A novel analytical method for the simultaneous determination of three anti depressive disorder drugs by RP-HPLC

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Abstract:

Although many methods were proposed for the estimation of Fluoxetine Hcl, Alprazolam, and Olanzapine individually or in their dual combinations, no method was proposed for simultaneous estimation of three drugs. This method serves ideal for the estimation three drugs simultaneously in bulk and can be used to estimate any dual combination of two drugs which are formulated. Instrument used for analysis was RP-HPLC, Agilent technologies, 1260 infinity with PDA detector. The detection was done at 227nm with a flow rate of 0.9ml. The mobile phase consists of Acetonitrile and phosphate buffer in the ratio of 70:30 and the pH was adjusted to 6. Phenomenex C18 column (4.6 × 250 mm, 5µ) was used as stationary phase. System suitable parameters were in limits and this method was specific and precise. Retention times were 4.02, 4.37, 5.61 for Fluoxetine Hcl, Alprazolam, and Olanzapine respectively. Resolution between them is more than 2 and signal to noise ratio above 10. The results were accurate and robust. This method was validated according to ICH guidelines**.**

Keywords: Fluoxetine HCL, Olanzapine, Alprazolam, PDA detector, RP-HPLC, ICH.

I. Introduction:

Fluoxetine HCl, named according to IUPAC as *N*-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propane-1-amine; hydrochloride. Its brand name is Prozac, a prescribed medicine to treat symptoms of major depressive disorder, obsessive-compulsive disorder, panic disorder, premenstrual dysphoric disorder (PMDD,) and bulimia nervosa.[1] Prozac belongs to the Antidepressants, Selective Serotonin Reuptake Inhibitor (SSRI) class of drugs, and it inhibits the presynaptic reuptake of the neurotransmitter serotonin.[2] As a result, 5-hydroxytryptamine (5-HT) levels in various parts of the brain increase. Further, fluoxetine is 5-HT selective as it has a high affinity for 5-HT transporters, a low affinity for noradrenaline transporters, and no affinity for dopamine transporters.[3]i

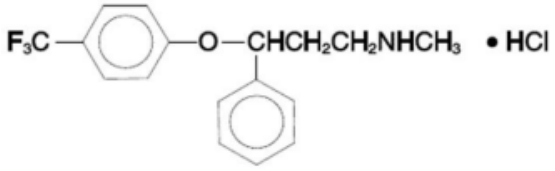

Fig.1: Structure of Fluoxetine HCL

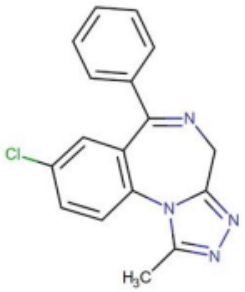

Fig.2: Structure of Alprazolam

Alprazolam, IUPAC name is 8-chloro-1-methyl-6-phenyl-4H- [1,2,4] triazole [4,3, -α]- [1,4] benzodiazepine. it is a triazole benzodiazepine with intermediate onset commonly used to treat panic disorders.[4] Alprazolam is a benzodiazepine that binds to GABA -A receptor. In CNS GABA-A receptor is made up of Ttwoalpha-1 subunits, two beta-2 subunits, and one gamma-2 subunit. Between the alpha-1 and gamma-2 subunits is the benzodiazepine binding site. GABA-A receptors are coupled with benzodiazepine binding sites and improves the effects of gamma-aminobutyric acid (GABA) by increasing its affinity at the receptor site. Then the primary inhibitory neurotransmitter GABA mediates the relaxing or inhibitory effects of alprazolam on the human nervous system.[5][6]

Olanzapine IUPAC name is 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno-[2,3b] [1,5] benzodiazepine. it is a thienobenzodiazepine antipsychotic that is classified as atypical or second-generation.[7] Second-generation antipsychotics were first released in the 90s and quickly gained popularity due to their high efficacy, low side effects, and low sensitivity to drug-drug interactions.[8] Olanzapine is remarkably similar to clozapine, except two additional methyl groups and the lack of a chloride moiety. It was discovered by Eli Lilly scientists and approved to be marketed in the United States in 1996.[9] Olanzapine is a second-generation antipsychotic drug that affects dopamine and serotonin receptors. It acts as an antagonist on dopamine D2 receptors in the mesolimbic pathway, preventing dopamine from acting at the post-synaptic receptor. Olanzapine binds to the receptor loosely and dissociates quickly, enabling normal dopamine neurotransmission to occur.[10]

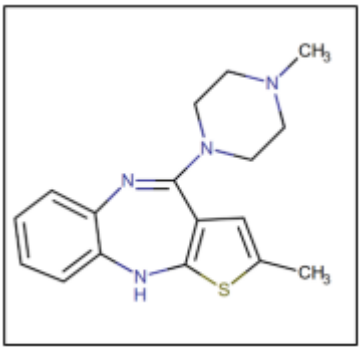

Fig.3: Structure of Olanzapine

From the Literature survey, it was inferred that there existed RP-HPLC [17][22][23], HPLC and HPTLC methods [14], Spectrophotometry (D2) [15] methods for the simultaneous estimation of Fluoxetine HCl and Alprazolam, HPLC and HPTLC methods [11-13] [20,21] [24,25] [16], Spectrophotometry (D1) [18], TLC-Spectro densitometry [19] methods for the simultaneous estimation of Fluoxetine HCl and Olanzapine and individual methods for estimating each drug respectively. A literature review revealed that only a few analytical methods that are relatively expensive have been reported, so the objective of this work is to develop a new single RP-HPLC method for the estimation of individual drugs as well as Fluoxetine HCl and Alprazolam, Fluoxetine HCl and Olanzapine combinations. Although many methods were proposed for the estimation of Fluoxetine Hcl, Alprazolam, and Olanzapine individually or in their dual combinations, no method was proposed for simultaneous estimation of three drugs. This method serves ideal for the estimation three drugs simultaneously in bulk and can be used to estimate any dual combination of two drugs which are formulated. The proposed method is simple, sensitive, and precise and could be easily applied in quality control laboratories with a high degree of accuracy and precision for the simultaneous determination of Fluoxetine HCl, Alprazolam, and Olanzapine.

II. Materials And Methods:

Instruments Used: HPLC - Agilent technologies 1260 infinity, Digital PH meter - Elico LI-120, Weighing machine, UV-Visible spectrophotometer - PG instruments T60, Ultrasonic cleaner - Labcopanianusp-20 xho70062-11002a.

Chemicals and Reagents: Potassium dihydrogen orthophosphate anhydrous Loba Chemie, Sodium hydroxide Loba Chemie, Acetonitrile Merck Water Merck, Methanol for HPLC Loba Chemie, Orthophosphoric acid Loba Chemie.

Preparation of mobile phase:

Preparation of 0.2 M NaOH: Take 0.8gm of sodium hydroxide in a 100ml volumetric flask and make the volume up to 10ml with water.

Preparation of 0.2M KH2PO4: Take 2.72gm of potassium dihydrogen orthophosphate anhydrous in a 100ml volumetric flask and dissolve it in a few

ml of water and make up the volume up to 100ml with water.

Preparation of p^H 6 phosphate buffer solution: Take 8.1ml of 0.2m NaOH and 50ml of KH2PO4 in a 200ml volumetric flask and make up the volume up to 200ml with water and sonicate it for 10min and filter the buffer by using vacuum filtration and now adjust the p^H of the buffer by using 1.0% orthophosphoric acid and again sonicate it for 10min.

Preparation of Diluent: The mobile phase was used as the diluent.

Preparation of stock solutions: the solutions were prepared by dissolving 10mg of Fluoxetine HCL, Alprazolam, and Olanzapine in three separate volumetric flasks and making the volume up to 10 ml with diluent and sonicate for 10min to get a concentration of 1000µg/ml. now take 1ml from each of the above stock solutions and add to a 10ml volumetric flask and made the volume up to 10ml with diluent and sonicate for 10 min to get a concentration of 100µg/ml.

Preparation of working standard solutions: from the stock solution take 1.2ml,2.5ml & 0.2ml in three separate volumetric flasks and make the volume up to 10ml with diluent and sonicate for 10min to get a concentration of 12 µg/ml,25 µg/ml& 2 µg/ml. now take 1ml from each of the above stock
solutions and add to a 10ml volumetric flask and make up the volume up to 10ml with diluent, sonicate it for 10min.this solution contains 1.2 µg/ml,2.5 µg/ml&0.2 µg/ml concentrations of the Fluoxetine HCL, Alprazolam & Olanzapine respectively (standard solution).

III. Method Development:

UV- Visible Spectroscopy (Spectral scan)

Fluoxetine HCL, Alprazolam, and olanzapine were dissolved in Acetonitrile: Phosphate buffer P^H 6 (70:30) Spectral scan is done at 200-400nm. The maximum absorbance (λ max) is 227nm.

HPLC METHOD DEVELOPMENT:

The purpose of this experiment was to optimize the assay method for simultaneous estimation of the literature survey made of fluoxetine HCL, Alprazolam, and olanzapine. The trials described here explain how the optimizations performed.

Table No:1. Trails for developing the method

Conditions to chromatography:	Trail 1	Trail 2	Trail 3
Mobile phase	Acetonitrile: Phosphate buffer PH 6.5 (70:30)	Acetonitrile: Methanol: Phosphate Buffer pH 6.7 (50: 20: 30)	Acetonitrile: Phosphate buffer PH 6.7 (70:30)
Stationary phase	Hemo chrom intsil C18-5u 250×4.6	Hemo chrom intsil C18-5u 250×4.6	Hemo chrom intsil C18-5u 250×4.6
Flowrate	0.9 ml/min	0.9 ml/min	0.9 ml/min
Detection wavelength	227nm	227nm	227nm
The temperature in the column	ambient	ambient	ambient
Injection volume	20µl	20µl	20µl
Run time	10min	10min	8 min
Retention time	Fluoxetine HCL for 3.90 min, Alprazolam for 4.31min and Olanzapine for 4.68 min	Fluoxetine HCL for 2.72 min, Alprazolam for 4.89 min and Olanzapine for 5.82 min	Fluoxetine HCL for 2.42 min, Alprazolam for 3.92min and Olanzapine for 4.76 min

Table No:2. Optimised Chromatographic Conditions

Parameters	Method
Stationary phase	Hemo Chrom intsil C18-5u 250×4.6
Mobile phase	Acetonitrile: phosphate buffer (p^H6) (70:30)
Flow rate (ml/min)	0.9ml/min
Run time (min)	8 min
Column temperature (°c)	Ambient
Injection volume	20µl
Detection wavelength	227 nm
Drug RT (min)	4.02 for Fluoxetine HCL, 4.37 for Alprazolam, and 5.61 for Olanzapine.

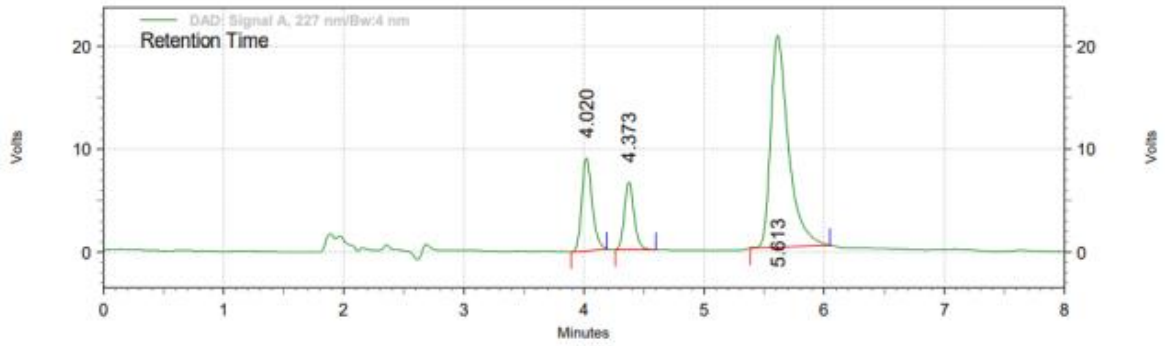

Fig No: 4 Optimised chromatogram

IV. Results And Discussion

Method Validation: The Developed Method Was Validated Based On Ich Guidelines To Detect And Quantitate Fluoxetine Hcl, Alprazolam & Olanzapine In Its Bulk Form With The Use Of The Hplc System.

1. System Suitability:

The Above-Prepared Working Standard Solution Was Injected Into The Hplc Device Six Times.

Table No:3. System suitability of Fluoxetine HCL

S.no.	Injection	RT	Area	Theoretical Plates	Tailing factor
1	1	4.027	108174	10541	0.967
2	2	4.033	107402	10836	1.013
3	3	4.027	108150	10342	1.140
4	4	4.013	107422	10620	1.231
5	5	4.107	104951	11660	1.260
6	6	4.067	103010	10936	1.302
	Average	4.04567	106518.167		
	SD	0.03199	1904.64279		
	%RSD	0.7908	1.78809197		

Table No:4. System suitability of Alprazolam

S.no.	Injection	RT	Peak Area	Theoretical Plates	Tailing factor
1	1	5.613	413240	7577	1.432
2	2	5.633	430347	7511	1.132
3	3	5.62	418003	7560	1.225
4	4	5.607	413606	7489	1.098
5	5	5.687	414964	7684	1.154
6	6	5.667	430847	8200	1.089
	Average	5.63783	420167.833		
	SD	0.02937	7533.02674		
	%RSD	0.52101	1.79286136		

Table No:5. System suitability of Olanzapine

Injections	RT (Fluoxetine HCL)	Area	Theoretical Plates	Tailing factor
1	4.02	112530	10581	1.189
2	4.013	115041	10368	1.017
3	4.007	112071	10306	1.056
4	4.027	114470	10210	1.132
5	4.026	113570	10310	1.287
6	4.025	112990	10599	1.009
Average	4.019666667	113445.333		
Standard deviation		1044.47685		
%RSD		0.92068736		

2. Specificity:

Specificity was performed by injecting one blank and one sample injection into the HPLC system and No peaks were detected at the retention time of Fluoxetine HCL, Olanzapine, and Alprazolam in the chromatograms of blank preparation**.**

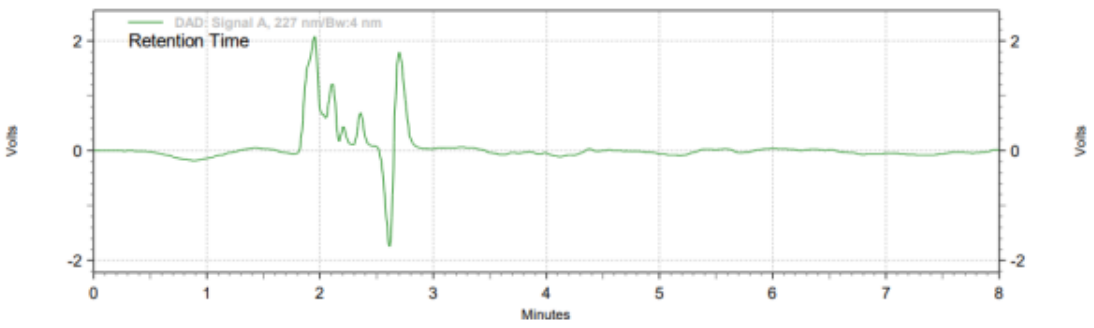

Fig No:5 Showing blank chromatogram

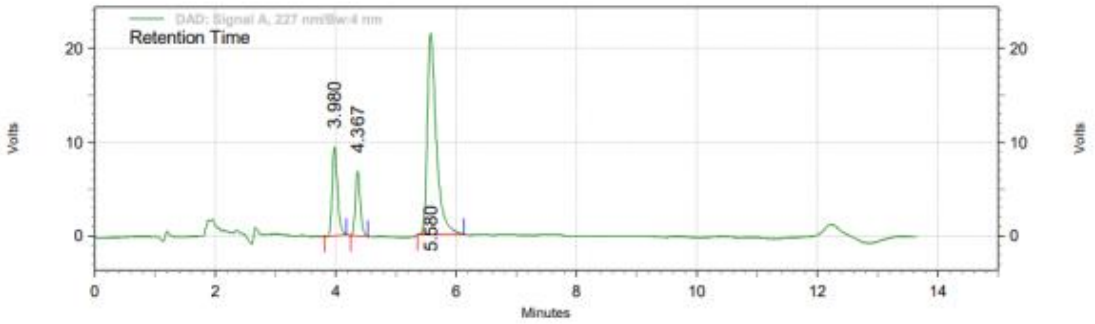

Fig No:6 Chromatogram Showing Fluoxetine Hcl, Alprazolam & Olanzapine

3. Precision:

The working standard solution was prepared and injected six times into the HPLC device. The relative standard deviation percentage of individual Fluoxetine Hcl, Alprazolam & Olanzapine drugs should be NMT 2%. The % RSD was found to be less than the acceptance criteria for retention periods and peak areas.

Table No:6. Precision Results of Fluoxetine HCL

Injections	RT (Fluoxetine HCL)	Area	Theoretical Plates	Tailing factor
1	4.02	112530	10581	1.189
2	4.013	115041	10368	1.017
3	4.007	112071	10306	1.056
4	4.027	114470	10210	1.132
5	4.026	113570	10310	1.287
6	4.025	112990	10599	1.009
Average	4.019666667	113445.333		
Standard deviation		1044.47685		
%RSD		0.92068736		

Table No:7. showing Data for Precision Results of Alprazolam

Injections	RT(Olanzapine)	Area	Theoretical Plates	Tailing factor
1	5.613	441258	7602	1.302
2	5.607	448148	7440	1.314
3	5.613	425273	7527	1.280
4	5.627	437524	7635	1.270
5	5.625	436524	7620	1.315
6	5.627	436575	7620	1.332
Average		437550.333		
Standard deviation		6816.59218		
%RSD		1.55789898		

Table No:8. Precision Results of Olanzapine

Injections	RT(Alprazolam)	Area	Theoretical Plates	Tailing factor
1	4.373	77923	14074	1.103
2	4.373	79606	13861	1.250
3	4.387	76293	14025	1.243
4	4.42	79862	14245	1.204
5	4.41	79860	14215	1.192
6	4.39	78456	14123	1.809
Average		78666.6667		
Standard deviation		1288.85785		
%RSD		1.63837862		

4. Accuracy:

The accuracy was performed for all three drugs the mean percentage recovery at each spiking level should be NLT 98.0% and NMT 102.0%. The percentage recovery was found to be within the limits.

Table No:9. Accuracy Results for Fluoxetine HCL

sl.no.	spiked %	injection no.	Amount added (microgram/ml)	RT	Area	Area average	% Recovered
1	50%	1	0.5	4.16	133731	143316	110%
2		2	0.5	4.113	133521		
3		3	0.5	4.147	132696		
4	100%	1	1	4.233	173049	195011.66	119%
5		2	1	4.093	173830		
6		3	1	4.107	173156		
7	150%	1	1.5	4.107	208988	234696.667	117.8%
8		2	1.5	4.133	206609		
9		3	1.5	4.28	208493		

Table No:10. Accuracy Results for Alprazolam

sl.no	spiked %	injection no.	Amount added(microgram/ml)	RT	Area	Area average	% Recovered
1	50%	1	0.5	4.427	115301	115370.3	99.40%
2		2	0.5	4.387	116332		
3		3	0.5	4.447	114478		
4	100%	1	1	4.527	153536	154179.6	100.23%

5		2	1	4.387	154964		
6		3	1	4.387	154039		
7		1	1.5	4.38	192667		
8	150%	2	1.5	4.387	191739	192212.3	99.75%
9		3	1.5	4.587	192231		

Table No:11. Accuracy Results for Olanzapine

5. Linearity regression coefficient shall be not less than 0.990

sl. no.	spiked %	injection no.	Amount added(microgram/ml)	RT	Area	Area average	% Recovered
1	50%	1	0.5	5.72	609184	623378	112%
2		2	0.5	5.673	573084		
3		3	0.5	5.733	609997		
4	100%	1	1	5.84	804583	795692	112%
5		2	1	5.653	788928		
6		3	1	5.66	763565		
7	150%	1	1.5	5.653	979735	1006407.67	115.3%
8		2	1.5	5.68	981894		
9		3	1.5	5.913	982094		

The linearity was performed over the concentration range of 0.72-1.68µg/ml for Fluoxetine HCL,1.5-3.5µg/ml for Alprazolam, and 0.2-0.28µg/ml for Olanzapine. the correlation coefficient and regression coefficient are found to be within the limits. for Fluoxetine HCL, Alprazolam, and Olanzapine. the correlation coefficient and regression coefficient are found to be within the limits.

Table: 12. Linearity Results for Fluoxetine HCL

sl.no.	linearity	concentration	Area
1	I	0.6	67918
2	II	0.8	82427
3	III	1	99327
4	IV	1.2	110063
5	V	1.4	124836

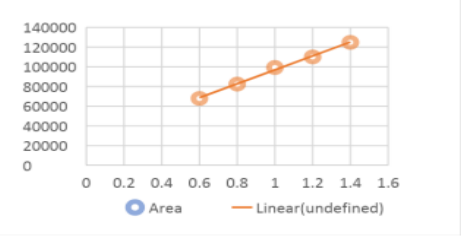

Fig No 7: Linearity graph for FLX HCL

Table :13 Linearity Results for Alprazolam

s.no.	linearity	concentration	Area
1	I	0.6	276513
2	II	0.8	330263
3	III	1	405506
4	IV	1.2	482853
5	V	1.4	545527

Table :14 Linearity Results for Olanzapine

s.no.	linearity	concentration	Area
1	I	0.6	44480
2	II	0.8	52402
3	III	1	61924
4	IV	1.2	71900
5	V	1.4	81627

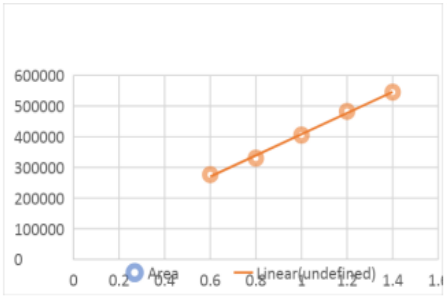

Fig NO 8: Linearity graph for ALP

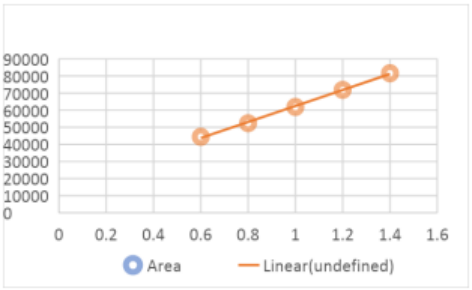

Fig No 9: Linearity graph for Olanzapine

6. Assay:

The assay was performed and the percentage assay was calculated for the individual drugs the % assay for fluoxetine HCL is 95.7%, Alprazolam is 103.6% and olanzapine is 102.2%. hence the % assay was found to be within the acceptable limits.

7. Limit Of Detection (Lod) And Limit Of Quantification (Loq):

The LOD and LOQ of the drug were derived by calculating the signal-noise ratio. The LOD & LOQ values for Fluoxetine HCL, Alprazolam & Olanzapine were found to be within the acceptance criteria

In this method, the LOD and LOQ of the drug were calculated by the following equation.

$$LOD = \frac{3.3 \times \text{Standard deviation}}{\text{Slope}}$$

$$LOQ = \frac{10.3 \times \text{Standard deviation}}{\text{Slope}}$$

Table no: 15. LOD and LOQ of three drugs:

s.no.	Name of the drug	LOD	LOQ
1.	Fluoxetine HCL	0.04	0.15
2.	Alprazolam	0.09	0.28
3.	Olanzapine	0.06	0.20

8. Ruggedness

The ruggedness study was carried out by repeating the complete experiment with different analysts, on different days in the same laboratory by preparing the working standard solution and six replicate injections were given. The relative standard deviation percentage of individual Fluoxetine Hcl, Alprazolam & Olanzapine drugs should be NMT 2%. The % RSD was found to be less than the acceptance criteria for retention periods and peak areas.

Table no: 16. Fluoxetine HCL

Analyst	RT	Peak Area	AVG	SD	%RSD
Swathi	4.107	104951	104505.3333	1085.731806	1.038924782
	4.013	105555			
	4.067	103010			
prapul	4.027	108150	107285	1223.294731	1.140229045
	4.013	105555			
	4.027	108150			
Thanuja	4.027	108174	107916.6667	363.9242901	0.337227141
	4.027	108174			
	4.033	107402			

Table no: 17. Alprazolam

Analyst	RT	Peak Area	AVG	SD	%RSD
Swathi	4.373	75304	75725.33333	300.4944074	0.396821505
	4.393	75984			
	4.42	75888			
prapul	4.4	73862	73116.33333	767.1680969	1.04924312
	4.38	73426			
	4.4	72061			
Thanuja	4.373	75304	75963.33333	466.2190711	0.61374225
	4.387	76293			
	4.387	76293			

Table no: 18. Olanzapine

Analyst	RT	Peak Area	AVG	SD	%RSD
Swathi	5.613	413240	420596.6667	7299.698274	1.735557805
	5.667	430547			
	5.62	418003			
prapul	5.613	441258	439917.6667	7328.768443	1.665940924
	5.607	448148			
	5.633	430347			
Thanuja	5.633	430347	432806	3337.128806	0.77104495
	5.627	437524			
	5.667	430547			

9. Robustness

The Robustness was performed by varying the flow rate, mobile phase composition, and wavelength, and the results have shown little change as the % RSD for fluoxetine HCL, Alprazolam & Olanzapine was found to be less than 2% and within the acceptable limits indicating the robustness of the method.

Table no: 19. Robustness for Fluoxetine HCL

Variations	RT	Area	AVG	SD	%RSD For Area
Flow Rate					
(1ml/min)	3.766	83298	41650.883	16.04853749	0.038531086
	3.768	83305			
	3.768	83335			
(0.9ml/min)	4.013	115041	113860.6667	1286.77642	1.130132519
	4.007	112071			
	4.027	114470			
(0.8ml/min)	4.723	107822	53913.3615	0.816496581	0.001514461
	4.707	107823			
	4.711	107824			
Mobile phase Composition	**RT**	**Area**	**AVG**	**SD**	**%RSD For Area**
ACN: PH 6 Phosphate buffer					
68:32	4.229	98866	98873.33333	89.2263040	0.090243042
	4.234	98788			
	4.213	98966			

70:30	4.027	108150	106841	1369.08022	1.281418394
	4.013	107422			
	4.107	104951			
72:28	3.856	11087	11101.66667	16.8027775	0.151353649
	3.897	11120			
	3.877	11098			
Wavelength	**RT**	**Area**	**AVG**	**SD**	**%RSD for Area**
225	4.147	91271	91287.66667	15.27525232	0.016733095
	4.142	91301			
	4.15	91291			
227	4.233	72732	72737	4.358898944	1.281418394
	4.241	72740			
	4.234	72739			
229	4.193	82813	82816.66667	3.511884584	0.151353649
	4.12	82820			
	4.191	82817			

Table no: 20. Robustness for Alprazolam

Variations	**RT**	**Area**	**AVG**	**SD**	**%RSD for Area**
Flow Rate					
(1ml/min)	3.96	55162	55158.33333	7.234178138	0.013115295
	3.94	55150			
	3.96	55163			
(0.9ml/min)	4.373	79606	78753.66667	1390.334332	1.765421714
	4.387	76793			
	4.42	79862			
(0.8ml/min)	4.94	76342	76341.33333	11.01514109	0.014428804
	4.92	76352			
	4.96	76330			
Mobile phase Composition	**RT**	**Area**	**AVG**	**SD**	**%RSD for Area**
ACN: PH 6 Phosphate buffer					

68:32	4.528	72919	72935.66667	55.89573627	0.07663704
	4.578	72890			
	4.51	72998			
70:30	4.4	73862	75018	851.475582	1.135028369
	4.373	75304			
	4.42	75888			
72:28	4.311	77975	77620.33333	542.356279	0.698729644
	4.309	77890			
	4.312	76996			
Wavelength	**RT**	**Area**	**AVG**	**SD**	**%RSD for Area**
225nm	4.413	75590	75611	20.0748599	0.026550184
	4.41	75630			
	4.44	75613			
227nm	4.42	54829	54829.33333	0.577350269	0.001052995
	4.423	54830			
	4.219	54829			
229nm	4.6	57642	57645.66667	4.041451884	0.007010851
	4.58	57645			
	4.61	57650			

Table no: 21. Robustness for Olanzapine

Variations	**RT**	**Area**	**AVG**	**SD**	**%RSD for Area**
Flow Rate					
(1ml/min)	5.153	369242	369227.3333	32.57811126	0.008823321
	5.15	369190			
	5.152	369250			
(0.9ml/min)	5.607	448148	445215	5146.063253	1.155860259
	5.613	439273			
	5.627	448224			
(0.8ml/min)	6.433	474498	474552.3333	50.93459859	0.010733189
	6.431	474560			
	6.44	474599			

Mobile phase Composition	RT	Area	AVG	SD	%RSD for Area
ACN: PH 6 Phosphate buffer					
68:32	5.81	398581	398294.6667	567.5388386	1.424922014
	5.815	397641			
	5.802	398662			
70:30	5.62	418003	415524.3333	1838.274976	0.442398875
	5.607	413606			
	5.687	414964			
72:28	5.478	447899	447080.3333	748.1860286	0.167349349
	5.434	446432			
	5.446	446910			
Wavelength	**RT**	**Area**	**AVG**	**SD**	**%RSD for Area**
225nm	5.713	389965	389937.6667	35.16153201	0.009017219
	5.71	389898			
	5.715	389950			
227nm	5.78	415680	415652.3333	47.05670338	0.011321169
	5.778	415679			
	5.781	415598			
229nm	5.733	373334	373401.3333	73.65686209	0.019725924
	5.731	373480			
	5.734	373390			

V. Conclusion:

The present study focused to develop and validate an RP-HPLC Method for the simultaneous estimation of Fluoxetine HCL, Alprazolam & Olanzapine in bulk form. From the comparative study, it was inferred that there existed different HPLC methods for the simultaneous estimation of Fluoxetine HCl and Alprazolam, Fluoxetine HCl and Olanzapine, and individual methods for estimating each drug respectively. The advantages of the proposed HPLC method over the reported ones are a single RP-HPLC method is useful for estimating individual drugs and simultaneously Fluoxetine HCl and Alprazolam, Fluoxetine HCl, and Olanzapine combinations. The proposed method is simple, sensitive, and precise and could be easily applied in quality control laboratories with a high degree of accuracy and precision for the simultaneous determination of FLX HCL, ALP & OLZ. The method was developed and validated as per ICH guidelines and all the validation parameters met the required acceptance criteria.

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