



# Spectrofluorimetric Method for Determination of Febuxostat in Bulk and in Dosage Form

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## ABSTRACT

Febuxostat is a xanthine oxidase inhibitor approved by the US FDA for the management of hyperuricemia in gout patients. The presence of extended unsaturation in planar orientation makes the drug molecule amenable to fluorimetric estimation. The present report describes the validation of simple, rapid, sensitive and cost-effective spectrofluorometric methods based on the native fluorescence of the drug febuxostat in basic medium. Fluorescence characteristics of the drug were found to significantly differ in ethanol ( $\lambda_{\text{excitation}}$  245 nm and  $\lambda_{\text{emission}}$  385 nm) and alkaline borate buffer (pH 10.4) ( $\lambda_{\text{exc}}$  371 nm and  $\lambda_{\text{em}}$  635 nm) and both methods were validated as per the ICH guidelines. The two methods were extremely sensitive, precise and accurate demonstrating excellent linearity in concentration ranges of 0.1-10  $\mu\text{g/ml}$ . The LOD and LOQ values were found to be 0.1255  $\mu\text{g/ml}$  & 0.3803  $\mu\text{g/ml}$  (ethanol) and 0.2183  $\mu\text{g/ml}$  & 0.6616  $\mu\text{g/ml}$  (borate buffer). The proposed methods were used to quantify the drug in its marketed tablet formulation with good recoveries suggesting their applicability to routine analysis of the drug in bulk as well as in formulations.

**Key Words:** Febuxostat; Spectrofluorimetric; Validation; Analysis; Fluorescence.

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## INTRODUCTION

Hyperuricemia is a degenerative disease characterized by increased uric acid levels above the normal range of the body, resulting from uric acid crystal deposition in bone and kidney [1]. Hyperuricemia is noticed more in patients with metabolic syndrome [2-4], and this could be explained by the inflammation occurring in metabolic syndrome [5]. Febuxostat FEB (Uloric<sup>®</sup> by Takeda Pharmaceuticals America, Inc.) (CAS number: 144060-53-7) (N-[2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5-carboxylic acid] (Figure 1) is a xanthine oxidase (XO) inhibitor drug approved by the US FDA for the chronic management of hyperuricemia in patients with gout [6]. It is a non-purine selective XO inhibitor that inhibits both oxidized and reduced types of XO and does not require dosage adjustment in patients with mild or moderate renal impairment. It is a more effective alternative to the prototypic XO inhibitor allopurinol in reducing serum urate levels in gout patients [7].

Some reports are there in the literature on the development of stability-indicating methods for febuxostat by UPLC, RP-HPLC [5-10], and spectrofluorimetric [11] methods. The reverse-phase chromatographic methods are used for the estimation of febuxostat in biological fluids such as dog and human plasma [12-14]. Metabolism and excretion of <sup>14</sup>C-febuxostat in humans have been studied through LC-MS by Grabowski et al [15]. Studies have also been reported on the chromatographic estimation of genotoxic impurities [16] and process-related impurities [17] in the febuxostat drug substance. In another study, the stress-degradation products of febuxostat have been identified through LC-MS studies [18]. Spectrofluorimetry is a highly sensitive, economical and simple technique that can be useful for the development of analytical methods for the estimation of drugs present in extremely low amounts. Considering the amenability of febuxostat molecule to spectrofluorimetric estimation, the present work was designed to develop simple, rapid, and reproducible spectrofluorimetric methods for the quantification of

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febuxostat in bulk as well as in its marketed tablet dosage forms.

## EXPERIMENTAL

### MATERIALS AND METHODS

#### Chemicals and reagents

All chemicals and materials were of analytical grade purchased from Merck India Pvt. Ltd., Mumbai and all solutions were freshly prepared in triple distilled water. Febuxostat (Batch number D8001247, Material Code 310299) was kindly gifted by Cipla Pharma Ltd, Mumbai (India). Sodium hydroxide, hydrochloric acid, hydrogen peroxide (30%) and methanol, used for sample preparation, were of AR grade and were procured commercially from Merck India Pvt. Ltd., Mumbai. All the glassware including volumetric flask, pipette, measuring cylinder, beakers, round bottom flask were of Class A apparatus from Borosil. Tablet formulation containing febuxostat 40 mg (Febutax® 40, manufactured by Leeford Healthcare Pvt. Ltd) with batch no. 4399001 was purchased from a local market.

#### Instruments

All absorption and emission spectra were recorded using a Hitachi spectrofluorimeter F2500 equipped with a 150W xenon lamp in self-deozonating lamp housing, grating excitation and emission monochromators, 1 cm pathlength cell, wavelength drive speed of 12,000 nm/min, and a resolution of 2.5 nm. Slit widths for excitation and emission monochromators were set at 5 nm. Melting point apparatus (model T0603160; EIE Instruments Pvt. Ltd., Ahmadabad, India) was used for the determination of the melting point of febuxostat. Digital pH meter (Eutech Instruments, model GC7252101B) was used to adjust the pH of the buffer solution.

#### Preparation of solutions

##### Preparation of borate buffer pH 10.4

Borate buffer pH 10.4 was prepared by dissolving 26.64 g of boric acid in about 900 ml of triple distilled water and adding sufficient 10M NaOH solution to adjust the pH to 10.4. The final volume was made to 1000 ml with triple distilled water. The pH of the buffer solution was adjusted using a digital pH meter.

##### Sample preparation and analysis

Standard Stock solution (1000.0 µg/ml) was prepared daily by dissolving 10.0 mg of febuxostat in 10 ml of the solvent (borate buffer pH 10.4 or absolute ethanol). This was diluted 1 in 10 to obtain the stock solution (100 µg/ml). Further, different aliquots of working standard solutions, ranging from 0.1 µg/ml to 100 µg/ml of febuxostat were prepared by serial dilutions of the stock solutions with an

appropriate solvent. Fluorescence intensities of these solutions were recorded taking 245 nm and 385 nm as the excitation and emission wavelengths respectively against the reagent blank.

#### Validation of the method

The optimized method was validated with respect to various parameters outlined in the ICH guideline Q2(R1).

- **Linearity and range**

The working standard solution (100.0 µg/ml) was serially diluted with appropriate reagent (borate buffer pH 10.4 or absolute ethanol) to prepare solutions with concentrations ranging from 0.1-100.0 µg/ml. The fluorescence intensity of all these solutions, prepared in triplicate, was noted at the selected wavelength.

- **Precision**

Solutions of six different concentrations of the drug (0.5, 4.0 and 20.0 µg/ml) were analyzed on the same day (to determine intra-day precision) and three samples were analyzed on three consecutive days (to determine inter-day precision). The precision was expressed as % RSD of each calculated concentration of the analyte.

- **Accuracy**

The pre-analyzed solution of the pure drug was appropriately diluted to obtain the unfortified solutions of the drug (10.0 µg/ml). The unfortified solution was mixed separately with equal volumes of the standard drug solutions having concentrations 20.0, 30.0, and 40.0 µg/ml so that the drug concentration was fortified by 5.0, 10.0, and 15.0 µg/ml (50%, 100%, and 150%) respectively. The drug concentration in each fortified and unfortified solution was determined (n=3) and accuracy was expressed as the percent recovery of the fortified drug concentration with reference to the unfortified one.

- **Robustness**

Robustness was assessed by carrying out deliberate changes in the method variables including voltage,  $\lambda_{excitation}$ ,  $\lambda_{emission}$ , analyst performing the study and studying their impact on the recovery of the drug in the test solutions.

#### Analysis of pharmaceutical formulation

Twenty tablets of febuxostat (Febutax® 40, Leeford Healthcare Pvt. Ltd) with a label claim of 40 mg per tablet were weighed, crushed, and powdered. Powder weight equivalent to 10 mg of febuxostat was suspended in ethanol/borate buffer, sonicated for 5 minutes and filtered. The volume was made up to 100 ml (final drug solution 100 µg/ml). The solution was suitably diluted and fluorescence intensity was noted.

## RESULTS AND DISCUSSION

In this report, a reliable and reproducible spectrofluorometric method of analysis for febuxostat has been developed and validated and its stability-indicating potential was also assessed.

### Febuxostat as a fluorophore

Febuxostat is a thiazole-carboxylic acid substituted with a phenyl group which creates an extended conjugated system of unsaturation in the molecule in a planar orientation. Hence, systematic analysis of fluorescence characteristics of the drug in various solvents/buffers revealed that the drug possesses a good native fluorescence in the alkaline medium as well as in ethanol without any requirement of any type of fluorimetric enhancers. Based on preliminary absorption/fluorescence studies and solubility characteristics of the drug, borate buffer (pH 10.4) and absolute ethanol were selected as media for carrying out the fluorimetric analysis of febuxostat. The excitation and emission spectra for the working standard solutions of febuxostat ranging from 0.1 µg/ml to 20.0 µg/ml were recorded over the range 210-400 nm and 400-800 nm. In absolute ethanol, the excitation and emission wavelengths for spectrofluorimetric analysis were selected as 245 nm and 385 nm respectively. The fluorescence intensity was determined after taking 245 nm and 385 nm as the excitation and emission wavelength respectively against the reagent blank. The UV absorption and fluorescence characteristics of the drug were significantly changed in borate buffer (pH 10.4) attributable to the ionization of the carboxylic acid group. Fluorescence intensity in borate buffer was determined taking 371 nm and 635 nm as the excitation and emission wavelengths respectively. Figures 2 and 3 show the emission/excitation scans in absolute ethanol and borate buffer respectively.

### Validation of the analytical method

The methods were validated with respect to linearity and range, accuracy and precision, the limit of detection (LOD) and limit of quantification (LOQ), and robustness. The various validation parameters are summarized in Tables 1 and 2. Stability indicating the nature of the assays was assessed by fortifying a mixture of degraded solutions with three known concentrations of the drug. The recovery of the added drug was then determined.

- **Linearity and Range**

A strictly linear relation was observed between the fluorescence intensity and the concentration of febuxostat (in absolute ethanol) in the concentration range of 0.1-20.0 µg/ml. The corresponding calibration curve was described by the equation  $y = 22.203 x - 3.9224$  ( $n=3$ ,  $r^2 = 0.9938$ ) (Figure 4). Linearity range for fluorimetric method in borate buffer was 0.1-10.0 µg/ml and the corresponding calibration

curve was described by the equation  $y = 3.2405 x - 0.0107$  ( $n=3$ ,  $r^2 = 0.9963$ ) (Figure 4).

- **Limits of Detection (LOD) and Quantification (LOQ)**

The LOD and LOQ were calculated using the formulas ( $3.3 \sigma/s$ ) and ( $10 \sigma/s$ ), respectively, where  $\sigma$  is the standard deviation of the response (calculated from the standard deviation of intercept) and  $s$  is the slope of the calibration curve. The slopes and intercepts of calibration plots for three sets of fluorescence intensities in linearity studies were calculated and taken for calculation of LOD and LOQ values.

Solutions of the drug having concentrations corresponding to LOD and LOQ values were prepared and analyzed six times ( $n = 6$ ) for the recovered amount determined from the corresponding calibration curve. The LOD and LOQ values in ethanol were found to be 0.1255 and 0.3803 µg/ml respectively whereas, in borate buffer, these values were found to be 0.2183 and 0.6616 µg/ml respectively.

- **Accuracy**

The accuracy of the proposed methods was assessed by preparing different concentration levels of drug for analysis from independent stock solutions. Further assessment of the accuracy of the developed methods was done by spiking excess drug (50%, 100%, and 150 %) to pre-analyzed drug solution samples (10 µg/ml) (Tables 1 and 2). Accuracy was determined as the mean % recovery of the spiked drug concentration. Good recoveries in both the methods, i.e., 93.17%-97.02% (absolute ethanol) and 90.33%-102.07% (borate buffer) respectively indicated good accuracies.

- **Precision**

Both methods were found sufficiently precise with low % RSD values for the intra-day and inter-day precision (below 1.30 % and 1.80 % respectively in absolute ethanol; below 1.80 % and 1.90 % respectively in borate buffer) (Tables 1 and 2). This showed that the method was sufficiently precise for determining the drug concentrations.

- **Robustness**

The results from the robustness studies for both the methods are shown in Table 3. The methods were found to be robust as no significant changes in fluorescence intensity were observed after deliberate changes in the method variables including the excitation wavelength, emission wavelength, voltage and analyst performing the study. The % RSD values in all cases were found to be less than 1.70 % (absolute ethanol) and 1.34% (borate buffer pH 10.4).

### Stability

The responses with fluorescence measurements were found to be stable for at least 7 hours at room temperature which

indicated the stability of the final sample solutions for at least 7 h.

### Analysis of pharmaceutical formulation

The final drug solution (100 µg/ml) obtained by sonication of tablet powder in ethanol/borate buffer was suitably diluted and fluorescence intensity of the resulting dilutions was noted. Tables 1 and 2 show the results of the assay by the proposed methods. Good recovery was obtained with both the methods, i.e., 97.13 % (absolute ethanol) and 97.26 % (borate buffer) showing a close agreement between the results obtained by the proposed methods and the label claim (40 mg per tablet).

### CONCLUSIONS

In this work, sensitive spectrofluorimetric methods have been proposed for the determination of febuxostat in bulk as well as in its marketed formulation (tablets) utilizing native fluorescence of the drug. The fluorescence characteristics of the drug were found to differ significantly in neutral (ethanol) and alkaline (borate buffer pH 10.4) medium. Computed validation parameters suggest the methods to be sufficiently precise, accurate, reproducible, and robust with solution stability for up to 7 hours. The proposed methods have been used to quantify the drug in its marketed tablet formulation with good recoveries suggesting their applicability to routine analysis of the drug in bulk as well as in formulations.

### Conflict of interest statement

The authors declared no conflict of interest.

### ACKNOWLEDGMENTS

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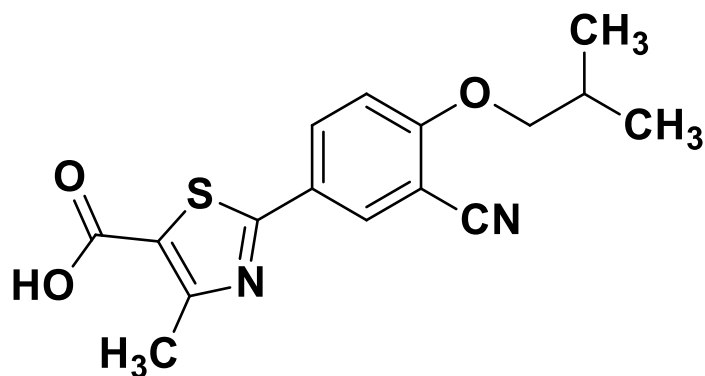


Fig. 1. Structure of Febuxostat.

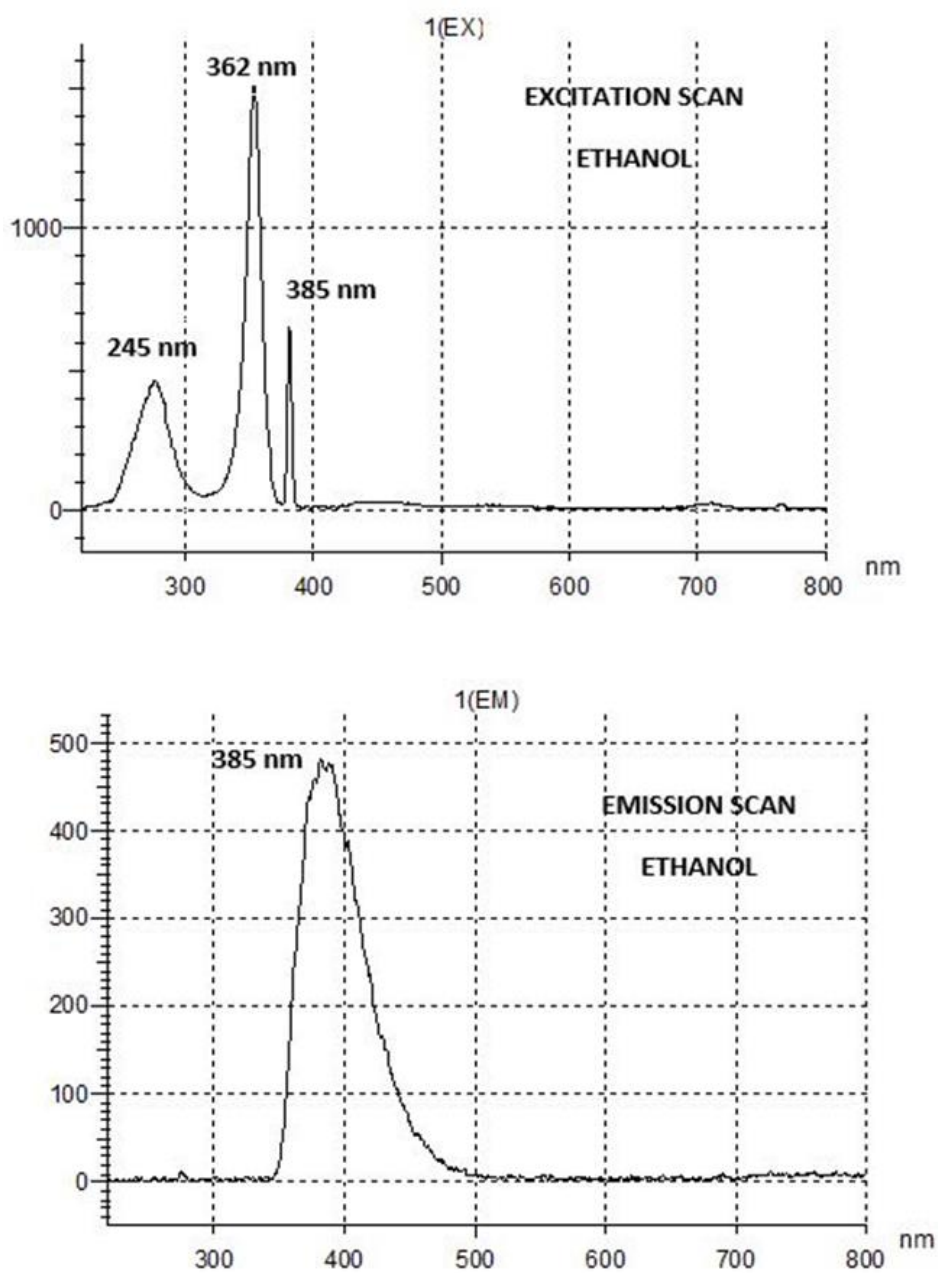


Fig. 2. Excitation and emission scans of febuxostat in absolute ethanol.

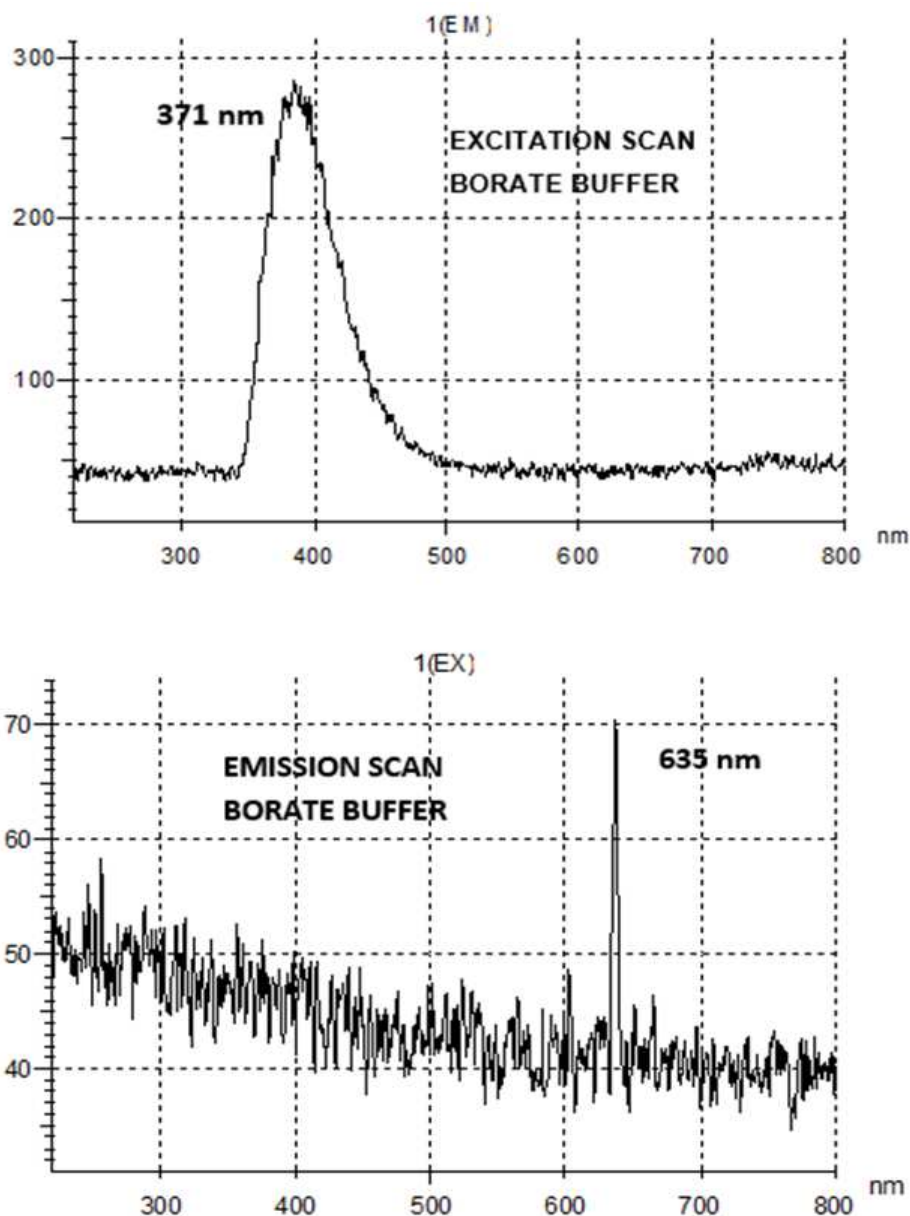


Fig. 3. Excitation and emission scans of febuxostat in borate buffer (pH 10.4).

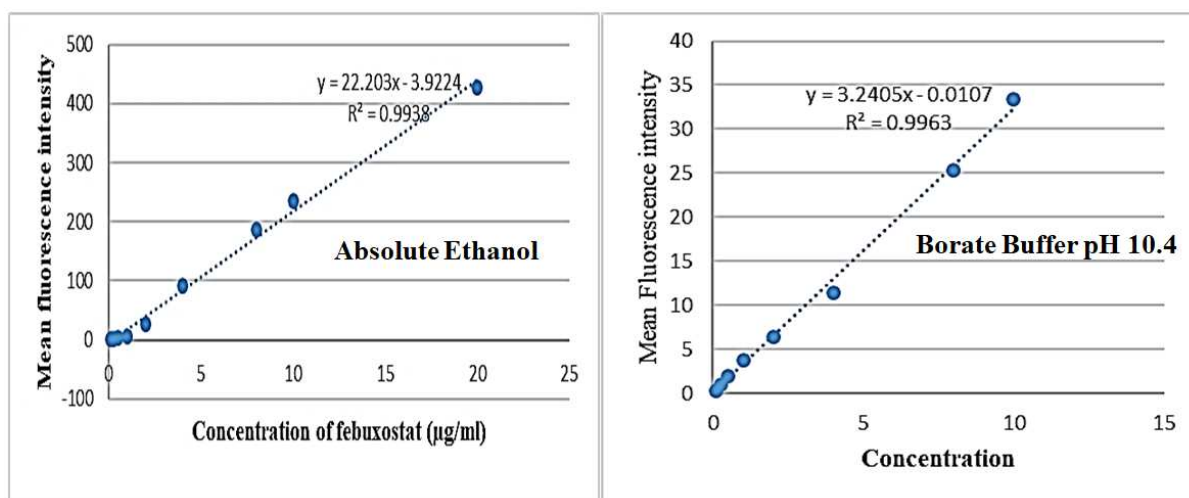


Fig. 4. Calibration curves for spectrofluorimetric analysis of febuxostat in absolute ethanol and borate buffer pH 10.4.

**Table 1.** Validation parameters for spectrofluorimetric determination of febuxostat in absolute ethanol.

Parameter	FEBUXOSTAT			
Accuracy	Concentration (µg/ml) ± S.D.; % RSD <sup>#</sup>			
	Concentration of drug taken (µg/ml)	Concentration of standard added(µg/ml)*	Calculated**	% Recovery
	10	5.0 (50%)	13.97± 4.84; 1.58%	93.17%
	10	10.0 (100%)	18.92± 6.92; 1.66%	94.61%
	10	15.0 (150%)	24.25± 11.58; 2.16%	97.02%
Precision	Calculated concentration (µg/ml) ± S.D.; % RSD			
	Concn. taken (µg/ml)	Intra-day (n = 6)	Inter-day (n = 3)	
	0.5	0.29± 0.03; 1.28%	0.38 ± 0.05; 1.22%	
	4.0	4.16 ± 0.62; 0.70%	4.21 ± 1.56; 1.73%	
	20.0	19.68 ± 0.88; 0.20%	19.39 ± 6.03; 1.41%	
Linearity	Range (µg/ml)	Slope	Intercept	Coefficient of correlation r <sup>2</sup>
	0.1 - 20.0	22.203	3.9258	0.9938
LOD & LOQ	0.1255 µg/ml & 0.3803 µg/ml			
Recovery (± SD); % RSD) in tablet samples (label claim 40 mg/tablet)			38.8520 ± 0.70 mg; 1.80%	
			97.13 ± 1.75%; 1.80%	

**Table 2.** Validation parameters for spectrofluorimetric determination of febuxostat in borate buffer pH 10.4.

Parameter	FEBUXOSTAT			
Accuracy	Calculated concentration (µg/ml) ± S.D.; % RSD			
	Concentration of drug taken (µg/ml)	Concn of standard added (µg/ml)*	Calculated**	% Recovery
	10.0	5.0 (50%)	13.55± 1.26; 2.88%	90.33%
	10.0	10.0 (100%)	20.41± 0.79; 1.19%	102.07%
	10.0	15.0 (150%)	23.97±0.81; 21.05%	95.91%
Precision	Calculated concentration (µg/ml) ± S.D.; % RSD			
	Concn. taken (µg/ml)	Intra-day (n = 6)	Inter-day (n = 3)	
	0.1	0.09 ± 0.005; 1.76%	0.11 ± 0.006; 1.89%	
	1.0	1.15 ± 0.057; 1.53%	1.16 ± 0.056; 1.50%	
	10.0	9.72 ± 0.029; 0.09 %	9.90 ± 0.55; 1.74%	
Linearity	Range (µg/ml)	Slope	Intercept	Coefficient of correlation r <sup>2</sup>
	0.1 - 10.0	3.2405	0.0107	0.9963
LOD & LOQ	0.2183 µg/ml & 0.6616 µg/ml			
Recovery (± SD); % RSD) in tablet samples (label claim 40 mg/tablet)			38.9056 ± 0.75 mg; 1.92%	
			97.26 ± 1.93%; 1.98%	

**Table 3.** Robustness of the proposed methods.

Parameter	Change	Fluorescence intensity			Mean	SD	% RSD
Method (Absolute ethanol)							
Optimized conditions	NA	245.2	240.46	238.67	241.44333	3.374230	1.39524



<b>Voltage (400 eV)</b>	700	259.89	265.41	260.13	261.81	3.12	1.191703
<b>Excitation <math>\lambda_{\max}</math> 245 nm</b>	250	238.88	236.79	238.09	237.92	1.055319	0.443560
<b>Excitation <math>\lambda_{\max}</math> (85:15)</b>	390	220.36	215.78	223.15	219.76333	3.721052	1.693209
<b>Analyst I</b>	Analyst II	236.47	238.91	239.58	238.32	1.636795	0.686805
<b>Method (Borate Buffer pH 10.4)</b>							
<b>Optimized conditions (10ug/ml)</b>	NA	32.45	32.56	33.03	32.68	0.30	0.94
<b>Voltage (400 eV)</b>	700	45.64	46.10	44.89	45.54	0.61	1.34
<b>Excitation <math>\lambda_{\max}</math> 315 nm</b>	320	22.4	22.31	22.82	22.51	0.27	1.20
<b>Emission <math>\lambda_{\max}</math> 635 nm</b>	640	26.31	25.89	25.94	26.04	0.22	0.88
<b>Analyst I</b>	Analyst II	32.88	32.06	32.56	32.5	0.41	1.27

### SPECTROFLUORIMETRIC DETERMINATION OF FEBUXOSTAT IN ABSOLUTE ETHANOL

**Table S1:** Fluorescence intensities in linearity studies for the proposed method.

<b>Drug conc. (µg/ml)</b>	<b>Fluorescence intensities</b>		
	<b>Set I</b>	<b>Set II</b>	<b>Set III</b>
0.1	0.6	1.95	2.08
0.25	1.6	3.13	3.34
0.5	2.55	4.06	5.88
1.0	5.49	5.29	5.37
2.0	30.08	26.32	25.43
4.0	87.79	94.32	95.01
8.0	201	178	183.44
10.0	245.2	225	235.38
20.0	432.88	426.02	420.87
<b>Slope</b>	<b>22.758</b>	<b>21.951</b>	<b>21.899</b>
<b>Intercept</b>	<b>4.0311</b>	<b>4.7073</b>	<b>3.0287</b>
<b>r<sup>2</sup></b>	<b>0.9910</b>	<b>0.9960</b>	<b>0.9927</b>

**Table S2:** Data for calculation of LOD and LOQ.

	<b>Set I</b>	<b>Set II</b>	<b>Set III</b>	<b>Mean</b>	<b>Std. Dev</b>
<b>Slope</b>	22.758	21.951	21.899	22.2026**	0.4816
<b>Intercept</b>	4.0311	4.7073	3.0287	3.9223	0.8845*

\*Standard deviation of the response.

\*\*Mean slope of calibration plot.

**Table S3:** Recovery in LOD and LOQ studies.

<b>Set No.</b>	<b>Fluorescence intensities for drug concentration</b>	
	<b>0.12 µg/ml*</b>	<b>0.38 µg/ml**</b>
<b>1</b>	0.84	84530
<b>2</b>	0.83	84326
<b>3</b>	0.85	84596
<b>4</b>	0.87	84257
<b>5</b>	0.86	84229

<b>6</b>	0.85	84653
<b>Mean</b>	0.85	3.076
<b>S.D.</b>	0.0141	0.045
<b>% RSD</b>	1.66	1.463

\*LOD value

\*\*LOQ value

**Table S4:** Recovery in LOD and LOQ studies.

Set No.	Fluorescence intensities for drug concentration	
	0.12 µg/ml*	0.38 µg/ml**
<b>1</b>	0.84	84530
<b>2</b>	0.83	84326
<b>3</b>	0.85	84596
<b>4</b>	0.87	84257
<b>5</b>	0.86	84229
<b>6</b>	0.85	84653
<b>Mean</b>	0.85	3.076
<b>S.D.</b>	0.0141	0.045
<b>% RSD</b>	1.66	1.463

\*LOD value

\*\*LOQ value

**Table S5:** Fluorescence intensities in the intra-day precision studies with pure drug febuxostat.

Concn (µg/ml)	Fluorescence intensity					
	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
0.5	2.55	2.56	2.57	2.60	2.61	2.62
4.0	87.79	87.73	87.81	88.94	88.94	88.95
20.0	432.88	433.31	434.85	431.94	431.98	432.54

**Table S6:** Fluorescence intensities in the inter-day precision studies with pure drug febuxostat.

Set No.	Fluorescence intensity								
	I			II			III		
	0.5 µg/ml	4.0 µg/ml	20.0 µg/ml	0.5 µg/ml	4.0 µg/ml	20.0 µg/ml	0.5 µg/ml	4.0 µg/ml	20.0 µg/ml
<b>1</b>	2.50	87.79	432.88	4.06	94.32	426.02	5.88	95.01	420.87
<b>2</b>	4.50	87.80	433.10	4.10	89.49	426.13	4.60	86.94	420.97
<b>3</b>	6.80	89.00	433.25	6.00	86.90	426.44	3.40	87.05	421.31

**Table S7:** Precision of the proposed method for analysis of febuxostat.

	Calculated concentration (µg/ml) ± S.D.; % RSD		
	Concn. taken (µg/ml)	Intra-day (n = 6)	Inter-day (n = 3)
	0.5	0.29 ± 0.03; 1.28%	0.38 ± 0.05; 1.22%
	4.0	4.16 ± 0.62; 0.70%	4.21 ± 1.56; 1.73%
	20.0	19.68 ± 0.88; 0.20%	19.39 ± 6.03; 1.41%

**SPECTROFLUORIMETRIC DETERMINATION OF FEBUXOSTAT IN BORATE BUFFER (pH 10.4)**

**Table S8:** Fluorescence intensities in linearity studies for the proposed method.

Drug concn. (µg/ml)	Fluorescence intensities		
	Set I	Set II	Set III
0.1	0.22	0.35	0.48
0.25	0.65	1.12	1.21
0.5	1.76	2.13	2.18
1.0	3.68	3.78	3.81
2.0	4.09	6.74	8.25
4.0	9.64	11.52	13.24
8.0	24.45	25.46	26.13
10.0	31.45	33.25	35.45
<b>Slope</b>	<b>3.1063</b>	<b>3.2256</b>	<b>3.3898</b>
<b>Intercept</b>	<b>0.5447</b>	<b>0.1212</b>	<b>0.3914</b>
<b>r<sup>2</sup></b>	<b>0.9893</b>	<b>0.9966</b>	<b>0.9957</b>

**Table S9:** Data for calculation of LOD and LOQ.

	Set I	Set II	Set III	Mean	Std. Dev
<b>Slope</b>	3.1063	3.2256	3.3898	3.2405 **	0.1423
<b>Intercept</b>	0.5447	0.1212	0.3914	0.3525	0.2144 *

\*Standard deviation of the response.

\*\*Mean slope of calibration plot.

**Table S10:** Recovery in LOD and LOQ studies.

Set No.	Fluorescence intensities for drug concentration	
	0.2183 µg/ml*	0.6616 µg/ml**
<b>1</b>	0.84	2.62
<b>2</b>	0.83	2.55
<b>3</b>	0.85	2.60
<b>4</b>	0.87	2.61
<b>5</b>	0.86	2.58
<b>6</b>	0.85	2.59
<b>Mean</b>	0.85	3.076
<b>S.D.</b>	0.0141	0.045
<b>% RSD</b>	1.66	1.463

\*LOD value

\*\*LOQ value

**Table S11:** Fluorescence intensities in the intra-day precision studies with pure drug febuxostat.

Conc. (µg/ml)	Fluorescence intensities					
	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
0.1	0.23	0.33	0.31	0.26	0.35	0.27
1.0	3.68	3.70	3.69	3.71	3.73	3.75
10.0	31.45	31.56	31.56	31.47	31.45	31.48

**Table S12:** Fluorescence intensities in the inter-day precision studies with pure drug febuxostat.

Set no.	Fluorescence intensities								
	I			II			III		
	0.1 µg/ml	1.0 µg/ml	10.0 µg/ml	0.1 µg/ml	1.0 µg/ml	10.0 µg/ml	0.1 µg/ml	1.0 µg/ml	10.0 µg/ml
1	0.22	3.68	31.45	0.35	3.78	33.25	0.48	3.81	31.45
2	0.36	3.70	31.47	0.36	3.75	31.0	0.29	3.81	31.49
3	0.51	3.71	34.0	0.37	3.79	33.30	0.35	3.80	31.44

**Table S13:** Precision of the proposed method for analysis of febuxostat.

Calculated concentration (µg/ml) ± S.D.; % RSD		
Conc. taken (µg/ml)	Intra-day (n = 6)	Inter-day (n = 3)
0.1	0.09 ± 0.005; 1.76%	0.11 ± 0.006; 1.89%
1.0	1.15 ± 0.057; 1.53%	1.16 ± 0.056; 1.50%
10.0	9.72 ± 0.029; 0.09 %	9.90 ± 0.55; 1.74%