Development and Validation of HPTLC Method of Fluoxetine Hydrochloride in Bulk and Pharmaceutical Formulation

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Abstract— A simple, accurate, low cost and specific HPTLC method for estimation of Fluoxetine hydrochloride in capsule has been developed. It was performed on Silica gel G_{60} F_{254} aluminium foil using Acetone: Methanol in the ratio of 5:4 as mobile phase. The mobile phase containing chamber was saturated for 10 minutes at room temperature. The Rf value of Fluoxetine was found to be 0.12. The plate was scanned and quantified at 226 nm. The calibration curve response was observed between 300-2100 ng. The linear regression data showed good linear relationship of r^2 = 0.999. The percent recovery was found to be 99.94 ±1.188. The developed method was validated for its accuracy and precision with suitable parameters.

Keywords: HPTLC, Fluoxetine Hydrochloride, Silica gel G_{60} F_{254}

I. Introduction

Fluoxetine hydrochloride, N-methyl-3-phenyl-3-(2, 2, 2-trifluoro-p-tolyloxy) propylamine hydrochloride (figure no.1) $C_{17}H_{18}F_3NO$, HCl, MW=345.8, is an antidepressant which differs structurally and pharmacologically from the tricyclic agents. It has been shown to selectively inhibit the reuptake of serotonin in presynaptic neurons[1]. Fluoxetine hydrochloride is also used in a variety of disorders in addition to depression[1,2]. Beneficial responses have been reported in obsessive compulsive disorders, pain syndromes including diabetic neuropathy and fibrositis, panic disorders and nervous bulimia (American Hospital Formulary Service, Drug Information 93)[3-5].

The estimation of fluoxetine hydrochloride by Gas chromatographic-mass spectrometric method[6], high performance liquid chromatography [HPLC][7], Micellar electrokinetic capillary chromatography[8] is reported in literature.

II. Materials and Method

A Camag, Linomat 5 sample applicator was used. The scanner used was Camag TLC Scanner 3 and CATS 4 software for interpretation of data. Fluoxetine Hydrochloride pure drug was procured from Wockhardt Pharma. Ltd. Aurangabad, as gift sample and was used without further purification. All chemicals and reagents used were of analytical grade.

A. Method:

1) Preparation of Standard Solution

10 mg of fluoxetine was weighed and transferred in 10 ml volumetric flask. The drug was dissolved in about 5 ml methanol by vigorous shaking and then volume was concentration of 1 mg/ml. 1 ml of the above solution was transferred to 10 ml volumetric flask and the volume was made up to the mark with methanol to get 100 ng/µl solution.

2) Selection of wavelength for densitometric evaluation:

The wavelength selected for densitometric determination was 226nm. The selection of wavelength was based on its high absorptivity at 226nm for better sensitivity of determination. Densitogram of fluoxetine shown in Fig. No.2

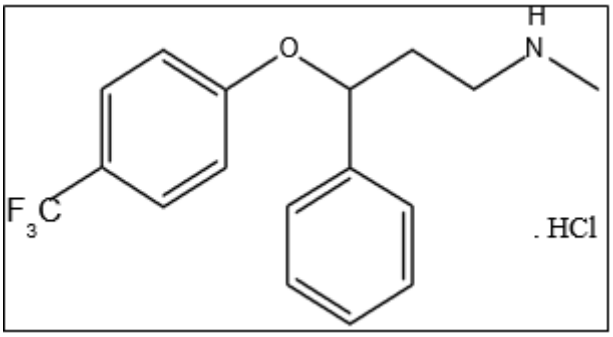

Fig. 1: Chemical structure of Fluoxetine Hydrochloride

3) Chromatographic conditions

Stationary phase- silica gel $G_{60}F_{254}$ TLC precoated plates (20×10), Mobile phase- Acetone: Methanol in ratio of 5:4, saturation time -10 Minutes, Migration distance-75 cm, Band width-7mm, Source of radiation- Deuterium lamp, Detection wavelength- 226nm using slit dimension 6 x 0.45 mm.

4) Calibration curve response

Aliquots of 3, 6, 9, 12, 15, 18, and 21 µl of standard solution of Fluoxetine were applied on the chromatographic plates. The plate was developed using Acetone: Methanol (5:4, v/v), dried and scanned at 226nm. Peak area was recorded for each concentration of drug. Concentration v/s response curve was constructed and it is depicted in Fig. No.3

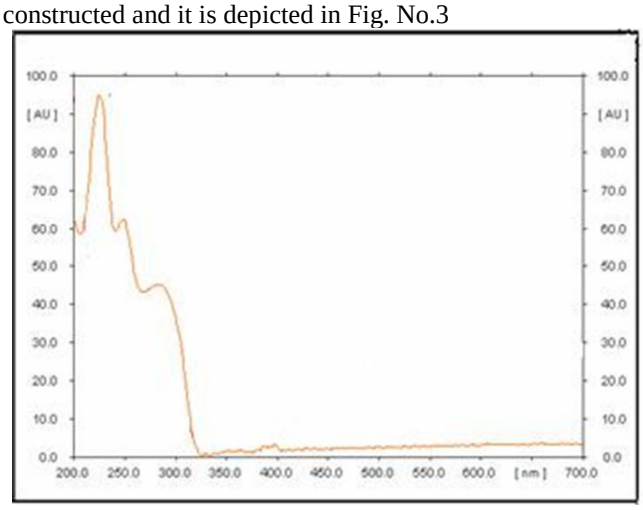

Fig. 2: Densitogram of fluoxetine

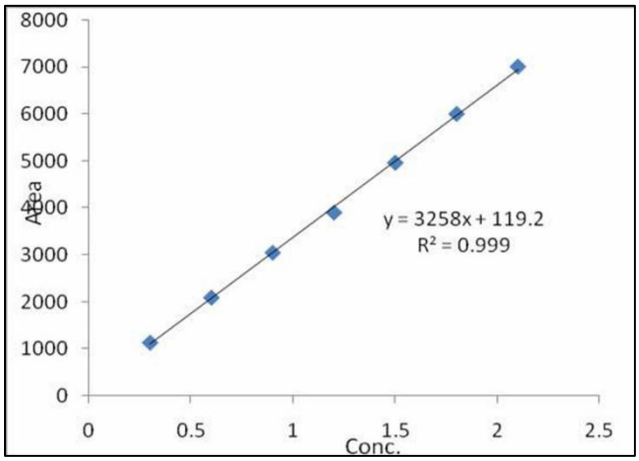

Fig. 3: Concentration- response curve for fluoxetine

5) Estimation of fluoxetine in capsules by proposed method:

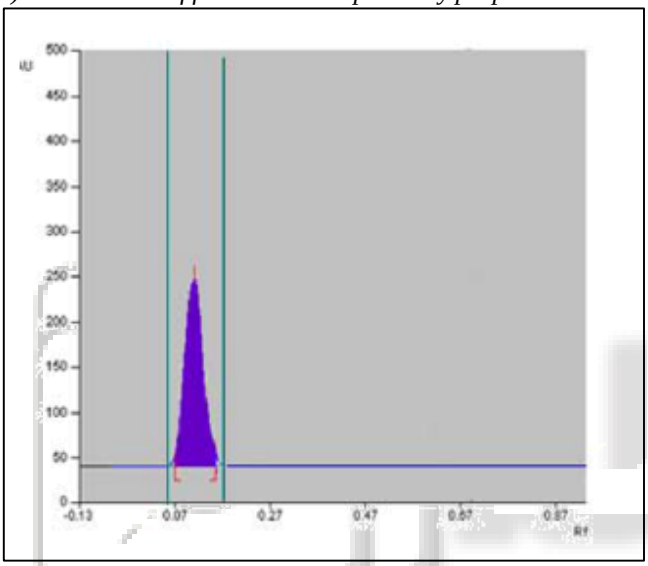

Fig. 4: Normal (Untreated)

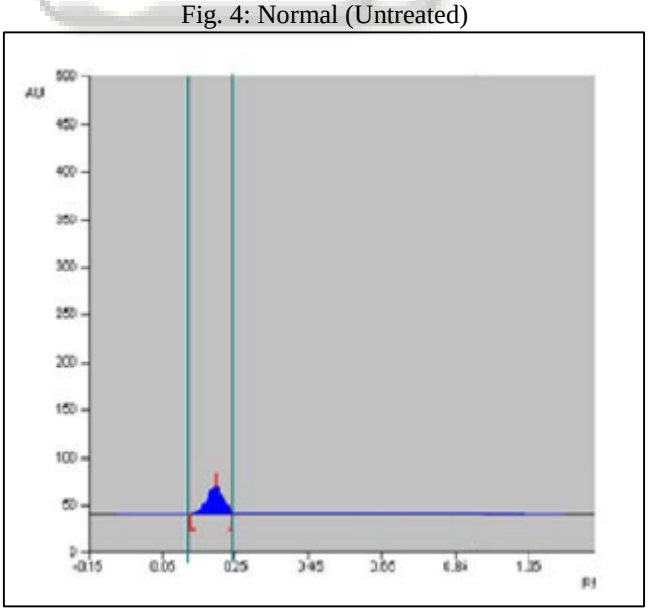

Fig. 5: NaOH treated

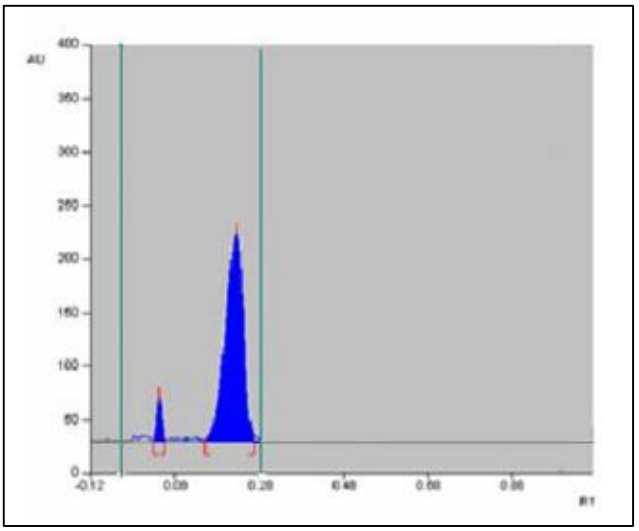

Fig. No. 6: Acid treated

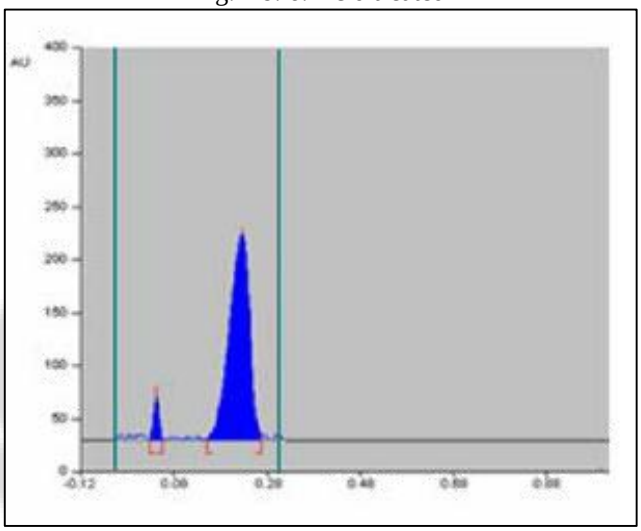

Fig. 7: H_2O_2 treated

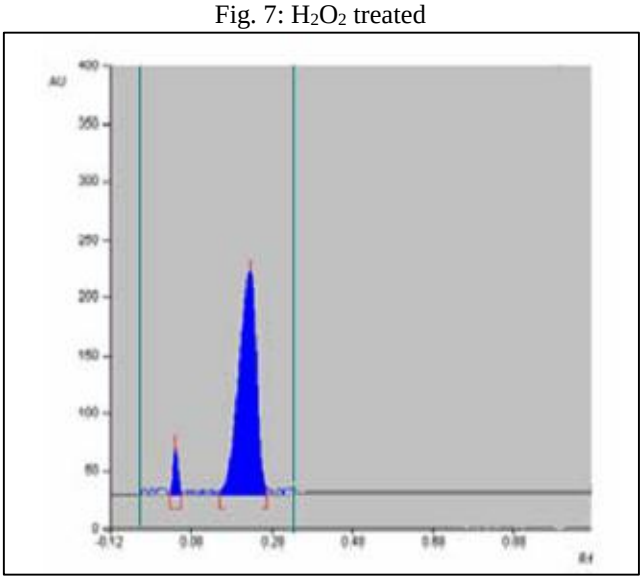

Fig. 8: UV radiations treated

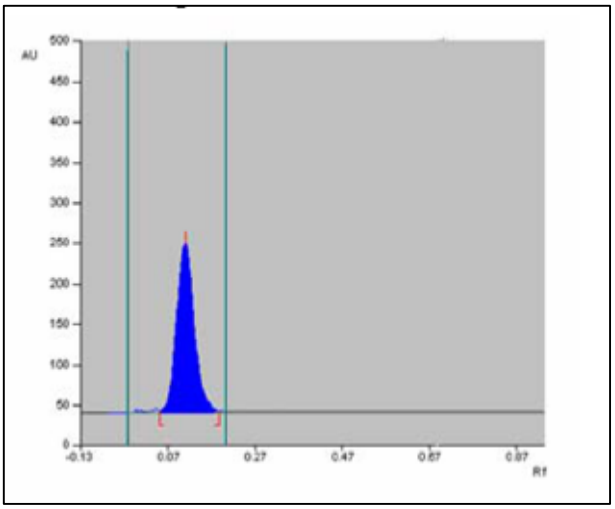

Fig. 9: 75% RH treated

6) Sample Solution:

Contents of twenty capsules were emptied and weighed. An accurately weighed quantity of capsule powder equivalent to 20 mg of fluoxetine was taken into 10 mL volumetric flask and about 5ml of methanol was added to it. The flask was shaken for 15 minutes and the volume was adjusted to 10 mL with methanol. Then the solution was sonicated for 15 min and filtered through Whatman filter paper No.41. The filtrate gives the concentration 2mg/ml for fluoxetine. 1 ml portion of the above filtrate was transferred to 10.0 mL volumetric flask and the volume was made up to the mark with methanol.

7) Procedure:

Two bands of standard solution and six bands of sample solution (5µl) were applied on TLC plate as 7mm band and the plate was developed and scanned as per the optimized chromatographic conditions.

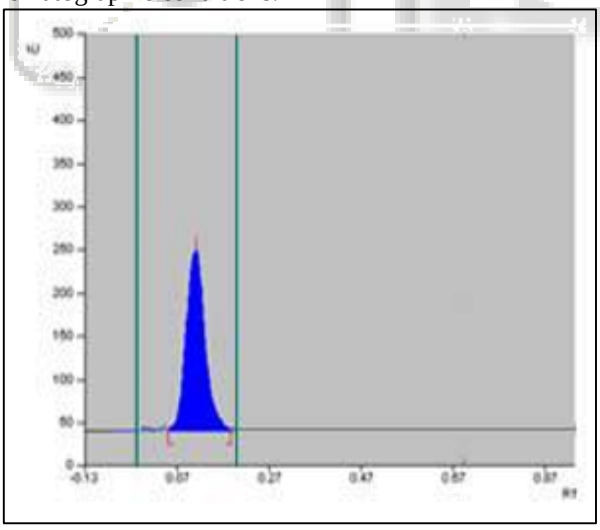

Fig. 10: 50ºC temp. Treated

Sr.no.	Parameter	Result
1	Absorption maxima (nm)	226
2	Linearity Range(µg/mL)	0.3-2.1
3	Standard regression equation	y = 3258× + 119.2
4	Correlation Coefficient (r2)	0.999
5	Accuracy (% recovery ±SD)	99.94± 1.188
6	Precision	99.64± 1.026
7	Specificity	Specific
8	Ruggedness	Rugged
9.	Robustness	Robust (99.29 ± 0.651)

Table 1: Validation parameters

III. RESULTS AND DISCUSSION

A. Precision:

Precision of any analytical method is expressed as SD and RSD of series of measurements. Precision of estimation of fluoxetine by proposed method was ascertained by analysis of homogeneous samples of capsules powder. Results are as shown in Table No.2

B. Accuracy (Recovery Test):

Accuracy of the Method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to capsule powder. The recovery was performed at five levels, 80, 90, 100, 110 and 120% of fluoxetine hydrochloride standard concentration. The recovery samples were prepared in afore mentioned procedure. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve (n=5). The recovery values for fluoxetine hydrochloride ranged from 99.94±1.188 (Table no.3)

Drug	Label claim mg/tab	Amount found mg/tab	% drug found
Fluoxetine	20	19.83	99.15
	20	19.66	98.30
	20	20.01	100.05
	20	19.93	99.65
	20	20.21	101.05
Mean: 99.64, SD1: 0.023, %RSD: 1.026			

Table 2: Results of Tablet analysis

Level of addition	Volume of tablet solution added (µl)	Volume of Standard solution added(µl)	Amount recovered(µg/ml)	% Recovery
80	3	2	0.1982	99.10
90	3	3	0.2968	98.93
100	3	4	0.4016	100.40
110	3	5	0.4974	99.48
120	3	6	0.6109	101.81
Mean: 99.94, SD: 1.188, %RSD: 1.188				

Table 3: Results of recovery study

C. Specificity:

Accurately weighed seven quantities of capsule powder each equivalent to about 20 mg of fluoxetine was transferred to 10 mL volumetric flask. All these solutions were stored for 24 hrs under following different conditions.

1) Normal
2) At 50^0C after addition of 1.0 mL of 0.1N NaOH (alkali)
3) At 50^0C after addition of 1.0 mL of 0.1N HCL (acid)
4) At 50^0C after addition of 1.0mL of 3% H_2O_2 (oxidation)
5) At 60^0C (Heat)
6) In UV chamber
7) At 75% RH

After 24 hours, the solutions of samples were prepared and analysed. The % labelled claim were calculated. The results shown in Table no.4

Sample	% Labeled claim
Normal	100.60
Acid	96.23
Base	38.96
Peroxide	96.50
UV	99.35
Heat	99.93
75% RH	100.20

Table 4: Results of Specificity study

D. Linearity and Range:

The linearity of the response of the drug was found to be between 0.3-2.1µg/ml concentration. The graphs plotted as the amount of drug applied v/s response (depicted in Fig. No.3) were found to be straight line. The correlation coefficient (r2) of determination was 0.999. The results are summarized in Table No.1

S.No.	Analyst	% labeled claim	Days	% Labeled claim*
1	I	98.80	Day I	99.09
2	II	99.84	Day II	99.34
3	III	99.59	Day III	99.19
Mean: 99.41,			Mean: 99.20,	
SD:0.54,			SD: 0.1258,	
%RSD: 0.5432			%RSD: 0.1268	

Table 5: Study of ruggedness of the proposed method

E. Ruggedness:

The ruggedness studies were performed by analyzing the capsule powder samples using proposed method by different analysts on different days. Results are as shown in Table no.5

Scanning wavelength(nm)	% labeled claim
200	99.52
205	98.57
210	99.80
Mean: 99.29, SD: 0.6446, %RSD: 0.6510	

Table 6: Study of robustness of the proposed method

F. Robustness

Repeatability is based on the results of the method operating over short time interval under same conditions. The low RSD values of precision (Table no.2), recovery (Table no.3), showed high repeatability. Making deliberate small changes in wavelength used tested the robustness of method (Table no.6)

IV. Conclusion

The proposed HPTLC method is simple, accurate, precise, specific and highly sensitive; developed and validated for the determination of fluoxetine hydrochloride in bulk and in dosage form. There are several methods existing for the estimation of Fluoxetine viz HPLC, GC-MS, and capillary zone electrophoresis. These methods are either costlier or cannot detect impurity whereas the HPTLC method developed can simultaneously run standards and formulation. Therefore it is concluded that the HPTLC method is cost effective and less time consuming. Hence, the proposed method can be successfully used for routine quality control analysis of drug in marketed preparations.

Acknowledgement

We are very much thankful to the Chairman, Mrs. Fatma Rafiq Zakaria, Maulana Azad Educational Trust and Dr. M. H. Dehghan, Principal, Y. B. Chavan College of Pharmacy for providing necessary facilities for the project work. We are thankful to Wockhardt Pharma.Ltd. Aurangabad for providing the gift sample of fluoxetine hydrochloride.

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