Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Olanzapine and Fluoxetine Hydrochloride in Bulk and Combined Tablet Dosage Form

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ABSTRACT: Stability indicating RP-HPLC method for evaluating Olanzapine (OLNZ) and Fluoxetine Hydrochloride (FLUX) in combination tablet dose form developed and validated. In this study, an isocratic reverse phase-high performance liquid chromatography (RP-HPLC) method for simultaneous quantification of OLNZ and FLUX in bulk and pharmaceutical dosage forms was developed and validated. Force degradation study are analyse by loaded the drug substances to stress condition Finally the separation and quantification of drugs from degradation products was determined on a Hypersil BDS C18 column (4.6 x 250 mm, 5μ) prepared mixture of phosphate buffer Ph 6 by OPA and acetonitrile in the proportion of 55:45 (v/v) mobile phase ,flow rate was 1.0 mL/min, temperature of coloum was designed at 25 0C, and also detection at 228 nm. Retention time of OLNZ 4.7min and FLUX 7.4 min achieved. The recovery evaluation was found satisfactory and the correlation coefficient were OLNZ (0.99) and FLUX (0.99) , which is indicates the linearity of methods was within the specification limits. The developed analytical method can used for regular simultaneous estimation and quantification of OLNZ and FLUX in bulk dosage form excluding any intervention of excipients.
KEYWORDS: Olanzapine, Fluoxetine Hydrochloride, HPLC, Validation.

I INTRODUCTION:

The Olanzapine is a yellow Crystaline powder having molecular wight 312.43 which is soluble in Acetonitrile, Methanol,Water,Chloroform. Olanzapine's act as antipsychotic which is not a known acitivity. It Antagonism of dopamine and serotonin receptors may be involved. Dopamine receptor antagonistism is linked to extrapyramidal consequences, such as tardive dyskinesia (TD), as well as therapeutic outcomes. Antagonism of muscarinic acetylcholine receptors is linked to anticholinergic another effects such dry mouth and constipation, as well as the suppression or reduction of extrapyramidal effects during the course of treatment, but it does not protect against the onset of tar dive dyskinesia..[1,2,3,4,7]

Fluoxetine HCl is a white crystalline powder with molecular weight 345.8 and which is soluble in acetonitrile,water,Trtrahydrofuran,dimethyl formamide. Fluoxetine HCl is dopamine reuptake at therapeutic doses and also a selective serotonin reuptake inhibitor (SSRI) It causes serotonin to last longer once it is produced by delaying its reuptake. A considerable rise in synaptic norepinephrine and dopamine has been observed in rats after large dosages. Dopamine and norepinephrine may thus play a role in Fluoxetine HCl's antidepressant activity in humans at supratherapeutic doses (60–80 mg). This impact could be mediated by 5HT2C receptors, which are blocked by Fluoxetine HCl at greater dosages.[6,8,9,10,14]

II EXPERIMENTAL WORK:
Standard solution of Olanzapine and Fluoxetine HCl
Weighed quantity about 50 mg of Olanzapine and Fluoxetine HCl and transferred into different two 100 ml volumetric flask, added 70 mL, water: acetonitrile (50:50) ,Sonicated for 10 Min , diluted upto the mark with the same solvent. Further diluted 10 ml of above solution to 50 ml of volumetric flask and diluted with using same solvent.
Determination of λ max of Olanzapine and Fluoxetine HCl
The 20μg/ml standard solution of Olanzapine was scanned in the scale of 200-400 nm and λ max was observed at 228 nm against Buffer Solution pH 6.0

acetonitrile (55:45). Same as, for Fluoxetine HCl also observed at same lambda i.e. 228 nm. Hence the isobestic point 228 nm was selected as wavelength for RP-HPLC analysis of same drugs. Simultaneous quantification of Olanzapine and Fluoxetine HCl in Bulk Drug and Tablet Dosage form by using RP-HPLC method.

Preparation of Solution:
Preparation of Buffer solution:
2.74 gm of Sodium dihydrogen phosphate monohydrate and 4ml Triethylamine was added to 1000 ml in Milli-Q water. The pH was adjusted to 6.0± 0.05 with OPA.
Mobile phase:
Prepare a mixture of Buffer pH 6.0 and acetonitrile in the proportion 55:45 v/v, respectively. Mix well and sonicated for 15 mins. Prepared mobile phase was then filtered through 0.45μm nylon membrane filter.
Diluents: Prepared mobile phase Buffer: Acetonitrile (55:45 %v/v)
Stock solution of Olanzapine and Fluoxetine HCl:

Accurately weighed quantity of Olanzapine and Fluoxetine HClof about 20mg and 80mg is added into each 100 ml volumetric flask, about 30 ml of diluent was added and sonicated to dissolved above drugs.
Standard preparation:
5 ml of stock solution of was diluted out and added to 50 ml volumetric flask and make volume up to up to the mark with diluents.(Concentration of Olanzapine:20ppm and of Fluoxetine HCl:80 ppm)
Sample preparation:
Accurately weighed twenty tablets; the average weight were determined. Then tablets were crushed to fine powder and an accurately weighed powder equivalent to 20 mg of Olanzapine was added into a 100volumetric flask. Afterwards, 70 ml of diluents was added, with intermittent swirling and sonicated for 20 minutes, stay it to cool and made up to volume with diluent and mix. Allowed to settle for 5 minutes, after that filter with 0.45μ syringe filter discarding first few ml. Further 5ml of filtrate was diluted to 50 ml with diluent and mix.
Method of validation:
System appropriateness was determined by injecting a standard solution (100 g/ml) six times and recording the chromatograms. The percent RSD for the area reaction from six duplicate injections of Standard solution should be less than 2.0 percent. In Standard solution, the tailing factor

for Olanzapine and Fluoxetine HCl should be less than 2.0. .[4,5,11,12]
Specificity: The ability to assess the analyte definitively in the presence of components that might be present is referred to as specificity. The capacity of an analytical procedure to quantify the analyte of interest precisely and particularly without influence from blanks or placebos is referred to as specificity. .[13,15,16]
There is no peak observed in blank and placebo at the retention time of Olanzapine and Fluoxetine HCl.From above data it is concluded that peak of Olanzapine and Fluoxetine HCl in standard and sample are pure.

FORCED DEGRADATION STUDY:
Tablets and placebos were submitted to various stress settings to conduct forced degradation tests in order to determine whether analytical method for the assay was stability-indicating or not. As specified in ICH Q1A, stress tests were conducted under acid/base hydrolysis, oxidation, and heat conditions (R2). Drug compounds and drug products were degraded in the solid state using thermal and UV radiation. Several trials were carried out with varying degrees of intensity for each stressful scenario, resulting in a degradation of up to 5-30 percent. .[17,18,19,20,21]
Photo degradation
20 Tablets, Olanzapine and Fluoxetine HCl 100mg drug were taken in separate Petri Plate and exposed it under UV light for 48 hrs and an integrated near UV energy of NLT 200 w/s.
Thermaldegradation
20 Tablets, 100 mg of each Olanzapine and Fluoxetine HCl drug were taken in separate Petri Plate and exposed it under 80℃ in hot air oven fora period of 48 hours for dry heat sterilization.
Acid degradation
Forced degradation was performed for Tablet, Olanzapine and Fluoxetine HCl drugs under acidic condition. 0.1N HCl (0.85ml concentrated HCl in 100ml of water) was prepared for this study.
Alkalidegradation
Forced degradation was performed for Tablet, Olanzapine and Fluoxetine HCl drugs under alkali condition. 0.1N NaOH (0.4gm NaOH in 100ml of water) was prepared for study.
Oxidation degradation
Forced degradation was performed for Tablet, Olanzapine and Fluoxetine HCl drugs under alkali condition. 10% H_2O_2 (33ml H_2O_2 in 100ml of water) was prepared.
Hydrolysis Degradation

Forced degradation was performed for Tablet, Olanzapine and Fluoxetine HCl drugs under alkali condition.

Linearity

To get the desired analyte concentrations, standard solutions were created by diluting known amounts of intermediate stock solution with the diluent, as described in Table. The regression coefficient 'r2', the y-intercept, and the slope of the regression were derived using a graph of Concentration (ppm) vs. Area. System suitability criteria should be fulfilled. Response should be linear. Co-relation coefficient (R) should not be less than 0.999% Limit of Y- intercept should be within ±2.0% of the corresponding Y-co-ordinate of the working level.[1,2,3,6,11,12,17,18,19]

Table 01. Preparation of linearity samples

Linearity Level%	Volume taken from Linearity stock solution (mL)	Total volume (mL)	Concentration(ppm)	
			Olanzapine	Fluoxetine HCl
50	2	50	10	40
75	3	50	15	60
100	4	50	20	80
125	5	50	25	100
150	6	50	30	120

Accuracy (Recovery Study): Accuracy was measured from 50% to 1500% of the sample concentration. A calculated amount of Olanzapine and Fluoxetine HCl was added to the placebo to achieve sample concentrations of 50 percent, 75 percent, 100 percent, 125 percent, and 150 percent.

Table 02: Sample preparations for accuracy

Concentration (%)	Olanzapine(mg)	Fluoxetine HCl(mg)	Placebo(mg)
Level 1 (50%)	10	45	1134
Level 2 (75%)	15	66.75	1134
Level 3 (100%)	20	90	1134
Level 4 (125%)	25	111.2	1134
Level 5 (150%)	30	133.5	1134

Acceptance criteria
System suitability criteria should be fulfilled. Mean recovery for 50% to 150% should be in the range of 99.83% - 100.66% and individual recovery for 50% to 150% should be in the range of 100.51% - 100.95%.
Precision:

Precision of System :

To guarantee that the analytical system was working properly, the precision of the system was tested using a standard substance. Six times a standard solution (100g/ml) was injected, and chromatograms were taken. Five determinations' retention time and area were measured, and the percent RSD was determined. The RSD of the peak area derived from six replicate injections should be less than 2.0%.

Precision of the method (Repeatability):
When the procedure is applied repeatedly to many samplings of homogenous samples, the precision of the analytical method is the degree of agreement

among individual test findings. Six replicate injections of assay concentration (100g/ml) of standard and sample solutions were analysed for method precision. The RSD for Olanzapine and Fluoxetine HCl percent Assay of six independent samples should not exceed 2.0 percent.

Robustness: The standard prepreation (100μg/ml) was injected six times for every varied conditions of flow rate, organic solvent, buffer pH, wavelength and chromatograms were recorded.System suitability criteria should be full filled.The absolute difference of % assay value in each modified condition should be within ± 2.0 when compared to the original condition.[13,19,20,21]

III RESULTS AND DISCUSSION:

For the determination of Olanzapine and Fluoxetine HCL from bulk and commercial formulations, a simple, precise, and cost-effective RP-HPLC method was devised and validated. Validation characteristics such as linearity, accuracy, precision, ruggedness, and robustness were used to validate the method according to ICH guidelines.

Selection of wavelength:
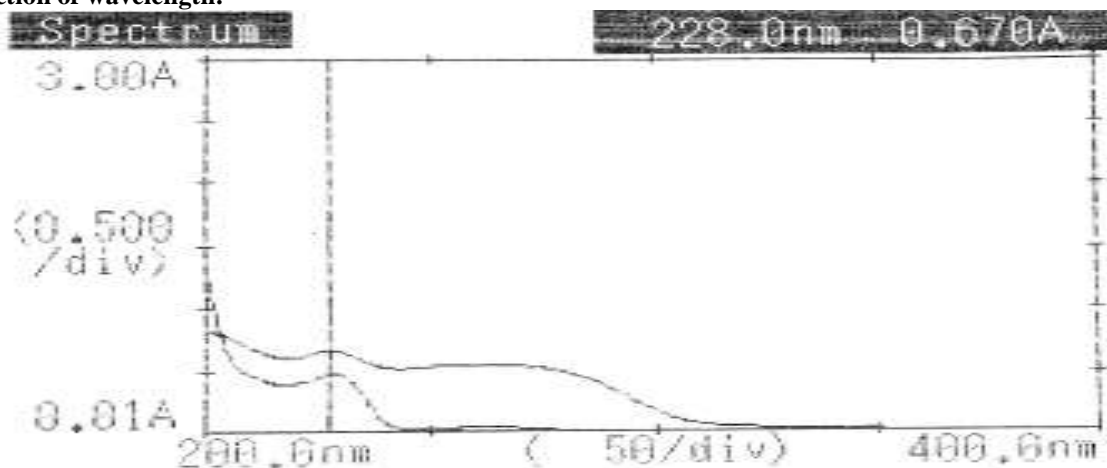
Fig 01:Overlain UVSpectra ofOlanzapine and Fluoxetine HCl Showingisobestic point at 228nm
Optimized method Table 01

Mobile phase	0.02M NaH$_2$PO$_4$+ 4ml TEA pH 6 by OPA (buffer) :Acetonitrile
Mobile phase concentration	55:45v/v
Injection volume	20μL
Diluent	Buffer : ACN(55:45)
Flow rate	1ml/min
Colum temperature	25^{0}C
Colum	Hypersil BDS (C18), 250 x 4.6 mm,5μ
Wavelength	228
Run Time	14 min
Elution	Isocratic

Fig 02:Chromatogram of Optimized method

METHOD VALIDATION
The validation study was carried out by International Conference on Harmonization.
System suitability:

Table 02: System suitability of Olanzapine

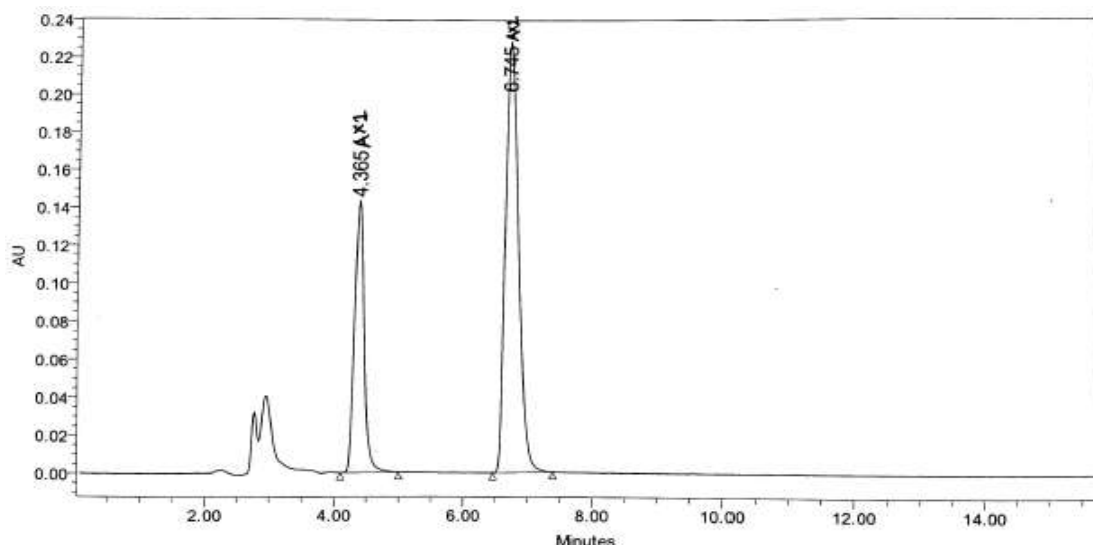| Injection No.	Injection Volume(µl)	RT (min)	Tailing Factor	Theoretical Plate	Peak Area
1	20	4.36	1.54	7739	735818
2	20	4.36	1.54	7789	733054
3	20	4.36	1.54	7856	735920
4	20	4.37	1.56	7935	739943
5	20	4.37	1.55	7991	734026
Mean		4.36	1.54	7862	735752
SD	2638.908				
%RSD	0.36				

Table 03: System suitability of Fluoxetine HCl

Injection No.	Injection Volume(µl)	RT (min)	Tailing Factor	Theoretical Plate	Peak Area
1	20	6.75	1.66	9280	1566935
2	20	6.75	1.66	9323	1565258
3	20	6.76	1.66	9322	1571773
4	20	6.76	1.66	9296	1574402
5	20	6.75	1.66	9296	1573986
Mean	20	6.75	1.66		1570471
SD	4158.782				
%RSD	0.26				

Specificity:

Table 04: Specificity of Olanzapine and Fluoxetine HCl

Component	Inj.Vol. (µL)	Retention Time (min)	Tailing Factor	Purity Threshold	Purity Angle	Peak Area	Peak Purity

Blank	20	-	-	-	-	-	-
Placebo	20	-	-	-	-	-	-
Standard Solution Olanzapine	20	4.36	1.49	0.318	0.132	739120	**Passes**
Standard Solution Fluoxetine HCL	20	6.75	1.47	0.339	0.137	1797765	**Passes**
Sample Solution Olanzapine	20	4.37	1.33	0.329	0.153	709969	**Passes**
Sample Solution Fluoxetine HCL	20	6.77	1.46	0.318	0.123	1758154	**Passes**

FORCED DEGRADATION STUDY:

Table 05: Degradation Study of Sample

| **Degradation Conditions**	**Purity Angle**		**Purity Threshold**		**Total Impurity Area**	**Total Area (including main peak)**	**Highest % Unknown Impurity**	**Total % Impurity**
	Olanzapine	**Fluoxetine HCL**	**Olanzapine**	**Fluoxetine HCL**				
Pure	0.153	0.123	0.329	0.318	8740	718709	0.855	1.216
Acid Stress	0.154	0.151	0.324	0.314	6855	729073	0.771	0.940
Alkali Stress	0.158	0.131	0.324	0.311	6852	731058	0.511	0.937
Oxidation	0.280	0.306	0.424	0.308	194171	612237	8.777	31.715
Heat Sol.	0.174	0.142	0.342	0.329	5996	717296	0.442	0.836
Thermal	0.155	0.137	0.324	0.318	4850	754973	0.270	0.642
Humidity	0.164	0.131	0.318	0.306	8457	734443	0.623	1.151
Photolytic	0.169	0.134	0.327	0.315	2043	746645	0.274	0.274
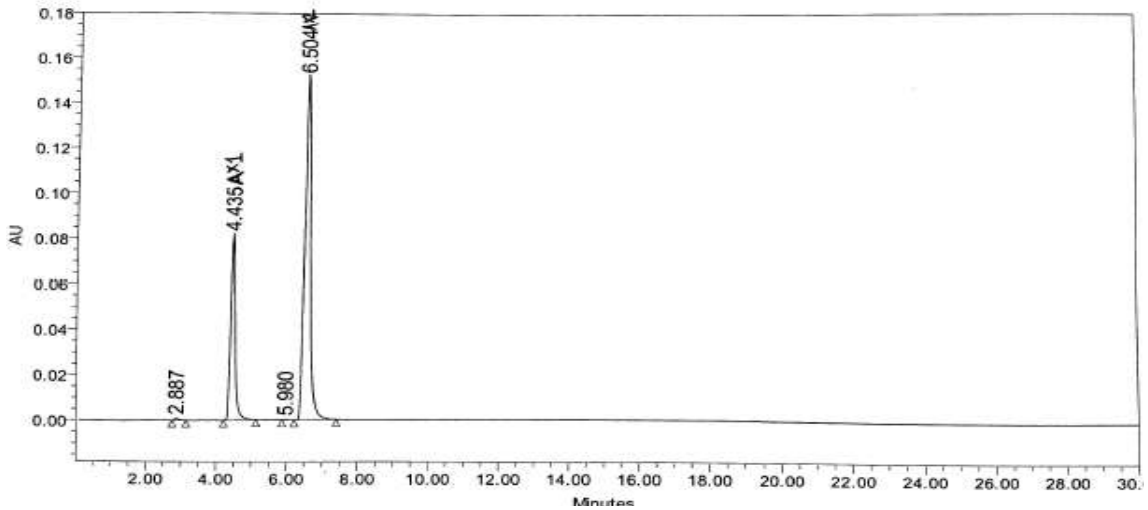
Fig 03: Chromatogram of Olanzepine and Fluoxetine HCL standard mixture
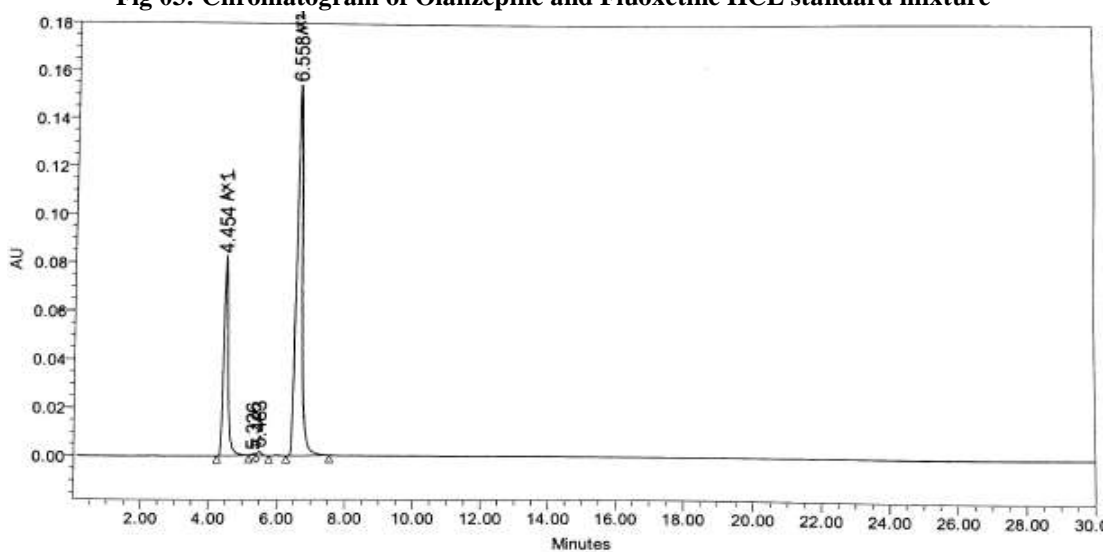

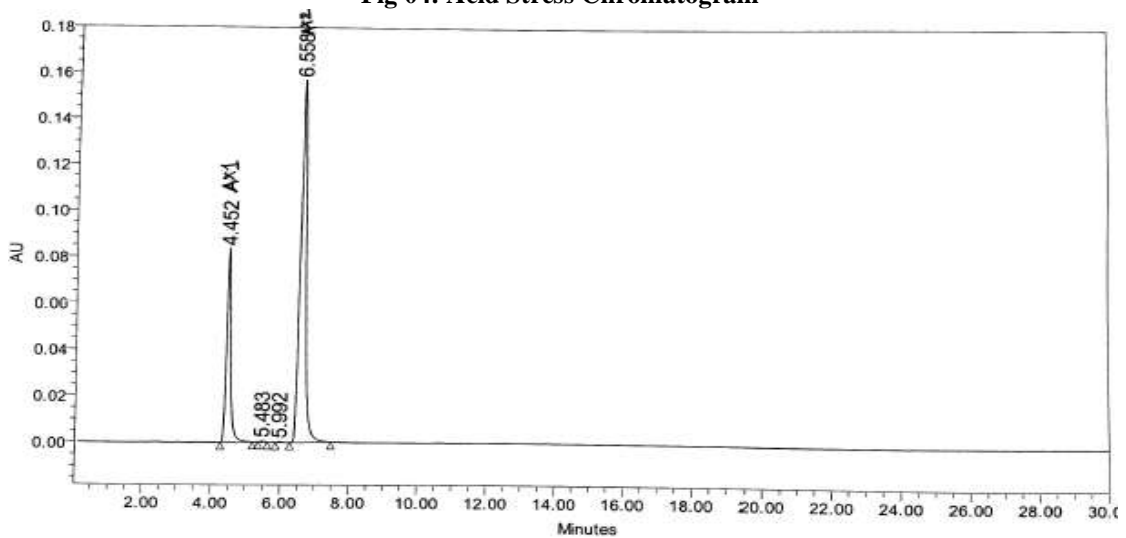**Fig 04: Acid Stress Chromatogram**

Fig 05: Alkali Stress Chromatogram
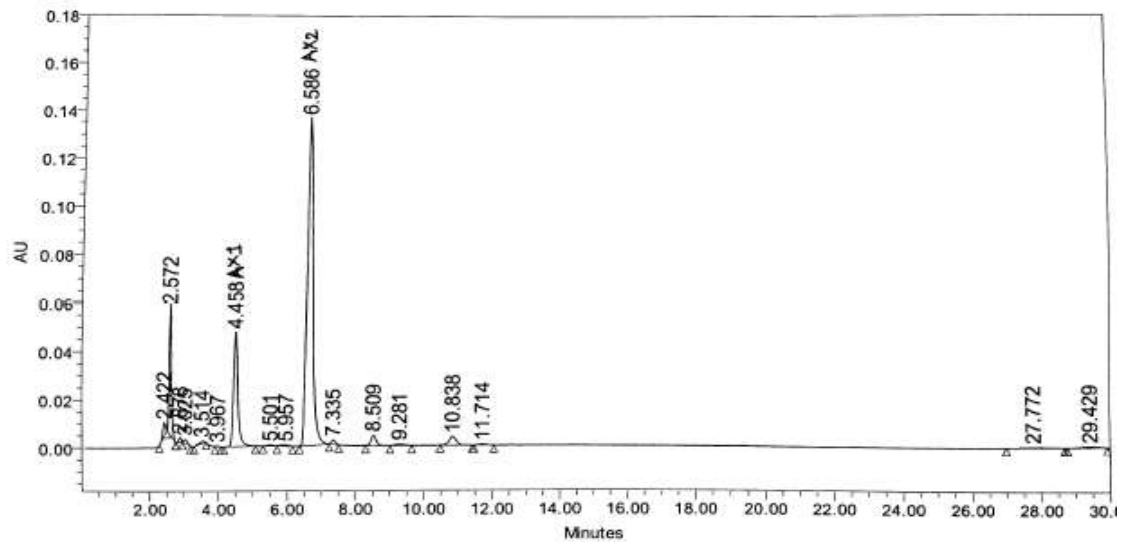
Fig 07: Oxidation Stress and Degradation Chromatogram
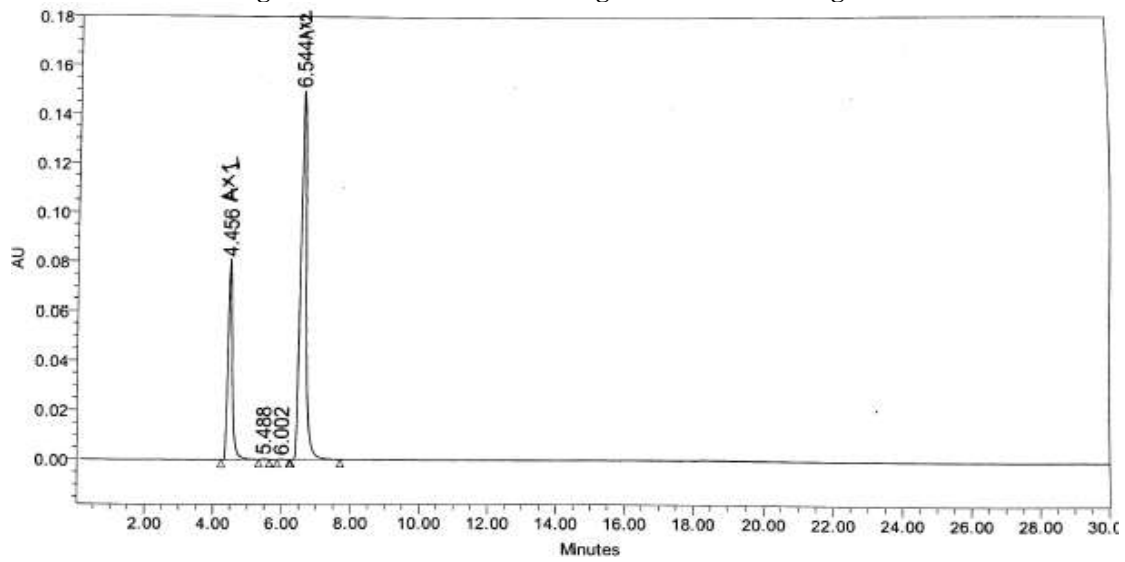
Fig 08: Chromatogram of Heat Solution State
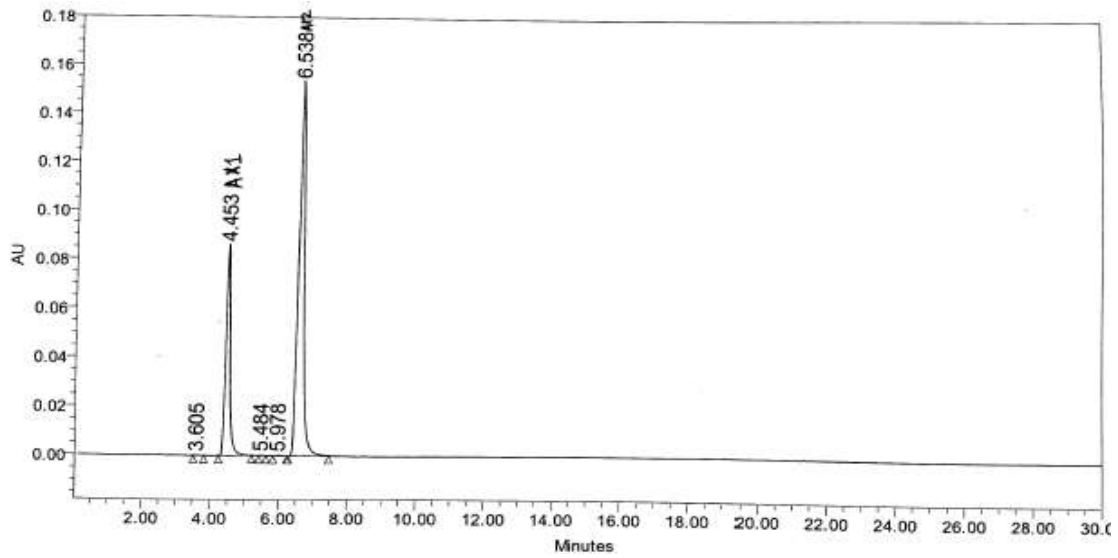
Fig 09: Chromatogram of Thermal State
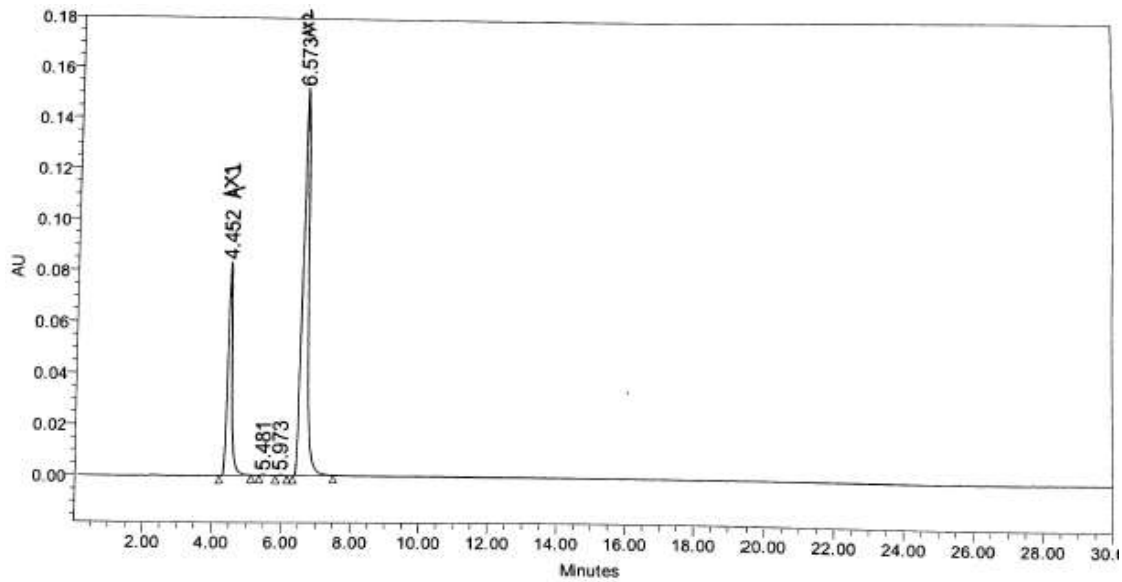
Fig 10: Chromatogram of Humidity State
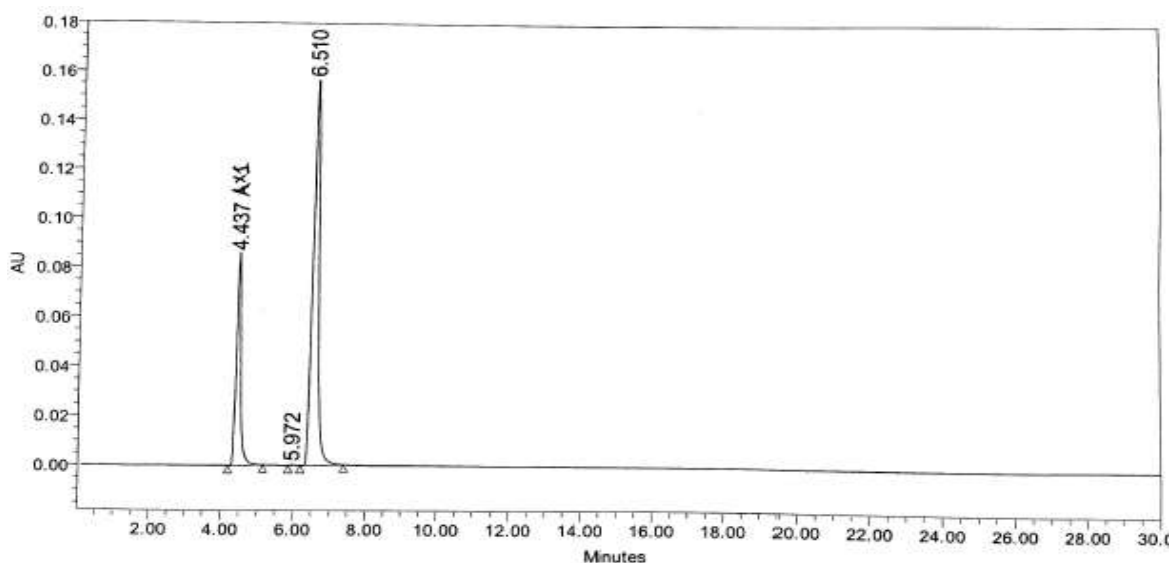
Fig 11: Chromatogram of Photolytic State

Linearity

Table 06: Linearity studies of Olanzapine

Linearity solution	Concentration (µg/ml)	Area of Olanzapine	Mean area of Olanzapine	Std. deviation	%RSD
Linearity L-1 (50%)	10	380868	380413	455.502	0.12
		379957			
		380415			
Linearity L-2 (75%)	15	552827	553343	1602.004	0.29
		552062			
		555139			
Linearity L-3 (100%)	20	737729	737664	343.595	0.05
		737293			
		737971			
Linearity L-4 (125%)	25	911956	912553	525.957	0.06
		912947			
		912757			
Linearity L-5 (150%)	30	1142898	1142395	596.370	0.05
		1141736			
		1142550			

Table 07: Regression studies of Olanzapine

Slope	Intercept	Regression Equation	Coefficient of correlation
37308	8070.9001	y=37308x-8070.9	0.996
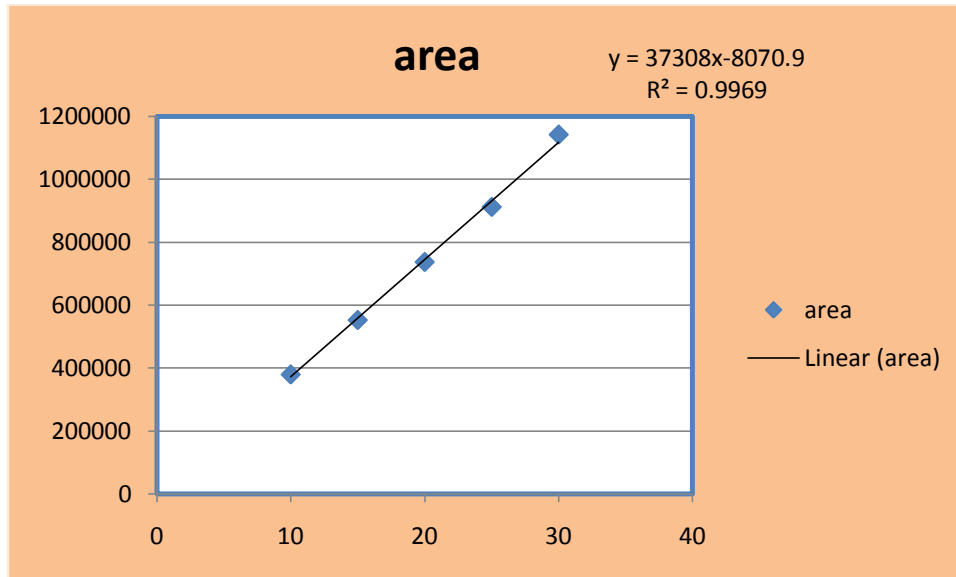
Fig. 12: Calibration curve of Olanzapine

Table 08: Linearity studies of Fluoxetine HCl

Linearity solution	Concentration (µg/ml)	Area of Olanzapine	Mean area of Olanzapine	Std. deviation	%RSD
Linearity L-1 (50%)	36	836080 836113 839410	837201	1913.121	0.23
Linearity L-2 (75%)	54	1211586 1211067 1221596	1214749	5934.7747	0.48
Linearity L-3 (100%)	72	1619268 1614866 1617419	1617184	2210.362	0.14
Linearity L-4 (125%)	90	1995630 1995661 1994377	1995223	732.533	0.04
Linearity L-5 (150%)	108	2495183 2504978 2498138	2499433	5024.269	0.20

Table 09: Regression studies of Fluoxetine HCL

Slope	Intercept	Regression Equation	Coefficient of correlation
22718	7273.90	y=22718x-7273.9	0.996
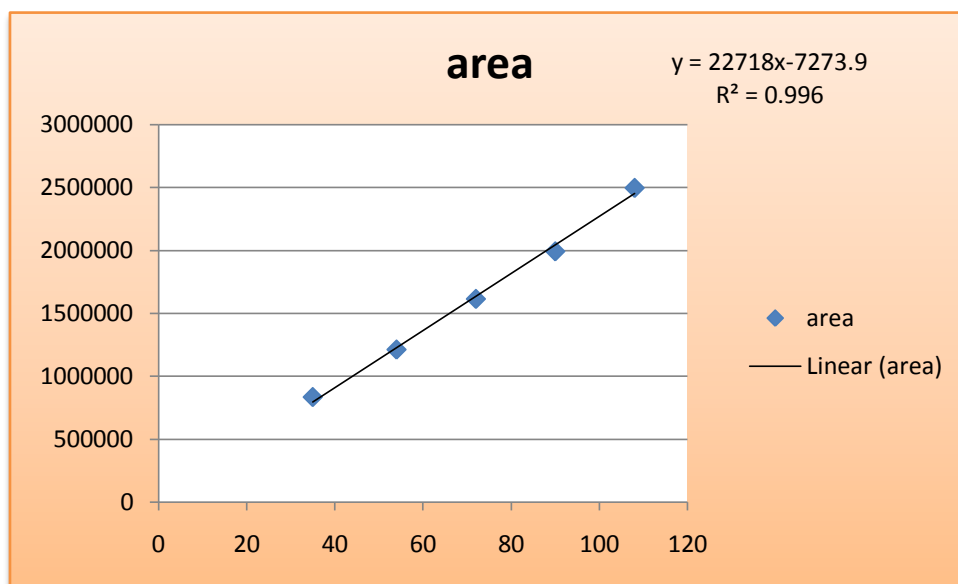
Fig. 13: Calibration curve of Fluoxetine HCL

Remarks:
➢ A linearity graph of the average area of Olanzapine and Fluoxetine HCL against the concentration in ppm is plotted and found to be a straight line graph.
➢ The correlation coefficient is 0.0998.
➢ In the range of 50 percent to 150 percent of the prescribed concentration of Olanzapineis 9.99- 29.96g/ml and 35.84- 107.52g/ml, the approach is found to be linear.

Accuracy
The evaluation obtained are shown in Table 8.11 and 8.12 respectively.
Table 10 Accuracy for Olanzapine

Sample preparation	Amount spiked (mg)	Area of Olanzapine		Mean Area	Amount recovered (mg)	% Recovery
		Inj-1	Inj-2			
At 50%-1	9.8	351222	349707	350465	9.86	100.61
At 50%-2	10	355202	354728	354965	9.99	99.90
At 50%-3	9.8	350403	349056	349730	9.84	100.41
					Mean	100.31
At 75%-1	15.7	560098	564316	562207	15.82	100.76
At 75%-2	15.8	562290	562086	562188	15.82	100.13
At 75%-3	15.9	560151	564471	562311	15.82	99.50
					Mean	100.13
At 100%-1	20.5	723542	724669	724106	20.37	99.37
At 100%-2	20.3	715538	721550	718544	20.22	99.61
At 100%-3	20.0	719281	709776	714529	20.10	100.50
					Mean	99.83
At 125%-1	24.7	866773	865139	865956	24.37	98.66
At 125%-2	25.9	923761	928500	926131	26.06	100.62
At 125%-3	25.7	926565	925802	926184	26.06	101.40

				Mean	100.23
At 150%-1	30.7	1094322	1094322	1094322	30.79	100.29
At 150%-2	30.0	1079473	1080016	1079745	30.38	101.27
At 150%-3	30.5	1086140	1100429	1093285	30.76	100.85
				Mean	100.66

Remark:

➤ **The mean recovery per concentration should be 99.83% - 100.66% for Olanzepine.**

Table 11: Accuracy for Fluoxetine HCl

Sample preparation	Amount spiked (mg)	Area of Olanzapine		Mean Area	Amount recovered (mg)	% Recovery
		Inj-1	Inj-2			
At 50%-1	45.0	875540	873274	874407	45.41	100.91
At 50%-2	45.4	880401	876809	878607	45.63	100.51
At 50%-3	45.2	868388	874782	871585	45.27	100.15
				Mean	100.52
At 75%-1	66.7	1303304	1308863	1306084	67.83	101.69
At 75%-2	67.2	1307711	1314542	1311127	68.10	101.34
At 75%-3	67.9	1300808	1308235	1304522	67.75	99.78
				Mean	100.94
At 100%-1	92.3	1793248	1789879	1791564	93.05	100.81
At 100%-2	91.5	1773065	1784732	1778899	92.39	100.97
At 100%-3	90.8	1774333	1759401	1766867	91.77	101.07
				Mean	100.95
At 125%-1	107.4	2048143	2037637	2042890	106.10	98.79
At 125%-2	111.8	2177804	2184311	2181058	113.28	101.32
At 125%-3	111.9	2190766	2179053	2184910	113.48	101.41
				Mean	100.51
At 150%-1	135.4	2650973	2650973	2650973	137.68	101.68
At 150%-2	135.8	2625730	2619046	2622388	136.20	100.29
At 150%-3	135.7	2639049	2653420	2646235	137.44	101.28
				Mean	100.94

Remark:

➤ **The mean recovery per concentration should be is 100.51% - 100.95% for Fluoxetine HCl.**

Precision:

Table 12: System Precision

Injection Number	Peak Area of standards	
	Olanzapine	Fluoxetine HCl
1	735818	1566935

2	733054	1562258
3	735920	1571773
4	739943	1574402
5	734026	1573986
Mean	73572	1570471
SD	2638.908	4158.782
%RSD	0.36	0.26
Tailing Factor	1.55	1.66

Remark:
➤ **The percent RSD of the peak areas of five duplicate injections of standard preparation should not exceed 2.0% USP tailing factor.**

Method Precision:

Table 13: Method Precision of Olanzapine

Sample Preparation	Sample wt (mg)	Area of Olanzapine		Mean Area	% Assay
		Inj-1	Inj-2		
Set-1	1253.6	721266	724962	723114	101.69
Set-2	1251.8	713993	717843	715918	100.82
Set-3	1254.5	706350	710252	708301	99.53
Set-4	1253.1	720218	717726	718972	101.15
Set-5	1252.4	715532	715992	715762	100.75
Set-6	1251.2	720496	723319	721908	101.71
				Mean	100.94
				SD	0.805
				%RSD	0.80

Table 14: Method Precision of Fluoxetine HCl

Sample Preparation	Sample wt (mg)	Area of Fluoxetine HCL		Mean Area	% Assay
		Inj-1	Inj-2		
Set-1	1253.6	1772976	1782591	1777784	101.36
Set-2	1251.8	1801385	1811859	1806622	103.15
Set-3	1254.5	1799652	1798896	1799274	102.51
Set-4	1253.1	1793925	1795593	1794759	102.37
Set-5	1252.4	1813589	1805224	1809407	103.26
Set-6	1251.2	1791392	1802195	1796794	102.64
				Mean	102.55
				SD	0.682
				%RSD	0.67

Remark:
➤ **% RSD for quaintified assay of multiple sample preparation not more than 2.0%**

Robustness:

Table 15: Robustness study of Olanzapine and Fluoxetine HCl

1)   **Change in Flow rate:**

Parameters	Changed values	
Flow rate	**0.9 ml/min**	**1.1 ml/min**

Table 16: Flow Rate: 0.9 ml/min (i.e. -0.1ml/min)

Sample	Area	Mean area	% Assay	% Assay From Method Precision	% Variance From Method Precision	% RSD
Olanzapine	**804962**	**805595**	**101.93**	**100.94**	**0.99**	**0.65**
	806227					
	797496	**797128**				
	796760					
Fluoxetine HCl	**1973002**	**1977647**	**101.00**	**102.55**	**1.55**	**1.18**
	1982292					
	2008554	**2008048**				
	2007542					

Table 17: Flow Rate: 1.1ml/min (i.e. +0.1ml/min)

Sample	Area	Mean area	% Assay	% Assay From Method Precision	% Variance From Method Precision	% RSD
Olanzapine	**660847**	**660897**	**100.88**	**100.94**	**0.06**	**0.58**
	660947					
	655620	**654516**				
	653411					
Fluoxetine HCL	**1624478**	**1624566**	**102.08**	**102.55**	**0.47**	**1.02**
	1624654					
	1648843	**1647360**				
	1645876					

Table 18: Change inWavelength

Parameters	Changed values	
Wavelength	**226nm**	**230nm**

Table 19: Change in Wavelength: 226nm (i.e. -2nm)

Sample	Area	Mean area	% Assay	% Assay From Method Precision	% Variance From Method Precision	% RSD
Olanzapine	**757696**	**757803**	**102.30**	**100.94**	**1.36**	**1.31**
	757909					
	734338	**734000**				

	733661					
Fluoxetine HCL	1856237	1860659	103.87	102.55	1.32	0.49
	1865081					
	1840842	1848647				
	1856452					

Table 20: Change in Wavelength: 230nm (i.e. +2nm)

Sample	Area	Mean area	% Assay	% Assay From Method Precision	% Variance From Method Precision	% RSD
Olanzapine	757696	757803	102.30	100.94	1.36	1.31
	757909					
	734338	734000				
	733661					
Fluoxetine HCl	1856237	1860659	103.87	102.55	1.32	0.49
	1865081					
	1840842	1848647				
	1856452					

Table 21: Change inpH:

Parameters	Changed values	
pH	5.8	6.2

Table 22: Change in Buffer pH: 5.8 (i.e. pH -0.2)

Sample	Area	Mean area	% Assay	% Assay From Method Precision	% Variance From Method Precision	% RSD
Olanzapine	743762	743536	101.23	100.94	0.29	0.57
	743309					
	739736	737117				
	734497					
Fluoxetine HCL	1810860	1810093	102.07	102.55	0.48	1.29
	1809326					
	1850441	1842313				
	1834185					

Table 23: Change in Buffer pH: 6.2 (i.e. pH +0.2)

Sample	Area	Mean area	% Assay	% Assay From Method Precision	% Variance From Method Precision	% RSD
Olanzapine	742635	749492	101.20	100.94	0.26	1.37
	756348					
	732575	732500				
	732424					
Fluoxetine	1797825	1796364	102.11	102.55	0.44	0.26

HCL	1794903					
	1797825	1796364				
	1794903					

Table 24: Change in mobile Phase Composition:

Parameters	Changed values	
Ratio of Organic Solvent	**+5%**	**-5%**

Table 25: Change in mobile phase composition: Organic solvent +5% (i.e. Buffer 52.7:ACN47.3)

Sample	Area	Mean area	% Assay	% Assay From Method Precision	% Variance From Method Precision	% RSD
Olanzapine	**7334453**	**733672**	**100.96**	**100.94**	**0.02**	**0.13**
	733891					
	732695	**735555**				
	738415					
Fluoxetine HCL	**1771927**	**1772913**	**101.04**	**102.55**	**1.11**	**1.76**
	1773898					
	1820007	**1825477**				
	1830947					

Table 26: Change in mobile phase composition: Organic solvent -5% i.e. Buffer: CAN (57.3: 42.7)

Sample	Area	Mean area	% Assay	% Assay From Method Precision	% Variance From Method Precision	% RSD
Olanzapine	**736415**	**737040**	**100.86**	**100.94**	**0.08**	**0.19**
	737665					
	731258	**734000**				
	736741					
Fluoxetine HCL	**1778050**	**1776251**	**101.33**	**102.55**	**1.22**	**1.75**
	1774451					
	1818520	**1818165**				
	1817809					

Remark:
➢ The % RSD should not be more than 2.0%
➢ The % RSD between results obtained with changed condition not be more than 2.0%.
Solution stability
Solution stability for Olanzapine and Fluoxetine HCL are shown in Table 8.28 and 8.29

Table 27: Solution stability for Olanzapine

Time interval (hrs)	Injection Volume(µg/ml)	Area of standard	Area of sample
Initial	**20**	**806487**	**756234**

	20	807982	753477
4 Hrs	20	776891	756173
	20	780271	751139
8 Hrs	20	779341	760122
	20	779341	761614
12 Hrs	20	788711	756308
	20	779372	756422
16 Hrs	20	767791	740467
	20	762720	736856
20 Hrs	20	766025	745644
	20	768462	745644
24 Hrs	20	766884	753513
	20	774377	747925
36 Hrs	20	777815	751721
	20	779734	750291
Mean	781029		751212
SD	13420.472		7117.529
%RSD	1.72		0.95

Table 28: Solution stability for Fluoxetine HCl

Time interval (hrs)	Injection Volume(µg/ml)	Area of standard	Area of sample
Initial	20	1739078	1909567
	20	1738486	1895748
4 Hrs	20	1669241	1902717
	20	1667874	1886141
8 Hrs	20	1664048	1912352
	20	1683847	1912996
12 Hrs	20	1683887	1901981
	20	1664544	1898856
16 Hrs	20	1639292	1864329
	20	1629497	1846515

	20	1642423	1873773
20 Hrs	20	1645878	1869781
24 Hrs	20	1635133	1881840
	20	1658172	1878740
36 Hrs	20	1667676	1896665
	20	1674488	1895485
Mean	1674009		1889093
SD	32844.676		18791.923
%RSD	1.96		0.99

Remark:
➢ **The % RSD of replicate standard injections from initial to 36 hours should be not more than 2.0%.**
➢ **The % RSD of sample preparation injections from initial to 36 hours should be not more than 2.0%.**

Table 29: Tablet Analysis of Olanzapine and Fluoxetine HCl

Tablet	Drug	mg/tab	% Drug obtained ± SD*	Std. error of estimation
Oleanz plus	**Olanzapine**	**20**	**98.86±0.16**	**0.105**
	Fluoxetine HCl	**80**	**99.35±0.12**	**0.112**

*Mean of three determinations

Table 30: Summary of validation parameters of HPLC method

Parameter	Olanzapine	Fluoxetine HCL
Linearity (µg/ml)	**10-30**	**36-108**
Linearity Intercept	**8070.90**	**7273.90**
Linearity Equation	**Y= 37308x-8070.9**	**Y=22718x-7273.9**
R^2	**0.996**	**0.996**
Oxidation degradation (%)	**30.00**	**31.24**
Precision(%RSD)	**0.80**	**0.61**
Accuracy	**100.31-100.66%**	**100.51-100.95%**
Robustness (%variance)	**0.06-1.36**	**0.29-1.55**
Solution stability (%RSD)	**1.72-0.95**	**1.96-0.99**

IV CONCLUSION

The devised HPLC method for determining stability was found to be simple, accurate, sensitive, precise, specific, and quick. This approach can be used to analyse Olanzapine and Fluoxetine HCl in bulk and pharmaceutical formulations such as tablets on a regular basis. This method was also utilised to verify the product's quality and stability following varying storage conditions and stress deterioration.

SOME OF THE ADVANAGES FROM THE ABOVE RESULTS
a)Robust analytical method
b)Quantification done by using less amount of organic solvents
c) Method can be applied for routine analysis of both of the drugs
d) Degradation study is also performed for better knowing of both drug.

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