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Research Article

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Development and validation of analytical method for estimation of fluoxetine hydrochloride in oral solution

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ABSTRACT

A simple, fast and precise reverse phase high performance liquid chromatographic method was developed for the estimation of Fluoxetine hydrochloride in oral solution with its major excipient benzoic acid. The chromatographic separation was achieved on Zorbax eclipse plus-C8 (250x4.6) mm; 5µm column with an isocratic mixture of diethyl amine buffer (pH 3.5) adjusted with orthophosphoric acid: acetonitrile in the ratio of 55:45 v/v, respectively. The mobile phase was kept at a flow rate of 1ml/min with injection volume of 20µl and wavelength of detection 227nm at room temperature. The retention times for Fluoxetine hydrochloride and Benzoic acid was found to be 3.417±0.1min and 2.919±0.1 min, respectively. The linearity was obtained in the range of 40-200µg/ml for Fluoxetine and 5-25µg/ml for benzoic acid with correlation coefficient 0.999 for both. On carrying out degradation studies it was found that degradation products did not interfere with the detection of Fluoxetine and benzoic acid. The proposed method was found to be linear, accurate, precise, stable, robust and specific and was successfully applied for the determination of investigated drug with an excipient in oral solution.

Keywords: Fluoxetine, Benzoic acid, RP-HPLC, Stability, Method validation.

INTRODUCTION

Fluoxetine hydrochloride (FLUOX) is chemically (±)-N-methyl-3-phenyl-3-[(α,α,α- trifluoro-p- tolyl)oxy] propylamine hydrochloride[1]. Fluoxetine is a selective serotonin-reuptake inhibitor (SSRI), it blocks the reuptake of serotonin at the serotonin reuptake pump of the neuronal membrane, enhancing the actions of serotonin on $5HT_1A$ autoreceptors and is used to treat depression. Benzoic acid (BA) is a preservative used in oral solutions. It inhibits the growth of fungi by suppressing their ability to grow or reproduce [2].

Rationale
Literature survey has revealed that a number of methods have been reported for estimation of Fluoxetine hydrochloride individually for example: HPLC method [3-5], HPTLC method [6], and in combination with other drugs for example: spectrophotometry [7], HPLC [8-12], HPTLC [12, 13], LC-MS [14]. But not a single method has reported for estimation of FLUOX in oral solution with its excipient. Therefore, an attempt has been made to develop an RP-HPLC method for estimation of FLUOX in oral solution.

In the proposed method forced degradation studies for the drug product will also be carried out under different stress conditions like acidic, basic, oxidative, thermal, and UV exposure and the stressed samples will be analysed by the developed and validated method.

EXPERIMENTAL SECTION

Materials

The solution of Fluoxetine hydrochloride, (label claim: Fluoxetine hydrochloride 400mg and Benzoic acid 50mg), manufactured by Thames laboratory Limited was procured from Advance Analytical Research & Training Institute. All the chemicals used are purchased from MERCK Chem. Ltd., Mumbai. HPLC instrument (Shimadzu) LC2010CHTwith SPDM20A diode detector was used for estimation of FLUOX. LC solution software was applied for data collecting and processing.), FTIR Spectrophotometer (Brukeroptics), Digital balance (Sartorius) (0.1 mg – 205 gm) pH meter (ELICO).

METHOD DEVELOPMENT

The chromatographic separation was performed with isocratic elution on a Zorbax Eclipse plus-C18 (250x 4.6) mm; 5µm as a stationary phase with mobile phase which is a mixture of diethyl amine buffer (pH 3.5) adjusted with OPA: acetonitrile (55:45v/v) pumped at a flow rate of 1ml/min. The samples were analyzed by a PDA detector at 227 nm with the injection volume of 20µL.

Preparation of working standard solution of FLUOX

Accurately weighed 100mg of standard FLUOX was transferred to a 100ml volumetric flask and dissolved in 100ml of solvent (diethyl amine buffer (pH 3.5) adjusted with OPA: acetonitrile in ratio of 55:45 v/v).The flask was then shaken and volume was made up to the mark with solvent to give a solution containing 1000µg/ml of FLUOX. From this 40ml of solution was taken out and diluted up to 100ml with solvent in volumetric flask to give a solution of 400µg/ml of FLUOX.

Preparation of working standard solution for BA

Accurately weighed 100mg of standard BA was taken and transferred to a 100ml volumetric flask and dissolved in 100ml of the solvent. The flask was then shaken and volume was made up to the mark with the solvent to give a solution containing 1000µg/ml of BA. From this 5ml of the solution was taken and diluted up to 50 ml in a volumetric flask to give a solution of 100µg/ml of BA. Further, 5ml of the solution was taken and diluted up to 10ml with the solvent to give a working standard solution containing 50µg/ml of BA.

Preparation of sample solution

The sample solution contains 20mg/ml of FLUOX and 0.5mg/ml of BA (label claim).One ml of this solution was taken and diluted with 10ml of solvent to obtain a final concentration of 400µg/ml of FLUOX and 50 µg/ml of BA.

Optimized method

The chromatographic separation was achieved with parameters shown in Table-1 and the final chromatogram is shown in Figure 1.

Calibration curve

Six different concentrations of FLUOX; 40µg/ml, 60µg/ml, 80µg/ml, 100µg/ml, 120µg/ml, 160µg/ml, 200µg/ml and BA; 5µg/ml, 7.5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml were prepared from working standard solution of FLUOX and BA, respectively. Calibration curves constructed were linear over the prepared concentration range of 40-200µg/ml for FLUOX and 5-25µg/ml for BA. Calibration curves were prepared using analyte peak area versus concentration of analyte. The calibration curves are shown in Figure 2 and 3.

METHOD VALIDATION [15]

The proposed method was validated in accordance with ICH guidelines. It was validated in terms of linearity, accuracy, precision, LOD, LOQ and % recovery.

Linearity

Linearity studies were carried out for FLUOX and BA at six different concentration levels. Calibration curves constructed were linear over the concentration range of 40-200µg/ml for FLUOX and 5-25µg/ml for BA. Evaluation

of drug was performed with UV detector at 227 nm and peak area was recorded for all the peaks. The correlation coefficient was found to be 0.999 both for FLUOX and BA.

Accuracy

The accuracy of the method was assessed by recovery studies of FLUOX and BA in solution dosage form at three concentration levels. A fixed amount of the pre-analyzed sample was taken and standard drug was added at 80%, 100% and 120% levels. The samples were then analysed and each level was repeated for three times. The percentage recoveries of FLUOX and BA were found to be 100.17% and 98.60%, respectively. This shows that there is no interference from excipients in the estimation of FLUOX. The lower values of %RSD of assay indicate that the method is accurate. The results are shown in Table 2.

Precision

The precision for the developed method was determined in terms of intraday and inter-day precision. For intraday precision evaluation a standard solution of fixed concentration was injected at various time intervals on a particular day and %RSD for FLUOX and BA were found to be 0.016% and 0.11%, respectively (limit %RSD <2.0%). The inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the %RSD for FLUOX and BA were found to be 0.85% and 1.69%, respectively (limit %RSD < 2.0%). The results are shown in Table-3 and 4.

Limit of detection and limit of quantification

The LOD and LOQ were determined by injecting progressively low concentration of the standard solutions using the developed HPLC method. The LOD for FLUOX and BA were found to be 6.76µg/ml and 0.62µg/ml, respectively. The LOQ for FLUOX and BA were found to be 20.48µg/ml and 1.88µg/ml, respectively.

Assay

Sample solution (20 µl) was injected and analysed. The peak area of FLUOX and BA and the amount of each drug in samples was computed. The results of the assay show presence of 99.65% and 99.14% for FLUOX and BA, respectively**.** The results of the assay are shows in Table-5.

STABILITY INDICATING STUDIES [16]

Force degradation studies

Whole stability indicating RP-HPLC assay method for simultaneous determination of FLUOX and BA were done using above developed method. In order to establish stability-indicating nature of the method, drug product and diluent were subjected to various stress conditions to conduct force degradation studies. Stress studies were carried out under the conditions of acidic, basic, oxidative, thermal and UV exposure. Several trials with different severity of each stressed condition were conducted. Results are shown in Table-6.

RESULTS AND DISCUSSION

A new RP-HPLC method was developed for estimation of FLUOX with its major excipient BA. The HPLC method was optimized with a view to develop an accurate assay method for estimation of FLUOX and BA in solution dosage form. The samples were analyzed by a PDA detector at 227 nm with the injection volume of 20µL, which resulted in peak with good shape and resolution. The method was found to be linear in the range of 40-200µg/ml for FLUOX and 5-25µg/ml for BA.

The percentage recoveries of FLUOX and BA were 100.17% and 98.60% which shows that there is no interference from excipients and the lower values of RSD of assay indicate the method is accurate. The %RSD of FLUOX and BA for intraday precision studies were found to be 0.016% and 0.11%, respectively (limit %RSD< 2.0%). and % RSD of FLUOX and B.A. for inter-day precision study were found to be 0.85% and 1.69%, respectively (limit %RSD < 2.0%).

The retention time of FLUOX and B.A. were found to be 3.417min and 2.919min respectively with an asymmetry factor of 1.93 for FLUOX and 1.34 for BA which indicates efficient performance of the column. The LOD for FLUOX and BA was found to be 6.76µg/ml and 0.62µg/ml, respectively. The LOQ for FLUOX and BA was found to be 20.48µg/ml and 1.88µg/ml, respectively which indicates good sensitivity of the proposed method. Assay studies of the proposed method indicate 99.65% and 99.14% recovery for FLUOX and BA, respectively.

Typical chromatogram of standard showing the separation of the drugs FLUOX and B.A. is shown in Figure 1(Optimized chromatogram).

Figure 1: Typical chromatogram of standard solution for FLUOX and BA

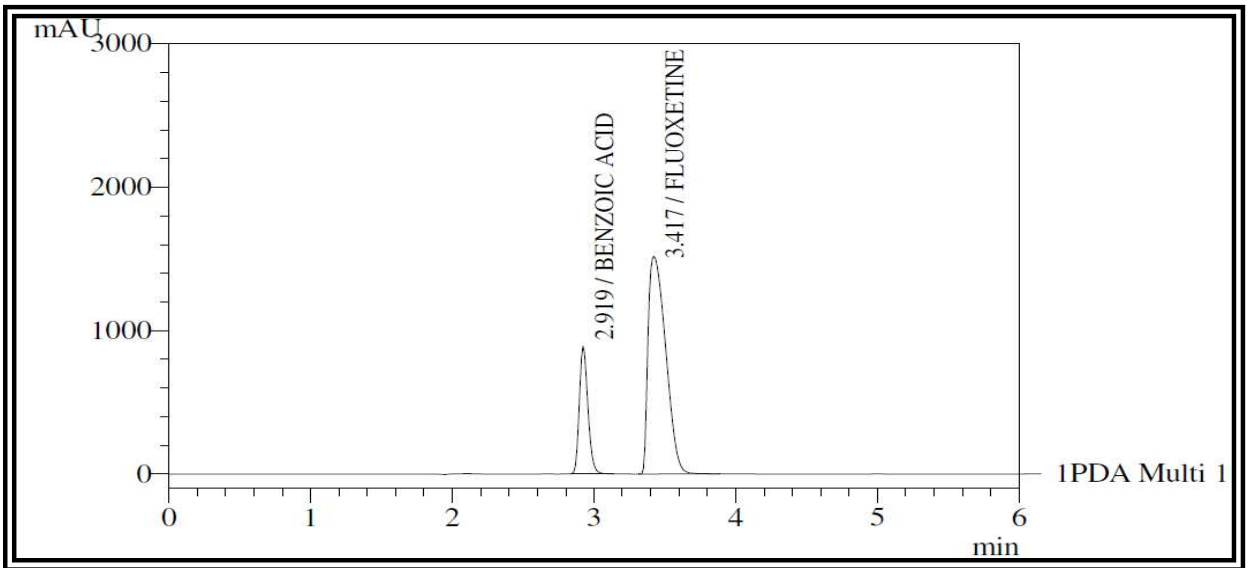

Figure 2: Calibration curve of FLUOX at 227nm

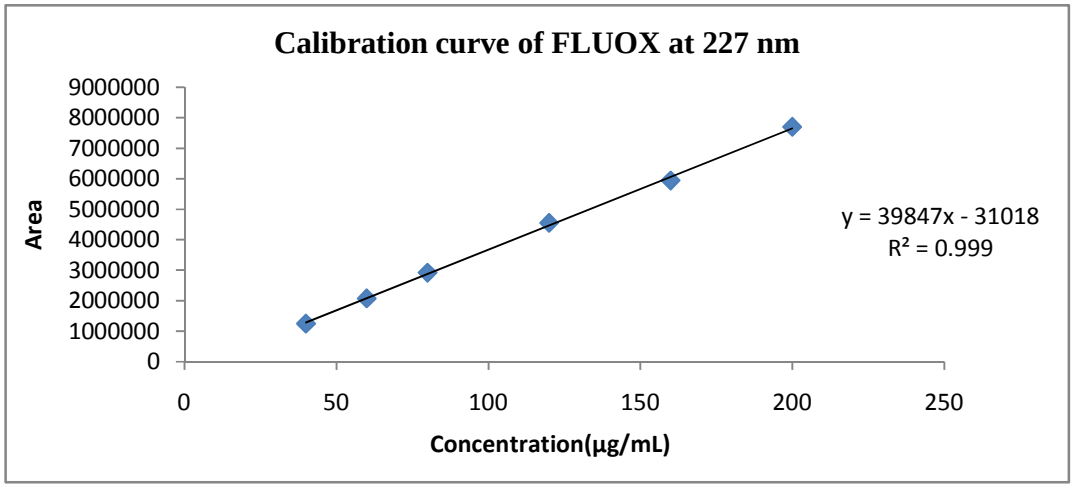

Table-1 Final optimized chromatographic condition

Sr. No.	Parameters	Condition
1	**Instrument**	Shimadzu LC 2010 A/CHT Auto sampler
2	**Stationary phase**	ZORBAX ECLIPSE PLUS C8 ,(250 x 4.6)mm ; 5 µm column
3	**Mobile phase**	Acetonitrile: Diethyl amine Buffer pH 3.5 (45:55v/v)
4	**Pump mode**	Isocratic
5	**Flow rate mL/min)**	0.8
6	**Run time (min)**	6
7	**Volume of injection (µl)**	20
8	**Detector**	UV
9	**Detection wavelength (nm)**	227
10	**Column temperature**	28˚ C
11	**Retention time (min)**	FLUOXETINE HYDROCHLORIDE: 3.417 ± 0.1 min BENZOIC ACID: 2.919± 0.1 min

Figure 3: Calibration curve of BA at 227nm

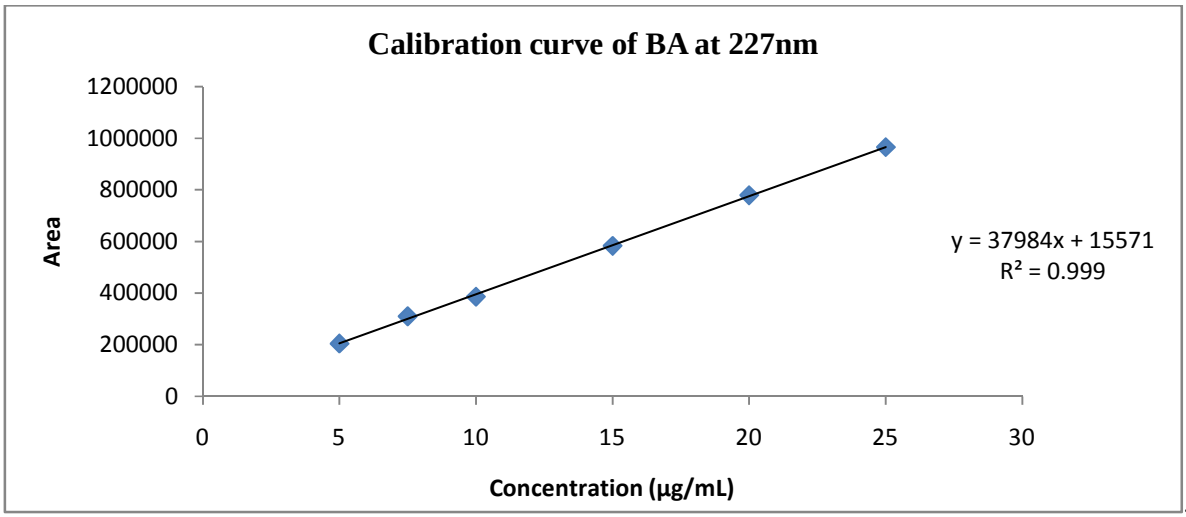

Table-2 Accuracy studies for the proposed method

Spiked level	Amount added (µg/ml)		Amount Found (µg/ml)		%Recovery	
	FLUOX	B.A.	FLUOX	B.A.	FLUOX	B.A.
80%	64	8	64.11	7.88	100.17%	98.60
100%	80	10	79.77	10.22	99.71%	102.22
120%	96	12	96.11	11.88	100.11%	99.06%

Table-3 Intra-day precision for the analysis of FLUOX and BA

Sr no.	Drug (µg/ml)	Peak Area			Mean	SD	%RSD
	FLUOX	1.	2.	3.			
1.	60	2081750	2082320	2080925	2081665	701.37	0.033
2.	80	2920908	2921753	2920896	2921186	491.36	0.016
3.	100	3697150	3696921	3697225	3697099	158.37	0.004
			Average % RSD				0.018
Sr no.	**BA**	**1.**	**2.**	**3.**	**Mean**	**SD**	**%RSD**
1.	7.5	329560	331095	328980	329878	1092.84	0.33
2.	10	396553	396731	395891	396391	442.63	0.11
3.	12.5	452937	454329	455691	454319	1377.02	0.30
			Average % RSD				0.24

Table-4 Inter-day precision for the analysis of FLUOX and BA

Sr no.	Drug(µg/ml)	Peak Area			Mean	SD	%RSD
	FLUOX	1.	2.	3.			
1.	60	2112750	2080753	2091175	2091175	18688.37	0.89
2.	80	2948908	2949816	2928450	2952391	25327.39	0.85
3.	100	3761150	3697605	3694857	3717904	37479.20	1.00
			Average % RSD				0.91
Sr No.	**BA**	**1.**	**2.**	**3.**	**Mean**	**SD**	**%RSD**
1.	7.5	335860	329753	329287	331633.33	3667.80	1.10
2.	10	397353	386720	385275	389782.70	6595.79	1.69
3.	12.5	462937	452889	452586	456137.30	5890.63	1.29
			Average % RSD				1.36

Table-5 Assay results of proposed methods

Sr No.	Label claim		Amount Taken		Amount Found		%Assay	
	FLUOX	BA	FLUOX	BA	FLUOX	BA	FLUOX	BA
1.	400mg	50mg	400mg	50mg	397.58	49.87	99.39%	99.74%
2.					398.66	49.12	99.66%	98.24%
3.					397.74	49.62	99.43%	99.24%
4.					397.49	49.51	99.37%	99.02%
5.					398.42	49.75	99.60%	99.5%
6.					398.31	49.43	99.57%	98.86%
SD							0.122	0.527
%RSD							0.123	0.532

Table-6 Force degradation study of FLUOX and BA

Degradation Condition	% ASSAY		% DEGRADATION	
	FLUOX	B.A.	FLUOX	B.A.
Acid Degradation	87.57%	87.84%	12.43%	12.16%
Base Degradation	89.85%	89.94%	10.15%	10.06%
Oxidative Degradation	80.61%	81.70%	19.39%	18.30%
Photo Degradation	89.90%	86.58%	10.10%	13.42%
Thermal Degradation	89.94%	89.80%	10.16%	10.20%

CONCLUSION

The developed RP-HPLC method is simple, specific, accurate and precise for the simultaneous estimation of FLUOX with BA in oral solution. The developed method provides good resolution between FLUOX and BA was successfully validated in terms of linearity, accuracy, precision, LOD, LOQ and recovery studies in accordance with ICH guidelines. Thus the described method is suitable for routine analysis and quality control of pharmaceutical preparation that is oral solution of Fluoxetine hydrochloride.

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REFERENCES

[1] British Pharmacopoeia, 6th edition, British Pharmacopoeia Commission, **2009**, 638, 2608.
[2] Drug Bank: Fluoxetine, www.drugbank.ca/drugs/DB00472
[3] MMN Fregonezi; MM Baracat; R Casagrande; HT Machado ; B Miglioranza ; EAS Gianotto; JC Dalmas. *Quimica Nova***, 2008,** 31(7), 1665-1669**.**
[4] R Mandrioli; V Pucci; D Visini; G Varani, MA Raggi. *Journal of Pharmaceutical and Biomedical Analysis*, **2002**, 29(6), 1127–1134.
[5] MT Maya; CR Domingos; MT Guerreio; JA Morais. *Journal of Pharmaceutical and Biomedical Analysis*, **2000**, 23(6), 989–996.
[6] MS Bhatia; ST Kumbhar. *International Journal of Pharmaceutical sciences and Research*, **2011**, 2(6), 1582-1587.
[7] MP Kavitha; A Rajasekhar. *International Journal of Pharmacy and Pharmaceutical Science Research*, **2013**, 3(4), 126-129.
[8] DF Freitas, CED Porto; EP Vielra; MEPB Siqueira. *Journal of Pharmaceutical and Biomedical Analysis*, **2010**, 51(1), 170-177.
[9] S Shukla; P Kumar; RS Shrivastava; SK Srivastava; P Trivedi. *Eurasian Journal of Analytical Chemistry*, **2010**, 5(3), 284.
[10] SK Tiwari; M Bapna; H Aarsad; C Dave; KD Bramhbhaat. *Pharma Science Monitor*, **2013**, 3(3), 180.
[11] Samanidou VF; Kourti PV. *Future Science*, **2009**, 1(5), 905-917.
[12] S Patel; NJ Patel. *Indian Journal of Pharmaceutical sciences*, **2009**, 71(4), 477-480.
[13] CR Shah; BN Suhagia; NJ Shah; DR Patel; NM Patel. *Indian journal of pharmaceutical science*, **2008**, 70(2), 251-255.

[14] S Gopinath; RS Kumar; S Alexander; P Danabal. *Biotechnology and Biopharmaceuticals*, **2012**, 26(9), 1077-1082.
[15] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology. Q2 (R1), **2005**. See www.ICH.org
[16] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Stability Testing of New Drug Substances and Products. Q1A (R2), **2005**. See www.ICH.org