The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(2): S.I (2), 745-763, 2026 www.thebioscan.com

A QbD-GUIDED DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF FEBUXOSTAT

Krushna Jagzap[1], Prashik B. Dudhe*[1], Rupali Wagh[1], Tanvi Baste[1], Nivrutti Datir[1], Sunil Kumar Patnaik[1], Mahavir Narwade[1], Sumit R Deore[1] and Abhijeet D. Kulkarni[1]

[1]School of Pharmaceutical Sciences, Sandip University, Nashik, Maharashtra 422213, India

***Address for correspondence:** Prashik B. Dudhe

Email: dudhe.prashik@gmail.com

DOI: https://doi.org/10.63001/tbs.2026.v21.i02.S.I(2).pp745-763

KEYWORDS
RP-HPLC;
Febuxostat;
Quality by Design;
Method Development;
Validation;
Central Composite Design

Received on:
14-04-2026
Accepted on:
03-05-2026
Published on:
12-05-2026

Abstract

A novel, simple, sensitive, suitable, precise, and powerful RP-HPLC method was developed and validated for febuxostat, which is of high quality in both bulk and tablet dosage forms. Chromatographic conditions were optimized using Design Expert software employing a Central Composite Design (CCD) approach. Separation was carried out with an HPLC system equipped with Chromeleon 7.3 software and an Agilent Zorbax C18 column (250 mm × 4.6 mm; 5 µm). Detection was performed at 315 nm. The RP-HPLC method was developed using acetonitrile:phosphate buffer (50:50 v/v) as the mobile phase at a flow rate of 1.0 mL/min. The developed method was validated as per ICH Q2(R2) guidelines. The method exhibited excellent linearity with a correlation coefficient (r^2) of 0.9997. The method precision was less than 2.0% RSD. The percentage recovery for febuxostat was 100.17%. The proposed approach was both predictive and reliable, demonstrating its suitability for routine quality control analysis of febuxostat in pharmaceutical formulations.

1. INTRODUCTION

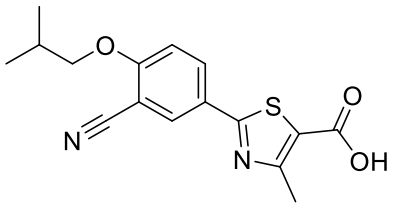
Gout is an acute arthritis caused by inflammation due to the presence of monosodium urate crystals in or around a joint and is closely associated with hyperuricemia [1]. Febuxostat is a selective xanthine oxidase (XO) inhibitor that has been used in the treatment of hyperuricemia and management therapy of gout, where it has been found to have high inhibitory potential against XO/xanthine dehydrogenase (XDH) [2]. Febuxostat can inhibit both the oxidized and reduced forms of XO, whereas allopurinol and oxypurinol can only bind to one form of the enzyme [3].

This unique mechanism of action makes febuxostat a valuable therapeutic option for patients with gout and chronic hyperuricemia.

Quality by Design (QbD) is a systematic approach to pharmaceutical development that emphasizes product and process understanding and process control, based on sound science and quality risk management [4]. Central Composite Design (CCD) is an excellent optimization tool applied to the optimization of major factors in analytical method development [5]. The preferred value of the variable that will lead to the preferred and optimal response is determined through CCD, which defines process conditions that are unlikely to undergo computational changes in the setting of factors. It also implies that there is a mathematical model linking the response to the critical variables, and thus it can be used to forecast the response with the smallest possible error [6].

The application of Analytical Quality by Design (AQbD) principles in method development has gained significant attention in pharmaceutical analysis, as it provides a systematic framework for understanding the relationship between method parameters and analytical performance [7], [8]. Recent studies from our research group have successfully applied AQbD-based RP-HPLC methods for the quantitative estimation of azilsartan [9] and pioglitazone [10], demonstrating the robustness and reliability of this approach for pharmaceutical quality control.

The literature review revealed that analysis of febuxostat can be performed using various HPLC methods [11], [12], [13], [14], [15], [16], [17]. However, a comprehensive QbD-guided approach with systematic optimization using CCD has not been extensively reported. The present study aims to develop and validate a robust, accurate, and precise RP-HPLC method for the quantitative estimation of febuxostat in bulk and tablet dosage forms using the QbD approach with CCD optimization.



2-(3-cyano-4-isobutoxyphenyl)-4-methyl- 1,3-thiazole-5-carboxylic acid

Figure 1: Chemical structure of febuxostat

2. MATERIALS AND METHODS

2.1. Materials

The drug febuxostat was provided by Arni Analytical Laboratory, Nashik, India. HPLC-grade solvents such as acetonitrile and methanol were purchased from Thermo Ultimate 3000. Potassium dihydrogen orthophosphate (analytical grade) and other reagents were of analytical grade. Ultrapure water was obtained from a Milli-Q water purification system.

2.2. Instrumentation

Method development and validation were performed using an HPLC Thermo Ultimate 3000 system equipped with Chromeleon 7.3 software and an Agilent Zorbax C18 column (250 mm × 4.6 mm, 5 µm particle size). The HPLC system consisted of a quaternary pump, autosampler, column oven, and UV-Vis detector. Design-Expert software (Version 11, Stat-Ease Inc., Minneapolis, MN, USA) was used for experimental design and statistical analysis.

2.3. Preparation of Solutions

2.3.1. Preparation of Buffer

Potassium dihydrogen orthophosphate buffer (0.01 M) was prepared by dissolving an appropriate amount of potassium dihydrogen orthophosphate in distilled water. The pH was adjusted if necessary, and the solution was filtered through a 0.45 µm membrane filter.

2.3.2. Preparation of Mobile Phase

The mobile phase was prepared by mixing acetonitrile and phosphate buffer in the ratio of 50:50 (v/v). The mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication for 15 minutes before use.

2.3.3. Preparation of Standard Solution

Accurately weigh 40 mg of febuxostat and transfer into a 100 mL volumetric flask. Add 60 mL of methanol and sonicate for 5 minutes to dissolve completely. Make up the volume to 100 mL with methanol to obtain a stock solution of 400 µg/mL. From this stock solution, pipette 5 mL into a 50 mL

volumetric flask and dilute to volume with methanol to obtain a working standard solution of 40 µg/mL.

2.3.4. Preparation of Sample Solution

Weigh and determine the average weight of 10 tablets. Crush the tablets into fine powder using a mortar and pestle. Accurately weigh powder equivalent to the average weight of one tablet into a 100 mL volumetric flask. Add 60 mL of methanol and sonicate for 10 minutes to ensure complete extraction. Make up the volume to 100 mL with methanol. Filter the solution through Whatman filter paper No. 41. Pipette 5 mL of the filtrate into a 50 mL volumetric flask and dilute to volume with methanol.

2.4. Method Development

Initial trials were performed to determine the mobile phase providing satisfactory separation and peak shape. During the initial stage of method development, a good peak was obtained with a 50:50 buffer-to-acetonitrile mixture; therefore, this mixture was selected as the mobile phase. The two factors chosen—percentage of acetonitrile and flow rate—were identified as critical method parameters that could significantly influence the chromatographic responses.

2.5. Design of Experiment (Central Composite Design)

The experimental variables were identified and optimized using Design of Experiment (DoE) software. The CCD must contain a k factor of 2^k factorial experiments, 2k axial experiments equally distributed on each variable axis at ±α, and at least one center point. These critical factors were combined to achieve the optimal level of desirable responses by creating a rotatable CCD (α = 1.68) comprising 13 random runs, including 5 center points.

After the identification of the chromatographic factors or independent factors that affect the responses of the HPLC system, the central composite design technique was applied to optimize the factors. Two-factorial design with extra or star points were added to facilitate the approximation of the second-order equation as characterized by Box and Wilson [18]. This allows the selection of coefficients in a quadratic regression model, enabling the prediction of nonlinear effects of variables.

The CCD model was designed using Design-Expert (Version 11, Stat-Ease Inc., Minneapolis, MN, USA). Thirteen trial runs were conducted during which the CCD was

performed, and the values of the independent variables and responses are presented in Tables 1 and 2, respectively.

Table 1: Central composite design showing 13 randomized trial runs

Run	% ACN	Flow rate (mL/min)	Rt Febuxostat (min)
1	50	1.0	3.060
2	50	1.0	3.087
3	35	1.0	3.613
4	50	0.7	2.713
5	64	1.0	2.535
6	60	1.2	2.820
7	50	1.0	3.063
8	60	0.8	3.135
9	50	1.0	3.068
10	50	1.2	2.940
11	50	1.0	3.073
12	40	0.8	3.878
13	40	1.2	3.220

Table 2: Experimental factors and levels used in central composite design

Name	Low (-1)	Medium (0)	High (+1)
Independent factor			
A: Acetonitrile (%)	40	50	60
C: Flow rate (mL/min)	0.8	1.0	1.2
Dependent factor			
R1	Retention time of Febuxostat		

Table 3: Experimental results and optimized conditions

Parameters	Description
Program	Isocratic
Column	Agilent Zorbax C18 (250 mm × 4.6 mm, 5 µm)
Mobile phase	Phosphate buffer:ACN (50:50 v/v)
Wavelength	315 nm
Injection volume	20 µL
Flow rate	1.0 mL/min

2.6. Method Validation

The developed method was validated according to ICH Q2(R2) guidelines [19], which included evaluation of system suitability, specificity, linearity, accuracy, precision (system precision, method precision, and intermediate precision), limit of detection (LOD), limit of quantitation (LOQ), robustness, and solution stability.

2.6.1. Specificity

Specificity is the ability of the analytical method to measure the analyte in the presence of components that may be expected to be present. The specificity of the analytical procedure was demonstrated by comparing the HPLC chromatograms of the blank, placebo, standard solution, and sample solution to ensure no interference at the retention time of febuxostat.

2.6.2. Precision

Precision expresses the degree of agreement (degree of scatter) of a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Precision was evaluated at three levels:

- **System Precision:** Six replicate injections of the standard solution were analyzed, and the percentage relative standard deviation (%RSD) of peak area was calculated.
- **Method Precision:** Six sample preparations were analyzed, and the %RSD of assay results was calculated.
- **Intermediate Precision:** The method precision study was repeated on a different day by a different analyst to evaluate intermediate precision.

2.6.3. Accuracy

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy was determined based on percentage recovery and was conducted at three levels: 80%, 100%, and 120% of the target concentration. Known amounts of febuxostat standard were spiked into the sample matrix, and the percentage recovery was calculated.

2.6.4. Linearity

The linearity of the developed method was evaluated in the range of 20–30 µg/mL (80–120% of the target concentration). Five different concentrations were prepared, and each concentration was injected in triplicate. Peak areas were plotted against concentrations to generate the calibration curve, and the correlation coefficient (r^2) was calculated.

2.6.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the linearity data using the following formulas:

$LOD = 3.3 \times (\sigma/S)$

$LOQ = 10 \times (\sigma/S)$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.6.6. Solution Stability

The stability of febuxostat in solution at room temperature was assessed by storing the working standard and sample solutions in tightly capped volumetric flasks for up to 24 hours. The solutions were analyzed at initial, 8 hours, and 24 hours, and the %RSD was calculated.

3. RESULTS AND DISCUSSION

3.1. Method Optimization and Design of Experiment

The two critical factors chosen for optimization were the percentage of acetonitrile in the mobile phase and the flow rate, which could evidently influence the chromatographic responses. Spectral analysis of febuxostat in the wavelength range of 200–400 nm revealed that febuxostat possesses a λmax at 315 nm. Therefore, the chromatographic detection was adjusted to 315 nm.

For every pair of variables, the area designated as the sweet spot (or the bright yellow area in the design space) was calculated, and the remaining factors were

kept constant. CCD selected two variables (concentration of acetonitrile and flow rate) at 5 levels with 13 experiments. The influence of these variables and the model's efficiency were confirmed through analysis of variance (ANOVA), as indicated by the p-value and F-value in Table 4.

A probability value ($p < 0.0001$) indicates that the effect of the independent factors on the response is significant ($p < 0.05$ is required for the model to be considered significant). The ANOVA results demonstrated that both acetonitrile concentration and flow rate had significant effects on the retention time of febuxostat.

Table 4: Statistical parameters obtained from ANOVA

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	0.9426	2	0.4713	9.09	0.0056	significant
A-Mobile Phase	0.8895	1	0.8895	17.15	0.0020	significant
B-Flow Rate	0.0531	1	0.0531	1.02	0.3353	-
Residual	0.5187	10	0.0519	-	-	-
Lack of Fit	0.5182	6	0.0864	766.38	< 0.0001	significant
Pure Error	0.0005	4	0.0001	-	-	-
Cor Total	1.46	12	-	-	-	-

The Model F-value of 9.09 indicates that the model is significant. There is only a 0.56% chance that such a large F-value could occur due to noise. P-values below 0.0500 indicate that model terms are significant. In this case, the mobile phase composition (A) is a significant model term.

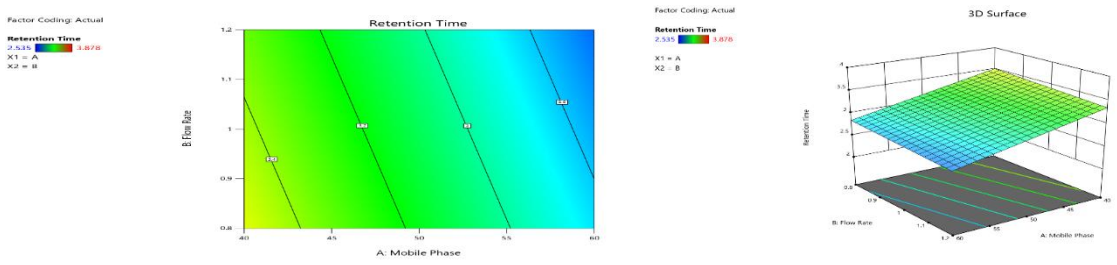


Figure 2: 2D and 3D curves of the interaction effects of critical factors on the retention time of febuxostat

Figure 2 shows the relationships between the dependent variable (retention time) and the two independent variables (mobile phase composition and flow rate) in both 2D and 3D plots. The graphs clearly demonstrate the effect of mobile phase composition and flow rate on retention time. As the percentage of acetonitrile increases, the retention time decreases due to increased elution strength. Similarly, an increase in flow rate results in decreased retention time due to faster analyte migration through the column.

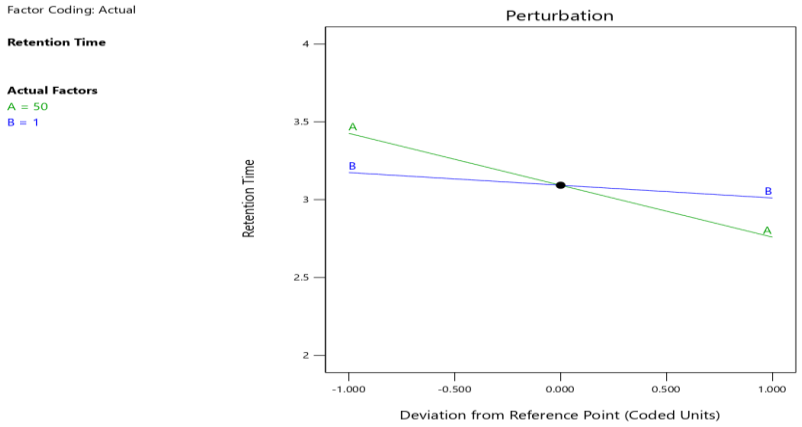


Figure 3: Perturbation plot showing effect of the factors on the responses

Figure 3 shows the perturbation plot, which illustrates the effect of each variable on the retention time. The plot indicates that the mobile phase composition has a more pronounced effect on retention time compared to flow rate, as evidenced by the steeper slope.

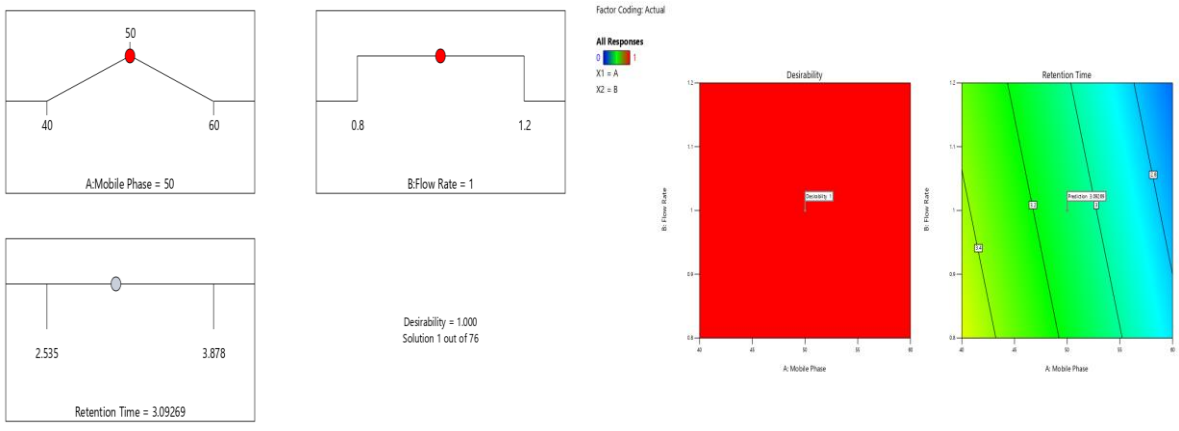


Figure 4: Graphical representation of constraints which were accepted to determine global desirability and achieve optimal conditions

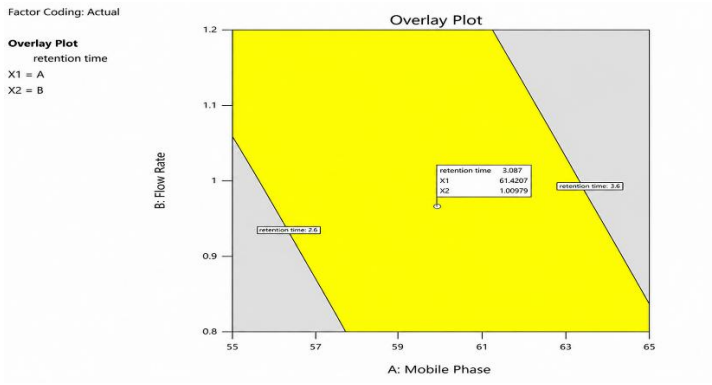


Figure 5: Overlay plot showing the sweet spot where the desired response meets

The overlay plot (Figure 5) displays the design space where all the desired criteria are simultaneously met. The yellow region represents the sweet spot, indicating the optimal combination of mobile phase composition and flow rate that provides the desired retention time with acceptable peak shape and resolution.

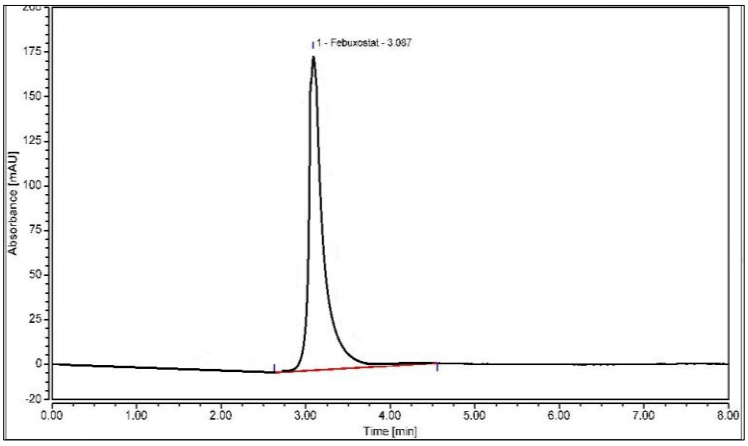


Figure 6: Optimized chromatogram of febuxostat

Figure 6 shows the optimized chromatogram of febuxostat obtained under the optimized conditions. The peak is sharp, symmetrical, and well-resolved, with a retention time of approximately 3.0 minutes, demonstrating the effectiveness of the optimization process.

The optimized chromatographic conditions (50:50 acetonitrile:phosphate buffer at 1.0 mL/min flow rate) provided an excellent balance between analysis time, peak shape, and resolution. This approach is consistent with the AQbD-based method development strategies successfully employed in our

previous studies on azilsartan [9] and pioglitazone [10], where similar CCD optimization approaches yielded robust and reliable analytical methods.

3.2. Method Validation

3.2.1. Specificity

Peak purity was maintained throughout the experiment, as confirmed by the peak purity index. The peaks were therefore considered to be pure, demonstrating that the method is specific. The blank solution did not show any interference at the retention time of febuxostat in the standard and sample solutions.

Table 5: Specificity results for febuxostat

Sr. No.	Solution Type	Retention Time (min)
1	Blank	0.000
2	Standard Solution	3.087
3	Sample Solution	3.060

The results demonstrate that there is no interference from the blank or excipients at the retention time of febuxostat, confirming the specificity of the method.

3.2.2. Precision

System Precision: The %RSD for six replicate injections of the standard solution was found to be 0.38%, which is well within the acceptance criterion of NMT 2.0%, indicating excellent system precision.

Method Precision: The assay values for six preparations of febuxostat 50 mg tablets were determined, with a mean assay of 100.49% (acceptance criteria: NLT 90.0% and NMT 110.0%). The %RSD was less than 2.0%, demonstrating that the method is precise.

Intermediate Precision: The assay values for six preparations of febuxostat analyzed on a different day by a different analyst showed a mean assay of 100.32% (acceptance criteria: NLT 90.0% and NMT

110.0%). The %RSD was less than 2.0%, confirming good intermediate precision.

The precision results demonstrate the reproducibility and reliability of the developed method, which is essential for routine quality control applications.

3.2.3. Accuracy

Accuracy was evaluated through recovery studies at three concentration levels: 80%, 100%, and 120% of the target concentration. Known amounts of febuxostat standard were spiked into the sample matrix, and the percentage recovery was calculated.

Table 6: Accuracy results for febuxostat

Sr. No.	Level	mg of drug spiked	Area	mg of drug Recovered	% Recovery
1	80%	0.040000	58.424	0.040264	100.66
2	100%	0.050000	72.109	0.049695	99.39
3	120%	0.060000	87.479	0.060288	100.48
				Average	**100.17**
				Std. dev	**0.69**
				%RSD	**0.69**

The recovery studies showed percentage recoveries ranging from 99.39% to 100.66%, with a mean recovery of 100.17% and %RSD of 0.69%. These results are within the acceptable range of 98–102%, demonstrating the accuracy of the method.

3.2.4. Linearity

The linearity of the method was evaluated over the concentration range of 20–30 µg/mL (80–120% of the target concentration). Five different concentrations were prepared and analyzed in triplicate. The calibration curve was constructed by plotting peak area against concentration.

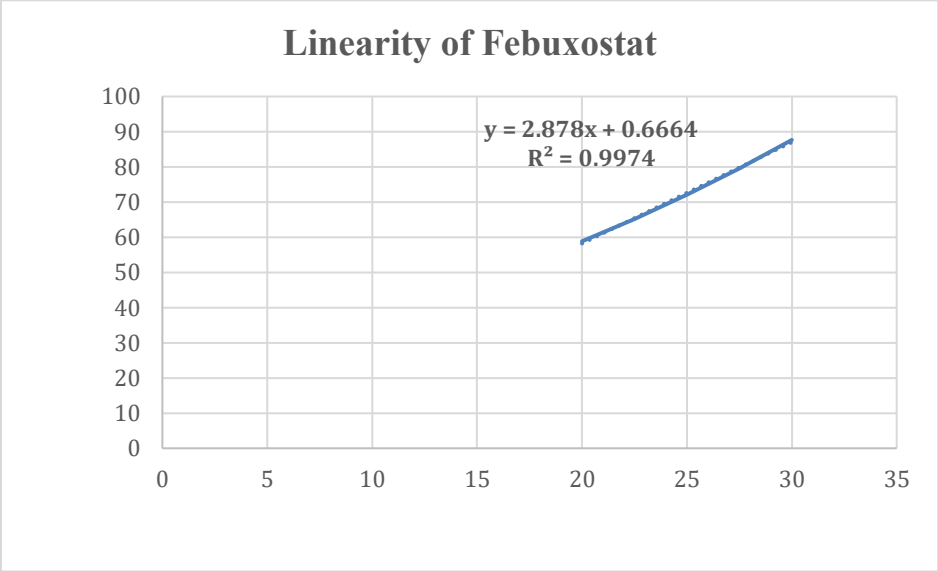


Figure 7: Calibration curve for febuxostat

Table 7: Linearity results for febuxostat

Sr. No.	Concentration (µg/mL)	Concentration of Solution (%)	Diluted to (mL)	Area
1	20.0	80	100	58.850
2	22.5	90	100	65.104
3	25.0	100	100	72.023
4	27.5	110	100	79.465
5	30.0	120	100	87.645

Linearity parameters: - Y-intercept: 0.6664 - Slope: 2.8780 - Correlation coefficient (r^2): 0.9997

The linear regression coefficient of 0.9997 demonstrates excellent linear correlation between peak area and concentration over the studied range. The high correlation coefficient confirms that the method is suitable for quantitative analysis of febuxostat.

3.2.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the linearity data using the standard deviation of the response and the slope of the calibration curve.

- **LOD:** 0.12 µg/mL
- **LOQ:** 0.35 µg/mL

These low LOD and LOQ values indicate that the method is sufficiently sensitive for the detection and quantitation of febuxostat at low concentrations, making it suitable for various analytical applications including impurity analysis and stability studies.

3.2.6. Solution Stability

The stability of febuxostat in solution was evaluated by storing the standard and sample solutions at room temperature for up to 24 hours. The solutions were analyzed at initial, 8 hours, and 24 hours.

Table 8: Solution stability results for febuxostat

Sr. No.	Time Point	Area	% Assay	% Assay Difference
1	Initial	72.000	100.32	-
2	8 Hour	72.300	100.02	0.30
3	24 Hour	71.610	99.54	0.78

The %RSD was found to be less than 2%, indicating that the sample solutions of febuxostat 50 mg are stable for at least 24 hours at room temperature. This stability allows for flexibility in the analysis schedule and confirms that the solutions can be prepared and analyzed within a working day without significant degradation.

3.2.7. Summary of Validation Results

Table 9: Summary of validation results for febuxostat

Parameters	Febuxostat
Specificity	No interference observed in Standard & Sample due to Blank
Linearity	
Range (µg/mL)	20–30 (80–120%)
Y-Intercept	0.6664

Slope	2.8780
Correlation coefficient (r^2)	0.9997
Accuracy	
% Recovery	100.17
Precision	
System Precision	RSD = 0.38%
Method Precision	% Assay = 100.49
Intermediate Precision	% Assay = 100.32
LOD (µg/mL)	0.12
LOQ (µg/mL)	0.35
Solution Stability	RSD < 2% (stable for 24 hours at room temperature)

The validation results demonstrate that the developed RP-HPLC method meets all the acceptance criteria specified in ICH Q2(R2) guidelines. The method is specific, linear, accurate, precise, sensitive, and robust, making it suitable for routine quality control analysis of febuxostat in bulk and pharmaceutical dosage forms.

The successful validation of this method is consistent with the robust analytical performance achieved in our previous AQbD-based method development studies for azilsartan [9] and pioglitazone [10]. The application of CCD in method optimization has proven to be an effective strategy for developing reliable analytical methods with enhanced understanding of method parameters and their interactions.

4. CONCLUSION

An effective and powerful RP-HPLC method was successfully developed and validated for the quantitative estimation of febuxostat in bulk and tablet dosage forms using the Quality by Design (QbD) approach. The study demonstrated the capability of multivariate regression analysis to establish the main influence of two critical variables—mobile phase composition and flow rate—on the retention time of febuxostat.

The optimization of chromatographic conditions was achieved by investigating

both the interaction and square effects of the key factors on the chosen responses using Central Composite Design (CCD). The optimized conditions (mobile phase: acetonitrile:phosphate buffer 50:50 v/v; flow rate: 1.0 mL/min; detection wavelength: 315 nm; column: Agilent Zorbax C18, 250 mm × 4.6 mm, 5 µm) provided excellent peak shape, resolution, and analysis time.

The developed RP-HPLC method was validated according to ICH Q2(R2) guidelines and demonstrated excellent performance characteristics including specificity, linearity (r^2 = 0.9997), accuracy (mean recovery = 100.17%), precision (RSD < 2.0%), sensitivity (LOD = 0.12 µg/mL; LOQ = 0.35 µg/mL), and solution stability (stable for 24 hours at room temperature).

The method is simple, robust, accurate, and precise, and can be successfully applied for routine quality control analysis of febuxostat in pharmaceutical formulations. The QbD approach employed in this study provided enhanced method understanding, improved robustness, and regulatory compliance. This systematic approach to method development, similar to that successfully applied in our previous studies on azilsartan [9] and pioglitazone [10], demonstrates the value of AQbD principles in pharmaceutical analysis and supports their adoption in routine quality control laboratories.

DECLARATIONS

Acknowledgement

The authors would like to acknowledge the Dean and faculty of the School of Pharmaceutical Sciences, Sandip University, Nashik, for facilitating and supporting the carrying out of this study.

Conflict of Interest

None

Funding

None

Ethics Statement

None

Informed Consent

None

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

[1] H. R. Schumacher Jr., R. Wortmann, M. A. Becker, et al., “A phase 2, long term

open-label safety and efficacy study of febuxostat, a novel non-purine, selective inhibitor of xanthine oxidase," *Arthritis Rheum.*, vol. 50, p. 800/180, 2004.

[2] K. Komoriya, Y. Osada, M. Hasegawa, et al., "Hypouricemic effect of allopurinol and the novel xanthine oxidase inhibitor TEI-6720 in chimpanzees," *Eur. J. Pharmacol.*, vol. 250, pp. 455–460, 1993. doi: 10.1016/0014-2999(93)90033-e.

[3] K. Okamoto, B. T. Eger, T. Nishino, et al., "An extremely potent inhibitor of xanthine oxidoreductase. Crystal structure of the enzyme-inhibitor complex and mechanism of inhibition," *J. Biol. Chem.*, vol. 278, pp. 1848–1855, 2003.

[4] International Conference on Harmonisation, "Pharmaceutical Development Q8(R2)," *ICH Harmonised Guideline*, pp. 1–28, 2009.

[5] N. P. Nadpara, R. V. Thumar, V. N. Kalola, and P. B. Patel, "Quality by design (QbD): A complete review," *Int. J. Pharm. Sci. Rev. Res.*, vol. 17, pp. 20–28, 2012.

[6] K. P. Jatte, D. D. Masne, M. A. Khachane, and M. S. Charde, "QbD approach in analytical method development: A review," *Int. J. Pharm. Pharm. Res.*, vol. 21, pp. 1–19, 2021.

[7] M. M. Sebaiy, S. M. El Adl, M. M. Baraka, A. A. Hassan, and H. M. El Sayed, "Quality by design approach for development and validation of a RP-HPLC method for simultaneous estimation of xipamide and valsartan in human plasma," *BMC Chem.*, pp. 1–13, 2022. doi: 10.1186/s13065-022-00864-4.

[8] D. Kavitha, S. K. Sahoo, V. R. P., M. Nagamani, and B. Ch, "Development and validation of RP-HPLC method for determination of metformin and sitagliptin in bulk and pharmaceutical dosage form," *Int. J. Pharm. Sci. Rev. Res.*, vol. 5, pp. 34–39, 2017.

[9] N. Datir, P. B. Dudhe, R. Wagh, T. Baste, K. Jagzap, and A. D. Kulkarni, "Analytical quality by design (AQbD)-based RP-HPLC method development and validation for the estimation of azilsartan," *School of Pharmaceutical Sciences, Sandip University, Nashik*, 2024.

[10] T. Baste, P. B. Dudhe, R. Wagh, N. Datir, K. Jagzap, and A. D. Kulkarni, "Analytical quality by design (AQbD)-based HPLC method development and validation for the estimation of pioglitazone," *School*

of Pharmaceutical Sciences, Sandip University, Nashik, 2024.

[11] S. Muvvala, V. N. Ratnakaram, and R. R. Nadendla, "A validated RP-HPLC method for the estimation of febuxostat in bulk drugs," *Int. J. PharmTech Res.*, vol. 4, no. 4, pp. 1686–1692, 2012.

[12] S. N. Darandale and S. S. Dhaneshwar, "Development and validation of RP-HPLC method for the determination of febuxostat in bulk and pharmaceutical dosage form," *Asian J. Res. Chem.*, vol. 10, no. 1, pp. 63–67, 2017.

[13] P. R. Kamble, N. V. Pandagale, O. D. Ghatage, P. B. Patil, and S. V. Patil, "Development and validation of RP-HPLC method for determination of febuxostat in bulk and pharmaceutical dosage formulations," *Asian J. Pharm. Anal.*, vol. 13, no. 1, pp. 7–12, 2023.

[14] S. Devidas, S. Bagade, and S. Jadhav, "Method development and validation of febuxostat in bulk and pharmaceutical dosage forms by RP-HPLC," *Int. J. Pharm. Res. Appl.*, vol. 9, no. 1, pp. 160–166, 2024.

[15] G. Ponnuveetil and K. Ilango, "Validated RP-HPLC and HPTLC methods for simultaneous estimation of febuxostat and diclofenac sodium in pharmaceutical dosage form," *Eur. J. Chem.*, vol. 5, no. 1, pp. 150–156, 2014.

[16] N. K. Alruwaili, "Analytical quality by design approach of reverse-phase HPLC method for atorvastatin: Method development, validation and application in pharmaceuticals," *Int. J. Anal. Chem.*, vol. 2021, Article ID 6645476, 2021.

[17] V. G. Napate and P. A. Napate, "Method development and validation for the febuxostat by RP-HPLC method," *Int. J. Pharm. Sci. Rev. Res.*, vol. 38, no. 1, pp. 204–208, 2016.

[18] D. C. Montgomery, *Design and Analysis of Experiments*, 10th ed. Wiley, 2020.

[19] International Council for Harmonisation, "Validation of Analytical Procedures Q2(R2)," *ICH Harmonised Guideline*, pp. 1–38, 2022.

[20] R. Khosravan, B. A. Grabowski, J. T. Wu, N. Joseph-Ridge, and L. Vernillet, "Pharmacokinetics, pharmacodynamics and safety of febuxostat, a novel non-purine selective inhibitor of xanthine oxidase, in a dose escalation study in healthy subjects,"

Clin. Pharmacokinet., vol. 45, no. 8, pp. 821–841, 2006.

[21] M. A. Becker, J. Kisicki, R. Khosravan, et al., "Febuxostat (TMX-67), a novel, non-purine, selective inhibitor of xanthine oxidase, is safe and decreases serum urate in healthy volunteers," *Nucleosides Nucleotides Nucleic Acids*, vol. 23, pp. 1111–1116, 2004.

[22] D. S. Wishart, Y. D. Feunang, A. C. Guo, E. J. Lo, A. Marcu, J. R. Grant, et al., "DrugBank 5.0: A major update to the DrugBank database for 2018," *Nucleic Acids Res.*, vol. 46, no. D1, pp. D1074–D1082, 2018. doi: 10.1093/nar/gkx1037. (Febuxostat record: https://go.drugbank.com/drugs/DB04854)

[23] K. G. Saag, D. Fitz-Patrick, J. Kopicko, M. Fung, N. Bhakta, S. Adler, et al., "Randomized, controlled trial of febuxostat in patients with gout and moderate-to-severe renal impairment," *Arthritis Rheumatol.*, vol. 69, no. 12, pp. 2386–2395, 2017. doi: 10.1002/art.40381.

[24] P. Ravisankar, C. Naga Navya, D. Pravallika, and D. N. Sri, "A review on step-by-step analytical method validation," *Int. J. Res. Pharm. Sci.*, vol. 2, no. 3, pp. 47–56, 2015.

[25] S. Sharma and D. Kumar, "High-performance liquid chromatography (HPLC) in pharmaceutical analysis," *PharmiWeb Journal*, 2020. Retrieved from https://www.pharmiweb.com/article/high-performance-liquid-chromatography-hplc-and-pharmaceutical-analysis