IJPSR (2011), Vol. 2, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 July, 2011; received in revised form 15 September, 2011; accepted 25 October, 2011

FIRST DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF FLUOXETINE HYDROCHLORIDE AND OLANZAPINE IN TABLETS

Vijaykumar K. Parmar*[1], Jatin N. Patel [2], Girish K. Jani [2], Laxman M. Prajapati [3] and Jagbir Gagoria [4]

Department of Pharmaceutical Sciences, Sardar Patel University [1], Vallabh Vidyanagar- 388 120, India
SSR College of Pharmacy [2], Silvassa, Union Territory of Dadara and Nagar Haveli, India
Shri B. M. Shah College of Pharmaceutical Education and Research [3], College Campus, Modasa 383 315, India
[4]RKSD College of Pharmacy, Kaithal- 136 027, Haryana, India

Keywords:
Simultaneous determination, Derivative spectrophotometry, Fluoxetine hydrochloride, Olanzapine, Tablets

Correspondence to Author:

Dr. Vijaykumar K. Parmar

Assistant Professor, Department of Pharmaceutical Sciences, Sardar Patel University, Vallabh Vidyanagar- 388 120, India

ABSTRACT

Fluoxetine hydrochloride (FH) in combination with Olanzapine (OZ) is used in treatment of depressive episodes associated with bipolar disorder. The first derivative spectrophotometric method for simultaneous determination of fluoxetine hydrochloride (FH) and olanzapine (OZ) from two component tablet dosage form has been developed and validated. Chromatographic methods viz. HPLC and HPTLC methods were reported in the literature for the simultaneous determination of FH and OZ from combined dosage forms. In the present investigation an attempt has been made to develop accurate, reproducible, rapid and cost-effective method for simultaneous determination of FH and OZ in pharmaceutical formulation. The wavelengths selected for determination of FH and OZ is 235.5nm and 296nm, respectively. The beer's law was obeyed in the concentration range of 8-80µg/mL and 2-20µg/mL for Fluoxetine and Olanzapine, respectively. The proposed methods were validated and successfully applied to the determination of FH and OZ in tablet formulations. The results suggested that the proposed procedures can be used for routine quality control of tablets containing FH and OZ.

INTRODUCTION: Fluoxetine hydrochloride (FH) in combination with Olanzapine (OZ) is used in treatment of depressive episodes associated with bipolar disorder [1]. This combination of FH and OZ produce robust and sustained increases of extracellular levels of dopamine and norepinephrine, which were significantly greater than with either drug alone [2]. This combination drugs are available in tablet dosage form in the market. The combination of FH and OZ is not official in any pharmacopoeia. The FH, a selective serotonin reuptake inhibitor, is chemically d, l- N- methyl- 3- phenyl-3-[α, α, α- trifluoro- p- tolyl) oxy] propyl amine hydrochloride (**Figure 1a**).

Chemically, olanzapine is 2-methyl-4-(4-methyl-1-piperazinyl)-10H [2, 3-b] [1, 5] benzodiazepine (**Figure 1b**).

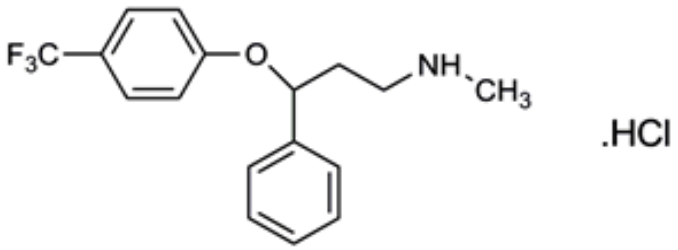

FIG. 1A: FLUOXETINE HYDROCHLORIDE

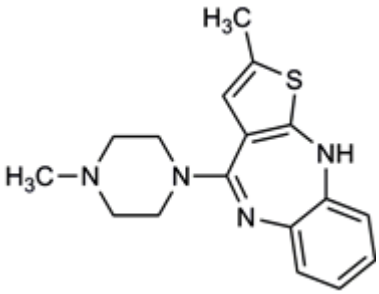

FIG. 1B: OLANZAPINE

FIGURE 1A & B: CHEMICAL STRUCTURES OF FLUOXETINE HYDROCHLORIDE AND OLANZAPINE

A survey of literature revealed that spectrophotometric [3, 4], spectrofluorimetric [5, 6], electrochemical [7], gas chromatography [8], liquid chromatography [9-11], capillary zone electrophoresis (CZE)[12] methods for determination of FH from pharmaceutical formulation and spectrophotometric [13-17], linear voltametric [16], HPLC [16-18], CZE [18] methods for determination of OZ from pharmaceutical dosage form whereas HPLC [19-22] and HPTLC [21, 22] methods for the simultaneous determination of FH and OZ from combined dosage forms were reported.

However, the methods reported for simultaneous determination of these two drugs are found to be comparatively expensive and time consuming. No spectrophotometric method has been reported for the simultaneous determination of FH and OZ from combined dosage form. In the present investigation an attempt has been made to develop a validated, rapid and cost-effective method for simultaneous determination of FH and OZ in tablet dosage forms. The developed method was validated as per the ICH guidelines [23]. The proposed method was successfully applied for simultaneous determination of FH and OZ in tablet formulations that are available in market.

MATERIALS AND METHODS:

Instrumentation: A double beam HEλIOS α UV/Visible spectrophotometer with two matched quartz cells of 1 cm path length was used for spectral measurements.

Chemicals and reagents: Fluoxetine hydrochloride (gift sample from Cadila Healthcare Pvt. Ltd., Ahmedabad, INDIA) and olanzapine (gift sample from Mangalam Organic Pvt. Ltd., Vapi, INDIA), hydrochloric acid (AR Grade, Finar Chemicals (India) Pvt. Ltd., Ahmedabad, India) and distilled water were used for the present study.

Preparation of Stock Solutions: FH powder (100 mg) was accurately weighed and transferred to a 100 mL volumetric flask. It was dissolved and diluted to 100 mL with 0.1 N hydrochloric acid solution to obtain a stock solution of FH with final concentration of 1 mg/mL.

OZ powder (100 mg) was accurately weighed and transferred to a 100 mL volumetric flask. It was dissolved and diluted to 100 mL with 0.1 N hydrochloric acid solutions to obtain a stock solution of OZ with final concentration of 1 mg/mL.

Preparation of Working Standard Solutions: Stock solution of FH (10 mL) was transferred to a 100 mL volumetric flask and diluted to 100 mL with 0.1 N hydrochloric acid solutions to obtain working standard solution of FH with final concentration of 100µg/mL.

Stock solution of OZ (10 mL) was transferred to a 100 mL volumetric flask and diluted to 100 mL with 0.1 N hydrochloric acid solutions to obtain working standard solution of OZ with final concentration of 100µg/mL.

Wavelength selection: Standard solutions of FH (16 µg/mL) and OZ (20 µg/mL) were scanned in the region of 200 nm to 400 nm against solvent blank using SCAN mode by UV/Visible spectrophotometer to get normal (zero order) spectrum. The normal spectrum was manipulated to first order spectrum using MANIPULATE option in the SCAN PAGE. The peaks, valleys and zero-crossing points were determined using the TRACK function. The zero-crossing points of OZ were identified where FH showed maximum amplitude values of peaks or valleys. The zero-crossing points of FH were identified where OZ showed maximum amplitude values of peaks or valleys.

Calibration curve of standard FH and OZ: Appropriate aliquots from the stock solutions of FH, OZ and mixture thereof were used to prepare three different sets of dilutions, Series A, B and C as follows.

Series A: Aliquots of the working standard solution of FH (100 µg/mL) were transferred to series of 25 mL volumetric flasks and diluted with 0.1 N hydrochloric

acid solution to get final concentration in range of 8-80 µg/mL.

Series B: Aliquots of the working standard solution of OZ (100 µg/mL) were transferred to series of 25 mL volumetric flasks and diluted with 0.1 N hydrochloric acid solution to get final concentration in range of 2-20 µg/mL.

Series C: Aliquots of 0.5, 1, 2, 3, 4 and 5 mL of the stock solution of mixture of FH (400 µg/mL) and OZ (100 µg/mL) were transferred to a series of 25 mL volumetric flasks. The volume was made up to the mark with 0.1 N hydrochloric acid solution to obtain the standard solutions of FH (8, 16, 32, 48, 64 and 80 µg/mL) and OZ (2, 4, 8, 12, 16 and 20 µg/mL).

The first derivative spectrum of each standard solution was obtained. The first derivative amplitudes were measured at the wavelength of 235.5 nm for series A, 296 nm for series B, 235.5 nm and 296 nm for series C. Calibration curves were constructed by plotting first derivative amplitude at selected wavelengths against corresponding concentration of FH and OZ. Regression equations for FH and OZ were calculated from corresponding calibration curves of FH and OZ.

Validation of the proposed method (Procedure I):

Linearity: The linearity for FH in the range of 8-80 µg/mL and for OZ in the range of 2-20 µg/mL was assessed in terms of slope, intercept, and correlation co-efficient values.

Accuracy: The accuracy was determined by standard addition method. To a fixed amount of pre-analyzed sample, increasing amount of standard drug solution was added in all the levels of calibration curve. The percent recoveries for the amount of FH and OZ were calculated at each level (n = 3)**.**

Precision: Repeatability of measurement of first order amplitude at 235.5 nm and 296 nm. The first order amplitude of the standard solution of mixture of FH (32µg/mL) and OZ (8µg/mL) was measured seven times and RSD was computed.

Interday and Intraday precision: The intraday precision (RSD) was determined by analyzing standard solution of mixture of FH and OZ over the entire calibration range for five times on the same day. The interday precision (RSD) was determined by analyzing standard solution of mixture of FH and OZ over the entire calibration range for five days.

Limit of Detection: Standard solutions of FH (2, 4 and 8 µg/mL) and OZ (0.5, 1 and 2 µg/mL) were analyzed using the developed method and minimum detectable limit was found.

Limit of Quantification: Standard solutions of FH (2, 4 and 8 µg/mL) and OZ (0.5, 1 and 2 µg/mL) were analyzed using the developed method and minimum quantifiable limit was determined for acceptable precision and accuracy.

Analysis of Sample: Twenty tablets were accurately weighed and finely powdered. Tablet powder equivalent to 5 mg of OZ was accurately weighed and transferred to 100 mL volumetric flask and 20 mL 0.1 N hydrochloric acid solution was added. The mixture was sonicated for 30 min, diluted to 100 mL with 0.1 N hydrochloric acid solution and filtered through whatman filter paper No. 41 (first 15 mL of filtrate were discarded. The first derivative amplitude of resulting solution was measured at 235.5 nm and 296 nm. The concentrations of FH and OZ were found by fitting values of absorbance in the corresponding regression equations for FH and OZ.

RESULTS AND DISCUSSION: The zero order spectra of FH and OZ showed wavelength maxima at 225 and 258 nm, respectively. Overlapping of both the spectra led to interference at respective wavelength maxima of the drugs. The first order derivatization of the normal spectra of FH and OZ was carried out to resolve the overlapping spectra (**Figure 2**).

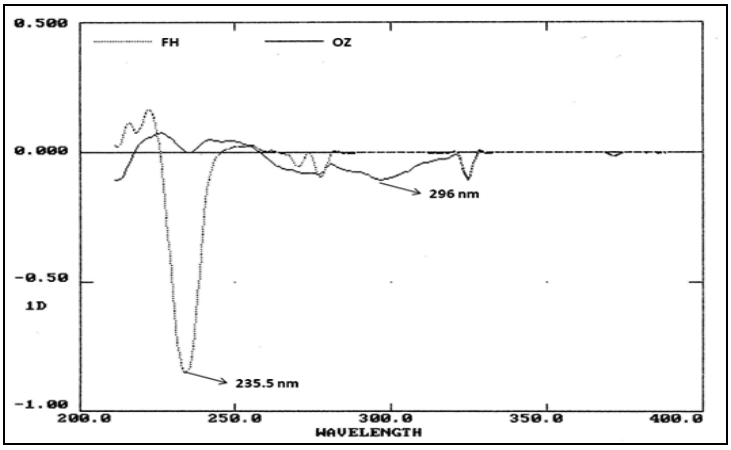

FIG. 2 FIRST ORDER DERIVATIVE OVERLAIN SPECTRA OF FH AND OZ

Wavelength selection: The peaks, valleys and zero-crossing points were observed for selection of wavelengths at which quantification of FH and OZ can be done. The zero-crossing point of OZ where FH showed maximum amplitude was found to be 235.5 nm and selected for analysis of FH. The zero-crossing point of FH where OZ showed maximum amplitude was found to be 296 nm and selected for analysis of OZ (Figure 2).

Calibration curve for FH and OZ: Calibration curve data of series A, B and C are shown in **Table 1 and 2**. Regression analysis of series A and C for FH shows no difference in the equations of straight line and thus indicates that there is no interference of OZ in determination of FH (**Table 3**). Regression analysis of series B and C for OZ shows no difference in the equations of straight line and thus indicates that there is no interference of FH in determination of OZ (Table 3).

TABLE 1: DETERMINATION OF FH ALONE AND FH IN PRESENCE OF OZ BY FIRST ORDER DERIVATIVE SPECTROPHOTOMETRY

Series A				Series C			
Composition of mixture (µg/mL)		First derivative amplitudes at 235.5 nm (Mean ± S.D.)	% C.V.	Composition of mixture (µg/mL)		First derivative amplitudes at 235.5 nm (Mean ± S.D.)	% C.V.
FH	OZ			FH	OZ		
8	0	0.1177±0.0040	2.26	8	2	0.1156±0.0041	3.55
16	0	0.2370±0.0071	3.32	16	4	0.2386±0.0067	2.82
32	0	0.4512±0.0147	2.58	32	8	0.4503±0.0149	3.31
48	0	0.6714±0.0199	1.95	48	12	0.6723±0.0192	2.85
64	0	0.8771±0.0162	1.99	64	16	0.8755±0.0157	1.79
80	0	1.0519±0.0393	2.11	80	20	1.0520±0.0389	3.69

(n=5)

TABLE 2: DETERMINATION OF OZ ALONE AND OZ IN PRESENCE OF FH BY FIRST ORDER DERIVATIVE SPECTROPHOTOMETRY

Series B				Series C			
Composition of mixture (µg/mL)		First derivative amplitudes at 296 nm (Mean ± S.D.)	% C.V.	Composition of mixture (µg/mL)		First derivative amplitudes at 296 nm (Mean ± S.D.)	% C.V.
FH	OZ			FH	OZ		
0	2	0.0156±0.0010	2.01	8	2	0.0160±0.0004	2.71
0	4	0.0321±0.0013	1.56	16	4	0.0334±0.0012	3.55
0	8	0.0687±0.0010	2.45	32	8	0.0661±0.0017	2.53
0	12	0.0946±0.0019	3.65	48	12	0.0956±0.0032	3.36
0	16	0.1291±0.0020	1.94	64	16	0.1260±0.0024	1.91
0	20	0.1598±0.0023	1.56	80	20	0.1616±0.0035	2.18
							2.85

(n=5)

TABLE 3: REGRESSION ANALYSIS DATA OF THE CALIBRATION CURVE OBTAINED USING SERIES A, B AND C

Series	Composition of the sample solution		Regression equation of the curve	Coefficient of correlation
	FH (µg/mL)	OZ (µg/mL)		
Series A	8-80	0	y=0.0131x±0.0274	0.9991
Series B	0	2-20	y=0.0080x±0.0009	0.9993
Series C	8-80	2-20	*y=0.0131x±0.0270	0.9991
			#y=0.0080x±0.0009	0.9997

y is first derivative amplitude and x is concentration in µg/mL.. *Regression equation for FH; #Regression equation for OZ

Method Validation: The proposed first order derivative spectrophotometric method was validated in terms of linearity, accuracy, precision, limit of detection and limit of quantification.

Linearity: The representative calibration curves for FH and OZ (Series C) were constructed by plotting absorbance at 235.5 nm and 296 nm against concentration range 8-80µg/mL and 2-20µg/mL, respectively (n = 5). They were found to be linear over above-mentioned range with correlation co-efficient of 0.9991 for FH and 0.9997 for OZ. The average linear regressed equations for the corresponding curves were y=0.0131x+0.0270 (FH) and y=0.0080x+0.0009 (OZ). The RSD for FH and OZ were found to be in the range of 1.79-3.69 % and 1.91-3.55 %, respectively.

Accuracy: The data of recovery studies for FH and OZ are shown in **Table 4 and 5**, respectively. The recovery of added sample was 98.32-100.09 % and 95.19-105.3 for FH and OZ, respectively.

TABLE 4: RECOVERY STUDIES FOR FH USING FIRST ORDER DERIVATIVE SPECTROPHOTOMETRY

Amount of sample (µg/mL)	Amount of sample added (µg/mL)	Average amount of FH found Mean ±S.D. (µg/mL)	% recovery Accuracy ± % C.V.
20	2	21.21±0.008	99.74±0.27
20	4	23.26±0.0053	98.32±1.63
20	6	25.26±0.0014	99.96±0.38
40	2	42.70±0.0044	100.09±0.75
40	4	44.24±0.0035	98.97±0.57
40	6	47.70±0.0058	98.90±0.91

(n = 5)

TABLE 5: RECOVERY STUDIES FOR OZ USING FIRST ORDER DERIVATIVE SPECTOPHOTOMETRY

Amount of sample (µg/mL)	Amount of sample added (µg/mL)	Average amount of FH found Mean ±S.D. (µg/mL)	% recovery Accuracy ± % C.V.
5	2	7.07±0.054	105.3±1.68
5	4	9.12±0.009	104.7±3.29
5	6	10.72±0.002	100.0±1.43
10	2	11.50±0.0018	95.19±1.91
10	4	13.68±0.0029	97.15±2.60
10	6	15.96±0.0028	99.25±2.21

(n = 5)

Precision: The RSD for measurement of first order amplitude for FH at 235.5 nm and OZ at 296 nm were found to be 1.13 and 0.71, respectively. Interday and intraday precision data for FH and OZ are given in **Table 6 and 7**, respectively. The interday precision (RSD, n=5) for FH and OZ were found to be in the range of 1.79-3.69 % and 1.91-3.55 %, respectively. The intraday precision (RSD, n=5) for FH and OZ were found to be 1.32-2.76 % and 0.65-2.94 %, respectively.

TABLE 6: INTERDAY AND INTRADAY PRECISION DATA FOR FH AT 235.5 nm

Concentration (µg/mL)	Interday precision		Intraday precision	
	Absorbance	RSD	Absorbance	RSD
8	0.1156±0.0041	3.56	0.1254±0.0032	2.56
16	0.2386±0.0067	2.82	0.2321±0.0064	2.76
32	0.4503±0.0149	3.31	0.4432±0.0066	1.47
48	0.6723±0.0192	2.85	0.6836±0.0156	2.35
64	0.8755±0.0157	1.79	0.8650±0.0115	1.32
80	1.0520±0.0389	3.69	1.0667±0.0242	2.27

(n = 5)

TABLE 7: INTERDAY AND INTRADAY PRECISION DATA FOR OZ AT 296 nm

Concentration (µg/mL)	Interday precision		Intraday precision	
	Absorbance	RSD	Absorbance	RSD
2	0.0160±0.0004	2.71	0.0159±0.0004	2.45
4	0.0334±0.0012	3.55	0.0322±0.0008	2.60
8	0.0661±0.0017	2.53	0.0627±0.0010	1.60
12	0.0956±0.0032	3.36	0.0970±0.0029	2.94
16	0.1260±0.0024	1.91	0.1251±0.0014	1.11
20	0.1616±0.0035	2.18	0.1593±0.0010	0.65

(n = 5)

Limit of detection: Limit of detection observed for FH and OZ were 2µg/mL and 0.5µg/mL, respectively.

Limit of quantification: Limit of detection observed for FH and OZ were 8µg/mL and 2µg/mL, respectively.

Analysis of Marketed Formulations: Quantitative determination of FH and OZ in tablets using proposed methods, simultaneous equation and absorbance ratio method was performed and the results were in good agreement with the labeled amount of FH and OZ in (**Table 8**).

TABLE 8: ASSAY OF FH AND OZ FROM COMBINED TABLET FORMULATIONS

Tablet Formulation	Labeled value (mg)		Content (%w/w)	
	FH	OZ	FH	OZ
OLEANZ®PLUS	20	5	102.13	107.5
OLEANZ®FORTE	20	10	104.75	98.5
OLAPADPLUS	20	5	101.52	95.5

CONCLUSION: The proposed three procedures were developed and validated for quantitative determination of fluoxetine hydrochloride and olanzapine in tablets. The developed methods were found to be simple, rapid, reproducible and economical. Results obtained for the analysis of marketed combined dosage forms were in good agreement with the labeled claim. The proposed methods can be utilized for the routine analysis of FH and OZ in tablet dosage form.

REFERENCES

1. Tohen M, Vieta E, Calabrese J, Ketter TA, Sachs G, Bowden C, Mitchell PB, Centorrino F, Risser R, Baker RW, Evans AR, Beymer K, Dube S, Tollefson GD, Breier A: Efficacy of Olanzapine and Olanzapine-Fluoxetine Combination in the Treatment of Bipolar I Depression. Arch. Gen. Psychiatry. 2003; 60, 1079.
2. Zhang W, Perry KW, Wong DT, Potts BD, Bao J, Tollefson GD and Bymaster FP: Synergistic Effects of Olanzapine and Other Antipsychotic Agents in Combination with Fluoxetine on Norepinephrine and Dopamine Release in Rat Prefrontal Cortex. Neuropsychopharmacology. 2000; 23, 250.
3. Naik MT, Rokad MD and Dhadke PM: Extractive spectrophotometric estimation of fluoxetine hydrochloride in pharmaceutical formulations. Indian J Pharm. Sci. 1999; 61, 156.
4. Mandrioli R, Pucci V, Visini D, Varani G and Raggi MA: Rapid methods for determination of fluoxetine in pharmaceutical formulations. J Pharm Biomed Anal. 2002; 29, 1127.
5. Prabhakar AH, Patel VB and Giridhar R: Spectrophotometric determination of fluoxetine hydrochloride in bulk and in pharmaceutical formulations. J Pharm Biomed Anal. 1999; 20, 427.
6. Berzas JJ, Alañón A and Lázaro JA: Cyclodextrin enhanced spectrofluorimetric determination of fluoxetine in pharmaceuticals and biological fluids. Talanta. 2002; 58, 301.
7. da Silva AR, Lima JC, Oliva Teles MT and Oliveira Brett AM: Electrochemical studies and square wave adsorptive stripping voltammetry of the antidepressant fluoxetine. Talanta. 1999; 49, 611.
8. Berzas Nevado JJ, Berzas Nevado MJ, Contento Salcedo AM and Aguas Nuevo EJ: Determination of Fluoxetine, Fluvoxamine, and Clomipramine in Pharmaceutical Formulations by Capillary Gas Chromatography. Chromatogr Sci. 2000; 38, 200.
9. El-dawy MA, Mokhtar MM and El-dawy FA: Liquid chromatographic determination of fluoxetine. j Pharm Biomed Anal. 2002; 30, 561.
10. United States Pharmacopoeia 30, NF 25, United States Pharmacopeial Convention, Rockville, MD. 2007, pp. 2165-2170.
11. British Pharmacopoeia. Stationery Office, London, 2007, Vol. III, pp. 2593-2594.
12. Berzas Nevado JJ, Contento Salcedo AM, Villaseñor Llerena MJ and Aguas Nuevo E: Method development and validation for the simultaneous determination of fluoxetine and fluvoxamine in pharmaceutical preparations by capillary electrophoresis. Anal Chim Acta. 2000; 417, 169.
13. Basavaiah K, Zenita O, Tharpa K, Nagaraju R, Anilkumar UR, Hiriyana SG and Vinay KB: Iodimetric assay of olanzapine in pharmaceuticals using iodate and Nile blue as reagents. Chem Ind Chem Eng Quarterly. 2009; 15, 95.
14. Krebs A, Starczewska B, Puzanowska-Tarasiewicz H and Sledz J: Spectrophotometric determination of Olanzapine by its Oxidation with N- Bromosuccinimide and Cerium(IV)sulfate. Anal Sci. 2006; 22, 829.
15. Jasińska A and Nalewajko E: Batch and flow-injection methods for the spectrophotometric determination of olanzapine. Anal Chim Acta. 2004; 2, 165.
16. Biryol I and Erk N: Voltammetric, spectrophotometric, and high performance liquid chromatographic analysis of olanzapine. Anal Lett. 2003; 36, 2497.
17. Raggi MA, Casamenti G, Mandrioli R, Izzo G and Kenndler E: Quantitation of olanzapine in tablets by HPLC, CZE, derivative spectrometry and linear voltammetry. j Pharm Biomed Anal. 2000; 23, 973.
18. Prameela RA and Bala SC: Development of HPLC method for the determination of Olanzapine in bulk and dosage forms. Int J Pharm Tech Res. 2009; 1, 654.
19. Reddy BV, Reddy S, Sreeramulu J and Kanumula GV: Simultaneous Determination of Olanzapine and Fluoxetine by HPLC. Chromatographia. 2007; 66, 111.
20. Phatak A and Rajput SJ: Development of a stability-indicating HPLC method for simultaneous determination of olanzapine and fluoxetine in combined dosage forms. J Chromatogr Sci. 2009; 47, 605.
21. Shah CR, Shah NJ, Shuagia BN and Patel N: Simultaneous assay of olanzapine and fluoxetine in tablets by column high-performance liquid chromatography and high-performance thin-layer chromatography. J AOAC Int. 2007; 90, 1573.
22. Patel S and Patel NJ. Simultaneous RP-HPLC and HPTLC estimation of fluoxetine hydrochloride and olanzapine in tablet dosage forms. Indian J Pharm Sci. 2009; 71, 477.
23. International Conference on Harmonisation for technical requirements for registration of pharmaceuticals for human use. ICH guidelines - Q2(R1) Validation of Analytical Procedures: Text and Methodology. Available at: http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html

****