ANALYTICAL METHOD METHOD DEVELOPMENT AND VALIDATION BY RP-HPLC FOR THE ESTIMATION TOPIROXOSTAT IN BULK AND DOSAGE FORM

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ABSTRACT

Background: Conventional analytical procedures for Topiroxostat require a validated, reliable, and reproducible chromatographic method to ensure quality control of pharmaceutical formulations. Topiroxostat is a selective xanthine oxidoreductase inhibitor used in the treatment of hyperuricemia and gout. Although several analytical approaches have been reported, there remains a need for a simple, rapid, accurate, and economical RP-HPLC method for routine estimation of Topiroxostat in bulk drug and tablet dosage forms.

Objectives: This study aimed to develop and validate a simple isocratic RP-HPLC method for the estimation of Topiroxostat in bulk and tablet dosage forms. The method was developed and validated in accordance with ICH Q2(R1) guidelines to demonstrate its suitability for routine pharmaceutical analysis.

Materials and Methods: Chromatographic separation was achieved using a Phenomenex C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of Acetonitrile and 0.1% Orthophosphoric Acid (30:70 v/v), delivered at a flow rate of 1.0 mL/min. Detection was carried out at 273 nm using a UV detector. The developed method was validated for system suitability, specificity, linearity, accuracy, precision, intermediate precision, and robustness according to ICH guidelines.

Results: Topiroxostat was eluted at a retention time of 3.56 min with a tailing factor of 1.1 and theoretical plate count of 9456. The method exhibited excellent linearity over the concentration range of 50–150% of the working concentration with a correlation coefficient (r^2) of 0.9999. Recovery studies demonstrated accuracy with mean recoveries ranging from 99.59% to 100.20%. Method and intermediate precision studies showed %RSD values below 1.0%, while robustness studies confirmed that deliberate variations in chromatographic conditions did not significantly affect assay results.

Conclusion: The developed RP-HPLC method is simple, rapid, precise, accurate, specific, and robust for the estimation of Topiroxostat in bulk drug and tablet dosage forms. The method is suitable for routine quality control analysis and can be effectively applied in pharmaceutical industries for assay determination of Topiroxostat.

Keywords: Topiroxostat, RP-HPLC, Method Validation, ICH Q2(R1), Hyperuricemia, Quality Control, Pharmaceutical Analysis.

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1. INTRODUCTION

The development of a reliable and validated chromatographic method is a fundamental requirement in pharmaceutical quality control to ensure the safety, efficacy, and consistency of drug products. High-performance liquid chromatography (HPLC) remains one of the most widely employed analytical techniques due to its high sensitivity, selectivity, reproducibility, and suitability for routine analysis of pharmaceutical compounds. For newly developed therapeutic agents, validated analytical methods are essential for raw material testing, formulation development, stability studies, and regulatory compliance.[1,10]

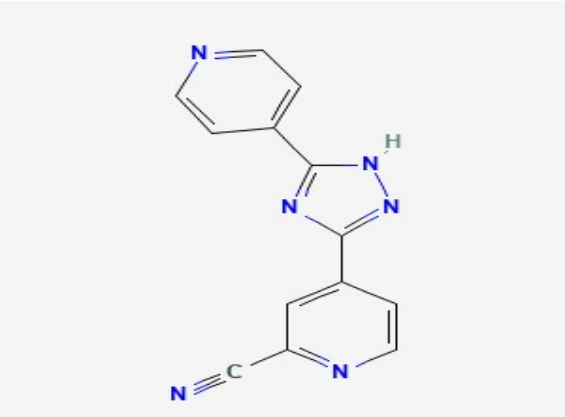


Fig 1:Structure of Topiroxostat

Topiroxostat is a novel non-purine selective xanthine oxidoreductase (XOR) inhibitor indicated for the treatment of hyperuricemia and gout. Chemically, Topiroxostat is 4-[5-pyridin-4-yl-1H-1,2,4-triazol-3-yl] pyridine N-oxide and possesses the molecular formula $C_{13}H_8N_4O$ with a molecular weight of 236.23 g/mol.[3] Unlike conventional urate-lowering agents, Topiroxostat exerts its pharmacological action through potent inhibition of xanthine oxidoreductase, the enzyme responsible for the oxidation of hypoxanthine to xanthine and subsequently xanthine to uric acid. By suppressing uric acid production, Topiroxostat effectively reduces serum urate concentrations and minimizes the risk of gout attacks and complications associated with chronic hyperuricemia.[3,5,8]

Hyperuricemia is a metabolic disorder characterized by elevated serum uric acid levels resulting from increased uric acid production, decreased renal excretion, or both. Persistent hyperuricemia is associated with gout, nephrolithiasis, chronic kidney disease, cardiovascular disorders, and metabolic syndrome.[5] Xanthine oxidoreductase inhibitors therefore represent an important therapeutic class in the management of urate-related disorders. Compared with allopurinol and febuxostat, Topiroxostat has demonstrated favorable efficacy and safety profiles in clinical studies and has emerged as a promising therapeutic option for long-term management of hyperuricemia.[5,8,9]

Topiroxostat exhibits significant ultraviolet absorption due to the presence of aromatic heterocyclic rings and conjugated nitrogen-containing functional groups within its molecular structure. UV spectral analysis performed during method development showed appreciable absorption in the range of 200–400 nm, with maximum absorbance observed at 273 nm. This wavelength was therefore selected for chromatographic detection as it provided optimum sensitivity, peak response, and reproducibility for quantitative analysis of Topiroxostat. The selected wavelength also minimized baseline noise and enhanced method performance during validation studies.[1,3,6]

The International Council for Harmonisation (ICH) guideline Q2(R1) outlines the validation characteristics required for analytical procedures intended for pharmaceutical applications. According to these guidelines, analytical methods must demonstrate acceptable specificity, linearity, accuracy, precision, robustness, and system suitability before being considered suitable for routine quality control use. Furthermore, regulatory authorities require validated chromatographic methods capable of producing accurate and reproducible results throughout the product lifecycle. Consequently, method development and validation remain critical components of pharmaceutical analytical research.[10,16,18,20]

A review of the published literature indicates that several analytical techniques have been reported for the determination of Topiroxostat in pharmaceutical formulations and biological matrices. UV spectrophotometric methods have been described for routine estimation of the drug; however, these methods often lack the specificity required for complex pharmaceutical matrices. Liquid chromatography coupled with mass spectrometry (LC–MS/MS) has been employed for pharmacokinetic and bioanalytical investigations due to its high sensitivity, although such techniques require sophisticated instrumentation and higher operational costs.[9] High-performance thin-layer chromatography (HPTLC) methods have also been reported for quantitative analysis, but they generally offer lower precision compared with modern HPLC techniques.[1],[5,6,]

Reverse-phase high-performance liquid chromatography (RP-HPLC) remains the preferred analytical approach for routine pharmaceutical quality control because of its robustness, accuracy, and ease of implementation. Nevertheless, limited information is available regarding simple, economical, and fully validated RP-HPLC methods suitable for routine assay determination of Topiroxostat in bulk drug and tablet dosage forms. Therefore, there remains a need for a rapid and reproducible chromatographic method that can be readily adopted in quality control laboratories.[2,18,20]

The present study addresses this requirement through the development and validation of a simple isocratic RP-HPLC method for the estimation of Topiroxostat in bulk drug and tablet dosage form. Chromatographic separation was achieved using a Phenomenex C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of Acetonitrile and 0.1% Orthophosphoric Acid (30:70 v/v) delivered at a flow rate of 1.0 mL/min. Detection was carried out

at 273 nm, and the method was validated according to ICH Q2(R1) guidelines with respect to system suitability, specificity, linearity, accuracy, precision, and robustness. The developed method is intended to provide a rapid, accurate, precise, and cost-effective analytical procedure suitable for routine quality control analysis of Topiroxostat in pharmaceutical formulations.[10]

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Topiroxostat working standard was procured from Vidisha Analytical Laboratory, Pune. Commercially available Topiroxo tablets containing 40 mg of Topiroxostat were obtained from Alkem Laboratories Ltd., India. HPLC-grade acetonitrile was purchased from Merck Life Science Pvt. Ltd., Mumbai, India. Orthophosphoric acid (OPA, AR grade) and HPLC-grade water were obtained from Merck and Rankem, respectively. Nylon membrane filters (0.45 µm) and PVDF syringe filters (0.45 µm) were used for filtration of mobile phase and sample solutions before chromatographic analysis.

2.2 Instrumentation

Chromatographic analysis was carried out using an Agilent 1260 Infinity II High-Performance Liquid Chromatography system equipped with a UV/PDA detector and OpenLab EZChrom software for data acquisition and processing. UV spectral studies were performed using a Jasco double-beam UV–Visible spectrophotometer with matched quartz cells of 10 mm path length. Samples were weighed using a calibrated analytical balance, and pH measurements were performed using a digital pH meter.

2.3 Chromatographic Conditions

Chromatographic separation was achieved using a Phenomenex C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of 0.1% Orthophosphoric Acid and Acetonitrile in the ratio of 70:30 (v/v), delivered in isocratic mode at a flow rate of 1.0 mL/min. Detection was performed at 273 nm using a UV/PDA detector. The column temperature was maintained at 40°C, while the sample compartment temperature was kept at 25°C. The injection volume was 20 µL and the total run time was 7 minutes.

2.4 Preparation of Mobile Phase

A 0.1% Orthophosphoric Acid solution was prepared by accurately transferring 1.0 mL of Orthophosphoric Acid into 1000 mL of HPLC-grade water and mixing thoroughly. The aqueous phase was filtered through a 0.45 µm nylon membrane filter and degassed by sonication for 15 minutes. The mobile phase was prepared by mixing 700 mL of 0.1% Orthophosphoric Acid solution with 300 mL of Acetonitrile (70:30 v/v). The resulting solution was filtered through a 0.45 µm membrane filter and sonicated before use.

2.5 Preparation of Diluent

The mobile phase consisting of 0.1% Orthophosphoric Acid and Acetonitrile (70:30 v/v) was used as the diluent for preparation of standard and sample solutions throughout the study.

2.6 Preparation of Standard Solution

Accurately weighed 40 mg of Topiroxostat working standard was transferred into a 100 mL volumetric flask. Approximately 70 mL of diluent was added and the solution was sonicated for 15 minutes to ensure complete dissolution. The volume was then made up to the mark with diluent to obtain the stock solution. Appropriate aliquots were further diluted with diluent to obtain the working standard concentration required for chromatographic analysis. The solution was filtered through a 0.45 µm nylon membrane filter, discarding the first 2 mL of filtrate.

2.7 Preparation of Sample Solution

Twenty tablets were accurately weighed and powdered. A quantity of powder equivalent to 40 mg of Topiroxostat was transferred into a 100 mL volumetric flask. About 70 mL of diluent was added and the mixture was sonicated for 30 minutes with intermittent shaking to achieve complete extraction of the drug. The solution was allowed to cool and diluted to volume with diluent. The resulting solution was filtered through a 0.45 µm nylon membrane filter, discarding the first 2 mL of filtrate. Suitable dilutions were prepared to obtain the required concentration for assay determination.

3. Results and Discussion

3.1 Drug Characterisation

3.1.1 Physical Characteristics

Topiroxostat was obtained as a white to off-white crystalline powder with no characteristic odour. The physical appearance of the drug complied with the specifications provided by the manufacturer and was consistent with the reported description of Topiroxostat in the literature.[3,5]

3.1.2 Solubility Studies

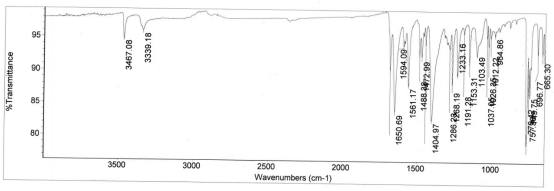
Solubility studies were performed in various solvents commonly used during chromatographic method development. Topiroxostat showed limited solubility in water but exhibited good solubility in Acetonitrile. Complete dissolution was achieved in the optimized diluent system consisting of Acetonitrile and 0.1% Orthophosphoric Acid, producing a clear and stable solution suitable for

chromatographic analysis. Therefore, the optimized mobile phase composition was selected as the diluent for all subsequent standard and sample preparations.

3.2 Infrared Spectroscopy

The identity of Topiroxostat was confirmed by FTIR spectroscopy. The spectrum showed characteristic absorption bands corresponding to the functional groups present in the drug molecule. Prominent peaks were observed for aromatic C–H stretching, C=N stretching of the triazole ring, C=C aromatic stretching, and N–O functional group vibrations. The obtained spectrum was consistent with the reported reference spectrum of Topiroxostat, confirming the authenticity and purity of the working standard. (Figure 2)

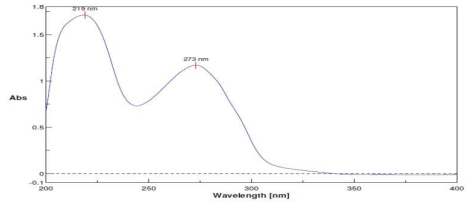
Figure 2: FTIR spectrum of Topiroxostat showing characteristic absorption bands at 1404.97cm^{-1} (C-H Bending), 1650.69cm^{-1} (C=N stretching), 3467.08cm^{-1} (N-H Stretching), and 984.86cm^{-1} (C=C Bending (At column width)



3.3 UV Spectroscopy and Wavelength Selection

The UV absorption spectrum of Topiroxostat was recorded over the range of 200–400 nm. The drug exhibited significant absorption throughout the UV region with a maximum absorbance (λmax) observed at 273 nm. The wavelength of 273 nm provided optimum absorbance response, minimal baseline interference, and satisfactory detector sensitivity. Consequently, 273 nm was selected as the analytical wavelength for all RP-HPLC investigations. (Figure 3)

Figure 3: UV absorption spectrum of Topiroxostat (10 µg/mL in Water: Acetonitrile 40:60 v/v) scanned from 200 to 400 nm, showing primary λmax at 273 nm (absorbance 1.3561 AU).



3.4 Method Development and Optimisation

Several chromatographic trials were conducted during method development by varying the mobile phase composition, flow rate, and chromatographic conditions to obtain optimum peak symmetry, retention, and column efficiency. Initial trials using different ratios of aqueous phase and organic modifier resulted in broad peaks, poor symmetry, and inadequate retention behavior.

The optimized chromatographic conditions were achieved using a Phenomenex C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of 0.1% Orthophosphoric Acid and Acetonitrile (70:30 v/v) delivered at a flow rate of 1.0 mL/min. Detection was carried out at 273 nm with a column temperature of 40°C and a run time of 7 minutes.

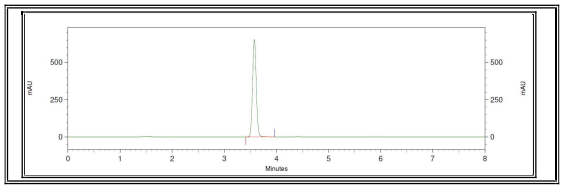
Under these optimized conditions, Topiroxostat was eluted at a retention time of 3.56 min with excellent peak symmetry. The tailing factor was found to be 1.1 and the theoretical plate count was 9456, indicating efficient chromatographic separation and good column performance. These optimized conditions were selected for method validation. (Table 2 and Figure 4)

Table 2: Final optimized RP-HPLC chromatographic conditions column, mobile phase, flow rate, detection wavelength, injection volume, column temperature, and run time.

Chromatographic Conditions:

Column	Phenomenex C18, 5µ, 4.6 x 250mm
Mobile Phase	Acetonitrile: 0.1% Orthophosphoric Acid (30:70 v/v)
Flow Rate	1.0 mL/min
Injection Volume	20 µL
Wavelength	273 nm
Column Temp	40ºC
Sample Temp	25ºC
Run Time	7.0 minutes

Seal Wash	Water: Acetonitrile (90:10) v/v
Needle Wash	Water: Acetonitrile (10:90) v/v



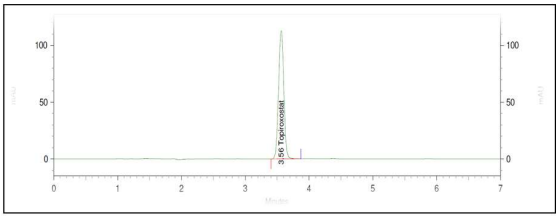


Fig. 4: Typical chromatogram for Trial- 6

3.5 Method Validation

3.5.1 System Suitability

System suitability was evaluated using five replicate injections of the standard solution. The mean retention time of Topiroxostat was 3.56 min. The percentage relative standard deviation (%RSD) of peak areas was 0.25%, which was well within the acceptance criterion of not more than 2.0%.

The tailing factor was 1.1 and the theoretical plate count was 9456, exceeding the minimum requirement of 2000 plates. These results demonstrated that the chromatographic system was suitable for routine analysis. (Table 3)

Table 3: System suitability parameters for Topiroxostat %RSD of peak area, tailing factor, theoretical plates, and resolution (n = 5 replicate injections)

Parameter	**Topiroxostat**
Tailing Factor	1.1
Theoretical Plates	9456
Retention Time (min)	3.56
Parameter	**Topiroxostat**
%RSD	0.25

Injection No.	**Area**
1	11,526,415
2	11,505,418
3	11,574,857
4	11,542,515
5	11,565,869
Mean	**11,543,015**
%RSD	**0.25**

3.5.2 Specificity

Specificity was evaluated by comparing chromatograms obtained from blank, standard, and sample solutions. No interfering peaks were observed at the retention time of Topiroxostat in the blank chromatogram.

Peak purity evaluation using PDA detection demonstrated that the purity angle was lower than the purity threshold for both standard and sample preparations, confirming the absence of co-eluting impurities and establishing the specificity of the developed method. The retention time of Topiroxostat remained consistent across all injections at approximately 3.56 min. (Table 4 and Figure 5–8)

Table 4: Specificity data retention times for identification, blank interference results, and PDA peak purity angles and thresholds for Topiroxostat in standard and sample solutions.

Component	**Retention time (min)**	**Tailing Factor**	**Purity angle**	**Purity threshold**
Blank	-	-	-	-
Standard solution				
Topiroxostat	3.56	1.1	2.34	3.68
Sample solution				
Topiroxostat	3.56	1.1	2.10	3.45

Individual peak identification				
Topiroxostat	3.56	1.1	2.27	3.48

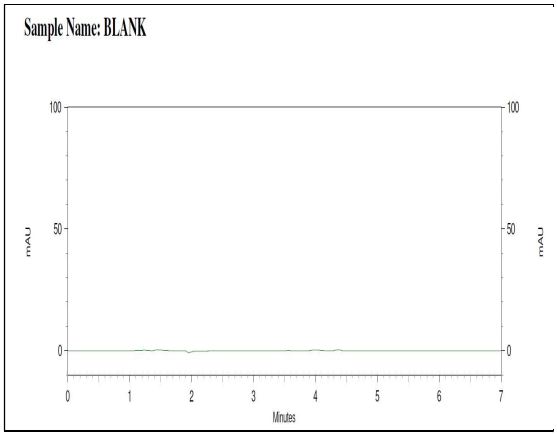


Fig.5: Chromatogram of Blank

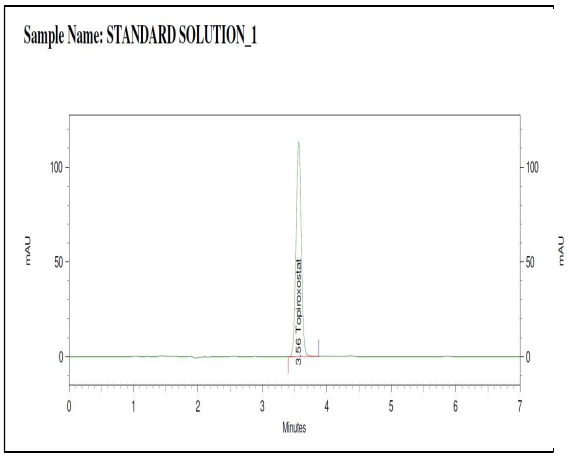


Fig.6: Chromatogram of Standard

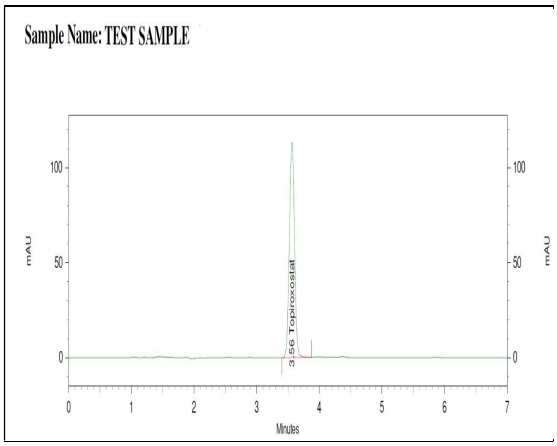


Fig.7:Chromatogram of Sample

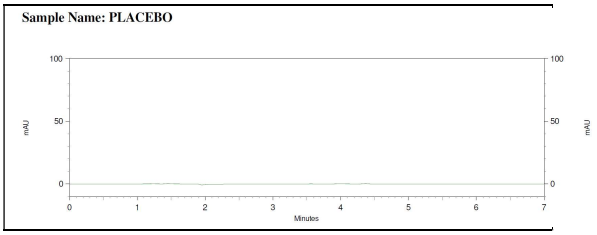


Fig.8: Chromatogram of Placebo

3.5.3 Linearity

The method exhibited excellent linearity over the concentration range corresponding to 50–150% of the target analytical concentration. Calibration solutions prepared at five concentration levels produced a linear relationship between concentration and peak area response.

The regression analysis yielded a correlation coefficient (r^2) of 0.9999, demonstrating excellent linearity across the studied concentration range. The calibration curve showed a direct proportional relationship between concentration and detector response, satisfying the acceptance criterion of $r^2 \geq 0.999$. (Table 5 and Figure 9)

Table 5: Linearity data for Topiroxostat concentration levels (30 µg/mL), peak areas, calibration curve parameters (slope, intercept, correlation coefficient r, % Y-intercept, RSS).

Linearity was evaluated in the range of 50 % to 150 % of Topiroxostat for working concentration. The working concentration of Topiroxostat in solution is 30 µg/mL. The data summarized in Table.

Level	Conc (µg/mL)	Area	Mean
50%	10	5763210	5772350
		5774185	
		5779654	
75%	15	8635412	8642926
		8642109	
		8651257	
100%	20	11524109	11511009
		11510351	
		11498567	
125%	25	14368451	14356016

		14359468	
		14340128	
150%	30	17402540	17402906
		17410524	
		17395653	
Corr. Coeff			0.9999
Intercept			119442
Slope			568040
% Y-intercept			1.04

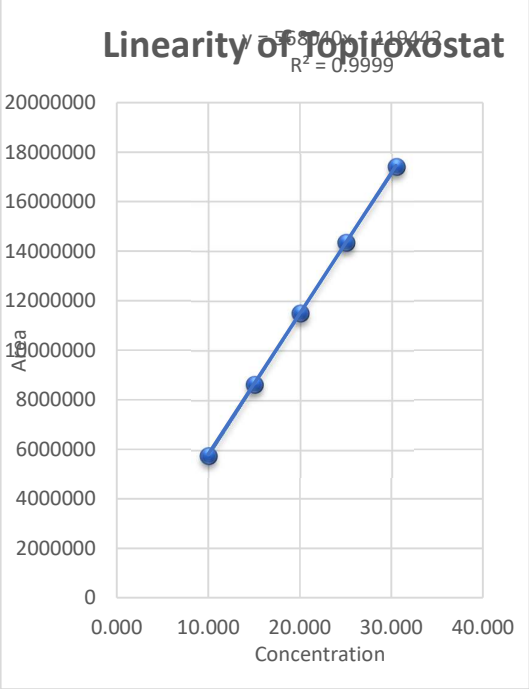


Fig. 9: Linearity of Topiroxostat

3.5.4 Accuracy

Accuracy was determined through recovery studies performed at 50%, 100%, and 150% concentration levels in triplicate.

The mean percentage recoveries obtained were 99.59%, 99.86%, and 100.20% at the respective levels. All recovery values were within the acceptance range of 98.0–102.0%, demonstrating the accuracy of the developed method for quantitative estimation of Topiroxostat. (Table 6)

Table 6: Accuracy (% Recovery) data for Topiroxostat at 50%, 100%, and 150% The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 98.0% - 102.0%.

Level (%)	Topiroxostat Added Conc (µg/mL)	Topiroxostat Recovered conc	Area	% Recovery	Mean % Recovery
50	10.01	9.98	5762514	99.73	99.59
	10.05	10.06	5803521	100.07	
	10.04	9.93	5732548	98.97	
100	20.03	19.98	11532540	99.75	99.86
	20.04	19.85	11456257	99.03	
	20.05	20.21	11665213	100.80	
150	30.06	30.18	17420429	100.41	100.20
	30.02	30.19	17426547	100.57	
	30.38	30.26	17465208	99.61	

3.5.5 Precision

Method precision was assessed using six independently prepared sample solutions. The mean assay value obtained was 98.28% with a %RSD of 0.484%, indicating excellent repeatability.

Single injection of blank (Diluent), Standard solution (five replicates) and sample solution (six preparations) was injected on the system. (Table 7)

Table 7: Method precision on pharmaceutical dosage form

Sample	Area	% Assay
Sample 1	11323635	97.95
Sample 2	11412023	98.61
Sample 3	11365219	98.41
Sample 4	11356234	97.77
Sample 5	11452109	99.01
Sample 6	11342022	97.95
Mean		**98.28**
STD DEV		**0.4753**
% RSD		**0.484**

Table 8: Method precision on Bulk(API)

Sample	Area	% Assay
Sample 1	11530419	99.74
Sample 2	11510235	99.46
Sample 3	11498567	99.56
Sample 4	11515471	99.14
Sample 5	11520315	99.60
Sample 6	11496538	99.29
Mean		**99.46**
STD DEV		**0.2172**
% RSD		**0.218**

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

Intermediate Precision:

Intermediate precision was evaluated by a second analyst on a different day using the same chromatographic conditions. The mean assay value obtained was 98.35% with a %RSD of 0.576%. The difference between method precision and intermediate precision results was negligible and within the predefined acceptance limits, confirming the ruggedness of the analytical procedure. (Table 9)

Table 9: Intermediate Precision

Sample	Area	% Assay
Sample 1	11420157	98.88
Sample 2	11406876	98.51
Sample 3	11282415	97.54
Sample 4	11432784	98.48
Sample 5	11275416	97.78
Sample 6	11433207	98.89
Mean		**98.35**
STD DEV		**0.5663**
% RSD		**0.576**

Table 10: Intermediate Precision pool Data

Parameter	Method Precision (Analyst-I)	Intermediate Precision (Analyst-II)
HPLC NO.	**AD/HPLC-02**	**AD/HPLC-04**
Column No.	**HPLC-33**	**HPLC-46**
Sample No.	**%Assay**	
1	97.95	98.88
2	98.61	98.51
3	98.41	97.54
4	97.77	98.48
5	99.01	97.78
6	97.95	98.89
Mean	**98.28**	**98.35**
Mean of Precision % Assay	**98.32**	

Absolute Mean difference % assay	**0.5**

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % Assay is of six samples is not more than 2.0
- The data shows that % Assay is within the acceptance criteria and hence the method is rugged.

3.5.6 Robustness

Robustness studies were conducted by deliberately varying chromatographic parameters including flow rate and detection wavelength.

Flow rate variation to 0.9 mL/min and 1.1 mL/min resulted in assay values of 98.91% and 97.31%, respectively. Similarly, wavelength variations to 271 nm and 275 nm produced assay values of 98.28% and 97.59%, respectively. All results remained within acceptable limits, and system suitability parameters were maintained under each modified condition.

The findings demonstrated that minor deliberate changes in chromatographic conditions did not significantly affect the analytical performance of the method, confirming its robustness for routine quality control applications. (Table 11)

Table 11: Robustness data for Topiroxostat % assay and system suitability parameters under deliberate variations in flow rate (±0.1 mL/min), detection wavelength (±2 nm), and column temperature (±2°C), with absolute difference from control condition.

Change in parameter	Condition	Area	% Assay	Absolute difference of % Assay
Control	As per method	11323635	97.95	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.1 ml/min	11250235	97.31	0.64
	0.9 ml/min	11435237	98.91	-0.96
Change in wavelength (±2 nm)	275 nm	11282415	97.59	0.36
	271 nm	11362105	98.28	-0.33

4. SUMMARY

A simple, rapid, and reliable RP-HPLC method was successfully developed and validated for the estimation of Topiroxostat in bulk drug and tablet dosage forms according to ICH Q2(R1) guidelines.

Chromatographic separation was achieved using a Phenomenex C18 column (250 × 4.6 mm, 5 µm) with a mobile phase comprising 0.1% Orthophosphoric Acid and Acetonitrile (70:30, v/v) at a detection wavelength of 273 nm. Topiroxostat eluted at a retention time of 3.56 min with excellent peak symmetry (tailing factor 1.1) and column efficiency (9456 theoretical plates). The total run time was 7 min, making the method suitable for routine quality control analysis.

System suitability results demonstrated good chromatographic performance with a peak area %RSD of 0.25%, confirming excellent repeatability. Specificity studies showed no interference from blank or excipient components at the retention time of Topiroxostat, indicating high method specificity.

The method exhibited excellent linearity over the studied concentration range (50–150%) with a correlation coefficient (r^2) of 0.9999. Accuracy studies produced mean recoveries ranging from 99.59% to 100.20%, demonstrating the accuracy of the method. Precision studies showed %RSD values of 0.484% for method precision and 0.576% for intermediate precision, confirming high reproducibility.

Robustness evaluation revealed that small deliberate changes in flow rate and detection wavelength did not significantly affect assay results or system suitability parameters. All validation results complied with ICH acceptance criteria.

Overall, the developed RP-HPLC method was found to be simple, accurate, precise, specific, and robust for the routine estimation of Topiroxostat in pharmaceutical dosage forms.

5. CONCLUSION

A simple, rapid, accurate, precise, and robust RP-HPLC method was successfully developed and validated for the estimation of Topiroxostat in bulk drug and tablet dosage forms. The optimized chromatographic conditions provided efficient separation with a retention time of 3.56 min and excellent system suitability parameters. Validation studies performed according to ICH Q2(R1) guidelines demonstrated satisfactory specificity, linearity (r^2 = 0.9999), accuracy (99.59–100.20% recovery), precision (%RSD < 1%), and robustness. The short analysis time, simple isocratic mobile phase, and use of readily available instrumentation make the method suitable for routine quality control analysis. Therefore, the developed RP-HPLC method can be successfully applied for the

quantitative determination of Topiroxostat in pharmaceutical dosage forms. The validated RP-HPLC method for Topiroxostat can be further applied in stability-indicating studies, degradation kinetics, and quality control analysis of pharmaceutical formulations. The method may also be adapted for bioanalytical applications in pharmacokinetic and bioavailability studies. Additionally, it can support the development of new dosage forms, excipient compatibility studies, generic drug development, and regulatory submissions. Its simplicity, accuracy, and robustness make it a valuable analytical tool for future pharmaceutical research and industrial applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The Topiroxostat working standard was provided by Vidisha Analytical Laboratory, Pune, and the tablet formulation was obtained from Alkem Laboratories Ltd. Neither organization had any role in study design, data collection, analysis, interpretation of results, manuscript preparation, or the decision to publish this work.

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