Analytical Method Development and Validation of Febuxostat using RP-HPLC

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ABSTRACT

Robust analytical method development and validation are indispensable pillars of pharmaceutical quality assurance, directly influencing the safety and efficacy of drug products released to patients. Febuxostat is a selective, non-purine xanthine oxidase inhibitor prescribed for the chronic management of hyperuricemia in gout patients. Reliable and well-characterized analytical methods are therefore essential for its quality control testing and regulatory submissions.

The present study focuses on the development and validation of a simple, rapid, precise, and economical Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the quantitative estimation of Febuxostat. Preliminary characterization of the drug was carried out using UV spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Differential Scanning Calorimetry (DSC). Various chromatographic conditions were investigated to obtain optimum peak shape, resolution, and retention characteristics.

The developed method was validated according to International Council for Harmonisation (ICH) guidelines for parameters including specificity, linearity, accuracy, precision, robustness, limit of detection, and limit of quantitation. The validated method demonstrated satisfactory analytical performance and can be successfully employed for routine quality control analysis of Febuxostat in bulk drug and pharmaceutical dosage forms.

Keywords: Febuxostat; RP-HPLC; Analytical Method Development; Method Validation; FTIR; DSC; UV Spectroscopy; ICH Q2(R1); Pharmaceutical Analysis; Quality Control.

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INTRODUCTION

Ensuring the quality, safety, and therapeutic efficacy of pharmaceutical products is inseparable from the availability of robust analytical methods **[1,2]**. Throughout a drug's lifecycle—from raw material characterization to finished product release—analytical chemistry serves as a critical backbone that underpins every quality decision **[3]**. Regulatory agencies such as the ICH and USFDA mandate that all analytical procedures be scientifically sound, reproducible, and thoroughly validated before their adoption in quality control laboratories **[3,4]**. Among the broad spectrum of analytical techniques currently in use, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has emerged as one of the most widely preferred approaches, owing to its exceptional selectivity, sensitivity, and ability to handle complex pharmaceutical matrices with high reproducibility **[5,6]**.

Gout is a well-recognized metabolic disorder characterized by persistently elevated serum uric acid concentrations, a condition referred to as hyperuricemia **[7,8]**. When uric acid levels surpass the threshold of physiological solubility, monosodium urate crystals begin to deposit in articular and periarticular tissues, triggering recurrent bouts of intense inflammatory arthritis, progressive joint destruction, and in severe cases, tophi formation **[8,9]**. Over recent decades, the global prevalence of gout has risen considerably, driven by shifts in dietary patterns, rising rates of obesity, widespread use of diuretics, and the

growing burden of comorbidities such as chronic kidney disease and metabolic syndrome **[7,9]**. Long-term reduction of serum urate to below the saturation threshold is widely accepted as the cornerstone of effective gout management to prevent disease progression and recurrent flares **[8]**. Xanthine oxidase (XO) is the terminal enzyme in the purine catabolic pathway, catalyzing the sequential oxidation of hypoxanthine to xanthine and xanthine to uric acid **[7,8]**. Pharmacological inhibition of this enzyme offers a rational and well-validated strategy for lowering uric acid biosynthesis in hyperuricemic patients **[9]**. Febuxostat (2-[3-cyano-4-(2-methylpropoxy)phenyl]-4-methyl-1,3-thiazole-5-carboxylic acid) is a potent, selective, non-purine xanthine oxidase inhibitor that was approved for the chronic management of hyperuricemia in gout patients **[7,10]**. Unlike allopurinol, febuxostat inhibits both the oxidized and reduced forms of xanthine oxidase and is primarily metabolized by glucuronidation, making it usable in patients with mild-to-moderate renal impairment **[8,10]**. Given its expanding clinical use and therapeutic significance, the establishment of sensitive, reliable analytical methods for the quantitative determination of febuxostat in bulk drug substances and pharmaceutical formulations is of considerable practical importance for quality control and regulatory compliance **[11–13]**.

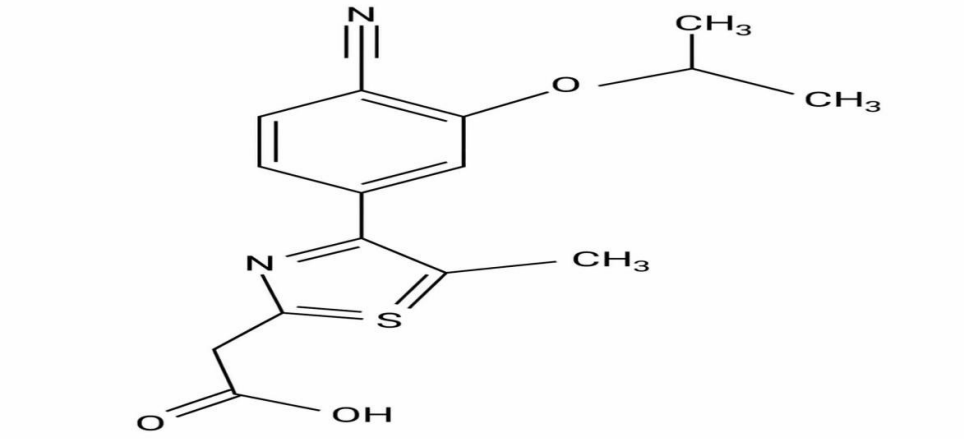

Figure 1: Chemical Structure of Febuxostat

Molecular Formula: $C_{16}H_{16}N_2O_3S$

IUPAC Name:

2-[3-cyano-4-(2-methylpropoxy)phenyl]-4-methyl-1,3-thiazole-5-carboxylic acid

Before embarking on chromatographic method development, a thorough physicochemical characterization of the drug substance is considered an important prerequisite, as it informs decisions regarding solvent selection, mobile phase optimization, and detection parameters **[1,2]**. In the present investigation, febuxostat was characterized using three complementary analytical techniques: UV spectroscopy, to identify the wavelength of maximum absorbance (λmax) suitable for detection; Fourier Transform Infrared Spectroscopy (FTIR), to confirm the integrity of characteristic functional groups present in the molecule; and Differential Scanning Calorimetry (DSC), to assess its thermal behavior, melting characteristics, and crystalline purity. The information gathered from these preformulation studies formed a sound scientific basis for the subsequent development and optimization of the RP-HPLC method.

Building on the preformulation data, an RP-HPLC method was systematically developed and optimized for the quantitative estimation of febuxostat. Mobile phase composition, flow rate, column selection, and detection wavelength were carefully varied to achieve acceptable peak symmetry, adequate retention, and robust analytical performance **[11,12,16]**. Once optimized, the method was rigorously validated in accordance with ICH Q2(R1) guidelines, covering the key performance characteristics of specificity, linearity and range, accuracy, intraday and interday precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ) **[3]**. The primary aim of the present study was to develop and validate a simple, accurate, rapid, and cost-effective RP-HPLC method for the quantitative determination of febuxostat in both bulk drug substance and pharmaceutical tablet dosage form. The method was designed to fulfill current regulatory requirements while offering a practical and accessible tool for routine quality control operations in pharmaceutical industries and analytical research settings **[3,6,13]**.

2. MATERIALS AND METHOD

2.1 Chemicals and Reagents

The following chemicals and reagents were used in this study:

Febuxostat API: Gift sample from Apex Laboratories Pvt. Ltd.

Ortho Phosphoric Acid: AR Grade

Methanol: HPLC Grade

Acetonitrile: HPLC Grade
HPLC Grade Water: Generated in-house
All chemicals and reagents — Methanol, Acetonitrile, and Ortho Phosphoric Acid — were purchased from LOBA CHEMIE PVT. LTD., Mumbai.

2.2 Instrumentation

All instruments were calibrated and performance-qualified before use. The instruments and equipment used in this study are listed in Table 1.

Table 1: Instruments and Equipment Used in the Study

Instrument / Equipment	Make and Model
HPLC Pump	JASCO PU-2080 Plus Intelligent HPLC Pump
UV-VIS Detector	JASCO UV-2075 UV-VIS Detector
Analytical Column	Rubitas C18 Column (250 × 4.6 mm, 5 µm particle size)
Sample Injector	Rheodyne injector with 50 µL loop
HPLC Software	Borwin Chromatography Software Version 1.50
Analytical Balance	Shimadzu AY-120
Sonicator / Ultrasonic Bath	PRAMA Solutions
Water Purification System	Extrapure Lab Link Water Purification System
DSC Instrument	Mettler Toledo STAR SW 19.00
FTIR Spectrometer	JASCO FT/IR-4600 typeA

EXPERIMENTAL WORK

3.1 Preformulation Studies

Preformulation studies were carried out to characterize the drug substance in terms of physical appearance, solubility, melting point (thermal behaviour), and spectral properties before method development.

3.1.1 Physical Appearance

The Febuxostat sample was examined visually for its organoleptic characteristics. The drug was found to be a white to off-white, odourless, crystalline powder, free from extraneous matter, consistent with the pharmacopoeial description of the drug substance.

3.1.2 Solubility

The solubility of Febuxostat was determined by visual solubility testing at room temperature. Acetonitrile was selected as the primary solvent for preparing standard and sample solutions due to its good solubility profile.

3.1.3 Melting Point (Differential Scanning Calorimetry)

The melting point of Febuxostat was determined by Differential Scanning Calorimetry (DSC) using a STARe System (Mettler Toledo, SW 19.00). A 5.7000 mg sample was heated at a rate of 10.00 °C min^{-1}. The DSC thermogram showed a sharp endothermic peak with onset temperature of 208.14 °C, peak temperature of 209.51 °C, and endset temperature of 213.26 °C, with an enthalpy of fusion (ΔH) of −110.07 J g^{-1} and a narrow peak width (FWHM: 2.85 °C), confirming the high purity and crystalline nature of Febuxostat.

3.1.4 UV Spectroscopy – Selection of Analytical Wavelength (λmax)

The standard stock solution (1000 µg/ml) of Febuxostat was prepared in Acetonitrile. Further dilutions were prepared using mobile phase and the solution was scanned over the range of 200–400 nm. A 10 µg/mL solution of Febuxostat showed maximum absorbance at 314 nm, which was therefore selected as the analytical wavelength for HPLC method development and validation studies.

3.1.5 Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectrum of Febuxostat was recorded using a JASCO FT/IR-4600 typeA spectrophotometer (Serial No. F193061786) fitted with an ATR PRO ONE accessory. The spectrum showed characteristic absorption bands at 3527.17 cm^{-1} (N-H/O-H stretch), 2961.16 cm^{-1} (C-H stretch), 1680.66 cm^{-1} (C=O stretch of carboxylic acid), 1602.58 cm^{-1} (C=N stretch), 1468.53 cm^{-1} (C=C aromatic), and 1280.5 cm^{-1} (C-O stretch), confirming the identity of Febuxostat.

3.2 Preparation of Standard Solutions

3.2.1 Mobile Phase Preparation

The mobile phase was prepared by mixing Acetonitrile and 0.1% Ortho Phosphoric Acid (OPA) in the ratio of 60:40 v/v. Both solvents were of HPLC grade and were filtered separately through a 0.45 µm membrane filter under vacuum to remove particulate matter. The filtered solvents were then mixed in the required proportion and degassed by sonication for 15 minutes prior to use.

3.2.2 Preparation of Standard Stock Solution

Standard stock solution of Febuxostat was prepared by dissolving an accurately weighed 10 mg of Febuxostat in 10 mL of acetonitrile to obtain a concentration of 1000 µg/mL. From the standard stock solution, 1 mL was pipetted into a 10 mL volumetric flask and the volume was made up with acetonitrile to obtain a concentration of 100 µg/mL. Further dilutions were prepared from the stock

solution to obtain concentrations of 5–30 µg/mL for method development and validation studies.

3.2.3 Preparation of Working Standard Solution

An aliquot of 1 mL was withdrawn from the standard stock solution (1000 µg/mL) and transferred into a 10 mL volumetric flask. The volume was made up with acetonitrile to obtain a working standard solution containing 100 µg/mL of Febuxostat. Further dilutions were prepared to obtain concentrations of 5–30 µg/mL. The 10 µg/mL solution was used for system suitability testing, assay, precision, and other validation parameters.

3.2.4 Preparation of Sample Solution (Tablet Assay)

Tablets of FEBUGOOD 80 (Torrent Pharmaceuticals Ltd., label claim 80 mg/tablet) were accurately weighed and the average tablet weight was calculated. The tablets were finely powdered, and a quantity of powder equivalent to 10 mg of Febuxostat was transferred into a 10 mL volumetric flask. The drug was dissolved in methanol with sonication and the volume was made up to the mark with methanol to obtain a sample stock solution containing 1000 µg/mL. The resulting solution was filtered through Whatman filter paper No. 41. An appropriate aliquot of the filtrate was further diluted to obtain a final concentration of 10 µg/mL for assay determination.

3.3 Chromatographic Method Development and Optimization

Chromatographic conditions for Febuxostat were optimized by testing various mobile phase compositions on a Rubitas C18 column (250 mm × 4.6 mm, 5 µm). Various mobile phase systems — Methanol:Water and Acetonitrile:Water at proportions of 70:30, 80:20, and 90:10 — were initially tried but did not yield appropriate peak shape. Using Acetonitrile:0.1% OPA (60:40 v/v) at a flow rate of 1.0 mL/min with detection at 314 nm, a good peak shape was obtained with appropriate system suitability parameters. The retention time of Febuxostat was found to be 4.971 min.

Table 2: Trials for Mobile Phase of Febuxostat

Sr. No.	Mobile Phase	Observation
1.	MeOH: Water (90:10 v/v)	Peak shape was not proper. Peak splitting. RT = 4.500 min.
2.	ACN: Water (90:10 v/v)	Peak shape was not proper. RT = 4.458 min.
3.	Acetonitrile: 0.1% OPA (60:40 v/v)	Good peak shape. RT at 4.968 min. Selected.

3.4 Analytical Method Validation

The developed RP-HPLC method was validated as per ICH Harmonized Guideline Q2 (R1) Validation of Analytical Procedures. The following validation parameters were examined: system suitability, specificity, linearity and range, accuracy, precision (intraday and interday), limit of detection (LOD), limit of quantification (LOQ), and robustness.

RESULTS AND DISCUSSION:

4.1 Physical Appearance

The Febuxostat bulk drug was visually inspected and found to be a white to off-white crystalline powder. The observed appearance was consistent with the description reported in the literature and confirms the identity of the procured drug substance.

4.2 Solubility Profile

The solubility of Febuxostat was assessed in several solvents during method development. Acetonitrile was selected as the primary solvent for preparing standard and sample solutions due to its good solubility, which supported the development of a simple, reproducible RP-HPLC method.

4.3 Differential Scanning Calorimetry (DSC)

The DSC thermogram of Febuxostat showed a sharp endothermic peak with an onset temperature of 208.14 °C and a peak temperature of 209.51 °C, corresponding to its melting point. The narrow peak width (FWHM: 2.85 °C) and enthalpy of fusion (ΔH = −110.07 J g^{-1}) confirmed the crystalline nature and high purity of the drug sample. The DSC thermogram is shown in Figure 2.

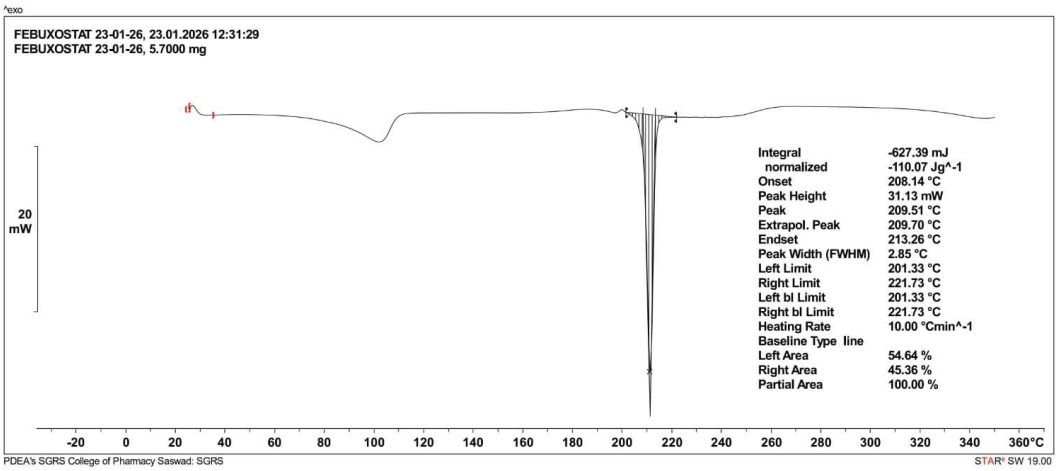

Figure 2: DSC Thermogram of Febuxostat

4.4 FTIR Spectroscopy

The FTIR spectrum of Febuxostat was recorded on a JASCO FT/IR-4600 typeA spectrophotometer in ATR mode over 4000–650 cm^{-1}, showing characteristic absorption bands corresponding to the functional groups present in the drug molecule. These peaks matched the reported structural features of Febuxostat, confirming the identity and purity of the drug substance. The bands are summarized in Table 3, and the spectrum is shown in Figure 3.

Table 3: FTIR Spectral Interpretation of Febuxostat

Sr. No.	Functional Group	Standard IR Range (cm^{-1})	Observed IR Peak (cm^{-1})
1	N-H / O-H Stretching	3650–3200	3527.17
2	C-H Stretching (Aliphatic)	3000–2850	2961.16
3	C=O Stretching (Carboxylic acid)	1750–1680	1680.66
4	C=N Stretching	1650–1580	1602.58
5	C=C Aromatic Stretching	1600–1450	1468.53
6	C-O Stretching	1300–1000	1280.5

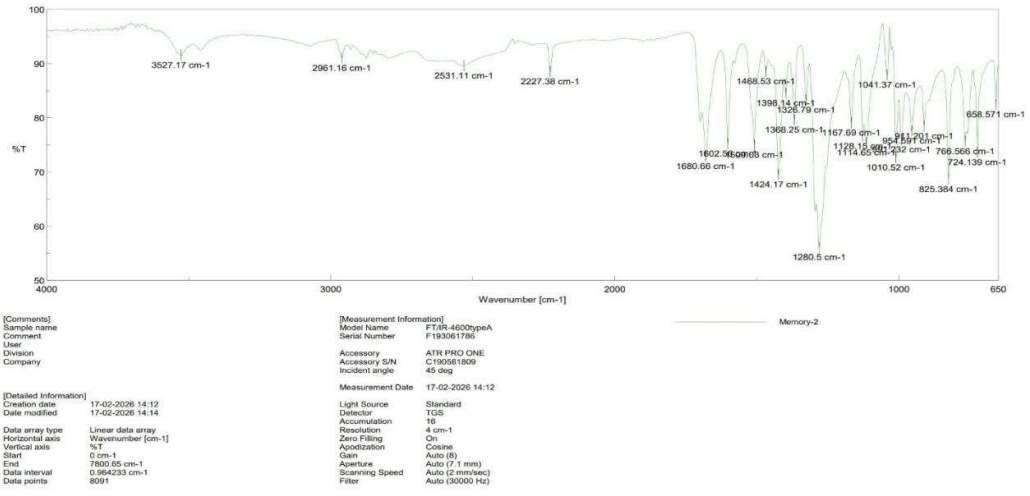

Figure 3: FTIR Spectrum of Febuxostat

4.5 UV Spectroscopic Analysis

A standard solution of Febuxostat (10 µg/mL) was prepared in acetonitrile and scanned in the wavelength range of 200–400 nm using a UV-Visible spectrophotometer. The UV absorption spectrum exhibited a characteristic maximum absorbance (λmax) at 314 nm. Therefore, 314 nm was selected as the analytical wavelength for the RP-HPLC method development and validation studies.

4.6 Optimized Chromatographic Conditions for Febuxostat

The final optimized chromatographic conditions for the determination of Febuxostat are presented in Table 5.

Table 5: Optimized Chromatographic Conditions for Febuxostat

Chromatographic Parameter	Optimized Condition
Column	Rubitas C18 Column (250 × 4.6 mm, 5.0 µm)
Mobile Phase	Acetonitrile : 0.1% OPA (60:40 v/v); Isocratic
Flow Rate	1.0 mL/min
Detection Wavelength	314 nm
Injection Volume	50 µL
Column Temperature	Ambient
Retention Time (Febuxostat)	4.965 ± 0.165 min
Run Time	10 min

4.7 System Suitability

System suitability testing was performed by injecting the standard solution of Febuxostat (10 µg/mL) under the optimized chromatographic conditions. The chromatogram showed a sharp, symmetrical peak at a retention time of 4.965 min, with a theoretical plate count of 2461.36 and an asymmetry factor of 0.99. The obtained values were within the acceptable limits prescribed by ICH guidelines (theoretical plates > 2000 and asymmetry factor between 0.8 and 2.0), confirming that the chromatographic system was suitable for the intended analysis.

Table 6: System Suitability Parameters of the Developed RP-HPLC Method

Parameter	Obtained Values
Concentration (µg/mL)	10
RT (min)	4.965
Asymmetry Factor	0.99
Theoretical Plates (N)	2461.36

4.8 Specificity

Specificity is the ability of an analytical method to unequivocally assess the analyte in the presence of components that could reasonably be present, such as impurities, degradation products, excipients, or other matrix components. To evaluate specificity, the blank solution (mobile phase) was checked for interference at the retention time of Febuxostat. The blank chromatogram showed no interfering peaks at the retention time of the analyte (4.965 min), confirming that the method is specific for Febuxostat.

4.9 Linearity and Range

Linearity is the ability of an analytical procedure to give results that are directly proportional to the concentration of analyte over a given range. The linearity of the developed RP-HPLC method for Febuxostat was established by preparing six concentrations (5, 10, 15, 20, 25, and 30 µg/mL) from the working standard solution and analyzing in six replicates. The peak area for each concentration was recorded and a calibration curve was plotted. The results are summarized in Table 7.

Table 7: Linearity of Febuxostat

Sr. No.	5	10	15	20	25	30
1	214360.11	421837.64	573297.78	746390.98	880581.56	1059673.97
2	220303.30	420545.19	578414.26	775375.75	909051.77	1106263.55
3	218812.26	420975.61	571846.01	757798.12	924276.13	1093722.06
4	220538.07	420315.71	573297.83	756099.91	907460.77	1085339.01
5	219557.78	422640.45	570428.88	766951.96	925599.14	1099992.80
6	220654.81	425299.70	582429.47	771679.69	907460.77	1098726.73
AVG	219037.723	21935.715	574952.370	762382.735	909071.690	1090619.685
SD	2394.537	1860.240	4549.249	10884.469	16255.519	16694.055

% RSD	1.093	0.441	0.791	1.428	1.788	1.531

The calibration curve for Febuxostat exhibited good linearity over the concentration range of 5–30 µg/mL. The regression equation obtained was: y = 34324x + 62325 with a correlation coefficient (R^2) of 0.9984. The %RSD values for all concentration levels were below 2%, indicating acceptable repeatability of the analytical response. These results demonstrate that the developed RP-HPLC method provides a linear response over the studied concentration range and is suitable for quantitative analysis of Febuxostat.

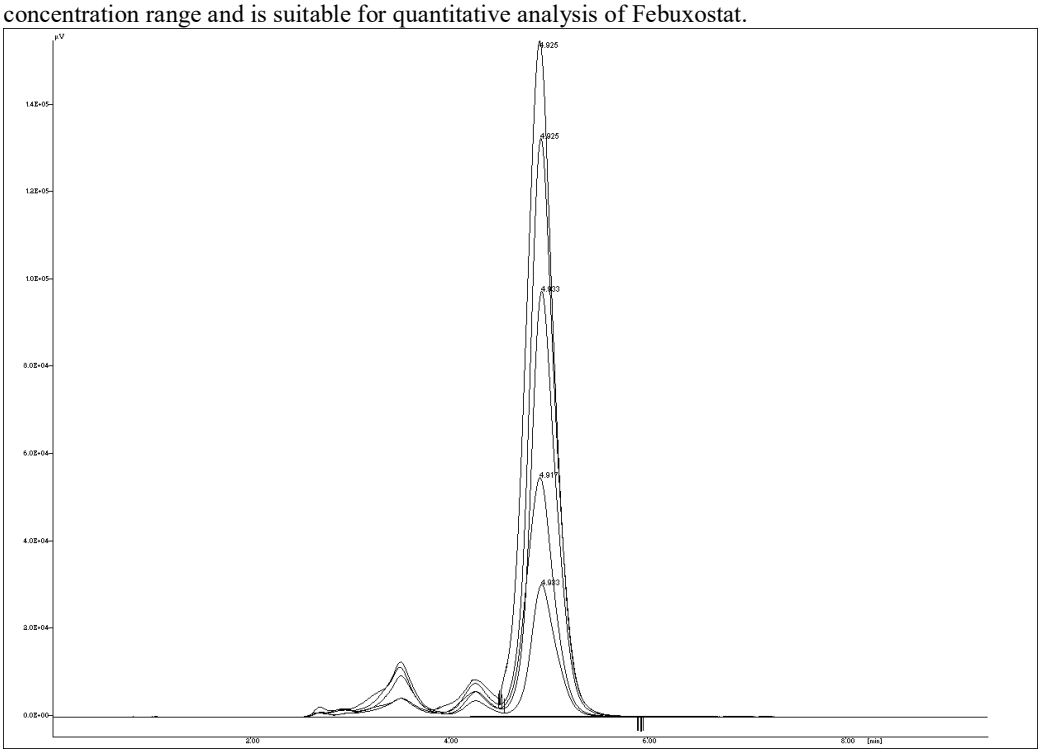

Figure 4: Overlain chromatogram of Febuxostat (5-30 µg/ml)

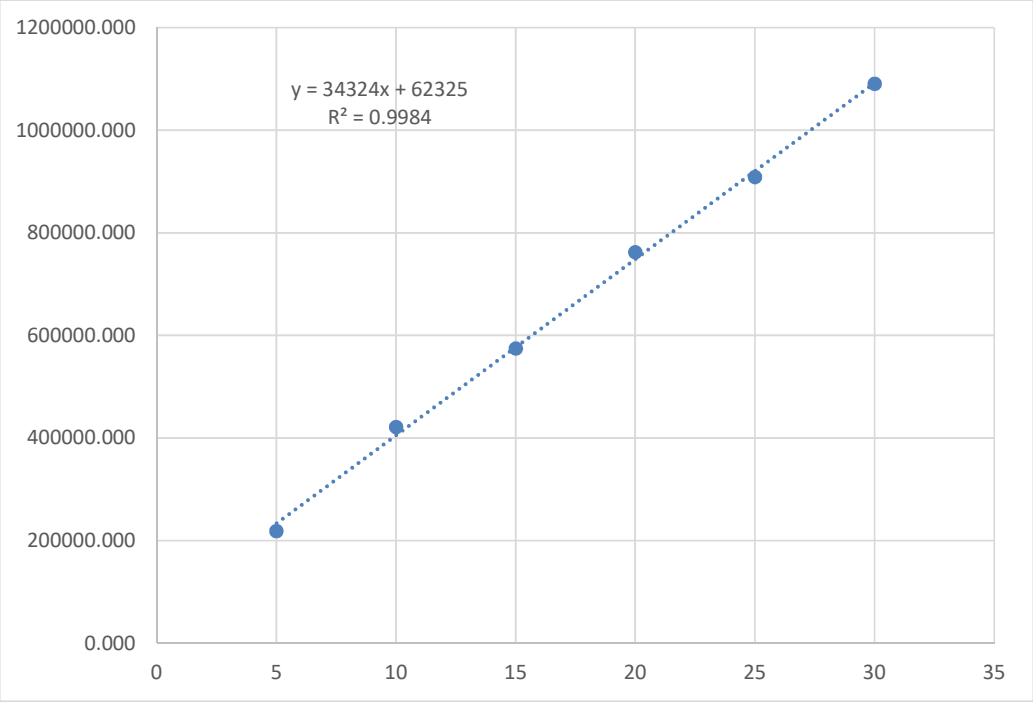

Fig 5: Calibration Curve of Febuxostat (linearity 5-30 µg/ml)

4.10 Accuracy

Accuracy expresses the closeness of agreement between the value accepted as a true value and the value found using the analytical method. The accuracy of the developed RP-HPLC method for Febuxostat was assessed by the standard addition method at three spiking levels — 50%, 100%, and 150% of the target concentration. The study was performed in triplicate, and the results were expressed as percentage recovery and percentage relative standard deviation (%RSD). The results are summarized in Table 8.

Table 8: Results of Accuracy of Febuxostat

Spiked Level (%)	Conc. of Drug Added (µg/mL)	Conc. of Standard Spiked (µg/mL)	Area	Amount Recovered (µg/mL)	% Recovery	Mean ± %RSD
50	10	5	575198.699	14.942	99.614	99.616 ± 0.251
			576501.625	14.980	99.867	
			573929.298	14.905	99.368	
100	10	10	745668.943	19.909	99.543	99.864 ± 0.466
			746412.617	19.930	99.651	
			751538.472	20.080	100.398	
150	10	15	925534.392	25.149	100.595	100.201 ± 0.522
			923861.782	25.100	100.401	
			917059.075	24.902	99.608	

The percentage recovery values obtained at 50%, 100%, and 150% spiking levels were 99.616%, 99.864%, and 100.201%, respectively. The %RSD values at all concentration levels were less than 2%, indicating excellent accuracy and reproducibility of the developed method. The recovery results were within the acceptable limits of 98–102%, demonstrating that the method is accurate and suitable for the quantitative determination of Febuxostat in pharmaceutical formulations.

4.11 Precision

Precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The precision of the developed RP-

HPLC method for Febuxostat was evaluated in terms of intraday and interday precision at three concentration levels (10, 20, and 30 µg/mL). The results were expressed as percentage recovery and percentage relative standard deviation (%RSD). The acceptance criterion for precision was %RSD not more than 2%. The results obtained are summarized in Table 9 and Table 10.

Table 9: Intraday Precision Studies

SN	Theoretical Conc (µg/mL)	Area	Practical Conc (µg/mL)	%Recovery	AVG	SD	%RSD
1	10	407067.895	10.044	100.438			
2	10	405436.124	9.996	99.962	100.189	0.239	0.238
3	10	406133.012	10.017	100.165			
4	20	743490.948	19.845	99.226			
5	20	751958.848	20.092	100.459	99.845	0.617	0.618
6	20	747781.491	19.970	99.851			
7	30	1095229.908	30.093	100.309			
8	30	1092153.293	30.003	100.011	100.404	0.448	0.447
9	30	1101230.779	30.268	100.892			

Table 10: Interday Precision Studies

SN	Theoretical Conc (µg/mL)	Area	Practical Conc (µg/mL)	%Recovery	AVG	SD	%RSD
1	10	406784.386	10.036	100.355			
2	10	405271.635	9.991	99.915	100.123	0.221	0.221
3	10	405901.739	10.010	100.098			
4	20	747620.748	19.965	99.827			
5	20	751750.636	20.086	100.429	100.129	0.301	0.300
6	20	749697.713	20.026	100.130			
7	30	1093099.286	30.031	100.102			
8	30	1089115.654	29.915	99.716	99.978	0.228	0.228
9	30	1093252.402	30.035	100.117			

The developed RP-HPLC method demonstrated excellent precision for the determination of Febuxostat. The %RSD values obtained for intraday precision were 0.238%, 0.618%, and 0.447% at concentrations of 10, 20, and 30 µg/mL, respectively. Similarly, the %RSD values for interday precision were 0.221%, 0.300%, and 0.228% at the corresponding concentration levels. Since all %RSD values were well below the acceptance limit of 2%, the method was found to be precise and reproducible for routine quantitative analysis of Febuxostat.

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

The limit of detection (LOD) is the lowest concentration detectable by the analytical method, but not necessarily quantifiable with accuracy. The limit of quantification (LOQ) is the lowest concentration that can be quantified with acceptable accuracy and precision. LOD and LOQ were calculated using the following formulae based on the calibration curve data:

LOD = 3.3 × σ/S LOQ = 10 × σ/S

Where σ = standard deviation of the y-intercept and S = slope of the calibration curve. The results are summarized in Table 10.

Table 11: Results of LOD and LOQ Studies of Febuxostat

		S.D. of Y-intercept (σ)	Slope (m)	Concentration (µg/mL)
LOD	3.3 (σ/S)	5708.735	34324.275	0.549
LOQ	10 (σ/S)	5708.735	34324.275	1.663

The LOD and LOQ were found to be 0.549 µg/mL and 1.663 µg/mL, respectively, indicating adequate sensitivity of the developed RP-HPLC method for the determination of Febuxostat.

4.13 Assay

Assay of Febuxostat was performed on the marketed formulation FEBUGOOD 80 (Torrent Pharmaceuticals Ltd., label claim 80 mg/tablet). Tablet powder equivalent to 10 mg of Febuxostat was dissolved in methanol, filtered, and diluted to obtain a concentration of 10 µg/mL. The procedure was repeated six times. The amount of Febuxostat was determined by extrapolation of peak area from the linearity equation. The results are summarized in Table 11.

Table 12: Assay Results of Febuxostat Commercial Tablet (FEBUGOOD 80)

Sr. No.	Peak Area	Amount Recovered (µg/mL)	% Recovery
1	404961.097	9.982	99.824
2	405626.604	10.002	100.018
3	404878.507	9.980	99.800
4	405660.156	10.003	100.028
5	405228.815	9.990	99.902
6	406521.233	10.028	100.279
AVG	405479.402	9.998	99.975
SD	605.353	0.018	0.176
% RSD	0.149	0.176	0.176

The mean assay value of Febuxostat in FEBUGOOD 80 tablets was found to be 99.975 ± 0.176%, which is within the acceptance limit of 98–102%. This confirms that the method is accurate and suitable for quantitative analysis of Febuxostat in its pharmaceutical dosage form without interference from excipients.

4.14 Robustness

Robustness is a measure of the capacity of the method to remain unaffected by small but deliberate variations in method parameters. The acceptance criterion is no significant change in results. Robustness was evaluated by deliberately varying the mobile phase ratio (±2 mL: ACN:0.1% OPA 58:32 and 62:28 v/v), flow rate (±0.1 mL/min: 0.9 and 1.1 mL/min), and detection wavelength (±1 nm: 313 and 315 nm), while keeping all other parameters constant. The results are summarized in Table 13.

Table 13: Results of Robustness Study

Parameter	Variation	AVG Area	SD	% RSD
Flow Rate (mL/min)	0.9	424296.524	5616.192	1.324
	1.0 (nominal)	416018.142	3938.144	0.947
	1.1	425880.085	5354.164	1.257
Wavelength (nm)	313	427340.894	3284.665	0.769
	314 (nominal)	420730.252	2240.904	0.533
	315	420527.577	4285.915	1.019
Mobile Phase (v/v)	58:42	416773.844	4700.586	1.128
	60:40 (nominal)	429807.911	2421.205	0.563
	62:38	437456.463	1975.872	0.452

All %RSD values obtained during robustness testing were below 2.0%, demonstrating that small deliberate variations in flow rate, detection wavelength, and mobile phase composition did not significantly affect the analytical performance of the method. These results confirm the robustness of the developed RP-HPLC method for Febuxostat.

5. CONCLUSION

A simple, rapid, accurate, precise, and cost-effective RP-HPLC method was successfully developed and validated for the quantitative estimation of Febuxostat in bulk drug and pharmaceutical dosage forms. Preliminary characterization studies using UV spectroscopy, FTIR spectroscopy, and Differential Scanning Calorimetry (DSC) confirmed the identity, purity, and physicochemical characteristics of the drug substance. The optimized chromatographic separation was achieved on a C18 column using Acetonitrile:0.1% Orthophosphoric Acid (60:40 v/v) as the mobile phase at a flow rate of 1.0 mL/min with UV detection at 314 nm. Febuxostat exhibited a well-resolved and symmetrical peak with a retention time of approximately 4.97 minutes.

The developed method demonstrated excellent linearity over the concentration range of 5–30 µg/mL with a correlation coefficient (R^2) of 0.9984. Validation studies performed according to ICH Q2(R1) guidelines confirmed the reliability of the method. Accuracy studies showed recoveries ranging from 99.61% to 100.20%, while precision studies produced %RSD values below 2%, indicating good repeatability and reproducibility. The method also exhibited satisfactory sensitivity, with LOD and LOQ values of 0.549 µg/mL and 1.663 µg/mL, respectively. Robustness testing demonstrated that minor deliberate changes in chromatographic conditions did not significantly affect analytical performance.

The assay of the marketed tablet formulation showed a mean recovery of 99.98%, confirming the suitability of the method for routine pharmaceutical

analysis. Overall, the developed RP-HPLC method is reliable, selective, and regulatory compliant, making it suitable for routine quality control, assay determination, and analytical applications involving Febuxostat in pharmaceutical industries and research laboratories.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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