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MULTIVARIATE CALIBRATION TECHNIQUE AIDED UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF EPALRESTAT IN PHARMACEUTICAL DOSAGE FORM: ASSESSMENT OF GREENNESS PROFILE

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ABSTRACT

The present work aims to develop a eco-friendly UV spectrophotometric method using a multivariate calibration technique for the estimation of Epalrestat in pharmaceutical tablets. For more accurate measurements, the multivariate calibration approach measures the sample absorbance at different wavelengths. The UV spectrophotometric method was developed and method validation was performed. All the validation parameters comply with ICH norms. The proposed Multivariate Calibration technique can be applied to the estimation of Epalrestat. The results were treated statistically. The analytical Eco scale, Agree metrics, and Green analytical procedure index was used to assess the method's greenness scores.

Keywords: Epalrestat, Multivariate calibration technique, Pharmaceuticals formulations, ICH guidelines, Validation, Greenness

INTRODUCTION

Epalrestat **(Figure 1)** It is an aldose reductase inhibitor **[1]**. It is approved for the improvement of subjective neuropathy symptoms, abnormality of vibration sense, and abnormal changes in heart beat associated with diabetic peripheral neuropathy **[2]**.

Epalrestat is chemically (5Z)-5-[(2E)-2-Methyl-3-phenyl-2-propenylidene]-4-oxo-2-thioxo-3-thiazolidineacetic acid. The molecular formula is $C_{15}H_{13}NO_3S_2$

with the molecular weight of 319.4 gm/mol **[3]**.A literature survey revealed RP-HPLC **[4–7]**,UV **[8][9]**, HPTLC **[10]** and LC **[11]** of few publications are reported for estimation of Epalrestat in pharmaceutical preparations and review referred to the Multivariate Calibration technique (MVC) using UV spectrophotometry, which haven't reported. Hence, the present method deals with the development of the eco-friendly UV spectrophotometric aided MVC technique for the estimation of Epalrestat.

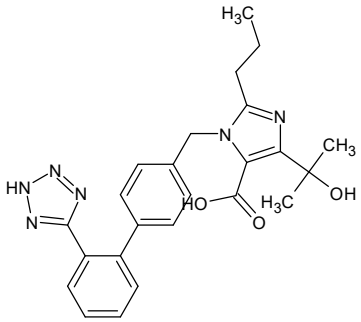


Figure 1: **Chemical structure of Epalrestat**

The MVC approach for Epalrestat was used to decrease instrumental error and increase efficiency. The method is easy, inexpensive and can be applied to bulk chemical and pharmaceutical dosage forms. For exact findings, MVC employs straight regression algorithms ranging between the wavelengths of 5-10nm **[12]**. In this study, we discussed the use of a UV spectral MVC approach with minimal mathematical content for estimating Epalrestat in pharmaceutical dosage forms. As a result, to ensure the sensitivity in comparison to the traditional ultraviolet (UV) approach, five distinct wavelengths were chosen. The algorithm techniques of MVC's statistics multivariate data are converted into univariate data using the following equations **[13]**. If the absorbance of a sample(x) is measured at five different wavelengths (λ), that is, at 383,386,389,392 and 395nm, the following equation can be produced for each selected wavelength.

$A_{\lambda 283} = a \times C_x + k_1$ ……. (1)

$A_{\lambda 286} = b \times C_x + k_2$ ……. (2)

$A_{\lambda 289} = c \times C_x + k_3$ ……..…..... (3)

$A_{\lambda 292} = d \times C_x + k_4$ ……...…. (4)

$A_{\lambda 295} = e \times C_x + k_5$ ……..……...…. (5)

Whereas,

- A_λ= Absorbance of the sample;
- a, b, c, d, e = Slope of the straight regression functions of a sample;
- k_1, k_2, k_3, k_4, k_5 = Intercept of the straight regression;
- C_x = Concentration of the sample

The above five equations can be rearranged as:

$A_T = a \times C_x + b \times C_x + c \times C_x + d \times C_x + e \times C_x + K_T$……. (6)

Equation (6) can be re- arranged as:

$A_T = C_x (a + b + c + d + e) + K_T$ ……. (7)

Whereas,

- A_T = Sum of the absorbances acquired

- K_T = Sum of intercepts of regression equation

The concentration of the sample (X) in a solution can be calculated by using the equation

$$Cx = \frac{A_T - K_T}{(a+b+c+d+e)} \quad \ldots\ldots\ldots\ldots\ldots (8)$$

Greenness evaluation techniques

The analytical eco scale **[14]** is constructed on allocating penalty points determined by the number of pictograms with associated signal words as established by "The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)", as well as the quantity. Every reagent, its type and quantity, potential occupational exposure, and energy depletion, including waste, are all part of the analytical eco scale approach. Penalty points are deducted from a starting score of 100.

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

The Green Analytical Procedure Index **[15]** (GAPI) is also a pictorial representation that constitutes five pentagrams which unique colour coding. The colour coding in the pictogram involves three levels of assessment at each stage of an analytical procedure. The colour coding used by GAPI to assess greenness ranges from green to yellow to red, signifying the low, medium, and high environmental impact associated with the analytical procedure, respectively. A brief description of GAPI was well described and reported by J. Płotka-Wasylka in the year 2018 **[15]**. AGREE metrics, **[16]** unique software for quantifying the greenness profile, are used in the second assessment methodology. The software's output is a circular diagram containing numbers on the edges ranging from 1 to 12 in a clockwise orientation. These numbers depict the 12 ideologies of green analytical chemistry. The outcomes of all these 12 principles are given a rating of 0 to 1 based on the inputs and their weightage. This aggregate scale is colour coded as red, yellow, and green, with red denoting zero, dark green denoting one or near to one, and yellow denoting a number between red and dark green. The sum of the 12 principles and the core generates a score that reflects the extent of greenness.

MATERIALS AND METHODS

Instruments used

A LABINDIA UV 3092 model double beam UV-VISIBLE spectrophotometer (Gurugram, India) was used. It consists of an automatic eight-cell charger, Czerny-Turner monochromator optics sealed and coated with quartz, a deuterium lamp, and a tungsten lamp were used as a detector, which has a wavelength of 190-900 nm and aspectral bandwidth of 0.1-5.0nmwith a 0.1nm interval. The software used to run this instrument andproduce data output is UV Win Lab

Version 5.1.1. For weighing the materials, an analytical balance (AS 245, Mettler Toledo, India) was used, and for sonication purposes, a Labman Digital Ultra Sonicator (model LMUC-3, Thebarton, Australia) was used.

Reference Samples

Epalrestat was kindly supplied by Ideal Analytical and Research Institution (Pondicherry, India)

Preparation of solutions

Standard stock solution preparation of Epalrestat

Weigh accurately 50 mg of Epalrestat and transfer it into a 100 ml volumetric flask. Dissolve it in 50 ml of ethanol, sonicate for 20 minutes, and then increase the volume to 100 mL with ethanol. The solution was filtered through Whatman grade 42 circular filter paper.

Working solutions of Epalrestat

rom the above stock solution 3.5-6.5µg mL^{-1}solutions were prepared by using Ethanol as a solvent.

Selection of wavelength for MVC

Across the wavelength range of 200 to 400 nm, the Epalrestat working standard solutions were scanned against ethanol as the blank solution, which has maximum absorption at 389 nm. Thus, the wavelength for MVC approach was around these absorption maxima, i.e., 383,386,389,392,395nm.

Stability of the solution

Solution stability studies were performed for Epalrestat by storing prepared sample solutions at room temperature for 0-12 hours. The absorbance was measured at regular intervals of 0, 6, and 12 hrs.

Linearity

Linearity of Epalrestat was performed by suitable dilution of the stock solution with Ethanol to achieve concentrations ranging from 3.5-6.5µg mL^{-1} (3.5,4.0,4.5,5.0,5.5,6.0,6.5) in order to analyse linearity and spectral region. The absorbance of linearity solutions at the appropriate wavelength was measured and analysed for the MVC method.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were estimated for Epalrestat based on the calibration curve slope and standard deviation of responses for a particular wavelength using the following formulae.

$$\mathbf{LOD} = \frac{\mathbf{3.3 \times standard\ deviation}}{\mathbf{Slope}} \quad \textbf{..... (9)}$$

$$\mathbf{LOQ} = \frac{\mathbf{10 \times standard\ deviation}}{\mathbf{Slope}} \quad \textbf{..... (10)}$$

Precision

The precision was evaluated and analysed for repeatability through intraday and interday precision. To test different levels of accuracy, a typical standard solution of Epalrestatat a concentration of 5µg$mL^{-1}$was used. Six solutions at five

distinct wavelengths were analysed for the repeatability study. In the scenario of intravariation, at a specified time interval,the absorbance of prepared solutions was evaluated three times on a comparable day. Further, intravariation was accomplished by utilising the absorbance on three subsequent days.

Accuracy

The accuracy of the methodology for Epalrestat was tested at 80%, 100%, and 120% of the pre-analysed sample solutions, and the percentages of recovery values were estimated.

Assay

Weigh accurately a quantity of Epalrestat capsules equivalent to 10 mg of Epalrestat, add 25 ml of ethanol and sonicate for 20 minutes. Add sufficient ethanol and make upto 100 mL. The solution obtained above is filtered and diluted with ethanol to attain 5 µg mL^{-1} concentration of Epalrestat. The absorbance of the resulting solution is measured at 389 nm and the content of Epalrestat is quantified.

RESULTS AND DISCUSSION

These standard solutions of Epalrestat were scanned initially between 200-400 nm.The highest absorbance was recorded at 389 nm for Epalrestat. To perform MVC, the wavelength was chosen at 389 nm and the UV spectrum was recorded for standards and samples of Epalrestat by taking ethanol as a blank. The spectra of 5µg mL^{-1} standard Epalrestat are represented in **Figure 2**.

Stability of solution

The results of solution stability of Epalrestat is not major changes in the absorbance values as well the spectrum obtained at using the solution measured at 0, 6 and 12 hrs. The absorbance difference between the fresh standard solution and stored solutions were negligible and found to be less than 2%.

Linearity

The developed method linearity findings for Epalrestat were identified a concentration range of 70 - 130% for 5 µg mL^{-1} (3.5-6.5 µg mL^{-1}) according to ICH Q2 R1 guidelines. The spectra for Epalrestat were represented in **Figure 3**. The calibration curve was developed by measuring the absorbance of diluted standard solutions at five distinct wavelengths (383, 386, 389, 392, 395).

Limit of Detection and Limit of Quantification

The LOD and LOQ for Epalrestat was calculated from the linearity slope, which has been confirmed by different sample analyses. The LOD for Epalrestat was calculated from the average of all the absorbance, which was found to be 0.0666 µg mL^{-1}. The LOQ for Epalrestat was calculated from the average of all the

absorbance, which was found to be 0.2019 µg mL^{-1}.

Precision

The system precision spectra for Epalrestat are represented in **Figure 4**. The interday precision spectra for Epalrestat are represented in **Figure 5**. The intraday precision spectra were represented in Figure 6 for Epalrestat. The % RSD of system precision, interday and intraday precision, was determined for Epalrestat. It was found to be less than 2%, which shows that the approach method is precise. The outcomes are represented in **Table 2** for Epalrestat. The proposed method shows good precision compared to the values obtained from various precision methods.

Accuracy

The accuracy of Epalrestat was tested at 80, 100, and 120%. The overlay spectra for Epalrestatis in **Figure 7**.The results are shown in **Table 3** for Epalrestat and the obtained results were found to be within limits.

Assay of marketed formulations:

The quantification of Epalrestat in the capsules formulation was examined using the suggested spectrophotometric method. For three replicates, the commercial capsules UV absorption spectrum was achieved. The pharmaceutical formulation does not have a significant loss in terms of high analytical recovery values after the extraction and filtration process. The findings, which demonstrate that the new approach performs better than the prior methods, are shown in **Table 4** for Epalrestat.

Evaluation of Greenness Profile

The results of greenness profile for the proposed methods were evaluated. The results of analytical scale is shown in **Table 5**, while the results agree metrics and GAPI is depicted in **Figure 9** and **Figure 10**.

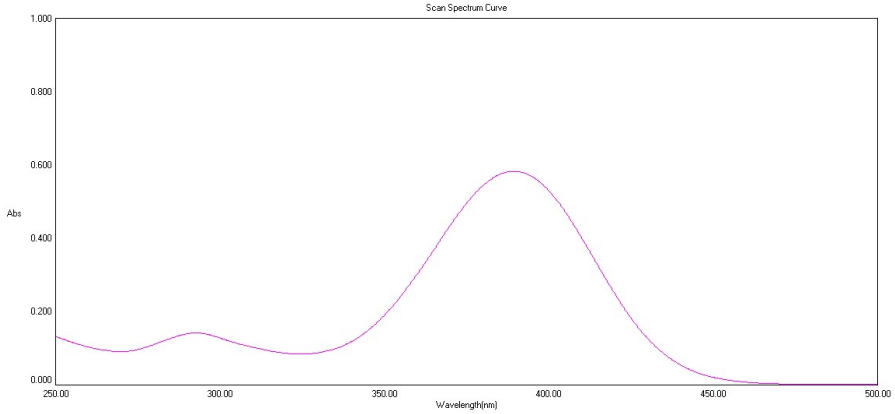


Figure 2: UV spectrum of standard Epalrestat (5 µg mL^{-1}) using ethanol as a blank

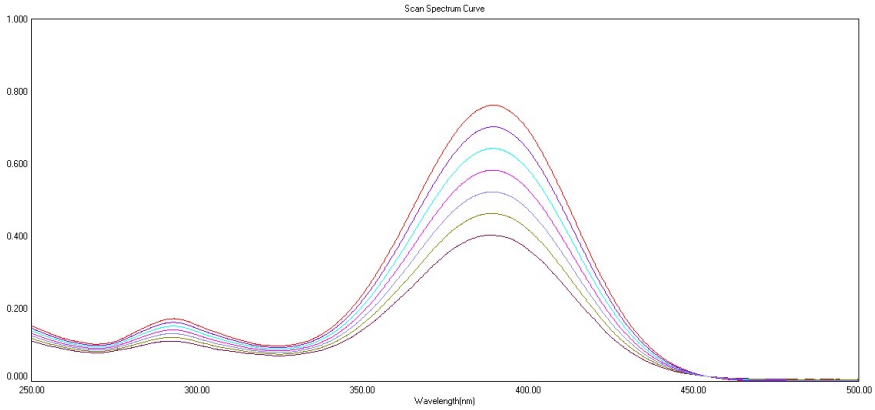
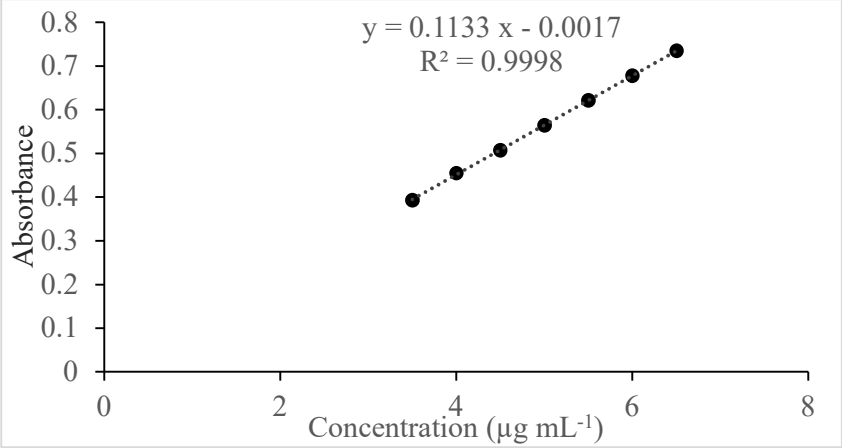
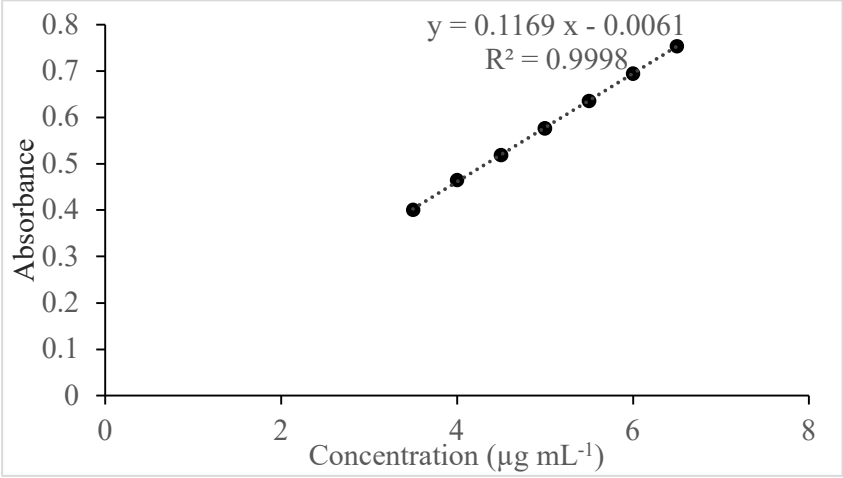


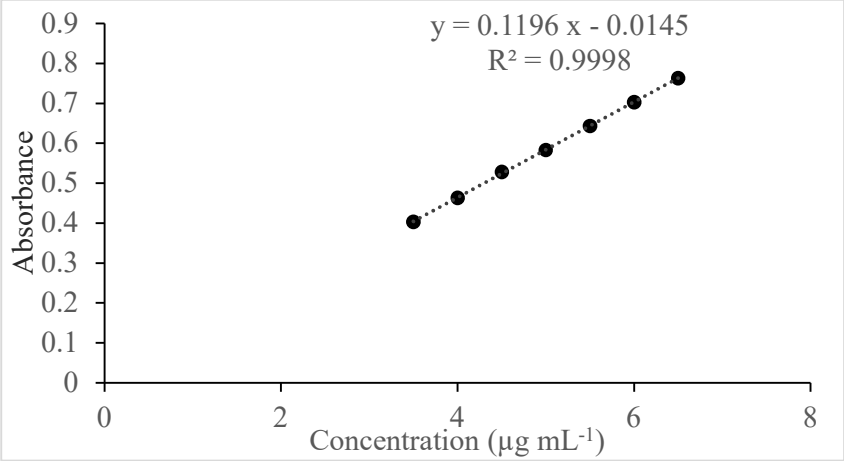
Figure 3: Linearity spectrum of Epalrestat (3.5-6.5 µg mL^{-1}) using ethanol as a blank



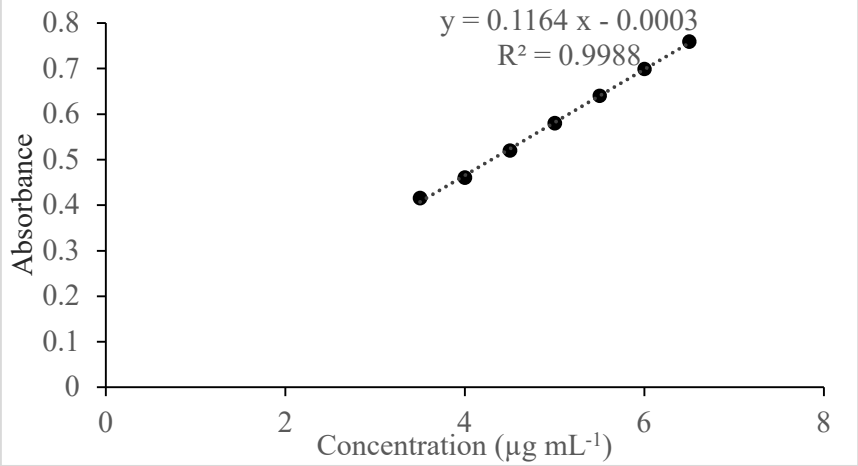
Multivariate calibration graph at 383 nm



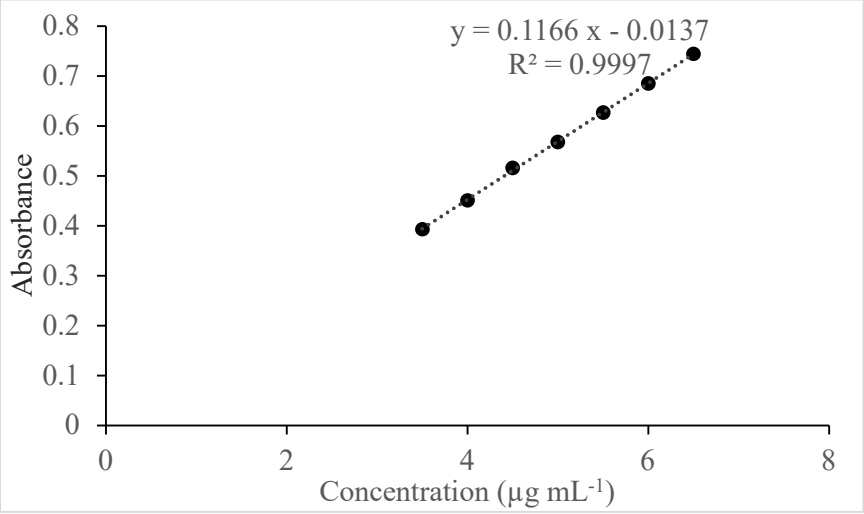
Multivariate calibration graph at 386 nm



Multivariate calibration graph at 389 nm



Multivariate calibration graph at 392 nm



Multivariate calibration graph at 395 nm

Table 1: Linearity values for proposed method of Epalrestat

Best-fit values	383 nm	386 nm	389 nm	392 nm	395 nm
Slope	0.1133	0.1169	0.1196	0.1164	0.1166
Y-intercept when X=0	0.0017	0.0061	0.0145	0.0003	0.0137
R^2	0.9998	0.9998	0.9998	0.9988	0.9997

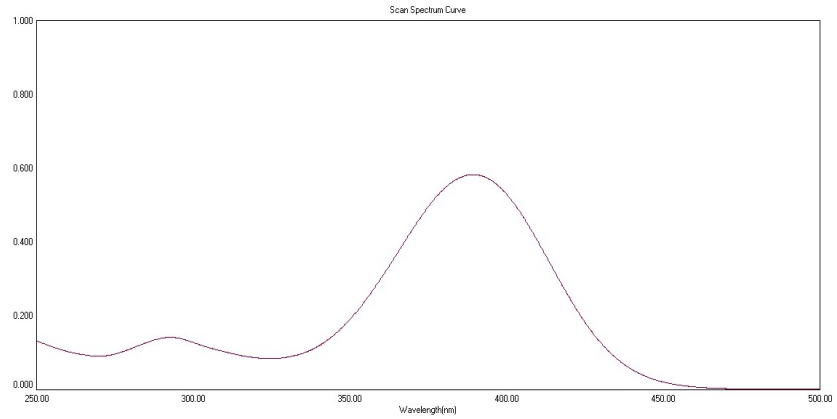


Figure 4: System precision overlay spectra of Epalrestat

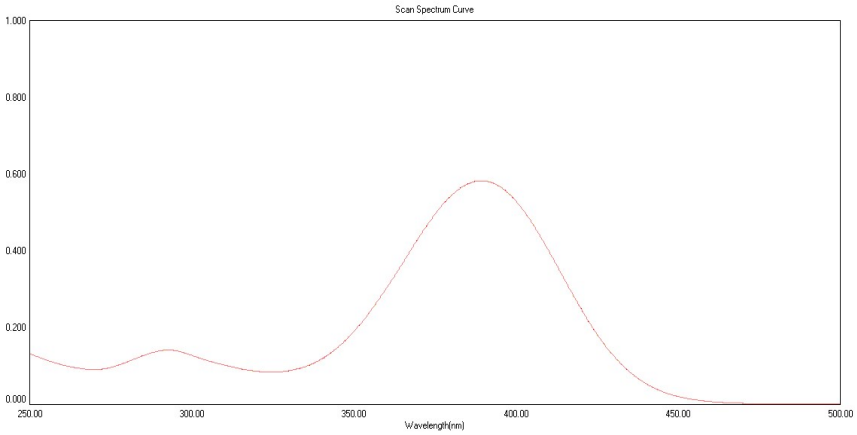


Figure 5: Interday precision overlay spectra of Epalrestat

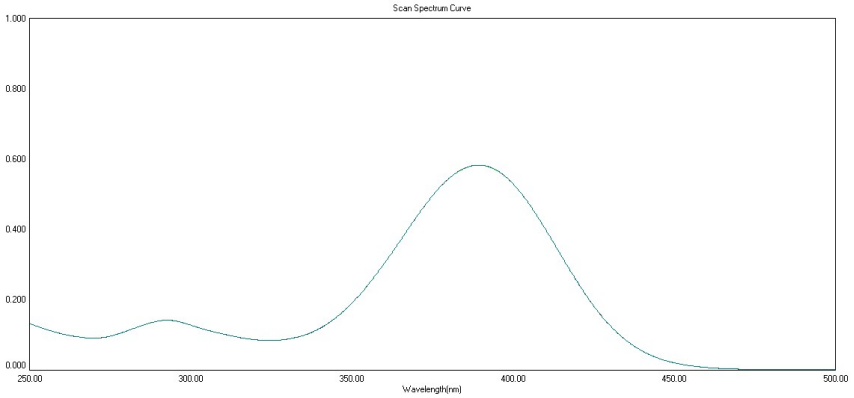


Figure 6: Intraday precision overlay spectra of Epalrestat

Table 2: System precision, Interday and Intraday precision data for the proposed method of Epalrestat

	System precision	Intraday and interday precision		
	Absorbance of standard for 5 µg mL^{-1}	% Recovery of sample equivalent to 5 µg mL^{-1} of sample		
		Day 1	Day 2	Day 3
1	413.732	99.12	98.01	98.3
2	412.254	98.85	99.2	99.49
3	415.234	99.32	99.1	99.32
4	416.719	99.25	99.47	99.5
5	414.743	99.35	99.11	98.48
6	415.869	99.29	98.16	99.5
Mean	414.759	99.20	98.84	99.10
SD	1.589	0.19	0.60	0.56
% RSD	0.38	0.19	0.61	0.56
CI	1.2717	0.1503	0.4825	0.4448

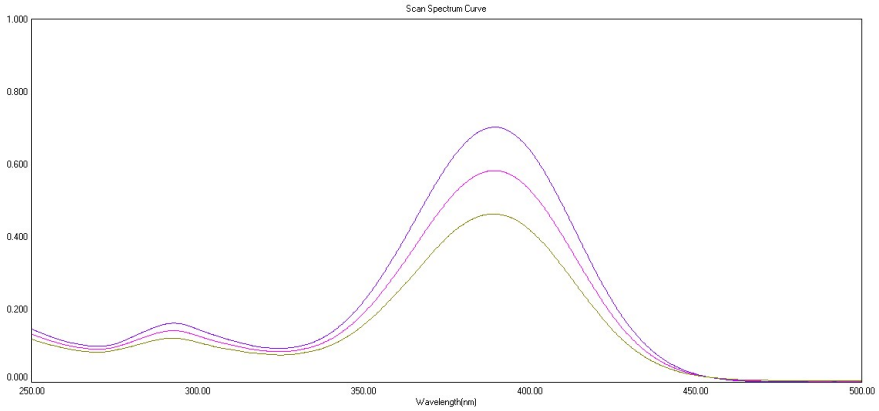


Figure 7: Overlay spectra of accuracy of Epalrestat at 80, 100, 120 % spiking

Table 3: Accuracy data for proposed method of Epalrestat

Concentration levels (%)	Amount present	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	Mean % Recovery	SD
80	50	3	4.9	98.00	1.9767
100	50	5	9.9	99.00	1.9000
120	50	7	14.7	98.00	1.9400

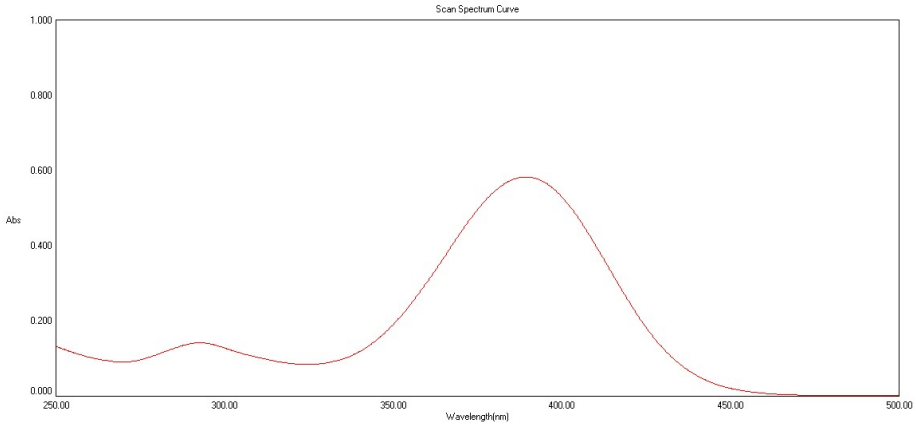


Figure 8: UV spectrum of sample Epalrestat (5 µg mL^{-1}) using ethanol as a blank

Table 4: Assay results for marketed formulation of Epalrestat

Marketed formulation	Label claim (mg)	Mean±SD(n=3)	% RSD
Batch - 1	50	49.90 ± 0.02	0.1542
Batch - 2	50	49.92 ± 0.05	0.5040

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 9: Agree metrics output for the proposed method

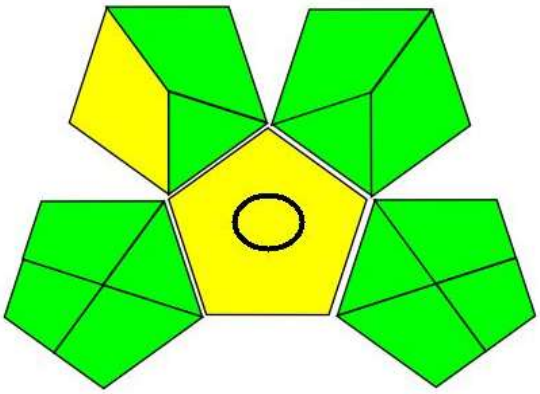


Figure 10: GAPI Pictogram for the proposed method

CONCLUSION:

The proposed multivariate calibration approach was a simple, novel, accurate, precise technique for the estimation of Epalrestat. It is highly recommended to develop a new strategy for routine Epalrestat analysis. When compared to ICH recommendations, all validation parameters were assessed and found to be within acceptable bounds. The proposed method possess an ideal greenness profile assessed by analytical ecoscale, GAPI and agree metrics. The proposed method shall be used for routine simultaneous determination of Epalrestat as an alternative to time-consuming and expensive separation techniques.

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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