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Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Fluoxetine Hcl and Olanzapine in Combined Tablet Dosage Form

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A B S T R A C T

A simple, Accurate, precise method was developed for the simultaneous estimation of the Fluoxetine Hcl and Olanzapine in Tablet dosage form. Chromatogram was run through Inertsil C_{18} ODS column (250mm 4.6mm, 5µ). Mobile phase containing Acetonitrile and methanol in the ratio of 90:10A was pumped through column at a flow rate of 1ml/min. Optimized wavelength for Fluoxetine Hcl and Olanzapine was 233nm. Retention time of Fluoxetine Hcl and Olanzapine were found to be 2.9 min and 3.5 min. %RSD of the Fluoxetine Hcl and Olanzapine were and found to be 0.12 and 0.36 respectively. %Recover was Obtained as 98.0% and 101.5% for Fluoxetine Hcl and Olanzapine respectively. LOD, LOQ values are obtained from regression equations of Fluoxetine Hcl and Olanzapine were 0.41ppm, 0.16ppm and 1.25ppm, 0.48ppm respectively. Regression equation of Fluoxetine Hcl is y = 18305.x + 366.7, and of Olanzapine is y = 5411x + 159.5

Keywords: Fluoxetine Hcl, Olanzapine, RP-HPLC.

A R T I C L E I N F O

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1. Introduction

Pharmaceutical Analysis plays a very vital role in the quality assurance and quality control of bulk drugs and their formulations [1,2,3]. Pharmaceutical analysis is a specialized branch of analytical chemistry which involves

separating, identifying and determining the relative amounts of components in a sample of matter [4,5,6]. It is concerned with the chemical characterization of matter both quantitative and qualitative [7,8]. In recent years, several analytical techniques have been evolved [9].

Olanzapine (OLZ) is a benzodiazepine chemically named as 2-methyl-4-(4-methylpiperazin-1-yl)-5H-thieno [3,2-c][1,5] benzodiazepine[10,11]. It is used in the treatment of depression. It improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis [12, 13]. It decreases the gluconeogenesis while increasing the glucose uptake by muscles and fat cells. Fluoxetine (FLU) is chemically 2-methyl-4-(4-methylpiperazine 1-yl) 5H-thieno [3,2-c] benzodiazepine [14,15]. Literature survey revealed that few analytical techniques are available for estimation of OLZ alone as well as in combine dosage form such as UV, HPLC [16].Similarly few analytical methods are available for estimation of FEN alone and its combination with drugs such as UV and HPLC [9-12]. Keeping this objective in mind an attempt has been made to develop and validate the RP-HPLC method for the simultaneous estimation of Olanzapine and Fluoxetine which would be highly sensitive having good resolution reproducible and cost effective.

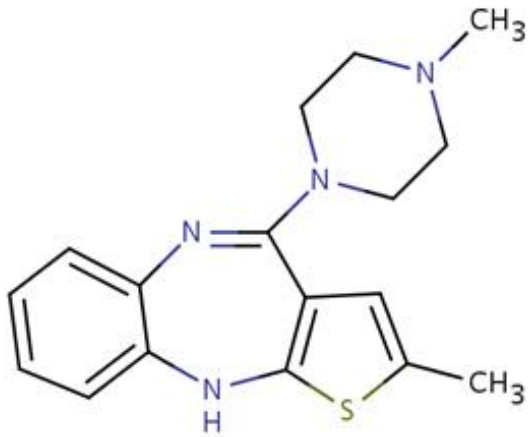

Figure 1: Structure of Olanzapine

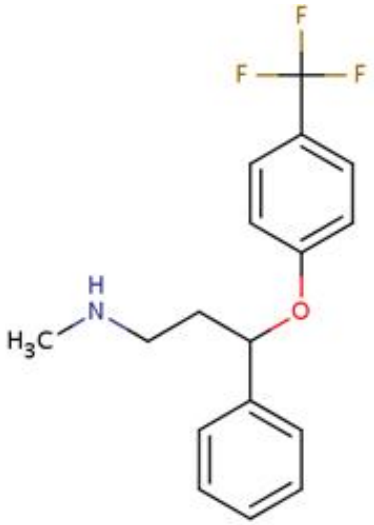

Figure 2: Structure of Fluoxetine Hcl

2. Materials and Methods

Instruments: HPLC–WATERS Model NO.2690/5series Compact System Consisting of

- Inertsil- C18 ODS column.
- Electronic balance (SARTORIOUS)
- Sonicator (FAST CLEAN)

Chemicals:

- Purified water HPLC Grade
- HPLC Grade Methanol
- HPLC Grade Acetonitrile

Raw Material: Fluoxetine Hcl and Olanzapine Working Standard.

Method Development FOR HPLC:

The objective of this experiment was to optimize the assay method for estimation of Fluoxetine hcl and Olanzapine based on the literature survey made. So here the trials mentioned describes how the optimization was done.

Trial: 1

Mobile Phase: Degassed Methanol 100%.

Preparation of Standard Solution: 10mg of Fluoxetine hcl and Olanzapine RS drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from this and diluted to 10 ml with mobile phase

Chromatographic Conditions:

Flow rate : 1.0ml/min
Column : Inertsil- C18 ODS column
Detector wavelength : 233nm
Column temp : Ambient
Injection volume : 20µl
Run time : 8min
Retention time : 2.7min For Fluoxetine Hcl and 3.3min for Olanzapine

Observation: Two peaks are not separated completely merged. The trial 1 chromatogram result was shown in Fig:1.

Trail: 2

Mobile Phase Degassed Methanol and Water in the ratio of 90:10 V/V.

Preparation of Standard Solution:

10mg of Fluoxetine hcl and Olanzapine RS drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

Chromatographic Conditions:

Flow rate : 1ml/min
Column : Inertsil-C18 ODS column
Detector wavelength : 233nm
Column temp : Ambient
Injection volume : 20µl
Run time : 6min
Retention time : 2.8min For Fluoxetine Hcl and 3.2min for Olanzapine

Observation: Peaks got separated but the resolution is very less. The trial 2 chromatogram result was shown in Fig:2.

Trail: 3

Mobile Phase: Degassed Methanol and Water in the ratio of 50:50 V/V.

Preparation of Standard Solution:

10mg of Fluoxetine Hcl and Olanzapine RS drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

Chromatographic Conditions:

Flow rate : 1.0ml/min
Column : Inertsil- C18 ODS column
Detector wavelength : 233nm
Column temp : Ambient
Injection volume : 20µl
Run time : 7min
Retention time : 2.2min For Fluoxetine Hcl and 3.6min for Olanzapine.

Observation:

Peak shapes are not good. The trial 3chromatogram result was shown in Fig:3

Optimized Method

Mobile Phase: Degassed Methanol and Acetonitrile in the ratio of 90:10 V/V.

Preparation of stock solution:

10mg of Fluoxetine hcl and Olanzapine RS drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 2 ml. was taken from this and diluted to 10ml.with mobile phase.

Preparation of working standard solution:

The stock solution equivalent to 20ppm to 80ppm were prepared, sonicated and filtered through 0.45µ membrane.

Preparation of sample drug solution for pharmaceutical formulations: Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 40 mg Fluoxetine hcl and 10 mg Olanzapine was weighed and dissolved in the 70 mL mobile phase with the aid of ultrasonication for 20 min. The content was diluted to 100 mL with mobile phase to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to give a test solution containing 40 µg/mL Fluoxetine hcl and 10 µg/mL Olanzapine.

Table 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil- C18 ODS, 250×4.6mm, 5µ
Mobile Phase	ACN: Methanol -10:90
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	7min
Column temperature (°C)	Ambient
Volume of injection loop (ml)	20
Detection wavelength (nm)	233nm
Drug RT (min)	2.955min for Fluoxetine Hcl and 3.538min for Olanzapine

Method Validation

1. System Suitability:

A Standard solution was prepared by using Fluoxetine Hcl and Olanzapine working standard as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Fluoxetine Hcl and Olanzapine**,** retention times and peak areas.

2. Specificity:

Fluoxetine Hcl and Olanzapine identification:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

3. Precision:

Repeatability:

System precision: Standard solution prepared as per test method and injected five times.

Method precision:

Prepared six sample preparations individually using single as per test method and injected each solution.

4. Accuracy (Recovery):

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Fluoxetine Hcl and Olanzapine into each volumetric flask for each spike level to get the concentration of Fluoxetine Hcl and Olanzapine equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Fluoxetine Hcl and Olanzapine was calculated.

5. Linearity of Test Method:

A Series of solutions are prepared using Fluoxetine hcl and Olanzapine working standard at concentration levels from 20ppm to 80 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

6. Ruggedness of Test Method:

a) System to system variability:

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 test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

b) column to column variability:

Column to column variability study was conducted by using different columns. Six samples were prepared and each was analyzed as per test method.

7. Robustness:

a) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. Fluoxetine hcl and Olanzapine was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

b) Effect of variation of temperature:

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were

evaluated and found to be within the limits for a temperature change of 20ºc. Similarly sample solution was chromatographed at 25ºC temperature. Fluoxetine Hcl and Olanzapine were resolved from all other peaks and the retention times were comparable with those

8. Limit of Detection and Quantitation (LOD and LOQ): From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\ \sigma}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\ \sigma}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyte.

3. Results and Discussion

Method development*:*

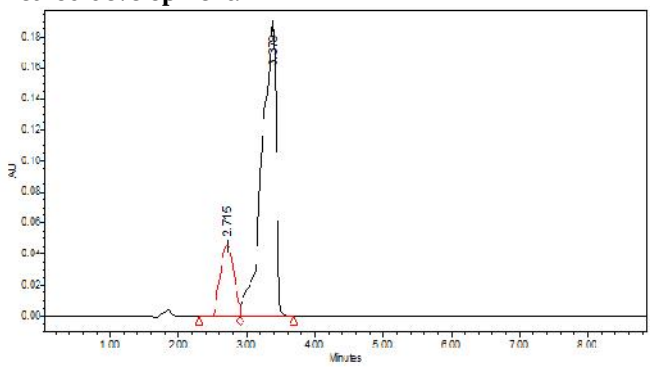

Figure 3: Chromatogram of Trial 1

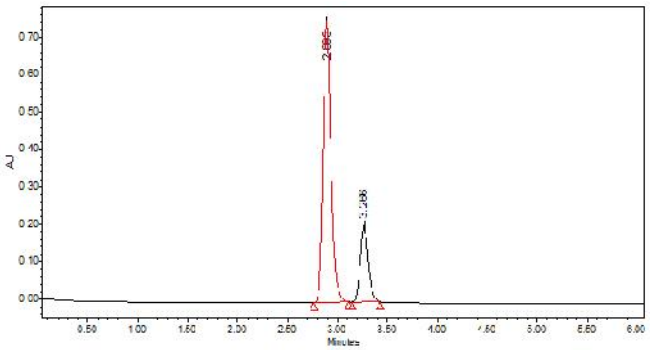

Figure 4: Chromatogram of Trial 2

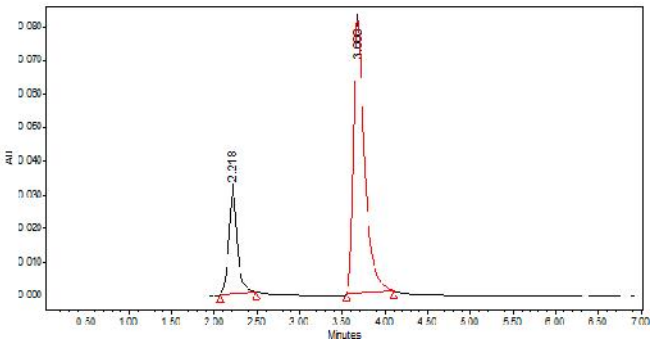

Figure 5: Chromatogram of Trial 3

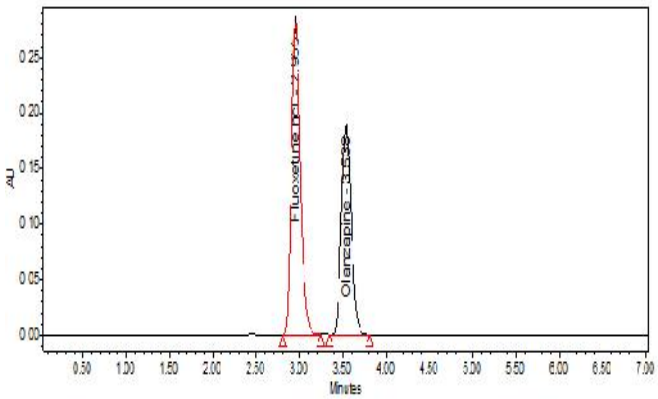

Figure 6: Chromatogram of standard

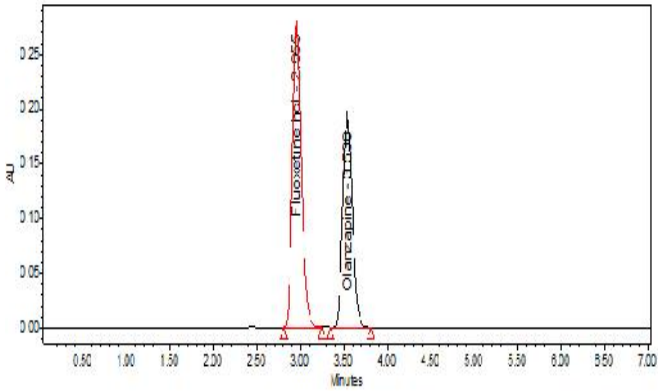

Figure 7: Chromatogram of sample

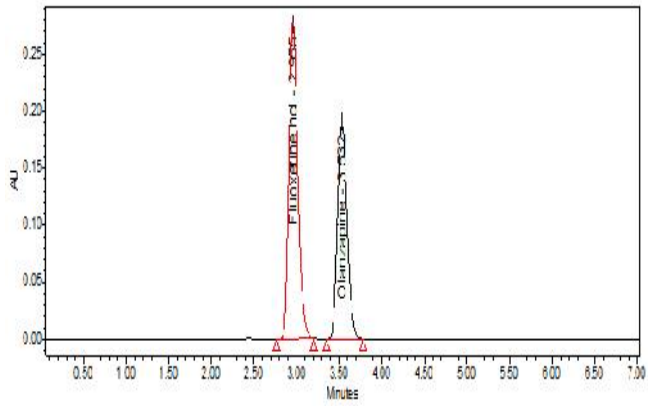

Figure 8: Chromatograms of system precision

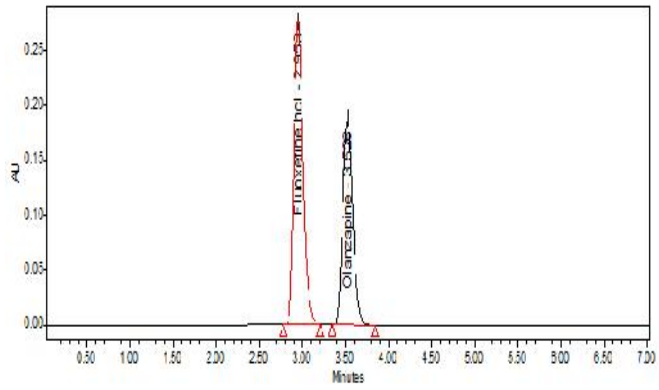

Figure 9: Chromatograms of Repeatability

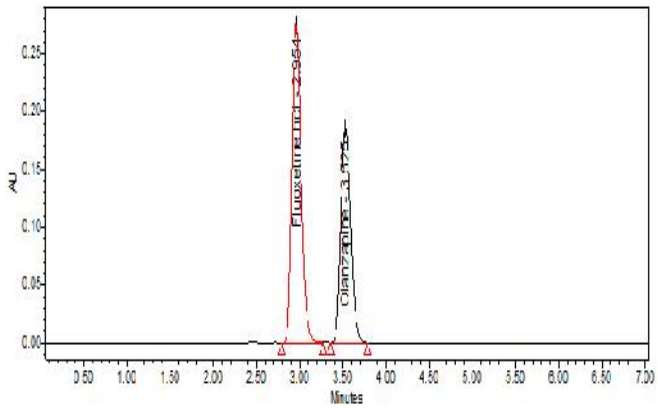

Figure 10: Chromatograms of Intermediate precision

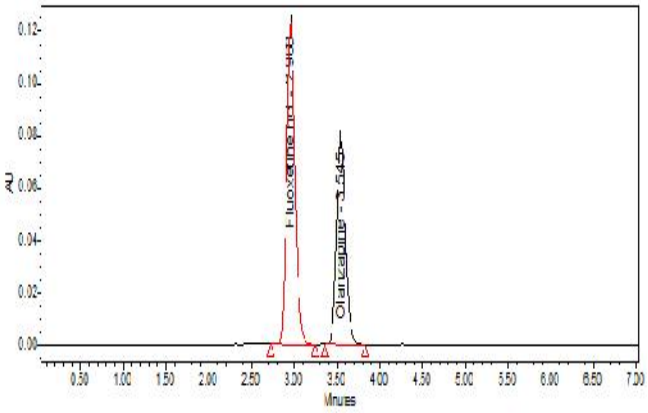

Figure 11: Chromatograms for accuracy (50%)

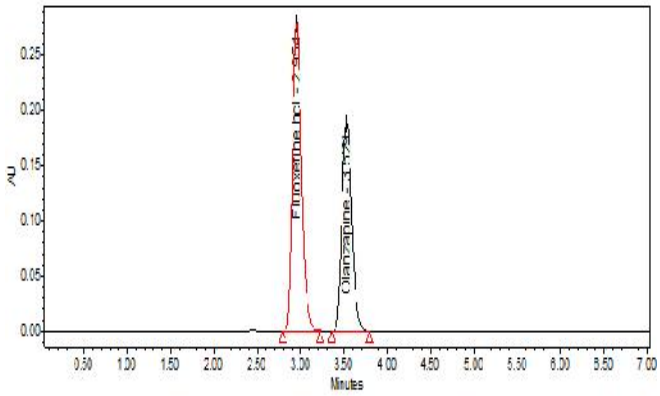

Figure 12: Chromatograms for accuracy (100%)

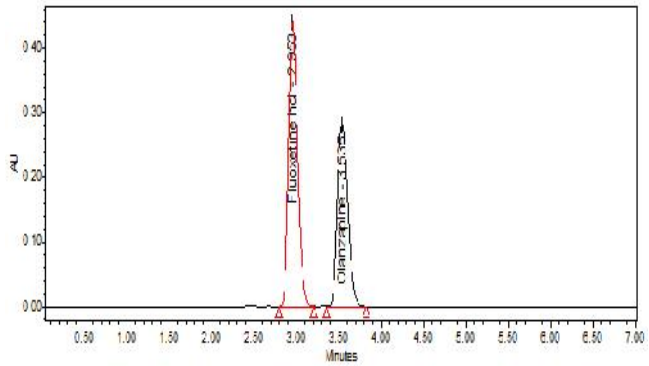

Figure 13: Chromatograms for Accuracy (150%)

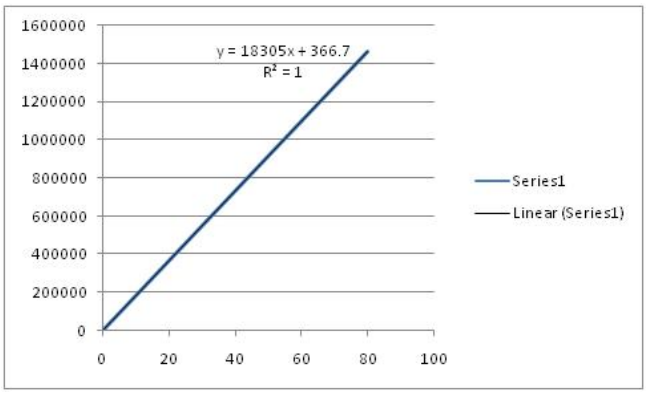

Figure 14: Linearity Plot (Concentration Vs Response) for Fluoxetine Hcl

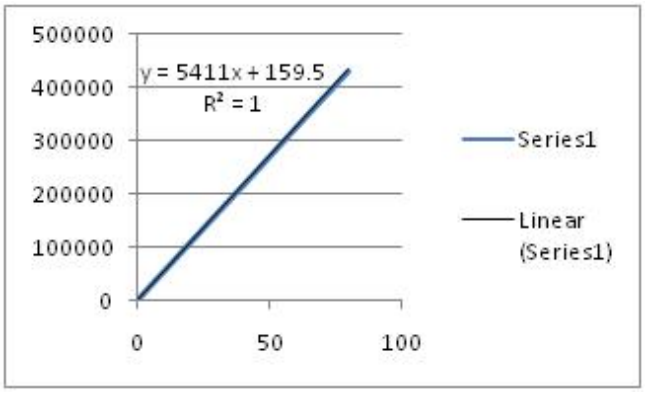

Figure 15: Chromatograms for 20 ppm

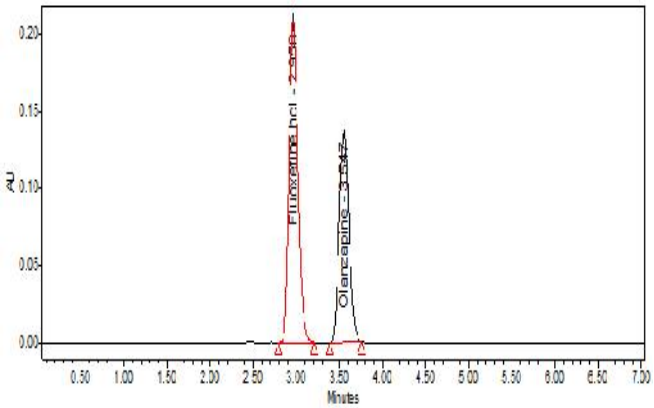

Figure 17: Chromatograms for 30ppm, 40 ppm

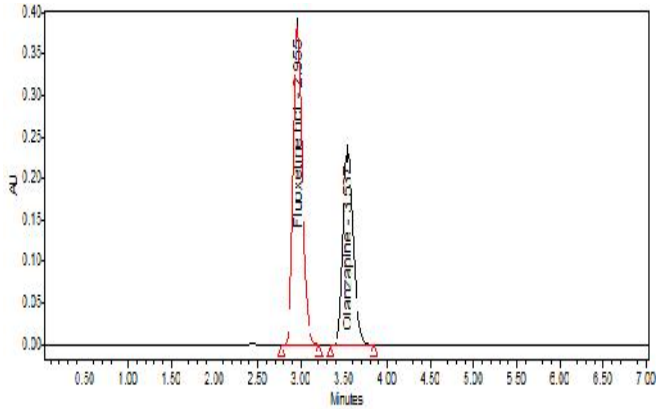

Figure 18: Chromatograms for 50 ppm, 60 ppm

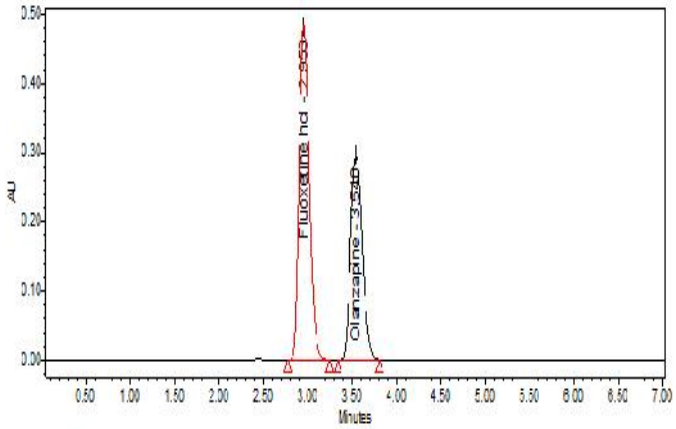

Figure 19: Chromatograms for 70 ppm, 80 ppm

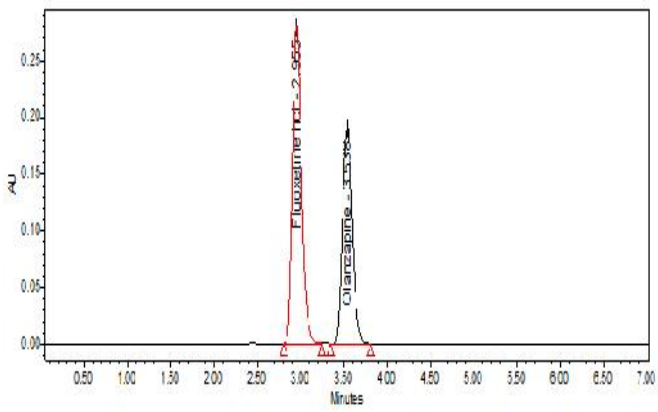

Figure 20: Chromatograms for Limit of detection and Limit of Quantification

Table 2(A): Data of Repeatability (System precision)

	Injection	Peak Areas of Fluoxetine Hcl	% Assay
Concentration 40ppm	1	731458	99.85
	2	732034	99.93
	3	732192	99.95
	4	732454	99.98
	5	733777	100.17
Statistical Analysis	**Mean**	**732383**	**99.97**
	SD	860.54	0.118
	% RSD	0.11	0.12

Table 2(B): Data of Repeatability (System precision)

	Injection	Peak Areas of Olanzapine	%Assay
Concentration 40ppm	1	216835	100.10
	2	216759	100.07
	3	215852	99.65
	4	216283	99.85
	5	217735	100.53
Statistical Analysis	**Mean**	216692	100.04
	SD	704.5681	0.32
	% RSD	0.325	0.32

Table 3(A): Data of Repeatability (Method precision)

	Injection	Peak Areas of Fluoxetine Hcl	% Assay
Concentration 40ppm	1	731458	99.84
	2	732034	99.93
	3	732192	99.95
	4	732454	99.98
	5	733777	100.17
***Statistical* Analysis**	**Mean**	733495	100.13
	SD	732568	100
	% RSD	893.6036	0.12

Table 3(B): Data of Repeatability (Method precision)

	Injection	Peak Areas of Olanzapine	% Assay
Concentration 40ppm	1	216835	100.11
	2	216759	100.07
	3	215852	99.65
	4	216283	99.85
	5	217735	100.53
***Statistical* Analysis**	**Mean**	216925	100.15
	SD	216731	100.06
	% RSD	637.2747	0.29

Table: 4(A): Data of Intermediate precision (Analyst 2)

	Injection	Peak Areas of Fluoxetine Hcl	% Assay
Concentration 40ppm	1	733495	100.15
	2	732045	99.93
	3	733992	100.19
	4	732590	100
	5	731085	99.79
Statistical Analysis	**Mean**	731458	99.85
	SD	732444	99.98
	% RSD	1140.219	0.16

Table 4(B): Data of Intermediate precision (Analyst 2)

	Injection	Peak Areas of Olanzapine	% Assay
Concentration 40ppm	1	216925	100.15
	2	215907	99.68
	3	217042	100.20
	4	215465	99.48
	5	216567	99.98
***Statistical* Analysis**	**Mean**	216835	100.10
	SD	216456	99.93
	% RSD	632.8976	0.28

Table 5(A): Data of Accuracy of Fluoxetine Hcl

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	20	20.06	100.31	**MEAN**	100.11
50% Sample 2	20	19.95	99.75		
50% Sample 3	20	20.05	100.29	**%RSD**	0.31
100 % Sample 1	40	40.06	100.15		
100 % Sample 2	40	39.87	99.68	**MEAN**	100.01
100% Sample 3	40	40.08	100.20	**%RSD**	0.29
150% Sample 1	60	59.87	99.78		
150% Sample 2	60	60.22	100.37	**MEAN**	100.16
150% Sample 3	60	60.20	100.34	**%RSD**	0.33

Table 5(B): Data of Accuracy of Olanzapine

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	20	20.07	100.40	**MEAN**	100.08
50% Sample 2	20	19.93	99.65		
50% Sample 3	20	19.99	99.95	**%RSD**	0.37
100 % Sample 1	40	40.05	100.12		100.08
100 % Sample 2	40	39.97	99.93	**MEAN**	0.13
100% Sample 3	40	40.07	100.19	**%RSD**	
150% Sample 1	60	59.97	99.94	**MEAN**	99.98
150% Sample 2	60	60.01	100.02	**%RSD**	
150% Sample 3	60	59.99	99.99		0.40

Table 6(A): Data of Linearity of Fluoxetine Hcl

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	18305
20	366469	y-Intercept	366.7
30	549948	Correlation Coefficient	1
40	732444		
50	916214		
60	1098544		
70	1281360		
80	1464675		

Table 6(B): Data of Linearity of Olanzapine

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	5411
20	108506	y-Intercept	159.5
30	162477	Correlation Coefficient	1
40	216456		
50	270647		
60	325353		
70	379178		
80	432497		

4. Conclusion

The proposed RP-HPLC (Reverse Phase High performance Liquid Chromatography) method has been evaluated for the accuracy, precision and linearity. Retention time of Fluoxetine Hcl and Olanzapine were found to be 2.9min and 3.5min. %RSD of the Fluoxetine Hcl and Olanzapine were found to be 0.12 and 0.36 respectively. %Recover was Obtained as 98.0% and 101.50% for Fluoxetine hcl and Olanzapine respectively. LOD, LOQ values are obtained from regression equations of Fluoxetine Hcl & Olanzapine were 0.41ppm, 0.16ppm and 1.25ppm, 0.48ppm respectively. Regression equation of Fluoxetine Hcl is y = 18305x + 366.7, and of Olanzapine is y = 5411.x + 159.5 Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries. The method was found to be precise, accurate and linear over the linear concentration range. The method developed is unique in determining the impurities even at low levels than that of specifications. The analytical method validation of Fluoxetine Hcl and Olanzapine in Tablet dosage form by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Fluoxetine Hcl and Olanzapine in tablet dosage form. Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, forced degradation studies, ruggedness, robustness & solution stability. Therefore, this HPLC method can be used as a routine analysis of these drugs in pharmaceutical formulations.

5. Acknoledgement

I gives my immense pleasure to express my sincere thanks to my guide Dr.M. Gobinath, M.Pharm, Ph.D, FIC, Ratnam institute of pharmacy, for giving guidance at all stages of my work.

6. References

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