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A NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF FLUOXETINE AND OLANZAPINE

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Abstract: A novel RP-HPLC method is developed and validated for the simultaneous determination of Olanzapine and Fluoxetine HCl in pure and formulations. The chromatographic separation is achieved by using an Inertsil C_{18} ODS Column (5µ, 250mm x 4.5mm ID) at ambient temperature; Methanol:Acetonitrile in the ratio 90:10v/v, 1.0 ml/min; and a PDA detector at 233nm. Olanzapine and Fluoxetine are eluted at 3.538 min and 2.955 min respectively. The system suitability parameters and resolution are found to be within the acceptable limits. The method is linear 20-80 µg/ml for both Olanzapine and Fluoxetine. The LOD and LOQ of Olanzapine and Fluoxetine are found to be 0.41&1.25 and 0.16&0.48 µg/ml respectively. The % assay estimated for Olanzapine and Fluoxetine in tablet dosage forms is within the range 98 to 102%. Olanzapine and Fluoxetine are stable under a variety of degradation conditions. This method can be used for quality control in any pharmaceutical industry.

Index Terms **- RP-HPLC, Isocratic, Olanzapine, Fluoxetine HCl, Validation, Linearity, LOD, LOQ and Degradation**

I. INTRODUCTION

1.1 Profile of the Drugs

Fluoxetine HCl is an anti-agent, and is a selective serotonin-reuptake inhibitors (SSRIs) which are potent inhibitors of neuronal serotonin reuptake. It is present in the racemic mixture of R&S enantiomers having equivalent pharmacologic activity and similar pharmacological activity. During acute use, SSRIs block serotonin reuptake and increase serotonin stimulation of somatodendritic 5-HT1A and terminal autoreceptors. Fluoxetine was discovered by Eli Lilly and company in 1970 by using 3-Phenoxy-3-phenylpropylamine as a starting material and Molloy synthesized a series of dozens of its derivatives[1]. The first article about fluoxetine was published in 1974[2]. The drug is approved by the Food and Drug Administration (FDA)[3]. The molecular formula and molecular weight of Fluoxetine hydrochloride are $C_{17}H_{18}F_3NO$ and 309.3261 g/mol respectively. It is a white crystalline powder soluble in water, methanol

and acetonitrile, dimethly formamide and tetrahydrofuran. IUPAC's name of the compound is methyl ({3-phenyl-3-[4-(trifluoromethyl) phenoxy] propyl}) amine. The structure of Fluoxetine is presented in Fig.-1.

Olanzapine is an anti-psychotic agent and its activity is likely due to a combination of antagonism at D2 receptors in the mesolimbic pathway and 5HT2A receptors in the frontal cortex. Olanzapine was patented in 1991 and approved for medical use in the United States in 1996[4-5]. In 2020, it was the 164th most commonly prescribed medication in the United States, with more than 3 million prescriptions[6-7]. Lilly also markets olanzapine in a fixed-dose combination with fluoxetine as olanzapine/fluoxetine (Symbyax)[8]. It is chemically known as 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 having molecular formula $C_{17}H_{20}N_4S$ and weight 312.432 g/mol respectively. It is a yellow crystalline powder soluble in water, methanol, acetonitrile and chloroform. The chemical structure of the Olanzapine is given in Fig.-2.

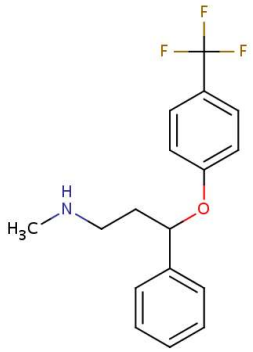

Fig.-1: Structure of Fluoxetine

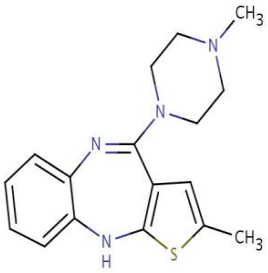

Fig.-2: Structure of Olanzapine

1.2 Literature Survey

The study of literature indicates that, a few spectrophotometric methods are reported for the determination of fluoxetine[9-12] and Olanzapine[13-15]in single dosage forms. A few RP-HPLC methods[16-18] are found to determine olanzapine in plasma or serum and dosage forms[19-20.] Several HPLC and HPTLC methods[21-23] are reported for simultaneous estimation of fluoxetine HCl in plasma and tablet dosage forms[24] respectively. Fluoxetine HCl and olanzapine are determined simultaneously by a few HPLC methods[25-30] in plasma and tablet dosage forms respectively. As spectrophotometric methods are less sensitive, and the reported HPLC methods are not economic, hence there is a scope to develop a fast, simple and economic RP-HPLC method for the consecutive determination of Fluoxetine HCl and Olanzapine in pure and formulations.

1.3 Aim and Objectives of the Investigation

The aim of the present investigation is determine the percent degradation of the drug components Fluoxetine and Olanzapine consecutively in acidic, basic, peroxide, thermal and light conditions and the assay of formulations by developing a new HPLC method. The following are main objectives of the present work. To develop a simple, accurate, fast and cost effective alternative HPLC method, to validate the developed method in terms of precision, accuracy, sensitivity, linearity, LOD, LOQ, ruggedness and robustness, to apply the developed method to determine the assay of Fluoxetine HCl and Olanzapine. And to study the stability or percent degradation of drug molecules under different forced degradation conditions

II. RESEARCH METHODOLOGY

2.1 Materials and Methods

HPLC grade methanol (Merck), acetonitrile (Merck), hydrogen peroxide (Merck), hydrochloric acid (Merck), sodium hydroxide (Merck) and purified water of HPLC grade are used in the present work. Active pharmaceutical ingredients such as Fluoxetine HCl and Olanzapine are procured from Active Pharmaceutical Labs, Hyderabad, Telangana.

2.2 Instrumentation

HPLC – WATERS Model NO.2690/5 series compact cystem consisting of an Inertsil- C_{18} ODS column. 2695 waters pump, Digital Electronic balance (Sartorius) and **a** sonicator (Fast Clean) are used in the investigation. Empower 2.0 software is used for data acquisition

2.3 Method development

The selection of stationary phase and mobile phase in any chromatographic method depends upon the nature of the drug molecules to be separated and quantified. The drugs present in the formulation are polar and soluble in the polar solvent methanol. As the components are polar, a reversed phase HPLC mode of separation is chosen, in which bonded phase non polar columns and polar mobile phases are chosen in trials. The absorption spectra of 40µg/ml working standard solution of Olanzapine and Fluoxtine are recorded by scanning in the UV region 200 to 400 nm and it is observed that wavelength 233 nm is an isosbestic point, therefore, wavelength 233 nm is selected as the detection wavelength of Olanzapine and Fluoxtine for RP-HPLC method development. The proposed method is optimised by changing one of the chromatographic parameters at a time while keeping the others constant, and chromatograms are obtained under a set of chromatographic conditions. The system suitable parameters of Fluoxtine and Olanzapine are evaluated by the software. Different trials are performed until a set of valid system suitable parameters is obtained.

Standard Solution: The stock solution (1mg/ml) of Fluoxetine HCl and Olanzapine are prepared by accurately transferring 10mg of Fluoxetine HCl and 10 mg of Olanzapine into two separate 10 ml volumetric flasks, dissolved in the minimum amount of degassed methanol (mobile phase), and sonicated for 20 min. The working standard solution is prepared by transferring 1 ml of each stock solution into 10ml standard flask and made up to the mark with mobile phase.

Sample solution: Oleanz Plus Tablet, Fluoxetine (20mg) and Olanzapine (5mg) manufactured by Sun Pharmaceutical Industries Ltd. Mumbai are purchased from the local market. Twenty tablets are weighed accurately, and average weight is calculated, then ground into a homogeneous powder. An amount of the fine powder equivalent to the average weight is accurately weighed, transferred into a 10 ml standard flask, dissolved in the mobile phase, sonicated and filtered through a 0.45 µm Nylon syringe filter. Further, 1.0 mL of the filtrate is diluted into 100.0 mL to give a test solution of concentration of 20 µg/mL Fluoxetine HCl and 5µg/mL Olanzapine.

Chromatographic separation-Trial-1: Chromatographic separation is carried out by injecting about 20µl of working standard solution into the Inertsil- C18 ODS column at ambient temperature, mobile phase is allowed to flow through the column for a run time of 8 minutes at a flow rate of 1.0ml/min. The components are monitored at 233 nm using a PDA detector. The two peaks are very close to each other at retention time 2.7 and 3.33 of min., with resolution less than 2.0. The method is rejected and preceded for second trial.

Trail-2: The polarity of the mobile phase is increased by preparing a 90% methanol solution in water (Methanol: Water, 90:10v/v ratio). Chromatographic parameters are determined by injecting 20µl of working standard solution into the Inertsil- C18 ODS column at ambient temperature, elution is carried out by passing the mobile phase at a flow rate of 1.0ml/min for a run time of 6 minutes, and the components are monitored at 233 nm using a PDA detector. The time of elution of Fluoxtine and Olanzapine are found to be 2.8 and 3.2 min., respectively. The two peaks were separated, but the resolution is less than 2.0, hence, the method again is rejected, and we proceeded with the third trial by changing parameters.

Trail-3: The polarity of the mobile phase is further increased by increasing the ratio of water (Methanol: Water, 50:50v/v ratio). The mobile phase is prepared by mixing equal volumes of methanol and water and is used in trial-3. Chromatographic separation is achieved by injecting 20µl of the same working standard solution into the Inertsil- C18 ODS column at ambient temperature, mobile phase allowed to flow through the column for a run time of 7 minutes at a flow rate of 1.0ml/min. The components are monitored at 233 nm using a PDA detector. Retention times of Fluoxtine and Olanzapine are found to be 2.2 and 3.6 min., respectively. The components separated but shape of peaks is not good, therefore, the method is rejected, and preceded for new trial.

Chromatographic separation (Optimized Method) : The components and composition of the mobile phase are changed in trail-4. 90:10 v/v ratio of methanol and acetonitrile is used as the mobile phase in the optimized method.

Stock standard solution: The stock solution (1mg/ml) of Fluoxetine HCl and Olanzapine is prepared by accurately transferring 10mg of Fluoxetine HCl and 10 mg of Olanzapine into two separate 10 ml volumetric flasks, dissolved in the minimum amount of degassed methanol (mobile phase), and sonicated for 20 min. Further, an intermediate stock solution is prepared by diluting 2ml of each stock solution to 10ml with mobile phase in two separate 10ml standard flasks, and the concentration of each solution is 200µg/ml.. The working standard solution of 40 µg/ml Fluoxetine HCl and Olanzapine is prepared by transferring equivalent volumes of intermediate stock solution (2 ml of Fluoxetine HCl and 2ml of Olanzapine into 10ml volumetric flask) into a 10 ml standard flask, diluting up to the mark, and filtered through a 0.45µ membrane filter.

Chromatographic separation: The chromatographic separation is carried out by injecting 20µl of working standard solution into the Inertsil- C_{18} column at ambient temperature, using mobile phase at a flow rate of 1.0ml/min for a run time of 8 minutes. The components are monitored at 233 nm using a PDA detector. The typical chromatogram of optimised method (trial-4) is presented in Fig.-3.

System suitability parameters: The system suitability parameters such as USP plate count, USP tailing factor, retention time, and peak area of Fluoxtine and Olanzapine are found to be within the limits. Retention time, peak area, USP plate count and USP tailing factor of Fluoxetine HCl / Olanzapine are found to be 2.955min, 733495, 10953.609752, and 1.604407, and 3.538min, 216925, 9423.123707, and 1.046295 respectively. The resolution between the two peaks of Fluoxtine and Olanzapine is found to be greater than 2.0.

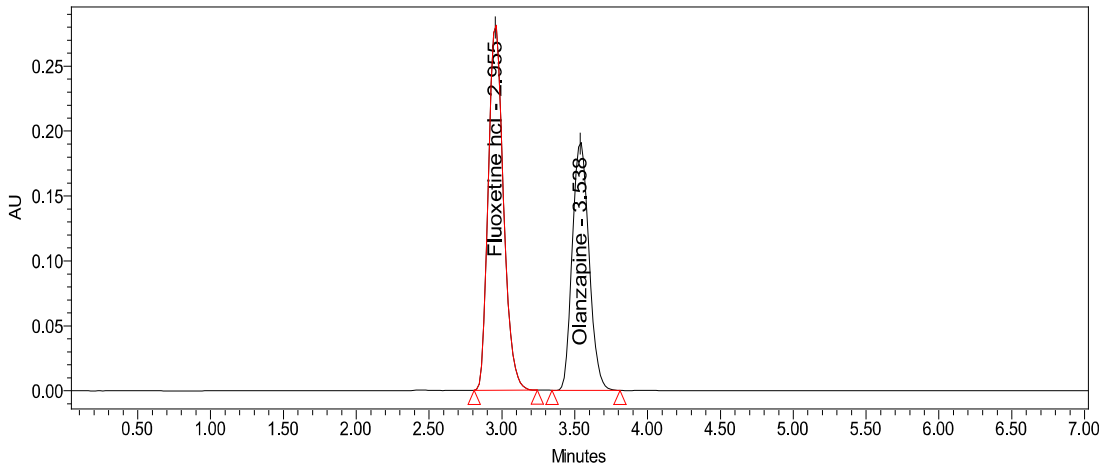

Fig.-3: HPLC Chromatogram of system suitability

2.4 Method Validation

2.4.1 System suitability: A working standard solution (40 µg/ml of Fluoxetine HCl and Olanzapine) is prepared, chromatograms are obtained for five replicates under optimised chromatographic conditions, and the system suitability parameters are evaluated using Empower 2.0 software. The typical chromatogram for system suitability is presented in Fig.-6. Time of retention, peak area are determined, and %RSD is calculated on five replicate measurements. The HPLC chromatogram for system suitability is presented in Fig.-3.

2.4.2 Specificity: The contents of the mobile phase are filtered before use through a 0.45micron membrane filter and pumped from the respective solvent reservoirs into the column at a flow rate of 1ml/min. Prior to injection of the standard or sample solutions; the column is equilibrated for at least 30min with the mobile phase flowing through the system. The response of the PDA detector is recorded at 233 nm. It is observed that the base line is parallel to x-axis and no peaks are found in the chromatogram. Chromatograms are obtained for standard and sample solutions under the optimized chromatographic conditions, and then the blank chromatogram is compared with the standard and sample chromatograms. Typical chromatograms of blank/standard/sample are presented in Fig.-4-6 respectively. The chromatograms of standard and sample are identical with same retention time.

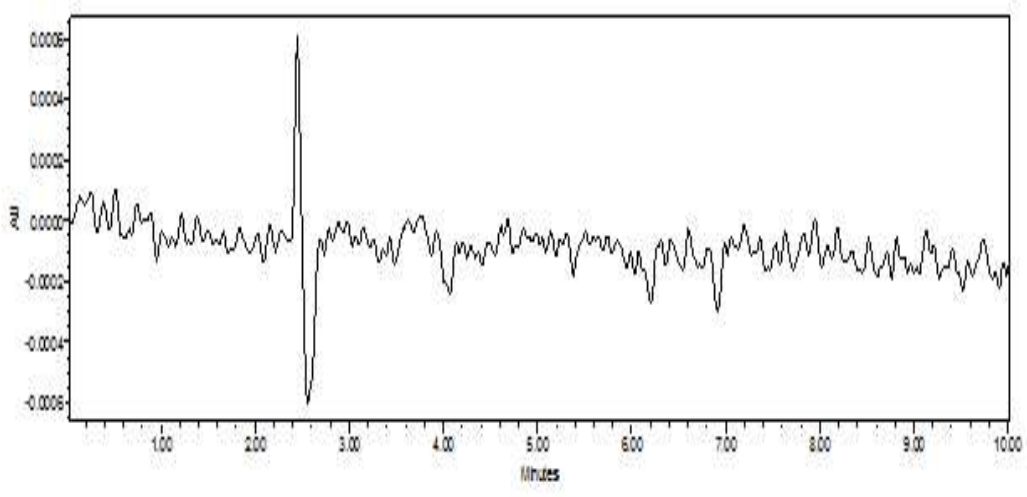

Fig.-4: HPLC Chromatogram of blank solution

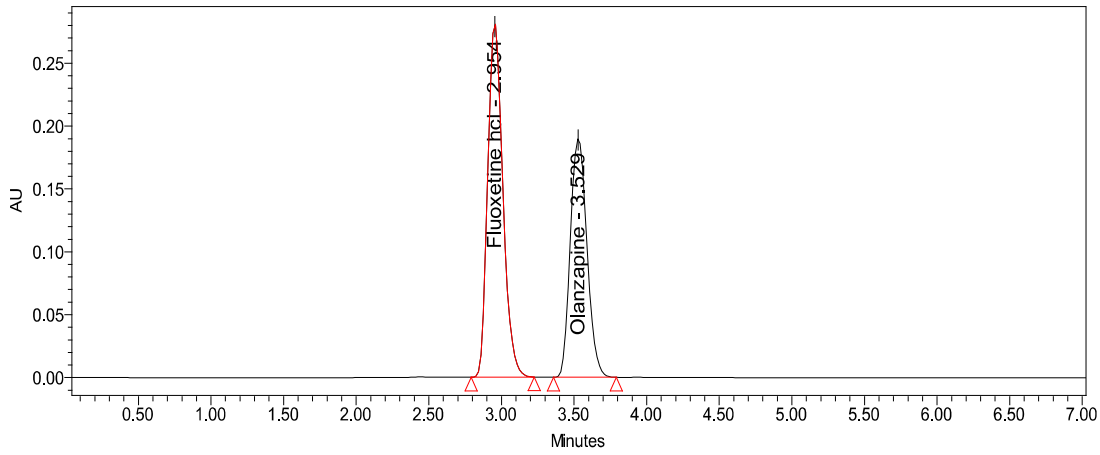

Fig.-5: HPLC Chromatogram of standard

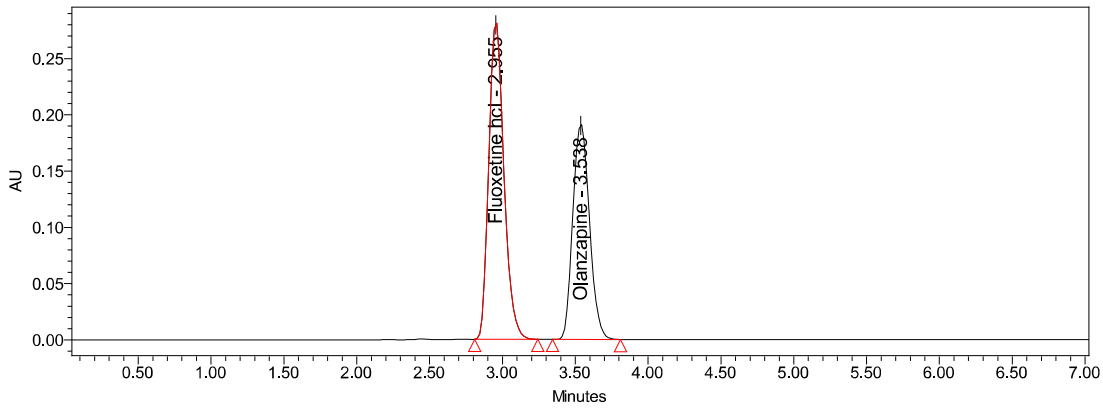

Fig.-6: HPLC Chromatogram of sample

2.4.3 Precision: In system precision, a standard solution of 40 µg/ml mixture of Fluoxetine HCl and Olanzapine is prepared, chromatograms for five replicate injections of the standard solution are obtained, and system suitability parameters are determined. The % RSD of five replicates is less than 2.0. Method precision is determined by preparing six standard solutions of target concentration, chromatograms for each individual solution are obtained under developed chromatographic conditions by spiking each standard solution into the chromatographic system. For method precision, the percent of assay of Fluoxetine HCl and Olanzapine should be between 98-102.0%. HPLC chromatograms of system precision and method precision are presented in Fig.-7-8 respectively.

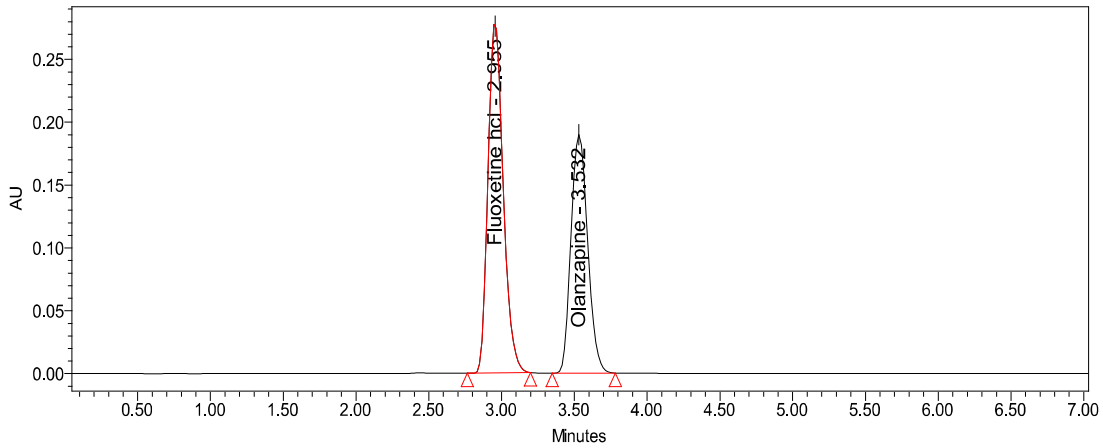

Fig.-7: HPLC Chromatogram of standard in system precision

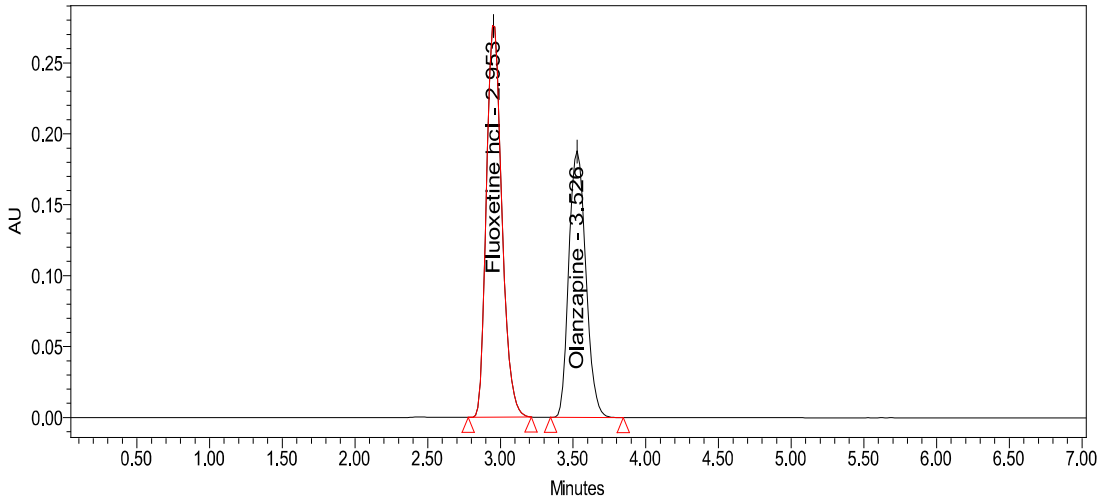

Fig.-8: HPLC Chromatogram of standard in method precision

2.4.4 Accuracy: The accuracy of developed method is tested at three different concentration levels. An amount of 20, 40 and 60 µg/ml equivalent to 50%, 100%, and 150% of the target concentration is added to the standard solution, dissolved, and diluted up to the mark. Each solution is injected in triplicate as per the test method, chromatograms are recorded, and the average percent recovery of Fluoxetine HCl and Olanzapine is calculated from the peak areas. The HPLC chromatograms in accuracy study are presented in Fig.-9-11 respectively. The percent recovery of the added amount is calculated three times at each concentration. Acceptance criteria of accuracy is the mean % recovery of triplicate measurements at each spike level should not be less than 98.0% and not more than 102.0%.

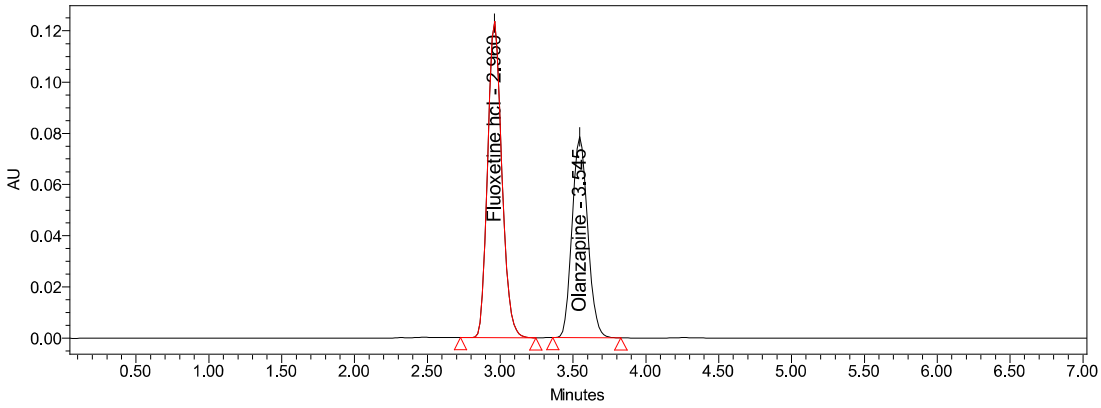

Fig.-9: HPLC Chromatogram of 50% spiked level in accuracy study

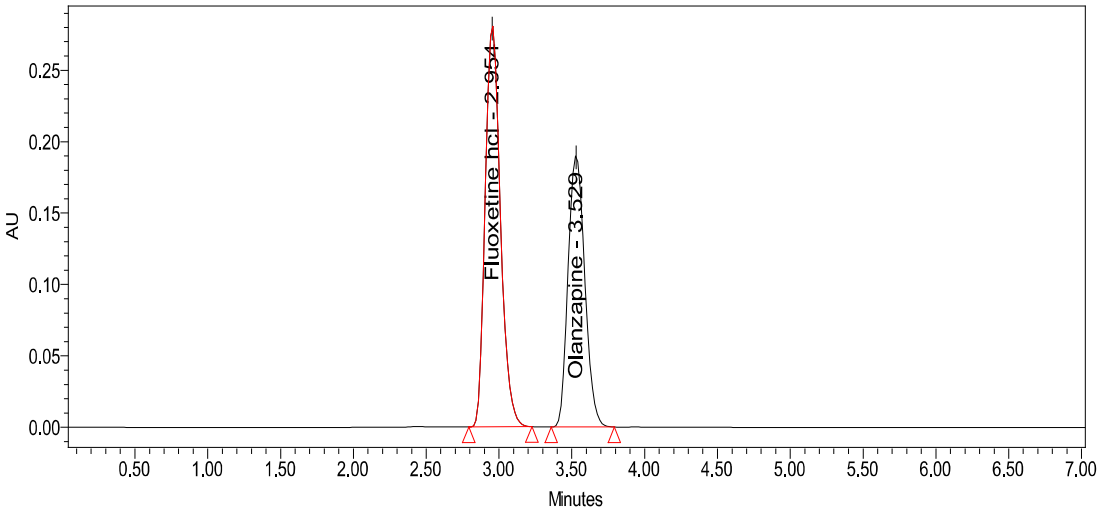

Fig.-10: HPLC Chromatogram of 100% spiked level in accuracy study

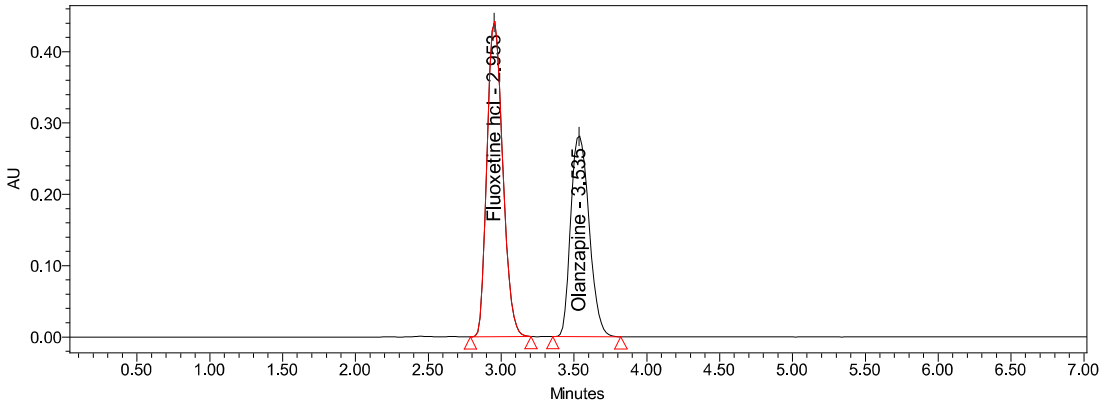

Fig.-11: HPLC Chromatogram of 150% spiked level in accuracy study

2.4.5 Linearity: A series of standard solutions ranging from 20 µg/ml to 80 µg/ml of Fluoxetine HCl and Olanzapine are prepared, each solution is introduced into the column, and chromatograms are obtained (six replicate measurements at levels-1 & 6, two measurements from levels-2-5). The area of the peaks in each chromatogram is determined. The acceptance criteria for linearity are that the correlation coefficient should be greater than 0.9890, % y- Intercept should be ±2.0., and the % RSD for level 1and 6 should be less than 2.0%. Linearity plots of Fluoxatine and Olanzapine are given in Figs.-12-13 respectively.

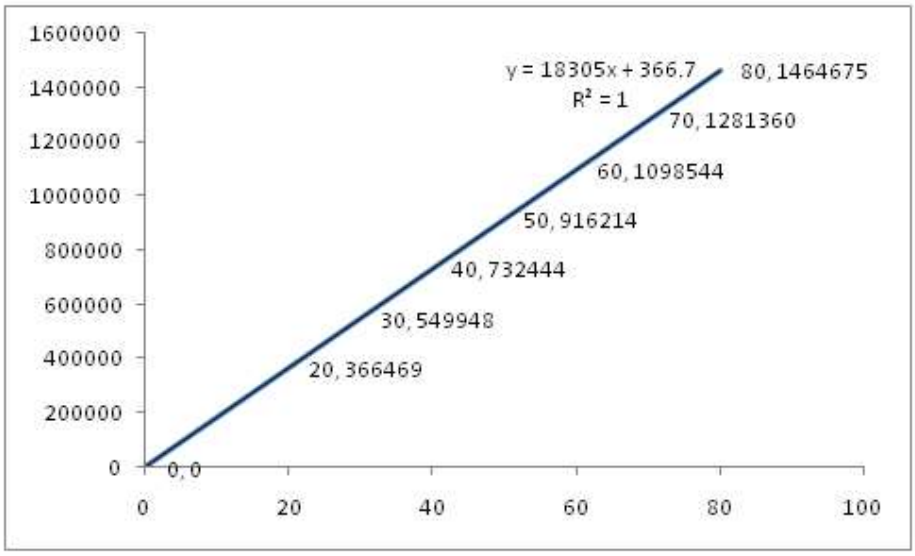

Fig.-12: Linearity plot –peak area vs concentration of Fluoxatine in µg/ml

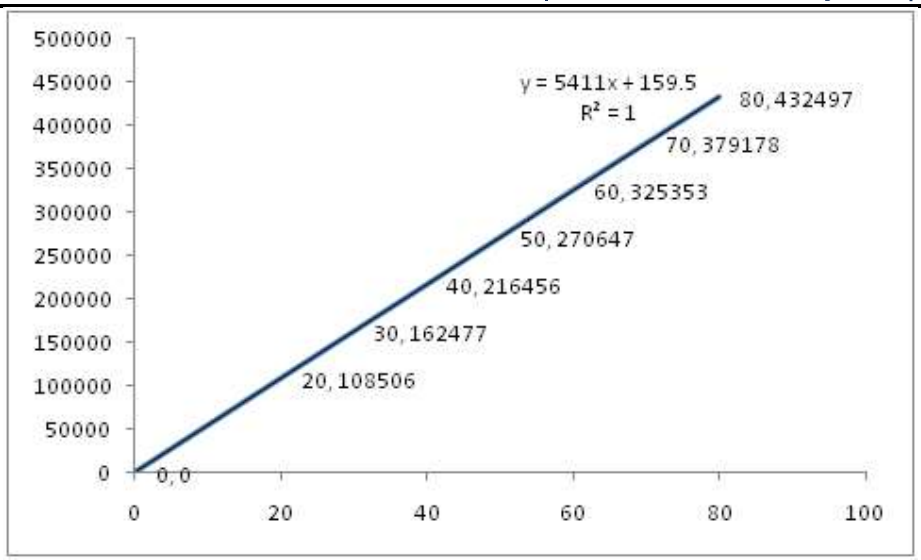

Fig.-13: Linearity plot –peak area vs concentration of Olanzapine in µg/ml

2.4.6 Ruggedness: A study of ruggedness is a study of the variation between of results between two analysts and two systems. Six sample solutions were prepared as per the test method, and each analyzed by both analysts as per test method. System to system variation: System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared, and each was analyzed as per the test method. A Comparison of both the results obtained on two different HPLC systems, shows that the assay test method is passes for ruggedness for system to system variability. Acceptance criteria: The individual assays of Fluoxetine HCl and Olanzapine should be not less than 98% and not more than 102%, and the %RSD of the assay should be NMT 2.0% by both analysts.

2.4.7 Robustness: Ruggedness is the study of the effect of variation of optimised parameters such as composition of the mobile phase, flow rate and detection wavelength on system suitability. A standard solution is prepared as per the test method. Chromatograms are obtained for each variation, and suitability parameters are determined. In flow rate variation, a study was conducted to determine the effect of variation in flow rate 1.0±0.2. In the study of the composition of mobile phase, two mobile phases of different compositions, 85:15 methanol: acetonitrile and 95:05 methanol: acetonitrile are prepared and chromatograms are recorded. In the study of variation of wavelength, chromatograms are recorded at wavelengths 233±5 nm. The system suitability parameters were evaluated in each case and found to be within limits.

2.5 Degradation studies

Stress degradation tests were conducted under acid and base hydrolysis, oxidation, and thermal and UV light conditions. Drug compounds were degraded in the solid state using thermal and UV radiation. In photo/thermal degradation, accurately 40 mg of Olanzapine and Fluoxetine HCl are taken in separate Petri dishes and exposed under UV energy of NLT 200 w/s / the thermal energy of 80°C in a hot air oven for 48 hrs. In acid/Base degradation, degradation for Olanzapine and Fluoxetine HCl drugs under acidic/basic conditions 0.1N HCl/0.1N NaOH solutions for 36 hours is tested. After 36 hours, the solutions are neutralized with 0.1N NaOH/0.1N HCl respectively. In case of oxidation degradation, 10% H_2O_2 is added to Olanzapine and Fluoxetine HCl and kept aside for 36 hours. After degradation, standard working solutions are prepared as per the test method, chromatograms are obtained, and the percent of degradation is calculated.

III. RESULTS AND DISCUSSIONS

3.1 System suitability

Statistical analysis is applied to the data obtained, mean, standard deviation, and the percent of relative standard deviation (% RSD) are calculated for retention time and peak area of five replicate injections. It is observed that, the %RSD for retention times and peak areas is found to be less than 2.0. The results are presented in Table-1

Table- 1: Results of System Suitability of developed HPLC method

	Fluoxetine HCl		Olanzapine	
Statistical Parameter	RT	Peak Area	RT	Peak Area
Mean*	2.9542	733030	3.530	216381
SD	0.000837	876.64	0.005244	676.6064
%RSD	0.028321	0.119	0.148556	0.312

** Mean, SD and %RSD are calculated for five replicate measurements*
SD: Standard deviation, RSD: Relative standard deviation

3.2 Specificity

The chromatograms of the blank, standard, and sample are obtained under optimised chromatographic conditions, and compared. It is found that no peaks are observed for blank, and peaks at 2.954 & 3.529 min and 2.955 & 3.538 min in the standard and sample are identical. In the sample chromatogram, no additional peaks are observed. The results of specificity are given in Table-2.

.Table-2: Specificity results of Fluoxetine HCl and Olanzapine

	Fluoxetine HCl		Olanzapine	
Parameter	RT	Peak Area	RT	Peak Area
Blank	--	--		--
Standard	2.954	733495	3.529	216925
Sample	2.955	731085	3.538	216567

3.3 Precision

System precision is calculated for six replicate measurements; the %RSD is calculated for peak area and %assay, and is found to be 0.11 & 0.12 and 0.325&0.32 for Fluoxetine HCl and Olanzapine respectively. Method precision is also calculated for measurements of six standard solutions of the target concentration, %RSD is found to be 0.12 & 0.12 and 0.29&0.28 for Fluoxetine HCl and Olanzapine respectively. The results of system precision and method precision are presented in Table-3&4 respectively.

Table-3: Statistical evaluation of system precision data

Statistical Parameters*	Fluoxetine HCl		Olanzapine	
	Peak Area	%Assay	Peak Area	%Assay
Mean	732383	99.97	216692	100.04
SD	860.54	0.118	704.5681	0.32
% RSD	0.11	0.12	0.325	0.32

** Statistical parameters are evaluated for five replicate measurements*

Table-4: Statistical evaluation of method precision data

Statistical Parameters*	Fluoxetine HCl		Olanzapine	
	Peak Area	%Assay	Peak Area	%Assay
Mean	732568	100	216731	100.06
SD	893.6036	0.12	637.2747	0.29
% RSD	0.12	0.12	0.29	0.28

** Statistical parameters are evaluated for six replicate measurements*

3.4 Accuracy

The accuracy of the developed method is determined at three different concentration levels (20, 40, and 60 g/ml), and the percentage of drug recovered is calculated by using the formula % Recovery = (Amount recovered/Amount added)*100. The mean percent of recovery, %RSD, is calculated for three measurements. The mean % recovery is found to be between 98 and 102%. The results for accuracy are presented in Table-5.

Table-5: Accuracy studies of developed HPLC method

Concentration % of spiked level	Fluoxetine HCl				Olanzapine			
	Amount added (µg/ml)	Amount found* (µg/ml)	% Recovery*	%RSD	Amount added (µg/ml)	Amount found* (µg/ml)	% Recovery*	%RSD
50%	20	20.2	100.11	0.31	20	19.96	100	0.37
100%	40	40.0	100.01	0.29	40	40.03	100.08	0.13
150%	60	60.09	100.16	0.33	60	59.99	99.98	0.40

** Statistical evaluation on three measurements*

3.5 Ruggedness

Ruggedness is the study of variation in results between two analysts and two systems. The percent of assay for six replicate measurements is calculated for analyst and system variation. The mean percent of assay, and %RSD are calculated and are acceptable. The results of ruggedness for analyst and system variation are reported in Table-6&7 respectively.

Table-6 : Statistical evaluation of intermediate precision data
(Analyst to Analyst variation)

Statistical Parameters*	Fluoxetine HCl		Olanzapine	
	Peak Area	%Assay	Peak Area	%Assay
Mean	732444	99.98	216456	99.93
SD	1140.219	0.16	632.8976	0.28
% RSD	0.15	0.16	0.29	0.28

** Statistical parameters are evaluated for six replicate measurements*

Table-7: Statistical evaluation of intermediate precision data
(System to System variation)

Statistical Parameters*	Fluoxetine HCl		Olanzapine	
	Peak Area	%Assay	Peak Area	%Assay
Mean	732350.8	99.97	216580.5	99.99
SD	795.100	0.109	709.438	0.330
% RSD	0.108	0.109	0.327	0.330

3.6 Robustness

Robustness is the study of the effect on results of a deliberate variation in chromatographic conditions such as flow rate, composition of the mobile phase, temperature. Chromatographic parameters are determined by changing one parameter at a time while keeping other parameters constant. The mean, SD, and %RSD are calculated on peak area and tailing factor for six replicate measurements, and the results are acceptable. The results of robustness for low rate variation and mobile phase composition are presented in Table-8&9 and Table-10&11 respectively.

Table-8: Results of the effect of flow rate variation for Fluoxetine

Flow rate	0.8ml		1.0ml		1.2ml	
Parameter	Peak Area	Tailing factor	Peak Area	Tailing factor	Peak Area	Tailing factor
Mean*	1046027	1.424556	732612	1.595979	620821	1.399278
SD	972.0292	0.006854	660.8453	0.01043	659.4844	0.011945
%RSD	0.091	0.48	0.090	0.65	0.11	0.85

** Statistical calculation on six replicate measurements*

Table-9: Results of the effect of flow rate variation for Olanzapine

Flow rate	0.8ml		1.0ml		1.2ml	
Parameter	Peak Area	Tailing factor	Peak Area	Tailing factor	Peak Area	Tailing factor
Mean*	266608	1.429049	216515	1.131168	152593.6	1.266551
SD	1197.261	0.006352	850.2783	0.015936	772.7346	0.010619
%RSD	0.45	0.44	0.39	1.40	0.51	0.83

** Statistical calculation on six replicate measurements*

Table-10: Results of the effect of mobile phase composition for Fluoxetine

MeOH: ACN	85:15		90:10		95:5	
Parameter	Peak Area	Tailing factor	Peak Area	Tailing factor	Peak Area	Tailing factor
Mean*	1046415	1.424656	732412	1.595471	620895	1.399778
SD	972.0221	0.006154	660.5453	0.01013	659.4247	0.011345
%RSD	0.0928	0.432	0.090	0.634	0.106	0.810

* Statistical calculation on six replicate measurements

Table-11: Results of the effect of mobile phase composition for Olanzapine

MeOH: ACN	85:15		90:10		95:5	
Parameter	Peak Area	Tailing factor	Peak Area	Tailing factor	Peak Area	Tailing factor
Mean*	266684	1.399049	217519	1.131168	152993.6	1.246551
SD	1197.295	0.016352	850.2783	0.016937	775.7387	0.011619
%RSD	0.448	1.16	0.39	1.49	0.50	0.93

** Statistical calculation on six replicate measurements*
MeOH: Methanol, ACN: Acetonitrile

3.7 Linearity

A series of six different concentration (20-80 µg/ml) solutions are prepared, and chromatograms are recorded under standard experimental conditions. A linear plot is drawn of the peak area against concentration. The slope, intercept, and correlation coefficient for six measurements are calculated from the linear plot. The results of linearity are presented in Table-12

Table-12: Linearity studies and results of regression analysis of the developed method

	Fluoxetine		Olanzapine	
S.No.	Concentration (µg/ml)	Average Area	Concentration (µg/ml)	Average Area
1	0	0	0	0
2	20	366469	20	108506
3	30	549948	30	162477
4	40	732444	40	216456
5	50	916214	50	270647
6	60	1098544	60	325353
7	70	1281360	70	379178
8	80	1464675	80	432497
	Slope	18305		5411
	y-Intercept	366.7		159.5
	Correlation Coefficient	0.9998		0.9999

3.8 Limit of Detection and Limit of quantification

The limits of detection and quantification are determined from the standard deviation and slope for both the drugs. LOD and LOQ for Fluoxetine and Olanzapine are found to be 0.41&1.25 and 0.16&0.48 µg/ml respectively

3.9 Degradation studies

The stability of the drugs Fluoxetine and Olanzapine are studied under forced degradation by exposing the drugs to degradation conditions such as 0.1 N HCL, 0.1N NaOH, 10% hydrogen peroxide, thermal, and photo light. The results of the stability test are presented in Table-13.

Table-13: Results of stability of Fluoxetine and Olanzapine

Degradation condition	% Degradation of Fluoxetine	% Degradation of Olanzapine
Acid Hydrolysis	9.4	8.9
Base Hydrolysis	10.27	10.86
Peroxide degradation	9.07	9.05
Thermal degradation	10.18	10.96
Photo light degradation	9.24	10.41

IV. CONCLUSIONS

A simple, rapid, precise, accurate, sensitive, and economic isocratic RP-HPLC method is developed, and it is linear in the range of concentrations 20-80 µg/ml. The percent of recovery is found to be 98.0-101.50. The developed method is successfully applied for the assay analysis of pharmaceutical formulations and degradation studies, and hence it is suggested to employ it for routine quality control analysis in any pharmaceutical industry.

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