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ORIGINAL ARTICLE

Development and Validation of HPLC Method for The Simultaneous Estimation of Olanzapine and Fluoxetine HCl in Bulk and Tablet Dosage Form

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ABSTRACT
The HPLC method was developed and validated for simultaneous estimation of Olanzapine and Fluoxetine HCL. The mobile phase was consisting of pH 5.5 phosphate: ACN. The linearity range of Olanzapine was found to be 5-25 µg/ml and Fluoxetine HCL 20-100 µg/ml. The calibration curve was plotted, and regression equation of Olanzapine was found to be y = 2,79,185.26x + 88,989.90 with correlation coefficient (r2) of 0.9991 and Fluoxetine HCL y = 36,427.78x - 48,373.20 with correlation coefficient (r2) of 0.9998. Detection was done at 235 nm and the retention time of Olanzapine was found to be 3.2 min and Fluoxetine HCL 5.2 min with the flow rate of 0.8 ml/min. From accuracy study % recovery of Olanzapine was found in the range of 98.98-100.51% and Fluoxetine HCL is 98.86-101.38% which is in the limits accordingly the ICH guidelines. The limit of detection and limit of Quantitation of Olanzapine is 0.12µg/ml – 0.36µg/ml and Fluoxetine HCL is 0.10 – 0.31 respectively. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD was found within limit as per ICH guidelines. The result showed that proposed chromatographic method was suitable for the accurate, precise and rapid simultaneous determination of Olanzapine and Fluoxetine in its bulk form and pharmaceutical dosage form.
KEYWORDS: *Olanzapine, Fluoxetine, HPLC method, ICH Guidelines*

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INTRODUCTION

An antipsychotic medication called olanzapine is used to treat the agitation brought on by schizophrenia, bipolar disorder, and other related diseases. Thienobenzodiazepine olanzapine is categorized as an atypical or second-generation antipsychotic drug. Due to their outstanding effectiveness, decreased risk for extra pyramidal side effects, and decreased sensitivity to drug-drug interactions, second-generation antipsychotics, which were first launched in the 1990s, swiftly gained popularity. Olanzapine and clozapine are quite similar to one another; the major differences are two extra methyl groups and the lack of a chloride moiety. Eli Lilly scientists made the discovery, and in 1996 the US FDA approved its marketing. The Structure of Olanzapine is shown in **Figure 1**.

A selective serotonin reuptake inhibitor called fluoxetine is used to treat bipolar I, premenstrual dysphoric disorder, major depressive disorder, bulimia, OCD, and panic disorder. The Structure of Fluoxetine HCL is shown in **Figure 2** .A selective serotonin reuptake inhibitor (SSRI), fluoxetine is a second-generation antidepressant. Although it was first developed to treat depression, it received FDA clearance in 1987, and now, in addition to managing a number of other disorders, it is often given [1-4].

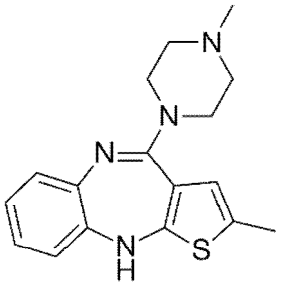

Figure 1: Structure of Olanzapine

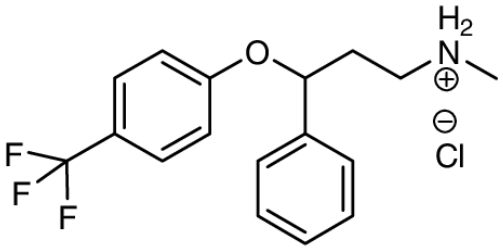

Figure 2: Structure of Fluoxetine

MATERIAL AND METHODS

Instruments:

The chromatographic method was performed Analytical Technologies HPLC system coordinated with avariable wavelength programmable UV identifier and a Rheodyne injector outfitted with 20µl fixed circle. An opposite stage Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron) was utilized. Wenser High Precision Balance Model: PGB 100 electronic equilibrium were utilized for Spectrophotometric judgments and gauging purposes individually.

Reagents and chemicals

Olanzapine and Fluoxetinewas procured from PharmaTech Solutions. HPLC grade Acetonitrile and water were acquired from Merck specialities private restricted, Mumbai.

Chromatographic conditions

Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron) was utilized for the chromatographic method at wavelength of 235 nm. pH 5.5 Phosphate buffer: ACNwas chosen as mobile phase for elution and samesolvent was utilized in the preparation of standard and sample solutions. The elution was checked by infusing the 20µl and the flow rate was changed in accordance with 0.8 ml/min.

Preparation of Standard Stock solutions

Accurately Weighed and transferred 5 mg of Olanzapine and 20 mg of Fluoxetine HCL working Standards into a 100ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. and the final concentration of Olanzapine is 50 µg/mL and 200 µg/mL is of Fluoxetine HCL. The working standard solutions of these drugs were obtained by appropriate dilution of the respective stock solution with mobile phase.

Preparation of Mobile Phase A (pH 5.5 Phosphate buffer):

Solution I. Dissolve 13.61 g of potassium dihydrogen phosphate in water and dilute to 1000.0 ml with the same solvent.

Solution II. Dissolve 35.81 g of disodium hydrogen phosphate in water and dilute to 1000.0 ml with the same solvent.

Mix 96.4 ml of solution I and 3.6 ml of solution II.

Mobile phase was filtered through 0.45µm membrane filter and degassed by sonication for 20 min.

Preparation of Mobile Phase B: 100 % ACN

Selection of mobile phase

Standard solutions of Olanzapine (5µg/mL) and Fluoxetine HCL (20µg/mL) were injected into the RP-HPLC system and run in different solvent systems. Different mobile phases systems like Phosphate buffer and ACN were initially tried in the isocratic mode in order to determine the best conditions [5-8].

HPLC Method Development

Optimisation of RP-HPLC method

The HPLC method was developed for the simultaneous estimations of Olanzapine and Fluoxetine. Different mobile phases were gone after for the method optimisation, however satisfactory retention times, hypothetical plates and good resolution were seen with pH 5.5 Phosphate buffer: AC Nusing Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron) by gradient method.

Table 1: Optimized Chromatographic Conditions

Mobile phase	pH 5.5 Phosphate buffer: ACN
Selection of column	Cosmosil C18 (250mm x 4.6mm ID, Particle size: 5 µm)
Injection volume	20 µL
Flow rate	0.8 ml/min
Column temperature	Room Temperature
Detection wavelength	235 nm
Run Time	8.0 minutes
Retention time	Olanzapine (3.2 min) and Fluoxetine HCL (5.2 min)

Table 2: Gradient Programme for Optimized Trial

Time (min)	pH 5.5 Phosphate Buffer (% v/v)	ACN (% v/v)
0	40	60
2.0	30	70
4.0	20	80
5.0	30	70

Table 1 gives Optimized Chromatographic Conditions and **Table 2** contains Gradient Programme for Optimized Trial.

Validation of RP-HPLC method

Validation of the optimized RP-HPLC method was performed in accordance with the ICH Q2 (R1) guidelines.

Linearity

Test solutions of different concentration were injected separately and the chromatograms were recorded. A series of test preparations of Olanzapine and Fluoxetine HCL were prepared by taking 1 ml - 5 ml from the stock solution containing Olanzapine (50µg/mL) and Fluoxetine HCL (200µg/mL) in five 10 ml volumetric flask and final volume make up to the mark with mobile phase. A 20 µl volume of each concentration was injected into HPLC, three times under the optimized chromatographic conditions.

Accuracy

Samples are prepared normally covering 50 % to 150 % of the nominal sample preparation concentration. These samples are analyzed and the recoveries of each are calculated.

Precision

Intraday precision study was carried out by preparing test solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for two different days to determine interday precision. The result was reported as %RSD.

Limit of Quantitation (LOQ) & Limit of Detection (LOD)

The LOD and LOQ were analysed from the slope(s) of the calibration curve and the standard deviation (SD) of the peak areas using the formula LOD = 3.3 s/s and LOQ = 10 s/s.

Robustness

Robustness was measured by altering the chromatographic circumstances such as mobile phase composition, detection wavelength, flow rate, and so on, and the % RSD should be given. Small modifications were permitted in the optimal conditions, and the method's robustness was determined. Individual deviations of ± 2 nm in detecting wavelength and ± 0.1 ml/min in flow rate were attempted. In triplicate, solutions of 100% test concentration with the necessary alterations in the optimum conditions were injected into the system.

Ruggedness:

Ruggedness is the investigation of the influence of external factors on the approach. To assess the robustness of the suggested approach, factors were purposefully altered. These factors included system variation, various analysts, and atmospheric fluctuations. Two distinct analysts prepared the test solution

according to the test procedure and injected three concentrations of test solution into the HPLC system at a flow rate of 0.8 ml/min.

Assay of marketed formulation

20 tablets of marketed formulation (FOSTERA-5) of Intas Pharmaceutical were taken, weighed individually and crushed into fine powder. Average weight of tablet sample was weighed and transferred to 100 mL volumetric flask & diluent was added to make up the volume. Sonicate for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter, The prepared stock solution is of 50 µg/ml of Olanzapine and 200 µg/ml of Fluoxetine HCL. For Analysis 3 ml solution was withdrawn and diluted upto 10 ml and injected into system.

System suitability

To validate the system, technique, and column performance, system suitability characteristics were examined. Six times a standard solution of Olanzapine and Fluoxetine was injected into the system, and system suitability characteristics were examined [9-11].

RESULT AND DISCUSSION

Linearity

It was clarified from the analytical method linearity as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The peak area obtained from the HPLC chromatograph was plotted against corresponding concentrations to obtain the calibration graph. **Table 3** contains the data of results of Linearity. Olanzapine was found to be linear in the concentration range of 5-25µg/ml and Fluoxetine is in the range of 1-5µg/ml as shown in **Figure 3:** Calibration curve for Olanzapine, Figure.**4:** Calibration curve for Fluoxetine and **Figure 5:** Chromatograph of Olanzapine and Fluoxetine respectively [12, 13].

Table 3: Summary of results of Linearity

Sr. No.	Olanzapine		Fluoxetine	
	Concentration(µg/ml)	Area	Concentration (µg/ml)	Area
1	5	1402364	20	692544
2	10	2965416	40	1382646
3	15	4302154	60	2154813
4	20	5698413	80	2859640
5	25	7015497	100	3596825

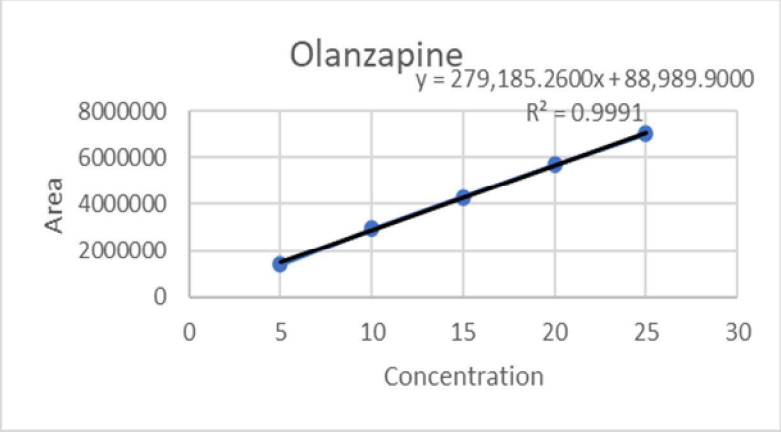

Figure 3: Calibration curve for Olanzapine

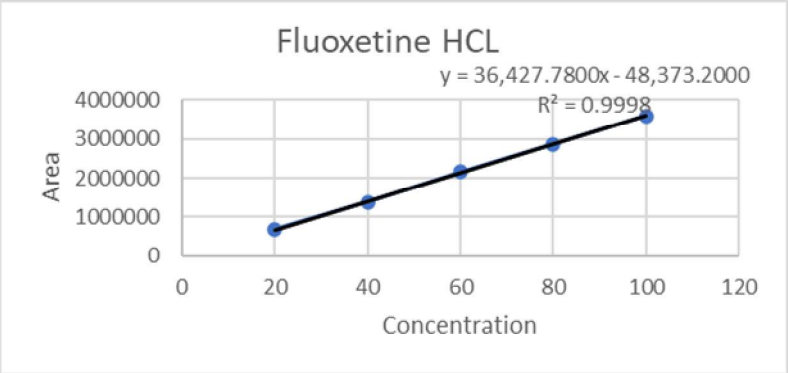

Figure.4: Calibration curve for Fluoxetine

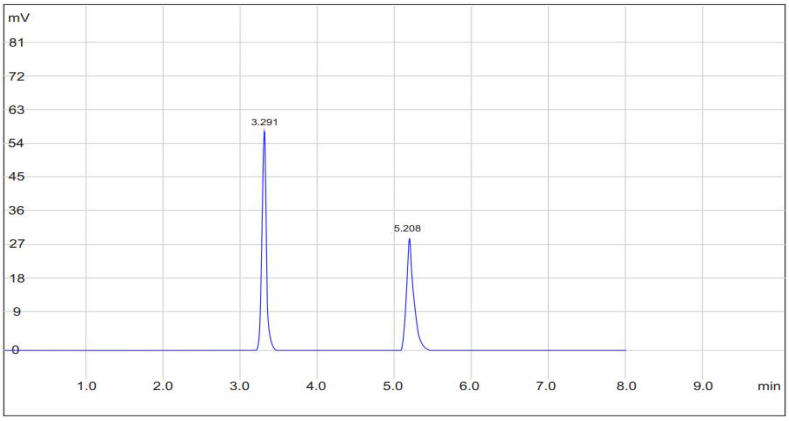

Figure 5: Chromatograph of Olanzapine and Fluoxetine

Accuracy

The accuracy of the method determines the closeness of results obtained by that method to the true value. From the results of accuracy testing, it was showed that the method is accurate within the acceptable limits. The % RSD is calculated for the Olanzapine and Fluoxetine and all the results are within limits. Acceptable accuracy was within the range and not more than 2.0% RSD as given in **Table 4-** Statistical validation for accuracy of Olanzapine and Table **5**: Statistical validation for accuracy of Fluoxetine [13, 14].

Table 4: Statistical validation for accuracy of Olanzapine

Level of addition	% Mean recovery*	SD	% RSD
50%	99.60	0.30	0.30
100%	99.58	0.55	0.55
150%	100.09	0.50	0.50

Table 5: Statistical validation for accuracy of Fluoxetine

Level of addition	% Mean recovery*	SD	% RSD
50%	100.39	1.34	1.34
100%	99.50	0.32	0.32
150%	99.41	0.44	0.44

Precision

Intraday and inter day precision assures the repeatability of results. The % RSD found was below 2 for both Olanzapine and Fluoxetine as calculated in Table 6: Data for intraday precision, Table7: Data for interday precision of Olanzapine and Table 8: Data for intraday precision, Table9: Data for interday precision of Fluoxetine [15, 16].

Table 6: Data for intraday precision of Olanzapine

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	1408417	1396971.67	10069.63	0.72
2	5	1393024			
3	5	1389474			
4	15	4302154	4304044.00	30506.15	0.71
5	15	4320154			
6	15	4289824			
7	25	7014258	6988571.00	31659.27	0.45
8	25	6953201			
9	25	6998254			

Table7: Data for interday precision of Olanzapine

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	1406825	1397168.67	10480.06	0.75
2	5	1398657			
3	5	1386024			
4	15	4275814	4286486.67	29792.03	0.70
5	15	4263501			
6	15	4320145			
7	25	7054814	7018206.33	44794.29	0.64
8	25	7031548			
9	25	6968257			

Table 8: Data for intraday precision of Fluoxetine

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	20	691248	694339.00	3917.67	0.56
2	20	693024			
3	20	698745			
4	60	2156484	2164673.33	18256.71	0.84
5	60	2163021			
6	60	2174515			
7	100	3596824	3586784.33	21497.24	0.60
8	100	3562104			
9	100	3601425			

Table 9: Data for interday precision of Fluoxetine

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	20	692457	692245.00	1130.01	0.16
2	20	691024			
3	20	693254			
4	60	2145714	2154094.00	8415.22	0.39
5	60	2162544			
6	60	2154024			
7	100	3601425	3595298.00	5857.30	0.16
8	100	3594715			
9	100	3589754			

Robustness

Robustness was studied by different deliberate variations in the chromatographic conditions i.e. Change in flow rate and wavelength. From robustness study % RSD was found to be within limit of 2 % for the Olanzapine and Fluoxetine as given in **Table 10**: Data for Robustness study of Olanzapine and Fluoxetine.Hence it is robust and complies per ICH guidelines [15, 16].

Table 10: Data for Robustness study of Olanzapine and Fluoxetine

Sr.No	Parameter	Condition	Olanzapine				Fluoxetine			
			Area	Mean	SD	% RSD	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.7	4302154	4306961	11575.03	0.27	2154813	2155433	10116.2	0.47
2		0.8	4320165				2145642			
3		0.9	4298564				2165846			
1	Change in Wavelength (nm)	233	4279856	4286635	12911.10	0.30	2132052	2141501	13124.8	0.61
2		235	4301524				2156487			
3		237	4278526				2135964			

Ruggedness

Ruggedness was studied by different analysts. **Table 11:** Data for ruggedness study of Olanzapine and Fluoxetinestudy showa % RSD was found to be within limit of 2 % for the Olanzapine and Fluoxetine. Hence it is complying as per ICH guidelines [16, 17].

Table 11: Data for ruggedness study of Olanzapine and Fluoxetine

Sr.No	Analyst	Olanzapine				Fluoxetine			
		Area	Mean area*	SD	% RSD	Area	Mean area*	SD	% RSD
1	Analyst-I	4302155	4287751	15435.56	0.36	2156498	2165135.3	9226.82	0.43
		4289642				2164052			
		4271458				2174856			
2	Analyst-II	4302525	4318235	14839.43	0.34	2186469	2165007.6	18719.48	0.86
		4320165				2152046			
		4332015				2156508			

Specificity

Excipients and impurities were not interacting with the standard drugs. Hence the method is specific from data in **Table 12:**Data for specificity study of Olanzapine and Fluoxetine [18, 19].

Table 12: Data for specificity study of Olanzapine and Fluoxetine

Drug	Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Area	Mean	SD	%RSD
Olanzapine	5	10	15	1402364			
	5	10	15	1394582	1405700.00	13108.34	0.93
	5	10	15	1420154			
	10	10	20	2965416			
	10	10	20	2971402	2974277.33	10595.76	0.36
	10	10	20	2986014			
	15	10	25	4302154			
	15	10	25	4320165	4318112.67	15037.91	0.35
	15	10	25	4332019			
Fluoxetine	20	40	60	692544			
	20	40	60	693214	693260.67	741.10	0.11
	20	40	60	694024			
	40	40	80	1382646			
	40	40	80	1365850	1368173.33	13462.21	0.98
	40	40	80	1356024			
	60	40	100	2154813			
	60	40	100	2174501	2173008.00	17496.34	0.81
	60	40	100	2189710			

% Assay of Marketed formulation

The % Assay (FOSTERA-5 marketed formulation of Intas Pharmaceutical was calculated as shown in **Table 13.** Data of % Assay of marketed formulation [20].

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Table 13. Data of % Assay of marketed formulation

Sr. NO.	Drug	Area of Sample	Area of Standard	% Assay
1	**Olanzapine**	4286571	4302154	**99.64**
2	**Fluoxetine**	2125014	2154813	**98.62**

System Suitability Parameters:

Table 14 has System suitability parameters were measured to verify the system, method and column performance. Standard solution of Olanzapine and Fluoxetine was injected into the system for six times and system suitability parameters were checked [20, 21].

Table 14: System suitability parameter

Sr. No.	Olanzapine			Fluoxetine		
	Retention Time (min)	Theoretical plates	Asymmetry Factor	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	3.284	9316	1.09	5.249	8975	1.11
2	3.291	8745	1.1	5.201	9974	1.13
3	3.402	9364	1.11	5.313	9152	1.14
4	3.384	9542	1.12	5.294	9034	1.12
5	3.296	9024	1.09	5.219	9231	1.13
6	3.204	9150	1.08	5.274	8754	1.12
Mean	3.31	9190.17	1.10	5.26	9186.67	1.13
SD	0.07	281.86	0.01	0.04	419.14	0.01
%RSD	2.20	3.07	1.34	0.83	4.56	0.93

SUMMARY

The Olanzapine was found to be linear in the concentration range of 5-25 µg/ml and Fluoxetine HCL is 20-100 µg/ml. From Accuracy study % recovery of Olanzapine was found in the range of 98.98-100.51% and Fluoxetine HCL is 98.86-101.38% which is in the limits accordingly the ICH guidelines. Intraday and Interday precision assures that % RSD was within limits of ICH guidelines i.e NMT 2 for both Olanzapine and Fluoxetine HCL. Limit of detection and limit of Quantitation of Olanzapine is 0.12µg/ml – 0.36µg/ml and Fluoxetine HCL is 0.10 µg/ml – 0.31 µg/ml respectively. Robustness was studied by deliberate variation i.e. change in Flow rate and change in Wavelength which was within 2 % of RSD as per ICH guidelines. ruggedness study gives results within the limits of 2% in which variation in Analyst was studied. The % assay of FOSTERA-5 was found to be Olanzapine (99.64%) – Fluoxetine HCL (98.62%).

CONCLUSION

The proposed chromatographic method was found to be simple, precise, accurate, rapid and specific for determination of Olanzapine and Fluoxetine from pure and its dosage forms. The mobile phase used for method development is very simple to prepare and economical also. The sample recoveries in the formulation were showing good results. This method is economical and run time is relatively short which enables rapid analysis among all the developed methods and hence, all the analysed validation parameters showed acceptable data with satisfactory correlation co-efficient and lower % RSD as per the ICH guidelines. The developed method can be utilized by industry for quantitative simultaneous estimation of Fluoxetine and Olanzapine as bulk and in tablet dosage form.

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