Research Paper

STABILITY INDICATING ECO-FRIENDLY HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FLUVOXAMINE

Running title: Development and Validation of HPLC Method for Determination of Fluvoxamine

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ABSTRACT

Quantitative analysts are interested in greening their approaches to decrease environmental effect and increase safety and health. OCD patients can use fluvoxamine. It's an SSRI. For tablet FVX quantification, a green HPLC technique was designed, optimized, verified, and used. Experiments were done on the Capcell Pak C8, 4.6 X 150 mm, 5 µm. A 30:70 v/v/v mobile phase of Ethanol and water was used for isocratic elution. An analytical wavelength of 234.0 nm was used. At 12.0 minutes, an experiment ended. Each drug's calibration graphs were rectilinear from 35 to 65 µg/mL. Both drugs were subjected to acidic, alkaline, oxidative, thermal, and photolytic stress. International Conference on Harmonization (ICH) guidelines guided validation studies. The examination confirmed the approach's greenness in solvent use, chemical compounds, energy use, and waste creation. No formulation additions have created chromatographic or spectrum issues. The AGREE Indicator was used to assess the environmental, safety, and sustainability of analytical methods in this study, harmonizing with the UN Sustainable Development Goals. These proven methodologies for quantitative FVX analysis in tablet formulations promote safer and greener pharmaceutical analytical lab procedures.

Keywords: RP-HPLC, Assay, Green analytical chemistry, Validation, Fluvoxamine and Forced Degradation Studies.

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Introduction

When developing a new analytical method for detecting a specific analyte, two crucial factors must be addressed. First, the method's metrological value must be validated to ensure accuracy, precision, and reproducibility. Second, environmental sustainability must be prioritized by incorporating safer, less harmful chemicals and processes, and adhere to the guidelines for green chemistry. The application of these principles is critical yet still underutilized in many fields, including pharmaceutical analysis **[1-2]**. High-Performance Liquid Chromatography (HPLC) remains a cornerstone technology in pharmaceutical quality control, owing to its robust and reliable performance. Traditionally, reverse-phase HPLC utilizes a polar mobile phase combined with a hydrophobic stationary phase. The mobile phase often consists of a mixture of water, modified with additives, and organic solvents like ethanol. In reverse phase separation mode, mobile phases primarily composed of water and aqueous buffers may be utilized, depending on the analyte's solubility. However, using only water and aqueous buffers in the mobile phase can lead to prolonged analysis times. Consequently, mobile phases that combine aqueous buffers with organic modifiers are preferred for more efficient separation. These organic solvents are preferred due to their favourable chromatographic characteristics, such as miscibility with water, low viscosity, and chemical stability, which ensure high-quality analytical performance. Despite these advantages, both acetonitrile and ethanol pose significant environmental and health risks. The widespread use of these solvents in HPLC has resulted in substantial amounts of waste, leading to significant environmental and disposal challenges **[3-5].**

Depression is one of the most common mental disorders, triggered by the interplay of social, psychological, and biological factors, and is characterized by persistent depressed mood or

loss of interest in daily activities. It is estimated that 1.1 % of adolescents aged 10–14 years and 2.8 % of those aged 15–19 years globally experience depression, while 3.6 % of adolescents aged 10–14 years and 4.6 % of those aged 15–19 years suffer from anxiety disorders. The incidence of depression and anxiety significantly increases during adolescence, particularly in early adolescence, with approximately 3.1 % of adolescents experiencing depressive disorders by the age of 14 and 38 % suffering from anxiety disorders **[6-10]**. Fluvoxamine (FVX) is a selective serotonin reuptake inhibitor (SSRI) belonging to a new chemical series, the 2-aminoethyl oxime ethers of aralkylketones. It is chemically designated as 5-methoxy-4′-(trifluoromethyl) valerophenone (E)-*O*-(2-aminoethyl) oxime maleate. Fluvoxamine maleate is a white to slightly off-white, odourless, crystalline powder, sparingly soluble in water, freely soluble in ethanol and chloroform, practically insoluble in diethylether **[11-12]**.

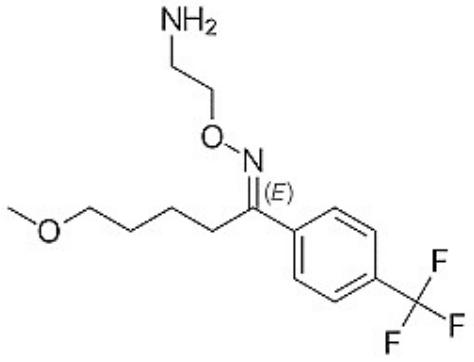

Figure 1: Chemical structure of Fluvoxamine (FVX)

In this study, we aim to address the growing need for environmentally sustainable analytical techniques by developing a green liquid chromatography (Green LC) method utilizing ethanol as the mobile phase solvent. Ethanol's favourable environmental profile and chromatographic performance make it an ideal candidate for promoting greener analytical practices **[13-14]**. The literature reveals a wide range of analytical methods for quantifying FVX in pharmaceutical and biological samples, including spectrofluorimetric, spectrophotometric, and liquid chromatography techniques **[15-19]**. Many different analytical methods have been developed for the quantification of FVX in biological fluids, bulk, and dosage forms. For example, the Liquid chromatographic-tandem mass spectroscopy method for the quantification of FVX in human plasma [**20-21**]; the RP-HPLC/UV technique for the quantification of FVX in rat plasma and its application to a pharmacokinetic study in rats **[22-23]**; the LC-MS/MS-ESI method for the determination of FVX in rat and human plasma and its application to a pharmacokinetic study **[24]**; Liquid chromatography coupled with ultraviolet detection and electron ionization mass spectrometry method for the analysis of FVX stress degradation behaviour **[25]**; FVX in human plasma was determined using an ultra-high-performance liquid chromatography-tandem mass spectrometry technique; and FVX in human plasma was determined using an HPLC-fluorescence technique **[26-27]**. While effective, these methods often require expensive equipment, toxic solvents, and complex procedures, limiting their accessibility and increasing their environmental impact. The introduction of greener methods for FVX analyses is, therefore, timely and necessary. In this context, our study presents a novel, environmentally friendly green LC method using ethanol, as well as a Simplified Spectrophotometric Method that employs ultrapure water as the solvent. These methods aim to reduce the environmental burden associated with traditional HPLC techniques while maintaining robust analytical performance.

Materials and methods

Materials:

All the chemicals and reagents used in this study were of analytical grade. API FVX received as a Gift sample from Glenmark Pharmaceuticals R and D and HPLC grade acetonitrile (Thermo Fisher Scientific India Pvt Ltd Mumbai). Marketed formulation Felbamate Tablet (600 mg) was procured from local pharmacy. HPLC-grade solvents such as Methanol, Ethanol and Acetonitrile were procured from Thermo Fisher Scientific India Pvt Ltd Mumbai.

Method Development

HPLC instrumentation

A Waters-HPLC (Model-2489) outfitted with an autosampler, a UV detector, and a column oven was used to perform chromatographic separation. Throughout the Empower-3programming process, data was collected. A C18 column of 5 µm column of Capcell Pak C8, 4.6 X 150 mm was used.

Chromatographic conditions

The description goes over the methodology used in the chromatography experiment. Chromatographic data were collected and processed using Empower 3 software (Waters Corporation, USA) to determine retention times, peak areas, and resolution parameters. In the process of separation, an Capcell Pak C8, 4.6 X 150 mm, 5µm, Ethanol and water (30:70) was used for the mobile phase, with a flow rate of 1.0 millilitres per minute. The pore size impacts how much interaction the analytes have with the stationary phase. The 150 Å pore size allows FVX to access enough surface area within the stationary

phase to interact effectively. Eluent and run time were recorded at a wavelength of 237 nm for ten minutes. The wavelength of 237 nm is likely chosen because it is close to the absorbance maximum of FVX. In UV–visible spectroscopy, each compound has a characteristic absorption spectrum, which shows the wavelengths at which it absorbs light most strongly. The column was conditioned by letting the mobile phase go through the system for at least half an hour before starting the experiment. 20 µl of injection volume was utilized for the sample analysis. Liquid chromatography forces the mobile phase through the column, simplifying the process of separating the constituent parts of the sample. This serves as the experiment's foundation. Among these famous reversed-phase columns is C18. Chromatographic condition as shown in Table 1.

Preparation of reference standard solution

Weighed accurately about 50.0 mg of Fluvoxamine Maleate working standard / reference standard and transferred into a 100 mL volumetric flask. Added about 70 mL of diluent and sonicated for 5 minutes to dissolved (Ensure the working standard / reference standard dissolve completely). Allowed the flask to attain room temperature and dilute with diluent to volume and mixed well. Pipetted out 5.0 mL this solution and transferred into 50 mL volumetric flask, diluted with diluent to volume and mix.

Preparation of Sample Solution:

Weighed and crushed 20 tablets to fine powder. Accurately weighed and transferred crushed powder equivalent to 500 mg of Fluvoxamine Maleate, into 500 mL volumetric flask. Add 300 mL of diluent, sonicate for 30 minutes with intermittent shaking. Allowed to cool at room temperature and diluted with diluent to volume and mix. Filtered the solution through 0.45 µm PTFE syringe filter discarded first few mL of filtrate. Pipetted out 5 mL of filtrate to 100 mL volumetric flask, dilute with mobile phase to volume and mix.

Table 1: Chromatographic condition optimization

Instrument	HPLC (Waters 2489)
Flow Rate	1 ml/min.
Column	Capcell Pak C8, 4.6 X 150 mm, 5 µm
Mobile Phase	Ethanol and water (30:70)
Detector, Wavelength	UV, 234 nm
Run Time	12 min
Volume of Injection	20 µl
Temperature of Column	Ambient Temperature
Diluents	Ethanol and water (30:70)
Mode of Separation	Isocratic Mode

Trial and error studies were made using different solvents with different Columns, different diluents again with different ratios, and different flow rates for getting the sharpest peak and with the least retention time.

Proposed technique validation

Method validation is documented evidence which provides a high degree of assurance for a specific method that the process used to confirm the analytical process is suitable for its intended use. The developed HPLC method for estimation FVX was validated as per ICH Q2 (R1) guidelines **[28-32]**.

System suitability studies

The system suitability was evaluated by six replicate analyses of FVX. The retention time, column efficiency, peak asymmetry, and theoretical plates were calculated for standard solutions.

Linearity

The linearity of FVX was evaluated by analyzing 5 independent levels concentration range of 35–65µg/ml. The calibration curve was constructed by plotting peak area on y axis versus concentration on x-axis. The regression line equation and correlation coefficient values were determined.

Precision

The precision of an analytical method is the closeness of agreement between series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed condition. The system precision was determined by injecting six replicates of the standard solution as per test procedure from the same HPLC vial. Reproducibility expressed the precision under the same operating condition over a short interval of time. Method precision was carried on Felbamate Tablets 600 mg. Prepared six sample solutions and injected into the chromatograph. Intermediate precision expressed within the laboratory variation on a different day, by a different analyst, using different HPLC system, different column of same make and same lot of samples as specified under repeatability. Intermediate precision was carried on Felbamate Tablets, USP 600 mg. prepared six sample solutions and injected into the chromatograph. Calculated % Assay, mean assay and overall RSD

of assay values obtained in the intermediate precision and method precision study.

Accuracy

The accuracy of the method was determined by calculating by recovery study from marketed formulation by at three levels 50%, 100%, and 150% of standard addition. The % recovery of FVX was calculated. The acceptance limit for % recovery as per ICH guidelines was 98–102% of standard addition.

Specificity

The specificity of the method has been established by comparing the chromatograms obtained by injecting blank (mobile phase), solution of FVX (20 µg/ml), and sample solution of FVX Tables. The effect of excipients used in film was checked. The parameters retention time, tailing factor, and resolution were calculated in order to prove that the method chosen was specific.

Forced degradation study

Acid degradation:

Weigh accurately about 354.78 mg of placebo powder, 852.68 mg Sample powder of Fluvoxamine Maleate Extended-Release Tablets 150 mg and 501.64 mg of Fluvoxamine Maleate API using suitable weighing boat and transferred to three different 500 mL volumetric flask. Added 120 mL of diluent and place the magnetic bar into the flask stirred the sample solution on magnetic stirrer at 500 rpm for 60 minutes. Then added 180 mL of diluent and stirred at 500 rpm for 30 minutes. Added 50 mL of diluent and sonicate for 60 minutes with intermittent shaking. Carefully removed the magnetic bar from flask with help of magnet and rinsed magnetic bar with diluent at neck of the volumetric flask. Added 50 mL of 1 N hydrochloric acid solution. Kept the flask on water bath at 80°C for 2 hours. Cooled and neutralized with 1 N of sodium hydroxide solution. Allowed the flask to attain room temperature and diluted with diluent to volume and mixed. Filtered the solution through 0.45 µm Pre-filter+PTFE, discard first 3-4 mL of filtrate. Pipetted out 5.0 mL of clear filtrate and transferred into 100 mL volumetric flask, diluted with diluent to volume and mixed.

Base degradation:

Weigh accurately about 354.78 mg of placebo powder, 852.68 mg Sample powder of Fluvoxamine Maleate Extended-Release Tablets 150 mg and 501.64 mg of Fluvoxamine Maleate API using suitable weighing boat and transferred to three different 500 mL volumetric flask. Added 120 mL of diluent and place the magnetic bar into the flask stirred the sample solution on magnetic stirrer at 500 rpm for 60 minutes. Then added 180 mL of diluent and stirred at 500 rpm for 30 minutes. Added 50 mL of diluent and sonicate for 60 minutes with intermittent shaking. Carefully removed the magnetic bar from flask with help of magnet and rinsed magnetic bar with diluent at neck of the volumetric flask. Added 50 mL of 1 N of sodium hydroxide solution. Kept the flask at room temperature on bench top for 20 minutes hours. Cooled and neutralized with 1 N hydrochloric acid solution. Allowed the flask to attain room temperature and diluted with diluent to volume and mixed. Filtered the solution through 0.45 µm Pre-filter+PTFE, discard first 3-4 mL of filtrate. Pipetted out 5.0 mL of clear filtrate and transferred into 100 mL volumetric flask, diluted with diluent to volume and mixed

Oxidation degradation:

Weigh accurately about 354.78 mg of placebo powder, 852.68 mg Sample powder of Fluvoxamine Maleate Extended-Release Tablets 150 mg and 501.64 mg of Fluvoxamine Maleate API using suitable weighing boat and transferred to three different 500 mL volumetric flask. Added 120 mL of diluent and place the magnetic bar into the flask stirred the sample solution on magnetic stirrer at 500 rpm for 60 minutes. Then added 180 mL of diluent and stirred at 500 rpm for 30 minutes. Added 50 mL of diluent and sonicate for 60 minutes with intermittent shaking. Carefully removed the magnetic bar from flask with help of magnet and rinsed magnetic bar with diluent at neck of the volumetric flask. Added 50 mL of 30 % hydrogen peroxide solution. Kept the flask on water bath at 80°C for 3 hours. Allowed the flask to attain room temperature and diluted with diluent to volume and mixed. Filtered the solution through 0.45 µm Pre-filter+PTFE, discard first 3-4 mL of filtrate. Pipetted out 5.0 mL of clear filtrate and transferred into 100 mL volumetric flask, diluted with diluent to volume and mixed.

Hydrolysis degradation:

Weigh accurately about 354.78 mg of placebo powder, 852.68 mg Sample powder of Fluvoxamine Maleate Extended-Release Tablets 150 mg and 501.64 mg of Fluvoxamine Maleate API using suitable weighing boat and transferred to three different 500 mL volumetric flask. Added 120 mL of diluent and place the magnetic bar into the flask stirred the sample solution on magnetic stirrer at 500 rpm for 60 minutes. Then added 180 mL of diluent and stirred at 500 rpm for 30 minutes. Added 50 mL of diluent and sonicate for 60 minutes with intermittent shaking. Carefully removed the magnetic bar from flask with help of magnet and rinsed magnetic bar with diluent at neck of the volumetric flask. Added 50 mL of water. Kept the flask on water bath at 80°C for 5 hours. Allowed the flask to attain room temperature and diluted with diluent to volume and mixed. Filtered the solution through 0.45 µm Pre-

filter+PTFE, discard first 3-4 mL of filtrate. Pipetted out 5.0 mL of clear filtrate and transferred into 100 mL volumetric flask, diluted with diluent to volume and mixed.

Photo degradation: Fluorescence

Transferred 2 g of placebo powder, 80 Fluvoxamine Maleate Extended-Release Tablets 150 mg and 2 g Fluvoxamine Maleate API into a three different petri dishes and kept in the photo stability chamber at NLT 1.2 million lux hours for degradation Weigh accurately about 352.31 mg of placebo powder, 851.72 mg powder of Fluvoxamine Maleate Extended-Release Tablets 150 mg and 502.12 mg of Fluvoxamine Maleate API (which was previously charged at NLT 1.2 million lux hours for degradation) using suitable weighing boat and transferred to three different 500 mL volumetric flask. Added 120 mL of water and stir at 500 rpm for 30 minutes. Added 50 mL of diluent and sonicated for 60 minutes with intermittent shaking. Carefully removed the magnetic bar from flask with help of magnet, rinsed magnetic bar with diluent at neck of the volumetric flask. Allowed the flask to attain room temperature and diluted with diluent to volume and mixed. Filtered the solution through 0.45 µm Pre-filter+PTFE, discard first 3-4 mL of filtrate. Pipetted out 5.0 mL of clear filtrate and transferred into 100 mL volumetric flask, diluted with diluent to volume and mixed

Photo degradation: UV

Transferred 2 g of placebo powder, 80 Fluvoxamine Maleate Extended-Release Tablets 150 mg and 2 g Fluvoxamine Maleate API into a three different petri dishes and keep in the photo stability chamber at NLT 200 watts' hours' / sq. meter for degradation. Weigh accurately about 352.31 mg of placebo powder, 851.72 mg powder of Fluvoxamine Maleate Extended-Release Tablets 150 mg and 502.12 mg of Fluvoxamine Maleate API (which was previously charged at NLT 200 watts hours / sq. meter for degradation) using suitable weighing boat and transferred to three different 500 mL volumetric flask. Added 120 mL of acetonitrile and place the magnetic bar into the flask stirred the sample solution on magnetic stirrer at 500 rpm for 60 minutes. Then added 180 mL of water and stirred at 500 rpm for 30 minutes. Added 50 mL of diluent and sonicate for 60 minutes with intermittent shaking. Carefully removed the magnetic bar from flask with help of magnet and rinsed magnetic bar with diluent at neck of the volumetric flask. Allowed the flask to attain room temperature and diluted with diluent to volume and mixed. Filtered the solution through 0.45 µm Pre-filter+PTFE, discard first 3-4 mL of filtrate. Pipetted out 5.0 mL of clear filtrate and transferred into 100 mL volumetric flask, diluted with diluent to volume and mixed

Humidity degradation:

Transferred 2 g of placebo powder, 80 Fluvoxamine Maleate Extended-Release Tablets 150 mg and 2 g Fluvoxamine Maleate API into a three different petri dishes and keep in the desiccator at 25°C / 97% RH for 24 hours for degradation. Weigh accurately about 352.31 mg of placebo powder, 851.72 mg powder of Fluvoxamine Maleate Extended-Release Tablets 150 mg and 502.12 mg of Fluvoxamine Maleate API (which was previously charged at 25°C/97% RH for 24 hours for degradation) using suitable weighing boat and transferred to three different 500 mL volumetric flask. Added 120 mL of acetonitrile and place the magnetic bar into the flask stirred the sample solution on magnetic stirrer at 500 rpm for 60 minutes. Then added 180 mL of water and stirred at 500 rpm for 30 minutes. Added 50 mL of diluent and sonicate for 60 minutes with intermittent shaking. Carefully removed the magnetic bar from flask with help of magnet and rinsed magnetic bar with diluent at neck of the volumetric flask. Allowed the flask to attain room temperature and diluted with diluent to volume and mixed. Filtered the solution through 0.45 µm Pre-filter+PTFE, discard first 3-4 mL of filtrate. Pipetted out 5.0 mL of clear filtrate and transferred into 100 mL volumetric flask, diluted with diluent to volume and mixed

Thermal degradation:

Transferred 2 g of placebo powder, 80 Fluvoxamine Maleate Extended-Release Tablets 150 mg and 2 g Fluvoxamine Maleate API into a three different petri dishes and keep in the oven at 60°C for 24 hours for degradation. Weigh accurately about 352.31 mg of placebo powder, 851.72 mg powder of Fluvoxamine Maleate Extended-Release Tablets 150 mg and 502.12 mg of Fluvoxamine Maleate API (which was previously charged at 60°C for 24 hours for degradation) using suitable weighing boat and transferred to three different 500 mL volumetric flask. Added 120 mL of acetonitrile and place the magnetic bar into the flask stirred the sample solution on magnetic stirrer at 500 rpm for 60 minutes. Then added 180 mL of water and stirred at 500 rpm for 30 minutes. Added 50 mL of diluent and sonicate for 60 minutes with intermittent shaking. Carefully removed the magnetic bar from flask with help of magnet and rinsed magnetic bar with diluent at neck of the volumetric flask. Allowed the flask to attain room temperature and diluted with diluent to volume and mixed. Filtered the solution through 0.45 µm Pre-filter+PTFE, discard first 3-4 mL of filtrate. Pipetted out 5.0 mL of clear filtrate and transferred into 100 mL

volumetric flask, diluted with diluent to volume and mixed

Robustness studies

To assess the robustness of the HPLC method, small deliberate variations in the detection wavelength and flow rate were introduced. The actual detection wavelength was set at 234 nm, and robustness was evaluated by varying the Temperature by ±5 nm (i.e., 30°C and 20°C). The actual flow rate was set at 1.0 mL/min, and small deliberate variations of ±0.2 mL/min (i.e., 0.8 mL/min and 1.2 mL/min) were introduced.

Assessment of the greenness of the developed method

Assessment of the proposed method greenness was carried out using the Analytical GREenEss (AGREE) tool **[26-27]**.

Results:

Method Development

Initially, a mobile phase Methanol (100%) and Acetonitrile (100%), was tried; the split peak and distorted peak of FVX was observed at Rt 3.2 respectively. The further mobile phase tried was Ethanol and water (30:70). The improvement of peak shape and symmetry was done by changing the column as shown in Figure 2. The system suitability test parameters were satisfied with optimized chromatographic condition. The optimized mobile phase consisting of Ethanol and water (30:70), and Column Capcell Pak C8, 4.6 X 150 mm, 5 µm.

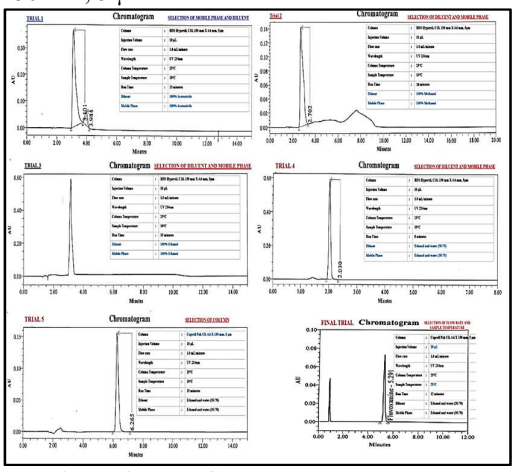

Figure 2: HPLC Method Development Chromatograms

The chosen solvent was Ethanol and water (30:70) as it is a polar aprotic solvent that effectively dissolves lipophilic compounds like FVX. It is compatible with the HPLC system and UV detectors at 234 nm, minimizing interference. It provides good peak resolution and reproducibility during separation. It has low viscosity, making it easier to handle in high-pressure HPLC systems.

Validation Results:

System suitability studies

A system suitability test is an essential first step in ensuring that the chromatographic system is suitable for the required analysis. This test's main objective is to repeatedly inject the sample and assess various metrics, such as theoretical plate height, peak area, tailing factor, and plate area. Each of these attributes must satisfy the standards established by the United States Pharmacopoeia (USP). The test's results indicate that the system is appropriate for the analysis because the RSD values fell between the generally accepted, 2 % upper limit as given in Table 2.

Table 2: System suitability data of FVX.

Parameter	Tailing factor (Limit = NMT 2.0)	Theoretical plates (Limit = NLT 2000)	% RSD (Limit = NMT 2.0)
Specificity			
Blank, placebo and impurities interference	1.10	9462	0.2
Forced degradation_1	1.10	9475	0.3
Forced degradation_2	1.10	9266	0.3
Linearity	1.10	9533	0.2
Accuracy	1.10	9470	0.3
Precision			
System Precision	1.10	9533	0.2
Method Precision	1.10	9536	0.1
Intermediate Precision	1.10	9656	0.3
Filter Study	1.10	9804	0.2
Solution Stability	1.10	9804	0.2
Robustness			
High Temperature (HT): 30°C	1.10	9807	0.2
Low Temperature (LT): 20°C	1.10	9240	0.2
High Flow rate (HF):	1.10	8944	0.8

1.1 mL/minute			
Low Flow rate (LF): 0.9 mL/minute	1.10	10345	0.4

Linearity

To create a calibration curve, we can plot the observed peak areas against the appropriate drug concentration using the recorded peak regions as a reference. Peak area and concentration have a linear relationship within the range that is being studied as shown in Figure 3 and Table 3. The analytical calibration curve is linear in the range of 35–65 µg/ml and indicates the closeness of the correlation coefficient (R^2) to 1 (0.998514). The linear regression equation is y = 11,819.422x + 4,422.213.

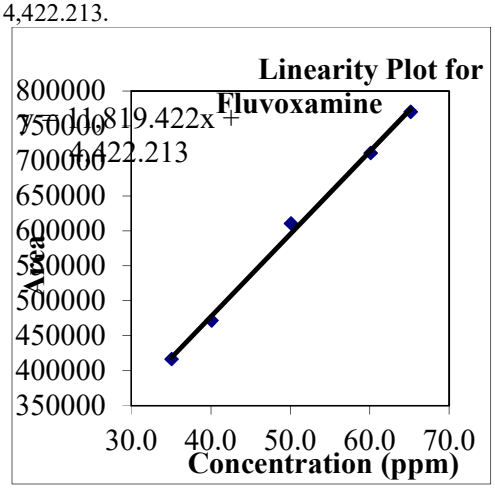

Figure 3: Linearity Curve of FVX by HPLC

Table 3: Linearity data for FVX

Level	% Concentration of Fluvoxamine w.r.t test concentration	Concentration of Fluvoxamine (in ppm)	Replicates	Peak area	Mean Peak area
1	70	35.0671	1	417650	417155
			2	417404	
			3	416410	
2	80	40.0767	1	472363	472165
			2	472465	
			3	471666	
3	100	50.0959	1	593921	611016
			2	610998	
			3	628128	
4	120	60.1151	1	711744	711624
			2	711966	
			3	711161	
5	130	65.1247	1	766689	770674
			2	772846	
			3	772486	
Correlation coefficient (R)					0.998514
Y intercept					4422.213
% Y intercept					0.724
Slope					11819.422
Residual Sum of squares					271446658.146155

Precision

Reproducibility expressed the precision under the same operating condition over a short interval of time. Method precision was carried on Felbamate Tablets 600 mg. Prepared six sample solutions and injected into the chromatograph. Statistical analysis for repeatability, intermediate precision, and reproducibility of FVX are given in Tables 4. The values of %RSD were found very well and within the 2% limit, indicating that the current method is precise and reproducible.

Table 4: Comparison between method precision and intermediate precision

Sample No.	Felbamate Tablets	
	Method Precision	Intermediate Precision
1	99.9	100.0
2	99.9	99.5
3	99.6	99.6
4	100.1	99.5
5	100.0	99.3
6	99.8	99.3
Mean	**99.9**	**99.5**
SD	**0.10**	**0.26**
% RSD	**0.1**	**0.3**
Absolute Difference	**0.4**	

Accuracy

To assess the accuracy of FVX, a standard working sample with varying concentrations (40, 50 and 60 µg/ml) was prepared and subjected to RP-HPLC spectrophotometer analysis in triplicate. The accuracy can be calculated using the percentage of the recovered analyte from a known amount added as shown in Table 5.

Table 5: Accuracy Results

Level	Concentration of Fluvoxamine w.r.t test concentration (%)	Amount of Fluvoxamine Maleate Added (mg)	Amount of Fluvoxamine Maleate recovered (mg)	% Recovery	Mean % Recovery	% RSD	Overall % recovery and overall % RSD
1	70	350.4090	352.3288	100.5	100.2	0.3	Overall % recovery = 100.2 Overall % RSD = 0.3
		351.6975	351.8077	100.0			
		351.3379	351.6525	100.1			
2	100	502.0768	506.1768	100.8	100.3	0.4	
		502.5163	502.1832	99.9			
		501.1179	502.6714	100.3			
3	130	651.9267	654.5218	100.4	100.1	0.3	
		654.9731	654.6955	100.0			
		650.1389	649.6250	99.9			

Specificity and selectivity

For FVX, at the retention time the market formulation it is 5.2 mins, the chosen chromatographic conditions are allowed. The specificity and selectivity of the procedure were assessed by contrasting the mobile phase chromatogram of a blank sample with one that had been spiked with FVX. During the retention time, no peaks were detected that could interfere with the analyte as shown in Figure 4 and Figure 5.

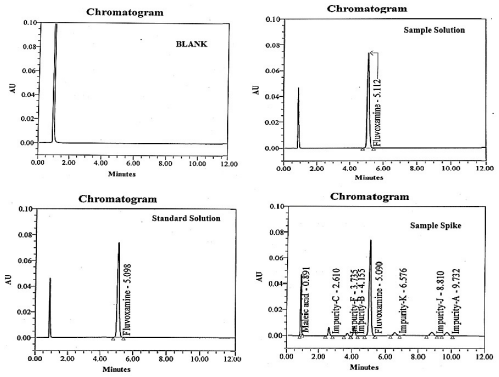

Figure 4: Chromatograms of Blank, Standard, Sample and sample spike

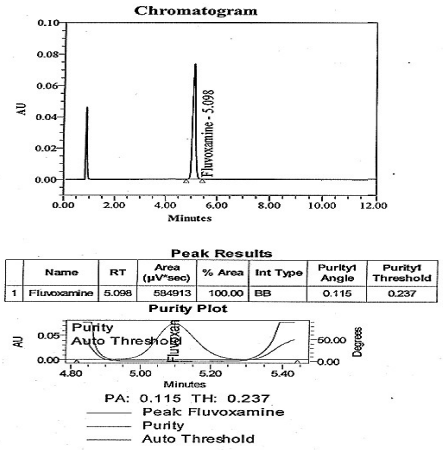

Figure 5:Peak purity of FVX for specificity study

Forced degradation study

Forced degradation studies were carried out to indicate the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions like water hydrolysis, acid hydrolysis, oxidative degradation, and thermal degradation to evaluate the ability of the proposed method to separate degradation products from active ingredients (Figure 6).

Table 6: Peak purity data for FVX in sample solution

Sample	% Assay	% Degradation of Fluvoxamine	Purity angle	Purity threshold	Peak purity
Control sample	100.3	NA	0.239	1.068	Pass

Acid degradation	88.5	11.8	0.298	1.082	Pass
Base degradation	84.5	15.8	0.338	1.089	Pass
Oxidation degradation	88.5	11.8	0.444	1.143	Pass
Hydrolysis degradation	101.3	No Degradation	0.282	1.094	Pass
Control sample-1	100.6	NA	0.293	1.132	Pass
Control sample-2	100.2		0.255	1.074	Pass
Photolytic degradation: Fluorescence	100.0	0.4	0.264	1.079	Pass
Photolytic degradation: UV	100.9	0.5	0.272	1.091	Pass
Humidity degradation	100.7	0.3	0.288	1.102	Pass
Thermal degradation	100.3	No Degradation	0.288	1.086	Pass

In forced degradation, it was observed that FVX is susceptible to degradation in acid and Base stress conditions, whereas FVX is found to be stable under all stress conditions. In every case, the peak purity was 99.96–100.00%. The results acquired from the peak purity tool confirmed that the active components' peak response was pure proving no other substances in the same retention time.

Robustness

The method's robustness was calculated by subjecting the method to a minor change in the state of the method, such as pump flow rate and pH of mobile phase composition. The ruggedness studies were determined by changing the analyst as extraneous influencing factor. The acceptance limit for calculated %RSD of peak area was less than 2. It shows that method is robust which was done by changing in Temperature and flow rate as it is shown in Table 7.

Table 7: Tailing factor, Theoretical Plate, Resolution and % RSD for robustness study

Table 13: % Assay of Felbamate in robustness study

Sr. No.	M. P.	+ Tem	- Tem	+ Flo	- Flow
1	99.9	99.2	98.9	99.4	99.2
2	99.9	98.9	98.8	98.8	99.0
3	99.9	99.1	98.6	99.2	99.4
4	100.1				
5	100.0				
6	99.8				
Mean	99.9	99.1	99.1	99.1	100.0
% RSD	0.10	98.8	98.8	98.8	0.3
Overall % RSD		1.3	99.1	99.1	99.1

Assessment of the greenness of the developed method

Assessment of the proposed method greenness was carried out using the Analytical GREenEss (AGREE) tool. The greenness profiles of the developed method and a reported method using AGREE tool were compared and the results obtained are presented in figure 6. The scores calculated for the newly developed reversed phase HPLC method and the reported method were 0.85 and 0.40, respectively. Both methods are green having a score above 0.5; an AGREE score of more than 0.7 is considered excellent for eco-friendly analysis. Although both methods have equal waste and occupational hazards scores, the proposed method has the advantage of using less and greener organic solvents compared to the reported method

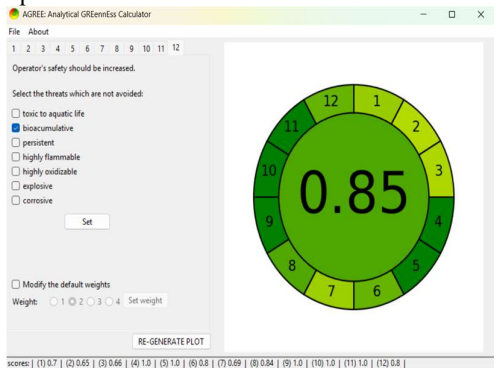

Figure 6: GREenEss (AGREE) tool

Discussion

The primary objective of this research project was to develop an HPLC technique that would be

Sr.	Paramete	(Limi	(Limit –	(Lim
1	Change in column oven temperature (± 5°C) of 30°C			
	High Temperature (HT): 35°C	1.10	9807	0.2
	Low Temperature (LT): 25°C	1.10	9240	0.4
2	Change in Flow rate (± 0.1 mL/minute) of 1 mL/minute			
	High Flow rate (HF): 1.1 mL/minute	1.10	8944	0.8
	Low Flow rate (LF): 0.9 mL/minute	1.10	10345	0.4

capable of testing FVX in a variety of dosage forms and mixtures consisting of four components and would be sensitive, accurate, and selective. In order to determine the reasons behind the satisfactory and unsatisfactory performance of high-performance liquid chromatography (HPLC) equipment, a significant amount of effort was required. The quantities of a particular component were altered while the chemical conditions were maintained at a constant level. The most effective part for the purpose of separation was determined by subjecting a number of different sections to a series of tests. The BDS Hypersil C18 (150 × 4.6 mm, 5 µm) are just a few of the options available. In comparison to C8, which showed the highest clarity and retention, C18 columns had a significant amount of peak overlap and very poor retention. In order to determine the most effective separation, a variety of mobile phases with varying compositions and ratios, as well as different washing techniques, have been experimented with and examined. Even after diluting the mixture with a new solvent and using a mobile phase composed of a combination of methanol, acetonitrile, and ethanol, the mixture was still unable to have the IBU removed. The mobile phase was purged, and isocratic elution was facilitated by the use of 30 percent ethanol. In conclusion, the Capcell Pak C8, 4.6 X 150 mm, 5 µm was employed. The flow rates that were examined were 0.8, 0.9, 1.0, and 1.2 milliliters per minute. When the flow rate was maintained at one milliliter per minute, the highest degree of separation and clarity was seen. The optimal range for ultraviolet light to detect objects was determined to be 234 nanometers due to the sensitivity of the medication being used. In order to determine the most effective environment for column separation, researchers investigated a variety of scenarios. They discovered that thirty degrees Celsius was the most temperature that they could tolerate. As shown in Figure 2, the necessary system elements for the approach were discovered, and the acceptable values were determined to fall within the specified ranges (22-25).

It was carried out on a Capcell Pak C8, 4.6 X 150 mm, 5 µm. The column featured a detection frequency of 238 nanometers and a mobile phase that consisted of 20 percent ethanol. FVX was discovered to be linear across the range of values between 100 and 300 µg/mL. The result for the linear regression of FVX was 0.998514. The length of time that FVX was discovered to remain in the brain was 5.2 minutes. The relative standard deviation, also known as the percent RSD, was determined to be 0.6 percent for the procedure precision and 0.4 percent for the intermediate accuracy of the FVX. Healing research was conducted to ensure that it was accurate. The rate of healing for FVX was shown to be somewhere in the range of 99.8 percent to 100.1 percent. When exposed to acidic conditions, fourteen percent of the FVX was shown to have degraded. The study revealed that 15.8 percent of the state was covered by basic condition. The oxidized condition was 11.8%, which was quite a shock for us to discover. The region was judged to be 0.0% hot. A humidity level of 0.3 percent was recorded (26-30).

The experts developed the Eco-Scale as a casual tool to determine the degree to which analytical methodologies are sustainable. This program takes into consideration a variety of factors, including the quantity and severity of the chemical components, the amount of energy that is used, and the amount of garbage that is generated, while it is determining the number of points that are assigned for penalties. Figure 6 demonstrates the ecologically beneficial nature of the HPLC process by using the AGREE number as a stand-in. When the score decreased, areas where the environment may potentially be harmed further were emphasized. A score, on the other hand, that is closer to one indicates that a method is more ecologically friendly. The AGREE tool, which was developed with the principles of green analytical chemistry in mind, was used to examine the portion of the process that defines the degree to which it may be considered "green." A technique or procedure is considered to be favorable for the planet and to comply with the principles of green analytical chemistry if it achieves a score of 0.85 or above. If a value is greater than 0.5, it indicates that the GAC principles are being followed with great fidelity. These concepts include the reduction of waste, the

minimization of energy use, the elimination or reduction of potentially hazardous compounds, and the enhancement of people's health and safety. This gives the impression that a firm commitment has been made to environmental responsibility and the protection of nature. The investigation came to the conclusion that HPLC technology used less energy, produced less waste, and was more environmentally friendly. Additionally, the analysis found that the manufactured stability had an AGREE score of 0.74 (31-35).

According to this study, a dependable, accurate, and consistent method for measuring FVX is discussed. Furthermore, ultraviolet analysis and high-performance liquid chromatography are two methods that use the same set of instruments in order to quantify other drugs. The return rates are rather high, and the samples are produced quickly and efficiently. This approach is particularly effective for pediatric samples since it has the capability to assess FVX in a volume of material that is around 100 to 300 microliters. Because modern technology only requires a single stage of methanol extraction, it does not need more difficult extraction techniques such as solid-phase extraction or liquid-liquid extraction. As a result, the amount of time that is required for setup is significantly decreased. It is possible that the technique will save costs since it only requires a single extraction step, a single column, and a homogeneous mobile phase. It also utilizes an internal standard, which may be purchased from retail establishments (36).

Conclusion

The proposed stability-indicating HPLC approach was shown to be accurate, sensitive, and not prohibitively expensive. In addition, a stability-indicating HPLC technique was developed and validated for FVX in a pharmaceutical dosage form. The approach that has been proposed is straightforward and user-friendly, as can be seen from the research that has previously been referenced. The conclusion was reached based on the findings and discussions that the percentage relative standard deviation (%RSD) value was less than 2%, which is within the acceptable limit established by the International Council for Harmonization (ICH). It was shown that the recommended chromatographic procedures were effective for the detection of FVX in dosage forms, laboratory-prepared mixtures, and stability degradants. They did not experience any interference, and they were both quite sensitive and precise. The description of the greenness was also substantiated by the use of a wide variety of distinct green grading techniques. There were a few of them: the analytical eco-scale, the analytical greenness measures, the National Environmental Method Index, and the green analytical process index. As a consequence, the recommended approach might be utilized to conduct routine checks of the medications that are specified in quality control laboratories. It is essential to keep in mind that this approach does not do any harm to the environment, making it a versatile option for testing FVX medicines. **[33]**.

Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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