160	159.81	0.374	159.5	0.253

TABLE-3
RESULTS OF THE RECOVERY STUDY

Amount of drug added (µg)	Recovery from drug solution		Recovery from tablet formulation	
	Mean amount found (n = 3)	Mean % revoery	Mean amount found (n = 3)	Mean % revoery
20	19.99	99.960	19.960	99.80
40	39.94	99.850	39.950	99.89
80	79.76	99.708	79.733	99.66

99.8 % of voriconazole could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

The drug content in tablets was quantified using the proposed analytical method and the results are shown in Table-4. The absence of additional peaks in the chromatogram indicates the non-interference of common excipients used in the tablets (Fig. 1). The tablets were found to contain 99.9-100.03 % of the labelled amount of drug. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of voriconazole in pharmaceutical dosage forms. The method was duly validated by evaluation of required parameters.

TABLE-4
ASSAY OF VORICONAZOLE IN TABLET DOSAGE FORMS

S. No.	Labeled amount of drug (mg)	Mean (± SD) amount (mg) by the proposed method (n = 5)	Mean (± SD) % labeled amount (n = 5)
Tablet-1	50	49.97±0.0380	99.94±0.0760
Tablet-2	50	49.56±0.0340	99.91±0.0600
Tablet-3	200	199.934±0.0480	99.967±0.0240
Tablet-4	200	200.07±0.0744	100.03±0.0372

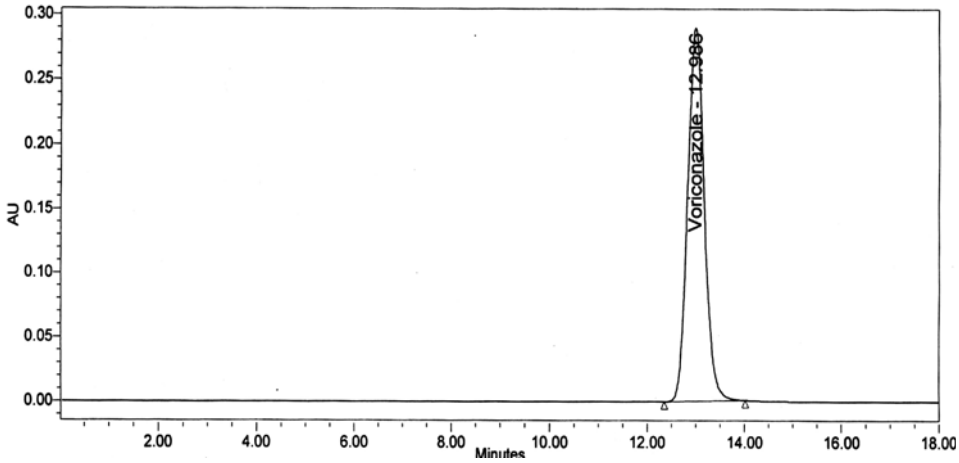

Fig. 1. Representative chromatogram of voriconazole

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