RESEARCH ARTICLE

Development of Validated Stability Indicating HPTLC Method for Estimation of Febuxostat in Bulk and Tablet Dosage Form by Using QBD Approach

Vrushali Kharate[1], Mohini Kuchekar[1*], Minal Harde[1], Bhushan Pimple[1], Vinita Patole[2], Manisha Salunkhe[1], Pooja Wadgave[1], Manish Bhise[3], Asmita Gaikwad[4], Harshal Tare[4]

[1]*Progressive Education Society's Modern College of Pharmacy, Savitribai Phule Pune University, Pune, Maharashtra, India.*
[2]*Dr. D. Y. Patil Vidya Pratishthan Society's Padmashree Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Savitribai Phule Pune University, Pune, Maharashtra, India.*
[3]*Shri Gurudatta Shikshan Prasarak Sanstha's Institute of Pharmacy, Akola, Sant Gadge Baba Amravati University, Maharashtra, India.*
[4]*Shri Gajanan Maharaj Shikshan Prasarak Mandal's Sharadchandra Pawar College of Pharmacy, Otur, Savitribai Phule Pune University, Pune, Maharashtra, India.*

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ABSTRACT

Objective: Development of a simple, reliable, time-tested, stability-focused HPTLC method for determining febuxostat (FEB) in bulk and pharmaceutical formulation.

Methods: Chloroform, methanol, and formic acid (6.7:2.9:0.1v/v/v) served as the mobile phase, and precoated aluminum TLC plates served as the stationary phase. Active drug FEB was subjected to a densitometric measurement (absorbance mode at 312 nm). The present study performed a comprehensive stress test of FEBas per ICH Q1A(R2) guidelines. FEB has undergone forced degradation by acid, alkali (0.5N HCl and NaOH), oxidative (3% H_2O_2), H_2O and photolytic effect.

Results: Calibration plots using HPTLC linear regression analysis indicated a strong correlation (r^2 = 0.999) between concentrations from 150 to 900 ng/spot. Accuracy, recoverability, robustness, and documentation are some of the aspects of the method that have been verified. The LoD was 189 ng and the LoQ was 395 ng per location. FEB was not degraded under acid, UV, water, peroxide and photolytic conditions but showed degradation under alkaline conditions.

Conclusion: Stability studies and quantification of FEB in bulk and pharmaceutical dose forms by QBD methodology have both been shown to benefit from the suggested HPTLC method. This technique can be utilized as a stability indicator because it successfully isolates the medication from the degradation product.

Keywords: QBD approach, Febuxostat, HPTLC method, Degradation study.

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INTRODUCTION

Febuxostat (FEB), a specific non-purine xanthine oxidase inhibitor, has been shown to be more efficacious than allopurinol 300 mg/day in the therapy of hyperuricemia in patients with chronic gout.[1] Gout is brought on by hyperuricemia, an unusually high quantity of uric acid in blood, which is also linked to arthritis and nodules.[2] FEB-(2-[3-cyano-4- (2-methylpropoxy) phenyl]-4-methyl-thiazole-5-carboxylic acid given in Figure 1.[3] FEB is It is estimated that about 1–6% of a single dose of xanthine oxidase is eliminated unaltered by the kidneys. As a result, changes in FEB pharmacokinetic profile are relatively unaffected by GFR reduction.[4] In 2011, it became the first treatment to reduce urine levels in treatment of hyperuricemia. The American College of Rheumatology (ACR) recommends xanthine oxidase inhibitors like allopurinol or FEB as the initial urate-lowering treatment for gout patients.[5] XO activation, endothelial dysfunction, and/or tubular injury have all been linked to hyperuricemia, which plays a role in the etiology of chronic kidney disease.[6] Myocardial energy competence, left ventricular ejection

**Author for Correspondence: mohini.kuchekar@gmail.com*

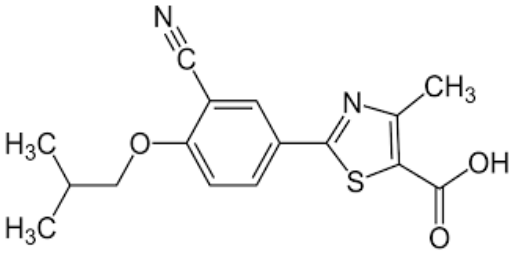

Figure 1: Chemical structure of febuxostat

fraction (LVEF), and plasma B-type natriuretic peptide (BNP) in patients with heart failure are all improved by the use of XO inhibitors, which are utilized therapeutically for indications of hyperuricemia.[7]

HPTLC is an advanced form of planar chromatography that has found widespread use in recent years for a variety of applications, including the detection of adulterants in formulation, the testing of goods and lichen substances, analysis of medicinal plants, including their identification, phytochemical analysis, and medical benefits. Using a low solvent volume allows for increased sensitivity and rapid throughput of several samples. This complicated instrumental method fully exploits the advantages of thin-layer chromatography.[8] This work aims to develop a responsive, specific, repeatable, and stability-indicating technique for detecting FEB in its presence of its degradation products using a QBD strategy, per ICH guidelines.

Analytical Quality by Design (AQbD) refers to applying the Quality by Design (QbD) philosophy to developing analytical techniques. It states that quality should be incorporated into design of the process rather than being tested based on the analytical process's final results.[9] The quality target method profile (QTMP) is defined as the first step in this procedure. "ATP" stands for analytical target profile. An expression that describes the method is QTMP or ATP. Aiming towards a certain result guides decision-making during a project's research and development phases.[10] Following the QTMP definition can help you identify the CAA. In the realm of product creation, CAAs are analogous to critical quality attributes (CQA). Limits, ranges, or distributions on CQAs (which ICH Q8 (R2) classifies as chemical, physical, biological, or microbiological properties) are necessary for ensuring the desired product quality. Ishikawa fishbone diagrams can be applied in a variety of situations identification and evaluation of risks.[11]

FEB can be analyzed using several ways, including simultaneous estimation using spectrophotometry, HPLC, HPLC using a diode array detector, HPLC mass spectrometry, UV HPLC, HPTLC, Micellar electrokinetic Chromatography, UPLC, TMS, UHPLC and LC-ESI-MS/MS.[12-32] Multiple HPLC, UPLC, and Capillary electrophoresis methods that can be used to determine FEB's stability have been published, either alone or in combination with other medications. In all of the above; HPTLC methods, it was found that the Rf values varied a lot and a specific degradation condition of photolytic; water; oxidative stress; acid and alkaline environment had not been studied. The goals of stability testing are to ascertain the appropriate storage conditions and time between retesting a drug substance. It also seeks to provide information on belongings of environmental factors like humidity, temperature and light on potency of a drug or drug product over time.[33] We were unable to find any published work that used design of experiments (DoE) technique to develop an AQbD-based TLC-densitometric strategy for quantitatively measuring FEB in commercially available formulations and, perhaps, resolving degradation products obtained in stress degradation investigations. Therefore, the current study represented an effective effort to establish an AQbD-based HPTLC approach for quantitative detection of FEB in commercial formulations using the Design of Experiments (DoE).

MATERIAL AND METHODS

Materials

FEB was obtained asa free sample from Mumbai;Cipla Pharmaceuticals, Pvt Ltd. All chemicals and reagents; including sodium hydroxide pellets, conc. hydrochloric acid, chloroform, methanol and formic acid are of analytical grade.

Method Development

Chromatographic parameters of HPTLC method were optimized in current study using the Box-Behnken design because it allows for parameter changes; alterations can be made to the experiment at any time. This work aimed to develop and verify HPTLC-based quality by design approach. Table 1 displays the software's suggested low, medium and high levels for the factors considered. The impact of the study factors was examined in an experimental design run concerning the CAAs.[34,35]

Instrumentation

Camag Linomat V was used to highlight the samples in 8 mm broad bands on precoated silica gel aluminum Plate 60 F-254 (20 cm x 10 cm, 0.2 mm thickness; E. Merck; Germany).

Both bands were spaced at 11.4 mm from one another, and the application rate was held constant at 150 nl/s. Scanning speed was kept at 5 mm/s, and slit size was maintained at 6 to 0.45 mm. The mobile phase was a mixture of chloroform, methanol, and formic acid (6.7:2.9:0.1 v/v). A glass chamber with twin troughs saturated with mobile phase linear ascending development was conducted. At room temperature, 20 minutes was the ideal chamber saturation period for mobile phase. The run of the chromatogram was around 70 mm long. TLC plate was developed and then air-dried afterward. The Camag TLC scanner III was used to do density scanning at 312 nm absorbance. The radiation source was a deuterium lamp.

Table 1: Experimental factors and levels used in experimental design

Factor	*Level(-1)*	*Level(+1)*
Band Length	7	9
Saturation Time	10	30
Solvent Front	60	80

Preparation of Solutions

Stock solution

A total of 10 mg of FEB was carefully weighed, then transferred to a 10 mL volumetric flask. To achieve a 1000 µg/mL concentration, methanol was employed as the solvent of choice. Further dilutions yielded a concentration of 100 µg/mL.

Mobile phase

A total of 6.7 mL of chloroform and 2.9 mL of methanol were mixed thoroughly and 0.1 mL formic acid was added to give chloroform: methanol: formic acid ratio of 6.7:2.9:0.1 v/v/v. A volume of 20 mL of the solution prepared above was used for each chromatography step. Using densitometric detection, the HPTLC-based approach is precise, accurate and specific for detecting deterioration components.

Analysis of marketed formulations

The marketed product Febutax 40 contained in FEB was carefully evaluated using the HPTLC technology. The 20 commercially available dose units were weighed in a balance before being finely crushed. A volumetric flask was filled with an equivalent dose (around 40 mg) (100 mL volume). A 50 mL amount of methanol was added; sonicated for 30 minutes and then diluted with the remaining substance. Centrifugation was carried out for 5 minutes and the supernatant was used as a source to calculate the amount of medication present. 20 mL of the volumetric flask was filled with 1-mL of above-mentioned material and final strength of 100 g/mL was obtained by appropriately diluting it with methanol. To achieve the desired concentration of 100 ng/spot FEB was spotted 3 times at a concentration of 1-µL and 3 times at a concentration of 1.5 µL using the developed chromatographic conditions. The HPTLC plate was eluted. Additionally, the potential for excipient intervention during the HPTLC analysis was looked into. The detecting wavelength used to calculate the spot peak regions was 312 nm. Using the multi-level calibration curve and a linear regression equation concentrations in samples were calculated on the corresponding plate under the same conditions.

Validation of method

The proposed HPTLC method has been properly validated according to the United States Pharmacopoeia (USP), ICH's Q2A and Q2B criteria, and the guidance of USFDA.[36]

Linearity and range

Using five measurements at ten different concentration levels (150 to 900 ng/band), linear association among peak area and drug concentration was evaluated.[37] Acceptance criteria are correlation term not any lower than 0.99.

Accuracy and recovery

The powder from already assessed tablets was mixed with medication solutions at 80, 100, and 120% of label claim to undertake recovery experiments. Consequently, 8;10, and 12 mg of FEB were added to tablet powder that had already been assessed to contain 40 mg of FEB.[38] The 98–102% range for an acceptable percentage of recovery is desirable. The recovery values for all nine preparations should have a percent RSD of less than 2.

Precision

Intermediate precision studies confirmed the method's precision. Repeatability studies were carried out using concentration analysis (300 ng/spot for Feb six times on same day). Studies were repeated over the course of three days to assess the method's intermediate precision.[39] Assay% must be between 95.0 and 105 and assay %RSD of 6 preparations must be ≤2.

Robustness

Minor but intentional changes were made to the optimized method's parameters to evaluate the proposed method's robustness. We investigated the effect of drugs on Rf values by introducing small changes in the duration of chamber saturation by the mobile phase. The method was tested for robustness in accordance with established protocols.[40] Acceptance Criteria: The suitability of the system must fall within the acceptance criteria.

LoD

LoD and measurement for purpose of calculating LoD and LoQ signal to noise technique was adopted. LoD is defined as level at which a signal-to-noise ratio of 3:1 is achieved. When calculating LoQ, however, a ratio of 10:1 is used instead. Table 4 shows determined LoD and LoQ values for analyte.[40]Acceptance Criteria: ≤ 2.

Stress degradation study of FEB by HPTLC

According to ICH recommendations stress tests were conducted (Q1A) (R2). The FEB was put through various stress conditions including hydrolysis; photolysis; oxidation; acidic and alkaline stress.[41]

Acidic stress degradation

After carefully measuring out 5 mg of FEB, we introduced it to 10 mL of 0.5 N hydrochloride (HCl) in a 50 mL volumetric flask for disintegration under acidic stress. The mixture stayed in a water bath at 70°C for the next eight hours. After 2, 4, 6, 8 hours, 0.5 mL of solution was put to 10 mL volumetric flask and filled with methanol.

Alkaline stress degradation

For alkaline stress degradation 5 mg of FEB was precisely weighed put into a 50 mL volumetric flask and 10 mL of 0.5 N NaOH was added. The mixture was then maintained in a water bath at 70°C for 8 hours. The volume of the solution in a 10 mL volumetric flask was then made up with methanol at 2, 4, 6, and 8 hours.

Oxidative stress degradation

For oxidative stress degradation 5 mg of FEB was precisely weighed put into a 50 mL volumetric flask and 10 mL of 3% H_2O_2 was added. Mixture was then maintained in a water bath at 70°C for 8 hours. The volume of the solution in a 10 mL volumetric flask was then made up with methanol at 2, 4, 6, and 8 hours.

Hydrolytic stress degradation

For hydrolytic stress degradation 5 mg of FEB was precisely weighed put into a 50 mL volumetric flask and 10 mL of H_2O was added.

The mixture was then maintained in water bath at 70°C for 8 hours. Then, 0.5 mL of the solution was added to a 10 mL volumetric flask and rest of volume was made up with methanol at 2, 4, 6, and 8 hours, in that order.

Photolytic stress degradation

Degradation under photolytic stress was performed by distributing 30 mg of FEB as a thin film in a petri dish and leaving it in a UV chamber with a spectral distribution of 254 nmfor 14 hours. Then, 5 mg of FEB was dissolved in methanol and final volume was brought up to 10 mL in a volumetric flask at 6, 8, 10, 12, and 14 hours.

RESULTS AND DISCUSSION

Method Development

While developing HPTLC, various solvents were tried, including both non-polar and moderately polar solvents. When the stationary phase is polar and the mobile phase uses non-polar solvents, chloroform ($CHCl_3$) is a good choice for normal phase chromatographic (NPC) applications. Since chloroform is a non-polar solvent, it is compatible with silica-packed columns. When using a mobile phase of chloroform, methanol, and formic acid, we were able to get FEB peaks with excellent sharpness and resolution (6.7:2.9:0.1 v/v).

Response Surface Modeling by Box-Behnken design (BBD)

Chromatographic conditions were screened and optimized using the Box-Behnken design. Band length; Saturation time; Solvent front were varied in the range of 7–9 mm; 10–30 minutes and 60–80, respectively. Table 1 shows the selected method responses and their levels. Table 2 lists the results of 17 experiments performed using the response surface method's 2^3 selected factorial designs. Table 3 displays the results of utilizing Design of Experiments software to create a quadratic model of the ANOVA of regression parameters for retention time. The F-value of the model, 9.01, indicates its significance. If the p-value for model terms is less than 0.0500, then they are significant.

This study's findings corroborate the reliability of a response surface model for estimating retention rate. The ultimate equation representing the regression model in terms of their coded components is presented below.

R^2=0.7000-0.0088A+0.0450.0112C+0.0075AB+0.0350AC +0.0075BC+0.0125A^2-0.0450B^2-0.0775C^2

Polynomial equations can be utilized for predicting the behavior of retention time and process variables using the equation in terms of actual elements.

Retention factor = -0.233750-0.468750 Band Length +0.011250 Saturation Time+0.077875 Solvent Front +0.000750 Band Length *Saturation time +0.003500 Band Length *Solvent front +0.012500 Band length^2 -0.000450 Saturation Time^2 -0.000775 Solvent Front^2

Method Optimization through DoE Software

The Design-Expert software used a numerical optimization method to assess the model's accuracy. 100 solutions were proposed but the software chose one based on desirability 1.0. The experiment was carried out under the optimal conditions specified by Design Expert. The Design-Expert recommended chromatographic settings were band length 8 mm; saturation time 20 minutes and solvent front 70. The model projected a method response of 0.7 for the retention factor as shown in Figure 2. The HPTLC equipment was operated under the same experimental conditions. Predicted values and observed values are compared and verified in Table 3. Figure 3 shows that a 0.999 correlation exists between the predicted and observed values. In this way, the model can confidently anticipate the technique's response between a 5 and 95% of the time. All

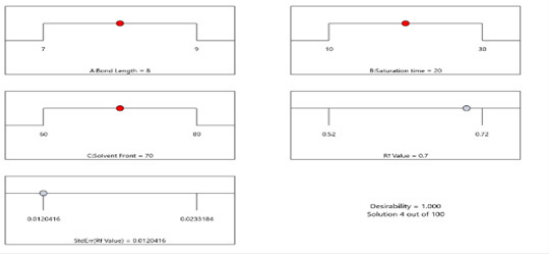

Figure 2: Optimization and prediction of method responses by model

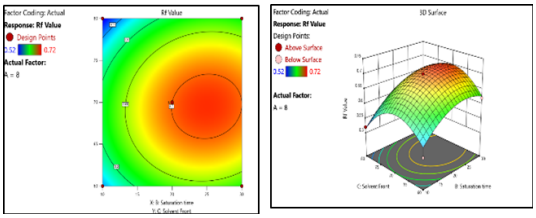

Figure 3A: Contour plots (a) 3 D Response Surface (b) for retension Factor as a function of Solvent front and Saturation time (constant Band length 8)

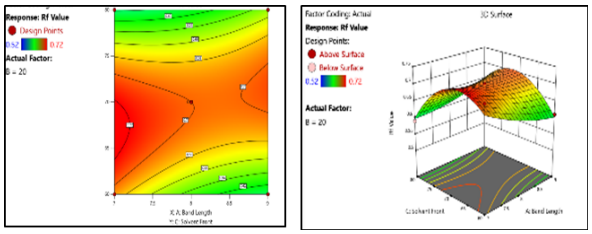

Figure 3B: Contour plots (a) 3 D Response Surface (b) for retension Factor as a function of Band Length and Solvent front (constant Saturation time 20)

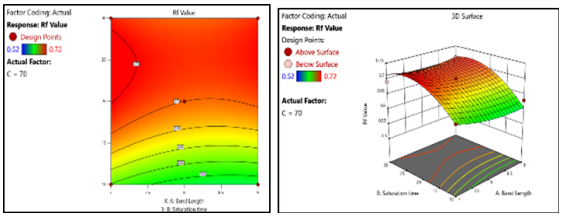

Figure 3 C: Contour plots (a) 3 D Response Surface (b) for retention Factor as a function of Band Length and Saturation time (constant Solvent front 70)

of these factors have been shown to significantly impact the retention factor as shown in Figure 2.

Linearity

Figure 3 displays the claimed 0.999 correlation coefficient among peak area and FEB concentration across range of 150–900 ng/band from the calibration curve. Three replicates of each concentration were analyzed. Cochran's C was used to analyze the homoscedasticity test results and establish the linearity of the calibration curve. Cochran's C for 4 replicates of standard FEB solutions at varying concentrations needs to be less than 0.597 to demonstrate homoscedasticity. The test

Table 2: Box Behnken design 3 Factors; 22 limits 17 Runs

Std	*Run*	*Band length*	*Saturation time*	*Solvent front*	*Rf value*
1	12	7	10	70	0.64
2	4	9	10	70	0.63
3	8	7	30	70	0.69
4	7	9	30	70	0.71
5	2	7	20	60	0.72
6	14	9	20	60	0.61
7	11	7	20	80	0.59
8	6	9	20	80	0.62
9	13	8	10	60	0.52
10	1	8	30	60	0.62
11	5	8	10	80	0.52
12	15	8	30	80	0.65
13	17	8	20	70	0.7
14	10	8	20	70	0.7
15	9	8	20	70	0.7
16	3	8	20	70	0.7
17	16	8	20	70	0.7

Table 3: Verification of experiment at optimumconditions

Method response	*Prediction*	*95% CI low for the mean*	*95% CI high for the Mean*	*Observed*
Rf Value	0.7	0.671526	0.728474	0.7

Table 3B: ANOVA for Quadratic model Response 1: Rf Value

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	0.0588	9	0.0065	9.01	0.0042	significant
A-Band Length	0.0006	1	0.0006	0.8448	0.3886	
B-Saturation Time	0.0162	1	0.0162	22.34	0.0021	
C-Solvent Front	0.0010	1	0.0010	1.40	0.2759	
AB	0.0002	1	0.0002	0.3103	0.5948	
AC	0.0049	1	0.0049	6.76	0.0354	
BC	0.0002	1	0.0002	0.3103	0.5948	
A^2	0.0007	1	0.0007	0.9074	0.3725	
B^2	0.0085	1	0.0085	11.76	0.0110	
C^2	0.0253	1	0.0253	34.88	0.0006	
Residual	0.0051	7	0.1007			
Lack of Fit	0.0051	3	0.1017			
Pure Error	0.0000	4	0.0000			
Cor Total	0.0638	16				

Table 4: Summary of linear regression and validation data

Parameters	*FEB*
Linearity range (ng/band)	150 - 900
Linear regression equation	y = 3.638x + 391.91
Slope	3.638
Intercept	391.91
Correlation coefficient (r^2)	0.9992
Standard error of slope	0.098
Standard error of intercept	0.081
Standard error of residual	0.099
Limit of detection (ng/band)	16.83
Limit of quantification (ng/band)	51.00
Intra-day precision (%mean" ± SD)	2055.00 ± 0.01
Inter-day precision (%mean ±SD)	2065.67 ± 0.01

Table 5: Results of Cochran's C test of FEB

Concentration (ng/band)	*S.D.*	*Square of S.D.*[a]	*Sum of square S.D.*	*C*[b]
150	± 406.51	1;65;250	52;52;970	0.597
300	± 671.267	4;50;599		
750	± 1411.76	19;93;066		
900	± 1625.44	26;42;055		

[a] S.D: Standard deviation of three replications
[b] C: Cochran's C value

Table 6: Results of accuracy study of FEB

Sr. no.	*Level (%)*	*Weight of Tablet powder taken*	*Amount of drug added (mg)*	*Amount of Drug Recovery (mg)*	*%Recovery of FEB*
1	80	322	8	8.04	100.5
2		322	8	7.85	98.125
3		322	8	8.21	102.625
4	100	322	10	10.03	100.3
5		322	10	10.04	100.4
6		322	10	9.97	99.7
7	120	322	12	12.3	102.5
8		322	12	12.05	100.4167
9		322	12	12.3	102.5

Table 7: Statistical validation for accuracy study (n=3)

Level of recovery	*%mean recovery of FEB*	*Standard-deviation*	*%RSD*	*S.E*
80	100.87	0.88	0.25	0.508083
100	100.18	0.42	0.09	0.242494
120	99.91	0.24	0.24	0.138568

Data were obtained from three replicates at each concentration

Table 8: Precisionstudy

Conc	Intraday			Interday		
	Mean ± SD	Amt Found	% Amt Found	Mean ± SD	Amt Found	% Amt Found
300	1524.67 ± 0.00	311.37	103.79	1513 ± 0.00	308.16	102.72
450	2055.00 ± 0.01	457.14	101.59	2065.67 ± 0.01	460.08	102.24
600	2579.33 ± 0.01	601.27	100.21	2568.33 ± 0.01	598.25	99.71

Table 9: Repeatability study

Sr No.	Conc	Area	Amt Found	%Amt Found
1	300	1500	304.59	101.53
2	300	1501	304.86	101.62
3	300	1480	299.09	99.70
4	300	1491	302.11	100.70
5	300	1490	301.84	100.61
6	300	1502	305.14	101.71
		Mean	302.94	100.98
		SD	2.36	0.79
		% RSD	0.78	0.78

Table 10: Robustness testing for the HPTLC method

Factors	Chromatographic changes		
Mobile phase composition	(± 0.1)	Peak area	Rf value
6.4:2.7:0.9 6.7:2.9:0.1 6.8:3.1:0.1	-1	700	0.526
	0	13550	0.597
	+ 0.1	8280	0.898
Amount of mobile phase (mL)	(± 0.1)		
9 10 11	-1	420	0.521
	0	17370	0.597
	+ 0.1	6560	0.881
Duration of chamber saturation (Min)			
10 20 30	-10%	530	0.516
	0	910	0.535
	+10%	490	0.500

Table 11: Parameters of system suitability of the developed HPTLC densitometric method for the determination of FEB

Parameters	FEB
Symmetry factor	1.04
Resolution (Rs)	-
Selectivity (ά)	1.53
Capacity factor (K)	0.86

was conducted at four different concentrations of the reference drug, two at the lowest (150 and 300 ng/band) and two at the highest (750 and 900 ng/band), the greatest and least amount of variation being represented by the two extremes of the calibration curve (heteroscedastic scenario). In Table 5, we can see the results of Cochran's C test. Cochran's C for the calibration curve was below the crucial value. Hence it passed the homoscedasticity test.

Table 12: Result of analysis of tablet formulation of FEB

Tablet strength 40 mg			
Sr no	Wt of tablet powder taken (mg)	Amount of drug estimated (mg/tablet)	% label claim
1	322	40.15	102.5
2	322	39.91	100.03
3	322	39.93	100.5
4	322	40.04	99.4
5	322	40.09	100.7
6	322	39.98	99.9

Table 13: Statistical validation of analysis of tablet formulation of FEB (n=6)

Drug	Amount of drug estimated (mg/tab)	% Labelled claim	SD	C.V	SE
Febuxostat	40	101.0933	0.65	0.6466	0.27

Data obtained from Six replicates at each concentration

Table 14: Results of degradation study of FEB

Sr. no.	Stress condition	Percent assay of active substance	Rf value of degraded product
1	Base (0.5 N NaOH)	80	0.45;0.58;0.83
2	Acid (0.5 M HCl)	100	-
3	3% v/v oxidative H_2O_2	100	-
4	Water	100	-
5	Photolytic	100	-

Accuracy

The results of the precision investigation are expressed as a percentage recovery. Tables 6 and 7 show that the suggested approach has a recovery percentage between 98 and 120% at each of the three levels tested (80, 100, and 120%).

Precision

The precision and repeatability study was completed according to the accepted methodology. Tables 8 and 9 show that the technique has a repeatability and precision of less than 2% for intraday and interday precision, respectively.

Robustness

Variation in mobile phase composition on the order of ± 1 mL. Saturation of the chamber took anything from ± 2.5 minutes and above. Table 10 displays the results of these alterations on RF value and peak area.

LoD and LoQ

Using the 3a/S phenomena and the 10a/S phenomenon, the limits of detection and quantification of the proposed method were established. The slope of the calibration curve is denoted

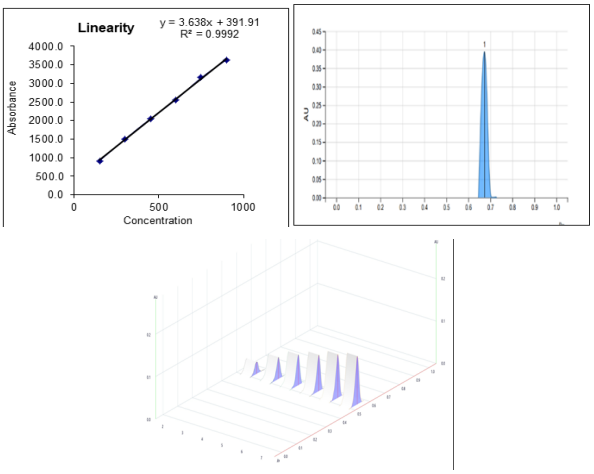

Figure 4: A) Densitogram of FEB B) Standard calibration curve of FEB C) 3D Linearity spectrum of FEB

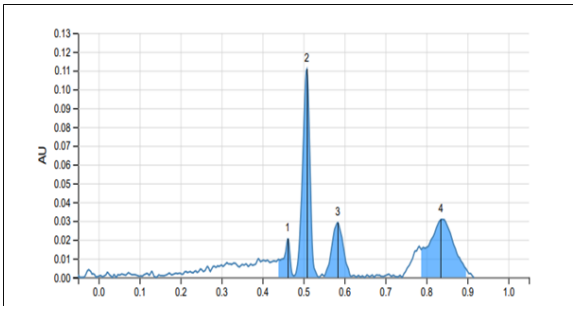

Figure 5: Chromatogram of 0.5 N NaOH treated FEB

by S, while the standard deviation of the intercept is denoted by an. Table 4 shows that the minimum detectable and quantifiable amounts were 8.08 and 12.16 ng/band.

System suitability

The system appropriateness test is an essential part of any chromatographic procedure. This technique is used to evaluate the chromatographic system's resolution, repeatability, and accuracy. Peak symmetry parameters were used to compute the resolution (Rs), capacity factor (K), and selectivity factor (a). The results of these calculations are reported in Table 11 for FEB, which has a selectivity larger than one and a known value for both the capacity factor and symmetry factor.

Marketed formulation analysis

Commercial pill FEB (40 mg) was quantified using the proposed HPTLC approach. Analyzing the pill formulation by contrasting the mean peak Evaluate the sample peak's size in relation to the standard band's. Tablet analytical results were consistent with the claims made on the label (Table 12). A statistical breakdown of available tablets is displayed in Table 13.

Degradation behavior of FEB

The effects of hydrolytic (basic, neutral and acidic), oxidative, thermal, and photolytic stress on FEB degradation were investigated using HPTLC. Drug degraded rapidly under alkaline stress conditions, whereas FEB remained stable under photolytic, acidic, oxidative and water stress conditions. Figure 4 shows the densitogram obtained for FEB under alkaline conditions while Figure 5 indicates the chromatogram.. Table 14 displays the findings of the forced deterioration studies. The developed HPTLC approach effectively separated FEB from its degradation products, demonstrating the technology's potential for indicating stability.

CONCLUSION

The HPTLC method developed and verified in this research can identify FEB in its undiluted and diluted forms. Box-Behnken designs and response surface methods can be used to collect crucial information about the sensitivity of Febuxostat RF values to various chromatographic factors. Utilizing a powerful experimental design instrument, we were able to fine-tune the band length, saturation duration, and solvent front all at once. Results from validating the method indicated that it is linear, accurate, specific, robust, and stable. The designed procedure has been validated in accordance with the ICH standards. As a result of the study described above we can conclude that under various stress situations like those listed above, the FEB degrades at different rates in basic conditions. It proves that using this technique may isolate a drug's degradation products from the drug.

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