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A Validated Inherent Stability–Indicating RP-HPLC-Dad Method for Estimation of Febuxostat in the Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT

A rapid, accurate, linear, and sensitive RP-HPLC method has been developed and validated for estimation of febuxostat in the bulk drug and marketed tablet formulation. The chromatographic separation was achieved on Kromasil C18 Column (250 mm × 4.6 mm, 5 µm particle size) using solvent methanol:water(65 : 35 v/v, pH 3.0 adjusted with OPA)as a mobile phase at flow rate of 1.0 ml/min and25°C column temperature was maintained and analysis were carried out at detection wavelength316 nm. The linearity study was studied in the concentration range 10-50 µg/ml for febuxostat and correlation coefficient was found to be 0.999. The percentage purity of febuxostat was found in the range of 98-101%. The limit of detection and limit of quantification were found to be1.22µg/ml and3.58µg/ml. The method was validated for linearity, precision, accuracy, specificity and selectivity. The obtained results indicates that the proposed method allows selective analysis of febuxostat, in the presence of their degradation products formed under a variety of stress conditions. The developed procedure is also applicable for the determination of instability of the drugs in commercial products.

Keywords: Febuxostat; Validation; Force degradation studies; HPLC-DAD.

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INTRODUCTION

Febuxostat chemically is 2- [3- cyano-4- (2- methlypropoxy) phenyl] - 4- methlythiazole- 5 - carboxylic acid. It is a non-purine selective inhibitor of xanthine oxidase and has very less effects on other enzymes of purine and pyrimidine metabolism that is indicated for use in the treatment of hyperuricemia and gout. It works by non competitively blocking the channel leading to the active site on xanthine oxidase. Xanthineoxidase is needed to successively oxidize both hypo xanthaine and xanthine to uric acid. Hence, Febuxostat inhibits xanthine oxidase, therefore reducing production of uric acid[1-2]. The stability-indicating assay method is mainly employed for the analysis of stability samples in Pharmaceutical companies. The revised stability test guideline Q1A(R2) issued by International Conference on Harmonization (ICH) suggests, stress testing on the drug substance should be carried out to establish the inherent stability characteristics and for supporting the suitability of the proposed analytical procedures. Febuxostat is not official in any pharmacopoeia. Literature survey reveals that few UV Spectrophotometric methods, difference Spectrophotometric method, RP-HPLC method for simultaneous estimation of Febuxostat and Ketorolac tromethamine in pharmaceutical formulations, estimation of related substances of Febuxostat in bulk and tablets by RP-HPLC method are present[3-8].Hence an attempt was made to develop novel HPLC method for estimation of febuxostat in bulk and pharmaceutical formulation with good precision, accuracy, linearity, reproducibility. Therefore, the focus in the present study was to develop an stability-indicating RP-HPLC-DAD method for the estimation of febuxostat, by degrading the drug under various stress conditions according to ICH guidelines[9-12]. The drug was well separated from degradation products using a reversed-phase HPLC column and analysis was also extended to marketed products. The results are discussed in this paper.

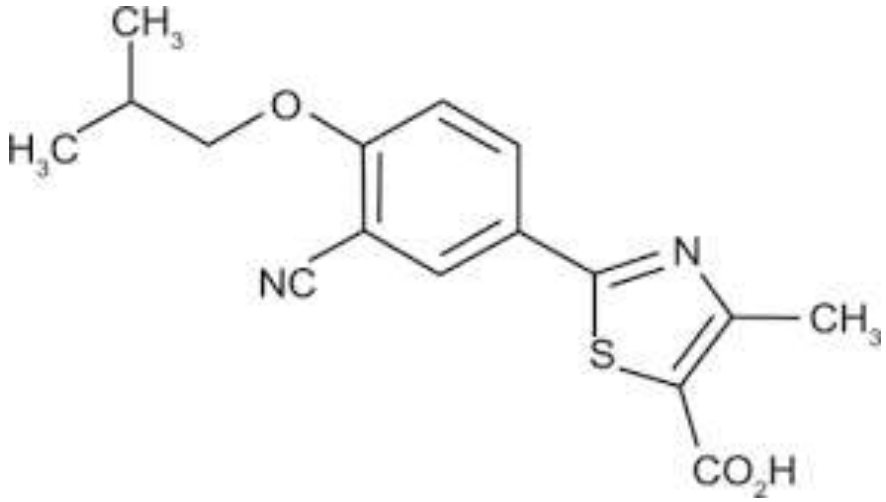

Figure 1: Structure of FBX

MATERIALS AND METHOD

Chemicals and reagents

Reference standard Febuxostat was obtained as gift sample from Ajanta Pharmaceuticals, Mumbai, India. Tablets were purchased from local pharmacy shop. HPLC grade water, methanol and acetonitrile were obtained from Merck specialties Pvt. Ltd., Mumbai (India).

Instrumentation and chromatographic conditions

The instrument used was Waters 510 HPLC system equipped with a rheodyne injector programmed at 20µl capacity per injection was used. The detector consisted of UV–Visible detector operated at wavelength 316 nm. Data acquisition was made with Data Ace software. The column used was Kromasil C-18 (250mm x 4.6mm, 5µm). Analytical balance used for weighing was Schimadzu AUX-220. Ultrasonicator used was Sonarex Super RK 102 (Berlin, Germany) equipment with thermostatically controlled heating (30–80 ◦C).

Preparation of stock solution

Accurately weighed quantity **(**25.0 mg) of FBX was transferred to 25.0 ml volumetric flask, dissolved and diluted up to the mark with mobile phase (Concentration 1000 µg/ml). The solution was filtered through 0.45 µ membrane filter.

Calibration standards of FBX

From standard stock solution, 5.0 ml was diluted to 50.0 ml with mobile phase. From above solution, 1.0, 2.0, 3.0, 4.0 and 5.0 ml were transferred individually to 10.0 ml volumetric flask and diluted to the mark with mobile phase (Concentration 10, 20, 30, 40 and 50µg/ml, respectively). The diluted solutions were filtered through 0.45µ membrane filter.

Force degradation studies:

In order to evaluate the stability indicating property of the developed HPLC method stress studies were carried out under ICH recommended conditions. Intentional degradation was tried by exposing the tablet sample to following stress conditions: acid (2 M HCl for 3h at 70^{o}C), base (0.5 M NaOH for 2 h at 70^{o}C), neutral hydrolysis (H_2O for 5.5 h at 70^{o}C), oxidation (30% H_2O_2 for 12h at 70^{o}C), and dry heat (70°C), and UV light (254 nm). Samples were withdrawn periodically and subjected to analysis after suitable dilution. Ability of the proposed method used to measure the analyte response in presence of its degradation products was studied using HPLC-DAD.

Method validation

The validated HPLC method proposed according to ICH guidelines (ICH 1994, 1996). The following parameters were used for validation of the developed method.

Linearity:

Linear relationship between peak area and concentration of the drugs were evaluated, making six measurements at concentration levels in the range of 5-25 µg/ml.

Accuracy:

Recovery studies were carried out by spiking three different known amounts of pure drug (at 80%, 100% and 120% of label claim) to the pre-analyzed powder (standard addition method). Hence, an accurately weighed quantity of pre-analyzed tablet 12 mg FBX to the pre-analyzed.

Precision:

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyzing the tablet sample six times at 100% of test concentration on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and quantitation:

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ) linear were separately determined based on the standard deviation (σ) of the response and the slope (S) of the calibration curve and using the formula LOD=3.3 σ /S and LOQ=10 σ /S, the LOD and LOQ for FBX was estimated.

Robustness:

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of change in flow rate and mobile phase ratio on retention time and tailing factor were studied. The solution containing 12 µg/ml of FBX was injected (in triplicate) into sample injector of HPLC three times under the varied conditions.

System suitability:

To ascertain resolution and reproducibility of proposed chromatographic system for estimation of FBX in Pharmaceutical dosage form, system suitability parameters like tailing factor (T), resolution (R) and column efficiency (number of theoretical plates, N) were studied. From stock solution D, appropriately diluted with mobile phase to obtain 12 µg/mlFBX. The diluted standard solutions were filtered through 0.2 µ membrane filter.

RESULTS AND DISCUSSION

Development and optimization of the stability-indicating HPLC method

A developed method was found necessary to optimize the separation of major degradation products formed under various stress conditions. Various mobile phases were tried containing

Acetate buffer, Phosphate buffer, Methanol and Acetonitrile in different ratio, and various pH were tried and finally methanol: water (65: 35 v/v, pH 3.0 adjusted with OPA) was selected as an appropriate mobile phase that resulted in good resolution and acceptable system suitability parameters. FBX was delivered at a flow rate of 1 ml/min with detection wavelength 316 nm for FBX. The injection volume was 20 µl. Analysis was performed at a temperature of 25°C and the obtained chromatogram is shown in Figure 2.

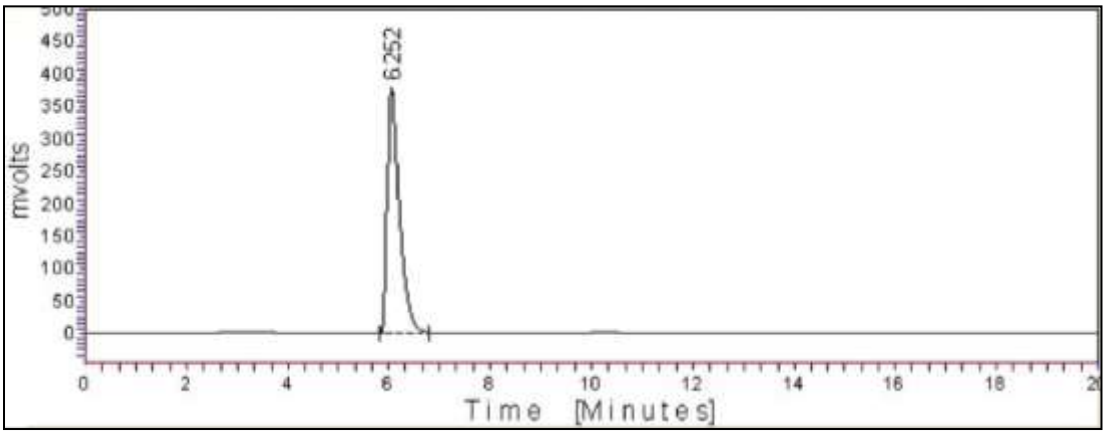

Figure 2: Typical Chromatogram of Febuxostat (Rt 6.252)

Assay of tablet:

Twenty tablets were weighed, average weight was calculated and crushed to obtained fine powder. Accurately weighed quantity of tablet powder equivalent to about 12.5 mg of FBX was transferred to 50.0 ml volumetric flask, dissolved and diluted up to the mark with mobile phase. From this solution, 1.0 ml was transferred to 10.0 mL volumetric flask and diluted to the mark with mobile phase (Concentration 25 µg/ml). The solution was filtered through 0.45 µ membrane filter. The corresponding chromatograms were recorded and area of each peak for FBX was measured at 316 nm. Amount of FBX in sample (mg) was calculated by comparing the mean peak area of standard and sample solution. The statistical analysis is shown in Table 1.

Table 1: Statistical Validation for Analysis of Tablet Formulation

Sr. No.	Drug	Amount of drug estimated (mg/tablet)*	% Label Claim*	S.D	C.V	S.E
1.	FBX	39.53	98.82	± 0.347	0.3513	0.141

***mean of six determinations

Force degradation studies:

In the forced degradation studies FBX were found to degrade majorly under acidic (2 M HCl for 3h at 70^{o}C) stress condition as compared to other stress condition. The results for forced degradation studies and selectivity were determined by checking peak purity of all the peaks,

including those of degradation products, using a PDA detector and results are included in Table 2. Peak purity spectra of a mixture of all stressed compound is represented in Figure 9. Typical chromatography obtained for FBX under different stress conditions are shown in Figures 3-8. The developed HPLC method could effectively resolve the drugs from their degradation products which confirm the stability indicating power of the developed method.

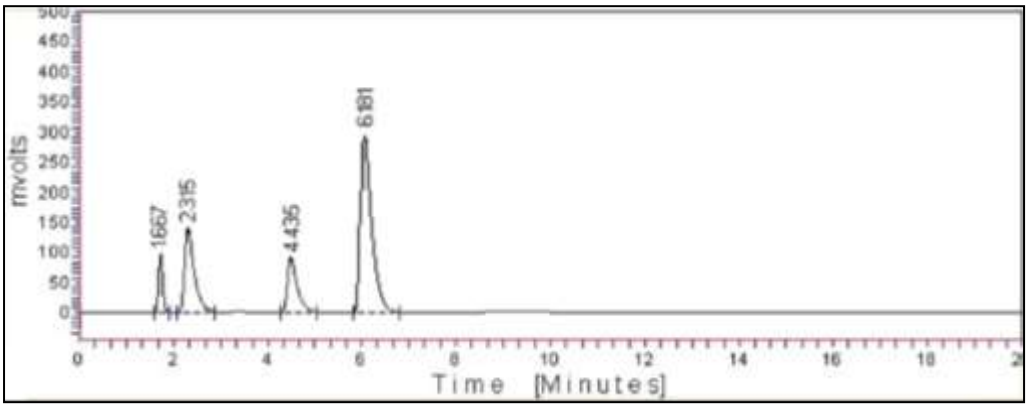

Figure 3: Chromatogram of Acid Hydrolysis

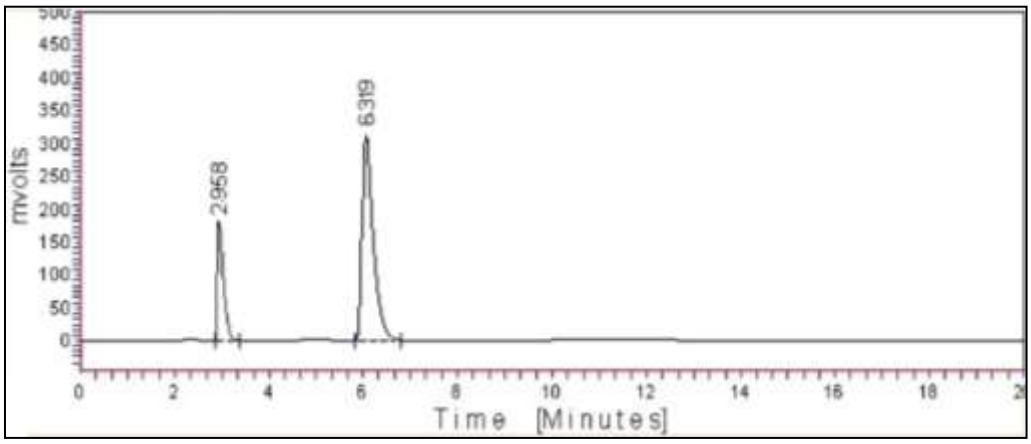

Figure 4: Chromatogram of Alkali Hydrolysis

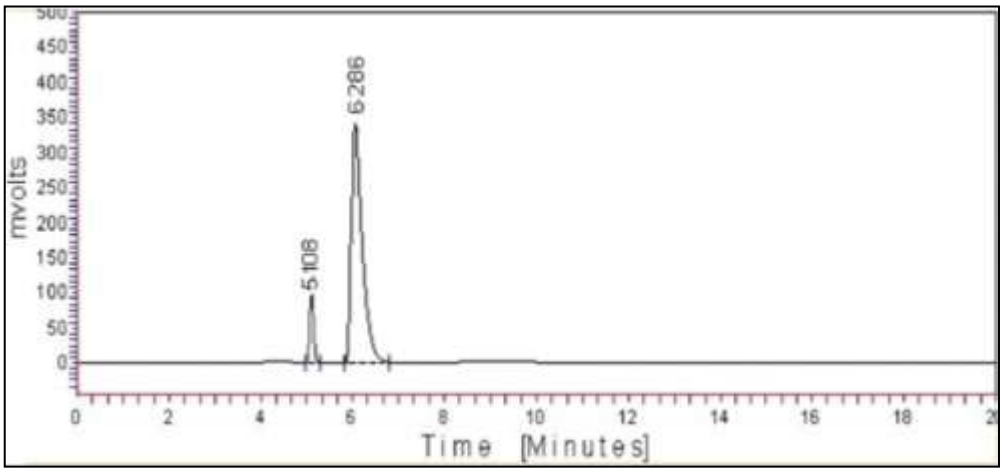

Figure 5: Chromatogram of Neutral Hydrolysis

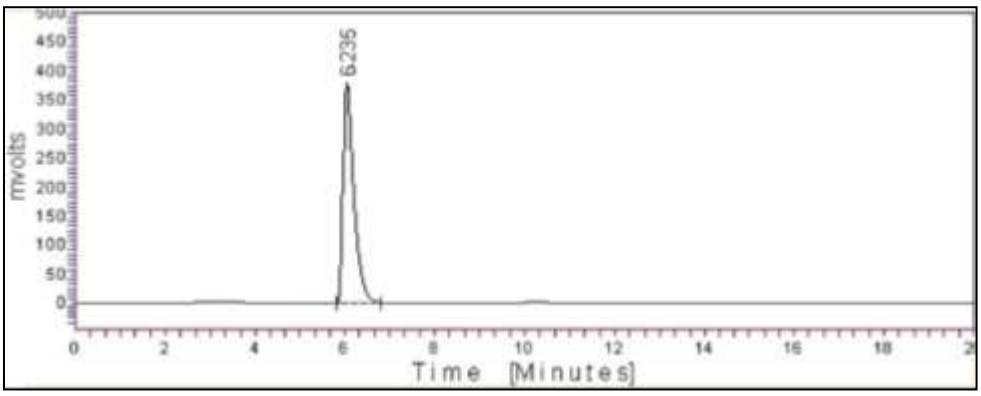

Figure 6: Chromatogram of Oxidative Degradation

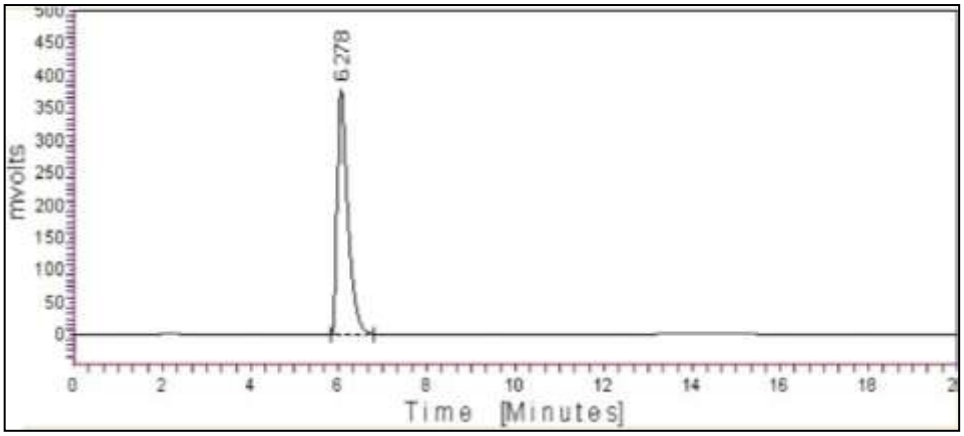

Figure 7: Chromatogram of Photolytic Degradation

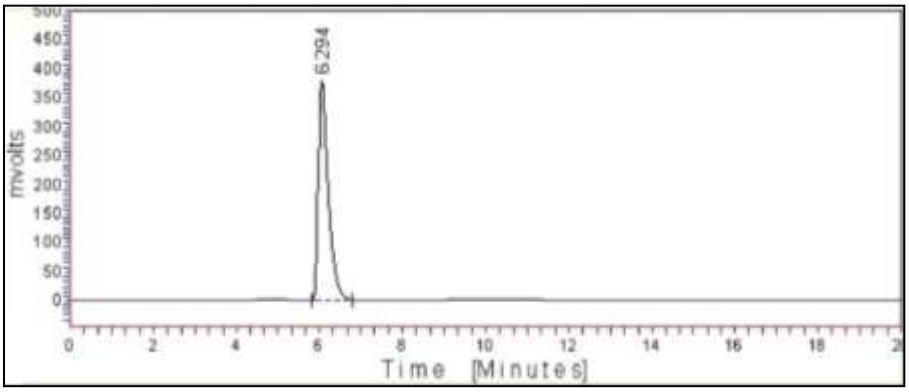

Figure 8: Chromatogram of Thermal Degradation

Table 2: Results of Forced Degradation Studies

Stress Condition	% degradation	% Assay	Mass Balance	RT in min.	Peak purity index
Acid	20.26	79.79	100.05	1.667, 2.315, 4.435	0.999987
Alkaline	18.94	79.81	98.75	2.315, 4.435	0.999993
Neutral	9.5	89.68	99.18	2.958	0.999851
Oxidative	0.0	99.2	99.2	-	0.999579
Photolytic	0.0	99.34	99.34	-	1.000000
Thermal	0.0	98.81	98.81	-	0.999999

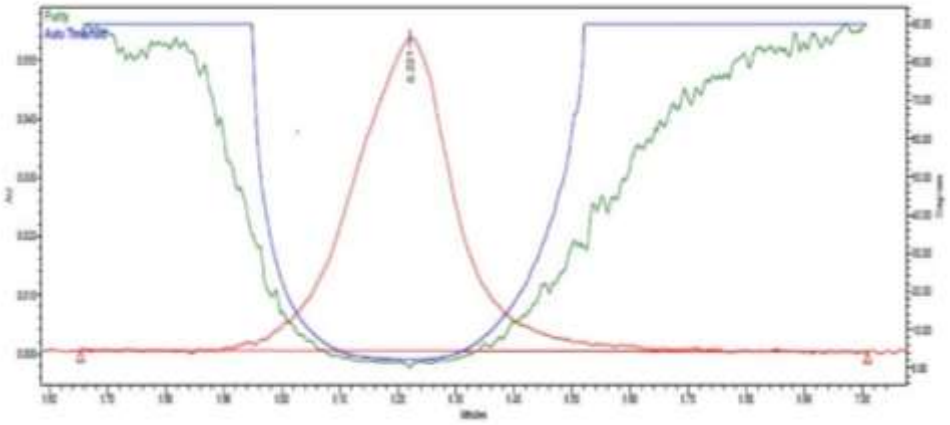

Figure 9: Peak Purity Spectra of A Mixture Of All Stressed Solutions

System suitability:

For system precision six replicate injections of standard solution were given, tailing factor (T), and column efficiency (number of theoretical plates, N) were studied. For FBX was recorded for each injection shown in Table 3.

Table 3: System Suitability Parameters

Sr. No.	Parameter	FBX
1.	Resolution	-
2.	Asymmetry factor (As)	1.10
3.	No. of theoretical plates (N)	6754

Validation of the method

Linearity of response: A linear relationship was observed between peak area and concentration in the range of 10-50 µg/ml FBX respectively. The correlation coefficients for the calibration curve were found to be 0.999, for FBX. Mean peak areas for FBX at selected wavelength are shown in Figure 10.

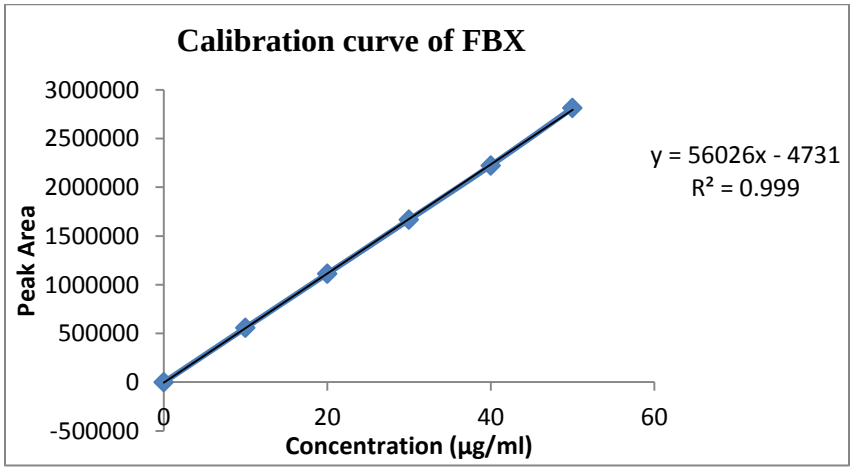

Figure 10: Standard Calibration Curve of FBXat 316nm.

Precision of the method:

Repeatability and reproducibility of the proposed method was determined by intra-day and inter-day precision studies. The Tablet was assayed three times on the same day (intra-day) and on three consecutive days (inter-day). The results of precision studies were expressed in terms of relative standard deviation (RSD) less than 2 of the percent label claim determined by developed method shown in Table 4 and 5.

Table 4: Intra-day Precision Data

Drug	Concentration (µg/ml)	Drug estimated (µg/ml)	S. D.	C. V.
FBX	20	19.96	± 0.020	0.201
FBX	25	25.16	± 0.026	0.209
FBX	30	29.98	± 0.043	0.285

* mean of six determinations

Table 5: Inter-Day Precision Data

Drug	Concentration(µg/ml)	Drug estimated (µg/ml)	S. D.	C. V.
FBX	20	19.97	± 0.045	0.451
FBX	25	24.44	± 0.052	0.418
FBX	30	29.99	± 0.135	0.900

* mean of six determinations

Limit of Detection (LOD) and Quantification (LOQ):

The limit of detection was1.22 µg/ml for FBX and limit of quantification was3.58µg/ml for FBX as shown in Table 6.

Table 6: LOD and LOQ of FBX

Parameter	FBX
Limit of Detection (µg/ml)	1.22
Limit of Quantification (µg/ml)	3.58

Accuracy:

The results of accuracy study are expressed in terms of percent recovery. The percent recovery at three levels (80 %, 100 % and 120 %) was found to be in the range of 98-102 %. Statistical analysis of recovery studies are shown in Table7.

Table 7: Statistical Validation for Recovery Study

Level of recovery	% Mean Recovery	Standard Deviation	% R.S.D.	S.E
80 %	99.46	± 0.9451	0.9506	0.5457
100 %	99.36	± 0.4233	0.4260	0.2444
120 %	99.44	± 0.3894	0.3915	0.2248

*mean of three determinations

Robustness:

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of change in flow rate, detection wavelength and mobile

phase ratio on retention time and tailing factor were studied. The solution of FBX was injected (in triplicate) into sample injector of HPLC under the varied conditions. Robustness data is given in Table 8.

Table 8: Result of Robustness Studies

Chromatographic Changes			
Flow Rate(ml/min)	**Level**	**Retention time**	**Tailing factor**
0.9	– 0.1	6.23	1.09
1.0	0	6.25	1.10
1.1	+ 0.1	6.26	1.11
	Mean	6.24	1.10
	S.D.	±0.015	± 0.010
Mobile Phase (v/v/v)	**Level**	**Retention time**	**Tailing factor**
64:36	**– 1.0**	6.23	1.12
65:35	**0**	6.25	1.10
66:34	**+1.0**	6.25	1.09
	Mean	6.24	1.10
	S.D.	± 0.011	± 0.015
wavelength (nm)	**Level**	**Retention time**	**Tailing factor**
315	**– 1.0**	6.25	1.10
316	**0**	6.25	1.10
317	**+1.0**	6.26	1.11
	Mean	6.25	1.10
	S.D.	± 0.005	± 0.005

CONCLUSION

A simple, rapid, accurate and precise stability-indicating HPLC analytical method has been developed and validated for the routine analysis of FBX in API and tablet dosage forms. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating. The obtained result indicates FBX is most instable in alkaline condition as compare to acidic, thermal and photolytic condition. Statistical analysis proved that the method is repeatable, reproducible, accurate and specific for the analysis of FBX. The developed HPLC method confirms the stability indicates power of the developed method. As the method in cost effective and less time consuming, thus, it can represent another good alternative for the already existing HPLC methods.

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