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Review Article

Development And Validation Of HPLC Method For Estimation Of Fluoxetine HCL In Bulk And Pharmaceutical Dosage Form

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ABSTRACT

A simple, sensitive and accurate RP-HPLC method has been developed for the determination of Fluoxetine HCL in bulk and marketed formulation. The method was developed after the several trials at the composition of Methanol: water (70:30), flow rate 1.0 ml/min and 264 nm wavelength of detection and retention time is 4.8 min. The method shows high sensitivity with linearity 5-25 µg/ml (regression equation: y = 203134x + 9713.5; r2 = 0.9997). The various parameters according to ICH guidelines and USP are followed for validating and testing of this method. The Detection limit and quantitation limit were found to be 0.008 µg ml–1 and 0.02 µg ml–1 respectively. The results demonstrated that the procedure is accurate, specific and reproducible (RSD <2%), and also being simple, cheap and less time consuming and appropriate for the determination of Fluoxetine HCL in bulk and Table dosage form.

INTRODUCTION

A selective serotonin reuptake inhibitor called fluoxetine is used to treat bipolar I, premenstrual dysphoric disorder, major depressive disorder, bulimia, OCD, and panic disorder. A selective serotonin reuptake inhibitor (SSRI), fluoxetine is a second-generation antidepressant. Despite being first developed to treat depression, it received FDA clearance in 1987 and is now often taken to treat both depression and a number of other illnesses..

The Chemical Structure of Fluoxetine HCL is shown in fig 1.

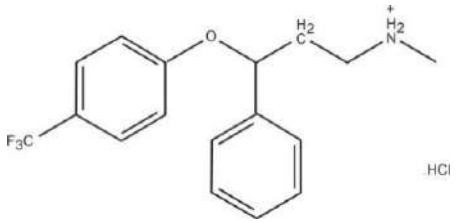

Fig 1: Structure of Fluoxetine HCL

Fluoxetine is a serotonin reuptake inhibitor that is used to treat severe depression, bulimia, OCD,

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premenstrual dysphoric disorder, panic disorder, and bipolar I disorder.

MATERIALS AND METHOD

Instruments:

The chromatographic analysis was performed on Analytical Technologies HPLC-3000 arrangement coordinated with a variable frequency programmable UV Detector and a Rheodyne injector outfitted with 20µl fixed circle. An stationary phase C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was utilized. Model - UV 2012 spectrophotometer and Wenser High Precision Balance Model: PGB 100 electronic equilibrium were utilized for Spectrophotometric analysis and gauging purposes individually.

Reagents and chemicals

Drug grade unadulterated Fluoxetine HCL sample was secured from Yarrow Chem, Mumbai. HPLC grade Methanol, ACN and water were acquired from Merck specialities private restricted, Mumbai.

Chromatographic conditions

C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was utilized for the chromatographic separation at a discovery frequency of 264 nm. Methanol: Water (70:30) was chosen as mobile phase for elution and same mobile phase was utilized in the preparation of standard and sample solutions. The elution was checked by injecting the 20µl sample and the flow rate was set to 1.0 ml/min.

Preparation of Standard solutions

Accurately 10.0 mg weighed quantity of Fluoxetine HCL was transferred to 10.0 mL volumetric flask. That was dissolved by adding 5.0 mL mobile phase and then the drug solution was diluted up to the mark with mobile phase to get the stock solution of 1000 µg/mL of Fluoxetine HCL. The working standard solutions of these drugs were obtained by appropriate dilution of the respective stock solution with mobile phase.

Optimization of Detection Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. For good response, optimization of wavelength was done at different wavelengths by UV detector. In the present study, drug solutions of 10µg/ml of each of Fluoxetine HCL were prepared in methanol. After observing UV spectra of the drug, wavelength of 264 nm was selected for further study.

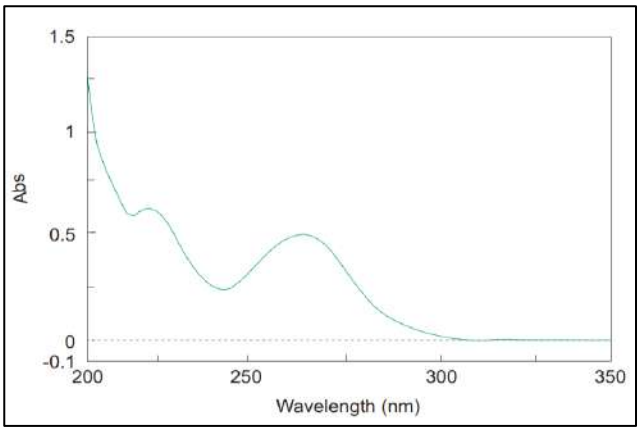

Fig. 2: UV Spectra of Fluoxetine HCL Showing λmax 264 nm

Optimisation of RP-HPLC method

Different mobile phases were gone after for the method optimisation, however satisfactory retention times, hypothetical plates and good resolution were seen with Methanol: Water (70:30) utilizing C18 column [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] and a run of the chromatograph of Fluoxetine HCL was appeared in figure 3.

Table 1: Optimized parameter

Parameter	Conditions
Column	Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol: Water (70:30)
Flow Rate	1.0 ml/min

Wavelength	264 nm
Injection Volume	20 µl
Detector	UV-3000-M
Retention Time	Approx. 4.8 min

Validation of RP-HPLC method

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

1. Linearity

Test solutions of different concentration were injected separately, and the chromatograms were recorded. A series of test preparations of Fluoxetine HCL (2-10 µg/ml) were prepared by taking 0.02 - 0.10 ml from the stock solution 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. The calibration curves of Fluoxetine HCL sample was shown in figure 2 and their related linearity parameters given in table 2.

2. Accuracy

To make sure the reliability and accuracy of the recovery study data were carried out by % recovery method which is also called as standard addition method. A known quantity of pure drug of Fluoxetine HCL was mixed to pre-analysed sample and contents again undergoes analysis by the optimised method and the % recovery was reported in table 3.

3. Precision

The repeatability study of the proposed method was verified by calculating the percentage RSD of three replica injections of 100% concentration i.e., 6µg/ml of Fluoxetine HCL on the same day and for intraday precision % RSD was calculated from repetition and also at different day for Interday precision. The results were shown in table 5.

4. 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.

5. Robustness

Robustness was calculated by changing the chromatographic conditions like compositions of mobile phase, detection wavelength, flow rate etc. and the % RSD should be reported. In the optimised conditions small changes were allowed and the extent to which the method was robust was determined. A deviation of ± 2 nm in the detection wavelength and ± 0.1 ml/min in the flow rate, were tried individually. Solutions of 100% test concentration with the specified n changes in the optimised conditions were injected to the system in triplicate. percentage RSD was shown in the table 7.

6. Ruggedness:

Ruggedness is the study to determine effect of external parameters on the method. To evaluate ruggedness of the developed method, parameters were deliberately varied. These parameters included variation of system, different analyst, Atmospheric changes. as per the test method and injected 3 concentrations of test solution into HPLC system with flow rate 1.0 ml/min by 2 different analysts.

7. Assay of marketed formulation

For the analysis of Fluoxetine HCL marketed formulation, Flunil-10 capsule was taken in a 100 mL volumetric flask, mobile phase was added in increments, the drug was dissolved by constant stirring and after complete dissolving of the cream base, remaining volume was made up with mobile phase and filtered. Sonicate for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter. Test sample of Fluoxetine HCL (6 µg/ml) was prepared

by from stock solution and diluted it upto 10 ml and injected into HPLC system.

8. Specificity

For chromatographic methods, developing a separation involves demonstrating specificity, which is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. as per the test method and injected 6 concentrations of 6 µg/ml of test solution into HPLC system with flow rate 1.0 ml/min.

9. System suitability

It was made sure that from the system suitability parameters, the method can give results of accuracy and precision**.** System suitability was performed with three replicate injections of solution of 6 µl/ml of Fluoxetine HCL into the chromatographic system. Tailing factor (T) Number of theoretical plates (N) obtained was reported in table 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. The results of linearity study (Figure 1) gave linear relationship over the concentration range of 2-10 µg/ml for Fluoxetine HCL. From the regression analysis, a linear equation was obtained y = 203134x + 9713.5, and the goodness-of-fit (r^2) was found to be 0.9997, indicating a linear relationship between the concentration of analyte and area under the peak.

Table 2: Summary of results of Linearity

Sr. No.	Conc. (µg/ml)	Area
1	2	415236
2	4	812365
3	6	1245947
4	8	1632548
5	10	2036481

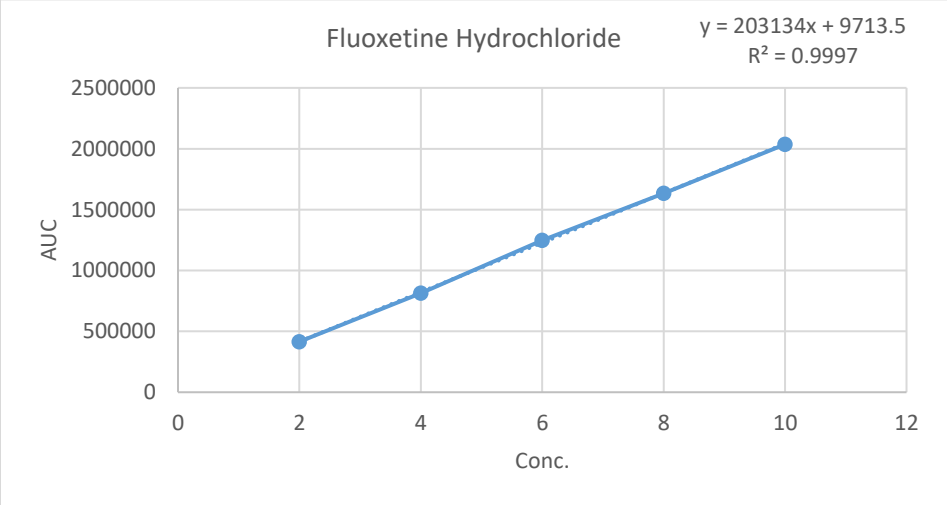

Figure 3: Linearity

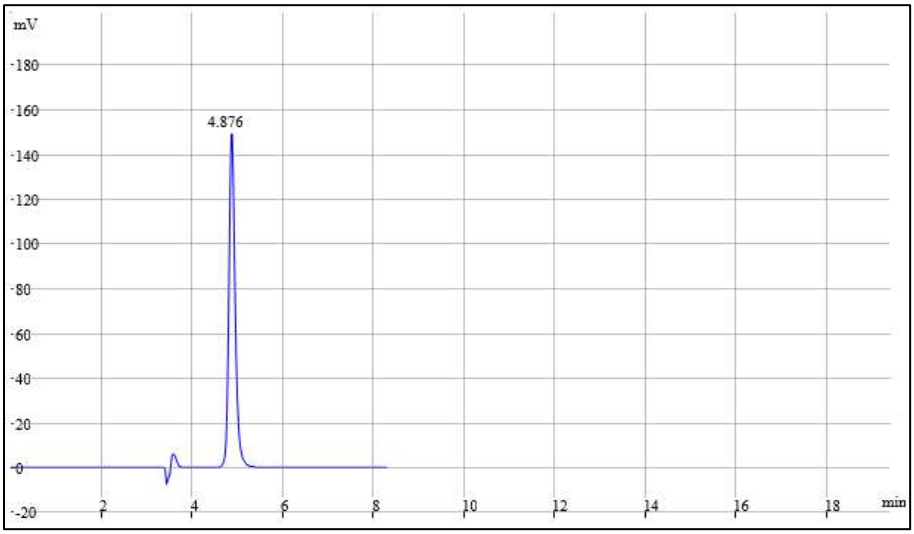

Figure 4: Typical chromatograph of Fluoxetine HCL

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 Fluoxetine HCL and all the results are within limits. Acceptable accuracy was within the range and not more than 2.0% RSD, as demonstrated in Table -3.

Table 3: summary of Results of Accuracy

Level of addition	Standard added (µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Area obtained*	Std Area	Drug recovered (µg/ml)	%Recovery
50%	2	4	6	1245568	1245947	6.00	99.97
	2	4	6	1243024		5.99	99.77
	2	4	6	1248876		6.01	100.24
100%	4	4	8	1645856	1632548	8.07	100.82
	4	4	8	1653264		8.10	101.27
	4	4	8	1632028		8.00	99.97
150%	6	4	10	2031255	2036481	9.97	99.74
	6	4	10	2036587		10.00	100.01
	6	4	10	2035654		10.00	99.96

Table 4: % recovery data

Level of addition	% Mean recovery*	SD	% RSD
50%	99.99	0.24	0.24
100%	100.7	0.66	0.66
150%	99.9	0.14	0.14

Precision

Precision is "the closeness of results obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is expressed in the form of relative standard deviation. The repeatability, intra-day and inter-day precision results are shown in the table 5. The RSD were calculated for all the results are within limits. Precision was not more than 2.0% RSD, as demonstrated in Table 5 and 6.

Table-5: summary of Intraday Precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	2	415356	415794.67	501.25	0.12
2	2	415687			
3	2	416341			
4	6	1243265	1247771.33	21021.71	1.68
5	6	1259784			
6	6	1240265			
7	10	2031248	2030564.00	6702.23	0.33
8	10	2036898			
9	10	2023546			

Table-6: summary of Interday Precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	2	415632	415869.33	739.15	0.18
2	2	416698			
3	2	415278			
4	6	1240526	1243146.00	4825.70	0.39
5	6	1248715			
6	6	1240197			
7	10	2030125	2035778.33	4899.58	0.24
8	10	2038416			
9	10	2038794			

LOD and LOQ

The LOD and LOQ were calculated by the equations $\text{LOD} = \frac{3.3 \times \text{std.Deviation}}{\text{slope}}$ and $\text{LOQ} = \frac{10 \times \text{std.Deviation}}{\text{slope}}$ where, std. Deviation taken from accuracy and slope is from linearity . Based on these equations, the calculated LOD and LOQ values for Fluoxetine HCL were 0.1094 and 0.3316 µg/ml, respectively.

Robustness

Robustness of the method reflects that the results are unaffected or reliable even if the minute changes in the method parameters. Here, the flow rate and wavelength were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. The results obtained with changes in the parameters on a 15µg/mL solution are as shown in Table No. 7

Table 7: robustness

Sr.No	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.9	1245678	1243948	3112.04	0.25
2		1	1245810			
3		1.1	1240355			
1	Change in Wavelength (nm)	262	1245163	1242110	5305.57	0.43
2		264	1235984			
3		266	1245184			

Ruggedness

Ruggedness was studied by different analyst.

Table 8: Data for ruggedness study of Fluoxetine HCL by HPLC method

Sr. No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	6	1245655	1243666.33	6355.79	0.51
			1248790			
			1236554			
2	Analyst-II	6	1245689	1247515.00	7933.21	0.64
			1256202			
			1240654			

% Assay:

The % Assay Flunil-10 marketed formulation of Intas Pharmaceutical was calculated and given in table.

Table 9: % Assay of Fluoxetine HCL by HPLC method

Sr. No.	Marketed Formulation	Area Obtained*	Area of Standard	% Assay
1	Flunil – 10 (Intas Pharmaceutical	1224125	1245947	98.22

Specificity:

Excipients and impurities were not interacting with the standard drug, hence method is specific. Results of specificity are shown in Table.

Table 10: Data for specificity study of Fluoxetine HCL by HPLC method

Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Area	Mean	SD	%RSD
2	4	6	415356	415455	203.45	0.05
2	4	6	415689			
2	4	6	415320			
4	4	8	813254	812451.667	694.86	0.09
4	4	8	812045			
4	4	8	812056			
6	4	10	1241005	1241079	4571.45	0.37
6	4	10	1236545			
6	4	10	1245687			

System Suitability Parameters:

System suitability was performed by injecting three replicate injections of 100% test concentration, number of theoretical plate, asymmetry factor are satisfactory. The chromatographs confirm the presence of Fluoxetine HCL at 4.1 min without any interference.

Table 11: System suitability parameter

Sr. No.	Conc. (µg/ml)	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	6	4.812	8646	1.23

2	6	4.816	8234	1.21
3	6	4.863	7982	1.23
4	6	4.875	8103	1.24
5	6	4.803	8956	1.25
6	6	4.835	8256	1.24
Mean		4.83	8362.83	1.23
SD		0.03	366.77	0.01
%RSD		0.61	4.39	1.11

CONCLUSION

In the present research work, a successful attempt was made for determination of Fluoxetine HCL in Bulk and dosage form by High performance liquid chromatography. The method was developed by experimentation, based on literature survey. The simplicity, rapidity, reproducibility and economy of the proposed method completely fulfill the objective of this research work. The HPLC method was developed and validated for estimation of Fluoxetine HCL. The mobile phase was consisting of Methanol: Water (70:30). Detection was done at 264 nm. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD was found within limit. The result showed that proposed method was suitable for the accurate, precise and rapid determination of in Fluoxetine HCL its bulk and Parenteral dosage form.

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No conflict of interest.

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