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Analytical method development and validation for simultaneous estimation of olanzapine and fluoxetine in pure and pharmaceutical dosage form by using RP-HPLC

Rohini. N[1]*, P. Sowjanya[1], B. Sravanthi[1], Dr. G. Vijay Kumar[1]

[1]*KGR Institute of Technology and Management, Rampally Village, Keesara Mandal, M M District, Telangana, Pin Code: 501301.*
[1]*Assistant Professor, Department of Pharmaceutical Analysis & KGR Institute of Technology and Management, Rampally Village, Keesara Mandal, M M District, Telangana, Pin Code: 501301.*
[1]*Associate Professor, KGR Institute of Technology and Management, Rampally Village, Keesara Mandal, M M District, Telangana, Pin Code: 501301.*
[1]*HOD of Department of Pharmacy, KGR Institute of Technology and Management, Rampally Village, Keesara Mandal, M M District, Telangana, Pin Code: 501301.*

***Corresponding Author: P. Sowjanya**
Email: sravanthi.bodike@gmail.com

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Olanzapine and Fluoxetine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Water (90:10) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 240 nm. The retention time of the Olanzapine and Fluoxetine was 2.256, 5.427 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Olanzapine and 25-125µg/ml of Fluoxetine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of pure and pharmaceutical formulations.

Keywords: Olanzapine, Fluoxetine, RP-HPLC, Validation.

INTRODUCTION

Olanzapine [26-28] is a synthetic derivative of thienobenzodiazepine with antipsychotic, antinausea, and antiemetic activities. As a selective monoaminergic antagonist, Olanzapine binds with high affinity binding to the following receptors: serotoninergic, dopaminergic, muscarinic M1-5, histamine H1, and alpha-1-adrenergic receptors; it binds weakly to gamma-aminobutyric acid type A, benzodiazepine, and beta-adrenergic receptors. The

antinausea and antiemetic effects of this agent appear to be due to the blockade of 5-HT2 and 5-HT3 receptors for serotonin. Although its exact mechanism of action in schizophrenia is unknown, it has been proposed that Olanzapine's antipsychotic activity is mediated through antagonism to dopamine D2 receptors with rapid Ligand-receptor dissociation kinetics that help to minimize extra pyramidal symptoms (EPS). Olanzapine may also stimulate appetite. The IUPAC Name of Olanzapine is 5-methyl-8-(4-methylpiperazin-1-yl)-4-thia-2,9-diazatricyclo[8.4.0.0^{3,7}]tetradeca-1(14),3(7),5,8,10,12-hexaene and the Chemical Formula is $C_{17}H_{20}N_4S$. The Chemical Structure of Olanzapine is shown in figure No-1.

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) widely used as an antidepressant. Fluoxetine therapy can be associated with transient asymptomatic elevations in serum aminotransferase levels and has been linked to rare instances of clinically apparent acute liver injury. Fluoxetine is a 2nd generation antidepressant categorized as a selective serotonin reuptake inhibitor (SSRI). It gained FDA approval in 1987 and although it was initially intended for the treatment of depression, today it is commonly prescribed to manage depression in addition to various other pathologies. The IUPAC Name of Fluoxetine [29-31] is methyl ({3-phenyl-3-[4-(trifluoromethyl) phenoxy] propyl}) amine and the Chemical Formula is $C_{17}H_{18}F_3NO$. The Chemical Structure of Fluoxetine is shown in figure No-2.

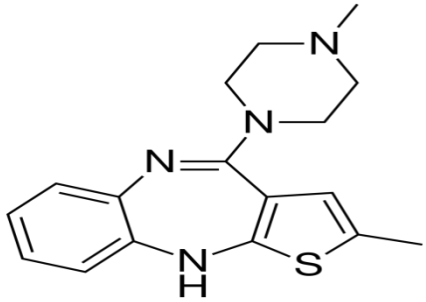

Fig-1: Chemical Structure of Olanzapine

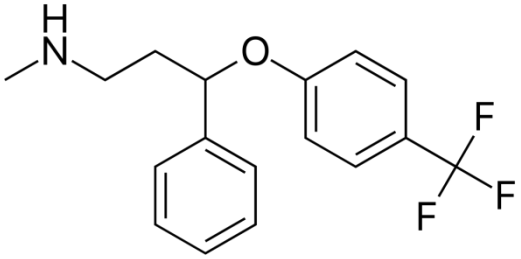

Fig-2: Chemical Structure of Fluoxetine

Literature Survey [16-25] reveals that few methods have been reported for the Simultaneous estimation of Olanzapine and Fluoxetine individually or in combination with other drugs in pharmaceutical dosage forms. And some method has been developed for estimation of Olanzapine and Fluoxetine in pure form and pharmaceutical dosage form. The present manuscript describes a sensitive, simple, precise and accurate isocratic RP-HPLC method for Simultaneous estimation of Olanzapine and Fluoxetine pure form and in pharmaceutical dosage form with subsequent validation as per ICH guidelines.

MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

The present study was carried out on a Waters HPLC system [Model: 2695] equipped with Photo Diode array detector, auto sampler integrated with empower 2.0 software and a column Phenomenex Gemini C18 (4.6mm×150mm, 5µm) using Methanol: Water in the ratio of 90:10%v/v as the mobile phase. The mobile phase is pump into the column at flow rate of 1ml/min and column oven

temperature is maintained at 40°C. The drugs were detected at a wavelength 240nm.The other instruments are used were PH meter, Ultra Sonicator.

Chemicals and Reagents

Olanzapine and Fluoxetine standard drugs were supplied as gift samples by Sura Pharma Labs, Hyderabad. The chemicals and reagents are used for development of the method were of AR grade and purchased from Merk. The solvents used were of HPLC grade and purchased from Merk.

PREPARATION OF STANDARD SOLUTIONS

Preparation of Working Standard Solution

Accurately weigh and transfer 10 mg of Olanzapine and Fluoxetine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15ml of the Olanzapine and 0.75ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Olanzapine and Fluoxetine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.15ml of the Olanzapine and 0.75ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

- Instrument Used: Waters HPLC with auto sampler and PDA 996 detector model.
- Temperature : 40ºC
- Column : Phenomenex Gemini C18 (4.6×150mm, 5µ)
- Mobile phase : Methanol: Water (90:10v/v)
- Flow rate : 1.0mL/min
- Wavelength : 240 nm
- Injection volume : 10 µl
- Run time : 10 minutes

METHOD VALIDATION PARAMETERS

System Suitability

Accurately weigh and transfer 10 mg of Olanzapine and 10mg of Fluoxetine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Olanzapine and 0.75ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Olanzapine and 10mg of Fluoxetine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Olanzapine and 0.75ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Olanzapine and Fluoxetine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.15ml of the Olanzapine and 0.75ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%\ ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY

Accurately weigh and transfer 10 mg of Olanzapine and 10mg of Fluoxetine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (5 µg/mL of Olanzapine & 25 µg/mL of Fluoxetine)

Pipette out 0.05ml of Olanzapine and 0.25ml of Fluoxetine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10 µg/mL of Olanzapine& 50 µg/mL of Fluoxetine)

Pipette out 0.1ml of Olanzapine and 0.5ml of Fluoxetine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15 µg/mL of Olanzapine& 75 µg/mL of Fluoxetine)

Pipette out 0.15 ml of Olanzapine and 0.75ml of Fluoxetine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20 µg/mL of Olanzapine& 100 µg/mL of Fluoxetine)

Pipette out 0.2 ml of Olanzapine and 1.0ml of Fluoxetine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25 µg/mL of Olanzapine& 125 µg/mL of Fluoxetine)

Pipette out 0.25ml of Olanzapine and 1.25ml of Fluoxetine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION REPEATABILITY

Preparation of Olanzapine and Fluoxetine Product Solution for Precision

Accurately weigh and transfer 10 mg of Olanzapine and 10mg of Fluoxetine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Olanzapine and 0.75ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

DAY 1

The standard solution was injected for six times and measured the area for all six injections in

HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

DAY 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ACCURACY

For preparation of 50% Standard Stock Solution

Accurately weigh and transfer 10 mg of Olanzapine and 10mg of Fluoxetine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.075ml of the Olanzapine and 0.37ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard Stock Solution

Accurately weigh and transfer 10 mg of Olanzapine and 10mg of Fluoxetine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Olanzapine and 0.75ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard Stock Solution

Accurately weigh and transfer 10 mg of Olanzapine and 10mg of Fluoxetine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.225ml of the Olanzapine and 1.12ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Olanzapine and Fluoxetine and calculate the individual recovery and mean recovery values.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard Solution

Accurately weigh and transfer 10 mg of Olanzapine and 10mg of Fluoxetine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Olanzapine and 0.75ml of the Fluoxetine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of Flow Conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Methanol: water was taken in the ratio and 85:15, 95:5 instead (90:10), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

HPLC Method Development

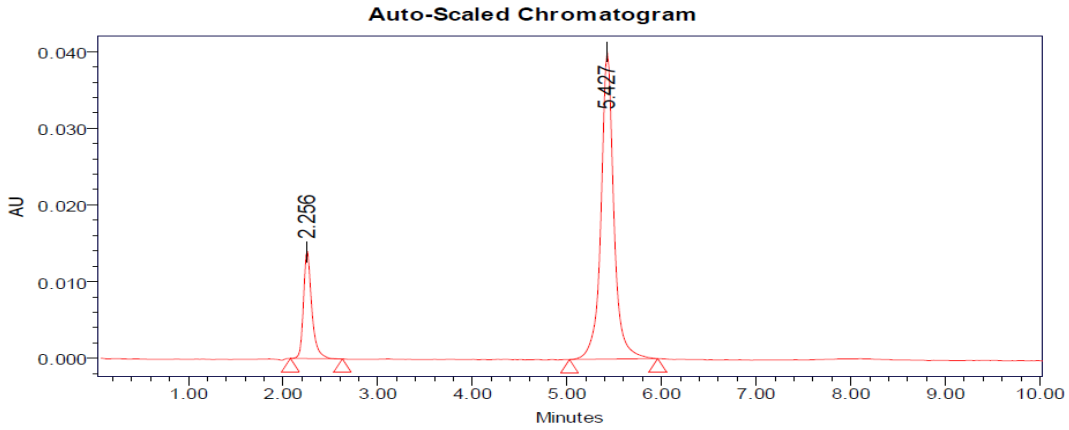

Fig-3: Optimized Chromatographic Condition

Table-1: Peak Results for Optimized Chromatogram

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Olanzapine	2.256	84994	13905		1.32	5535
2	Fluoxetine	5.427	377906	39948	16.27	1.03	9101

METHOD VALIDATION

System Suitability

To know reproducibility of the method, the system suitability test was done to establish the parameter such as retention time, tailing factor, theoretical plate, and peak area. This was performed by injecting the standard mixture. The System suitability parameters were found to be within the limits.

Linearity

The linearity of an analyte procedure is its ability (within a given range) to obtained test results which are directly proportional to the concentration of analyte in the sample. Linearity was evaluated by analyzing the plot of area as a function of concentration of analyte. The result was evaluated by calculating of regression coefficient (r2).

The standard calibration curve was obtained in the concentration range of 5–25µg/ml for Olanzapine with a correlation coefficient (r2) of 0.999. The linear regression equation was obtained y = 8893x + 3394 for Olanzapine. The standard calibration curve was obtained in the concentration range of 25–125µg/ml for Fluoxetine with a correlation coefficient (r2) of 0.999. The linear regression equation was obtained y = 8289x + 12813 for Fluoxetine. The results obtained for linearity are summarized in Table 2 and 3 and figure-4 and 5.

Table-2: Calibration Data of Olanzapine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	51080
66.6	10	92208
100	15	139140
133.3	20	180998
166.6	25	223920

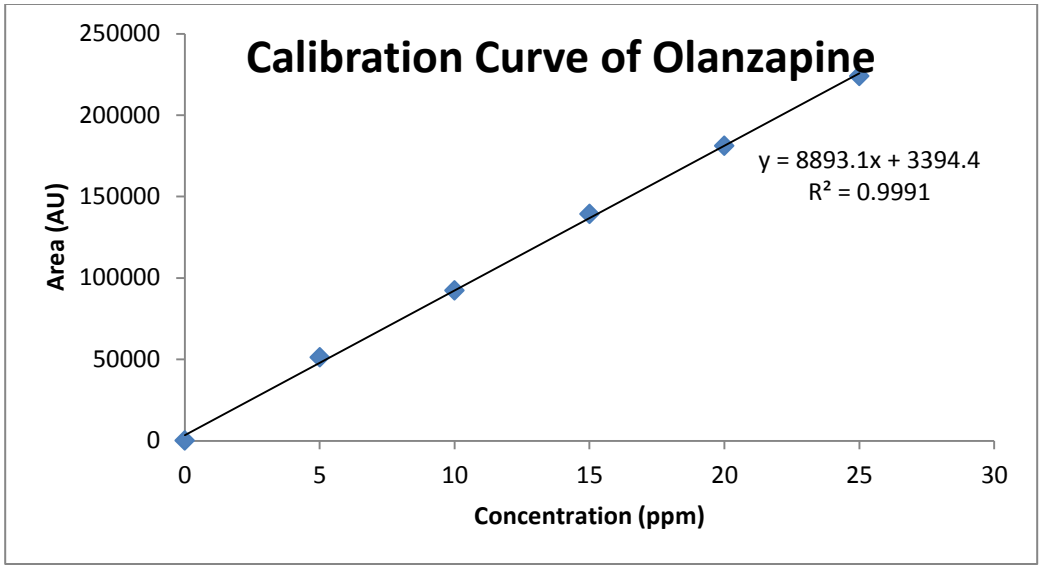

Fig-4: Calibration Curve of Olanzapine

Table-3: Calibration Data of Fluoxetine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	25	224573
66	50	441895
100	75	635379
133	100	842226
166	125	1041381

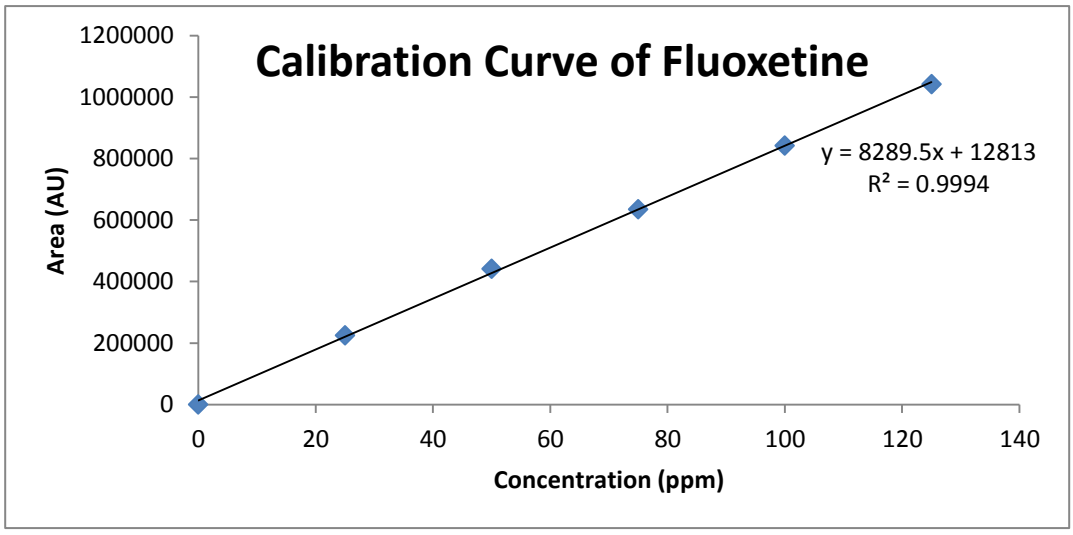

Fig-5: Calibration Curve of Fluoxetine

Precision

The precision of an analytical method is the closeness of replicating result obtained from analysis of the same homogeneous sample. Precision was considered at different levels, i.e., method precision and intermediate precision.

Method Precision

System precision was carried out with 5 replicates (n=5) of standard at working

concentration of 15µg/ml and 75µg/ml of Olanzapine and Fluoxetine respectively. The repeatability of sample applications and measurement of peak area were expressed in term of % relative standard deviation (%RSD).

The repeatability of sample applications and measurement of peak area were expressed in term of %RSD since their %RSD is <2.0%, and hence, the developed method was found to be precise. Data obtained from precision experiments for repeatability studies are shown as below Table 4 and 5.

Table-4: Results of Method Precision for Olanzapine:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Olanzapine	2.269	85148	13802	3405.7	1.4
2	Olanzapine	2.255	85369	13826	3338.4	1.4
3	Olanzapine	2.252	85451	13797	3474.5	1.4
4	Olanzapine	2.267	85812	13858	3422.2	1.4
5	Olanzapine	2.260	87007	14018	3326.6	1.3
Mean		2.264	87210	13986	3416.4	1.4
Std. Dev			85999.6			
% RSD			887.5 1.0			

Table-5: Results of Method Precision for Fluoxetine:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Fluoxetine	5.274	370076	40629	9075.5	1.1	15.4
2	Fluoxetine	5.266	370126	40937	9120.4	1.1	15.6
3	Fluoxetine	5.265	372484	41279	9212.4	1.1	15.3
4	Fluoxetine	5.278	376524	41454	8883.0	1.1	15.3
5	Fluoxetine	5.305	381812	41320	9041.5	1.1	15.3
Mean		5.319	382550	41133	8974.1	1.1	15.3
Std. Dev			375595.4				
% RSD			5620.2 1.5				

Intermediate Precision or Ruggedness

The ruggedness of the method was verified by analyzing six samples of the same batch used for method precision as per proposed method by different analyst.

The repeatability of sample applications and measurement of peak area were expressed in term of %RSD since their %RSD is <2.0 %, and hence, the developed method was found to be precise. Data obtained from intermediate are summarized in Table 6 and 7.

Table-6: Results of Intermediate Precision Analyst 1 for Olanzapine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Olanzapine	2.248	84029	13603	3519.3	1.4
2	Olanzapine	2.245	84202	13520	3372.9	1.4
3	Olanzapine	2.242	84745	13636	3411.8	1.4
4	Olanzapine	2.239	85442	13775	3323.5	1.3
5	Olanzapine	2.243	85535	13768	3433.4	1.4
6	Olanzapine	2.246	85699	13739	3336.9	1.3

Mean	84942
Std. Dev	720.3716
% RSD	0.8

Table-7: Results of Intermediate Precision Analyst 2 for Fluoxetine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Fluoxetine	5.284	366831	40102	9180.2	1.1	15.8
2	Fluoxetine	5.293	368856	40464	9155.6	1.1	15.5
3	Fluoxetine	5.306	370174	39977	9039.6	1.0	15.5
4	Fluoxetine	5.319	370603	40748	9119.3	1.1	15.8
5	Fluoxetine	5.346	372578	39772	9183.9	1.1	15.6
6	Fluoxetine	5.352	376550	40083	9009.1	1.1	15.9
Mean			370932				
Std. Dev			3349.09				
% RSD			0.9				

Accuracy

The accuracy of an analytical method is the closeness of the results obtained by that method to the true value of the sample. It is expressed as recovery (%), which is determined by the API method. The accuracy was evaluated by the recovery of Olanzapine and Fluoxetine at three different levels (50%, 100%, and 150%).

The % recovery was found to be 98.8 and 99.7 for Olanzapine and Fluoxetine respectively. %RSD was found to be <2, and hence, the method is said to be accurate. The results of accuracy studies are shown in Table 8 and 9.

Table-8: The Accuracy Results for Olanzapine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	69862.33	7.5	7.47	99.6	98.8%
100%	135467.7	15	14.8	98.6	
150%	199976	22.5	22.1	98.2	

Table-9: The Accuracy Results for Fluoxetine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	322954	37.5	37.3	99.7	99.7%
100%	632155	75	74.8	99.6	
150%	945870.3	112.5	112.5	100	

LOD AND LOQ

The LOD of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.

The LOQ of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The sensitivity of measurement of Olanzapine and Fluoxetine by use of the proposed method estimated in term of the LOQ and LOD. The results of LOD and LOQ are summarized in Table 10.

Table-10: LOD and LOQ Values

Drug Name	LOD	LOQ
Olanzapine	0.54	1.6
Fluoxetine	1.9	5.9

Robustness

The robustness of the method we determined by assessing the ability of the developed method to remain unaffected by the small changes in the parameters such as Flow rate (±1 nm), oven temperature (±1°C), detection wavelength (±0.2 ml/min).

The % assay was within the acceptance criteria in the condition described in this report, and hence, the method is robust. The results are summarized in Table 11 and 12.

Table-11: Results for Robustness for Olanzapine

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	84994	2.256	5535	1.32
Less Flow rate of 0.9 mL/min	89987	2.505	5891	1.27
More Flow rate of 1.1 mL/min	80653	2.046	5085	1.20
Less organic phase	89987	2.505	5098	1.20
More organic phase	80654	2.046	5123	1.27

Table-12: Results for Robustness for Fluoxetine

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	377906	5.427	9101	1.01
Less Flow rate of 0.9 mL/min	397680	5.599	9407	1.03
More Flow rate of 1.1 mL/min	327899	4.576	9584	0.98
Less organic phase	396750	5.599	9407	1.02
More organic phase	339025	4.576	9584	0.99

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Olanzapine and Fluoxetine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Olanzapine and Fluoxetine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: water (90:10) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This method can be used for the routine determination of Olanzapine and Fluoxetine in bulk drug and in Pharmaceutical dosage forms.

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REFERENCES

[1]. Shetti, P.D. High Performance Liquid Chromatography. 2001, 116.
[2]. The Merck Index, an Encyclopedia of chemicals, drugs and biological. Fourteenth Edn. USA; 2006.
[3]. Beckett, A.H.; Stenlake, J.B. Practical Pharmaceutical Chemistry, 4th Edn. C.B.S. Publications, 1.
[4]. Malik, V. Drugs and Cosmetics Act 1940, 16th Edn. Eastern Book Company, Lucknow, 5.
[5]. Willard, H.H.; Merit, L.L.; Dean, F.A.; Settle, F.A. Instrumental methods of analysis, 7th Edn. C.B.S. Publishers, New Delhi, 2002.
[6]. Mendham, J.; Denney, R.C.; Barnes, V; Thomas, M.J.K. Vogel's Text book of Qualitative Chemical Analysis, 6, 261-287.
[7]. Skoog, D.A.; West, D.M. Fundamentals of Analytical Chemistry, 7·
[8]. Sharma, B.K., Instrumental methods of Chemical analysis, 19, 2000.
[9]. Willard, H.H.; Merrit, L.L.; Dean. J.A., Instrumental methods of analysis, 7th Edn. CBS Publishers, New Delhi.
[10]. Khopkar, S.M. Basic concepts of analytical chemistry, 2nd edition, 2005.
[11]. Tips on Liquid Chromatography, Waters www.waters.com.
[12]. Validation of Analytical Procedures: Methodology, ICH Tripartite Guidelines 1996.
[13]. Validation of Analytical Procedures, ICH Harmonized Tripartite Guidelines 1994.
[14]. ICH, Q2A Text on validation of analytical procedures, Oct, 1994www.ich.org.
[15]. ICH, Q3B Validation of analytical procedures: methodology, Nov, 1996.
[16]. Mahmoud A. Tantawy, Nagiba Y. Hassan, Nariman A. Elragehy, and Mohamed Abdelkawy. Simultaneous determination of Olanzapine and Fluoxetine hydrochloride in capsules by Spectrophotometry, TLC-spectrodensitometry and HPLC. J Adv Res. 2013 Mar; 4(2): 173–180.
[17]. Sejal Patel and N. J. Patel. Simultaneous RP-HPLC and HPTLC estimation of Fluoxetine hydrochloride and Olanzapine in tablet dosage forms. 15 August 2009.
[18]. V.Pranitha, R.Saraswathi, Uma Maheshwar Rao V, Ajitha A. RP-HPLC method development and validation for Simultaneous estimation of Olanzapine and Fluoxetine in tablet dosage form. / Vol 4 / Issue 4 / 2014 /281-284.
[19]. A. Pathak and S.J. Rajput. Development of a Stability-Indicating HPLC Method for Simultaneous Determination of Olanzapine and Fluoxetine in Combined Dosage Forms. Journal of Chromatographic Science, Vol. 47, August 2009.
[20]. V. Navya Sree, G. Chiranjeevi, Rajashekhar Prahalad, Srinivasa Reddy Edara. Method development and validation for the simultaneous Estimation of Fluoxetine hydrochloride and Olanzapine in a Pharmaceutical formulation by RP-HPLC method. 2(4), 2013, 514 - 529.
[21]. Usha Rani U, Geethavani M, Vinod Kumar K, Sowjanya K, Bramarambha M. A New Validated UV Spectrophotometric Method for the Simultaneous Estimation of Fluoxetine Hcl and Olanzapine in Combined Tablet Dosage Form by Dual Wavelength Method. January - March 2016.
[22]. Vijaykumar K. Parmar, Jatin N. Patel, Girish K. Jani, Laxman M. Prajapati and Jagbir Gagoria. First derivative Spectrophotometric determination of Fluoxetine hydrochloride and Olanzapine in tablets. IJPSR, 2011; Vol. 2(11): 2996-3001.
[23]. B. Venkateswara Reddy, K. V. N. Suresh Reddy, J. Sreeramulu, G. V. Kanumula. Simultaneous Determination of Olanzapine and Fluoxetine by HPLC. 2007 > 66 > 1-2 > 111-114.
[24]. Mahmoud A. Tantawy; Nagiba Y. Hassan; Nariman A. Elragehy; Mohamed Abdelkawy. Simultaneous determination of Olanzapine and Fluoxetine hydrochloride in capsules by Spectrophotometry, TLC-spectrodensitometry and HPLC. Journal of Advanced Research, 4(2), 2013, 173-180(8).
[25]. Katayoun Mahdavi Ara, Farhad Raofie and Shahram Seidib. Simultaneous extraction and determination of trace amounts of Olanzapine and Fluoxetine from biological fluids: comparison of conventional hollow fiber supported liquid phase microextraction and pulsed electrically assisted liquid-phase microextraction techniques. Issue 18, 2015.
[26]. https://www.drugbank.ca/drugs/DB00334
[27]. https://en.wikipedia.org/wiki/Olanzapine

[28]. https://www.webmd.com/drugs/2/drug-1644-9274/olanzapine-oral/olanzapine-oral/details/list-contraindications
[29]. https://www.drugbank.ca/drugs/DB00472
[30]. https://en.wikipedia.org/wiki/Fluoxetine
[31]. https://www.webmd.com/drugs/2/drug-6997/prozac-oral/details/list-contraindications.