Research Paper

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

DEVELOPMENT OF ECO-FRIENDLY SENSITIVE HPLC METHOD FOR DETERMINATION OF FELBAMATE AND ASSESSMENT OF VALIDATION

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ABSTRACT

Epilepsy partial seizures are treated with felbamate. Experiments were conducted on a 150 mm x 4.6 mm BDS Hypersil C18 column with 5 µm particle size. An isocratic elution was performed using a 20:80 v/v ethanol-water mobile phase. Study wavelength was 210.0 nm. In 8.0 minutes, an experiment was finished. Calibration curves for each medicine were linear between 100-300 µg/mL. Validation experiments followed International Conference on Harmonization criteria. The assessment's green criteria confirmed the approach's solvent, chemical, energy, and waste sustainability. These reliable and ecologically friendly methods for quantitative FBM assessment in tablet formulations promote safer and greener pharmaceutical analysis lab practices.

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

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Introduction

Two key components must be addressed while developing a new analytical method for analyte detection. The method's metrological value must be verified for accuracy, precision, and repeatability. Following green chemistry principles, cleaner, less harmful chemicals and processes should be used to address environmental sustainability. Despite their importance, these principles are neglected in pharmacological analysis (1, 2). Due of its reliability, High-Performance Liquid Chromatography (HPLC) is essential in pharmaceutical quality control. Traditionally, reverse-phase HPLC uses polar mobile and hydrophobic stationary phases. The mobile phase is usually water with additions and organic solvents like ethanol. Mobile phases in reverse phase separation may be water and aqueous buffers, depending on analyte solubility. However, using only water and aqueous buffers in the mobile phase may prolong analysis. For better separation, mobile phases containing aqueous buffers and organic modifiers are preferred. These organic solvents are used for their water miscibility, low viscosity, and chemical stability, which provide excellent analytical performance. Despite these benefits, acetonitrile and ethanol pose environmental and health risks. HPLC waste from these solvents has caused environmental and disposal difficulties (3-5). Felbamate treats epilepsy. It is used to treat adult partial seizures, regardless of generalization. It replaces Lennox-Gastaut syndrome therapy for partial and generalized seizures. It slightly inhibits GABA receptor binding. Epilepsy partial seizures are treated with felbamate. It works alone or with additional anticonvulsants. Felbamate has no effect at the MK-801 receptor binding area of the NMDA receptor-ionophore complex in vitro. It also has minimal effect on benzodiazepine-GABA receptor binding. Unaffected by strychnine, felbamate blocks the NMDA receptor-ionophore complex's glycine recognition site (6, 7).

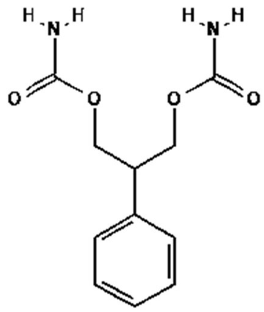

Fig. 1 Chemical structure of Felbamate

This study creates a green liquid chromatography (Green LC) method using ethanol as the mobile phase solvent to meet the growing need for environmentally friendly analytical methods. Ethanol is ideal for sustainable analytical methods due to its environmental benefits and chromatographic efficiency (8, 9). The literature describes spectrofluorometric, spectrophotometric, and liquid chromatography techniques for measuring FBM in pharmaceutical and biological products (10, 11). Biological fluids, bulk compounds, and dosage forms can be measured for FBM using various analytical methods. The liquid chromatographic-tandem mass spectrometry method quantifies FBM in human plasma (12-15); the RP-HPLC/UV method quantifies FBM in rat plasma and is used in a pharmacokinetic study in rats (16, 17); the LC-MS/MS-ESI method quantifies FBM in rat and human plasma and is used in a pharmacokinetic study (18-20); and liquid chromatography combined with ultraviolet detection and electron ionization mass spectrometry analyses FBM These methods work, but they need expensive equipment, dangerous chemicals, and complicated procedures, limiting their accessibility and environmental impact. Thus, sustainable FBM analysis methods are critical and necessary. An eco-friendly green liquid chromatography technology using ethanol and a Simplified Spectrophotometric technology using ultrapure water are presented in this paper. These methods reduce the environmental effect of HPLC while maintaining analytical performance.

Materials and Methods

Materials:

All chemicals and reagents utilized in this investigation were of analytical grade. API FEB was received as a gift sample from Glenmark Pharmaceuticals R&D, together with HPLC-grade acetonitrile from Thermo Fisher Scientific India Pvt Ltd, Mumbai. Felbamate Tablets (600 mg) were obtained from a nearby pharmacy. HPLC-grade solvents, including Methanol, Ethanol, and Acetonitrile, were obtained from Thermo Fisher Scientific India Pvt Ltd in Mumbai.

Method Development

Chromatographic conditions

Methods for the chromatographic experiment are described. Empower 3 software was used to analyse chromatographic data for retention lengths, peak areas, and resolution metrics. The separation process used a 150 mm x 4.6 mm BDS Hypersil C18 column with a particle size of 5 µm, a 20:80 ethanol-water mobile phase, and a flow rate of 1.0 ml/min. The stationary phase-analyte interaction depends on pore size. The 150 Å pore size allows FBM to efficiently interact with the stationary phase surface area. Eluent and run time were measured at 210 nm for 10 minutes. The 210 nm wavelength is likely chosen because it is near the FBM absorption maxima. In UV–visible spectroscopy, each molecule has a unique absorption spectrum that shows its maximum light absorption wavelengths. Before the experiment, the mobile phase circulated through the column for at least 30 minutes to equilibrate it. A 10 µl injection volume was used for sample analysis. Liquid chromatography separates sample components by moving the mobile phase through the column. This underpins the experiment. C18 is a famous reversed-phase column. Table 1 lists chromatographic conditions (21-24).

Preparation of reference standard solution

Accurately weigh about 50 mg of Felbamate working standard and place it into a 25 mL volumetric flask. Add 15 mL of diluent, sonicate to dissolve, and dilute with diluent to the mark, then mix thoroughly. Transfer 5 mL of Felbamate stock solution into a 50 mL volumetric flask, dilute to volume with the mobile phase, and mix thoroughly.

Preparation of Sample Solution:

Ground and pulverized 20 pills into a fine powder. Precisely weighed and put crushed powder amounting to 1000 mg of Felbamate into a 500 mL volumetric flask. Introduce 300 mL of diluent and sonicate for 30 minutes, incorporating occasional agitation. Permitted to cool to ambient temperature, then diluted with diluent to the desired amount and mixed thoroughly. The solution was filtered using a 0.45 µm PTFE syringe filter, and the initial few millilitres of filtrate were discarded. Transfer 5 mL of filtrate to a 50 mL volumetric flask, dilute with the mobile phase to the mark, and mix well.

Table 1 Chromatographic condition optimization

Instrument	HPLC (Waters 2489)
Flow Rate	1 ml/min.
Column	C18, 150 mm X 4.6 mm, 5 µm (BDS Hypersil is suitable)
Mobile Phase	Ethanol and water (20:80)
Detector, Wavelength	UV, 210 nm
Run Time	10 min
Volume of Injection	10 µl
Temperature of Column	Ambient Temperature

Diluents	Ethanol and water (20:80)
Mode of Separation	Isocratic Mode

Experiments employing trial and error were conducted utilizing various solvents, columns, diluents in diverse ratios, and flow rates to get the sharpest peak with little retention time.

Proposed technique validation

Method validation is recorded proof that offers a high level of confidence that the procedure employed to verify the analytical method is appropriate for its intended application. The established HPLC technique for the quantification of FBM was verified in accordance with ICH Q2 (R1) recommendations (25-28).

System suitability studies

The system appropriateness was assessed using six duplicate studies of FBM. The retention duration, column efficiency, peak asymmetry, and theoretical plates were computed for standard solutions.

Linearity

The linearity of FBM was assessed by analysing five separate concentration levels ranging from 100 to 300 µg/ml. The calibration curve was generated by graphing peak area on the y-axis against concentration on the x-axis. The equation of the regression line and the values of the correlation coefficient were established.

Precision

Analytical technique accuracy is the agreement between measurements from several homogenous sample samplings under defined conditions. The test approach involved injecting six standard solution duplicates from the same HPLC vial to assess system precision. Reproducibility is short-term consistency under similar operating conditions. Method accuracy was verified on 600-mg Felbamate Tablets. Six sample solutions entered the chromatograph. For repeatability, analysts utilizing various HPLC systems, columns, and samples from the same batch find intermediate precision on different days. Intermediate precision was employed on USP 600 mg Felbamate Tablets. Six sample solutions entered the chromatograph. Determined intermediate precision and process precision assay %, average, and relative standard deviation (29-31).

Accuracy

The method's accuracy was assessed using a recovery study of the marketed formulation at three levels: 50%, 100%, and 150% of standard addition. The percentage recovery of FBM was computed. The acceptability threshold for percentage recovery according to ICH criteria is 98–102% of the standard addition.

Specificity

The method's specificity has been determined by comparing the chromatograms derived from the injection of a blank, a solution of FBM (10 µg/ml), and the sample solution of FBM Tables. The impact of excipients utilized in the film was evaluated. The parameters of retention duration, tailing factor, and resolution were computed to demonstrate the specificity of the selected approach.

Forced degradation study

Acid degradation:

To measure acid degradation, weigh 1576.57 mg of powdered tablets and 1001.19 mg of API into two 500 mL volumetric flasks. Add 300 mL of diluent and sonicate for 30 minutes with occasional agitation. After cooling, 20 mL 5N hydrochloric acid was added. Cooled and neutralized with sodium hydroxide at the same volume and concentration. Let cool to room temperature, then diluted with diluent to desired quantity and blended well. Used a 0.45 µm PTFE syringe filter to filter the solution, discarding the first few millilitres. Put 5 mL of filtrate in a 50 mL volumetric flask, diluted with mobile phase, and mixed well (32).

Base degradation:

The base degradation process involved weighing 1577.78 mg of powdered tablets (1000 mg felbamate) and 1001.58 mg of felbamate API into two 500 mL volumetric flasks. After adding 300 mL of diluent, sonicated for 30 minutes with frequent agitation. After cooling, 20 mL 5N sodium hydroxide was added. Cooled and neutralized with same hydrochloric acid volume and concentration. Let cool to room temperature, then diluted with diluent to desired quantity and blended well. Used a 0.45 µm PTFE syringe filter to filter the solution, discarding the first few millilitres. Put 5 mL of filtrate in a 50 mL volumetric flask, diluted with mobile phase, and mixed well (33).

Oxidation degradation:

Weighed 1576.28 mg of powdered tablets and 1001.31 mg of API into two 500 mL volumetric flasks. Added 300 mL of diluent and sonicated for 30 minutes with frequent agitation. After cooling, 50 mL 30% hydrogen peroxide was added. The flask was kept in 80°C water for three hours. Let cool to room temperature, then diluted with diluent to desired quantity and blended well. Used a 0.45 µm PTFE syringe filter to filter the solution, discarding the first few millilitres. Put 5 mL of filtrate in a 50 mL volumetric flask, diluted with mobile phase, and mixed well (34).

Hydrolysis degradation:

To evaluate the hydrolysis degradation of Felbamate, 1577.45 mg of powdered tablets and 1001.47 mg of API were measured into two 500 mL volumetric flasks. Add 300 mL of diluent and sonicate for 30 minutes with occasional agitation. After cooling, 50 mL water was added. The flask was kept in 80°C water for three hours. Let equilibrate at room temperature, then diluted with a suitable diluent to the appropriate volume and combined. To filter the solution, a 0.45 µm PTFE syringe filter was used, discarding the first few millilitres. Put 5 mL of filtrate in a 50 mL volumetric flask, diluted with mobile phase, and mixed well (35).

Photo degradation: Fluorescence

Put 50 Felbamate tablets and 3000 mg of API on two petri plates in the photo stability chamber for 1.2 million lux hours of degradation. Measured 1576.17 mg of powdered tablets (1000 mg Felbamate) and 1000.38 mg of API into two 500 mL volumetric flasks. Add 300 mL of diluent and sonicate for 30 minutes with occasional agitation. Let cool to room temperature, then diluted and blended with a diluent. Used a 0.45 µm PTFE syringe filter to filter the solution, discarding the first few millilitres. Put 5 mL of filtrate in a 50 mL volumetric flask, diluted with mobile phase, and mixed well (36).

Photo degradation: UV

For degradation testing, put 50 Felbamate tablets and 3000 mg of API on two petri plates in the photo stability chamber at 200 watt-hours per square meter. Measured 1576.00 mg of powdered tablets and 1000.98 mg of API into two 500 mL volumetric flasks. Add 300 mL of diluent and sonicate for 30 minutes with occasional agitation. After cooling, diluted with a diluent to the required amount and properly mixed. Used a 0.45 µm PTFE syringe filter to filter the solution, discarding the first few millilitres. Put 5 mL of filtrate in a 50 mL volumetric flask, diluted with mobile phase, and mixed well (37).

Humidity degradation:

To test for humidity degradation, 50 Felbamate tablets and 3000 mg of API were placed in two petri dishes and held at 25°C and 90% relative humidity for 24 hours. Measured 1575.43 mg of powdered tablets and 1000.19 mg of API into two 500 mL volumetric flasks. Add 300 mL of diluent and sonicate for 30 minutes with occasional agitation. After cooling, diluted with a diluent to the required amount and properly mixed. Used a 0.45 µm PTFE syringe filter to filter the solution, discarding the first few millilitres. Put 5 mL of filtrate in a 50 mL volumetric flask, diluted with mobile phase, and mixed well (35, 36).

Thermal degradation:

Achieved thermal degradation by incubating 50 Felbamate tablets and 3000 mg of API on two petri plates at 60°C for 24 hours. Measured 576.06 mg placebo powder, 1575.93 mg powdered tablets, and 1000.35 mg API into two 500 mL volumetric flasks. Add 300 mL of diluent and sonicate for 30 minutes with occasional agitation. Let cool to room temperature, then diluted with diluent to desired quantity and blended well. Used a 0.45 µm PTFE syringe filter to filter the solution, discarding the first few millilitres. Put 5 mL of filtrate in a 50 mL volumetric flask, diluted with mobile phase, and mixed well.

Robustness studies

To evaluate the robustness of the HPLC process, minor intentional alterations in the detection wavelength and flow rate were implemented. The detecting wavelength was established at 210 nm, and resilience was assessed by altering the temperature by ±5 nm. The flow rate was established at 1.0 mL/min, with intentional minor fluctuations of ±0.2 mL/min implemented.

Assessment of the greenness of the developed method

The evaluation of the suggested method's sustainability was conducted utilizing the Analytical GREenEss (AGREE) tool (37).

Result

Method Development

In the first mobile phase of 100% Methanol and 100% Acetonitrile, a split peak and distorted peak of FBM were identified at Rt 3.2. The next mobile phase was 20:80 ethanol-water. Fig. 2 shows how changing the column improved peak shape and symmetry. The system suitability test parameters were fulfilled under optimal chromatographic conditions. The optimized mobile phase uses a 150 mm x 4.6 mm BDS Hypersil C18 column with a particle size of 5 µm and a 20:80 ethanol-water ratio. Being a polar aprotic solvent, ethanol and water (20:80) dissolved lipophilic compounds like FBM well. Compatible with HPLC and 210 nm UV detectors, it minimizes interference. It separates with high peak resolution and repeatability. Its low viscosity makes high-pressure HPLC handling easier.

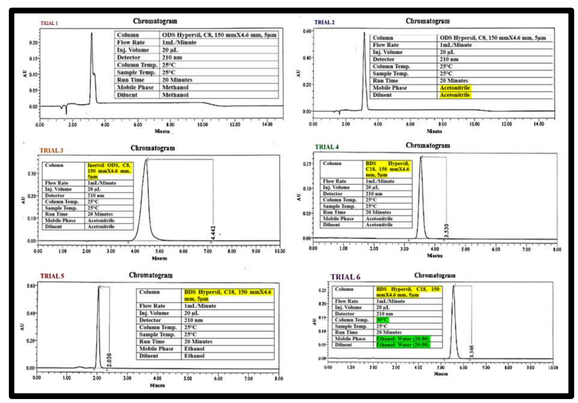

Fig. 1 HPLC Method Development Chromatograms

Validation Results:

System suitability studies

A system appropriateness test is a crucial initial step in verifying that the chromatographic system is appropriate for the intended analysis. The primary aim of this test is to repeatedly introduce the sample and evaluate many parameters, including theoretical plate height, peak area, tailing factor, and plate area. All of these features must comply with the requirements set out by the United States Pharmacopoeia (USP). The test results demonstrate that the system is suitable for analysis, since the RSD values remained under the commonly recognized upper range of 2%, as outlined in Table 2.

Table 2 System suitability data of FBM.

Para mete r	U S P	USP The oreti	Res olut ion	% R S

	(Lim	(Limit =	(Limit =	(Li
Specificity				
Blank, Plac	1.0	6721	7.15	0.3
Forced	1.0	6211	7.22	0.2
Forced	1.0	6142	7.14	0.3
Forced	1.0	6172	7.08	0.2
Forced	1.0	5846	7.02	0.3
Linearity	1.0	6248	7.22	0.1
Accuracy	1.0	6326	7.28	0.3
System	1.0	6215	7.22	0.4
Method	1.0	6215	7.22	0.4
Intermedi	1.1	4629	6.36	0.2
Filter	1.0	6353	7.30	0.2
Solution	1.0	6391	7.32	0.1
High Tem	1.0	5862	6.55	0.1
Low Tem	1.0	6370	7.90	0.1
High Flow	1.0	5812	6.94	0.3
Low Flow	1.0	6370	7.54	0.1

Linearity

To construct a calibration curve, we may graph the observed peak areas against the corresponding drug concentrations, utilizing the recorded peak regions as a reference. The peak size and concentration exhibit a linear correlation within the study range, as seen in Fig. 3 and Table 3. The analytical calibration curve exhibits linearity within the range of 100–300 µg/ml, with a correlation coefficient (R2) of 0.999, which is close to 1. The linear regression equation is y = 1793x + 87260.

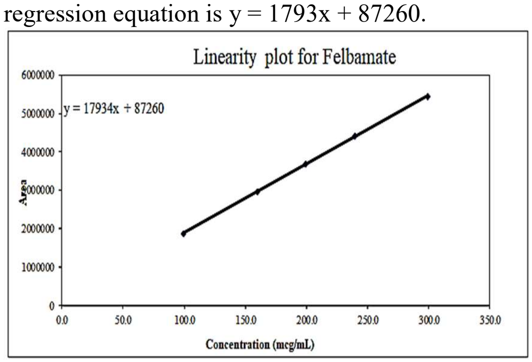

Fig. 2 Linearity Curve of FBM by HPLC

Table 3 Linearity data for FBM

	% concentration of Felbamate w.r.t standard concentration	**Concentration of Felbamate (in ppm)**	**Replicates**	**Area**	**Mean Area**
	50	99.7	1	18	1858879
			2	18	
			3	18	
	80	159.6	1	20	2962740
			2	20	
			3	20	
	100	199.5	1	36	3673008
			2	36	
			3	36	
	120	239.4	1	42	4391259
			2	42	
			3	42	
	150	299.2	1	54	5437728
			2	54	
			3	54	
Correlation coefficient (R)					0.99994
Y- Intercept					87260.3
Slope					17933.9
Residual Sum of squares					855271
% Y Intercept					2.38

Precision

Reproducibility denotes the precision achieved under identical operating conditions within a brief time frame. The accuracy of the method was conducted on Felbamate Tablets, 600 mg. Prepared six sample solutions and inserted them into the chromatograph. The statistical analysis for repeatability, intermediate precision, and reproducibility of FBM is presented in Table 4. The %RSD readings were determined to be satisfactory and within the 2% threshold, demonstrating that the present approach is exact and reproducible.

Table 4 Comparison between method precision and intermediate precision

Sample No.	Felbamate Tablets	
	Method Precision	Intermediate Precision
1	99.8	99.0
2	101.1	98.7
3	100.7	98.6
4	100.3	99.6
5	99.7	98.5
6	99.6	98.7
Mean	**100.2**	**98.9**
SD	**0.61**	**0.40**
% RSD	**0.6**	**0.4**
Absolute Difference	**1.3**	

Accuracy

To evaluate the precision of FBM, a standard working sample with differing concentrations (100, 200, and 300 µg/ml) was generated and analysed using RP-HPLC spectrophotometry in triplicate. The accuracy may be determined by calculating the percentage of the recovered analyte from a known quantity added, as illustrated in Table 5.

Table 5 Accuracy Results

	% concentration of Felbamate w.r.t. sample concentration	Amount of Felbamate Added (mg)	Amount of Felbamate recovered (mg)	% Recovery	Mean Recovery	[illegible]	Overall % recovery and overall % RSD
	50	99	10	10	100.9	[illegible]	
		99	10	10			
		99	10	10			
	100	19	19	10	100.4	[illegible]	
		19	19	10			
		19	19	10			
	150	29	29	99	99.8	[illegible]	
		29	29	10			
		29	29	99			

Specificity and selectivity

For FBM, the market formulation retention time is 5.2 minutes, and the selected chromatographic conditions are permissible. The procedure's specificity and selectivity were evaluated by comparing the mobile phase chromatogram of a blank sample to that of a sample spiked with FBM. No peaks that may interfere with the analyte were observed during the retention time, as seen in Fig. 4 and 5.

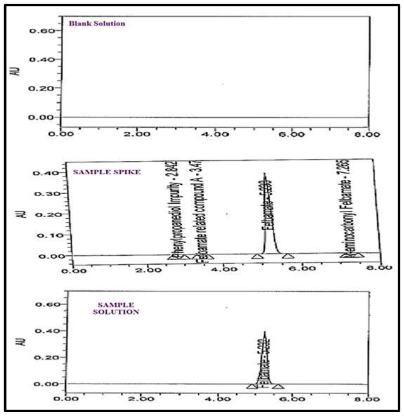

Fig. 3 Chromatograms of Blank, Sample and sample spike

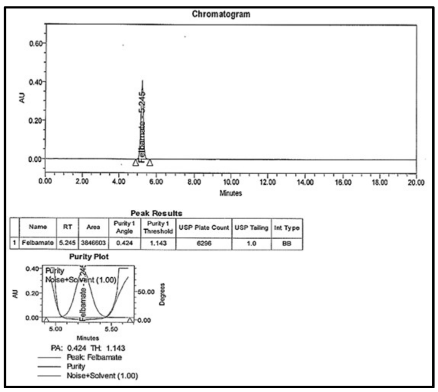

Fig. 4 Peak purity of FBM for specificity study

Forced degradation study

Forced degradation tests were conducted to demonstrate the stability-indicating capability and specificity of the suggested approach. Intentional degradation was conducted to simulate circumstances such as water hydrolysis, acid hydrolysis, oxidative degradation, and thermal degradation, in order to assess the efficacy of the proposed technique in isolating breakdown products from active components (Table 6).

Table 6 Peak purity data for Felbamate in sample solution and in API solution

Sample	% Assay	% Degradation of Felbamate	Purity angle	Purity threshold	Peak purity
Felbamate Tablets, USP 600 mg					
Co	9	NA	0	1.	P
Ph	1	No	0	1.	P
Ph	1	No	0	1.	P
Th	9	0.9	0	1.	P
H	9	0.7	0	1.	P
Co	9	NA	0	1.	P
O	1	No	0	1.	P
H	9	No	0	1.	P
Co	9	NA	0	1.	P
Ac	8	14.	0	1.	P
Ba	8	13.	0	1.	P
Felbamate API					
Co	9	NA	0	1.	P
Ph	1	No	0	1.	P
Ph	1	No	0	1.	P
Th	9	1.5	0	1.	P
H	9	1.9	0	1.	P
Co	9	NA	0	1.	P
O	9	0.1	0	1.	P
Co	9	NA	0	1.	P
H	9	0.1	0	1.	P
Co	1	NA	0	1.	P
Ac	8	14.	0	1.	P
Ba	8	16.	0	1.	P

In forced deterioration, it was noted that FBM is prone to degradation under acidic and basic stress conditions, whereas FBM demonstrates stability across all stress settings. The peak purity ranged from 99.96% to 100.00% in all instances. The data obtained from the peak purity instrument validated that the peak response of the active components was pure, indicating the absence of other compounds at the same retention time.

Robustness

The method's robustness was assessed by introducing a little alteration in the method's conditions, including pump flow rate and pH of the mobile phase composition. The toughness studies were conducted by altering the analyst as an external influencing element. The acceptable threshold for the estimated %RSD of peak area was below 2. The approach demonstrates robustness, as seen by the variations in temperature and flow rate presented in Table 7.

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

Table 8 % Assay of Felbamate in robustness study

Sr.	M.	+T	-T	+F	-F
1	9 9.	1 0	9 9.	9 9.	1 0
2	1 0	1 0	9 9.	1 0	9 9.

Sr. No.	Parameter	Tail (Lim	Theoretical (Limit = NL	Resolution (Limit = N	%RSD (Lim
1	Change in column oven temperature (± 5°C) of 30°C				
	High Temperature (HT): 35°C	1.0	5862	6.55	0.1
	Low Temperature (LT): 25°C	1.0	6370	7.90	0.1
2	Change in Flow rate (± 0.1 mL/minute) of 1 mL/minute				
	High Flow rate (HF): 1.1 mL/minute	1.0	5812	6.94	0.3
	Low Flow rate (LF): 0.9 mL/minute	1.0	6781	7.54	0.2

3	10	10	99.	10	99.
4	10				
5	99.				
6	99.				
Me	10	10	99.	10	10
%R	0.6	1.9	0.2	1.3	0.3
Overall % RSD		1.3	0.7	0.8	0.5

Assessment of the greenness of the developed method

The evaluation of the suggested method's sustainability was conducted utilizing the Analytical GREenEss (AGREE) tool. The greenness profiles of the created approach and a previously described method utilizing the AGREE tool were compared, with the results illustrated in Fig. 6. The scores obtained for the newly developed reversed phase HPLC technique and the previously published approach were 0.74 and 0.51, respectively. Both approaches are environmentally sustainable, with scores over 0.5; an AGREE score above 0.7 is deemed exceptional for ecological assessment. Despite both techniques exhibiting identical waste and occupational hazard scores, the suggested approach benefits from utilizing less and more environmentally friendly organic solvents in comparison to the published method.

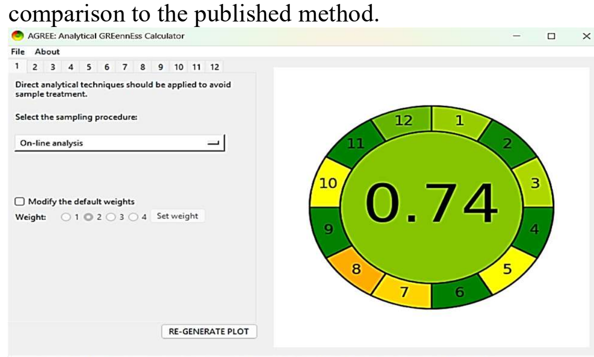

Fig. 5 GREenEss (AGREE) tool

Discussion

The primary objective of this research project was to develop an HPLC technique that would be capable of testing FBM 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 ODS Hypersil C8 (150 × 4.6 mm, 5 µm), the Inertsil ODS C8 (150 × 4.6 mm, 5 µm), and the BDS Hypersil C18 (150 × 4.6 mm, 5 µm) are just a few of the options available. In comparison to C18, which showed the highest clarity and retention, C8 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 20 percent ethanol. In conclusion, the BDS Hypersil C18 (150 × 4.6 millimeters, 5 micrometers) 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 210 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 BDS Hypersil C18 column that measured 150 × 4.6 millimeters and had a thickness of 5 µm. The column featured a detection frequency of 210 nanometers and a mobile phase that consisted of 20 percent ethanol. FBM was discovered to be linear across the range of values between 100 and 300 µg/mL. The result for the linear regression of FBM was 0.999. The length of time that FBM 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 FBM. Healing research was conducted to ensure that it was accurate. The rate of healing for FBM was shown to be somewhere in the range of 99.8 percent to 100.9 percent. When exposed to acidic conditions, fourteen percent of the FBM was shown to have degraded. The study revealed that 13.8 percent of the state was covered by acidic terrain. The oxidized condition was 0.1%, which was quite a shock for us to discover. The region was judged to be 1.5% hot. A humidity level of 1.9 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 0.61 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 FBM 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 FBM 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 (38).

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 FBM 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 FBM 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 FBM medicines.

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