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ECO-FRIENDLY MULTIVARIATE CALIBRATION AIDED SPECTROPHOTOMETRIC ESTIMATION OF TOPIROXOSTAT IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The primary objective of this study is to evolve and verify a reliable, simple, precise, and convenient UV-visible spectroscopic method in order to determine the quantity of Topiroxostat, following the recommendations outlined in ICH Q2 (R1). The multivariate calibration uses linear regression analysis to establish the association of concentration with absorbance at five specific wavelengths that are evenly distributed. Using ethanol as the solvent, Topiroxostat displayed λ_{max} at 272 nm. A linear plot was obtained with a regression coefficient 0.999 for the concentrations between 8-12 µg mL^{-1}. The % RSD for inter-day and intra-day precision was found to be 0.285 and 0.234, respectively, and the assay was determined to be 99.52 % w/w.

Keywords: ICH guideline, UV-visible spectrophotometry, Topiroxostat, Multivariate calibration

INTRODUCTION

Topiroxostat is chemically known as 4-[5-(4-Pyridinyl)-1H-1,2,4-triazol-3-yl] pyridine-2-carbonitrile. The molecular formula and molecular weight were found to be $C_{13}H_8N_6$ and 248.24 g mol^{-1} respectively **[1]**. Topiroxostat **(Figure 1)** is a non-purine, selective xanthine oxidase inhibitor that acts as an anti-gout medication. It regulates purine metabolism and inhibits the activity of xanthine oxidase or xanthine

oxidoreductase (XOR) resulting in the reduction of serum urate levels. It decreases the concentration of insoluble urates and uric acids in tissues, plasma, and urine hence exhibiting potent anti-hyperuricemic effects

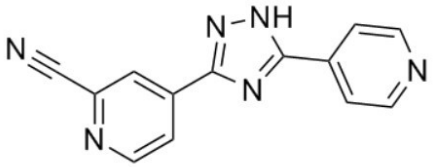

Figure 1: Chemical structure of Topiroxostat

The multivariate technique provides clear accuracy in results, so the results have shown increased accuracy and precision than conventional UV methods. This method simplifies and converts the result to "m" value as a reliant variable. The absorbance of an analyte (X), i.e., Topiroxostat, is examined at five diverse wavelengths surrounding its absorbance maxima (λ = 266, 269, 272, 275, 278 nm). The following formula can be expressed by,

"$A_{\lambda 266} = a\ X\ C_x + \ k_1$ ---------- (1)

$A_{\lambda 269} = b\ X\ C_x + \ k_2$ ---------- (2)

$A_{\lambda 272} = c\ X\ C_x + \ k_3$---------- (3)

$A_{\lambda 275} = d\ X\ C_x + \ k_4$---------- (4)

$A_{\lambda 278} = e\ X\ C_x + \ k_5$---------- (5)"

Equation system from (1-5) represent the analyte's absorbance at specific wavelengths i.e., 266, 269, 272, 275, 278 nm, linear regression slopes at (a, b, c, d, e), intercepts at (k_1, k_2, k_3, k_4, k_5), and concentration (C_x) **[2]**. Literature surveys demonstrate various techniques for estimating Topiroxostat, like UV-Visible Spectroscopy (UV) **[3, 4]**, High Performance Liquid Chromatography (HPLC) **[5]**, and Mass Spectroscopy **[6]**. respectively. A_T and K_T are the combination of absorbance from the regression equations at five selected wavelengths **[7-14]**.

$$A_T = a\ X\ C_x + \ b\ X\ C_x + \ c\ X\ C_x + \ d\ X\ C_x + \ e\ X\ C_x + \ K_T \text{---------- (6)}$$

The above equation can be further condensed to

$$A_T = C_x\ (a + b + c + d + e) + \ K_T \text{---- (7)}$$

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)}$$

Greenness Evaluation Techniques

The Globally Harmonised System of Classification and Labelling of Chemicals (GHS) developed the analytical eco scale. It is based on the assignment of penalty points based on both quantity and number. Additionally, the GHS produced a set of pictograms and associated signal words **[15]**. The analytical eco-scale method takes into account every reagent, taking into account its sort and quantity, waste, potential occupational exposure, and energy depletion. From a base score of 100 points, penalty points are subtracted.

Analytical eco-scale = 100 - total penalty points……. (9)

Comprising five pentagons with separate colour coding, the Green Analytical

Procedure Index (GAPI) is a visual representation. At every step of an analytical procedure, the coding of the color in the pictogram corresponds to 3 levels of evaluation. GAPI's color coding for greenness runs from green to yellow to red, indicating the minimal, medium, and high environmental implications related with the analytical method, respectively. J. Potka Wasylka provided a succinct overview of GAPI in the year 2018 **[16]**. The third way of assessment uses the unique software developed by AGREE metrics **[17]** to evaluate the greenness profile. The software produces a circle with clockwise-oriented numerals ranging from 1 to 12 around its periphery. The twelve green analytical chemistry ideologies are represented by these figures. Each of these 12 principles has an output rating ranging from 0 to 1, depending on the inputs and their respective weights. The hues red, yellow, and green are used in this aggregate scale to indicate different numbers. Yellow denotes a number in between red and dark green, dark green denotes one or very nearly one, and red denotes zero. The core and the 12 principles are added to provide a score that indicates the degree of greenness.

MATERIALS AND METHODS

Materials and reagents required

- Ethanol
- Topiroxostat was exgratis by Ideal Analytical Laboratory Pvt. Ltd, Pudhucherry.
- The dosage form Topiroxo 20 mg tablets manufactured by ALKEM LABORATORIES LTD, acquired from a local medical shop.

Instrumentation

- UV-Visible double beam spectrophotometer [LAB INDIA 3092]
- Analytical balance
- Micropipette

Analytical method development

Solvent selection

Topiroxostat shows high solubility in ethanol (EtOH). Further dilutions of the standard and sample mixtures were made by employing ethanol as the solvent.

Standard stock solution

Solubilizing 25 milligrams of the active pharmaceutical ingredient in (EtOH) to obtain 1000 µg mL^{-1} serves as the standard stock solution of Topiroxostat. This was used to make an aliquot of solutions with concentrations ranging from 8-12 µg mL^{-1}.

Determination of Absorption maxima

From the standard stock solution, 10 µg mL^{-1} was prepared and scanned in UV spectrophotometry in the region from 200 to 400 nm, to identify the maximum absorbance. The λ_{max} was at 272 nm and is presented in **Figure 2**. The linearity was

acquired in concentration limits of 8-12 µg mL^{-1}. The solutions were scanned over various wavelengths about 272 nm in order to reduce the oscillations of the instrument and improve the correlation, wavelengths including 266, 269, 272, 275, and 278 nm, respectively.

Preparation of sample solution

Ten Topiroxostat tablets were precisely measured and pulverized. In order to obtain 1000 µg mL^{-1}, a weight identical to 20 mg was transferred to a 25 ml standard flask, and it was then further dissolved, diluted, and made up to the mark with ethanol. The resulting filtrate is used for further analysis after filtering.

Method Validation

The above method has been evaluated in accordance with ICH Q2 (R1) guidelines for precision, accuracy, sensitivity, and linearity **[18]**.

Linearity

The standard stock solution of Topiroxostat was used to prepare different concentrations ranging from 8-12 µg mL^{-1}. To eliminate instrumental errors and enhance correlation prepared solutions were examined over the range of wavelengths around their respective maximum absorbance at 266, 269, 272, 275 and 278 nm. The absorbances were recorded, and a concentration versus absorbance graph was used to obtain the results **(Table 1)**. The extent of detection and quantification were assessed using the formula below to determine the method's sensitivity.

Table 1: UV Calibration data at five distinct wavelengths

Concentration (µg mL-1)	Absorbance				
	266 nm	269 nm	272 nm	275 nm	278 nm
8	0.169	0.171	0.187	0.172	0.168
9	0.324	0.336	0.353	0.339	0.332
10	0.502	0.525	0.533	0.527	0.508
11	0.678	0.719	0.727	0.720	0.693
12	0.851	0.911	0.919	0.912	0.871

[#]Average of 5 determinations; UV= Ultra Violet

$$LOD = 3.3\ \sigma/S \quad \text{..... (8)}$$

$$LOQ = 10\ \sigma\ /S \quad \text{..... (9)}$$

Where, S stands for standard curve slope, and σ for the standard deviation (SD) of the lowest concentration.

Precision

10 µg mL^{-1} solution was examined in the Ultra-Violet region from 200 to 400 nm on six various days for interday and six times over a short time period for intraday to assess the precision studies.

Accuracy

Assessing the recovery study at 80%, 100%, and 120% was done using the conventional addition technique. To three 10 ml standard flasks, 0.5 ml of the reference solution was pipetted. Sample solutions of 0.3, 0.5, and 0.7 ml were added to the same flasks and made up to the mark. After UV scanning

these solutions, the recovery percentage was computed.

Assay

On measuring the absorbance of the extracted tablet solution at 272 nm, the amount of Topiroxostat that is present in the tablet dosage form has been determined.

RESULTS AND DISCUSSION

Using distilled water as a solvent, the λ_{max} **of Topiroxostat** was found to be 272 nm (**Figure 2**).

This approach is linear within the applied range of concentration from 8 to 12 µg mL^{-1}. This linear regression analysis demonstrates a strong linear relationship with R^2 = 0.999-0.9996. The % RSD values were 0.234 and 0.285 for intra-day and inter-day precision. The LOD and LOQ values were 0.1912 and 0.5795 g mL^{-1}, respectively. Hence, the obtained values were within the ICH validation parameter limits.

Linearity

Linearity at 266, 268, 272, 275 and 278 nm was recorded with a concentration range from 8-12 µg mL^{-1} (**Figure 3**), with low relative standard deviation values demonstrating the method's accuracy and precision. LOD and LOQ were calculated. The calibration plots and residual plots were shown in **Figures 4a to 8a and 4b to 8b** with the data presented in **Table 2**.

Precision

The technique's specificity is demonstrated by the low standard deviation (SD) readings; the percentage RSD for inter-day and intra-day precision was determined to be 0.285 and 0.234, respectively. At each wavelength, they vary by less than 2 %. The low percentage of relative standard deviation suggests that this approach is precise as well as accurate. (**Figure 9, 10**).

Recovery

The percentage recovery of Topiroxostat was from 98.82% to 101.79 % w/w, as per ICH guidelines (**Table 3, Figure 11**).

Assay

Assay ultraviolet absorbance of the selected dosage form was reported at 272 nm. The amount and assay results were 19.90 mg and 99.52 % w/w, respectively with % RSD values as in **Table 4**.

Evaluation of Greenness Profile

For the suggested techniques, the outcomes of the greenness profile were assessed. **Table 5** displays the analytical scale results; the results agree metrics and the GAPI are shown in **Figure 12 and Figure 13**.

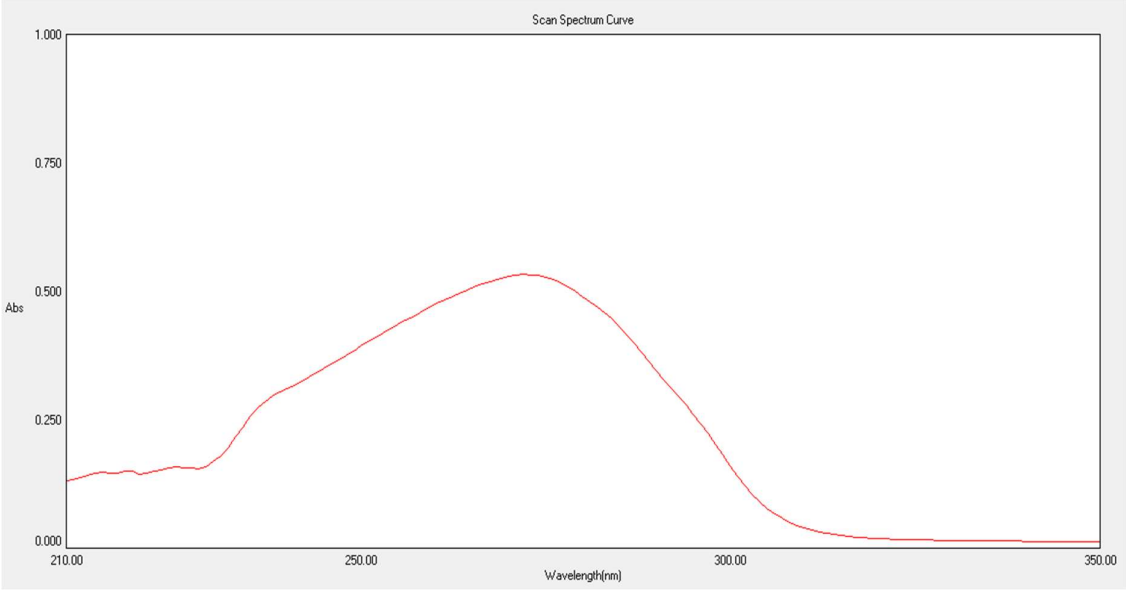

Figure 2: UV spectrum of Topiroxostat (10 µg mL^{-1}), λ_{max} at 272 nm

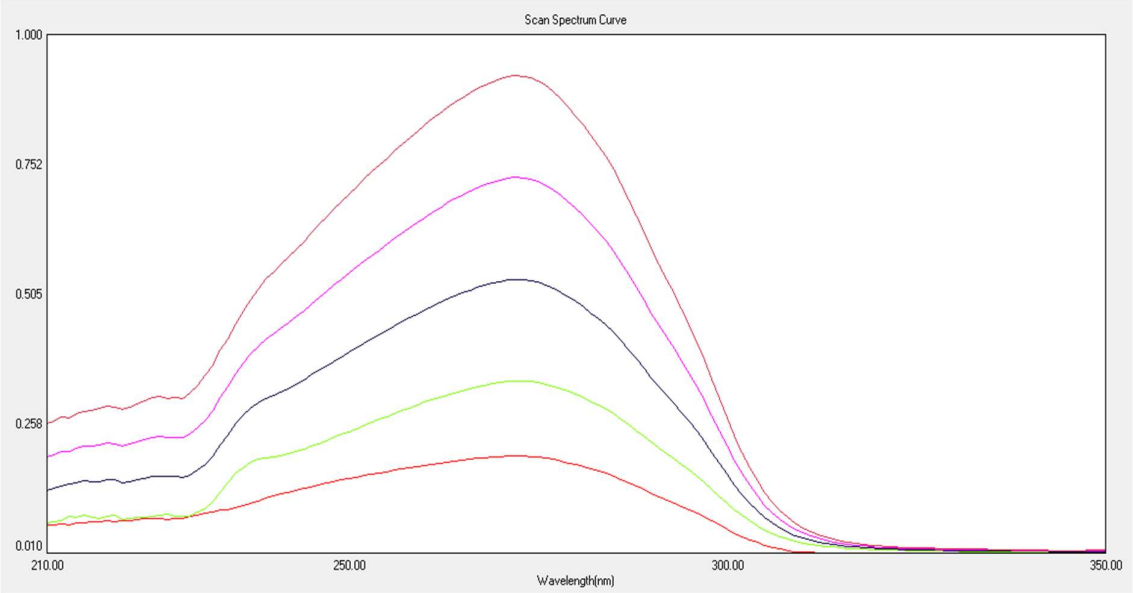

Figure 3: UV Spectrum of Topiroxostat showing linearity at 272 nm

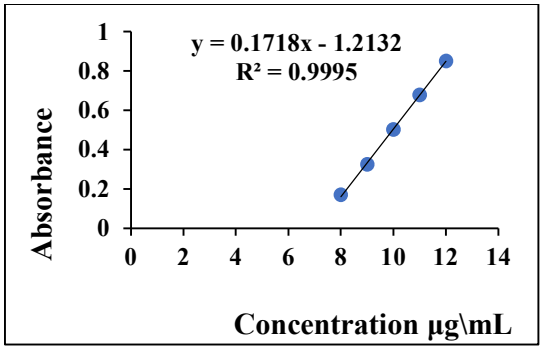

Figure 4a: Calibration curve at 266 nm

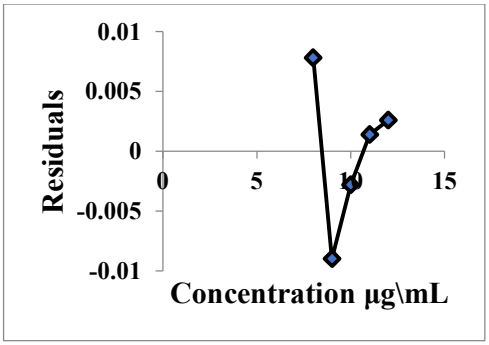

Figure 4b: Residual plot for 266 nm

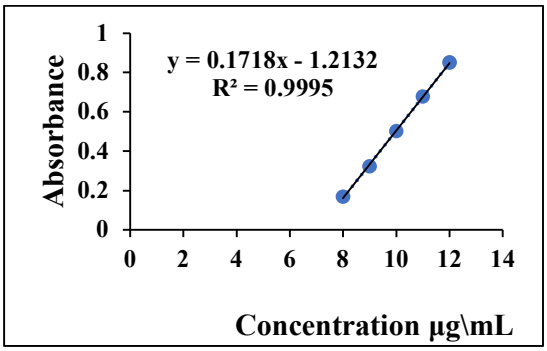

Figure 5a: Calibration curve at 269 nm

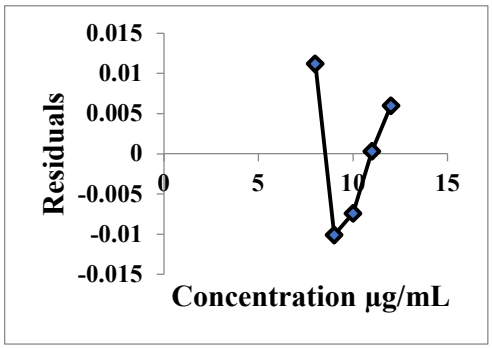

Figure 5b: Residual plot for 269 nm

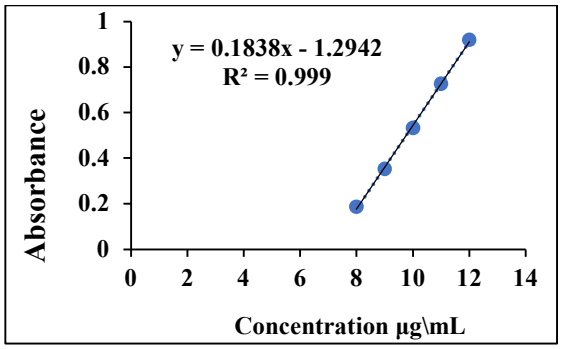

Figure 6a: Calibration curve at 272 nm

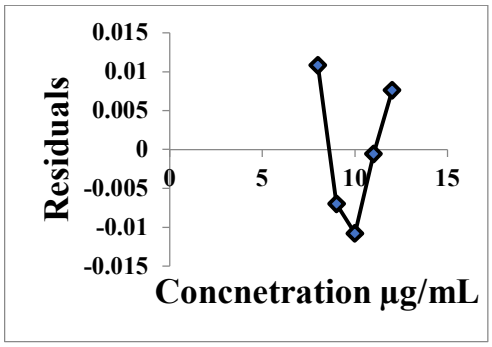

Figure 6b: Residual plot for 272 nm

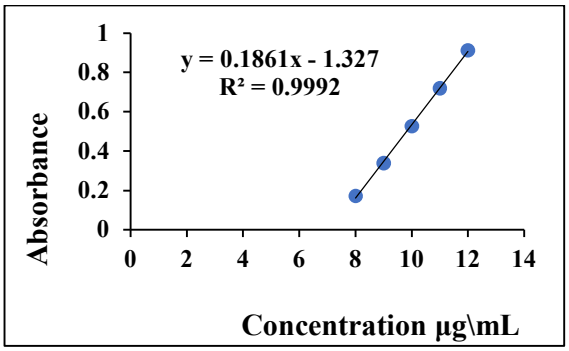

Figure 7a: Calibration curve at 275 nm

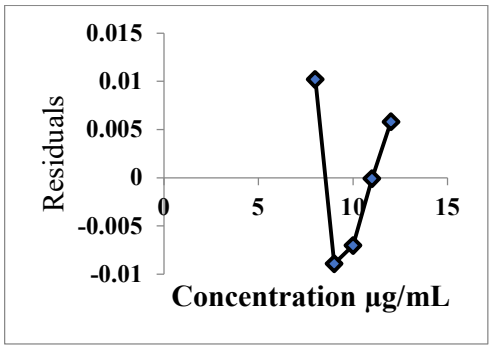

Figure 7b: Residual plot for 275 nm

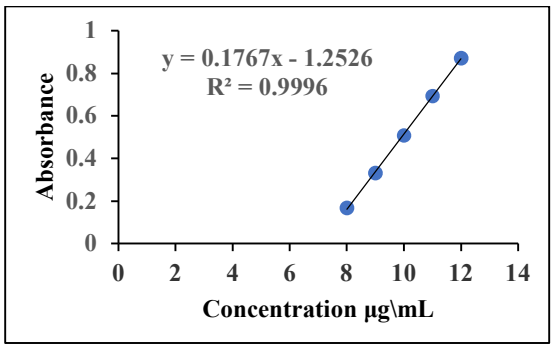

Figure 8a: Calibration curve at 278 nm

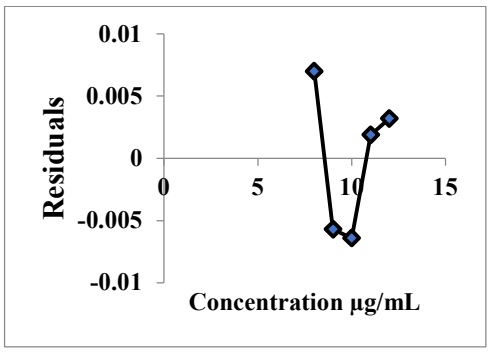

Figure 8a: Residual plot for 278 nm

Table 2: Linearity data with LOD and LOQ at selected five wavelengths.

Wavelength (nm)	Regression equation	R^2	LOD (µg mL^{-1})	LOQ (µg mL^{-1})	% RSD
266	y = 0.1718x-1.2132	0.9995	0.1395	0.4229	1.4394
269	y = 0.1718x-1.12132	0.9995	0.1824	0.5528	1.9347
272	y = 0.1838x – 1.2942	0.999	0.1912	0.5795	1.9588
275	y = 0.1861x – 1.327	0.9992	0.1669	0.5058	1.7630
278	y =0.1767x – 1.2526	0.9996	0.1258	0.3814	1.3103

*nm = nanometre; µg mL^{-1} = Microgram per millilitre

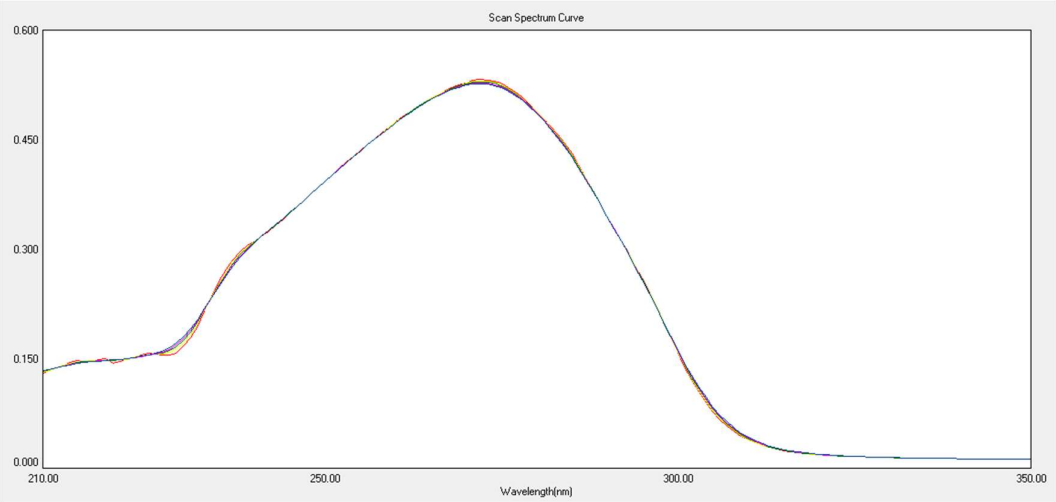

Figure 9: UV spectra showing intraday precision

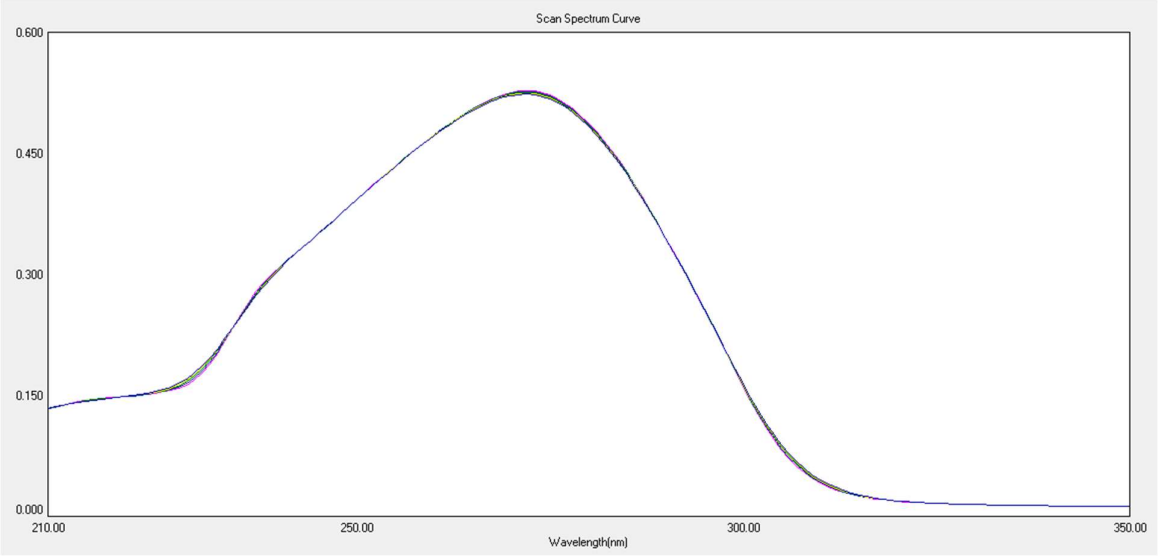

Figure 10: UV spectra showing interday precision

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg mL^{-1})	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	% Recovery
266 nm	5	3	7.90	98.82
		5	9.98	99.80
		7	12.02	100.24
269 nm	5	3	8.09	101.17
		5	9.92	99.24
		7	12.05	100.44
272 nm	5	3	8.08	101.07
		5	10.03	100.38
		7	12.07	100.65
275 nm	5	3	8.13	101.74
		5	9.92	99.24
		7	11.98	99.89
278 nm	5	3	8.14	101.79
		5	10.07	100.79
		7	12.09	100.80

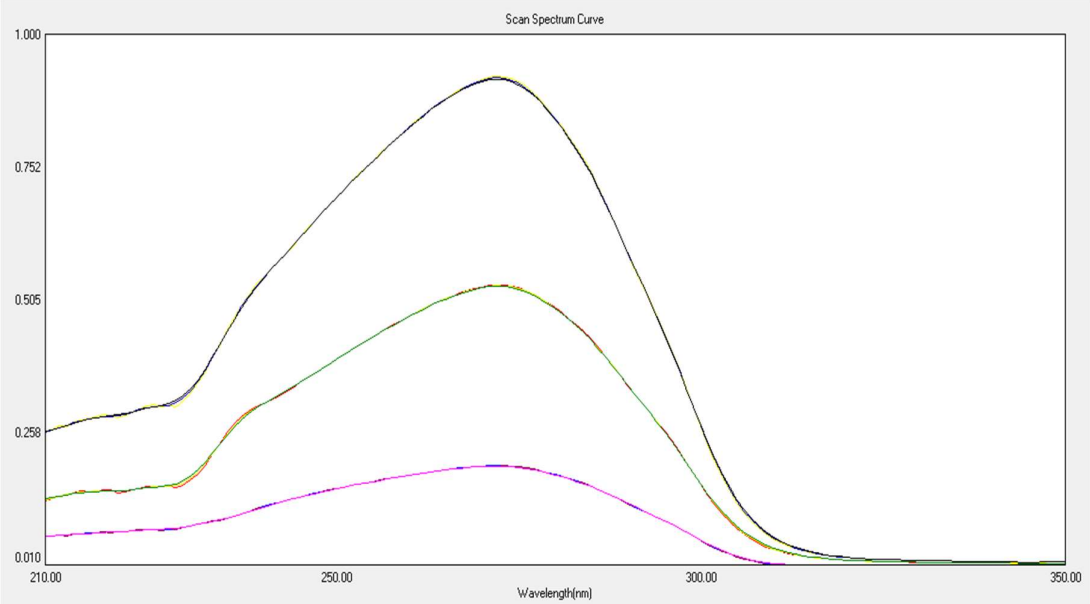

Figure 11: UV spectra showing recovery studies

Table 4: Assay of Topiroxostat

Label claim (mg)	Amount obtained (mg)	% Assay
20	19.98	99.90
20	20.08	100.40
20	19.65	98.25
Average	19.90	99.52
SD		1.1251
% RSD		1.1306

Table 5: Summary of Eco scale penalty points for the proposed method

Description	Penalty points	Total Penalty Points	Score
Ethanol	4	4	96
Instrument	0		
Occupational hazard	0		
Waste	0		

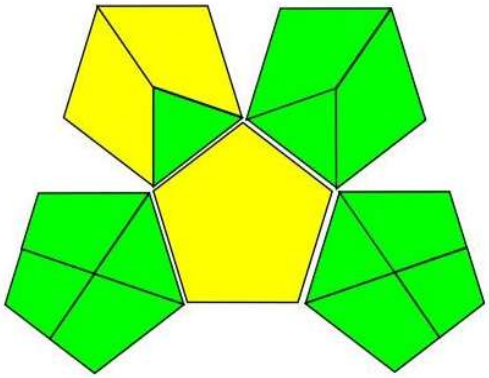

Figure 12: GAPI Pictogram for the proposed method

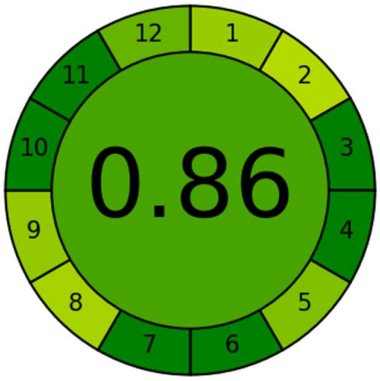

Figure 13: AGREEmetrics output for the proposed method

CONCLUSION

This multivariate analysis is more precise, accurate, sensitive, and economical than a conventional UV-Visible spectrophotometry method for estimating Topiroxostat. It has been revealed that this multilinear regression analysis is useful for testing both the conventional medication and different dosage forms of Topiroxostat. This method is validated in accordance with ICH requirements, and the values fall within the validation bounds. This is a basic working process that can be utilised for routine analysis of Topiroxostat in bulk and pharmaceuticals, especially when compared to expensive and advanced techniques like HPTLC and HPLC.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

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