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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF OLANZAPINE AND FLUOXETINE IN BULK DRUG AND TABLETS BY RP-HPLC METHOD

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ABSTRACT

A simple, sensitive, precise, accurate, linear, reproducible, simple, economic, ecofriendly and rapid HPLC method as per ICH guidelines has been developed. Reverse phase hplc method has been developed for simultaneous estimation of olanzapine and fluoxetine hydrochloride in bulkdrug and dosage forms. The mobile phase used is 55:45:0.03v/v (0.02M Phosphate buffer: Acetonitrile: Triethylamine) delivered at a flow rate of 1.0ml/min at a wavelength detection at 235 nm. The retention time of olanzapine and fluoxetine were 2.40 min and 5.71min respectively. The developed method was validated according to ICH guidelines. The proposed method can be used for determination of these drugs in bulk and combined dosage forms.

KEY WORDS: Olanzapine, Fluoxetine hydrochloride, RP-HPLC, Validaton.

INTRODUCTION

Fluoxetine HCl (FLX) is chemically, N-Methyl-γ-[4-(trifluoromethyl) phenoxy benzene propanamine hydrochloride. Fluoxetine hydrochloride is a selective serotonin reuptake inhibitor used as an antidepressant with non-sedating properties. Olanzapine (OLZ) is chemically **,** 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b] [1,5]-benzodiazepine. It is an antipsychotic agent, used in schizophrenia. FLX is official in BP and USP and both describe an LC method for the estimation of fluoxetine. A literature survey indicated spectrophotometric methods of FLX in formulations; HPLC and LC-MS methods of FLX

with norfluoxetine in plasma. Literature survey also indicated HPLC, HPLC-MS/ESI, capillary GC methods for simultaneous estimation of FLX in pharmaceutical formulation with drugs like fluvoxamine, clomipramine, citalopram and paroxetine. OLZ is official in IP, which described an HPLC method for its estimation. Literature survey indicated spectrophotometric, derivative spectroscopy and solid phase extraction methods for estimation of OLZ. Literature survey also indicated HPLC and LC-MS methods for determination of OLZ in biological fluids. FLX and OLZ are formulated together in the form of a tablet. SYMBYAX (olanzapine and fluoxetine HCl capsules) combines an atypical antipsychotic and a selective serotonin reuptake inhibitor, olanzapine (the active ingredient in Zyprexa, and Zyprexa Zydis) and fluoxetine hydrochloride (the active ingredient in Prozac, Prozac Weekly, and Sarafem). The literature survey has been carried out and it revealed that only few analytical methods which are comparatively costly have been reported and so the objective of work is to develop a new HPLC method for estimation of olanzapine and fluoxetine .Hence a precise, accurate, linear, reproducible, simple, economic, ecofriendly and rapid HPLC method as per ICH guidelines has been developed.

CHEMICALS AND REAGENTS

Glacial acetic acid-AR grade

Potassium dihydrogen phosphate - HPLC grade.

Acetonitrile-HPLC grade

Methanol-HPLC grade

Purified water-milli-Qgrade

Olanzapine working standard (obtained from ISP INDIA PVT.LTD)

Flouxetine working standard (obtained from ISP INDIA PVT.LTD)

INSTRUMENTATION

Analysis of drug was carried out on younglin, auto chrome 3000 software system equipped with u.v detector, degasser, manual sampler and a reverse phase HPLC column was used.

Equipments

Analytical balance: shimadzu

Uv : LAB INDIA

Sonicator : ultrasonic

PH meter : LAB INDIA

MATERIALS AND METHODS

Reagents and Solutions

Pure samples of Olanzapine, Fluoxetine Hcl and other reagents such as Acetonitrile, Methanol, Potassium dihydrogen Phosphate and water used were of HPLC and milli-q grade. All other chemicals like glacial acetic acid used were of AR grade. Optimized chromatographic conditions are listed in Table. 1.

Buffer Preparation

Buffer was prepared by dissolving 2.7218g of Potassium dihydrogen orthophosphate in 1L of water and adjusts the pH 4.2 ± 0.02 with Ortho Phosphoric acid followed by the degassing of the solution.

Diluent Preparation (Mobile Phase)

1L of diluent was prepared by mixing 550ml of Potassium Buffer (0.02M), 450ml of Acetonitrile and 0.3ml Triethylamine was added and sonicated upto 15 mines.

Stock Solution Preparation

Accurately weigh about 10mg of Olanzapine, Fluoxetine Hcl and transfer it into a 10ml of two separate volumetric flasks. Add 5ml of Methanol and kept in an ultrasonic bath until it dissolved completely. Make up to the mark with the methanol and mix. This yielded solution of 1000µg/ml concentrations for both Olanzapine and Fluoxetine Hcl.

Standard Solution Preparation

Spiked accurately about 1ml of Olanzapine and Fluoxetine Hcl stock solutions and transfer it into a 10ml volumetric flask. Make up to the mark with the mobile phase and mix. This yielded solution of 100µg/ml concentration for both Olanzapine and Fluoxetine Hcl.

Validation

Validation experiments were performed to demonstrate System suitability, precision, linearity, Accuracy study of analytical solution Limit of detection and Limit of quantification.

Precision

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample (100µg/ml).

Injection Precision

Standard solution were prepared as per test method and injected six times.

Method Precision

Six preparations were prepared individually using single batch of Olanzapine, Fluoxetine Hcl working standard as per test method and injected each solutions.

Accuracy

Accuracy for the assay of Olanzapine, Fluoxetine Hcl determined by applying the method in triplicate samples to which known amount of Olanzapine and Fluoxetine Hcl standard was added at different levels (50%, 100%, and 150%).

Linearity & Range

The Linearity of detector response is established by plotting a graph to concentration versus area of Olanzapine and Fluoxetine Hcl standards and determining the correlation coefficient. A series of solution of Olanzapine, Fluoxetine Hcl standard solution in the concentration ranging from about 10µg/ml to 200µg/ml level of the target concentration were prepared for both and injected into the HPLC system.

Limit of Detection (LOD)

Method Procedure

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The various concentrations ranging from 0.1 to 1µg/ml of Olanzapine and Fluoxetine Hcl were injected and peaks were recorded. 0.15µg/ml concentration was detected for both Olanzapine and Fluoxetine Hcl.

Limit of Quantification (LOQ)

Method Procedure

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The various concentrations ranging from 0.1 to 1µg/ml of Olanzapine and Fluoxetine Hcl were injected and peaks were recorded. 0.5µg/ml concentration was detected for both Olanzapine and Fluoxetine Hcl.

Table No. 1: Developed Chromatographic Conditions.

Parameters	Method
Stationary phase (column)	Phenomenix C_{18} (250 × 4.6 mm, 5 µm)
Mobile Phase	55: 45: 0.03v/v, (0.02M Phosphate buffer: Acetonitrile: Triethylamine)
Ph	4.2 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	8.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	235
Drugs RT (min)	2.40 & 5.71

DEVELOPED CHROMATOGRAM

Analysis

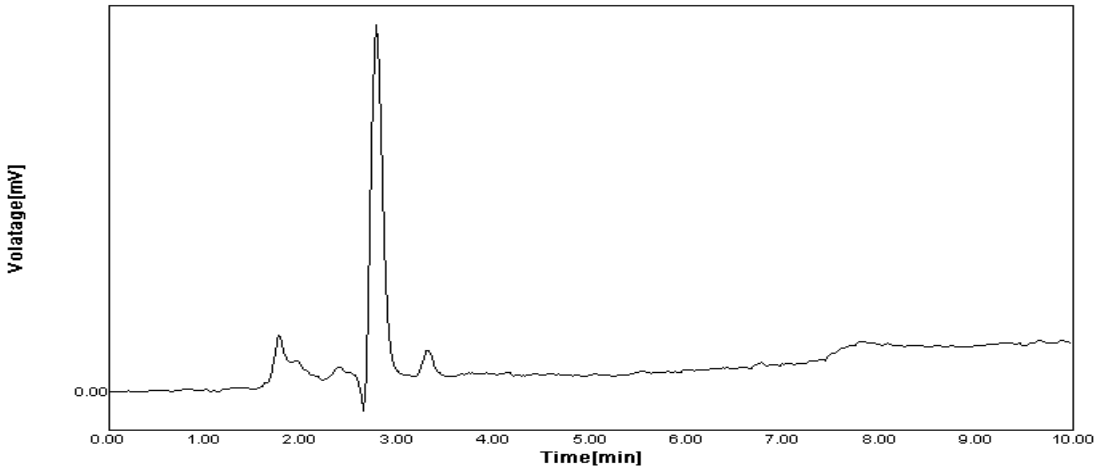
Sample: Blank

File: 0278.RAW

Scale Factor: 1.0000

Channel: 1. UV730D A

Blank Chromatogram



RESULT

No.	Name	RT[min]	Area[µV*s]	TP	TF	Resolution
Sum			0.0000			

Standard Chromatogram

Analysis

Sample Name: Olanz-Fluox-STD

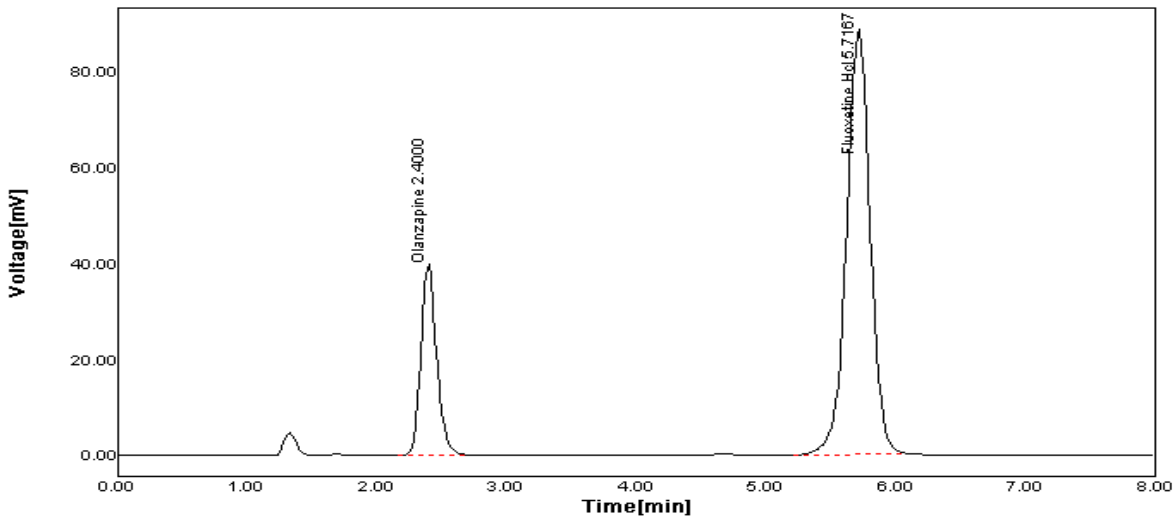
Sample ID:

File: 0500.RAW

Date: 2004-02-15 AM 03:00:19

Channel: 1. UV730D A

Chromatogram



Result

No.	Name	RT[min]	Area[µV*s]	TP	TF	Resolution
1	Olanzapine	2.4000	332440	2796.6	1.1250	0.0000
2	Fluoxetine Hcl	5.7167	1124680	3860.3	0.9643	11.1819
Sum			1457120			

Table No. 2: System Suitability for Olanzapine and Fluoxetine Hcl.

Conc. of Olanz. & Fluox.	Injection	Area of Olanz.	RT	Area of Fluox.	RT
100 & 100ppm	Inj-1	333861	2.366	1123829	5.71
	Inj-2	335624	2.34	1125267	5.7
	Inj-3	334725	2.368	1124072	5.68
	Inj-4	333910	2.352	1121279	5.69
	Inj-5	336541	2.36	1123785	5.71
	Inj-6	334762	2.366	1123547	5.73
Statistical Analysis	Mean	334903.8	2.358667	1123630	5.703333
	SD	1032.11	0.010857	1301.756	0.017512
	% RSD	0.308181	0.460287	0.115853	0.307047
	Tailing Factor	1.12		0.9643	
	Plate Count	2796.6		3860.3	

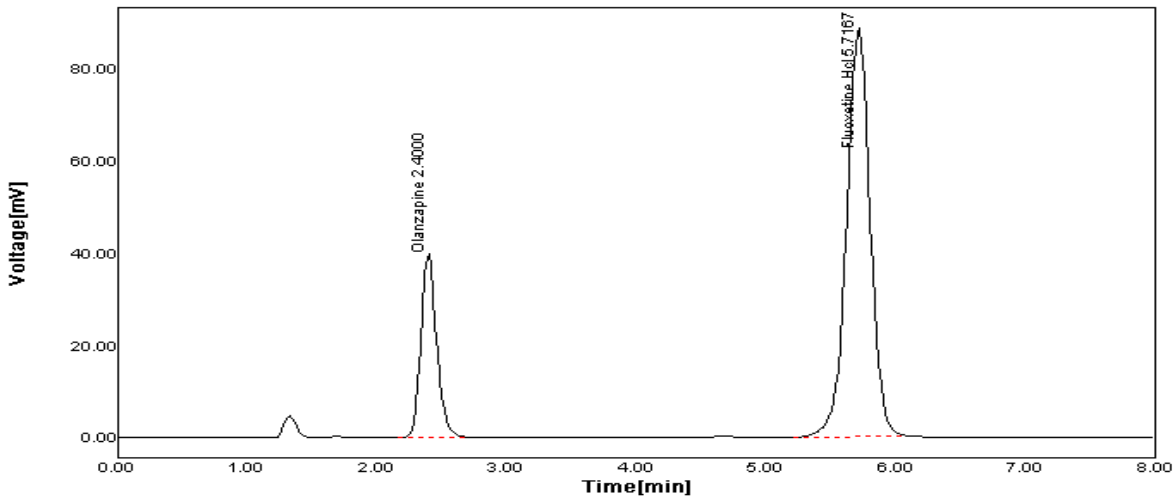
Table.3: Summary of results of Method Precision parameter for Olanzapine and Fluoxetine Hcl.

	inj-1	inj-2	Avg	MEAN	SD	% RSD
OLANZAPINE						
MP-1	339173	338252	338712.5	336701.75	1986.3122	0.58993225
MP-2	339737	336726	338231.5			
MP-3	337826	335926	336876			
MP-4	336738	337826	337282			
MP-5	332936	334728	333832			
MP-6	334826	335727	335276.5			
FLUOXETINE HCL						
MP-1	1118836	1120876	1119856	1122599.3	1870.2828	0.16660288
MP-2	1122816	1123925	1123370.5			
MP-3	1121274	1124680	1122977			
MP-4	1120914	1123817	1122365.5			
MP-5	1122924	1124680	1123802			
MP-6	1124624	1121826	1123225			

Table No.4: Summary of results of Injection Precision parameter for Olanzapine and Fluoxetine Hcl.

	Olanzapine	Fluoxetine Hcl
I.P-1	335674	1125624
I.P-2	337648	1123624
I.P-3	336864	1124680
I.P-4	337648	1123728
I.P-5	338763	1124936
I.P-6	335825	1122937
Mean	337070.3333	1124254.833
SD	1189.59601	993.132502
% RSD	0.352922192	0.088336956

Chromatogram-1



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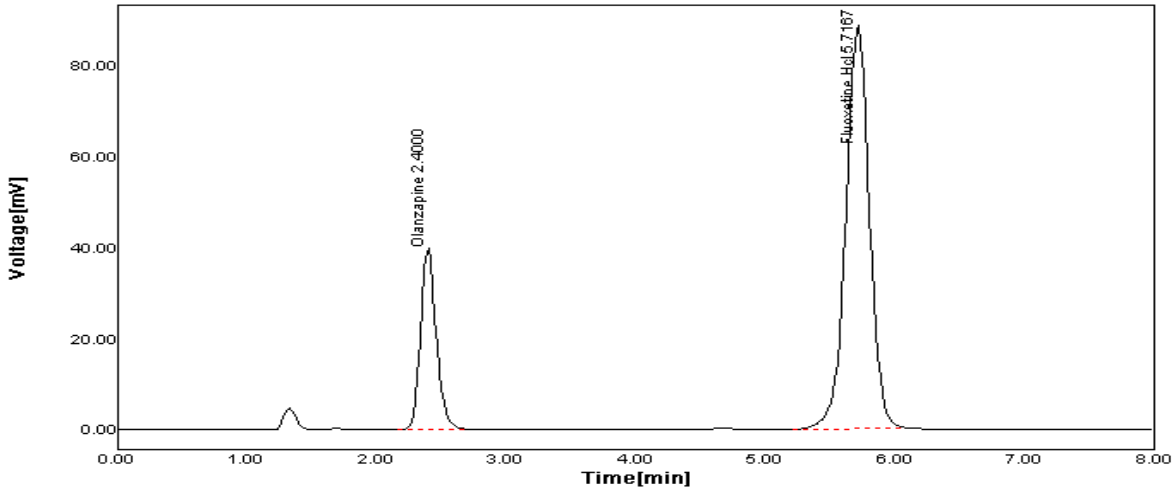

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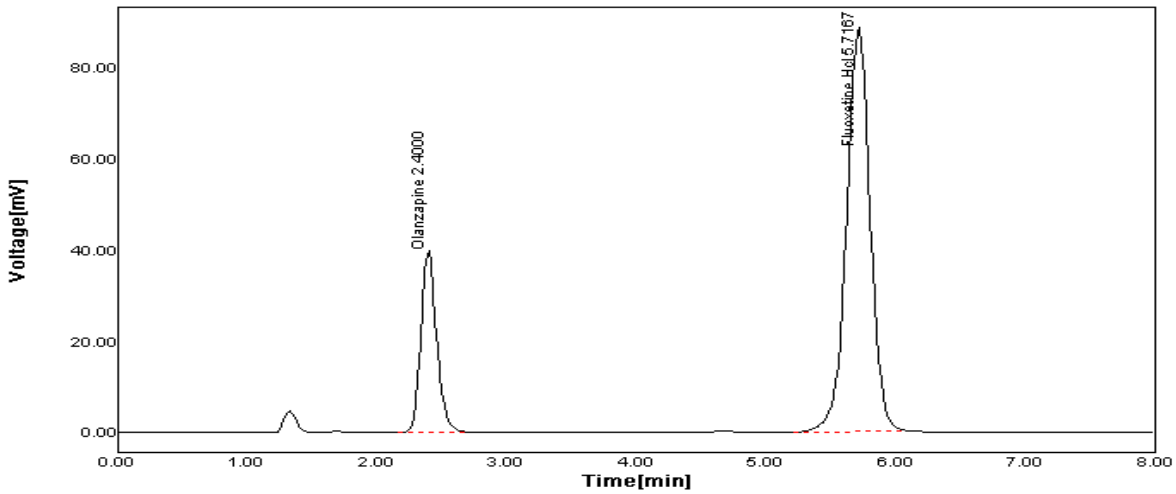

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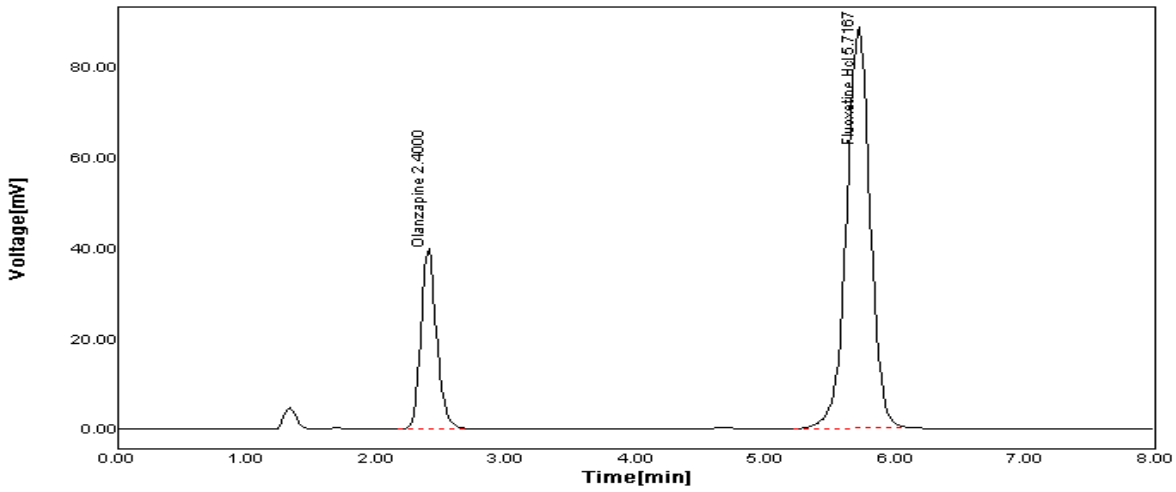

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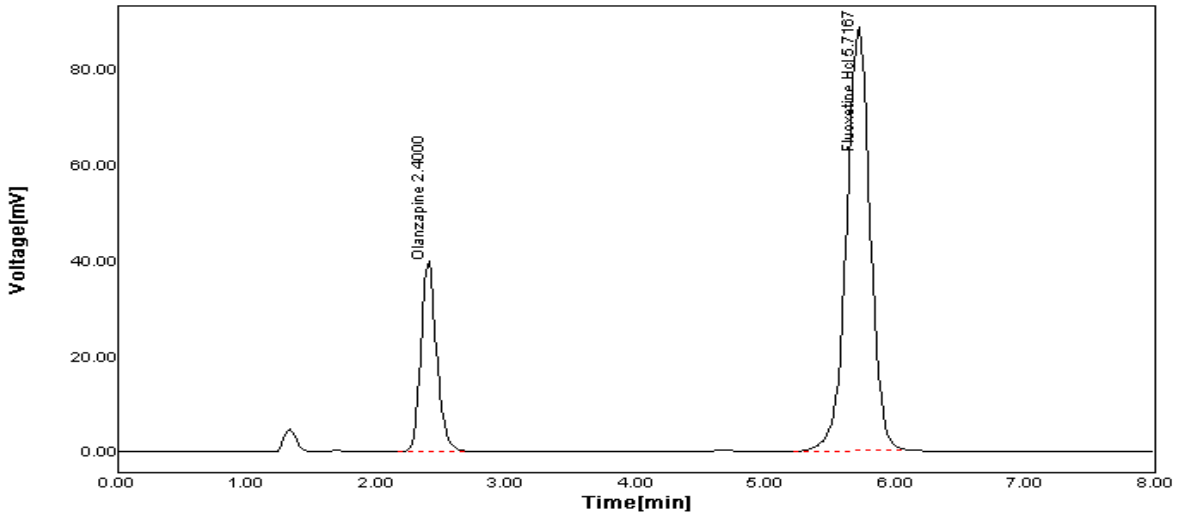

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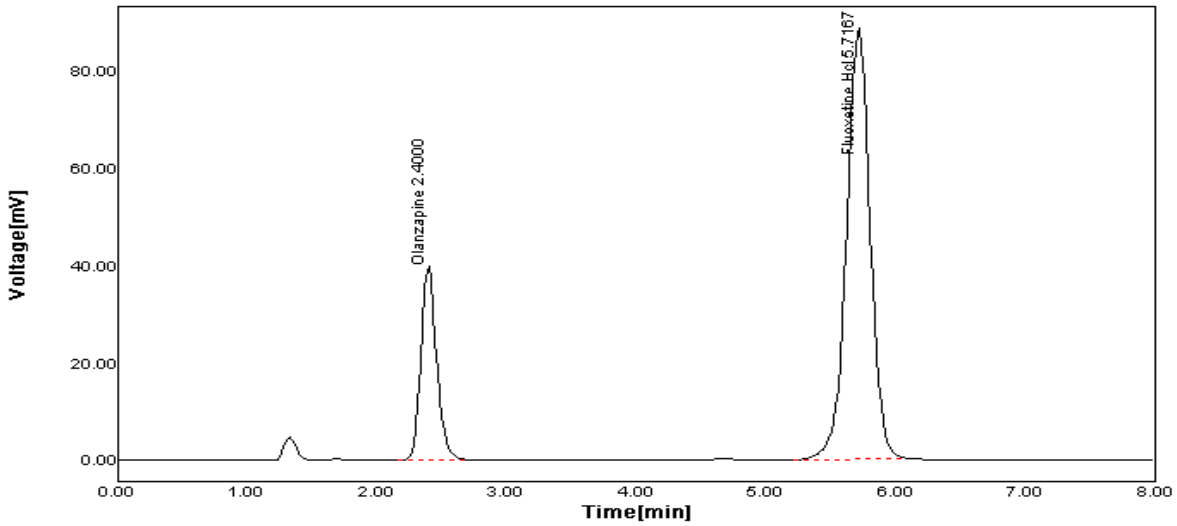
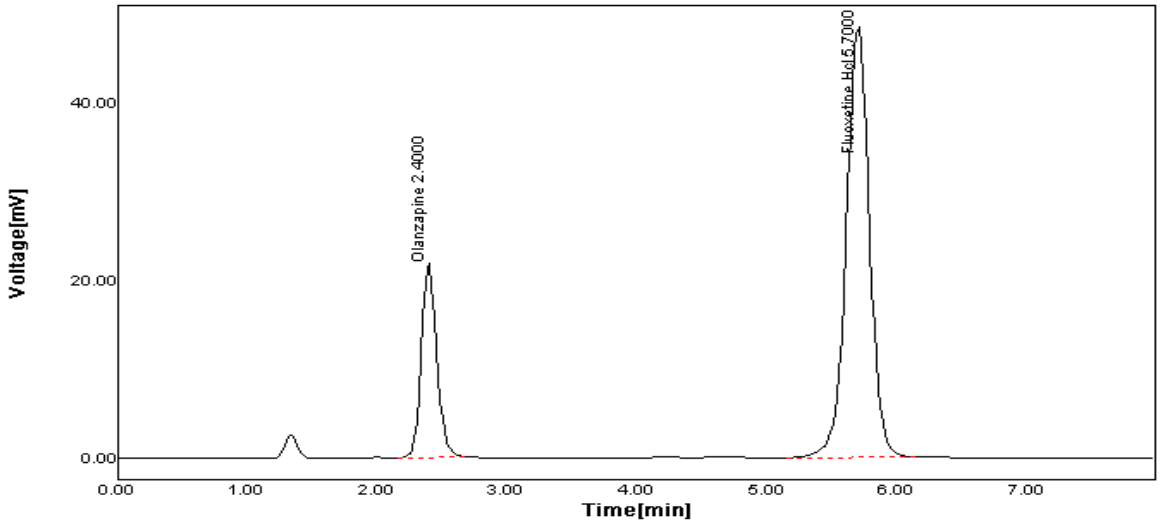
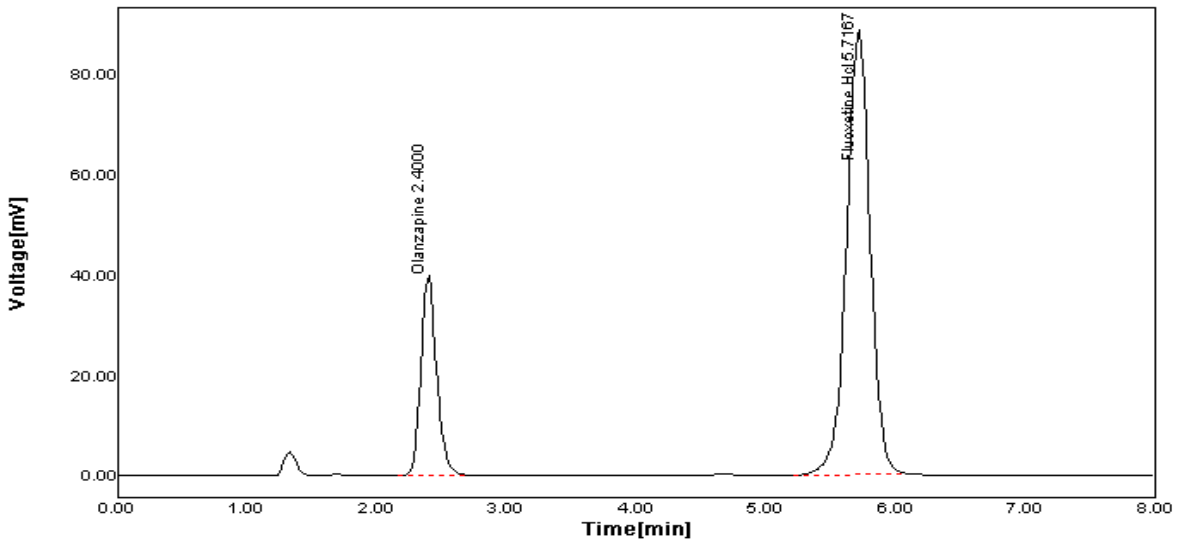


Table No. 5: Summary of results of Accuracy parameter for Olanzapine and Fluoxetine Hcl.

Olanzapine								
Conc.		**inj-1**	**inj-2**	**inj-3**	**Mean**	**% Recovery**	**STD**	**% RSD**
50pm	50%	162054	163726	164026	163268.7	98.22444	1062.573	0.650813
100ppm	100%	331908	332099	332281	332096	99.89652	186.5181	0.056164
150ppm	150%	503927	504836	504437	504400	101.1511	455.6281	0.090331
Fluoxetine Hcl								
50ppm	50%	552286	558397	554680	555121	98.71626	3079.276	0.554704
100ppm	100%	1123798	1121937	1124873	1123536	99.89828	1485.432	0.13221
150ppm	150%	1717658	1707574	1726757	1717330	101.7966	9595.714	0.558758


Olanzapine 2.4000
Fluoxetine Hcl 5.7000
Voltage[mV]
Time[min]


Olanzapine 2.4000
Fluoxetine Hcl 5.7167
Voltage[mV]
Time[min]

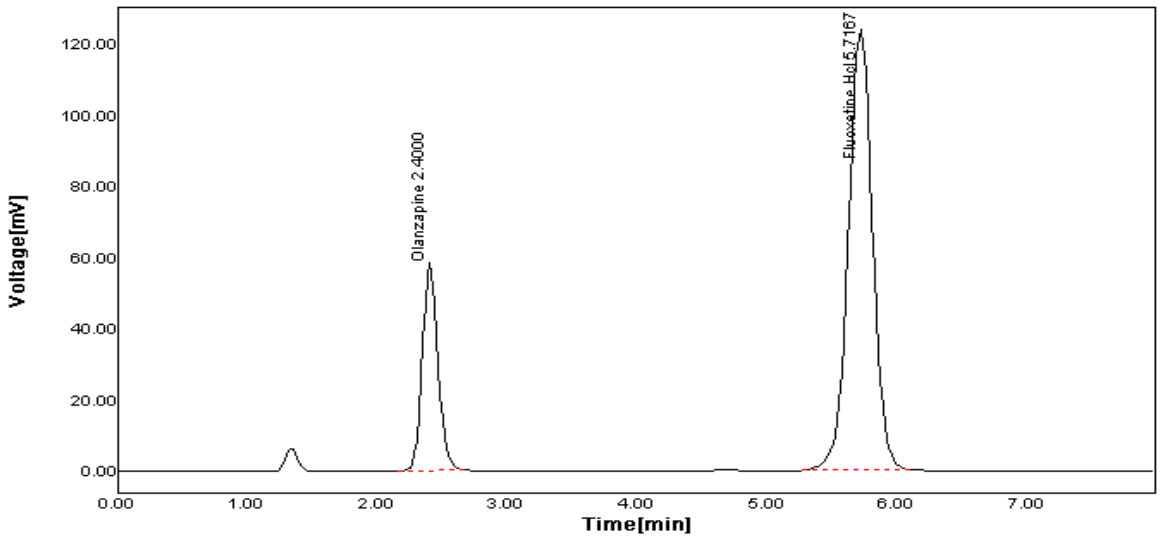

Olanzapine 2.4000
Fluoxetine Hcl 5.7167
Voltage[mV]
Time[min]

Table No.6: Summary of results of Linearity parameter for Olanzapine and Fluoxetine Hcl.

Olanzapine Conc. (ppm)	Average	Fluoxetine Hcl Conc. (ppm)	Average
10	48542	10	120868
20	72946	20	237895
50	170519	50	594273
100	332440	100	1124680
150	509364	150	1696443
200	674238	200	2196346

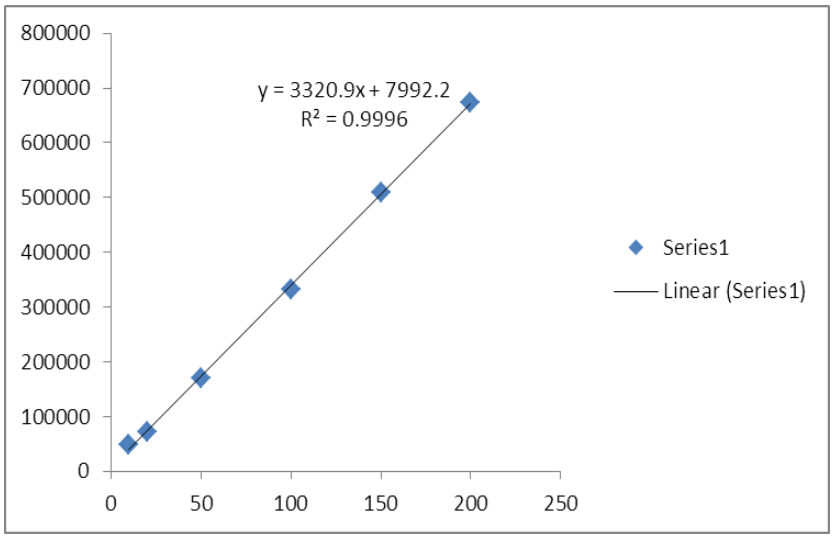


Fig. 1: Linearity Curve of Olanzapine.

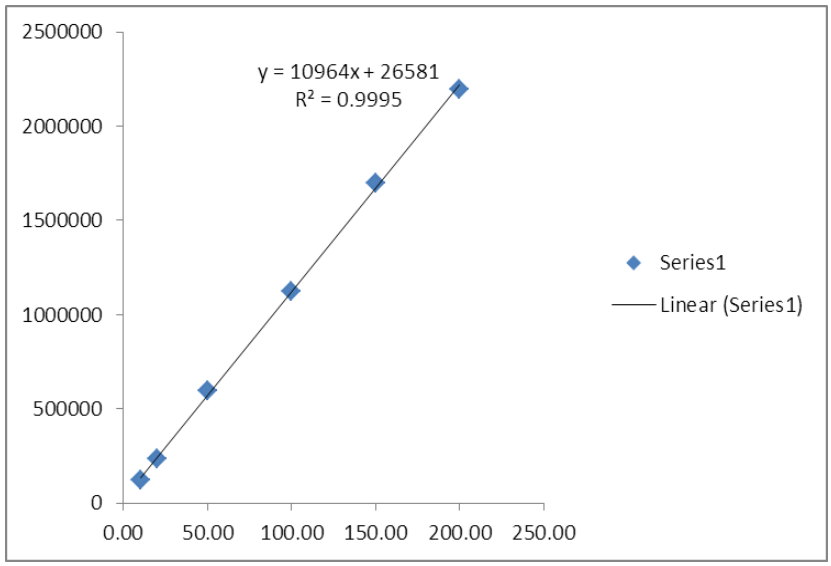
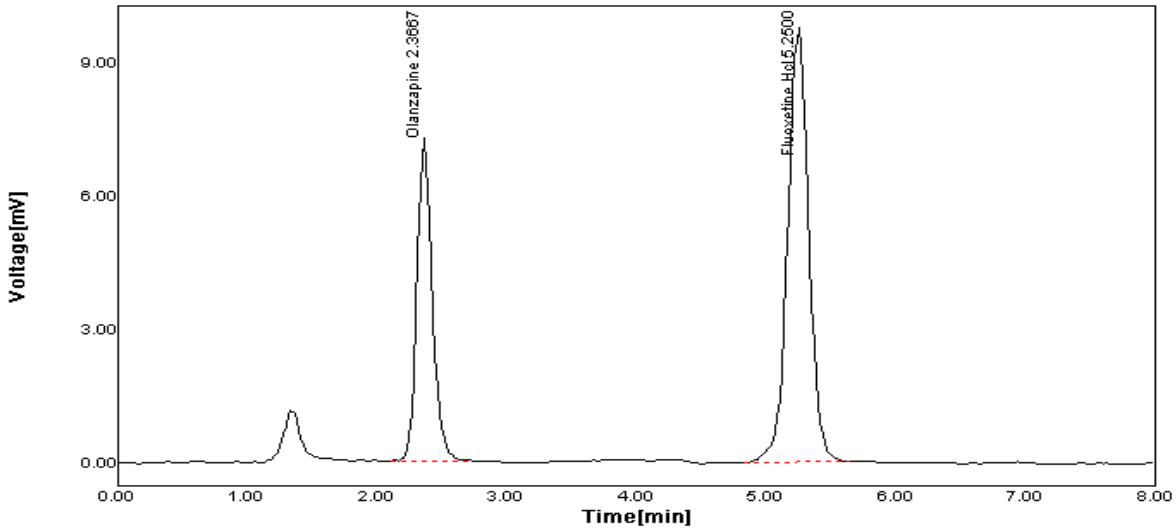
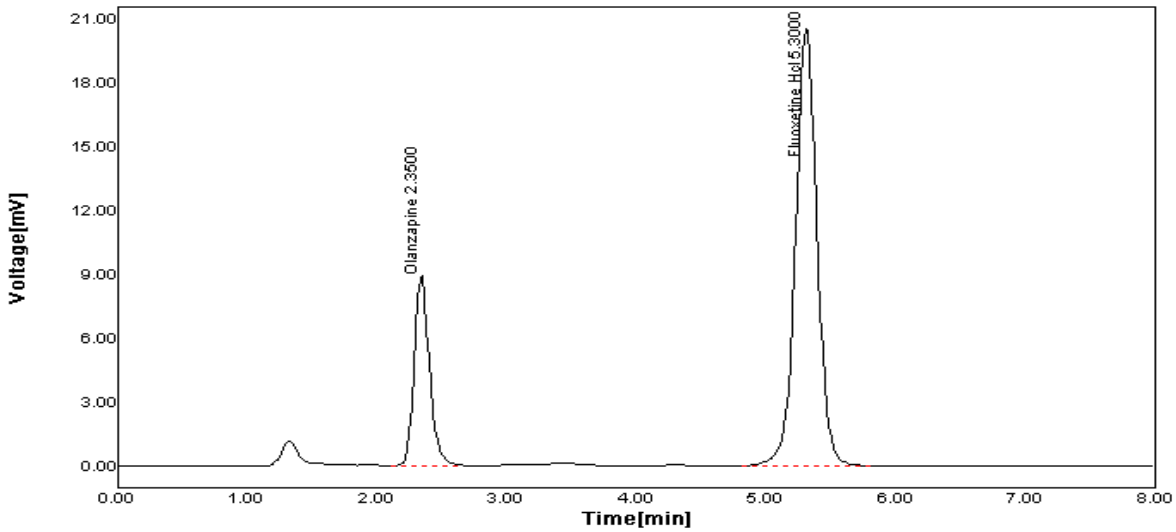
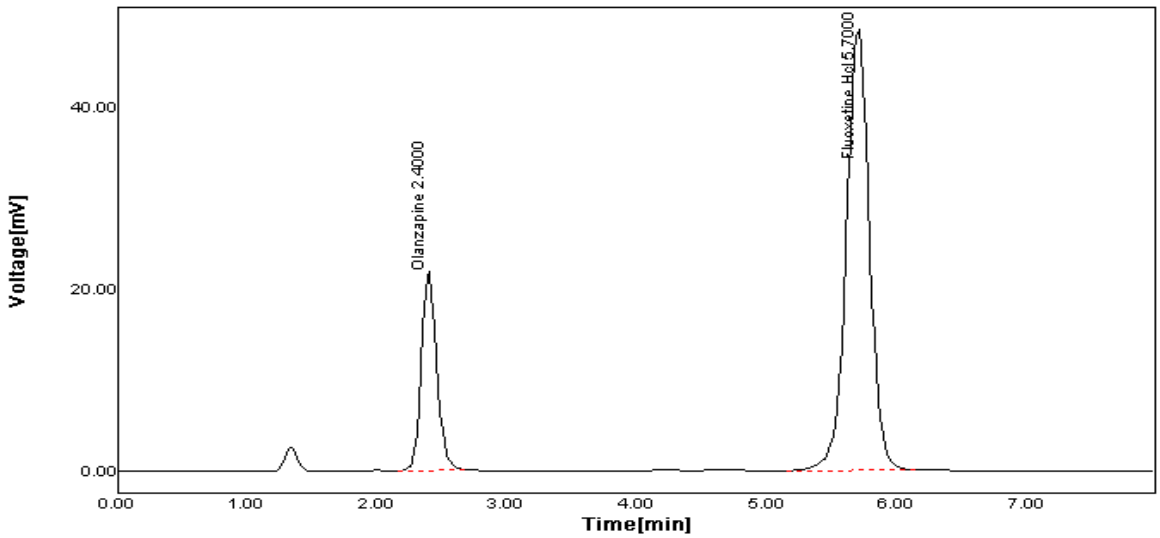
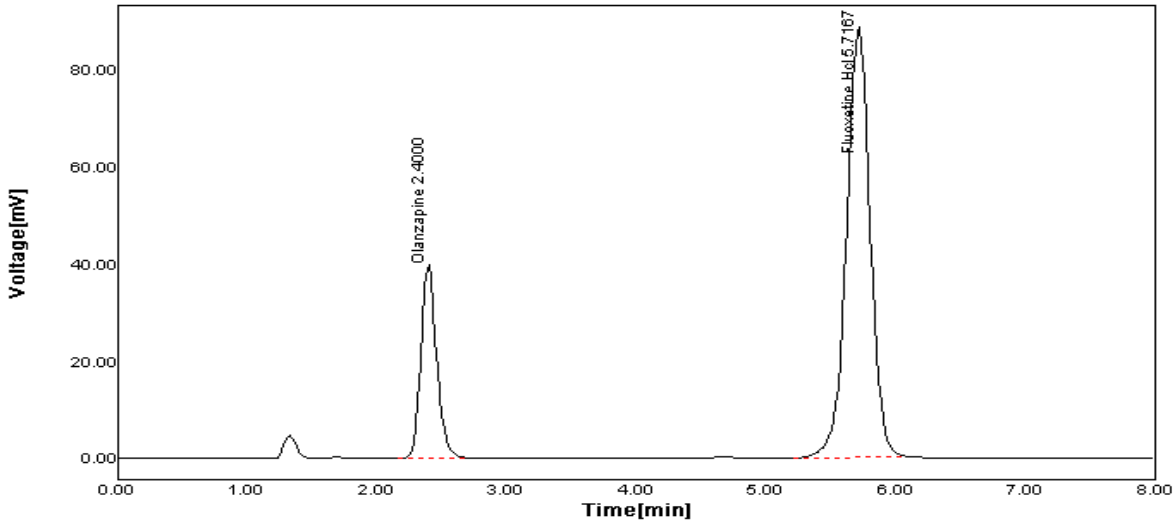


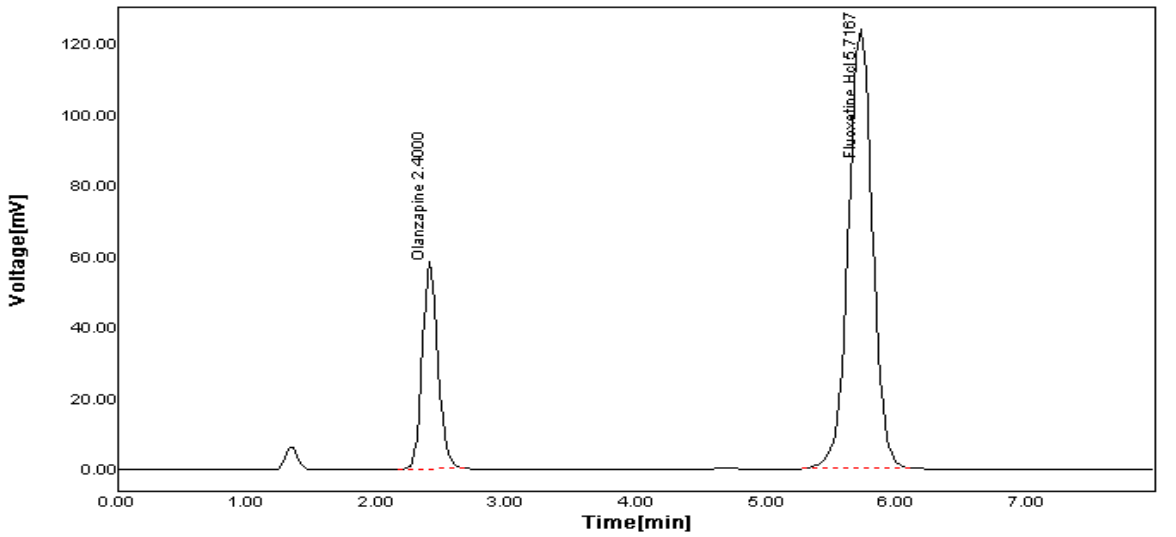
Fig. 2: Linearity Curve of Fluoxetine Hcl.


Olanzapine 2.3667
Fluoxetine Hcl 5.2500
Voltage[mV]
Time[min]


Olanzapine 2.3500
Fluoxetine Hcl 5.3000
Voltage[mV]
Time[min]


Olanzapine 2.4000
Fluoxetine Hcl 5.7000
Voltage[mV]
Time[min]


Voltage[mV]
80.00
60.00
40.00
20.00
0.00
Olanzapine 2.4000
Fluoxetine Hcl 5.7167
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Time[min]


Voltage[mV]
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Olanzapine 2.4000
Fluoxetine Hcl 5.7167
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
Time[min]

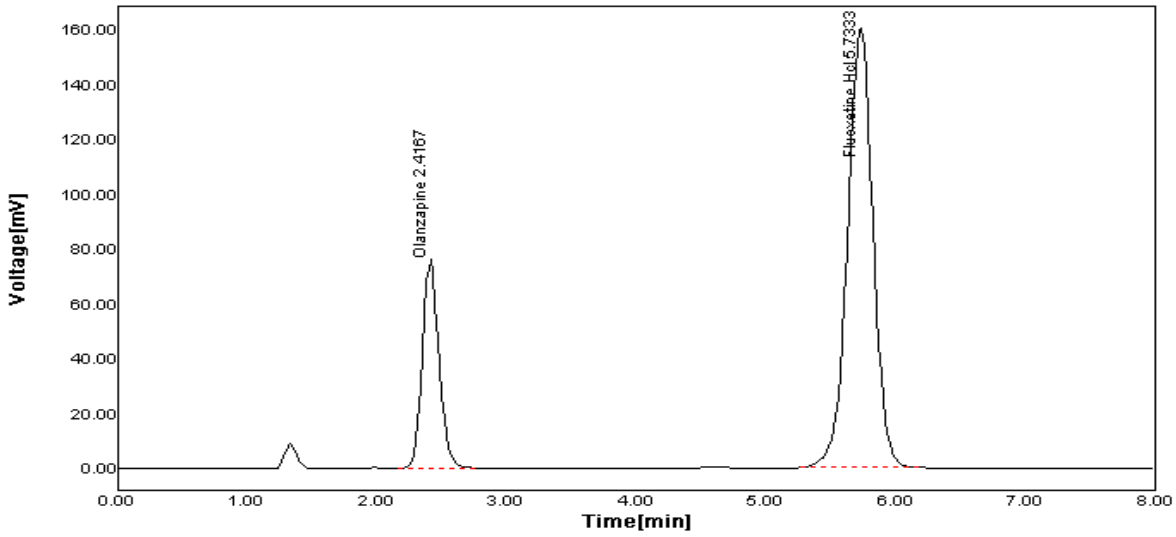
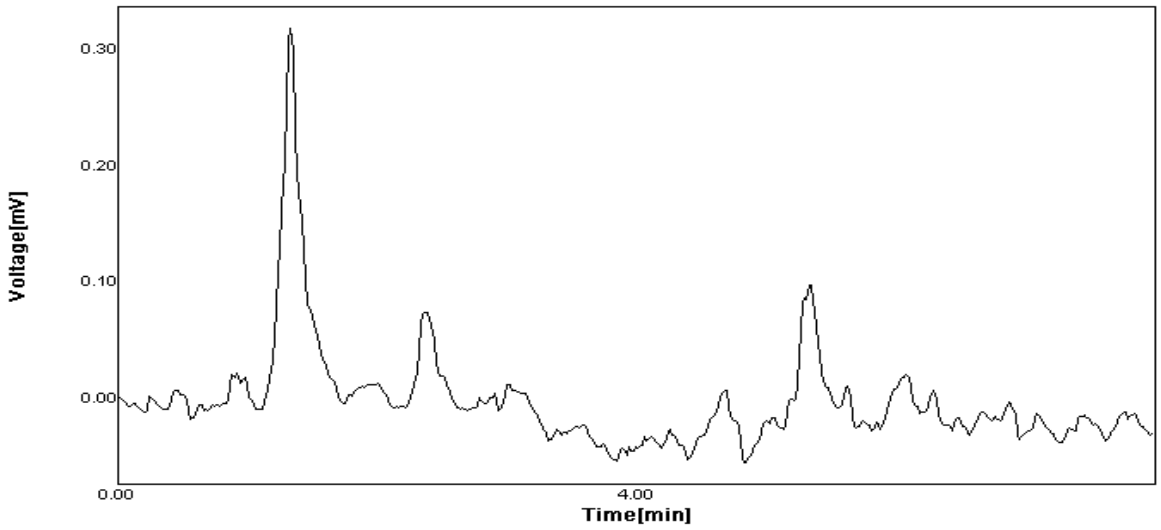

Voltage[mV]
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Olanzapine 2.4167
Fluoxetine Hcl 5.7333
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Time[min]

Table No. 7: Summary of results of LOQ for Olanzapine and Fluoxetine Hcl.

Injection	Area of Olanz. (0.5ppm)	Area of Fluox. (0.5ppm)
Inj-1	2574	5994
Inj-2	2573	5897
Inj-3	2605	5871
Inj-4	2581	5998
Inj-5	2573	5873
Inj-6	2617	5937
Mean	2587.167	5928.333
SD	19.08315	57.56967
% RSD	0.737608	0.971094

LOD: (0.15 & 0.15ppm)



LOQ: (0.5 & 0.5ppm)

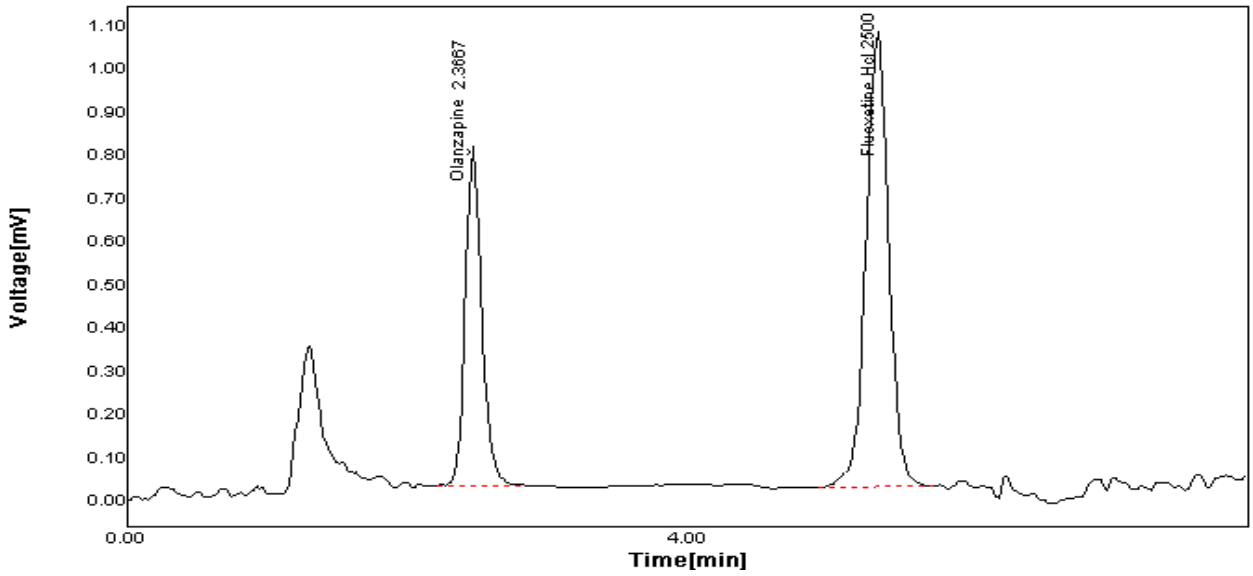


Table No. 8: Summary of results of Robustness parameter for Olanzapine and Fluoxetine Hcl.

Olanzapine					
Parameters	**Adjusted TO**	**Avg. Area[a]**	**RT**	**SD**	**% RSD**
Flow Rate As per method 1.0ml/min	0.9	413460	2.84	1357.18	0.33
	As it is	334903.83	2.36	1032.11	0.31
	1.1	245673.67	1.93	2675.91	1.09
Mobilephase comp[n] (55: 45: 0.03v/v, Buffer: Acetonitrile: Triethylamine)	50:50:0.03	385716.50	2.06	3784.67	0.98
	As it is	334903.83	2.36	1032.11	0.31
	60:40:0.03	296583.33	2.73	3097.23	1.04
Fluoxetine Hcl					
Flow Rate As per method 1.0ml/min	0.9	1570501.7	6.21	12826.87	0.82
	As it is	1123629.8	5.70	1301.76	0.12
	1.1	888153	5.12	7390.12	0.83
Mobilephase comp[n] (55: 45: 0.03v/v, Buffer: Acetonitrile: Triethylamine)	50:50:0.03	1247538.83	5.26	15528.59	1.24
	As it is	1123629.8	5.70	1301.76	0.12
	60:40:0.03	977340.17	6.15	8126.60	0.83

[a]Avg. Area = Six Repeatable injections.

CONCLUSION

An isocratic reverse phase High Performance Liquid Chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of olanzapine and fluoxetine in bulk and its pharmaceutical formulation.Seperation was achieved with a Phenomenix C_{18} (250 × 4.6 mm, 5 µm) and 55: 45: 0.03v/v, (0.02M Phosphate buffer: Acetonitrile: Triethylamine) as eluent at flow rate 1ml/min. uv detection was performed at 235nm. The method is simple rapid and selective. The described method is linear over a range of10µg/ml to 200µg/ml. The method precision for the determination of assay for olanzapine and fluoxetine respectively was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations. As a very little amount of organic solvents are used for elution the method is proved to be economic and ecofriendly too.

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