

Original Article

FORCE DEGRADATION STUDY AND RP-HPLC METHOD DEVELOPMENT FOR ESTIMATION OF FLUVOXAMINE MALEATE IN TABLET

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ABSTRACT

Objective: To evaluate stability of drug by force degradation studies and to develop validated RP-HPLC method for quantitative estimation of fluvoxamine maleate in pharmaceutical dosage form.

Methods: The optimized chromatographic conditions utilizes reversed-phase C18 Hyperchrome ODS column (250X4.6 mm, 5 μ), mobile phase comprised of Methanol and Phosphate buffer (pH-2.5) in the ratio of 70:30 v/v, flow rate of 1 ml/min at ambient temperature and UV detection at 250 nm.

Results: In the proposed method the retention time of fluvoxamine was found to be 5.94 min. The linearity of the proposed method was tested in the range of 10-50 μ g/ml and correlation coefficient was found to be 0.998. The accuracy of the proposed method was carried out by standard addition method and found to be 99.62%. The % RSD of precision study was calculated as 0.69. The standard and marketed formulation exposed to hydrolytic and oxidative stress conditions indicates significant degradation with good resolution between the peaks corresponds to degradation products and analyte. The proposed force degradation study shows that fluvoxamine is labile molecule to acid, alkali, neutral and oxidative conditions and also susceptible to degradation when exposed to UV light, and humidity conditions while it was stable under dry heat (50 $^{\circ}$ C).

Conclusion: The proposed validated HPLC method for the quantitative estimation of fluvoxamine maleate in tablet dosage form is accurate, precise, economic, and robust. The developed stability indicating method can be recommended for analysis of drug and its degradation products in stability samples.

Keywords: HPLC, Fluvoxamine maleate (FLV), Forced Degradation, Validation, Stability indicating.

INTRODUCTION

Chemically Fluvoxamine maleate (FLV), is (*E*)-5-methoxy-1-[4-(trifluoromethyl) Phenyl] pentan-1-one O-2-aminoethyl oxime maleate (Fig.1), an antidepressant which functions as a selective serotonin reuptake inhibitor (SSRI). It is also prescribed to treat major depressive disorder (MDD) and anxiety disorders, such as panic disorder and post-traumatic stress disorder (PTSD). It has molecular weight 434.4 and soluble in ethanol, methanol and chloroform [1].

Literature survey reveals that RP-HPLC [2-5] methods are reported for the estimation of Fluvoxamine in plasma as the single dosage form or in combination with other drugs. Also some RP-HPLC [6-8] methods reported for analysis of said drug in tablets. A detailed literature also indicates UV-Spectrophotometric [9], Polarographic [10], HPTLC [11] and Capillary GC [12] methods are reported for estimation of Fluvoxamine. An attempt was made to develop and validate totally new, precise, accurate, economic, isocratic RP-HPLC method for the quantitative estimation of Fluvoxamine in presence of its degradation products as per ICH guidelines. This manuscript gives the report for the application of specific stability indicating with degradation kinetics and RP-HPLC method in estimation of drug from its pharmaceutical dosage form.

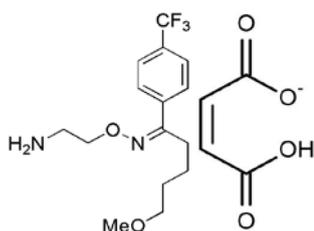


Fig. 1: Structure of Fluvoxamine maleate

MATERIALS AND METHODS

Chemicals and reagents

Analytical pure sample of Fluvoxamine maleate (Purity 99.27% w/w) was obtained as gift samples from Zydus Cadilla Healthcare Ltd., Ahmedabad. Marketed formulation containing 50 mg of Fluvoxamine maleate was purchased from local market. HPLC grade Methanol, Acetonitrile, and Potassium dihydrogen orthophosphate, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide of GR grade were used during the experimentation.

Instrumentation and chromatographic conditions

HPLC analysis was performed on Shimadzu HPLC series 1100 chromatograph equipped with binary pump LC-10ADvp, UV-Visible detector with manual injector 7725 I (Rheodyne) with 20 μ l loop and a reserved phase 5 μ Hyperchrome ODS C₁₈ column (250x4.6 mm) with pore size of 100 Å was used for chromatographic studies. Chromatographic separation was achieved using a mobile phase containing Methanol and Phosphate buffer (pH-2.5) in the ratio of 70:30 v/v, at a flow rate of 1 ml/min and the eluent was monitored using UV detector at a wavelength 250 nm.

The column was maintained at ambient temperature and injection volume of 20 μ l was used. The mobile phase was filtered through 0.45 μ m membrane filter prior to use.

Preparation of buffer (pH-2.5)

In 1000 ml volumetric flask, 10g potassium dihydrogen orthophosphate was dissolved in sufficient amount of double distilled water and pH 2.5 adjusted with 0.1NHCl and volume was made up to the mark with double distilled water.

Preparation of working standard solution

A stock solution of FLV was prepared in methanol having the concentration of 1 mg/ml. A 1.0 ml portion of this solution was

further diluted with mobile phase to get the final concentration 20 µg/ml of FLV.

System suitability parameters

The system suitability test is an integral part of chromatographic methods and used to verify that the reproducibility of the chromatographic system is adequate for the analysis to be performed. A RP-HPLC method was developed keeping in mind the system suitability parameters i.e. retention time, asymmetry, theoretical plates and percent Relative standard deviation of six injections were evaluated.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The specificity of the method was performed by injecting mix standard solution of FLV, marketed formulation and blank.

Calibration curve

Aliquots of the standard solution were diluted in the range 1.0 ml to 5.0 ml in a series of 10 ml volumetric flasks with the mobile phase to obtain the concentration range 10 µg/ml to 50 µg/ml for FLV. Calibration curve was constructed by plotting an area under curve against concentration.

Force degradation study

To evaluate intrinsic stability, FLV was subjected to force degradation as per International Community on Harmonization (ICH) guidelines to get an idea of how drug substance or product degrades, degenerate and behaves under changing condition, which helps in developing stability indicating method of analysis. This study was performed on standard FLV and its marketed formulation viz: Solution state degradation and Solid state degradation.

Solution state degradation

At concentration of 50 µg/ml for FLV, degradation studies in solution was carried out at 80 °C in an oven for 90 min. Standard and marketed formulation was degraded in acidic and alkaline medium using 0.1N HCl, 0.1N NaOH, neutral hydrolysis in distill water and Oxidative degradation in 3% H₂O₂. Analysis of exposed standard was done after 90 min while sample solutions were analyzed at an interval of 15, 30, 45, 60 and 90 min.

Solid state analysis

Solid samples of standard and marketed formulation exposed to Humidity studies (40 °C/75% RH), Photo stability studies (UV light) and Thermal studies (Dry Heat) over a period of 15 days. The samples were analysed on 1st, 4th, 7th and 15th day. Solid state analysis was performed at a drug concentration of 20 µg/ml of FLV. The study was performed for observing % un-degraded of the active ingredient and study of pattern of UV spectra of an exposed sample.

After specified time intervals, the stress solutions and solid stressed samples were allowed to cool at room temperature and diluted with methanol. The solution was filtered with whatmann filter paper (No.41). Further dilution with mobile phase of sample and standard solution were carried out. After equilibration of column with mobile phase 20 µL solution was injected to column under proposed conditions and chromatographed separately, AUC was noted and % un-degraded drug was calculated for each solution using

Formula for sample

$$\% \text{ Drug un-degraded} = \frac{A_{u(\text{expose})} \times W_{t.\text{std}(\text{unexposed})} \times A_{v.g.} \times W_{t.} \times \text{Potency}}{A_{S(\text{unexposed})} \times W_{\text{tab}(\text{expose})} \times L.C.} \dots \text{Eq. 1}$$

Formula for standard

$$\% \text{ Drug un-degraded} = \frac{A_{S(\text{expose})} \times W_{t.} \times \text{std}(\text{unexposed}) \times \text{Potency}}{A_{S(\text{unexposed})} \times W_{t.\text{std}(\text{exposed})}} \dots \text{Eq. 2}$$

Where, Au-area of unknown, As (expose)-area of exposed standard, As (unexposed)-area of unexposed standard, Wt. std (unexposed)-weight of standard taken, Wt. std (exposed)-weight of exposed standard taken, Wtab-weight of tablet powder taken, L. C.-label claim of drug

Application of proposed method to marketed formulation (precision)

The proposed method was applied to the determination of FLV in commercial dosage form, Fluvoxin tablets. An accurately weighed quantity of tablet powder equivalent to 10 mg of FLV was transferred to 10 ml volumetric flasks and volume was made up to the mark with methanol. The content was sonicated for 15 min and filtered through whatmann filter paper. A 1.0 ml portion of filtrate was diluted to 10 ml with the mobile phase. After that 2.0 ml portion of this solution were further diluted to 10 ml with the mobile phase.

After equilibration of the column with the mobile phase 20 µl volume of the final diluted solutions were injected in the system. Analysis of such six replicate of final diluted solution was carried out using optimized chromatographic conditions. AUC noted and % labeled claim was calculated.

Validation of proposed method

The developed method was validated as per the ICH guidelines with respect to various parameters such as linearity, accuracy, precision, and robustness, ruggedness, limit of detection and limit of quantification

Accuracy (% recovery)

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at five different levels (90%, 95%, 100%, 105%, and 110%). The amount of each drug contributed by the preanalysed tablet powder was deducted from total amount of respective drug estimated and resultant quantities were assured to be recovered from the pure drug added.

Intermediate precision

For intra-day, sample solution (20µg/ml) was prepared as per the procedure described under marketed formulation and analyzed at intervals of 0th, 2h, 6h and 8h and same solution analyzed at intervals of 1st, 3rd and 5thday.

Analyst to analyst variation

The sample solution was prepared by two different analysts in same manner to that of marketed formulation. Then sample solutions were analyzed using the proposed method.

Linearity and range

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110 and 120 % of label claim were taken separately and dilutions were made as described under marketed formulation. After equilibration of the column with mobile phase 20 µl volume of the final diluted solutions were injected in the system separately and chromatograms were recorded. Linearity curve was constructed by plotting the concentration level of drug verses corresponding peak area.

Sensitivity

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were calculated based on the equation:

$$\text{LOD} = 3.3 \times S/B \text{ and } \text{LOQ} = 10 \times S/B$$

Where, S is SD of peak areas of the drugs taken as a measure of noise and B is the slope of the corresponding calibration curve. The sensitivity of detection of FLV by use of the proposed method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ).

Robustness

Robustness of the proposed method was determined by small deliberate changes in the composition of mobile phase ratio ($\pm 10\%$) of mobile phase, pH (± 0.2) and detection wavelength (± 5 nm).

RESULTS AND DISCUSSION

Various mobile phases were tried by permutation and combinations and also by varying flow rate, column temperature and types of buffers with varying pH and solvents. The prepared different mobile phases were filtered through 0.45 μ m membrane filter paper prior to use.

The mobile phase composition at a ratio of 70:30 (v/v) of methanol and phosphate buffer pH 2.5 was found to be most suitable to obtain peak of FLV at 5.94 min is well defined and free, from tailing.

System suitability parameter

The optimized method developed resulted in the elution of FLV at 5.94 min. Fig. 2 represents the typical chromatogram of standard

FLV. System suitability parameters were evaluated for six replicate injections of standard at 10 μ g/ml. Results of system suitability parameters recorded in table 1.

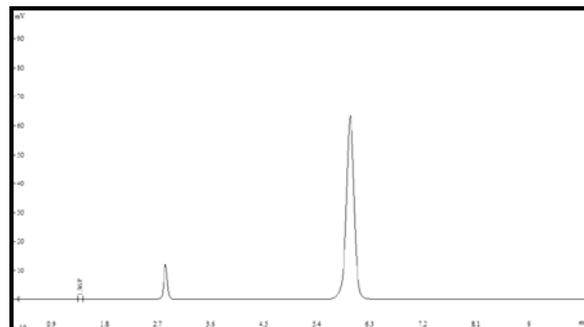


Fig. 2: Typical chromatogram of standard FLV

Table 1: System suitability parameters

Wt. of Std. taken (g)	Mean area (μ V)	\pm SD	Retention time	Asymmetry	Theoretical plates
0.010	601122.33	1927.56	5.939	1.05	8874

*Each observation is mean of six observations

Specificity

Chromatograms of standard FLV (fig. 2) and sample (fig. 3) reveal that the peak obtained in the standard solution and the sample solution at working concentration are only because of drug as blank has no peak at the retention time of FLV. No excipients were found to elute at the retention times of the said drugs in the sample solution shows developed method is said to be specific.

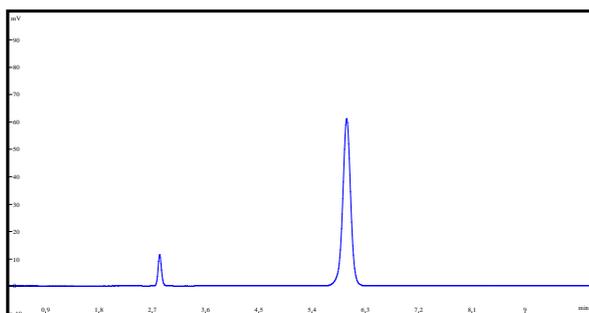


Fig. 3: Typical chromatogram of FLV from tablet formulation

Calibration curve

Calibration curve plot (fig. 4) of an area under curve against concentration shows that FLV was found to be linear over the range of 10-50 μ g/ml with correlation coefficient 0.998.

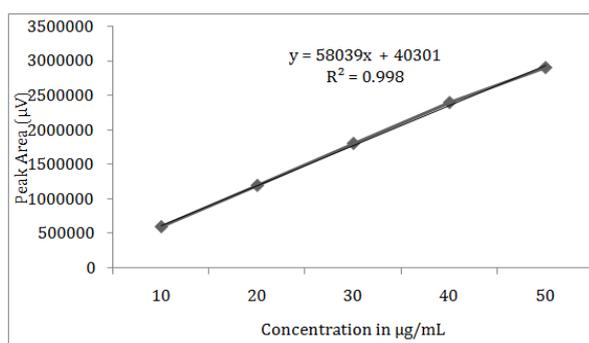


Fig. 4: Linearity curve of FLV

Force degradation studies

Chromatograms of base, acid, neutral and peroxide stressed marketed samples (fig. 5a-5d) and of Std. (fig. 6a-6d) are shown in respectively.

Similarly chromatograms of solid state analysis of the standard and sample are depicted in (fig. 7a-7c) and (fig. 8a-8c) respectively. The results of forced degradation study are given in table 2.

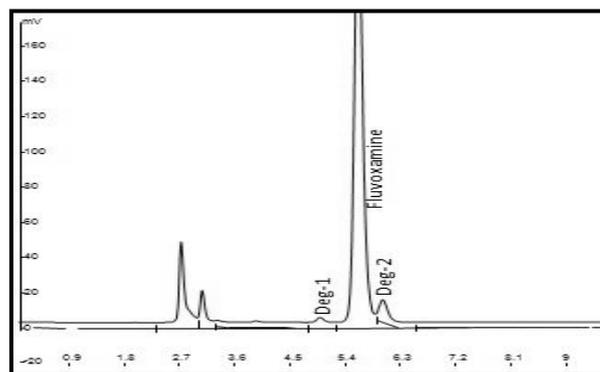


Fig. 5a: Chromatogram of base stressed sample

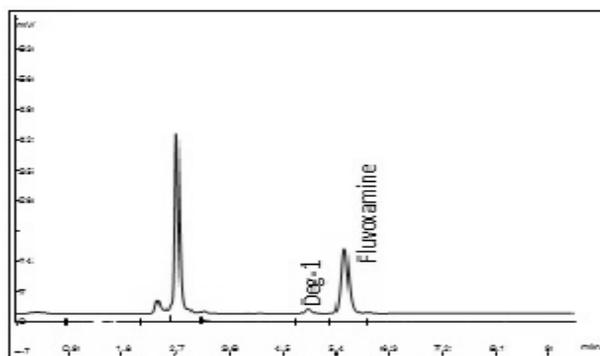


Fig. 5b: Chromatogram of acid stressed sample

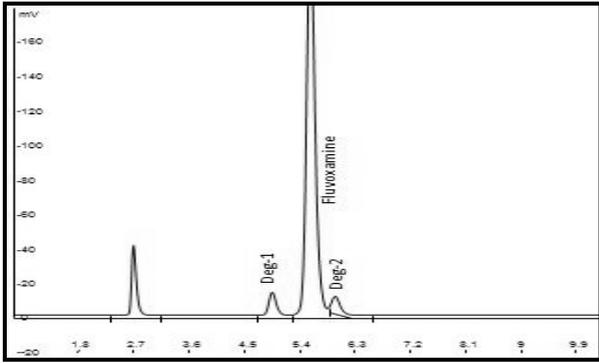


Fig. 5c: Chromatogram of water stressed sample

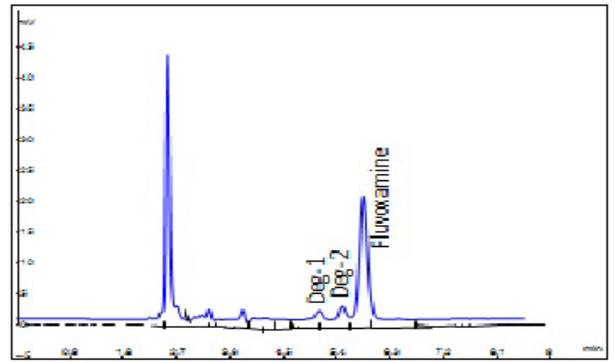


Fig. 6b: Chromatogram of acid stressed standard

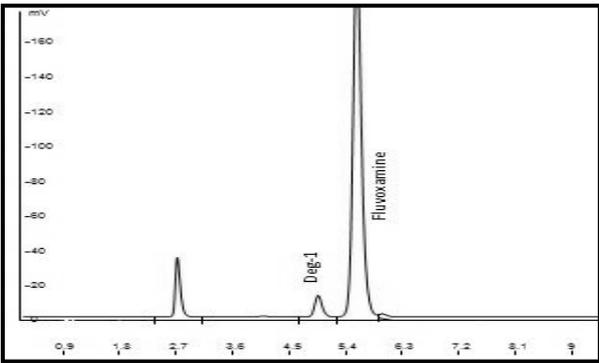


Fig. 5d: Chromatogram of peroxide stressed sample

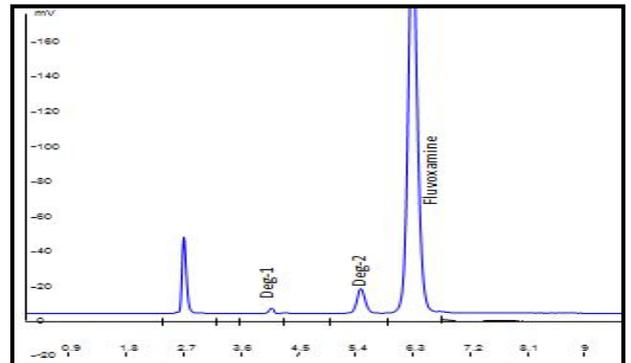


Fig. 6c: Chromatogram of distill water stressed standard

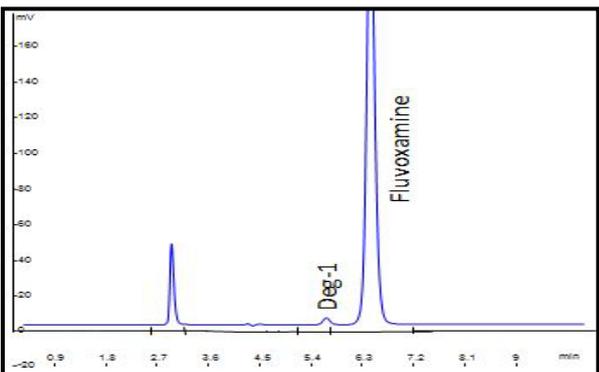


Fig. 6a: Chromatogram of base stressed standard

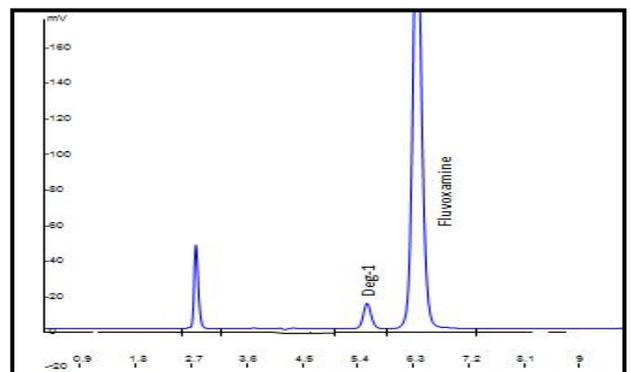


Fig. 6d: Chromatogram of peroxide stressed standard

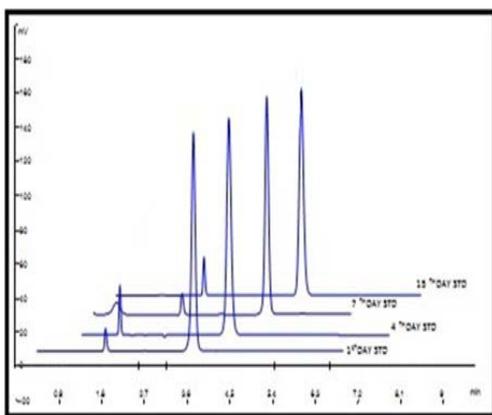


Fig. 7a: Chromatogram of exposed Std. to Humidity

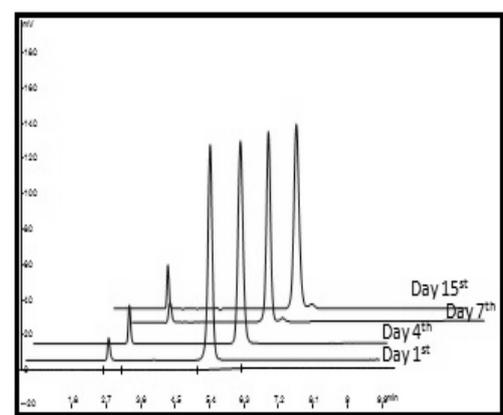


Fig. 8a: Chromatogram of exposed sample to Humidity

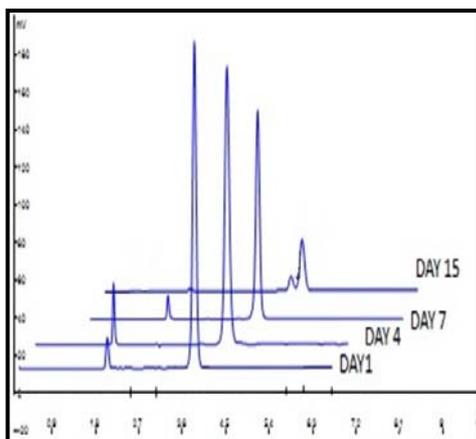


Fig. 7b: Chromatogram of exposed Std. to UV light

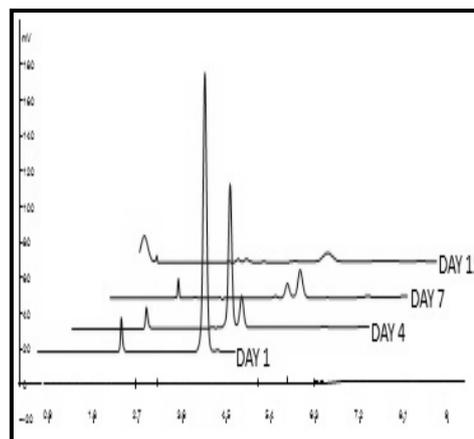


Fig. 8b: Chromatogram of exposed sample to UV light

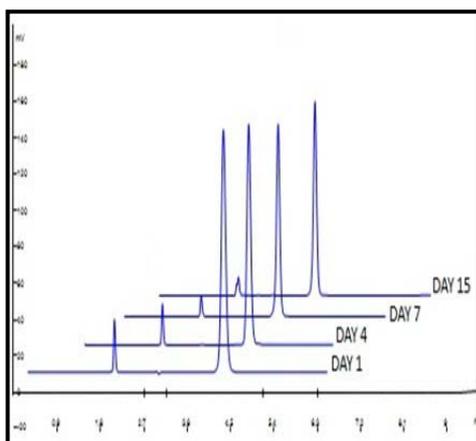


Fig. 7c: Chromatogram of exposed Std. to Heat

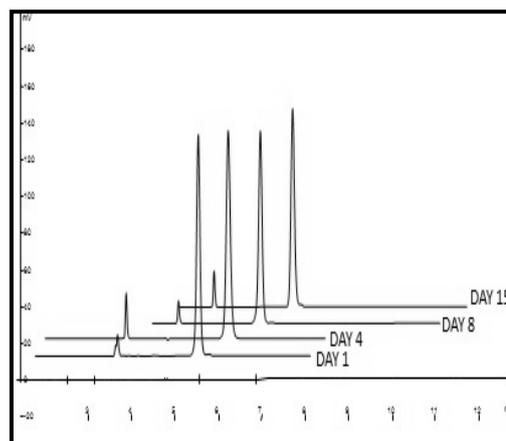


Fig. 8c: Chromatogram of exposed sample to Heat

Table 2: Force degradation study

Degradation parameters	Interval	% Drug un-degraded	
		Exposed Std.	Exposed MF
Solution state analysis			
Acid degradation (0.1 M HCl)	At 80 °C, 90 min	76.77	55.21
Base degradation (0.1 M NaOH)		88.97	84.26
Peroxide degradation (3% H ₂ O ₂)		86.75	80.25
Distill water degradation		79.93	77.75
Solid state analysis			
Humidity studies (40 °C/75% RH)	15Days	83.94	84.37
Photo stability studies (UV light)	*7day	86.45*	7.00*
Thermal studies (50 °C)		94.41	90.63

FLV was found to be labile to alkaline, neutral and oxidative condition as degradation was found to be around 20% in exposed standard and in sample while it was found to be 40% to acidic hydrolysis indicating extremely labile nature. Two additional degradants peaks [Deg1 (RRT-0.89); Deg2 (RRT-1.07)] were seen in chromatogram of sample under alkaline hydrolysis. One additional degradant peak [Deg1 (RRT-0.89)] was seen in chromatogram of sample under acidic hydrolysis. Two additional degradants peaks [Deg1 (RRT-0.74); Deg2 (RRT-0.99)] were seen in chromatogram of sample under neutral hydrolysis. One additional degradant peak [Deg1 (RRT-0.91)] was seen in chromatogram of sample under oxidative hydrolysis. The RRT of impurities reported in BP 2009[13] does not match with the degradant RRTs leading to formation of unknown impurities in sample under stress. Sample and standard FLV shows sufficient degradation when exposed to humidity, heat and light. Almost 93% degradation of sample on 7 day was seen under UV exposure (ICH Recommended) with additional peak seen in the chromatogram. There was complete

separation of degradation peaks and analyte peaks, which demonstrate the specificity of assay method for estimation of FLV in the presence of its degradation products; it can be employed as a stability indicating one and can be used to assess the stability of FLV in the bulk drug and in pharmaceutical dosage forms

Kinetics of solution state degradation studies

The kinetics of degraded samples was evaluated for all the hydrolytic conditions. The plot of regression coefficient (r) obtained and the best fit observed indicates the order of degradation reaction.

- Values of concentration against time (zero-order kinetics)
- Log of concentration verses time (first-order kinetics)
- Reciprocal of concentration verses time (second-order kinetics)

The observation and results of kinetics of degradation so recorded as shown in table 3.

Table 3: Kinetics of degradation studies

Degradation Study	Value of 'r' FLV	Order of reaction
Acid Hydrolysis	0.978	Second
Alkali Hydrolysis	0.984	Zero
Oxidation	0.977	Zero
Neutral Hydrolysis	0.832	Second

Precision (% Assay)

Precision is a measure of the reproducibility of the whole analytical method under normal operating circumstances. The precision was expressed as the relative standard deviation (RSD). The result shown in table 4 indicates that the method is selective for the assay of FLV without interference from the excipients used in these tablets. A low value of standard deviation (0.69) is indicative of high precision of the proposed method.

Table 4: Assay of FLV in marketed formulation

Marketed formulation-Fluvoxin			
Wt. taken (mg)	AUC of Standard (μV)	AUC of Sample (μV)	% Labeled claim*
51.67	1223172	1202530	99.31
51.72		1202705	99.23
50.92		1180403	98.92
52.10		1220698	99.98
51.98		1196205	98.20
50.43		1182163	100.03
		Mean	99.27
		±SD	0.68
		% RSD	0.69

*fluvoxamine mealate estimated as fluvoxamine

Intermediate precision and Analyst variations

% RSD value indicates the acceptable reproducibility and thereby the precision of the system. Summary of results are shown in table 5.

Table 5: Intermediate precision and Analyst variations

Parameters	Mean of % estimation of FLV	±SD	% RSD
Intraday	99.75	0.45	0.45
Interday	98.72	0.83	0.85
Analyst to Analyst	99.82	0.74	0.75

Accuracy

The % recovery was found within range, 98.34%-101.36% for FLV which indicates high accuracy of the method. Percent mean recovery was calculated as shown in table 6. The accepted limits of recovery 98%-102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 6: Recovery study

Wt. of tablet powder (mg)	Amt. of Std. FLV added (mg)	Amt. of FLV recovered (mg)	% Recovery of FLV
25.84	3.98	3.97	99.74
25.40	4.40	4.46	101.36
25.88	4.98	4.96	99.60
26.03	5.51	5.46	99.09
26.10	6.05	5.95	98.34
Mean % recovery			99.62
±SD			1.11
RSD			1.12

Linearity and range

The correlation coefficient of FLV was found to be 0.999 indicates excellent correlation between peak area and concentration level of drug (fig. 9).

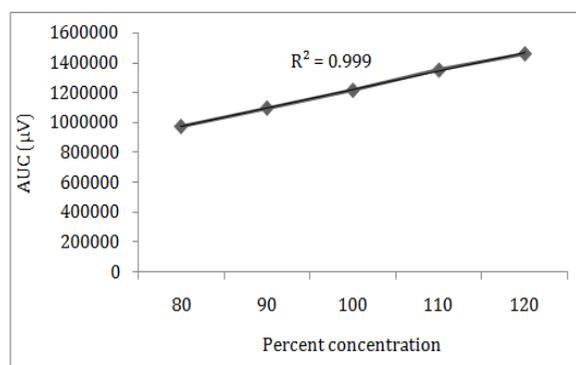


Fig. 9: Linearity and range plot of FLV

Sensitivity

The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 0.109 μg/ml and 0.332 μg/ml respectively. The results show very good sensitivity of the developed method.

Robustness

The content of the drug was not adversely affected by these changes as evident from the low value of mean RSD (1.55) indicating that the method was rugged and robust (table 7).

Table 7: Robustness data

Parameters	RT	Asymmetry	Theoretical plate
Mean	5.907	1.05	8743.57
RSD	1.74	0.93	1.98
Mean RSD	1.55		

CONCLUSION

The proposed HPLC method is stability indicating one, cost effective and less time consuming. The low percent RSD values of validation parameters indicates the method is accurate, precise and robust. Hence the proposed method is rapid, accurate and robust. Moreover the degraded peaks were well resolved from analyte peaks. So the

developed method may be used for analysis of stability samples of FLV in quality control laboratory.

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CONFLICTS OF INTERESTS

All authors have none to declare.

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