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ESTIMATION OF VORICONAZOLE IN PHARMACEUTICAL DOSAGE FORMS BY ZERO AND FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHODS

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ABSTRACT

Voriconazole is a broad-spectrum antifungal agent used to treat invasive fungal infections that are generally seen in patients who are immuno-compromised. In this study two spectrophotometric methods have been developed for the determination of Voriconazole in its tablet dosage forms. Method A involves zero order (λ_{max} 258 nm) and method B involves first derivative (maxima at 247 nm) spectrophotometric methods in 0.1 N HCl. Linearity was followed 5-80 µg ml^{-1} for both the methods and the methods are validated. The method is more precise and accurate as the RSD values are less than 2.0 %.

Keywords: Derivative Spectroscopy, Voriconazole and Zero order spectrocopy.

INTRODUCTION

Voriconazole (VOR) is a triazole anti-fungal agent indicated for use in the treatment of fungal infections including invasive aspergillosis[1], esophageal candidiasis, and serious fungal infections caused by *Scedosporium apiospermum* (asexual form of *Pseudallescheria boydii*) and *Fusarium* spp. including *Fusarium solani*. Fungal plasma membranes are similar to mammalian plasma membranes, differing in having the nonpolar sterol ergosterol, rather than cholesterol, as the principal sterol. Membrane sterols such as ergosterol provide structure, modulation of membrane fluidity, and possibly control of some physiologic

events. Voriconazole effects the formation of the fungal plasma membrane by indirectly inhibiting the biosynthesis of ergosterol[2]. This results in plasma membrane permeability changes and inhibition of growth. It is used to treat invasive fungal infections that are generally seen in patients who are immunocompromised[3]. The primary mode of action of voriconazole is the inhibition of fungal cytochrome P-450-mediated 14 alpha-lanosterol demethylation, an essential step in fungal ergosterol biosynthesis. The accumulation of 14 alpha-methyl sterols correlates with the subsequent loss of ergosterol in the fungal cell wall and may be responsible for the antifungal activity of voriconazole. Voriconazole has been shown to be more selective for fungal cytochrome P-450 enzymes than for various mammalian cytochrome P-450 enzyme systems.

Voriconazole[4] is chemically, (2R, 3S)-2-(2, 4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1, 2, 4-triazol-1-yl) butan-2-ol having molecular weight of $C_{16}H_{14}F_3N_5O$. The chemical structure is shown in Figure 1. Literature survey reveals that Voriconazole was assayed by RP-HPLC[5-12] and spectrophotometric methods[13, 14] in pharmaceutical formulations and biological fluids. In the present study, two methods have been described for the determination of VOR involving Zero order (0.1 N HCl) showing λ_{max} at 258 nm and a first derivative method showing maxima at 247 nm.

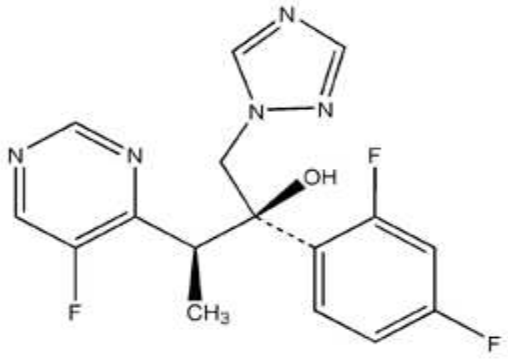

Figure 1: Chemical structure of Voriconazole (VOR)

Materials and Methods

A Shimadzu UV-VIS spectrophotometer 1800 was employed for all the spectral measurements. 0.1 N HCl (AR grade- Merck Ltd.) was used as the solvent for the two methods. A gift sample of Voriconazole was obtained from Mepro Pharmaceuticals, India and marketed formulations, VORIPRO and VORIZOL were purchased from local market.

Linearity

According to the solubility characteristics of drug, the standard stock solution (1000µg/ml) of the drug was initially prepared in methanol and further dilutions were made with 0.1N HCl for both method A and B. Twenty tablets were weighed and powdered. The tablet powder equivalent to 25 mg of VOR was transferred into 25 ml volumetric flask containing methanol, sonicated for 15 min and then made up to the mark. The solution was filtered through Whatman filter paper No. 41. and dilutions were made with 0.1N HCl for both the methods.

Method A

Aliquots of VOR (5-80 µg/ml) were prepared using 0.05 – 0.8 ml from the stock solution in 10 ml volumetric flasks and diluting with 0.1N HCl. Two of these dilutions, (i.e. 10µg/ml and 20µg/ml) were scanned in UV region (200-400nm) against the reagent blank and the λ_{max}, was observed at 258 nm (Figure 2). Absorbance of the entire sample solutions were measured at 258 nm and a calibration curve was plotted by taking concentration (µg/ml) on x-axis and the corresponding absorbance on y-axis. The marketed formulations were analyzed from the calibration curve.

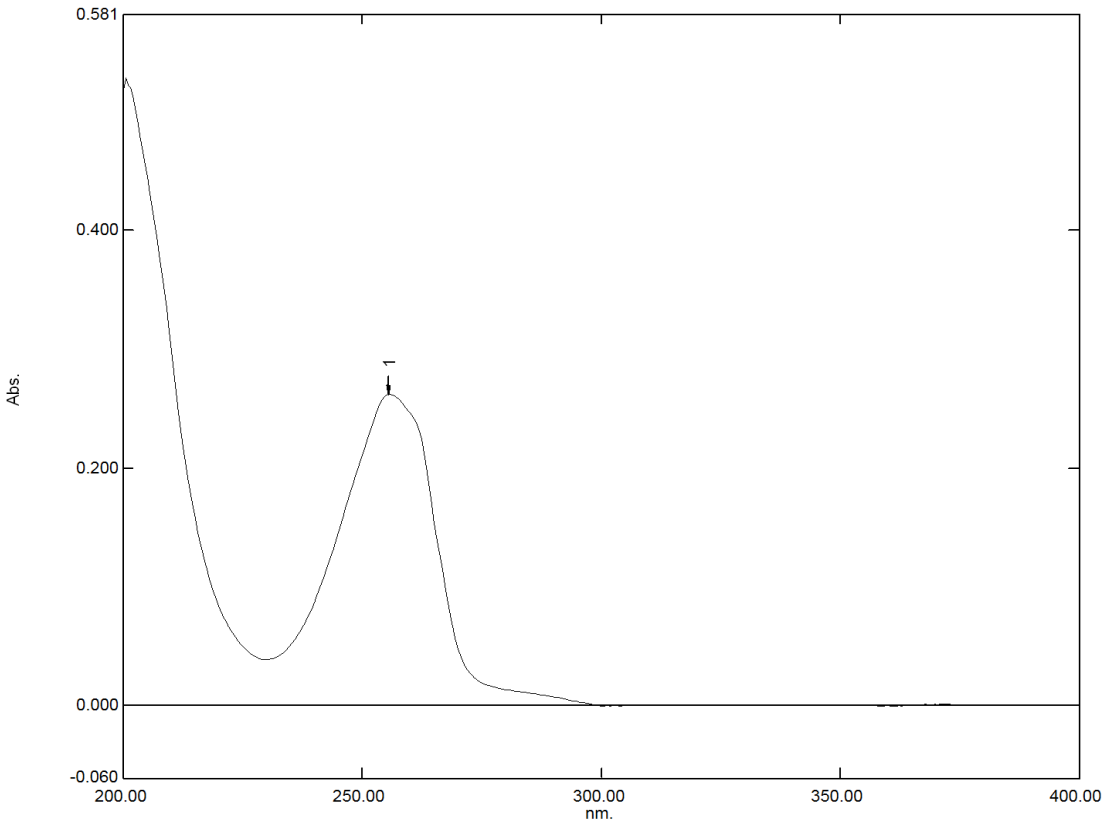

Figure 2. UV Absorption Spectrum (D_0) of Voriconazole (10 µg/ml) in 0.1 N HCl (λ_{max}=258nm)

Method B

0.05 – 0.8 ml of the stock solution was diluted with 0.1 N HCl in 10 ml volumetric flask and scanned over the wavelength region 200-400 nm. The spectrum obtained was transformed to first order derivative (Figure 3). The maxima and minima were found out to be at 247 and 265 nm respectively and the maxima values (at 247 nm) was chosen for the estimation. Calibration curve was plotted by taking concentration (µg/ml) of the drug on the x-axis and the corresponding dA/dλ values (first derivative absorbance) on the y-axis. The marketed formulations were analyzed from the calibration curve.

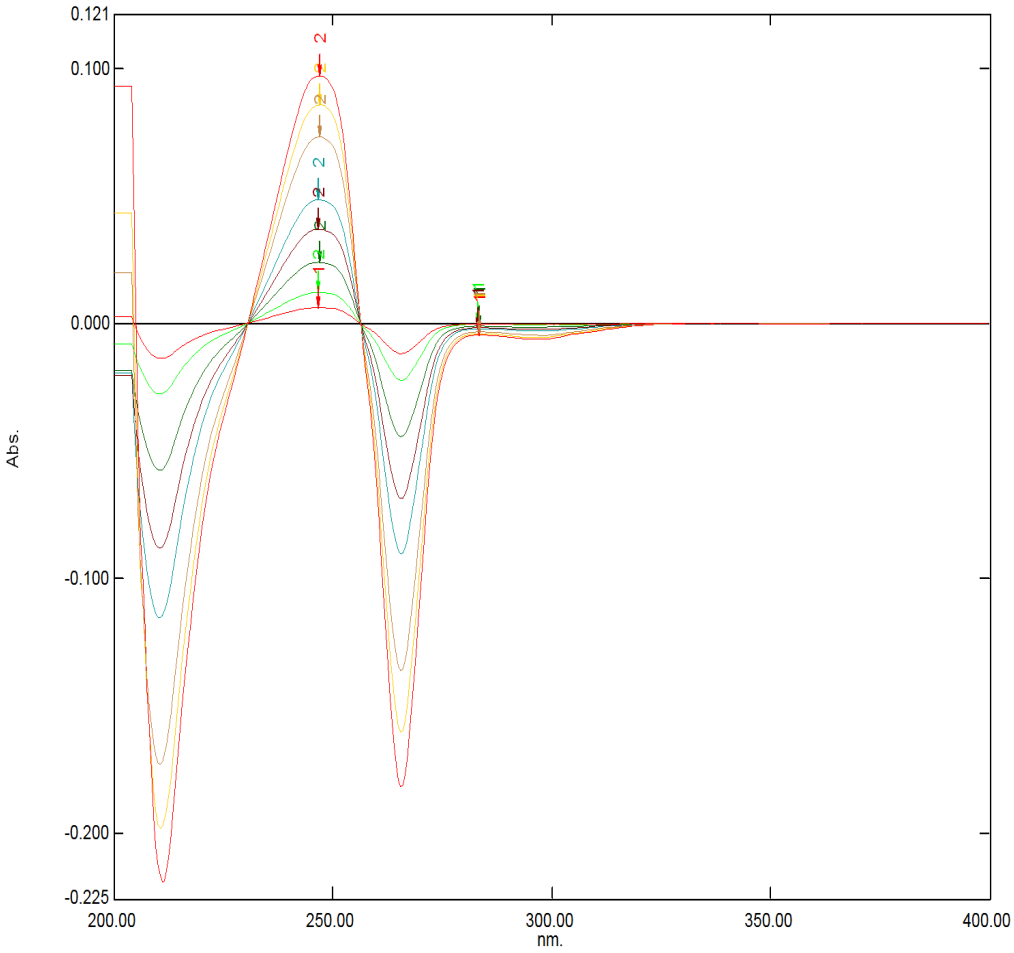

Figure 3. First Derivative spectra (D_1) of Voriconazole Solutions [5, 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml] in 0.1 N HCl (maxima at 247nm) [Method B]

Precision

The precision of the proposed method was ascertained by actual determination of absorbance of eight replicates of fixed concentration of the drug within the Beer's range

Accuracy

To determine the accuracy of the proposed method recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of VOR to the pre-analyzed formulation and the percentage recovery was calculated.

RESULTS AND DISCUSSION

The straight line observed in Figure 4 and 5 shows that VOR obeys linearity in the concentration range of 5-80 µg/ml for both methods A and B respectively and the optical characteristics were shown in Table 1. The % RSD from the recovery studies (Table 2) for both method A and B. The proposed methods were validated as per the ICH guidelines[15-16]. In precision and accuracy studies the % RSD was found to be less than 2.0. Thus it can be concluded that the methods proposed in the present investigation are simple, sensitive, accurate, rapid and precise and can be successfully applied for the estimation of Voriconazole in any pharmaceutical dosage forms.

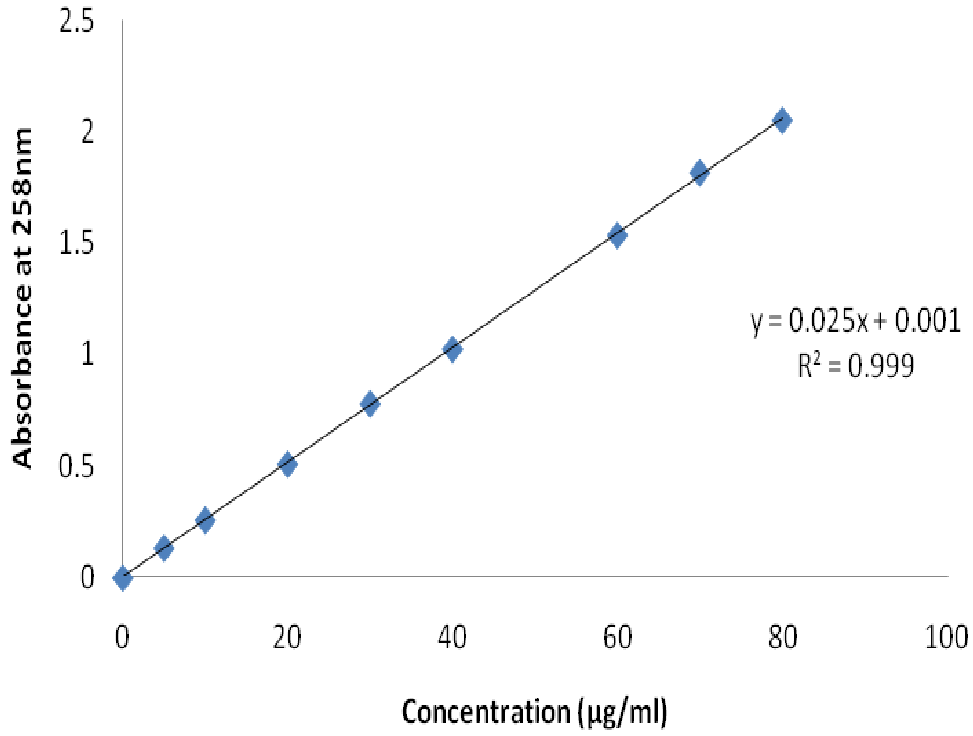

Fig 4: Linearity of Voriconazole (D_0) (Method A)

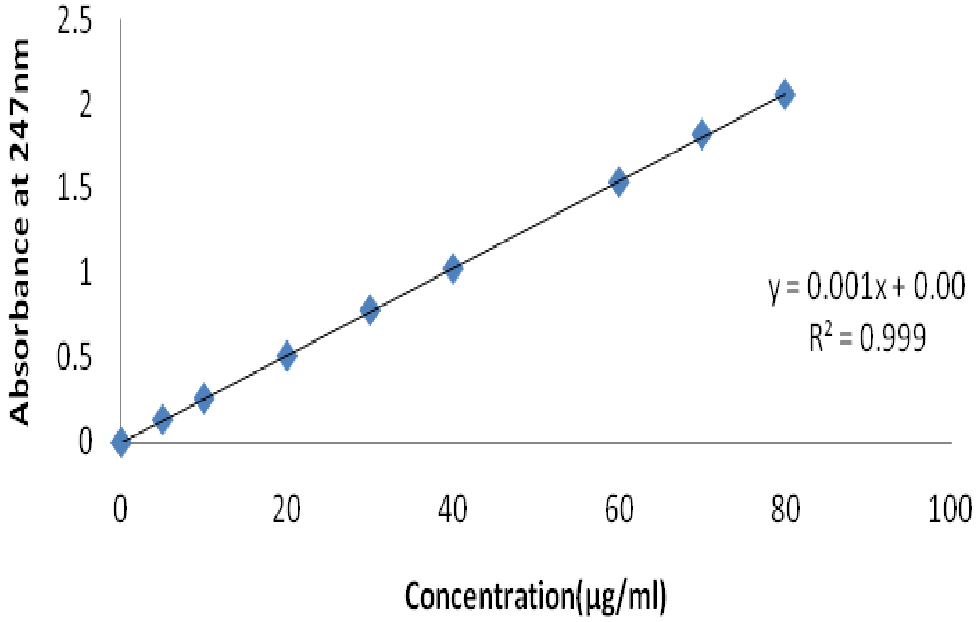

Fig 5: Linearity of Voriconazole (D_1) (Method B)

Table 1. Optical Characteristics.

Parameter	(Method A)	(Method B)
Wavelength (nm)	258	247
Linearity range (µg ml^{-1})	5 – 80	5 – 80
Regression equation	y = 0.025x + 0.001	y = 0.001x + 0.00
Sandell's sensitivity (µg/cm^2 / 0.001absorbance unit)	0.03861	–
Molar extinction coefficient ($mole^{-1}$ cm^{-1})	0.0904×10^5	–
Correlation coefficient (r^2)	0.9999	0.9998

Table 2: Recovery Studies.

Formulation	Labeled amount (mg)	Method A			Method B		
		Amount recovered* (mg) ± sd	%Drug recovered ± sd	% RSD	Amount recovered* (mg)	%Drug recovered ± sd	% RSD ± sd
Voripro-50	50	50.56±0.29	101.72±0.57	0.26	50.77±0.13	101.55±0.27	0.27
Voripro-200	200	202.62±0.17	101.34±0.07	0.152	201.24±0.32	100.62±0.17	0.53

* Each value is average of three determinations ± standard deviation.

Conclusion

It can be concluded that the proposed methods are simple, accurate, precise, reproducible and can be employed successfully for the determination of voriconazole in bulk and pharmaceutical dosage forms.

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