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Research Article

New Spectrophotometric Methods for the Determination of Voriconazole – An Anti-Fungal Agent

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Abstract

Five new zero order and first derivative spectrophotometric methods have been proposed for the quantification of Voriconazole in tablets. These spectrophotometric techniques are developed by using the reagents - sodium hydroxide, phosphate buffers (pH 2.0, 4.0, 6.8, and 7.0). Shimadzu UV-1800 Model UV-VIS spectrophotometer double beam was used for the study. Voriconazole has shown linearity 5-60 µg/ml in all the reagents and all the methods were validated as per ICH guidelines. These methods are precise, simple, accurate and economical. These techniques can be applied successfully for the determination of Voriconazole in tablets.

Keywords: Voriconazole, zero order spectroscopy (D0), first order derivative spectroscopy (D1), validation, ICH guidelines.

Introduction

Voriconazole (Figure 1) is a triazole anti-fungal agent and chemically known as (2R, 3S)-2-(2,4-difluro phenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4 triazol1-yl)-2-butanol [1]. Voriconazole is used to treat serious fungal infections. Voriconazole has a very low aqueous solubility and its maximum solubility is in acidic conditions [2]. It is used to treat invasive fungal infections that are generally seen in immunocompromised patients. These include invasive candidiasis, invasive aspergillosis and emerging fungal infections [3]. Voriconazole is an extended spectrum triazole developed specifically to target Aspergillus [4]. Voriconazole was determined by HPLC [5-12], LC-MS [13], HPTLC [14-16] and spectrophotometry [1-3,17,18]. In the present study the authors have proposed five zero order (Method I, II, III, IV and V) and five first order derivative (Method VI, VII, VIII. IX and X) spectrophotometric methods for the assay of Posaconazole in various reagents and buffer solutions of different pH and all the methods were validated as per ICH guidelines [19].

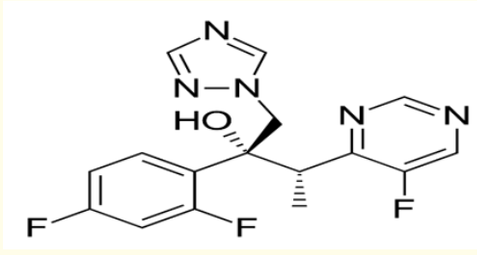

Figure 1: Chemical structure of Voriconazole.

Materials and Methods

Voriconazole is available in market with brand names Voraze (Sun Pharma), Vorizol (Natco Pharma), Vonaz (United Biotech Pvt. Ltd.), Voritek (Cipla Limited) and Vosicaz (Glenmark Pharmaceutical Ltd) (Labelled claim 200 mg) as tablets and Vfend (Pfizer) injections. Model No. UV-1800 double beam UV-VIS spectrophotometer (Shimadzu) with quartz cells is used for the entire study and all the solutions were scanned 200-400 nm.

Preparation of solutions

Reagents such as sodium hydroxide (0.1 N), phosphate buffers pH 2.0, pH 4.0, pH 6.8 and pH 7.0 were prepared as per IP 2010. Stock solution of Voriconazole was prepared by dissolving 25 mg of Voriconazole in 25 ml volumetric flask with methanol (1000 µg/ml) and further working standard solutions (100 µg/ml) were prepared by diluting the stock solution with respective reagents for the proposed I, II, III, IV and V methods. Voriconazole tablets of two different brands were procured and extracted with methanol followed by dilutions and assay was performed.

Method validation

Linearity

Zero order Spectroscopy (D_0)

A series of Voriconazole solutions 5-60 µg/ml were prepared from the stock solution, diluted with the respective reagents and scanned (200-400 nm) against reagent blank. The zero order spectrum so obtained has shown λ_{max} at about 256 ± 1 nm in all the reagents for Method I, II, III, IV and V respectively. The absorbance of

all these solutions were noted at their λ_{max} and calibration curves were drawn by plotting concentration on the x-axis and the corresponding absorbance on the y-axis respectively.

First order derivative Spectroscopy (D_1)

The individual zero order absorption spectra of Voriconazole obtained in Method I, II, III, IV and V was converted in to their first order derivative spectra with the help of inbuilt software of the instrument and the maxima value of the resultant derivative spectra was recorded for Method VI, VII, VIII, IX and X against the concentration and a calibration curve was constructed.

Precision and accuracy studies

The intra-day and inter-day precision studies were executed at three different concentration levels and accuracy studies were carried out by standard addition method (80%, 100%, and 120%) and the % recovery was calculated for all techniques.

Assay of Voriconazole tablets

Twenty Voriconazole tablets were weighed accurately, powdered and powder equivalent to 25 mg of Voriconazole was extracted with methanol in a 25 ml volumetric flask and dilutions were made using the five reagents for all the methods. The assay was carried out using the above analytical techniques and the percentage recovery was calculated.

RESULTS AND DISCUSSION

Five different reagents were used to develop two different analytical techniques i.e. Zero order and first order derivative spectroscopy for the determination of Voriconazole in pharmaceutical formulations i.e. Tablets. A detailed summary of the previously published liquid chromatographic methods was given in Table 1 and that of the spectrophotometric methods was given in Table 2.

HPLC Methods				
Mobile phase	**Column / Stationary phase**	**Flow (mL/min)**	**Detection (nm)**	**Ref**
Acetonitrile: Water (40:60)	C18 hypersil BDS	1.0	256	5
Acetonitrile: Water (7:3)	Microsorb MV- C18	1.0	255	6
Ammonium acetate: Acetonitrile: Methanol (40:20:40)	Microsorb MV- C18	1.0	250	7
Water: Acetonitrile: Methanol (50:25:25)	C18 hypersil BDS	1.5	256	8
Acetonitrile: Water: Acetic acid (55:45:25)	Diamonsil C18	1.0	256	9
Acetonitrile: Water (60:40)	C18G column	1.0	256	10
Acetonitrile: Ammonium phosphate dibasic buffer (48:52)	Agilent Zorbax SB-C18	1.0	250	11
Methanol: Water (45:55)	C_8 and C_{18}	1.0		12
LC-MS Methods				
Acetonitrile: 0.1% Formic acid in 10mm Ammonium acetate (50:50)	C18	0.5	-	13
HPTLC Methods				
Acetonitrile: Water (60:40)	Silica gel 60RP-18F_{254}s	-	257	14
Toluene: Methanol: Triethylamine	Silica gel 60 F_{254}	-	254	15
Butanol: Water: Acetic acid (8:2:1)	Silica gel 60 F_{254}	-	254	16

Table 1: Summary of previously published liquid chromatographic methods.

Reagent	**Linearity (µg/mL)**	**λ_{max} (nm)**	**Ref**
Water	5-80	252	17
Water	5-35	255	18
0.1N HCl	10-60	256	19
0.1N HCl	10-70	256	20
Methanol	5-30	256	21
Sodium Hydroxide Phosphate buffer pH 2.0 Phosphate buffer pH 4.0 Phosphate buffer pH 6.8 Phosphate buffer pH 7.0	5-60	256	Present method

Table 2: Comparison of the previously published spectrophotometric methods with the present method.

An analytical chemist has to perform the validation and the parameters must be statistically verified and for that during the linearity determination the correlation coefficient and the % RSD have to be determined.

The correlation coefficient is a statistical parameter which calculates the strength of the relationship between the relative movements of two variables. The values range between -1.0 and 1.0. A calculated number >1.0 or <-1.0 means that there was an error in the correlation measurement. A correlation of 0.0 shows no relationship between the movements of the two variables. The residual standard deviation is a statistical term used to describe the difference in standard deviations of observed values versus predicted values as shown by points in a regression analysis. Regression analysis is a method used in statistics to show a relationship between two different variables, and to describe how well you can predict

the behaviour of one variable from the behaviour of another. The relative standard deviation is the deviation measurement and it helps the scattering of a particular data around the average value. In general the correlation coefficient must be near to unity and that of the % RSD should be less than 2.

Zero order Spectroscopy (D_0)

The absorption spectra obtained in zero order spectrophotometric technique in sodium hydroxide solution (Method I), phosphate pH 2 (Method II), phosphate pH 4 (Method III), phosphate pH 6.8 (Method IV) and phosphate pH 7.0 (Method V) were shown in Figure 2. Voriconazole obeys Beer-Lambert's law (Figure 3) over the concentration range 5-60 µg/ml in all the reagents and the results were shown in Table 3. The optical characteristics of the five methods were given in Table 4. The percentage RSD in precision and accuracy studies were found to be less than 2 in all the methods indicating that the methods are precise and accurate (Table 5).

Conc. (µg/ml)	Methods (λ_{max} 256nm)				
	I	II	III	IV	V
0	0	0	0	0	0
5	0.113	0.124	0.132	0.137	0.168
10	0.227	0.248	0.265	0.228	0.393
15	0.374	0.374	0.387	0.366	0.514
20	0.46	0.499	0.53	0.456	0.704
25	0.58	0.651	0.678	0.57	0.905
30	0.682	0.78	0.827	0.69	1.06
40	0.9	0.99	1.06	0.941	1.382
60	1.381	1.499	1.59	1.38	2.077

Table 3: Linearity of Voriconazole (Zero order spectroscopy).

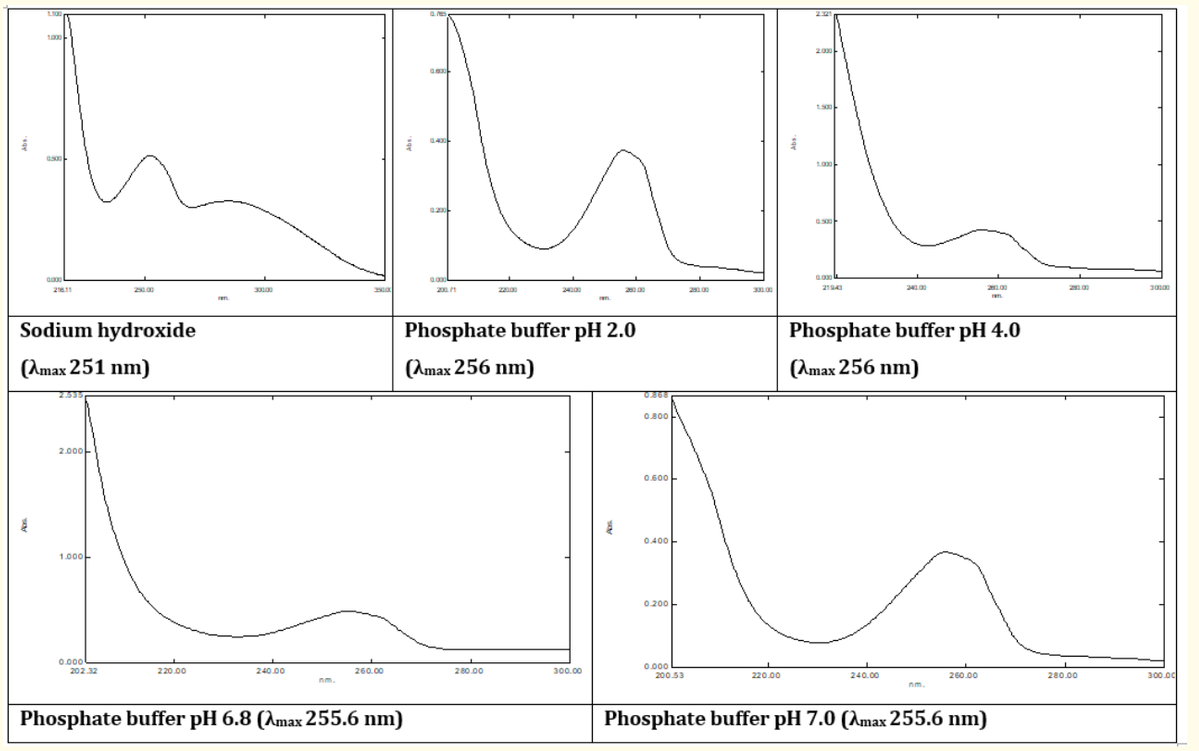

Figure 2: UV Absorption spectra of Voriconazole (15µg/mL).

Parameters	I	II	III	IV	V
Linearity range (µg /ml)	5-60	5-60	5-60	5-60	5-60
λ_{max} (nm)	256	256	256	256	256
Molar extinction coefficient (Litre/mole/cm) x 10^3	7.9294 x 10^3	8.6629 x 10^3	9.2567 x 10^3	7.9643 x 10^3	13.7279 x 10^3
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.0441	0.0377	0.0439	0.0205	0.0255
Slope	0.0344	0.0228	0.025	0.0266	0.0229
Intercept	0.013	0.0043	0.0035	0.0012	0.0122
Correlation coefficient	0.9991	0.9991	0.9991	0.9994	0.9991
Precision (%RSD)	0.25-0.97	0.83-0.98	0.33-0.86	0.51-0.75	0.31-0.87
Accuracy (%RSD)	0.53-0.92	0.72-0.84	0.53-0.91	0.35-0.90	0.54-0.91
Assay (%)	99.97	99.89	99.67	99.85	99.68

Table 4: Optical characteristics of Voriconazole - Zero order spectroscopy.

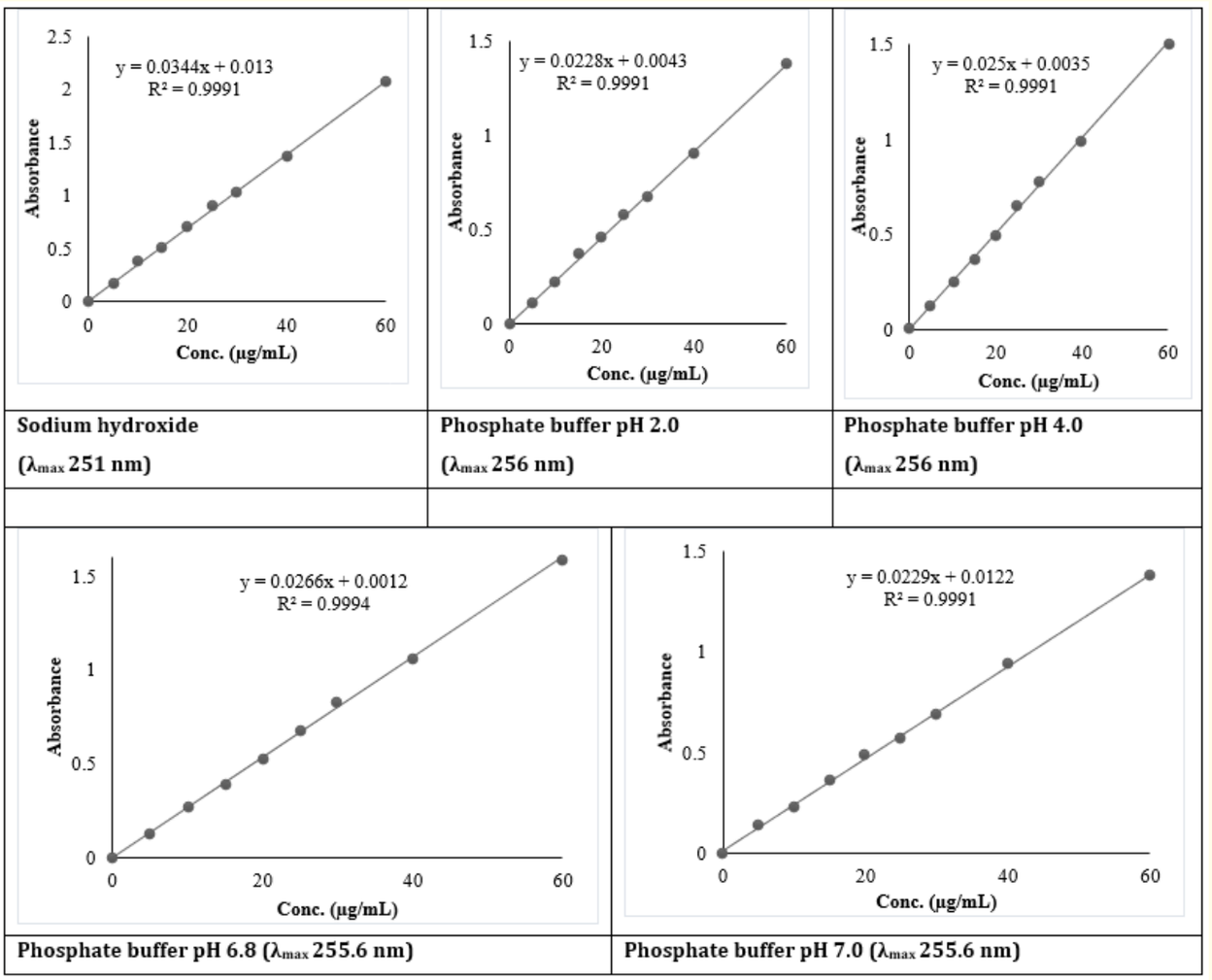

Figure 3: Calibration curves of Voriconazole (D0).

Conc. (µg/ml)	Methods				
	VI (Maxima at 210.70nm)	VII (Maxima at 246.40nm)	VIII (Maxima at 246.40nm)	IX (Maxima at 210.34nm)	X (Maxima at 240.30nm)
0	0	0	0	0	0
5	0.015	0.005	0.005	0.018	0.004
10	0.031	-	-	0.036	0.009
15	0.052	0.016	0.016	0.056	0.014
20	0.064	0.022	0.022	0.072	0.020
25	0.087	0.028	0.028	0.09	0.025
40	0.135	0.046	0.046	0.145	0.043
60	0.206	0.070	0.070	1.38	0.066

Table 5: Linearity of Voriconazole (First order derivative spectroscopy).

First order derivative Spectroscopy (D_1)

The overlay absorption spectra obtained in first order derivative spectrophotometric technique with different reagents was shown in Figure 4. The zero crossing points observed are nothing but the λmax values in zero order spectroscopy. The maxima value of the derivative spectra was chosen against concentration to draw the calibration curve and Voriconazole obeys Beer-Lambert's law (Figure 5) over the concentration range 5-60 µg/ml in all the Methods VI, VII, VIII, IX and X. The percentage RSD in precision and accuracy studies were found to be less than 2 in all the methods indicating that the methods are precise and accurate.

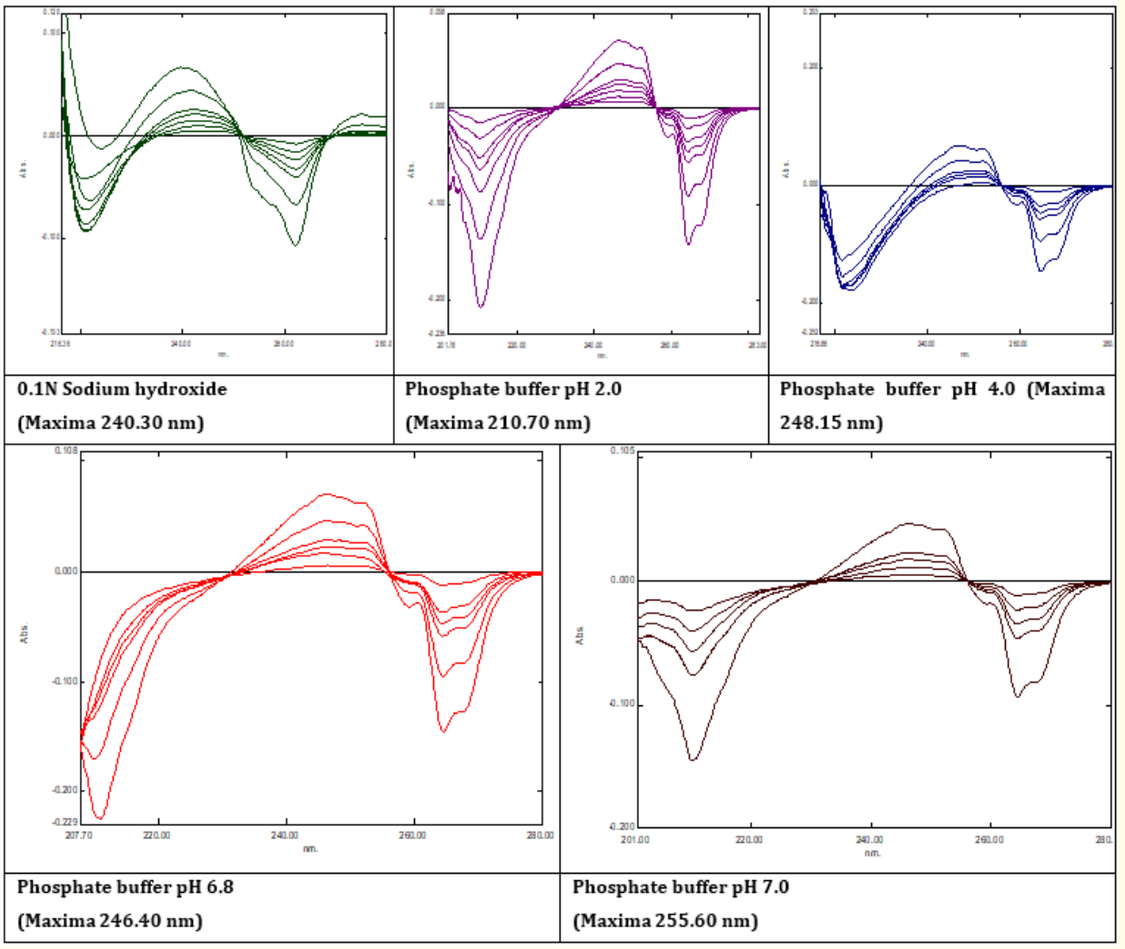

Figure 4: Overlay first order derivative spectrum of Voriconazole (D1).

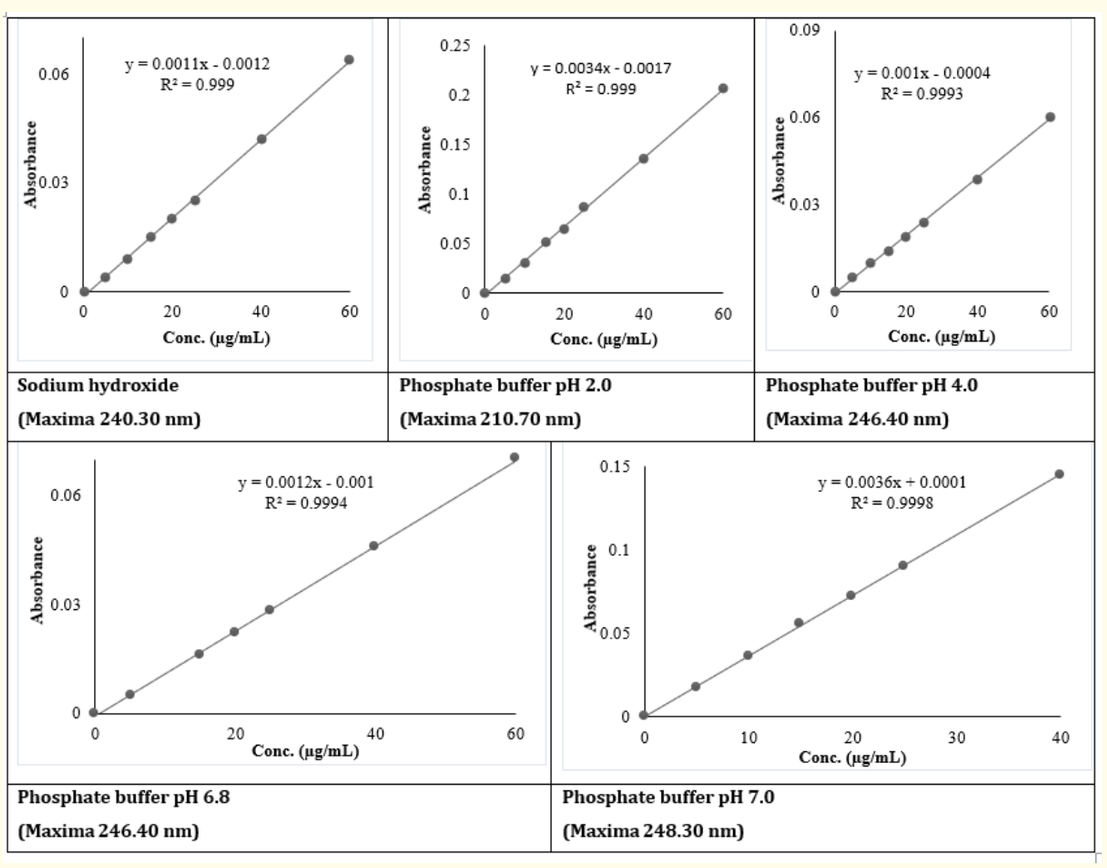

Figure 5: Calibration curves of Voriconazole (D1)

Assay of Voriconazole tablets

Assay was performed by extracting the Voriconazole with methanol from three brands and it was found that Voriconazole was 99.69-99.97 in the two marketed formulations selected in all the spectrophotometric techniques.

Conclusion

The spectrophotometric techniques were validated as per ICH guidelines and found to be simple, precise, accurate and economical for the routine analysis of Voriconazole formulations.

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