CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com Research Article

AN EMERGING PARADIGM EXOSOMES: COMPOSITION, A RAPID ECONOMICAL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FLUOXETINE IN API AND MARKETED FORMULATION

Byasabhusan Das *, Yasmin Afroze, Niranjan Panda, K. S. Rao.

Department of Pharmaceutical Analysis and Quality Assurance,

Anwarul Uloom College of Pharmacy, Hyderabad, India.

Abstract:

The objective of the current study is to develop a more sensitive, rapid, precise and economic analytically validated RP-HPLC method for the estimation of frequently prescribed anti-Depressant FLUOXETINE which is a selective serotonin reuptake inhibitor. The different analytical performance parameters such as linearity, precision, accuracy, range, LOD, LOQ were detected according to ICH guidelines. Chromatographic separation was carried out by quaternary system using Zorbax SB C-18 (150mm × 4.6mm id, µ5m particle size) utilizing AGILENT 1260 Infinity Quaternary LC System equipped with G1314F VW detector using Zorbax C_{18} column. The mobile phase consisted of Methanol:Acetonitrile: formic acid in Water (0.15 v/v) in the ratio 15:55:30 v/v at a flow rate of 1ml/min and temperature of 35°C. Total run time was given 10 minutes using VWD detector at 227nm. The proposed method is accurate and precise with recoveries in the range of 98-102% and retention time was found to be 1.143 min. The linearity is observed in the concentration range of 5-50µg/ml, (r^2=0.999). The reliability of the method was done by evaluation of precision (interday and intraday) %R.S.D was < 2%. The LOD and LOQ was found to be 0.130µg/ml and 0.872µg/ml respectively. The high percentage of recovery and low percentage of coefficient of variance confirm the suitability of method for estimation of Fluoxetine in pure and marketed form. It was successfully validated in accordance with ICH guidelines.

Keywords: *Fluoxetine, Method Development, Method Validation, RP-HPLC.*

Corresponding author:

Byasabhusan Das,

Department of Pharmaceutical Analysis and Quality Assurance,

Anwarul Uloom College of Pharmacy,

Hyderabad, India.

byasabhusandas@gmail.com

QR code

Please cite this article in press as Byasabhusan Das *et al*, ***An Emerging Paradigm Exosomes: Composition, A Rapid Economical RP-HPLC Method Development and Validation for Estimation of Fluoxetine in API and Marketed Formulation***, *Indo Am. J. P. Sci, 2016; 3(10).*

INTRODUCTION:

Fluoxetine is chemically, N-Methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy propan-1-amine. Fluoxetine hydrochloride is a selective serotonin reuptake inhibitor which is clinically effective for the treatment of depression. Fluoxetine and its major metabolite norfluoxetine act as neuronal inhibitors of serotonin reuptake and results in both increased serotonin concentreation at the cleft and autoreceptor stimulation.

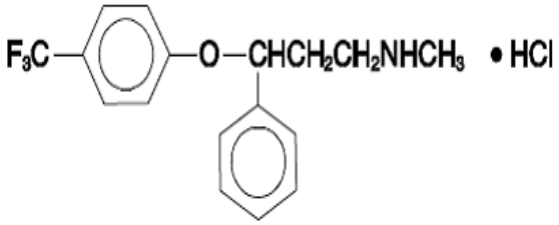

Fig.1: Chemical structure of Fluoxetine

Fluoxetine appears as white crystals or crystalline powder. It is soluble in ethanol, methanol and slightly soluble in water. Literature survey reveals a few HPLC and spectrophotometric methods for determination of Fluoxetine in bulk and tablet dosage form.

MATERIALS AND METHODS:

Instrument:

Chromatographic separation was performed on Agilent 1260 Quaternary LC system HPLC with ezechrome software and VW detector equipped with solvent delivery pump, automatic sample injector and column thermostat. Water, Acetonitrile, and methanol of HPLC grade was used.

Chemicals:

Fluoxetine was obtained from Dr. Reddy's laboratory and Tablets (120mg) manufactured by Sun pharmaceuticals ltd. were procured from local market.

Analytical Method Development:

Experimental:

Scanning and determination of maximum wavelength (λ_{max}):

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, different solutions of the drug (30µg and blank) were scanned using spectrophotometer within the wavelength region 200 – 400 nm against methanol as blank. By observing the spectra of standard solutions λmax 227nm was taken for trials to develop UV method.

Preparation of mobile phase:

The mobile phase solvents methanol, Acetonitrile and water were used in varied compositions (45:50:5, 50:45:5, 65:30:5, 60:30:10, 55:30:15, 50:30:20, 40:30:30, 15:55:30) at flow rate 1ml/min and detection wavelength of 227nm. Finally methanol: Acetonitrile: formic acid in water (0.15% v/v) in the ratio of (15:55:30) was selected and degassed in ultrasonic water bath for 5mins and filtered through 0.22µm membrane filter under vacuum filtration.

Preparation of standard solution:

Standard solution was prepared by dissolving 10mg of drug in 10ml of blank solution which is water: methanol (70: 30) to get concentration of 1mg / ml (1000µg/ml) solution.

From the prepared standard solution 0.5 ml solution is pipetted out and made the volume up to 10 ml in volumetric flask with solvent solution of methanol, acetonitrile and formic acid in water(0.15% v/v) in the ratio of 15:55:30 .This is working standard solution of 50µg/ml. For preparation of 5µg, 10µg, 20µg, 30µg and 40 µg per ml , 0.05, 0.1, 0.2, 0.3 and 0.4 ml is pipetted out respectively and the volume was made up to 10 ml with solvent solution of methanol : Acetonitrile: formic acid in water (0.15% v/v) in the ratio of (15:55:30) in 10ml volumetric flask.

Preparation of sample solution:

Twenty tablets were weighed, finely powdered and an accurately weight sample of powdered tablets equivalent to 25 mg of Fluoxetine was extracted with water and methanol in the ratio of 70:30 in a 25ml volumetric flask and the volume was made up after completely dissolving using using ultra sonicator. This solution was filtered through 0.22µm filter paper.

Further pipetted out 0.2 ml of Fluoxetine of the above solution into a 10 ml volumetric flask and makeup the volume with solvent solution.

Procedure: Inject 20 µl of the standard and sample solution into the chromatographic system and measure the areas for the Fluoxetine and calculate the assay percentage.

Method Validation:

1. Accuracy: (%Recovery):

Accuracy for the assay of Fluoxetine was determined by applying the method in triplicate samples to which known amount of Fluoxetine standard was added at different levels (80%, 100%, and 120%).

Table 1: Assay accuracy of fluoxetine

Concentration / Sample	Amount added (mg)	Amount found (mg)	% Recovery	Statistical Analysis	
80% Level Sample 1	159.4	159.3	99.9	Mean	100.0
80% Level Sample 2	159.0	159.3	100.2	SD	0.21
80% Level Sample 3	159.5	159.2	99.8	% RSD	0.2
100% Level Sample 1	199.0	197.3	97.1	Mean	97.9
100% Level Sample 2	198.8	198.7	97.9	SD	0.80
100% Level Sample 3	198.6	200.0	100.2	% RSD	0.8
120% Level Sample 1	238.4	240.2	102.8	Mean	101.9
120% Level Sample 2	238.7	240.7	101.8	SD	0.23
120% Level Sample 3	238.8	241.7	101.2	% RSD	0.2

Overall Statistical Analysis							
Mean	99.93	S.D	0.66	% RSD	0.7	95% Confidence Interval	± 0.5

2. Precision:

Intraday and inter day variations were determined by using three replicate injections of one concentration and analyzed on the same day and different days. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Table 2: Result of intraday precision

S. No.	Conc. (µg/ml)	Peak Area I	Peak Area II	Peak Area III	Mean ± S.D	% R.S.D
1	5	14329989	14348799	14339778	14339522.170 ±9407.613	0.250
2	10	24016598	24003638	24012894	24027474.667±43919.422	0.455
3	20	27910486	27872648	27919264	27910571.667±71340.070	0.354
4	30	43805292	43792604	43607840	43791192.333 ±44490.104	0.412
5	50	67398998	67651584	672882261	67400345.210 ± 237949.157	0.508

Table 3: Results of Inter day precision

S.No.	Conc. (µg/ml)	Peak Area I	Peak Area II	Peak Area III	Mean ± S.D	% R.S.D
1	5	14339787	14339652	14338698	14339379.667 ±5930.13557	0.194
2	10	18546892	18540150	18470120	18519054 ± 42511.95018	0.404
3	20	24066203	24075440	24211763	24117805 ±81501.23589	0.405
4	30	43687803	43965166	43980959	43877976 ± 164883.84445	0.551
5	50	66532259	67101925	66989816	66874666.667 ± 301785.295	0.663

3. Linearity:

Solutions were prepared containing 5µg/ml, 10µg/ml, 20µg/ml, 30µg/ml, 50µg/ml concentrations of Fluoxetine. Each solution was injected, linearity was evaluated by linear- regression analysis.

Table 4: Linearity table of fluoxetine

Concentration	Area
5	14329989
10	24016598
20	27910486
30	43805292
50	67398998

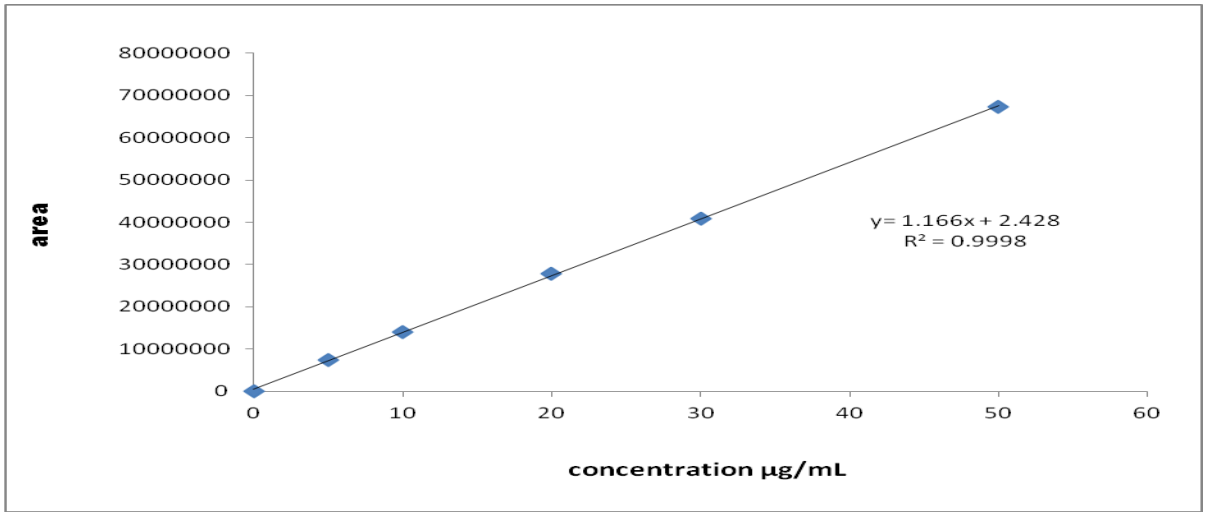

Fig 2: Linearity curve of fluoxetine

4. Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ was calculated from linear curve using formulae

LOD=3.3*σ /slope, LOQ=10*σ /slope (Where σ=the standard deviation of the response and S=Slope of calibration curve).

Table 5: Limit of detection and limit of Quantification of Analyte

DRUG	LOD (µg/ml)	LOQ (µg/ml)
Fluoxetine	0.130	0.872

5. **Robustness**

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors choosen for this study were the flow rate (±0.1ml/min), temperature and mobile phase composition.

RESULTS AND DISCUSSION:

The results suggest that a suitable, easy, less time consuming validated method has been developed for Fluoxetine.. Several mobile phase compositions were tried to resolve the peak Fluoxetine. The mobile phase containing methanol: Acetonitrile: Formic acid in water (0.1% v/v) (15:55:30) was found ideal to resolve the peak of Fluoxetine satisfactory. Retention time of Fluoxetine was found to be 1.143 min. The proposed method was found to be linear in concentration range 5-50µg/ml. The mean percentage recovery for Fluoxetine was found to be 98-102, which are well within the limit and hence the method was found to be accurate %. LOD and LOQ was found to be 0.130µg/ml and 0.872µg/ml respectively. Results of intraday and interday precision were shown. The robustness of the method was investigated by varying experimental conditions such as changes in flow rate, mobile phase composition and temperature.

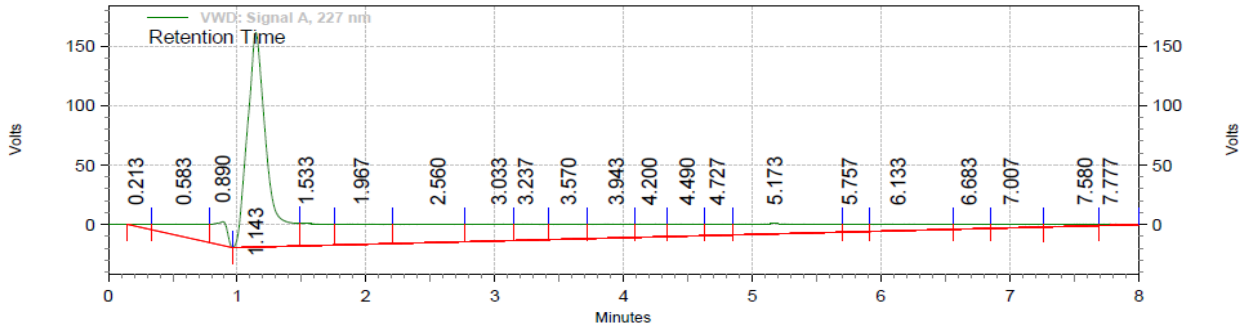

Fig 3: Standard Chromatogram of Fluoxetine

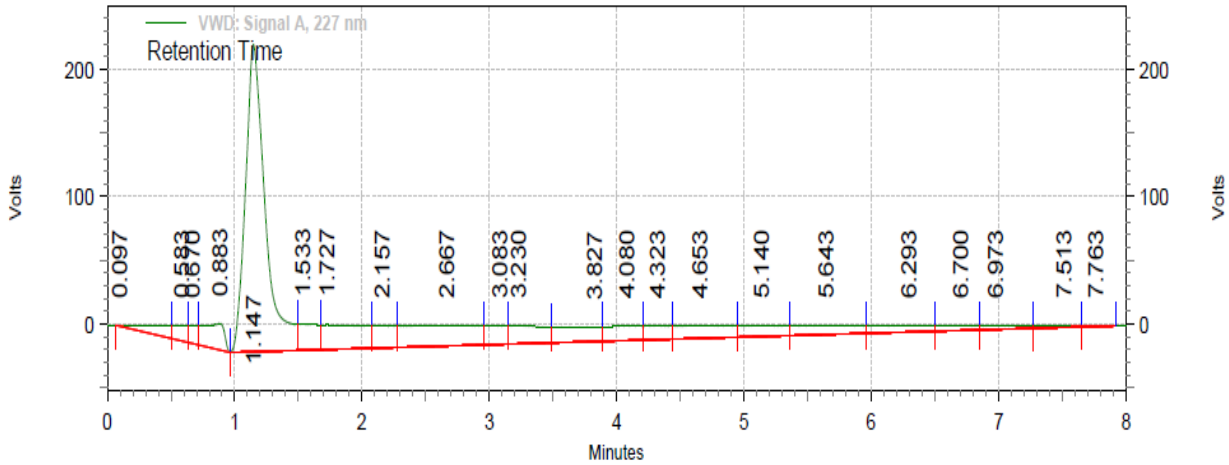

Fig 4: Sample Chromatogram of Fluoxetine

ACKNOWLEDGEMENT:
The authors are thankful to Dr. Reddys Laboratories Pvt. Ltd., Hyderabad, India, for providing the sample of pure Fluoxetine and the management of Adept pharma Pvt. Ltd., for providing the essential facilities to complete the work successfully.

REFERENCES:

1.http://en.m.wikipedia.org/wiki/Fluoxetine
2.Williard H.H, Merit L.L, Dean F.A. Settle F.A. Instrumentsl methods of analysis. 7th edition: CBS Publishers, New Delhi; 2002.
3. Vogel's Text book of Quantitative chemical analysis. 6th edition: Dorling Kindersley Pvt. Ltd;2005, Pg. 220-235.
4.Sharma B.K. Instrumental methods of chemical analysis. 22nd edition: Goal Publishing house. Meerut; 2000.
5.M.S Tswet. The new form of adsorption phenomena and its application in biochemical analysis. Warsaw;1903: Pg.20-39.
6. Beckett A.H and Stenlake J.B. Practical pharmaceutical chemistry. 4th edition: CBS publisher and distributor, New Delhi; 2002:pg.157-165.
7. L.R Snyder, J.J Kirkland. Introduction to modern liquid chromatography. 2nd edition: A WILEY Interscience Publication; 1988.
8. Chatwal G. R, Anand S K. Instrumental methods of chemical analysis. 5th edition: Himalaya Publishing House, Mumbai; 2004.
9.http://www.waters.com/waters/nrv.htm?cid=10048919&locale=en_us
10.Satinder Ahuja, Michael W. Dory. Handbook of pharmaceutical analysis. 2nd edition: Academy press; 2005: 50-54, 169, 233.
11. P.D.Sethi. HPLC: Quantitative analysis pharmaceutical formulations: CBS Publishers and distributors, New Delhi (India); 2001: pg.137-150.
12.Ranjit Singh. HPLC method development and validation: An overview. J Pharm educ res 2013; 5(1).
13.ICH, Q2(R1), Validation of Analytical Procedures: Text and Methodology; 2005.
14.ICH Harmonized Tripartite Guideline. Validation of analytical procedure methodology, Q2B; 1996: 1-8.
15.R. Green, R. Houghton, J. Scarth. Determination of fluoxetine and its major active metabolite norfluoxetine in human plasma by liquid chromatography-tandem mass spectrometry. Chromatographia; 2002. 55(1) S133-S136.
16.Sejal Patel, N. J. Patel. Simultaneous RP-HPLC and HPTLC estimation of fluoxetine hydrochloride and Olanzapine in tablet dosage form. Indian J pharm sci; 2009. 71(4): 477-480.