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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF FLUOXETINE IN ITS CAPSULE FORMULATION

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ABSTRACT

An HPTLC method for estimation of Fluoxetine in its capsule formulation has been developed. It employs aluminium backed silica gel 60 F_{254} TLC plates, (20 cm × 10 cm, layer thickness 0.2 mm) prewashed with methanol and mobile phase comprising of toluene: 2-propanol: ammonia 2:2:0.4 (v/v/v). The developing solvent was run upto 80 mm in Camag chamber previously saturated with 10.0 mL of solvent mixture for 30 min. Densitometric scanning was then performed with Camag TLC scanner-3 equipped with winCATS software Version 1.3.0 at λ_{max} 227 nm. The R_f value was found to be 0.74. The recovery of Fluoxetine was found to be 99.90% ± 1.68. The limit of detection and limit of quantitation were found to be 43.55 ng/spot and 131.99 ng/spot. The % RSD of intra-day variation and inter day variation were 0.54 and 0.41 respectively.

INTRODUCTION: Fluoxetine (FLX), N- methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy], propan-1-amine is a selective serotonin re-uptake inhibitor which is clinically effective for treatment of depression. It is official in United States Pharmacopoeia [2]. It is readily and completely absorbed from GI tract with peak serum levels occurring 6-8 hours after oral dosing with capsules.

The literature survey reveals that the drug has been determined by TLC using derivatization reagent [4], spectrophotometry [5], HPLC [6, 7, 8], GC [9, 10], thermoanalytical [11] and capillary electrophoresis [12], LC-MS [13, 15], HPLC-DAD [14],. The aim of this work was to develop simple, fast, precise, accurate HPTLC method for the estimation of FLX in its capsule formulation.

MATERIALS AND METHODS:

Instrument: CAMAG (Muttenz, Switzerland) HPTLC system including a linomat V applicator, Camag TLC scanner-3 and WinCATS (version 1.3.0) data processor was used.

Chemical and materials: FLX was kindly supplied by Cadila Pharmaceuticals Ltd., Ahmedabad. Toluene, isopropanol and ammonia used were of analytical grade from E-Merck Ltd., Capsule dosage form Prodep 10 manufactured by Sun Pharmaceutical Industries Ltd., were procured from market which contain FLX 10 mg.

Procedure:

Standard preparation: Accurately weighed quantity of about 40 mg FLX was transferred to 100 mL volumetric flask. It was dissolved in methanol and the volume was made up to mark with the same solvent to get concentration of 400 µg/mL of FLX.

Chromatographic conditions: The experiment was performed on a aluminium backed silica gel 60 F_{254} TLC plates, (20 cm × 10 cm, layer thickness 0.2 mm)

prewashed with methanol and mobile phase comprising of toluene: 2-propanol: ammonia 2:2:0.4 (v/v/v). The developing solvent was run upto 80 mm in Camag chamber previously saturated with 10.0 mL of solvent mixture for 30 min. Samples were applied as 6 mm wide bands and the distance between the bands was 11.6 mm. The developing solvent was run upto 80 mm, (distance to the lower edge was 10 mm) and the development was performed at 25±2°C. After development, the plate was dried at 50 ^{0}C in an oven for 5 minutes. Densitometric scanning was then performed with camag TLC scanner 3 equipped with winCATS software Version 1.3.0 at λ_{max} 227 nm, using deuterium light source and the slit dimensions were 6.00 × 0.45 mm.

Linearity of detector response: Standard solution 1-10 µl (400-4000 ng/spot) was applied on TLC plate with the help of microlitre syringe, using linomat V sample applicator. The plate was developed using mobile phase comprising toluene: 2-propanol: ammonia 2:2:0.4 (v/v/v) in twin trough chamber to a distance of 8 cm and scanned in the above established chromatographic conditions. Each concentration was spotted six times on the plate. Peak area was recorded for each concentration of drug; the observations are reported in **Table 1** and calibration curve was obtained by plotting peak areas against concentration of FLX **Figure1.** Regression equation data for Fluoxetine is shown in **Table 2.** A typical HPTLC chromatogram is shown in **Figure 2** and 3D Linearity spectra of Fluoxetine standard solution is shown in **Figure 3.**

TABLE 1: LINEARITY STUDY OF FLX

Concentration (ng/spot)	Area* (ng/spot)	% RSD
400	956.04	1.344
800	1972.4	1.030
1200	2868.15	0.964
1600	3570.39	0.913
2000	4780.26	1.015
2400	5736.31	0.373
2800	6692.36	0.312
3200	7648.41	0.501
3600	8598.18	0.399
4000	9560.52	0.497

*Average of six determinations

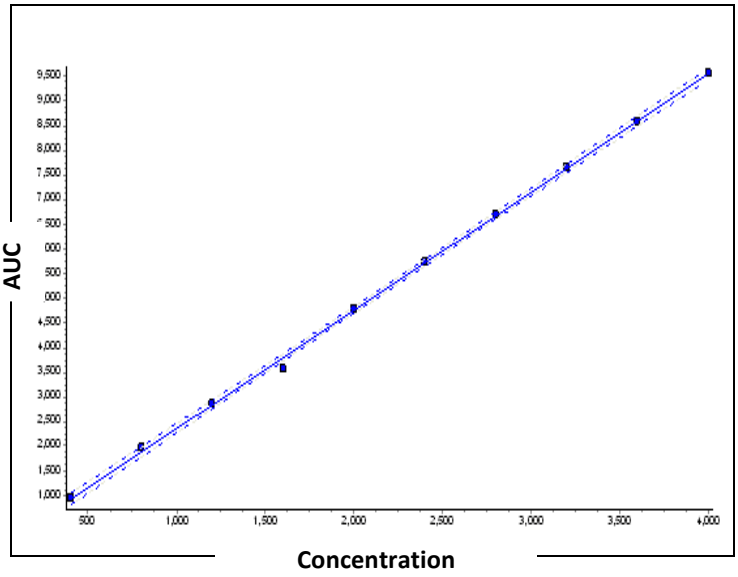

FIG. 1: CALIBRATION CURVE FOR FLX

TABLE 2: REGRESSION EQUATION DATA FOR FLX IN BULK SAMPLE

Regression equation data for Fluoxetine	
Coefficient of correlation	0.9992
Slope	2.3946
Intercept	29.833

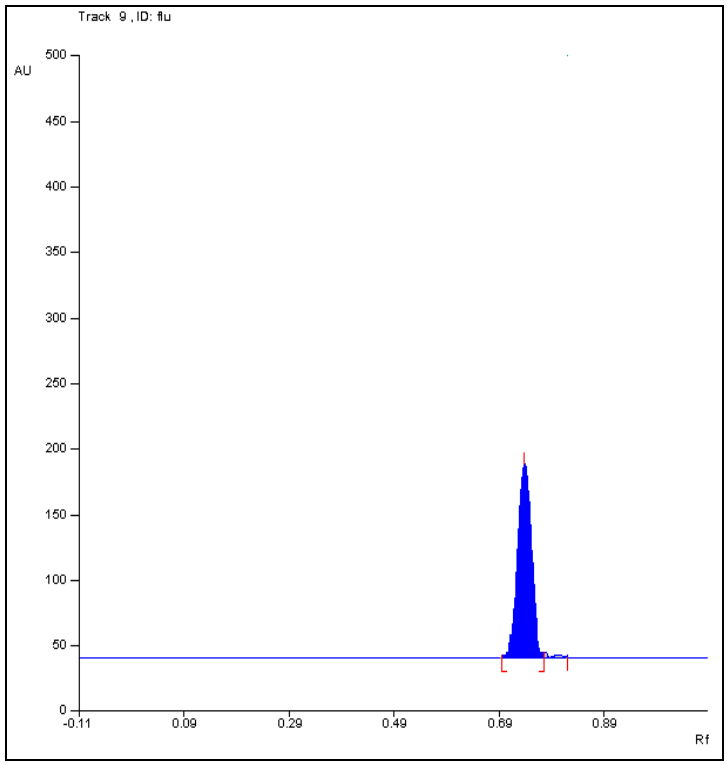

FIG. 2: DENSITOGRAM OF STANDARD FLX (R_F 0.74±0.02), MEASURED AT 227 nm

Mobile phase: Toluene: 2-Propanol: Ammonia 2:2:0.4 (v/v/v).

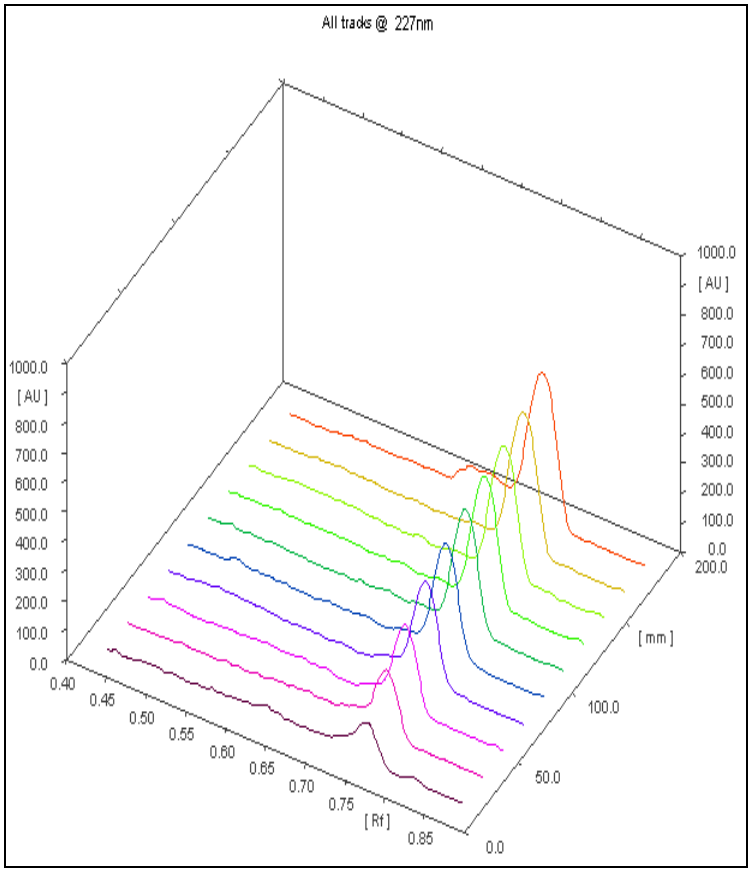

FIG. 3: 3D LINEARITY SPECTRA OF FLUOXETINE STANDARD SOLUTION

Assay of Fluoxetine in bulk sample: Accurately weighed quantity 40 mg (FLX) was transferred to 100 mL volumetric flask. It was dissolved in methanol and volume was adjusted to mark. The solution (2.5µL, containing 1000 ng) was spotted. After development and scanning the concentration was determined employing the regression equation; results are shown in **Table 3.**

Assay of Fluoxetine in capsule formulation: To determine the content of FLX; twenty capsule were weighed; average weight determined and crushed fine powder. An accurately weighed powder equivalent to 40 mg (FLX) was transferred to 100 mL volumetric flask containing 40 mL methanol, sonicated for 10 min. and volume was adjusted to mark with same solvent. The resulting solution was filtered using Whatmann filter paper 41. Appropriate solution of 2.5µL containing 1000 ng/spot was spotted for assay. The plate was developed using mobile phase comprising of toluene: 2-propanol: ammonia 2:2:0.4 (v/v/v) in twin trough chamber to a distance of 8 cm. The concentration was determined by regression equation y = 2.3946 X-29.833 and the results are shown in **Table 4.** Densitogram of FLX from capsule is shown in **Figure 4.**

TABLE 3: ASSAY OF FLX IN BULK SAMPLE

Component	Labeled claim (mg)	Amount Found (mg)	Amount found (%)	Mean ± SD	% RSD
FLX	10	9.96	99.63	100.39 ± 1.68	1.67
	10	10.16	101.67		
	10	9.86	98.67		
	10	10.16	101.62		
	10	9.84	98.43		
	10	10.23	102.30		

SD stands for Standard Deviation; RSD stands for Relative Standard Deviation

TABLE 4: ASSAY OF FLX IN CAPSULE SAMPLE

Component	Labeled claim (mg)	Amount Found (mg)	Amount found (%)	Mean ± SD	% RSD
FLX	10	9.74	97.49	99.75 ± 1.70	1.71
	10	10.17	101.71		
	10	10.07	100.79		
	10	9.90	99.03		
	10	9.83	98.32		
	10	10.11	101.15		

SD stands for Standard Deviation; RSD stands for Relative Standard Deviation.
Brand name: Prodep 10 (Sun Pharmaceutical industries); Batch no. AD 72033

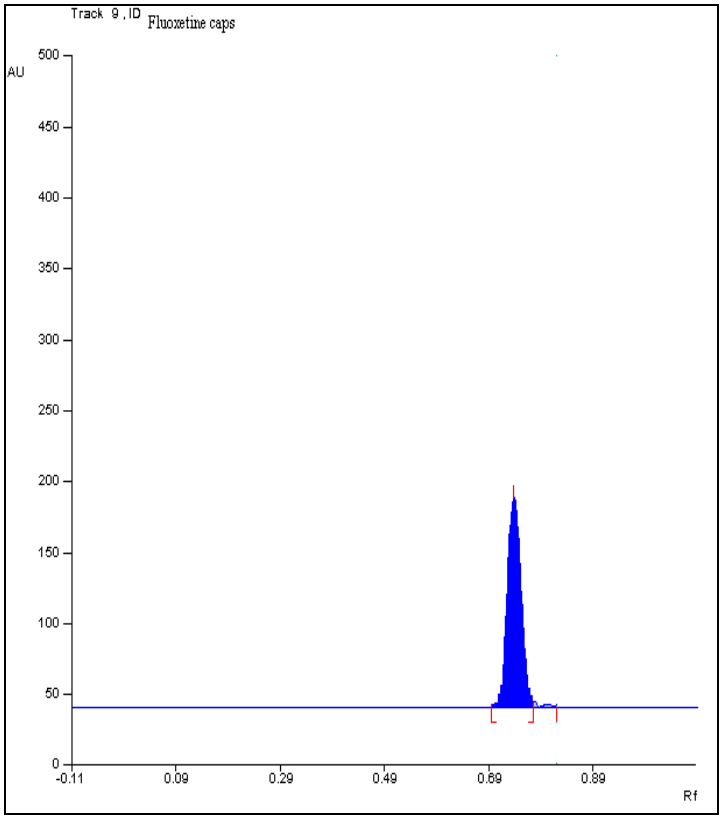

FIG. 4: DENSITOGRAM OF FLX FROM CAPSULE (R_F 0.74±0.02), MEASURED AT 227 nm

Mobile phase: Toluene: 2-Propanol: Ammonia 2:2:0.4 (v/v/v).

TABLE 5: RESULTS OF RECOVERY STUDIES:

Drug	Initial amount (mg)	Amount added (mg)	Amount recovered (ng/µL)	% Recovered (Mean ± SD)	Cumulative mean% RSD
FLX	10	0	10.00	100.04 ± 1.85	99.90 ± 1.68
	10	6	6.08*	100.56 ± 1.90	
	10	10	9.89*	99.49 ± 1.38	
	10	14	14.04*	100.19 ± 1.78	

*Average of three determinations; SD stands for Standard Deviation; RSD stands for Relative Standard Deviation

Repeatability of measurement of peak area was determined by spotting 2.5 µL (1000 ng/spot) of standard drug solution on TLC plate the spot was scanned 7 times without changing the positions of the plate. The sensitivity of measurements of FLX by the use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and limit of detection (LOD). Ruggedness of the method was checked, carrying out the analysis by two different analyst keeping same experimental and environmental conditions. Robustness of the method was ascertained by carrying out the analysis using same concentration but changing migration distance in the course of analysis.

Recovery study: Recovery experiment was done by using standard addition method at 80, 100 and 120 % level. Known amount of standard stock solution of FLX was added to pre-analyzed sample (1000 ng) and subjected to the proposed HPTLC method. The results are shown in **Table 5.**

Validation: The developed method was validated as per ICH Q2B guidelines[3] for specificity, repeatability, sensitivity, instrumental precision, accuracy, ruggedness and robustness (**Table 6).** The purity of FLX was tested by correlating the spectra of FLX at the peak start (S), peak apex (A) and at the peak end (E) positions (**Figure 5**). Thus, it can be concluded that no impurities or degradation products were found with the peaks of standard drug solutions. Intra-day precision was determined by analyzing 800, 1000, 1200 ng/spot of standard solution for three times on the same day. Inter-day precision was determined by analyzing 800, 1000, 1200 ng/spot of standard solution for three consecutive day over a period of a week.

TABLE 6: METHOD VALIDATION PARAMETERS

Parameters	Fluoxetine
Linearity range	400-4000 ng/spot
Correlation coefficient (r^2)	0.9992
Precision (n = 9) (%RSD)	Intraday-0.54, Interday-0.41
Specificity	Specific
Repeatability (n = 7) (%RSD)	1.71
Limit of detection (LOD)	43.55 ng/spot
Limit of quantitation (LOQ)	131.99 ng/spot
Ruggedness studies (n = 6) (%RSD)	Analyst I- 0.18, Analyst II-0.5
Robustness studies (n = 6) (%RSD)	1.0

RSD stands for Relative Standard Deviation

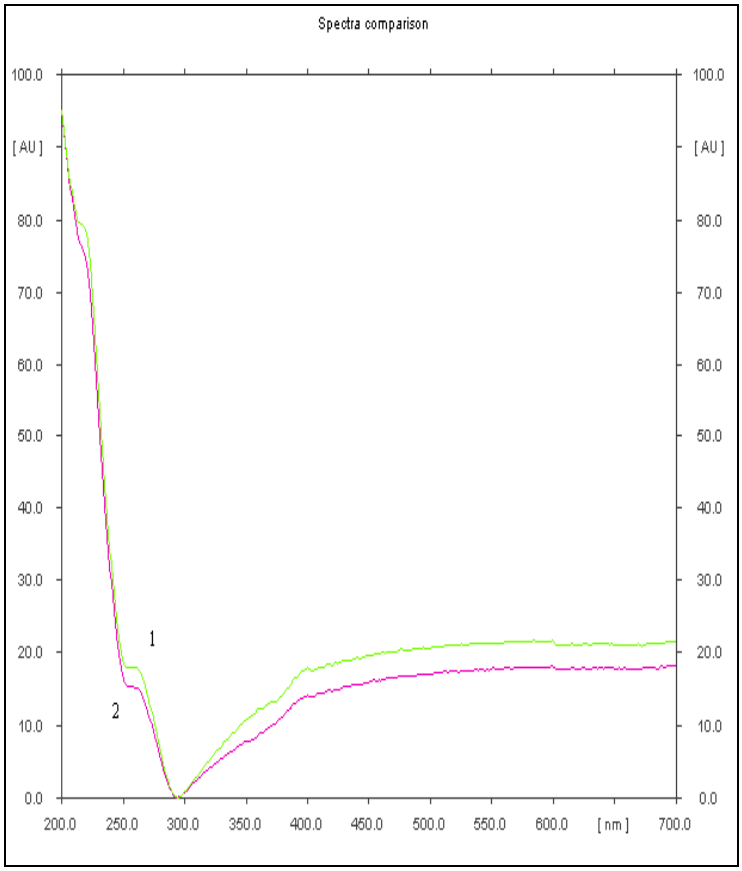

FIG. 5: PEAK PURITY SPECTRA OF 1. STANDARD FLX 2. EXTRACTED FROM A FLX CAPSULE, SCANNED AT THE PEAK-START, PEAK-APEX, AND PEAK-END POSITIONS OF THE SPOT
(Correlation > 0.99)

RESULT AND DISCUSSION: The peak area was observed to be dependent on the amount of the standard, FLX, and a linear relationship (r = 0.9992) was found between the peak areas of FLX at various concentrations over the range 400-4000 ng. The solvent system used for development of the plates produced no interfering peaks in the area under the curve. The R_f value of FLX under the conditions used was found to be 0.74±0.02 and spots were quantified at a wavelength of 227 nm. The proposed method can also be used to accurately determine FLX in capsules, the R_f values were found to be the same for capsules and standard FLX, and there was no interference from the excipients. The recovery of FLX was found to be 99.90% ± 1.68. The accuracy, precision and reliability of the procedure were found to be in agreement with the guidelines of ICH Q2B. The limit of detection and limit of quantitation were found to be 43.55 ng/spot and 131.99 ng/spot. The % RSD of intra-day variation and inter day variation were 0.54 and 0.41 respectively.

CONCLUSION: The developed HPTLC technique is simple, precise, specific and accurate. The result of analysis clearly indicates absence of interference from the excipients in the formulation. The statistical analysis proves that method is reproducible and selective for the analysis of FLX in bulk and capsule formulation.

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