IJPSR (2014), Vol. 5, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 February, 2014; received in revised form, 10 April, 2014; accepted, 13 June, 2014; published 01 August, 2014

DEVELOPMENT AND VALIDATION OF AN UV- SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF FLUOXETINE IN PURE AND TABLET DOSAGE FORMS

Avisek Mukhopadhyay, Kishanta Kumar Pradhan*, Rojalini Samanta

Department of Pharmaceutical Science and Technology, Birla Institute of Technology, Mesra, Ranchi - 835215, Jharkhand, India

Keywords:

Fluoxetine, UV spectroscopy, Validation, Degradation

Correspondence to Author:

Kishanta Kumar Pradhan

Department of Pharmaceutical Science and Technology, Birla Institute of Technology, Mesra, Ranchi -835215, Jharkhand, India

E-mail: kishantakumar@gmail.com

ABSTRACT: A rapid, precise, accurate, sensitive, simple, fast and reliable spectrophotometric method has been developed for determination of Fluoxetine in bulk and pharmaceutical dosage forms. The solubility of Fluoxetine was also examined in various solvents like distilled water, methanol, ethanol, acetonitrile, HCL, chloroform etc. For the determination of working wavelength different concentrations of Fluoxetine (10-60µg/ml) in water: methanol (9:1) was scanned using UV-VIS spectrophotometer within the wave length region of 200-400 nm against water and methanol mixture as blank. For the calibration curve the prepared concentrations were scanned at 216 nm in UV-VIS spectrophotometer. The R^2 value was found to be 0.999. Then the standard deviation was found to be 0.0065. Then the stability study was performed against neutral, acidic and basic condition at 60°C as per ICH guideline.

INTRODUCTION: Fluoxetine is chemically known as (D, L-N-methyl-3-phenyl-3-[(tri-fluoro-p-tolyl) oxy]propylamine and the structure is given in **Fig. 1**. Actually Fluoxetine is an antidepressantof the selective serotonin reuptake inhibitor (SSRI) class. Fluoxetine was first documented in 1974 by scientists from Eli Lilly and Company. It was presented to the U.S. Food and Drug Administration in February 1977, with Eli Lilly receiving final approval to market the drug in December 1987. Fluoxetine went off-patent in August 2001 [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(8).3418-24

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(8).3418-24

Fluoxetine is approved in the US for the treatment of major depression (including pediatric depression), obsessive-compulsive disorder (in both adult and paediatric populations), bulimia nervosa, panic disorder and premenstrual dysphoric disorder [3]. In addition, fluoxetine is used to treat trichotillomania if cognitive behaviour therapy is unsuccessful. Incombination with the atypical antipsychotic Fluoxetine it is known by a few brand names, including its US brand name Symbyax, which is approved for the treatment of depressive episodes as part of bipolar I disorder and in the treatment of treatment-resistant depression. A few methods were reported earlier for the determination of Fluoxetine in bulk and pharmaceutical dosage forms [4, 5]. The present study is designed to develop a simple, precise and accurate UV spectroscopic and reverse phase HPLC methods with good recoveries and shorter retention time for the estimation of fluoxetine in the tablet formulations.

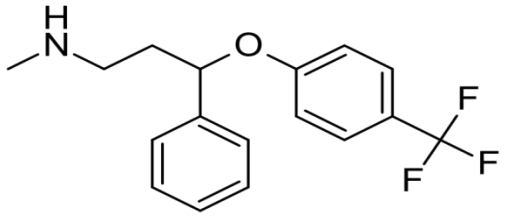

FIG. 1: CHEMICAL STRUCTURE OF FLUOXETINE.

MATERIALS AND METHODS:

Chemicals: The gift samples of Fluoxetine (pure drug) were procured from La Chemico, Kolkata. The required solvents like HPLC grade methanol, water were purchased from Sigma Aldrich Pvt. Ltd.

Instrument: The UV spectrophotometer used for the current study was UV spectrophotometer (SHIMADZU-1800) having 1 cm path length.

Determination of Working Wave Length: In order to determine the wave length of maximum absorption (λ_{max}) of the drug, different concentrations of Fluoxetine (10-60µg/ml) in water: methanol (9:1) was scanned using UV-VIS spectrophotometer within the wave length region of 200-400 nm against water: methanol (9:1) as blank. The working wavelength was found to be 216 nm.

Preparation of Calibration Curve: For preparation of calibration curve of Fluoxetine, a stock solution of 1000µg/ml was prepared. From it different concentrations ranging from 10-60µg/ml prepared and were scanned at 216 nm in UV-VIS spectrophotometer. Then the respective absorbances were noted, which are given in **Table 1**.

The calibration curve is shown in **Fig. 2**. From the calibration curve it was found that it shows linearity in the range of 10-60µg/ml with regression coefficient 0.999.

TABLE 1: ABSORBANCE OF FLUOXETINE AT 216 NM USING VARIOUS CONCENTRATIONS

Serial no.	Concentrations (µg/ml)	Absorbance
1	10	0.161
2	20	0.288
3	30	0.442
4	40	0.602
5	50	0.741
6	60	0.884

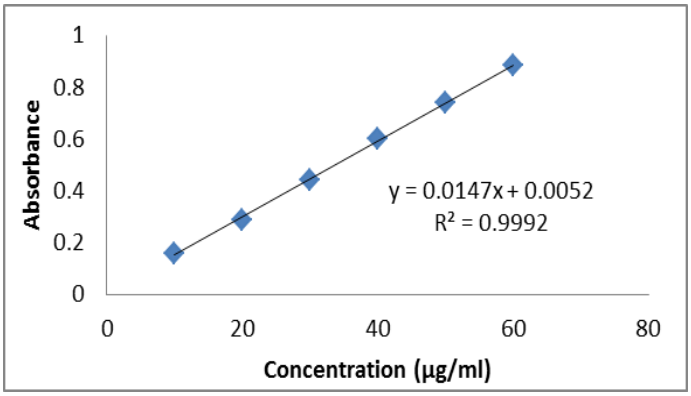

Fig. 2: Calibration curve of Fluoxetine using water: methanol 9:1

Optical characteristics: The optical characteristics like Beer's Law Limit, Sandell's Sensitivity, Standard Deviation, % Relative Standard Deviation, Correlation Coefficient, Regression equation, Slope, Intercept, and Absorption Maxima were determined and were given in **Table 2**.

TABLE 2: OPTICAL CHARACTERISTICS OF FLUOXETINE

Serial no.	Characteristics	Specifications
1	Beer's Law Limit	10-60 µg/ ml
2	Sandell's Sensitivity (µg/cm2/0.001absorbance unit)	0.01205
3	Standard Deviation	0.0065
4	% Relative Standard Deviation	0.0614
5	Correlation Coefficient	0.999
6	Regression equation (Y)	y = 0.014x + 0.005
7	Slope(a)	0.014
8	Intercept(b)	0.005
9	Absorption Maxima	216 nm

Validation parameters:

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy data are given in **Table 3**.

TABLE 3: ACCURACY DATA OF UV SPECTROPHOTOMETRIC METHOD FOR FLUOXETINE

Formulations	Concentration (µg/ml)		% Recovery	Statistical Analysis
	Pure	Formulation		
F_1:80%	24	30	99.18	Mean = 98.94S.D = 0.275 % R.S.D = 0.207
F_2:80%	24	30	97.64	
F_3:80%	24	30	99.89	
F_4:100%	30	30	100.01	Mean = 100.16S.D = 0.182 % R.S.D = 0.128
F_5:100%	30	30	100.37	
F_6:100%	30	30	100.11	
F_7:120%	36	30	98.39	Mean = 98.60S.D = 0.478 % R.S.D = 0.394
F_8:120%	36	30	97.70	
F_9:120%	36	30	99.64	

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. The precision data are given in **Table 4**. The intra-day precision data are given in **Table 5**. The inter-day precision data are given in **Table 6**.

TABLE 4: PRECISION DATA OF THE UV-VIS SPECTROPHOTOMETRIC METHOD FOR FLUOXETINE

Sl. No.	Concentration	Absorbance	Calculated amount	Statistical Analysis
1	40	0.605	29.04	Mean=29.37 S.D=0.215 %RSD=0.406
2	40	0.624	29.27	
3	40	0.601	28.74	
4	40	0.626	29.64	
5	40	0.619	29.48	
6	40	0.616	29.31	

TABLE 5: INTRA DAY PRECISION DATA FOR FLUOXETINE

Conc. (µg/ml)	Absorbance1	Absorbance 2	Absorbance 3	Statistical Analysis
40	0.621	0.614	0.604	Mean=29.68 S.D=0.421 %R.S.D=0.253
40	0.602	0.627	0.608	
40	0.616	0.623	0.610	
40	0.603	0.610	0.598	
40	0.622	0.603	0.603	
40	0.604	0.607	0.621	
Mean	0.613	0.624	0.606	
Calc. Amt. (µg/ml)	29.58	29.83	28.49	

TABLE 6: INTER DAY PRECISION DATA OF FLUOXETINE

Conc. (µg/ml)	Absorbance1	Absorbance 2	Absorbance 3	Statistical Analysis
40	0.621	0.602	0.601	Mean =29.48 S.D =0.426 %R.S.D =0.402
40	0.612	0.614	0.599	
40	0.598	0.599	0.592	
40	0.620	0.594	0.605	
40	0.618	0.619	0.609	
40	0.625	0.624	0.595	
Mean	0.625	0.615	0.598	
Calc. Amt. (µg/ml)	30.54	30.23	28.67	

Robustness/ Ruggedness: The definition for robustness/ruggedness applied is the robustness/ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained results, and a robustness test as an experimental set-up to evaluate the robustness of a method. The term ruggedness is frequently used as a synonym. Several definitions for robustness or ruggedness exist which are, however, all closely related. Robustness/ Ruggedness data are given in **Table 7 and Table 8**.

TABLE 7: RUGGEDNESS DATA OF FLUOXETINE

Analyst-1				Analyst-2			
Conc.	**Abs.**	**Calc.**	**Statistical**	**Conc.**	**Abs.**	**Calc.**	**Statistical**
40	0.608	29.13		40	0.617	29.63	
40	0.620	29.80		40	0.632	30.47	
40	0.622	29.91	Mean=29.78	40	0.613	29.41	Mean=30.14
40	0.637	30.74	S. D=0.693	40	0.639	30.82	S.D=0.074
40	0.614	29.47	%RSD=0.572	40	0.622	29.88	%RSD=0.059
40	0.619	29.69		40	0.626	30.13	

TABLE 8: ROBUSTNESS DATA OF FLUOXETINE

Water: Methanol (9:1)				Water: Methanol (8:2)			
Conc.	**Abs**	**Calc.**	**Statistical**	**Conc.**	**Abs.**	**Calc.**	**Statistical**
40	0.600	28.56		40	0.604	28.80	
40	0.625	30.07		40	0.597	28.41	
40	0.614	29.33	Mean=29.28	40	0.610	29.13	Mean=29.27
40	0.630	30.22	SD=0.079	40	0.636	30.58	SD=0.197
40	0.606	28.89	%RSD=0.065	40	0.612	29.24	%RSD=0.147
40	0.602	28.67		40	0.616	29.47	

Limit of Detection and Limit of Quantitation: The Limit of Detection (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value determined with statistical method by using Statistical formula. The limit of Detection (L.O.D.) was calculated as per below equation:

$$LOD = \frac{3.3 \text{ X } S.D}{Slpoe}$$

The Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with statistical method by using statistical formula. The limit of Quantification (L.O.Q.) was calculated as per below equation:

$$LOQ = \frac{10 \text{ X } S.D}{Slpoe}$$

The limit of detection (LOD) and limit of quantification (LOQ) data are given in **Table 9**.

TABLE 9: LIMIT OF DETECTION AND LIMIT OF QUANTITATION OF FLUOXETINE

Sl. No.	Parameters	S.D	Slope(b)	Formula	Calculation(µg/ml)
1	LOD	0.005	0.014	$3.3(S.D/b)$	1.178
2	LOQ	0.005	0.014	$10(S.D/b)$	3.571

Assay and control of impurities: Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution). Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test. The assay data are given in **Table 10**.

TABLE 10: ASSAY DATA OF FLUOXETINE FORMULATIONS

Formulation	Labeled claim	Observed Amount*	% Recovery	%R.S.D
Prozac®	2	1.90±0.063	98.15	0.614
Prodep®	2	1.92±0.069	99.45	0.893

Stability studies:

Hydrolytic degradation: Hydrolytic degradation usually means the cleavage of chemical bonds by the addition of water. Generally, hydrolytic degradation or saccharification is a step in the degradation of a substance. This can be performed in three conditions that are neutral medium, acidic medium and basic medium.

Samples were withdrawn according to protocol. From the drawn samples 25µg/ml solution were prepared and subjected for analysis. The representative UV-VIS spectrum indicates degradation after 5 hr at 60°C.

Hydrolytic Degradation in neutral condition: The neutral degradation data are given in **table 11** and UV spectrum are shown in **Fig. 3**.

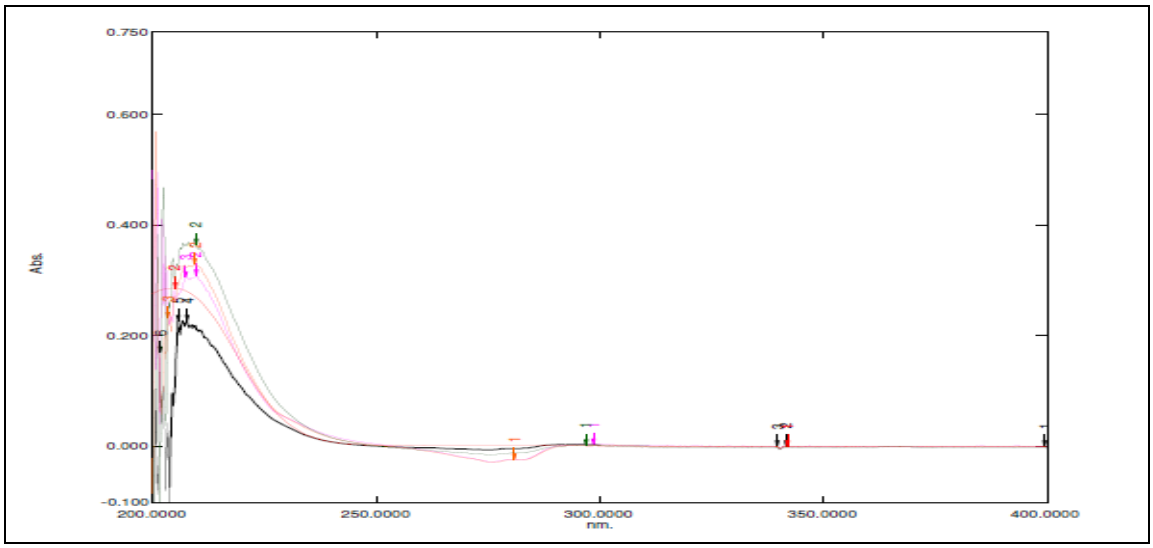

FIG. 3: UV-VIS SPECTRUM OF FLUOXETINE IN NEUTRAL DEGRADATION

TABLE 11: HYDROLYTIC DEGRADATION OF FLUOXETINE IN NEUTRAL CONDITION

Sl. no.	Name	Absorbance	Conc.	%Degradation
1	Drug	0.378	15	0
2	Degradation1	0.332	14.58	3.85
3	Degradation2	0.319	13.43	14.25
4	Degradation3	0.268	12.72	16.71
5	Degradation4	0.217	10.98	26.93

Hydrolytic Degradation in Acidic condition: The acidic degradation data are given in **Table 12 and UV spectrum is shown in Fig. 4**.

Hydrolytic degradation in Basic condition: The basic degradation data are given in **Table 13** and the UV spectrum are shown in **Fig. 5.**

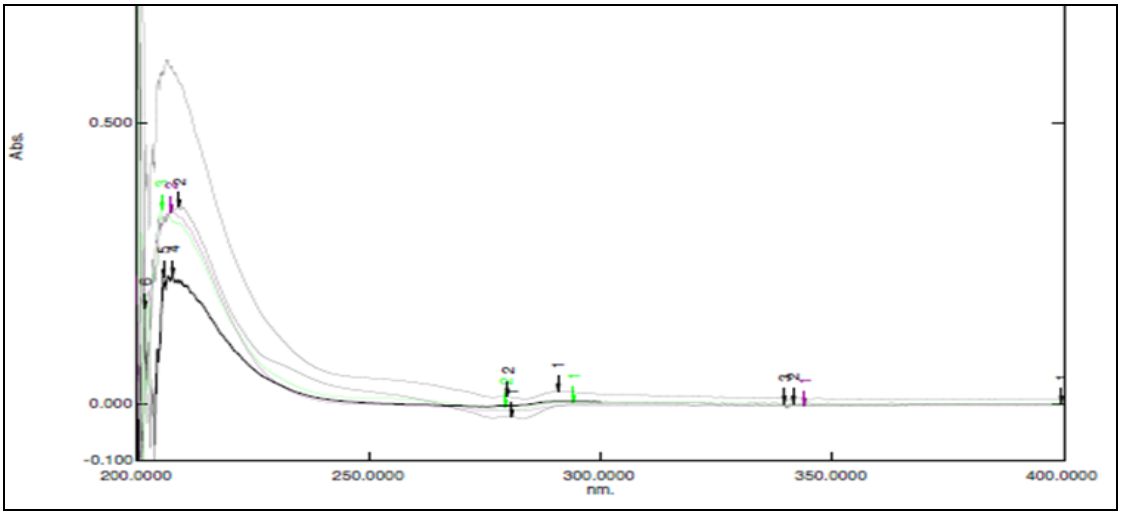

FIG. 4: UV-VIS SPECTRUM OF FLUOXETINE IN ACIDIC DEGRADATION

TABLE 12: HYDROLYTIC DEGRADATION OF FLUOXETINE IN ACIDIC CONDITION

S. No.	Name	Absorbance	Conc.	%Degradation
1	Drug	0.562	30	0
2	Degradation1	0.496	22.87	28.81
3	Degradation2	0.438	19.54	35.07
4	Degradation3	0.418	17.31	38.28

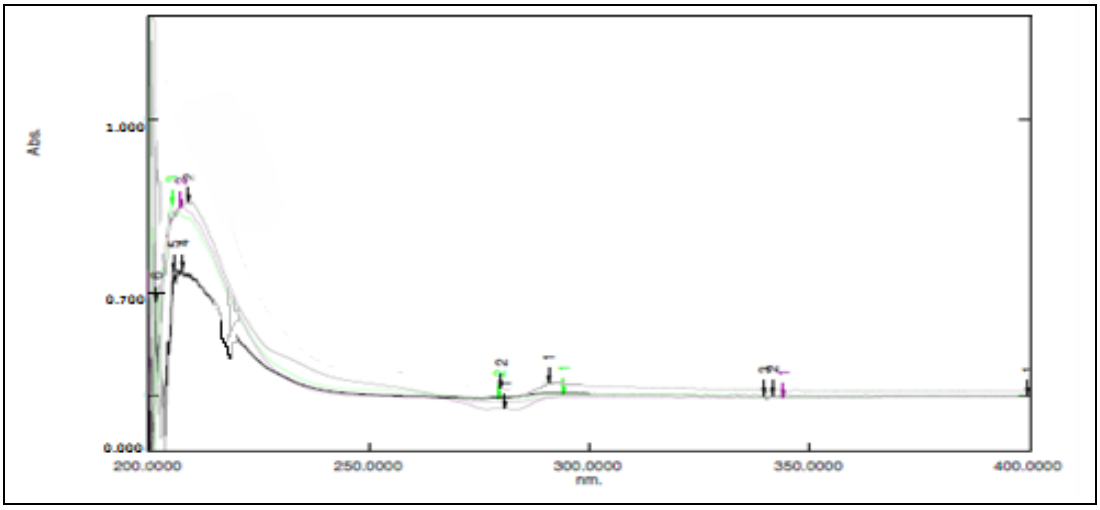

FIG. 5: UV-VIS SPECTRUM OF FLUOXETINE IN BASIC DEGRADATION

Table 13:Hydrolytic Degradation of Fluoxetine in Basic Condition

Sl no.	Name	Absorbance	Conc.	%Degradation
1	Drug	0.995	100	0
2	Degradation1	0.926	92.76	9.88
3	Degradation2	0.886	68.94	13.65
4	Degradation3	0.785	30.42	60.53

RESULTS AND DISCUSSION: The objective of the present work was development and validation of UV spectral study and degradation of Fluoxetine using UV spectrophotometer. The UV Spectra for Fluoxetine were recorded at the wavelength of 216nm (λmax).

The method was found to be simple and the accuracy, precision, intra-day precision, inter-day precision, repeatability and assay was performed and the results was tabulated below. With this study the degradation pattern were also studied and results were shown previously in the corresponding Tables and the Figures were also given.

ACKNOWLEDGEMENT: The authors are grateful to the Department of the Pharmaceutical Sciences, Birla Institute of Technology for providing the requirements and necessities for the present work. We are also thankful to the La Chemico Pvt. Ltd. for providing us the gift samples of Fluoxetine pure drug.

REFERENCES:

1. Parker G. Beyond major depression. *Psychol Med.* 2010; 35: 467-474.
2. Erman ME, McCloskey MS, Fanning JR, Schumacher JA, Coccaro EF. "Serotonin augmentation reduces response to attack in aggressive individuals". *Psychol Sci.* 2009; 20 (6): 714–20.
3. Msterdam JD, Shults J. Efficacy and Safety of Long-Term Fluoxetine Versus Lithium Monotherapy of Bipolar II Disorder: A Randomized, Double-Blind, Placebo-Substitution Study. *Am J Psychiatry*. 2008; 167(7):792–800.
4. Deepak S, Jain AK, Gunta S, Sanyal M, Pande UC and Shrivastav P. "Spectroscopic analysis of Fluoxetine in tablet dosage forms." *J. Pharm. Biomed Anal.* 2009, 26: 43-52.
5. Hai LZ, Wilson YL, Neil T. Fluoxetine: An overview of spectral data. *Int. J. Chem Tech Res* 2010; 2 (2): 1141-46.

How to cite this article:
Mukhopadhyay A, Pradhan KK and Samanta R: Development and validation of an UV- Spectrophotometric method for the estimation of Fluoxetine in pure and tablet dosage forms. Int J Pharm Sci Res 2014; 5(8): 3418-24.doi: 10.13040/IJPSR.0975-8232.5(8).3418-24