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Development and Validation of High-Performance Thin Layer Chromatography Method for Quantitative Estimation of Febuxostat in Marketed Tablets Formulations

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Abstract: Background: Febuxostat commonly used antigout drug in clinics, this study demonstrated the use of a simple validated High-Performance Thin-Layer Chromatography method has been proposed for the quantitative determination of Febuxostat in a tablet dosage form. **Objectives:** To develop a validated High-Performance Thin-Layer Chromatography method for chromophoric drug Febuxostat and its tablet formulations. **Methods:** The separation was achieved on silica gel 60 F254 coated aluminum sheet as stationary phase using n-Hexane: Ethyl acetate (7.0: 3.0 v/v) as mobile phase which gave compact spots with R_f value 0.49 ± 0.02. Quantitative densitometric evaluation was done in absorbance-reflectance mode at 315 nm. The developed method was validated with respect to linearity, limits of detection and quantitation, accuracy, precision, specificity and robustness. **Results:** The response was found to be linear over concentration range of 100-600 ng/spot with correlation coefficients 0.997 and 0.996 and mean percentage recovery of the drug was observed to be 98.84 ± 0.537 and 98.44 ± 0.556 by peak area and peak height, respectively. **Conclusion:** The method was validated for linearity, accuracy, range, precision and robustness according to International Council on Harmonization Q2 (R1) guidelines. The method is simple, accurate, precise and was successfully applied to the assay of drug in tablet formulation.

Keywords: Febuxostat, HPTLC, validation, assay, quality control

INTRODUCTION: Febuxostat, a nonpurine selective inhibitor of both the oxidized and reduced forms of xanthine oxidase/ xanthine dehydrogenase and used for the management of hyperuricemia in adults with gout. Chemically, it is 2 (3-cyano-4 [2-methyl propoxyl] Phenyl)-4-methylthiazole-5-carboxylic acid [1]. A literature survey revealed analytical methods like it has determined several impurities in Febuxostat drug substance. Impurities were identified with the help of LC-MS/MS and were characterized after synthesis by IR and NMR. Reverse phase gradient system was used with Kromasil C_{18}, 150 mm × 4.6 mm, 5 µm particle size column and eluent, was monitored by UV detector at 315 nm for the separation of impurities. Column oven temperature was 30^0C. The injection volume was 10 µL. Diluent was the mixture of water and acetonitrile in the ratio of (20:80) and sample concentration was 1mg/mL. Q-TOF mass spectrometer with electrospray ionization (ESI) source was used and operated in ESI positive mode. Four impurities were identified as amide, sec-butyl, des-cyano and des-acid in Febuxostat drug analog. These impurities were further confirmed by NMR and FT-IR spectral data [2]. It has studied a comprehensive stress testing of Febuxostat under different prescribed ICH stress conditions, and a stability-indicating reverse phase novel ultra-performance liquid chromatography (UPLC) assay was established. It was found capable of giving faster retention times, requiring minimal solvent and maintaining good resolution. The UPLC chromatographic separation was carried out on UPLC BEH C_{18} column using isocratic mode (Acetonirile: ammonium acetate buffer (pH 4.5), 70:30v/v) at flow rate of 0.2 mL/min. The drug showed degradation only in basic condition, while it was stable under other stress conditions. The response for the drug was linear (r^2 = 0.999) in the concentration range between 10 and 50 µg/mL. The retention time was found to be 2.046 min [3]. It has developed and validated a isocratic liquid chromatographic method for the determination of Febuxostat. Chromatographic separation was achieved on a C_{18} column using sodium acetate buffer (pH 4.0)- acetonitrile (40:60 v/v), with a flow rate 1.2 mL/min (ultraviolet detection at 254 nm). Linearity was observed in the concentration range of 0.1-200 µg/mL (r^2 =0.9999). The retention time was found to be 3.471 min [4]. It has developed a rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of Febuxostat and its three active metabolites in human plasma using a ZORBAX SB-C_{18} column (50 mm × 4.6 mm, 5 µm) and an optimized gradient mobile phase consisting of acetonitrile, water and formic acid. Plasma samples were spiked with the internal standard Losartan and then pre-treated using one-step protein precipitation with methanol. Mass spectrometric detection was performed by selective reaction monitoring mode via electrospray ionization source operating in positive ionization mode. The method exhibited good linearity over the concentration range of 10-20,000 ng/mL for Febuxostat,

1.0-270 ng/mL for 67M-1 and 67M-2, and 0.8-250 ng/mL for 67M-4, respectively. The method was successfully applied to a clinical pharmacokinetic study of Febuxostat in humans after oral administration of a single dose of Febuxostat at 40, 80 and 120 mg. The retention times of Febuxostat, 67M-1, 76M-2, 67M-4 and internal standard were 10.13, 8.98, 7.13, 8.00 and 8.96 min, respectively [5]. It has developed and validated a HPLC-MS/MS method for simultaneous determination of the active metabolites (67M-1, 67M-2 and 67M-4) in human plasma using Clopidogrel as the internal standard. The compounds were extracted by protein precipitation using acetonitrile and separated using a C_8 column by a gradient elution with the mobile phase consisting of acetonitrile (containing 0.1% formic acid) and 0.1% formic acid. Quantification was performed using multiple reaction monitoring in positive mode with m/z transitions of 333.1-261.0, 333.1-261.0, 347.0-261.0 and322.2-184.1 for 67M-1, 67M-2, 67M-4 and Clopidogrel (Internal Standard), respectively. The calibration curve was linear over the concentration range of 0.5-150 ng/mL [6]. It has developed and validated a simple, specific, accurate and cost-effective spectroscopic method for the estimation of Febuxostat in bulk as well as formulation. The optimum conditions for the analysis of the drug were established. The maximum wavelength was found to be 314 nm. The method shows high sensitivity with linearity in the range of 1-6 µg/mL with coefficient of correlation 0.999 [7]. There is no reported HPTLC method for its estimation in tablet formulation. A HPTLC method for estimation of FEB in tablet formulation is described in the present article.

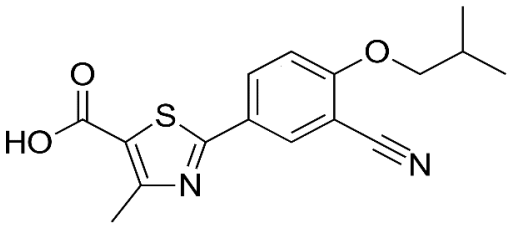


Fig. No. 1: Febuxostat

MATERIALS AND METHODS

Chemicals and Reagents

n-Hexane, ethyl acetate, methanol used were of AR grade, Merck India Ltd, Mumbai (India). A Milli-Q purification system from Merck Company (Darmstadt, Germany) was used to produce ultra-pure water. Standard drug sample of Febuxostat (99.89% pure, Fig.1) was obtained as a gift sample from Mylan Laboratories Limited, Hyderabad (India). The Febuxostat tablets used in this study with a declared content equivalent to 10 mg Febuxostat were procured from local market.

Instrumentation and Conditions

HPTLC was performed with Camag HPTLC equipment comprising of Linomat IV sample applicator, Linomat Microliter syringe (Hamilton- Bonaduz Schweiz) 100 µL, TLC Scanner-III with win CATS software version 1.4.1 for scanning and documentation, High-tech UV cabinet fitted with dual wavelength 254/ 366 nm, 8 volts UV lamps for visual inspection of HPTLC plates. 20 x 20 cm pre-coated Silica Gel 60 F254 TLC aluminum plates (E. Merck, Darmstadt, Germany) with layer thickness 0.2 mm were cut to required size (10 x 10 cm) at the time of use. The TLC plates were washed with methanol by over-run technique and activated at 110 ^{0}C for 5 min. The samples were applied with Linomat IV Sample applicator with the settings- band length, 4mm; distance between bands, 3mm; distance from the plate side edge, 10 mm and distance from the bottom of the plate, 10mm. Linear ascending development was performed in a 10 x 10 cm twin trough glass chamber with stainless steel lid, after its saturation with mobile phase vapour for 10 min. The distance traversed for development being about 8 cm. After development, the plates were dried in a current of warm air and densitometric scanning was performed with a TLC Scanner III at 315 nm in absorbance- reflectance mode.

Chromatographic conditions:

Optimization of mobile phase

Aliquot portions of working standard solution (5 µL) were applied on TLC plates in the form of band. Various pure solvents with varying polarity and their mixtures were tried for optimum movement of drug with sharp symmetrical peak. After trying several permutations and combinations, the mobile phase containing n-Hexane: Ethyl acetate (7.0: 3.0 v/v) was found to be most satisfactory as it gave sharp symmetrical peaks for the drug with R_f value 0.49 ± 0.02 (Fig. 2 a). The migrated band was scanned over the wavelength range 200- 400 nm in an absorbance/reflectance mode and an in-situ UV-absorption spectrum of drug was obtained. A 315 nm was selected as scanning wavelength as it gave maximum absorption for the drug (Fig. 2 b).

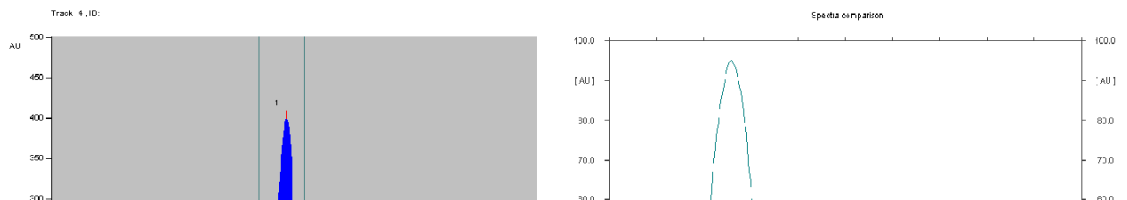
Preparation of Standard solutions: Solution A (Stock Standard solution)

Accurately weighed quantity (10.0 mg) of Febuxostat (FEB) was dissolved in small quantity of methanol and volume was made to 10.0 mL (Conc.: 1.0 mg/mL).

Solution B (Working standard solution)

Accurately measured quantity 0.6 mL of solution A was diluted to 10.0 mL with methanol (Conc.: 60.0 µg/mL).

After chromatographic development, bands were scanned over the range 200–400 nm and in situ spectrum were recorded and thus inferred that the estimations can be done at the maximum wavelength 315 nm. A Densitogram and *in situ* UV spectrum of standard FEB solution under optimized condition are shown in Fig. 2 (a) & (b).



(a) **(b)**

Fig. No. 2: HPTLC Densitogram of FEB Standard (a) and *in situ* UV spectrum of FEB Standard (b)

Assay of FEB in tablet by proposed method

Working standard solution B of FEB was freshly prepared (60.0 µg/mL) as described under preparation of standard solution.

Sample solution: Twenty tablets were weighed and average weight was calculated. The tablets were crushed to fine powder. An accurately weighed quantity of tablet powder equivalent to about 10 mg (~77 mg) of FEB was ultra-sonicated with about 6mL of methanol for 10 min and the volume was made up to 10.0 mL with methanol (Conc.: 1.0 mg/mL). The solution was filtered to get a clear solution. An aliquot portion (0.6 mL) of clear filtrates was diluted to 10.0 mL with methanol to get concentration about 60.0 µg/mL (on labelled claim basis).

Procedure: Two bands of standard solution and six bands of sample solution of equal volume (5 µL) were applied on TLC plate and the plate was developed and scanned as per optimized chromatographic conditions. A Densitogram and *in situ* UV spectrum of sample FEB solution under optimized condition are shown in Fig. 3 (a) & (b).

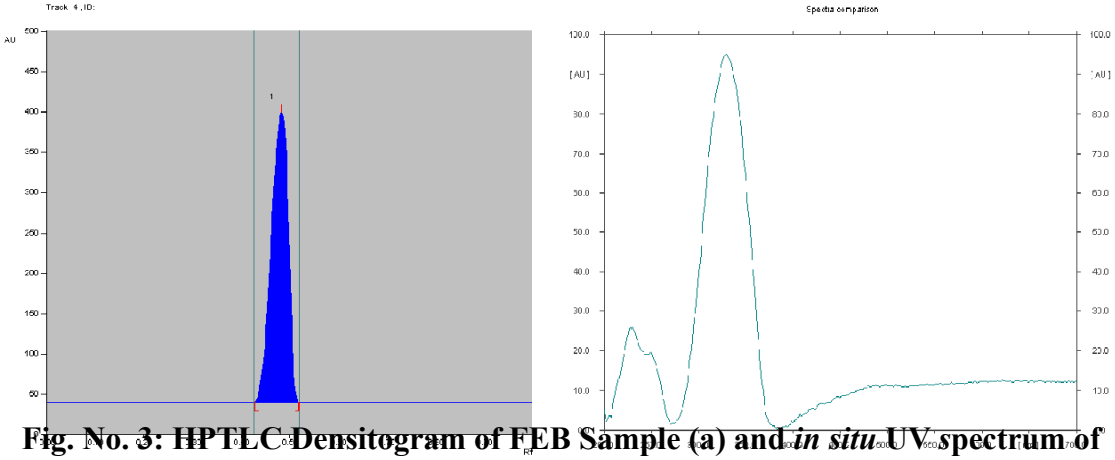


Fig. No. 3: HPTLC Densitogram of FEB Sample (a) and *in situ* UV spectrum of FEB Sample (b)

Calculation:

Percent of labelled claim were calculated using following formula-

$$\% \text{ of Labelled claim} = \frac{Au \times Wstd \times Wav}{Astd \times Ws \times Lc} \times 100$$

where,

Au = area/height of sample peak

Astd = area/height of standard peak

Wstd = standard weight (mg)

Ws = sample weight (mg)

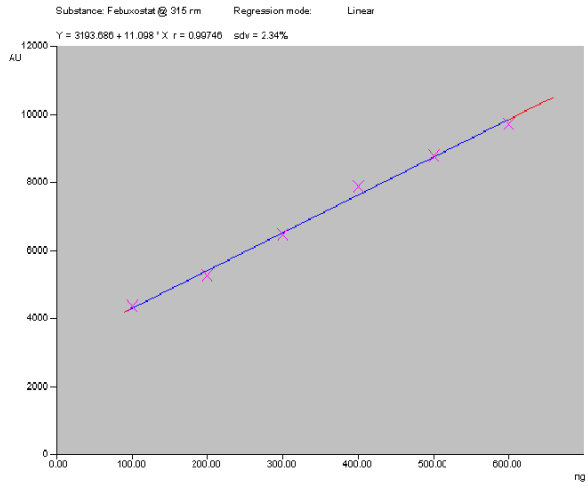
Wav = average weight of tablet (mg)

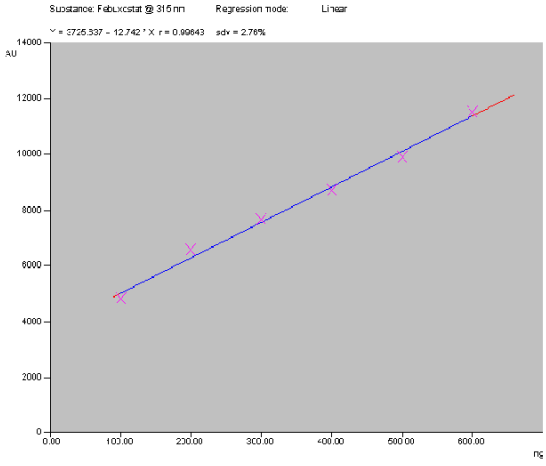
Lc = labelled claim (mg/tablet)

METHOD VALIDATION [8]:

Linearity of response

It was followed using stock standard solution A (0.2- 1.2 mL) diluted to 10.0 mL with methanol to get concentration from 20.0-120.0 µg/mL. Aliquots portions (5 µL) of series of standard solutions of six different concentrations of FEB 20.0, 40.0, 60.0, 80.0, 100.0, 120.0 µg/mL were applied in duplicate (100-600 ng/spot) on TLC plate and chromatograms were developed and scanned under optimized chromatographic conditions. The linear regression curves are depicted along with correlation coefficient; slope and y-intercept by peak height and area are shown in Fig. 4 (a) & (b). The data of linear regression study is given in Table 1.





(a) **(b)**

Fig. No. 4: Linear regression curves of FEB (a) by peak area and (b) by peak height

Table No. 1: Result of Linearity studies of FEB by Peak height & Peak area

Parameter	**Height**	**Area**
Concentration range	**20.0- 120.0 µg/mL (100-600 ηg/spot)**	
Equation for straight line	Y=12.742X+ 3725.637	Y=11.098X+ 3193.686
Slope	12.742	11.098
Y-intercept	3725.637	3193.686
Correlation coefficient	0.99643	0.99746

Table No. 2: Assay of FEB tablet by HPTLC method

FEBOXA tablet (Avg. wt.= 309.63 mg, Labelled claim: 40 mg per tablet)					
Sr. No.	**Wt. of tablet powder taken (mg)**	**Scanner response***		**% Labeled claim***	
		Peak Height	**Peak Area**	**By Height**	**By Area**
1.	77.40	139.1	3879.19	98.23	98.58
2.	77.39	137.8	3781.07	97.89	97.96
3.	77.38	141.7	3917.03	99.18	99.39
4.	77.41	140.2	3856.82	98.52	98.85

5.	77.39	141.1	3884.05	98.86	99.11
6.	77.42	134.3	3728.01	97.94	98.19
7.	Standard	147.4	3991.21	-	-
			Mean	**98.43**	**98.70**
			± SD	**0.5151**	**0.5452**
			% RSD	**0.5233**	**0.5523**

Precision:

Repeatability

Repeatability of results of assay by proposed method was ascertained by replicate analysis (n=6) of homogeneous sample of tablet powder. The results are shown in Table 3.

Intermediate precision

The samples were analysed by proposed method on same day in quick succession (intra-day), on different days (inter-day), and by different analyst. The results of study are given in Table 4.

Table No. 3: Results of Intermediate Precision

Sr. No.	**Observations**	**% of Labeled claim***					
		Intraday		**Interday**		**Different Analyst**	
		By Height	**By Area**	**By Height**	**By Area**	**By Height**	**By Area**
1.	I	99.97	99.99	98.87	99.83	98.99	99.99
2.	II	98.91	100.01	99.13	99.95	99.26	100.00
3.	III	99.99	99.99	98.91	99.97	98.93	99.98
	Mean	**98.56**	**98.88**	**97.91**	**98.07**	**98.12**	**98.58**

	±SD	**0.4805**	**0.5935**	**0.3975**	**0.3962**	**0.5359**	**0.6591**
	%RSD	**0.4875**	**0.6002**	**0.4041**	**0.4039**	**0.5461**	**0.6685**

***** Each value is mean of five observations.

Accuracy:

To check the accuracy of the method, recovery was measured by addition of standard drug at five different levels (70, 85, 100, 115 and 130% of labelled claim) to pre-analyzed sample. Accurately weighed quantities of pre-analyzed tablet powder equivalent to about 8.0 mg of FEB (~ 62 mg) were transferred to five different 10.0 mL volumetric flasks and accurately weighed 1.5, 3.0, 4.5 and 6.0 mg of standard FEB were added to 2nd, 3rd, 4th, 5th flask respectively (representing 70-130 % of labelled claim). This was followed by addition of about 6.0 mL of methanol in each flask and the contents were shaken and sonicated for 10 minutes. Sufficient was added to each flask to adjust the volume to 10.0 mL and filtered. A 0.6 mL of each of the filtrate was diluted to 10.0 mL with methanol. Resultant sample solutions were analyzed as described under assay method. The percent recovery was then calculated at different levels of sample concentration using the formula:

$$\text{Recovery \%} = \frac{T}{B + C}$$

where, T = total drug estimated (mg) B = amount of drug contributed by pre-analyzed tablet powder (mg)

C = weight of pure drug added (mg). The results of study are given in Table 4.

Table No. 4: Results of Recovery study of FEB

		FEBOXA tablet (Avg. wt.= 309.63 mg, Labelled claim: 40 mg per tablet)						
Sr. No.	**Wt. of tablet powder (mg)**	**Amount of standard added (mg)**	**Scanner response**		**Amount of drug recovered (mg)**		**% Recovery***	
			By Height	**By Area**	**By Height**	**By Area**	**By Height**	**By Area**
1.	61.92	2.01	138.1	3867.11	1.98	1.99	98.09	98.17
2.	61.91	2.03	135.8	3775.02	1.99	2.01	98.45	99.97

3.	61.93	2.05	141.3	3908.14	1.97	1.98	98.76	98.59
4.	61.94	2.09	139.5	3846.92	1.99	2.01	98.89	99.98
5.	61.90	2.02	140.7	3878.13	1.96	1.97	98.51	98.77
* Each value is mean of three observations.						**Mean**	**98.54**	**99.09**
						±SD	**0.3091**	**0.8314**
						% RSD	**0.3136**	**0.8390**

Range of method:

A graph was plotted as densitometric response (peak height or area) vs. percent of labelled claim on the basis of accuracy studies data (Table 5).

Table No. 5: Result of Accuracy studies over Range of Method studied

FEBOXA tablet (Avg. wt.= 309.63 mg, Labelled claim: 40 mg per tablet)					
Flask No.	**Wt. of tablet powder (mg) + Wt. of std. drug added (mg)**	**Amount of FEB drug recovered (mg)**		**% Recovery***	
		By Height	**By Area**	**By Height**	**By Area**
1.	54.18 + 0 (70%)	-	-	98.53	98.81
2.	54.19 + 1.5 (85%)	1.47	1.48	98.49	98.72
3.	54.17 + 3.0 (100%)	2.97	2.99	98.48	98.71

4.	54.18 + 4.5 (115%)	4.46	4.47	98.33	99.45
5.	54.16 + 6.0 (130%)	5.96	5.98	99.69	99.91
* Mean of five observations			**Mean**	**98.44**	**98.84**
			± SD	**0.5563**	**0.5378**
			%RSD	**0.5651**	**0.5441**

Table No.6: Results of Range of method by HPTLC

Parameter	**Result**	
	By Height	**By Area**
Range	70-130% of labelled claim	
Equation for straight line	y = 1.4863x - 1.7353	y =39.224x + 13.699
Slope	1.4863	39.224
Y-intercept	(-) 1.7353	13.699
Correlation coefficient	0.9994	0.9998

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

The LOD and LOQ were determined by the method based on standard deviation of the response and slope of calibration curve as per ICH guidelines [8].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ is the standard deviation of the response (estimated by measuring the response in term of peak height or peak area of standard solution of conc. 100 ng/spot for six times and S is the slope of calibration curve (obtained from calibration curve).

The results of study are given in Table 7.

Table No. 7: LOD and LOQ values of FEB

Sr. No.	Parameters	By Height	By Area
1.	**LOD(ηg/spot)**	0.1418	0.4650
2.	**LOQ(ηg/spot)**	0.4298	1.409

Robustness:

The samples were analyzed using proposed method by deliberate small change in the scanning wavelength (315 ± 2 nm) and mobile phases with different compositions (± 0.2 mL) of n-Hexane: Ethyl acetate (7.2: 2.8 v/v, 7.0: 3.0 v/v, 6.8: 3.2 v/v).

The results of study are given in Table 8.

Table No. 8: Results of Robustness study

Sr. No.	Parameters	% Labeled claim			
		Mean± SD		%RSD	
		Height	Area	Height	Area
1.	Change in wavelength				
	313.0 nm	98.66±1.269	98.91± 1.201	1.286	1.214
	315.0 nm	99.91±0.197	100.01±0.101	0.1971	0.100
	317.0 nm	99.14±0.923	99.24± 0.866	0.9310	0.8726

2.	Change mobile phase composition n-Hexane: Ethyl acetate (7.0: 3.0 v/v)				
	7.2 mL : 2.8 mL v/v	99.19±0.812	99.45± 0.968	0.8186	0.9733
	7.0 mL : 3.0mL v/v	99.93±0.701	100.02±0.101	0.7014	0.1009
	6.8 mL : 3.2 mL v/v	98.67±1.273	99.31± 0.862	1.290	0.8679
3.	Change in saturation time				
	10 min	99.23± 0.771	99.56± 0.502	0.7769	0.5042
	15 min	99.94±0.241	100.01±0.103	0.2411	0.1029
	20 min	98.96±1.097	98.97± 0.794	1.108	0.802

Specificity:

The specificity studies were carried out by attempting deliberated degradation of the tablet sample with exposure to stress conditions like acidic (0.1 M HCl), basic (0.1 M NaOH), normal, oxidizing (3% H_2O_2), dry heat (80 ^{0}C) and direct sunlight.

Sample solution: Accurately weighed quantities of tablet powdered equivalent to about 10.0 mg (~ 77 mg) of FEB were transferred to six different 10.0 mL volumetric flasks. The samples were then exposed to stress conditions as follows:

1) Normal (control) for 24 h at room temperature

2) Acidic: At room temperature for 24 h on addition of 1.0 mL of 0.1 M HCl

3) Basic: At room temperature for 24 h on addition of 1.0 mL of 0.1 M NaOH

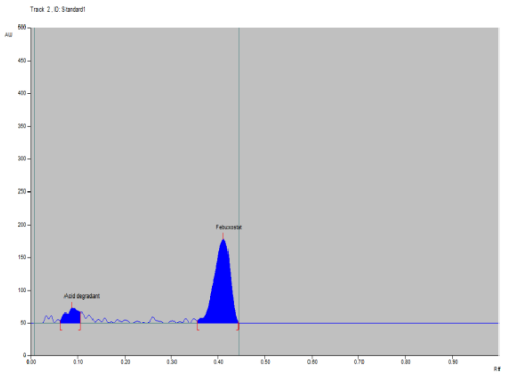
4) Oxidative: At room temperature in dark for 24 h on addition of 1.0 mL of 3 % H_2O_2

5) Dry heat: At 80 ^{0}C for 24 h

6) Sunlight: For 24 h in sunlight on three consecutive days

After stipulated time of each stress conditions the samples were dissolved in methanol and volume was made to 10.0 mL and sonicated for 15 minutes. The solutions were filtered, and 0.6 mL of each filtrate was diluted to 10.0 mL with methanol and analyzed in similar manner as described under assay method.

The typical densitograms and *in situ* spectra of principle spots (analyte) of sample exposed to stress conditions are shown in Fig. No. 5 and results of specificity study are shown in Table No. 9.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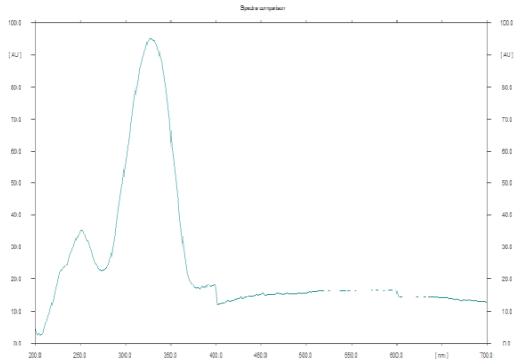
(a)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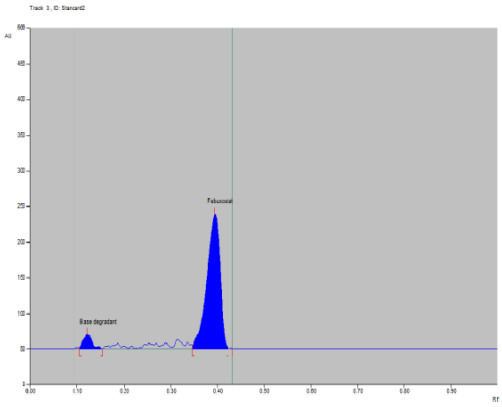
[nm]

(i)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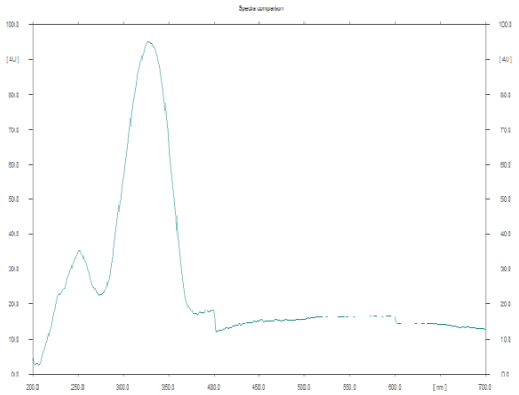
Base degradant
Rt

(b)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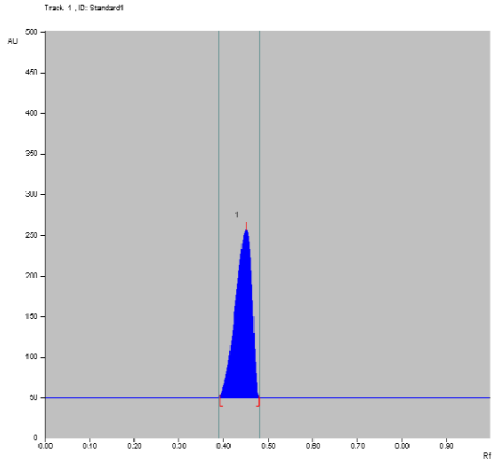
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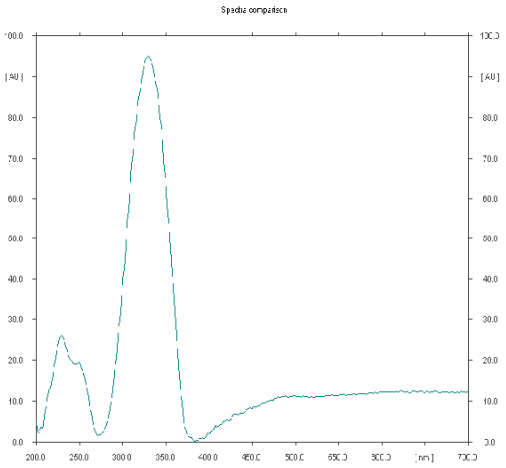
(c)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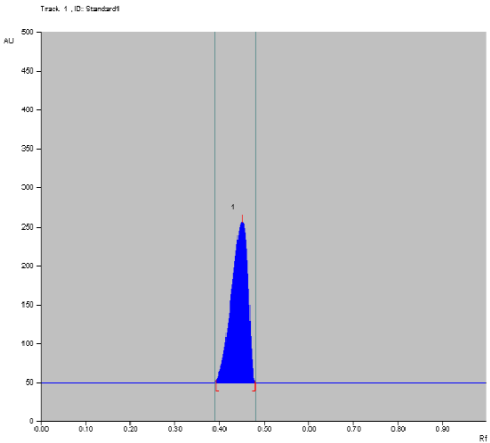
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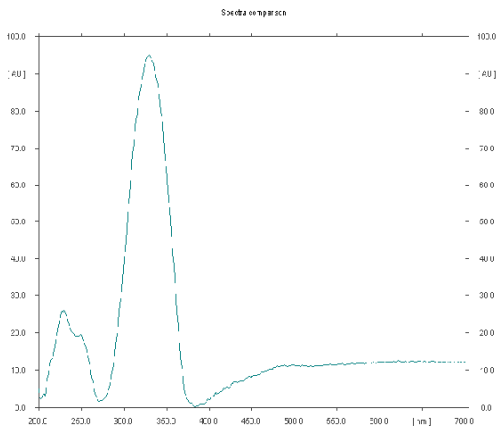
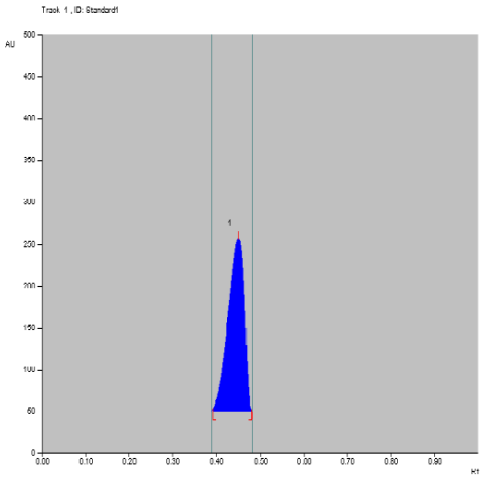
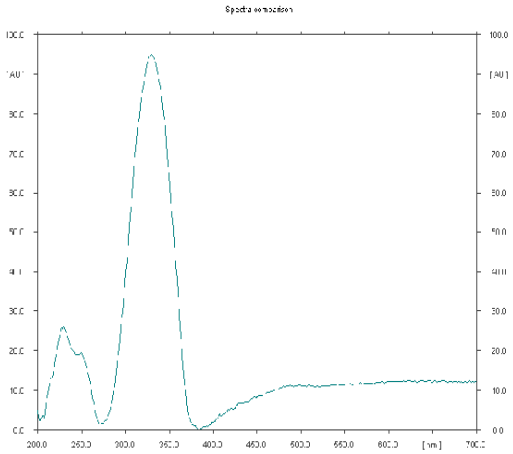
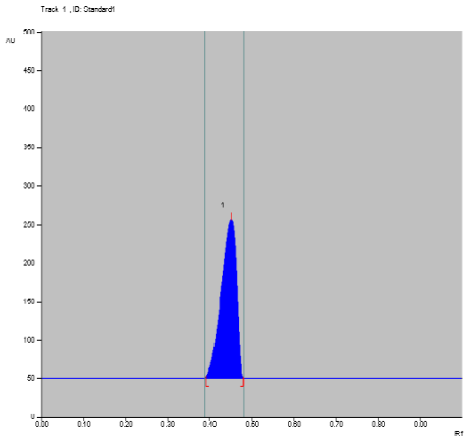

Spectra comparison
[AU]
[nm]

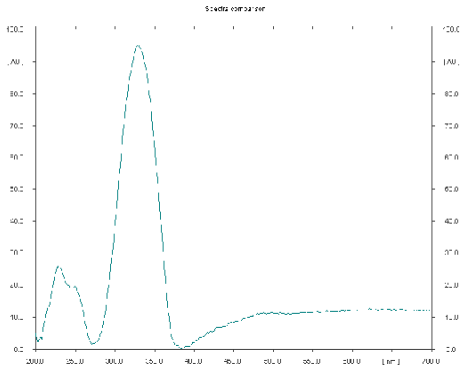
(d) **(iv)**





(e) **(v)**





(f) **(vi)**

Fig. No. 5: HPTLC Densitograms of Specificity studies in (a) acidic, (b) basic, (c) oxidative, (d) thermal (dry heat), (e) photolytic (sunlight) (f) and normal conditions, 24 and corresponding *in situ* UV spectra (i-vi) of FEB

Table No. 9: Results of Specificity study

Sr. No.	Stress Conditions	% Labeled claim*	
		By Height	**By Area**
1.	Acidic	87.16	87.23
2.	Basic	88.21	88.29
3.	Oxidative	97.48	96.61
4.	Dry Heat (60 ºC)	97.31	98.85
5.	Sunlight	98.93	99.84
6.	Normal	99.74	99.89

*Mean of five observations

RESULTS

Optimization of chromatographic conditions:

The mobile phase comprising of mixture of n- Hexane and Ethyl acetate in the ratio 7.0:3.0 v/v have repeatedly yielded sharp symmetrical peaks with Rf value 0.49 ± 0.02 for standard and sample (Fig. 2 a & 3 a). The in-situ UV spectra of developed standard and sample spots indicated 315 nm as suitable wavelength for quantitation of drug (Fig. 2 b & 3 b).

Linearity of response:

A graph plotted as peak height or peak area as a function of concentration of standard was found to be linear over the concentration range of 100-600 ng/spot (Table 1) and Fig. 4 a & b.

Precision and Accuracy:

The assay results of repeatability and intermediate precision studies were found to be quite precise. Accuracy studies over the range of 70-130 % of labelled claim had shown the recoveries of the drug from sample matrix close to about 99 % (Table 2, Table 3 and Table 5).

Range of the method:

A graph plotted on the basis of accuracy studies as response of analyte in sample solution (peak height or peak area) vs. % labelled claim was found to be linear over the range of 70-130 % of labelled claim (Table 6).

Robustness:

The deliberate minor changes in optimized chromatographic conditions did not have any significant effect on the results (Table 8).

Specificity:

Under the mild stress conditions of the sample, the assay results were not affected and were close to normal sample (Table 9). Moreover, no additional peaks were observed in the chromatograms of stress samples indicating the stability of FEB against the stress conditions studied (Fig. 5 A-F).

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD & LOQ values of proposed method are given in Table 7.

DISCUSSION

The results of the repeatability and the intermediate precision were quite reproducible with % RSD value well below 1.0 indicates high level of precision of the proposed method under the conditions studied (Table 2 and Table 3). The recovery studies performed by standard addition method over range of 70-130 % of labeled claim yielded the recovery close to 100 % indicating the capability of the method to accurately measure the drug contents free of interference of excipients. The linear response of the analyte concentration in sample matrix as a function of labeled claim indicates the wide range of accurate measurement of drug content over 70-130 % of labeled claim indicating non-interference of excipients (Table 4 and Table 5). The deliberate small changes in experimental conditions with respect to scanning wavelength and mobile phase composition have no significant effect on the results by the proposed method indicates reasonable robustness of the method (Table 8). Specificity of estimation with respect to degradation product does not appear to be big problem as drug appears to be stable to likely stress it may have to withstand during its shelf life as evident from results of specificity studies (Table 9 & Fig. 5. A-F). The LOD and LOQ values are indicative of sensitivity of the method to detect and to determine the drug content down to few nanograms (Table 7). Moreover, the proposed HPTLC is more sensitive, simpler and suitable alternative for other reported methods with the advantage such as several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis.

CONCLUSION:

The results of the various validation parameters indicate that the method is quite simple, precise, accurate, sensitive and rapid which may be used for routine assay of Febuxostat in tablet.

CONSENT FOR PUBLICATION:

Not applicable.

AVAILABILITY OF DATA AND MATERIALS:

The data and supportive information are available within the article.

FUNDING:

Not applicable.

CONFLICT OF INTEREST:

The authors declare no conflict of interest financial or otherwise.

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