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Hydrolytic degradation monitoring of voriconazole by derivative spectrophotometric method

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ABSTRACT

A derivative spectrophotometric method for the simultaneous determination of Voriconazole and its hydrolytic degradation product has been developed. The objective of this study was to evaluate the utility of derivative spectrophotometry for the determination of Voriconazole Working standard (VORI WS) in presence of its degradation product without prior separation. First derivative spectrophotometry allowed specific determination of VORI WS at 269.8nm and 234.2nm with no contribution by the products formed after alkali and acid treatment respectively. Similarly, both acid and alkali catalyzed hydrolytic products were determined at 325nm with no interference by VORI WS. The Beer's law is obeyed in the concentration range of 50-150mgmL^{-1} for both VORI WS and its hydrolytic product. The percentage recovery for VORI WS was found to be 99.16-101.23%. LOD and LOQ values were also determined. Intra and interday precision values were within range. The results indicate that proposed method is simple, rapid and specific.

KEYWORDS

Voriconazole;
Derivative
spectrophotometric method.

INTRODUCTION

Voriconazole is designated chemically as (αR,âS)-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[2-5]. Determination of drug in pharmaceutical dosage form has been reported by methods that includes spectrophotometric and chromatographic techniques as well[6-10]. However these methods are not claimed to be "stability-indicating" for the determination of the drug in presence of degradation products. No useful method for the analysis of voriconazole in presence of hydrolytic degradation product is yet reported.

In the present study, the hydrolysis of Voriconazole was effected by treating the drug separately with 10M Hydrochloric acid (HCl), 2M Sodium Hydroxide (NaOH) and water (H_2O). Upon hydrolysis, the product formed has a different $_m$ (wavelength of maximum absorbance). Accordingly, a rapid and accurate derivative spectrophotometric method for the analysis of Voriconazole in presence of its hydrolysis product in bulk is proposed. The present UV derivative method was validated as per the ICH guidelines.

EXPERIMENTAL

Chemicals

VORI WS was kindly provided by Alkem Laboratories Ltd., Mumbai. Methanol AR grade was purchased from Ashonuj Chem. Pvt. Ltd. Navi Mumbai. Hydrochloric acid (HCl) and Sodium hydroxide (NaOH) were purchased from Loba chemie Pvt. Ltd. Mumbai.

Instrumentation

The zero and first order derivative spectra were recorded in the wavelength range 200 - 400 nm using UV double beam spectrophotometer of make Jasco, model V-550 with 1cm matched quartz cells. The instrumental parameters optimized for the first order derivative spectrum were:
Bandwidth : 1nm
Scanning speed : 400 nm/min
Data pitch: 0.2 nm

Preparation of VORI WS solution

50mg of Voriconazole was dissolved in methanol and the volume was made up to 50ml mark. 1ml of this solution was then diluted to 10ml in volumetric flask using distilled water to obtain the concentration 100mcg/ml.

Conditions of hydrolysis

Alkaline hydrolysis

50mg of Voriconazole was weighed and transferred to 50ml volumetric flask. The volume was made up to 50ml with methanol. To 2ml of this solution, 4ml of 2M NaOH was added before making the volume upto 10ml by distilled water (200mgmL^{-1}). This solution was kept for 15 min at room temperature. After completion of the 15 min, appropriate dilution was done with distilled water to get a solution of 100mgmL^{-1}.

Acid hydrolysis

50mg of Voriconazole was weighed and transferred to 50ml volumetric flask. The volume was made up to 50ml with methanol. 2ml of this solution then transferred to 10ml volumetric flask. 5ml of 10 M HCl was added to this flask before making the volume upto 10ml by distilled water (200mgmL^{-1}). This solution was kept for 30 min at room temperature. After completion of the 30 min, appropriate dilution was done with distilled water to get a solution of 100mgmL^{-1}

Neutral hydrolysis

50mg of voriconazole was dissolved in methanol in 50ml volumetric flask. 5ml of this solution was then transferred to 50ml volumetric flask and volume was made up to the mark with distilled water to get concentration 100mgmL^{-1}. This solution was then heated on boiling water bath for 90 minutes.

Assay method

Twenty tablets, each containing 200mg Voriconazole were weighed and finely powdered. A quantity of powder equivalent to 50mg Voriconazole was weighed and transferred to a 50ml volumetric flask. Methanol was added to the same flask and sonicated for 5 minutes. The volume was made up to 50ml with methanol. The solution was filtered using Whatman filter paper no. 41. From the filtrate, appropriate dilution was done to get a solution of 60mgmL^{-1}. This was scanned in the range of 200-400 nm. Absorbance at 269.8 nm was noted and the concentration was calculated using the following regression equation:

$y = 0.0012x + 0.0060$ ($r^2 = 0.9975$)

Procedure

The goal of the procedure was the simultaneous determination of VORI WS and its hydrolytic product in the same order spectrum. Acid hydrolysis and alkali hydrolysis was carried out by treating the VORI WS with 10 M Hydrochloric acid (HCl) and 2 M Sodium Hydroxide (NaOH). Whereas the neutral hydrolysis was carried out by treating the drug with distilled water.

Firstly the samples of VORI WS, acid hydrolytic

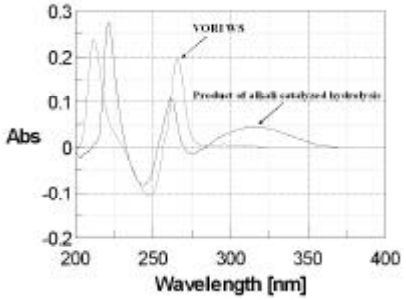

Figure 1: Overlain first derivative spectra of product of alkali catalyzed hydrolysis (100µgmL^{-1}) and VORI WS (100µgmL^{-1})

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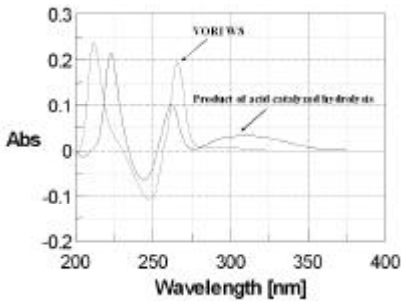

Figure 2: Overlain first derivative spectra of product of acid catalyzed hydrolysis (100μgmL^{-1}) and VORI WS (100μgmL^{-1})

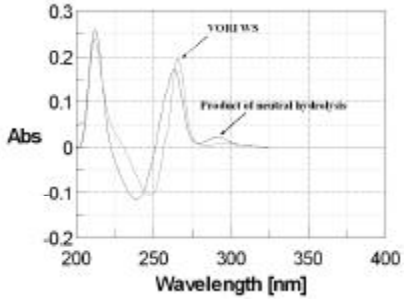

Figure 3: Overlain first derivative spectra of product of neutral hydrolysis (100μgmL^{-1}) and VORI WS (100μgmL^{-1})

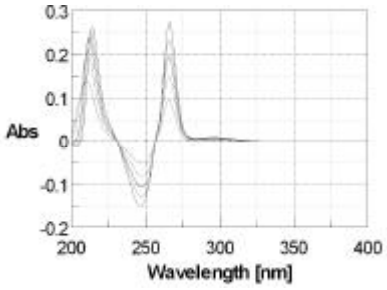

Figure 4: Linearity of first derivative spectrum of VORI WS (50-150μgmL^{-1})

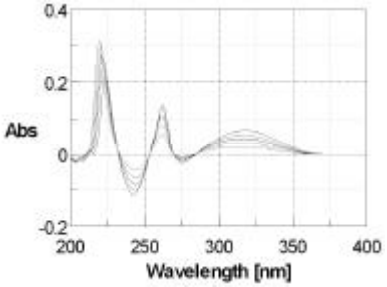

Figure 5: Linearity of first derivative spectrum of product of alkali catalyzed hydrolysis

product, alkali hydrolytic product and neutral hydrolytic product were scanned from 200-400 nm. The spectrum of VORI WS was overlaid on the spectrum of

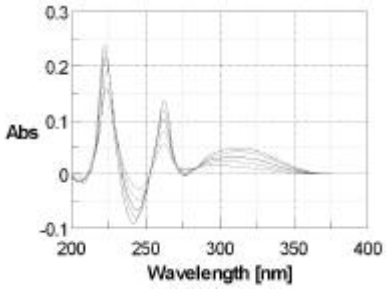

Figure 6: Linearity of first derivative spectrum of product of acid catalyzed hydrolysis

acid, alkali, and neutral hydrolytic products. Only the first derivative spectrum was found to provide a better resolution of overlapping absorption bands. Figure 1 shows the overlain spectrum of VORI WS(100mg mL^{-1}) and alkali hydrolytic product. VORI WS was determined at 269.8nm with negligible contribution by alkali hydrolytic product. In the same spectrum alkali hydrolytic product determined at 325 nm with negligible contribution by VORI WS. Figure 2 shows the overlain spectrum of VORI WS (100mgmL^{-1}) and acid hydrolytic product. VORI WS was determined at 234.2nm with negligible contribution by acid hydrolytic product. In the same spectrum acid hydrolytic product was determined at 325nm with negligible contribution by VORI WS. Figure 3 shows the overlain spectrum of VORI WS (100mgmL^{-1}) and neutral hydrolytic product. VORI WS was determined at 256.6 nm with negligible contribution by neutral hydrolytic product. In the same spectrum neutral hydrolytic product determined at 222 nm with negligible contribution by VORI WS.

Method validation

Linearity and range

The linearity for VORI WS and its hydrolysis products were determined at five concentration levels ranging from 50mgmL^{-1} to 150?gmL^{-1}. Figure 4 shows the linearity of VORI WS whereas the linearity of alkali hydrolytic product and acid hydrolytic product were shown in figures 5 and 6 respectively. The linear regression equations of the lines are:

VORI WS (269.8 nm) $y = 0.0012x + 0.0060$ ($r^2 = 0.9975$)

VORI WS (234.2 nm) $y = -0.0003x - 0.0002$, ($r^2 = 0.9981$)

Base catalyzed degradation product (325 nm) $y = 0.0005x - 0.0007$, ($r^2 = 0.9984$)

Acid catalyzed degradation product (325 nm) $y = 0.0003x - 0.0065$, ($r^2 = 0.9939$)

Precision

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies spectra of solution of VORI WS and degraded drug were repeated thrice in a day.

In the inter day variation studies spectra of solution of VORI WS and degraded drug repeated on three consecutive days.

From the data obtained, the developed UV method was found to be precise.

Accuracy

The accuracy of the method was determined by recovery experiment. The recovery studies were performed by the standard addition method, at 80%, 100%, 120% level, and percentage recovery was calculated. Recovery was within the range of 100 (2%, which indicates accuracy of the method. A known concentration of VORI WS was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovered was calculated by comparing the absorbance before and after the addition of the VORI WS. The percent recovery indicates the accuracy of the developed method.

Limit of detection and limit of quantitation

The limit of detection (LOD) is smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula:

LOD = 3.3σ/S

σ = Standard deviation of the response, S = slope of the calibration curve

The LOQ is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula:

LOQ = 10σ/S

σ= Standard deviation of the response, S = slope of the calibration curve

Robustness

Robustness of the method was determined by making slight deliberate changes in data pitch , such as from 0.2 to1nm. It was observed that there were no marked changes in the calibration data which demonstrated that the spectrophotometric method developed is robust.

TABLE 1: Validation parameters for VORI WS

Parameters	VORI WS (269.8nm)	VORI WS (234.2nm)
Linearity range (μgmL^{-1})	50-150	50-150
Correlation co-efficient	0.9975	0.9981
Linear regression equation	y = 0.0012x+ 0.0060	y = -0.0003x - 0.0002
LOD (μgmL^{-1})	1.38	8.03
LOQ (μg mL-1)	4.19	25.16
% Recovery	99.16-101.23	
Precision (% RSD)		
Intra day (n = 3)	0.56-1.22	0.02-0.04
Inter day (n = 3)	0.31-0.58	0.02-0.05

TABLE 2: Validation parameters for products of alkali and acid catalyzed hydrolysis

Parameters	Product of alkali catalyzed hydrolysis(325nm)	Product of acid catalyzed hydrolysis(325nm)
Linearity range (μgmL^{-1})	50-150	50-150
Correlation co-efficient	0.9984	0.9939
Linear regression equation	y = 0.0005x -0.0007	y = 0.0003x- 0.0065
LOD (μgmL^{-1})	6.38	3.53
LOQ (μg mL-1)	19.36	10.7
Precision(% RSD)		
Intra day (n = 3)	0.66-1.02	1.10-1.35
Inter day (n = 3)	1.19-1.39	0.72-1.39

RESULTS AND DISCUSSION

The proposed methods for simultaneous determination of VORI WS and its hydrolytic product in the first order spectrum were found to be simple, accurate, rapid and economical. The linearity for VORI WS and its hydrolysis products were determined from 50μgmL^{-1} to 150μgmL^{-1}. The values of percent RSD were not more than 1.5 and recovery was between 99.16 to 101.23%, indicating reproducibility and accuracy of methods. The summary of validation parameters of proposed derivative method is given in TABLES 1 and 2.

CONCLUSION

The validated spectrophotometric method employed here proved to be simple, fast and reliable. The low values of LOQ for the product obtained upon acid/base catalyzed hydrolysis, indicates that the method is sensitive to quantify hydrolytic degradation products.

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