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Research Article

New Zero Order and First Derivative Spectrophotometric Methods for the Determination of Posconazole Tablets

Rajani Kaibada and Mukthinuthalapati Mathrusri Annapurna*

Department of Pharmaceutical Analysis and Quality Assurance, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India

***Corresponding Author:** Mukthinuthalapati Mathrusri Annapurna, Department of Pharmaceutical Analysis and Quality Assurance, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India.

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Abstract

New zero order and first derivative spectrophotometric methods have been developed for the assay of Posconazole in tablets. These spectrophotometric techniques are developed by using the reagents - phosphate buffers (pH 2.0, 4.0, 6.8, and 7.0), sodium hydroxide and hydrochloric acid. Shimadzu UV-1800 Model UV-VIS spectrophotometer double beam was used for the study. Posconazole has shown linearity 1-40 µg/ml in all the phosphate buffers, 1-20 µg/ml in sodium hydroxide and 0.5-5 µg/ml in hydrochloric acid respectively 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 Posconazole in tablets.

Keywords: Posconazole; Zero Order Spectroscopy (D0); First Order Derivative Spectroscopy (D1); Validation; ICH Guidelines

Introduction

Posaconazole (Figure 1) is a synthetic triazole antifungal agent used for the prevention of invasive fungal infections. Posaconazole acts against fungal infections caused by aspergillosis and candida. Posaconazole inhibits 14α-ergosterol demethylase which is responsible for the convertion of lanosterol to ergosterol and finally blocks the cell membrane synthesis [1-2]. Posaconazole acts by inhibiting CYP 3A4 activity, which shows significant drug-drug interactions and elevations in plasma levels of other medications,that are metabolized by P450 enzyme [3]. Posaconazole is a white solid with molecular formula $C_{37}H_{42}F_2N_8O_4$ and molecular weight 700.792g/mol and it is insoluble in water. Literature survey reveals that Posconazole was determined by different analytical techniques such as HPLC [4-12], UPLC [13], UHPLC [14], UPLC-MS/MS [15], LC-MS [16] and spectrophotometric methods [17,18]. Nadia., *et al*. have published a comparative study of achiral and chiral separation and analysis of antifungal drugs by HPLC and CE [19]. In the present study the authors have proposed six zero order (Method A, B, C, D, E and F) and six first order derivative (Method G, H, I, J, K and L) 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 [20].

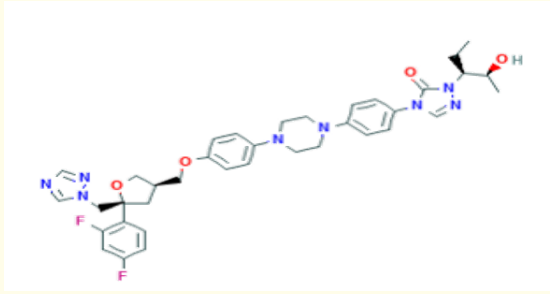

Figure 1: Chemical structure of Posconazole.

Materials and Methods

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. Posconazole is available with brand names Noxafil (Merck Sharp and Dohme Limited) as gastro resistant tablets (Labelled claim: 100 mg); oral suspension (Labelled claim: 40 mg/mL); Posconazole (Merck Ltd.) as delayed release tablets (Labelled claim: 100 mg) and intravenous injection (Labelled claim: 18 and 300 mg/mL).

Preparation of solutions

Buffer solutions such as phosphate buffers pH 2.0, pH 4.0, pH 6.8, pH 7.0 hydrochloric acid (0.1N) and sodium hydroxide (0.1N) were prepared as per IP 2010. Stock solution of Posconazole was prepared by dissolving 25 mg of Posconazole 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 buffers for the proposed A, B, C, D, E and F methods. Posconazole tablets of two different brands were procured and extracted with methanol followed by dilutions and assay was performed.

Method validation

Linearity

Zero order Spectroscopy (D0)

A series of Posconazole solutions 1-40 µg/ml in all the phosphate buffers, 1-20 µg/ml in sodium hydroxide and 0.5-5 µg/ml in hydrochloric acid respectively were prepared from the stock solution and scanned (200-400 nm) against reagent blank. The zero order spectrum so obtained has shown maximum absorbance (λ_{max}) at 224.20, 224.60, 224.60, 225.60, 219.6 and 222.80 nm in phosphate pH 2, phosphate pH 4, phosphate pH 6.8, phosphate pH 7.0, 0.1 N sodium hydroxide and 0.1 N hydrochloric acid for Method A, B, C, D, E and F respectively. The absorbance of all the solutions were noted at their λ_{max} and calibration curves were drawn taking concentration on the x-axis and the corresponding absorbance on the y-axis for Method A, B, C, D, E and F respectively.

First order derivative Spectroscopy (D1)

The individual zero order absorption spectra of Posconazole obtained in Method A, B, C, D, E and F 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 G, H, I, J, K and L against the concentration for the construction of calibration curve.

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 (50%, 100%, and 150%). The % recovery was calculated for all techniques Method A, B, C, D, E, F, G, H, I, J, K and L.

Assay of Posconazole tablets

Twenty Posconazole tablets were weighed accurately, powdered and powder equivalent to 25 mg of Posconazole was extracted with methanol in a 25 ml volumetric flask and dilutions were made using phosphate pH 2, phosphate pH 4, phosphate pH 6.8, phosphate pH 7.0, 0.1 N sodium hydroxide and 0.1 N hydrochloric acid. The assay was carried out using the above analytical techniques and the percentage recovery was calculated.

Results and Discussion

Two different analytical techniques Zero order and first order derivative spectroscopy have been developed for the determination of Posconazole in pharmaceutical formulations i.e. Tablets. A detailed summary of the previously published methods was given in Table 1.

Method	Reagent/ Mobile Phase/ Detection wave length (nm)	Flow rate (ml/min)	Linearity (µg/ml)	Ref
HPLC	Acetonitrile: Water (70: 30)/261	0.8	0.125 - 16	4
HPLC	Acetonitrile: Water (60: 40)/262	0.8	0.125-16	5
HPLC	Ammonium acetate 10mm (pH 5.5) and acetic acid: methanol: Methanol/220 (Gradient mode)	0.8	0.1– 0.75	6
HPLC	A-water: acetonitrile (95:5), B- acetonitrile: formic acid (100: 0.1) Excitation: 258; Emission: 350	1.2	0.02-3.0	7
HPLC	Sodium acetate buffer (pH3.3) and methanol (18: 82) Excitation: 240; Emission: 385	1.0	0.098 - 50	8
HPLC	Mobile phase A: Di Potassium hydrogen phosphate (p^H: 5.5) diluted in orthophosphoric acid and acetonitrile (90: 10) Mobile phase-B: Acetonitrile: Water (90:10)/210	1.0	-	9
HPLC	Ammonium acetate (0.1 M): water: acetonitrile: TFA (409: 590: 1)	1.1	0.1–10	10
HPLC	Methanol: water (75: 25)/260	1.0	5-60	
HPLC	Ammonium acetate (pH 8.0, 0.01M) and acetonitrile (35:65)/263	0.8	0.1-4.0	12

UPLC	0.1% Ortho phosphoric acid: Acetonitrile (Gradient mode)/210	0.5	1.04-4.44	13
HPLC-DAD & UHPLC-UV	Acetonitrile: 15mM potassium dihydrogen orthophosphate (45:55)/262	0.4	5-50 5-50	14
UPLC-MS/MS	Mobile phase A: water: 2 mM Ammonium acetate: 0.1% formic acid; Mobile phase B: Methanol: 2mM Ammonium Acetate: 0.1% formic acid	0.8	-	15
LC-MS	25 mM formic acid: Acetonitrile	0.51	-	16
Spectrophotometry	Methanol/260	-	5- 25	17
Spectrophotometry	Methanol/262	-	2.5-17.5	18
Spectrophotometry (Zero order & First order derivative methods)	Phosphate buffer pH 2 Phosphate buffer pH 4 Phosphate buffer pH 6.8 Phosphate buffer pH 7 0.1 N NaOH 0.1N HCl	-	1-40 1-40 1-40 1-40 1-20 0.5-5.0	Present method

Table 1: Comparison of previously published methods with the present method.

Zero order Spectroscopy (D_0)

The absorption spectra obtained in zero order spectrophotometric technique for phosphate pH 2 (Method A), phosphate pH 4 (Method B), phosphate pH 6.8 (Method C), phosphate pH 7.0 (Method D), 0.1 N sodium hydroxide (Method E) and 0.1 N hydrochloric acid (Method F) were shown in Figure 2. Posconazole obeys Beer-Lambert's law (Figure 3) over the concentration range 1-40 µg/ml in all the phosphate buffers (Method A, B, C and D) 1-20 µg/ml in sodium hydroxide (Method E) and 0.5-5 µg/ml in hydrochloric acid (Method F) respectively. The linearity results and the optical characteristics were given in Table 2 and Table 3 respectively. 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.

Conc (µg/ml)	Method (Zero order spectroscopy, D_0)						Method (First derivative spectroscopy, D_1)					
	A	B	C	D	E	F	G	H	I	J	K	L
0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	-	-	-	-	-	0.287	-	-	-	-	-	0.003
1	0.06	0.047	0.045	0.045	0.076	0.474	-	-	-	-	-	0.006
2	0.12	0.0956	0.096	0.190	0.152	1.094	-	-	-	-	-	0.013
3	-	-	-	-	-	0.155	-	-	-	-	-	0.019
4	-	-	-	-	-	0.159	-	-	-	-	-	0.026
5	0.213	0.239	0.232	0.243	0.38	2.290	0.004	0.004	0.004	0.004	0.027	0.033
10	0.436	0.479	0.464	0.487	0.76	-	0.009	0.007	0.009	0.009	0.069	-
15	0.644	0.718	0.696	0.7	1.18	-	0.013	0.011	0.013	0.013	0.139	-
20	0.872	0.959	0.929	0.97	1.52	-	0.018	0.015	0.018	0.017	0.217	-
30	1.35	1.437	1.363	1.5	-	-	0.027	0.023	0.028	0.025	0.279	-
40	1.744	1.919	1.287	1.89	-	-	0.036	0.03	0.037	0.034	0.348	-

Table 2: Linearity of Posconazole.

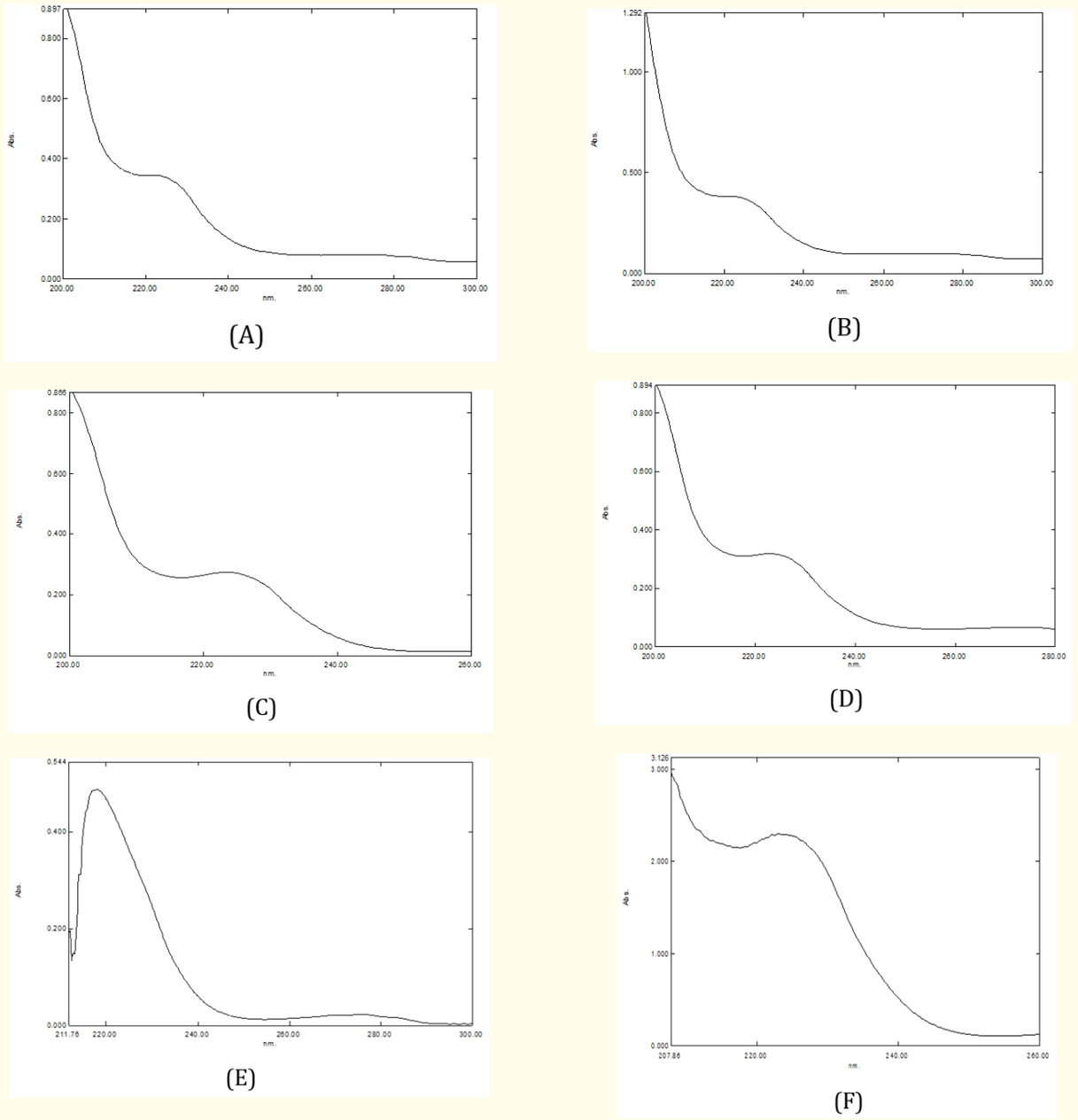

Figure 2: UV Absorption spectra of Posconazole (5µg/mL).
(A) Phosphate buffer pH 2 (B) Phosphate buffer pH 4 (C) Phosphate buffer pH 6.8
(D) Phosphate buffer pH 7 (E) 0.1 N NaOH (F) 0.1N HCl.

Parameters	Method					
	A	B	C	D	E	F
Linearity range (µg /ml)	1-40	1-40	1-40	1-40	1-20	0.5-5
λ_{max} (nm)	224.20	224.60	224.60	225.60	219.6	222.80
Molar extinction coefficient (Litre/mole/cm)	3.0555	3.3568	3.2517	3.4129	5.326	0.3209
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.0229	0.0209	0.0216	0.0205	0.0132	0.0022
Slope	0.0441	0.04730	0.0461	0.0483	0.0763	0.2742
Intercept	0.0051	0.00766	0.0016	0.0022	0.0024	0.0003
Correlation coefficient	0.9994	0.9996	0.9997	0.9991	0.9996	0.9994
Precision (%RSD)	0.21-0.93	0.78-1.01	0.33-1.07	0.45-1.12	0.35-0.98	0.29-1.29
Accuracy (%RSD)	0.62-0.98	0.76-1.03	0.65-1.09	0.29-1.24	0.63-1.63	0.58-1.31
Assay (%)	99.08	99.36	99.31	99.68	99.56	99.84

Table 3: Optical characteristics of Posconazole - Zero order spectroscopy.

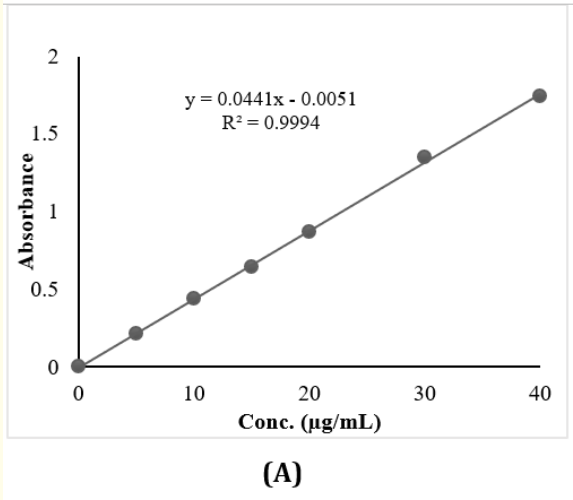

Figure 3: Calibration curves of Posconazole (D0).
(A) Phosphate buffer pH 2 (B) Phosphate buffer pH 4 (C) Phosphate buffer pH 6.8
(D) Phosphate buffer pH 7 (E) 0.1 N NaOH (F) 0.1N HCl.

First order derivative Spectroscopy (D1)

The overlay absorption spectra obtained in first order derivative spectrophotometric technique for different reagents such as phosphate pH 2 (Method G), phosphate pH 4 (Method H), phosphate pH 6.8 (Method I), phosphate pH 7.0 (Method J), 0.1 N sodium hydroxide (Method K) and 0.1 N hydrochloric acid (Method L) were shown in Figure 4. The zero crossing points are observed at 216.52, 223.72 nm (Figure 4A) in Method G, 217.09, 227.27 nm (Figure 4B) in Method H, 216.80, 223.77 nm (Figure 4C) in Method I, 216.50, 223.94 nm (Figure 4D) in Method J, 217.33, 211.58 nm (Figure 4E) in Method K and 218.33, 223.84 nm (Figure 4F) in Method L respectively. Posconazole obeys Beer-Lambert's law (Figure 5) over the concentration range 5-40 µg/ml in G, H, I, J and K Methods and 0.5-5.0 µg/ml in Method L respectively. 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.

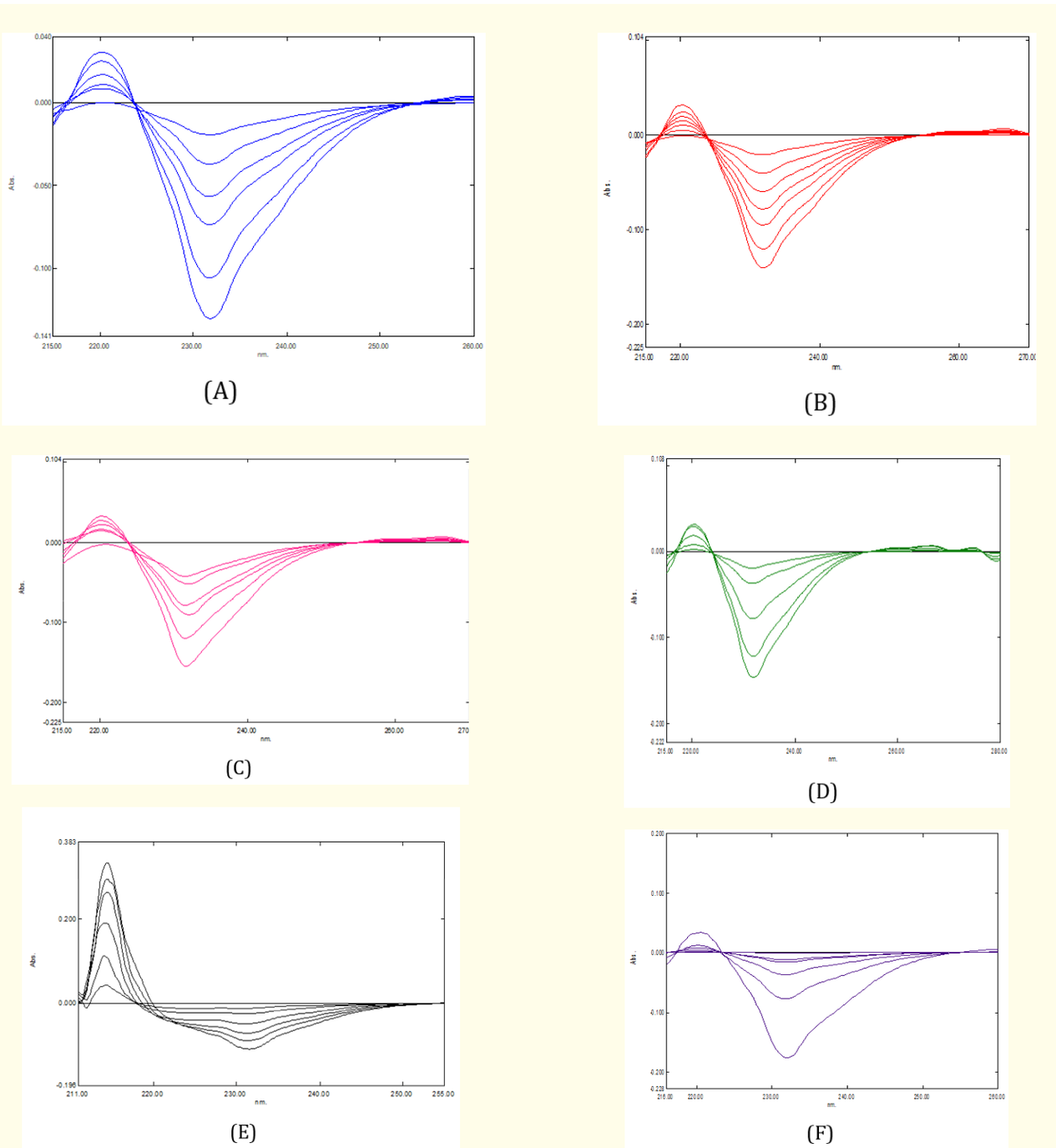

Figure 4: First derivative overlay absorption spectra of Posconazole (D1).
(A)Phosphate buffer pH 2 (B) Phosphate buffer pH 4 (C) Phosphate buffer pH 6.8
(D) Phosphate buffer pH 7 (E) 0.1 N NaOH (F) 0.1N HCl.

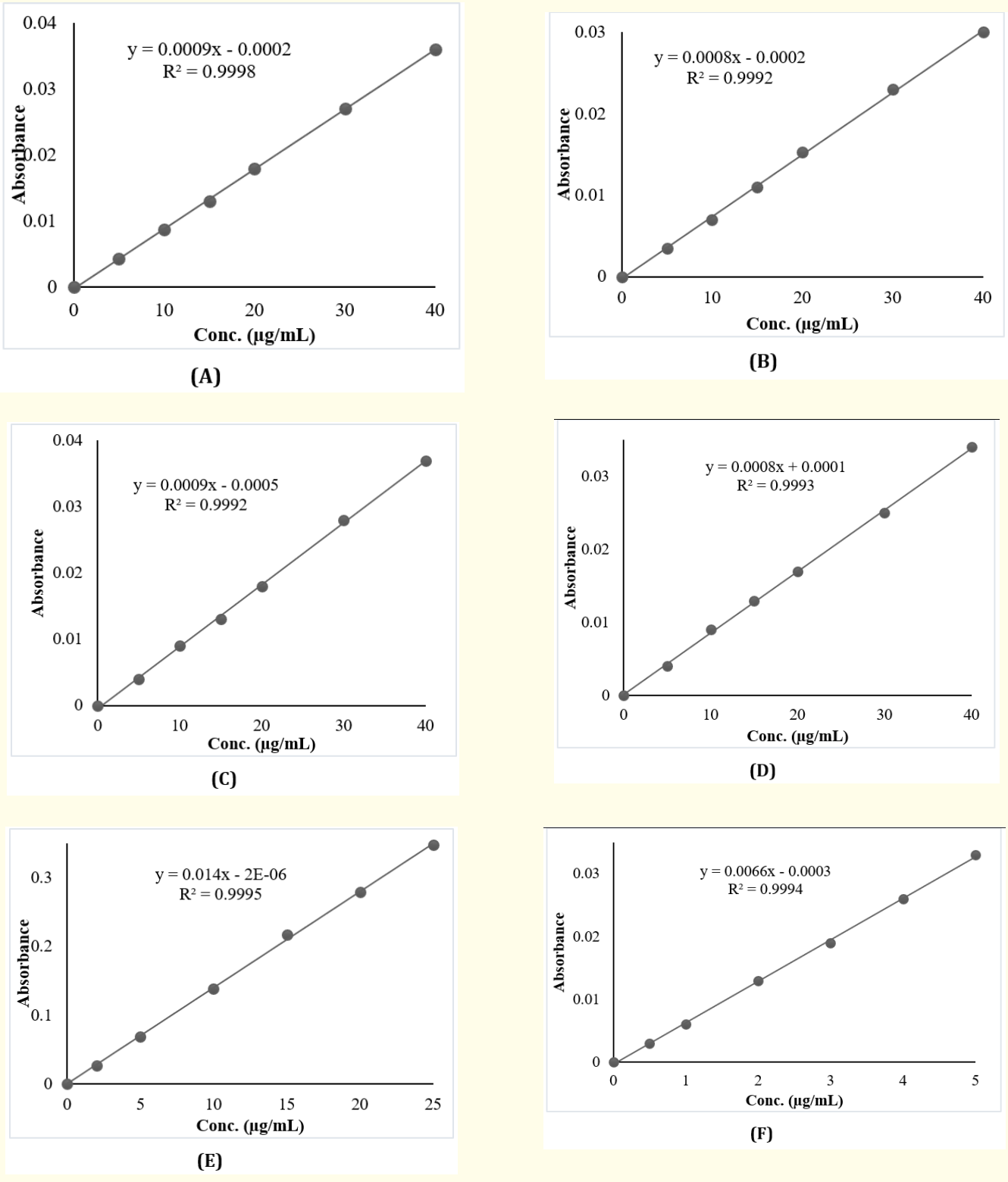

Figure 5: Calibration curves of Posconazole (D1).
(A) Phosphate buffer pH 2 (B) Phosphate buffer pH 4 (C) Phosphate buffer pH 6.8
(D) Phosphate buffer pH 7 (E) 0.1 N NaOH (F) 0.1N HCl.

Assay of Posconazole tablets

Assay was performed by extracting the Posconazole with methanol from two brands and it was found that Posconazole was 99.03-99.89 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 Posaconazole formulations.

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