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ORIGINAL ARTICLE

RP-HPTLC method for determination of Voriconazole in bulk and in cream formulation

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Abstract Voriconazole is used as an antifungal agent. A new rapid, simple, economical and environmental friendly Reversed -Phase High-Performance Thin-Layer Chromatography (RP-HPTLC) has been developed and validated for quantitative determination of voriconazole in bulk and in cream formulation. RP-HPTLC separation was performed on aluminium plates precoated with silica gel 60RP-18F-$_{254}$S as the stationary phase using Acetonitrile: Water (60:40% *v*/*v*) as mobile phase. Quantification was achieved by densitometric analysis at 257 nm over the concentration range of 200–1200 ng/band. The method was found to give compact and well resolved band for Voriconazole at Retention factor (R_f) 0.48 ± 0.02. The linear regression analysis data for calibration graph showed good linear relationship with r^2 = 0.999. The method was validated for precision, recovery, robustness, ruggedness and sensitivity as per International conference on Harmonization (ICH) guidelines. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 19.99 ng and 60.60 ng, respectively. The proposed developed RP-HPTLC method can be applied for identification and quantitative determination of Voriconazole in bulk and in cream formulation.

1. Introduction

Voriconazole is designated as a (αR,βS)-α-(2,4-Difluorophenyl)-5-fluoro-β-methyl-α-(1*H*-1,2,4-triazol-1-yl-methyl)-4-pyrimideethanol (Fig. 1). It is used as an antifungal agent (The Merck Index 2006) and its primary mode of action is by inhibition of the fungal cytochrome P450-dependent 14α-sterol demethylase, an essential enzyme in ergosterol biosynthesis (Block and Beale, 2004).

In the literature, several methods have been described for determination of Voriconazole in biological fluids including HPLC assay of voriconazole in human plasma (Pennick et al. 2003), Simultaneous determination of Voriconazole and Itraconazole and its hydroxy metabolite in human serum using HPLC (Khoschosour et al. 2005), and HPTLC method for

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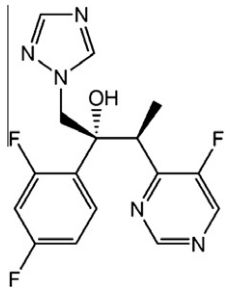

Figure 1 Chemical structure of Voriconazole.

determination of voriconazole in human plasma using toluene : methanol : triethylamine (6:4:0.1 *v*/*v*/*v*) as mobile phase. (Dewani et al. 2011). Some methods are reported for determination of Voriconazole in pharmaceutical preparations such as Liquid chromatography using prominence diode array detector and mobile phase of water : acetonitrile (35:65 % *v*/*v*) (Patel et al. 2009), HPLC method for determination of Voriconazole using UV–visible detector and mobile phase of buffer : acetonitrile (50:50 % *v*/*v*) (Bharati et al., 2010), Stability indicating HPLC method (Wamorkar et al. 2010, Eldin et al. 2010), Polarography using a 0.01 M KH_2PO_4 water solution (pH 4.5) as supporting electrolyte (Gianfranco et al. 2009), Comparison of Microbiological and UV-Spectrophotometric Assays for Determination of Voriconazole in Tablets (Adams et al. 2006), and HPTLC determination in bulk and formulation (Khetre et al. 2008).

So far no RP-HPTLC method for the analysis of Voriconazole has been reported. Therefore, in the present research paper a simple, accurate, sensitive and precise RP-HPTLC method has been developed for determination of Voriconazole in the bulk and in cream formulation.

2. Experimental

2.1. Materials and reagents

Voriconazole was supplied as a gift sample from Glenmark Pharmaceutical LTD, Sinnar, and Nasik, India. All chemicals and reagents used were of Analytical grade and were purchased from Merck Chemicals, India.

2.2. Chromatographic conditions

The plates were prewashed with methanol and activated at 100 °C for 5 min prior to chromatography. The drug standard and samples were spotted in the form of bands of 6 mm width with a Camag microlitre syringe on precoated silica gel aluminium plates 60 RP-18 F_{254} S(20 × 10 cm with 200 mm thickness, E. Merck), using a Camag Linomat 5 applicator. The slit dimension was kept at 6.00 × 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of Acetonitrile: Water (60:40 *v*/*v*), and 10 mL of mobile phase was used. Linear ascending development was carried out in a 20 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 20 min at room temperature (25 °C ± 2). The length of the chromatogram run was approximately 80 mm. After development; the HPTLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by winCATS software (Version 1.3.0).

2.3. Preparation of standard solution and linearity study

Stock standard solution was prepared by dissolving 10 mg of Voriconazole in 10 mL methanol to obtain concentration 1 mg/mL. Aliquots of standard solutions 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL of Voriconazole were transferred into six separate 10 mL volumetric flasks and volumes were made up to the mark using same solvent. An appropriate volume 10 µL was applied with the help of microlitre syringe, using Linomat 5 applicator on RP-HPTLC plate to obtain concentrations of 200, 400, 600, 800, 1000 and 1200 ng/band. The standard curves were assessed for within day and day-to-day reproducibility. Each experiment was repeated for six times.

3. Method validation

3.1. Precision

Precision can be performed at two different levels i.e. repeatability and intermediate precision. Repeatability of sample application and measurement of peak area were carried out using six replicates of the same band (600 ng/band of Voriconazole). The intermediate precision results from the variations such as different days, analysts and equipments. The intra-day variation experiments were studied using three different concentrations over the linearity range within same day. The inter-day variations in the methods were assessed by studying three different concentrations for three different days over a period of week. The intra and inter-day variation for the determination of Voriconazole was done at three different concentration levels of 400, 600, and 1000 ng/band.

3.2. Limit of detection (LOD) and limit of quantification (LOQ)

In order to determine limit of detection and limit of quantification, Voriconazole concentrations in the lower part of the linear range of the calibration curve were used. Voriconazole solutions of 200, 240, 280, 320, 360 and 400 ng/band were prepared and applied on RP-HPTLC plate. The LOD and LOQ were calculated using equation LOD = 3.3 × N/B and LOQ = 10 × N/B, where, '*N*' is standard deviation of the peak areas of the drugs ($n = 3$), taken as a measure of noise, and '*B*' is the slope of the corresponding calibration curve.

3.3. Specificity

The specificity of the method was determined by examining Voriconazole standard and Voriconazole extracted from the cream formulation. The spot for Voriconazole in sample was confirmed by comparing the R_f values and spectra. The peak-purity of Voriconazole was assessed by comparing the spectra at three different levels, i.e., peak- start (*S*), peak- apex (*M*) and peak- end (*E*) positions of the band.

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