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EVALUATING THE GREENNESS PROFILE AND QUANTIFYING THE LOBEGLITAZONE USING MULTIPLE CALIBRATION TECHNIQUES IN PHARMACEUTICALS USING UV SPECTROPHOTOMETRY

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ABSTRACT

The current study proposes the development and testing of a novel methodology to determine the amount of lobeglitazone in formulations and bulk materials. This analytical method is characterized by its sensitivity, precision, and rapid execution. Utilizing a UV spectrophotometer in conjunction with a multivariate calibration approach, the methodology leverages the established the connection among concentration and absorbance at five distinct wavelengths, including the absorption maxima at 250 nm. The procedure was validated in accordance with ICH Q2 (R1) guidelines, employing equations for linear regression along with other mathematical and statistical techniques. Validation parameters such as accuracy, linearity, and others specified in the guidelines were successfully met. This technology proves to be sensitive, cost-effective, and reliable for the routine examination of medications and formulations. The technique's greenness was assessed making use of the Green Analytical Procedure Index, AGREE metrics, and Analytical Eco-Scale.

Keywords: Lobeglitazone, ICH guidelines, pharmaceutical formulations, UV spectrophotometric analysis, multivariate calibration procedure, and validation

INTRODUCTION

The pharmacophore lobeglitazone is composed of ethoxy-benzyl N-methylamino group and a 2,4-thiazolidinedione group joined to it as a connecting element. Its structural formula is C24H24N4O5S. **Figure 1** depicts the structure of lobeglitazone **[1]**.

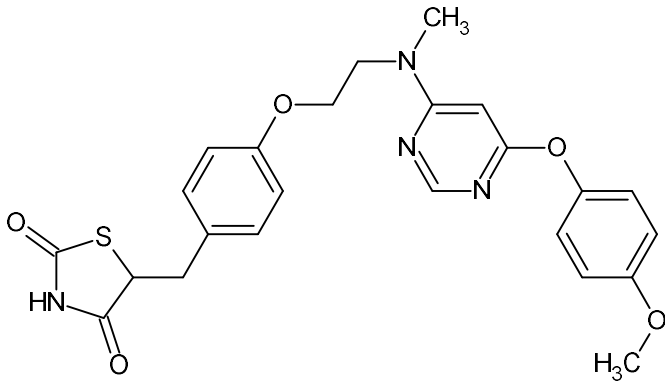

Figure 1: structure of Lobeglitazone sulfate

The TZD lobeglitazone was first created in South Korea in the 2000s, and it has been licensed for clinical usage there since 2013 at a dosage of 0.5 mg per day **[2]**. By maintaining β-cell activity and increasing insulin sensitivity, TZDs offer long-lasting glycaemic management. By binding and activating the nuclear receptor PPAR-γ, these medications, which are peroxisome proliferator-activated receptor gamma agonists (PPAR-γ), enhance insulin resistance and decrease the synthesis of glucose in the liver. Compared to metformin and sulfonylureas, PPAR agonists have a lower risk of hypoglycemia and gastrointestinal side effects, and its efficacy may last longer **[3]**.

A review of the literature indicates that there aren't many published techniques for locating lobeglitazone in pharmacological or biological formulations. There were descriptions of one UV **[4]** approach, a couple chromatographic techniques like HPLC **[5][6]**, HPTLC **[7]**, and one hyphenated technique LC-MS/MS **[8]** for lobeglitazone. There was no description of a multivariate assessment (MVC) by use of UV spectrophotometry for lobeglitazone sample. Thus, the current technique focusses on developing the UV spectrophotometric MVC for lobeglitazone measurement. Applied analytical technique provides a quantitative examination of investing admixtures under ideal conditions that is strong, quick, sensitive, and inexpensive.

The following equations may be generated for each chosen wavelength when a sample's absorbance (x) is calculated at various

wavelengths (λ), namely at 238, 243, 248, 253, and 258nm.

$A_{\lambda 238} = a \times C_X + k1$ …… (1)

$A_{\lambda 243} = b \times C_X + k2$…… (2)

$A_{\lambda 248} = c \times C_X + k3$ …… (3)

$A_{\lambda 253} = d \times C_X + k4$ …… (4)

$A_{\lambda 258} = e \times C_X + k5$ …… (5)

While,

- A_λ= Sample's absorbance;
- a, b, c, d, e = Slope of a sample's straight regression functions;
- k_1, k_2, k_3, k_4, k_5 = The straight regression's intercept;
- C_X = Sample concentration

The five previously mentioned equations can be put in the following order:

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

The aforementioned equation may be reduced even more to

$A_T = C_X (a+b+c+d+e) + K_T$…… (7)

While,

- A_T = Sum of the obtained absorbances
- K_T = Sum of the regression equation's intercepts

To determine the amount of analyte X inside a mixture, use the formula.

$$Cx = \frac{A_T - K_T}{(a+Ab+c+d+\ )} \quad \text{…… (8)}$$

Techniques for evaluating greenness

"The Globally Harmonised System of Classification and Labelling of Chemicals (GHS)" states that the eco analytical scale deducts points based on how many signal words are used in conjunction with the pictograms. Each and every reagent, including the kind, amount, waste, energy depletion, and occupational exposure risk is considered in The Eco-Scale Analysis Method. There are deductions of penalty points from the original 100-point total **[9]**.

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

Another illustration is the Green Analytical Procedure Index (GAPI), which has a unique scheme of colours and five pentagonal shapes. For every stage of an analytical procedure, the pictogram's colour coding employs three levels of assessment. The three colours that GAPI uses to represent the degrees of minor, moderate, and major environmental harm associated with the analytical technique are green, yellow, and red, respectively. J. Potka-Wasylka gave clear, precise, and educational reporting on GAPI in 2018 **[10]**. AGREE metrics, specialised software is employed during the second evaluation step In order to measure the greenness profile. The program creates a circle employing data ranging from 1 to 12 orientated clockwise around its periphery. These pictures represent the 12 green analytical chemistry philosophies. On a scale of 1 to 12, the outcomes of these 12 principles are ranked from 0 to 1, depending on the information

supplied and their weight. Red indicates a value of zero on this general scale, dark green indicates a value of one or a number that is near to 1, and A value in between is shown by yellow. A score indicating the degree of the greenness is obtained by combining the core and the twelve principles **[11]**.

MATERIALS AND TECHNIQUES:

Chemicals and solvents employed:

- Methanol
- LOBG® TABLETS – (Label claim – 0.5 mg of LBG), produced by Glenmark Pharmaceuticals Pvt. Ltd. The therapeutic formulations that were sold were obtained locally.

Solubility:

- Readily dissolving in methanol and DMSO.

Instrumentation:

- Lab India UV-3092, a UV-Vis double beam spectrophotometer.
- The Shimadzu AY-220H electronic balance.
- Soni clean sonicator (model 160T, The barton-Australia).

DEVELOPMENT OF METHODOLOGY:

Choice of solvent:

It was found that methanol, the solvent utilised to solubilise the drug throughout the analysis, was easily soluble.

Preparation of the standard solution

The lobeglitazone standard stock solution was made by the medication component, 0.5 mg, into 10 mL of methanol. We used this solution for additional research after adjusting its concentration to (3.5–6.5µg/mL).

Preparation of sample solution:

0.5 mg of lobegitazone were precisely measured and then added to a 10 ml volumetric flask. The mixture underwent a 15 minutes sonication after 7 ml of methanol was added. 10 ml (1000 µg/mL) was the net volume. Following that, the mixture had been diluted with the solvent to provide concentrations between 3.5–6.5 µg/mL.

λ_{max} estimation and wavelength selection for multivariate calibration:

The working standards for lobeglitazone were scanned in relation to methanol, blank solution, which, within the 200–400 nm wavelength range, exhibits a maximum absorption at 250 nm. Accordingly, Using the MVC technique, the wavelength was found at 238, 243, 248, 253, and 258 nm, which are in between these absorption peaks.

METHOD VALIDATION

In compliance with ICH guidelines, the linearity, accuracy, and precision of the suggested approach were confirmed **[12]**.

Linearity

After the stock solution was sufficiently diluted with methanol, concentrations between 3.5–6.5µg/mL were obtained, and

they were utilised for lobeglitazone's linearity and spectral area analysis. The absorbance of linearity solutions at the appropriate wavelength was measured and analysed for the MVC technique.

Limit of Detection and Quantification

By taking into account the slope of the calibration curve and the response standard deviation for a particular wavelength, The following formulas were used to determine the limits of quantification (LOQ) and limits of detection (LOD) for lobeglitazone.

$$\text{LOD} = \frac{3.3 \text{ x standard deviation}}{\text{Slope}} \quad \ldots\ldots (10)$$

$$\text{LOQ} = \frac{10 \text{ x standard deviation}}{\text{Slope}} \quad \ldots\ldots (11)$$

Precision

The repeatability of the precision was evaluated using intraday and interday precision. A standard lobeglitazone mixture containing 5 µg/mL was employed to assess different levels of precision. To evaluate repeatability, six solutions were analysed at five distinct wavelengths. The prepared solutions' absorbance was measured three separate times on similar day at various times for the intervariation scenario. To account for intravariation, the absorbance was used for three additional days.

Accuracy

The accuracy of the lobeglitazone technique was evaluated at 80, 100, and 120 percent of the concentrations of the previously investigated sample solutions, as well as the expected recovery value percentages.

Assay

Measure and powder 10 Tablets. Precisely measure out 0.5 mg of lobeglitazone of tablet powder, add 7 ml of methanol, and use a sonicator for 15 mins. Add upto 10mL by adding enough methanol. Lobeglitazone at a 5 µg/mL concentration is achieved by filtering and diluting the above-mentioned solution with methanol. Lobeglitazone concentration is determined by measuring the final solution's absorbance at 250 nm.

RESULTS AND DISCUSSION

The original scan range for the lobeglitazone standard solution was 200–400 nm. Lobeglitazone's maximum spectrum is 250 nm in wavelength. Using methanol as a blank and a wavelength of 250 nm for MVC, the lobeglitazone standards' and samples' UV spectra were noted. The typical lobeglitazone spectrum at 5 µg/mL is seen in **Figure 2**.

Linearity

The linearity results for the lobeglitazone developed methods were found to be within 70–130 percent concentration range for 5 µg/mL (3.5–6.5µg/mL), meeting the requirements of ICH Q2 R1. The lobeglitazone linearity spectrum is shown in **Figure 3**. The calibration curve was generated through the estimation of the absorbance of five distinct wavelengths of diluted reference solutions (238, 243, 248,

253, 258). The observed results are tabulated and shown in **Table 1**. It was found that every standard curve was linear in the concentration range that was selected. The regression analysis and calibration graphs are shown in **Table 2 and Figure 4-8**, respectively.

Limits of Detection and Limits of Quantification

The linearity slope was utilised for determining the lobeglitazone LOD and LOQ, and this approach has been validated by numerous sample studies. The LOD for lobeglitazone was determined to be 0.0338 µg/mL by averaging all of the absorbance. The LOQ for lobeglitazone was determined to be 0.1027 µg/mL by averaging all of the absorbances.

Precision

The **Figure 9** shows the lobeglitazone system precision spectra. The spectra of interday precision for lobeglitazone appears in **Figure 10**. The spectra of intraday precision for lobeglitazone appears in **Figure 11**. The intraday and interday precision of the system was computed as a percentage RSD for lobeglitazone. The approach method's precision was demonstrated by the finding that it was less than 2%. When compared to the outcomes of other accuracy approaches, the suggested method exhibits good precision.

Accuracy

As seen in **Figure 12**, The accuracy of the overlay spectra for lobeglitazone was confirmed at 80, 100, and 120%. **Table 3** displays the lobeglitazone study's findings, and it was concluded that the results fell within acceptable boundaries.

Assay of commercial formulations:

The suggested spectrophotometric approach was used to measure the amount of lobeglitazone in the tablet's formulation. Three tests were performed on a commercial medication's UV absorption spectrum. The pharmaceutical formulation's superb analytical recovery values did not change during the extraction and filtering process. The results are shown in **Table 4**.

Assessment of the Greenness Profile

The outcomes of the suggested approaches' greenness profile were assessed. Analytical scale findings are displayed in **Table 6**, whereas agree and GAPI metrics data are provided in **Figures 14 and 15**.

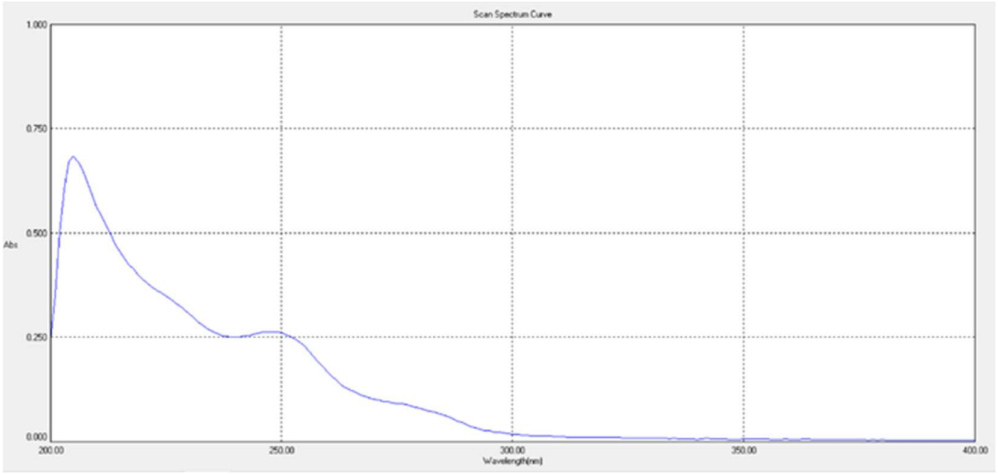

Figure 2: The standard lobeglitazone's UV spectrum (5 µg/mL) using methanol as blank

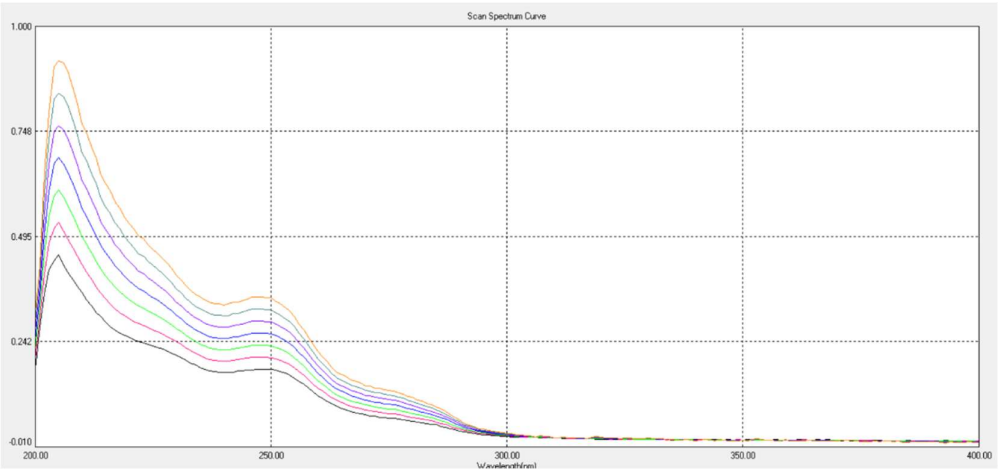

Figure 3: Lobeglitazone's linearity spectrum (3.5–6.5 µg/mL) utilizing methanol as blank

Table 1: Data on multivariate UV calibration at five chosen wavelengths

Concentration (µg/mL)	238nm	243nm	248nm	253nm	258nm
3.5	0.170	0.171	0.177	0.167	0.130
4.0	0.197	0.199	0.206	0.193	0.150
4.5	0.224	0.227	0.234	0.219	0.171
5.0	0.252	0.255	0.263	0.246	0.191
5.5	0.279	0.282	0.292	0.272	0.212
6.0	0.306	0.310	0.321	0.298	0.232
6.5	0.334	0.338	0.350	0.325	0.253

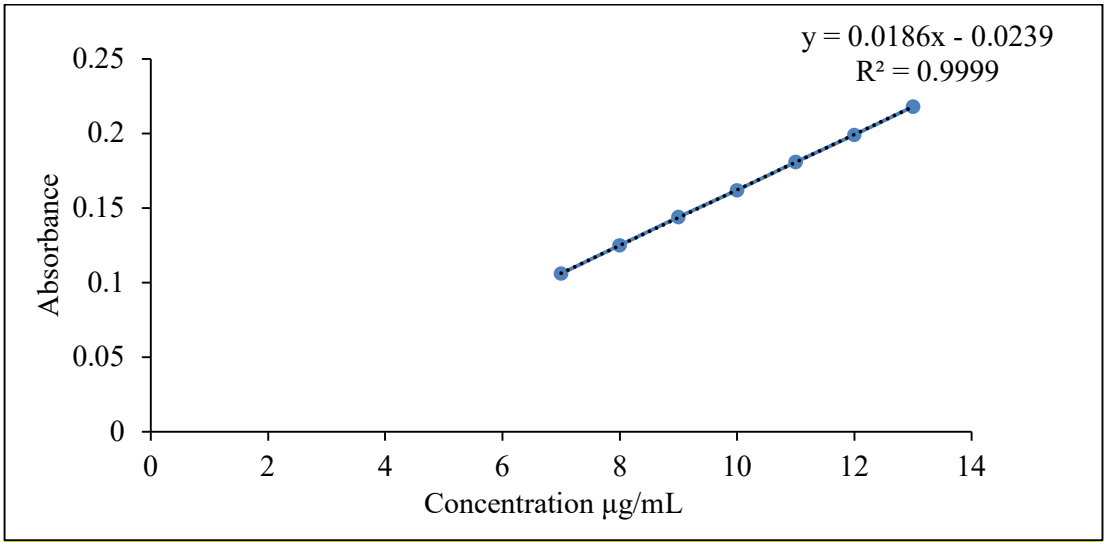

Figure 4: Calibration curve of lobeglitazone at 238nm

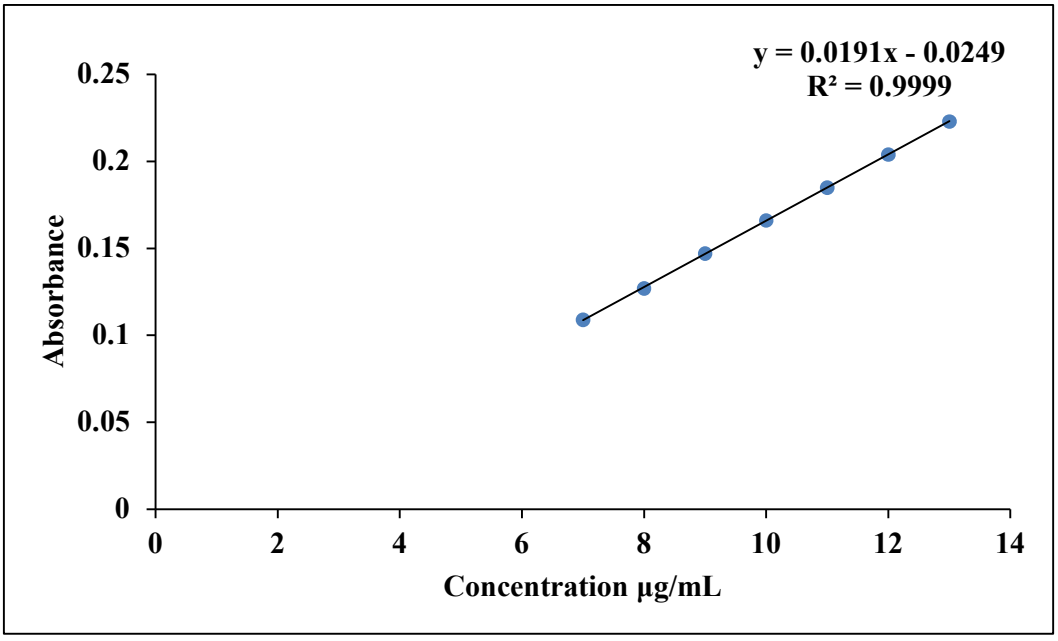

Figure 5: Calibration curve of lobeglitazone at 243nm

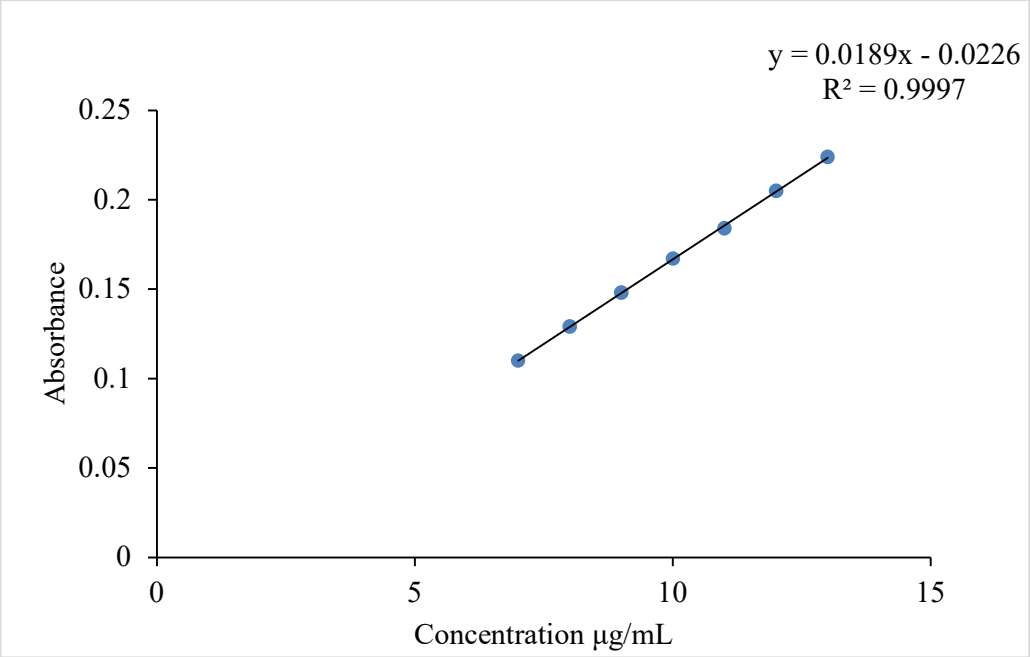

Figure 6: Calibration curve of lobeglitazone at 248nm

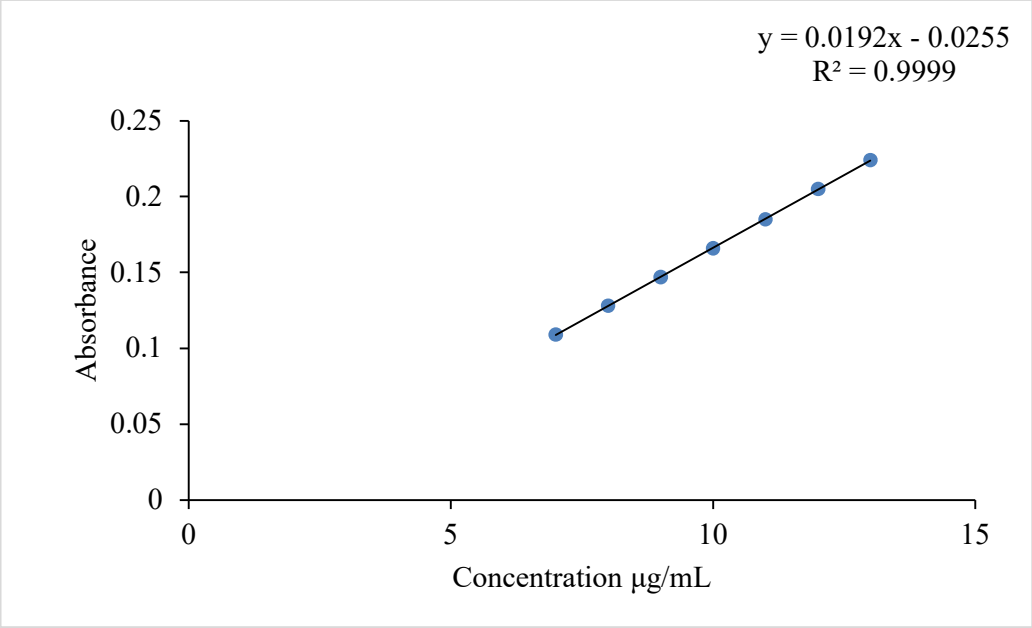

Figure 7: Calibration curve of lobeglitazone at 253nm

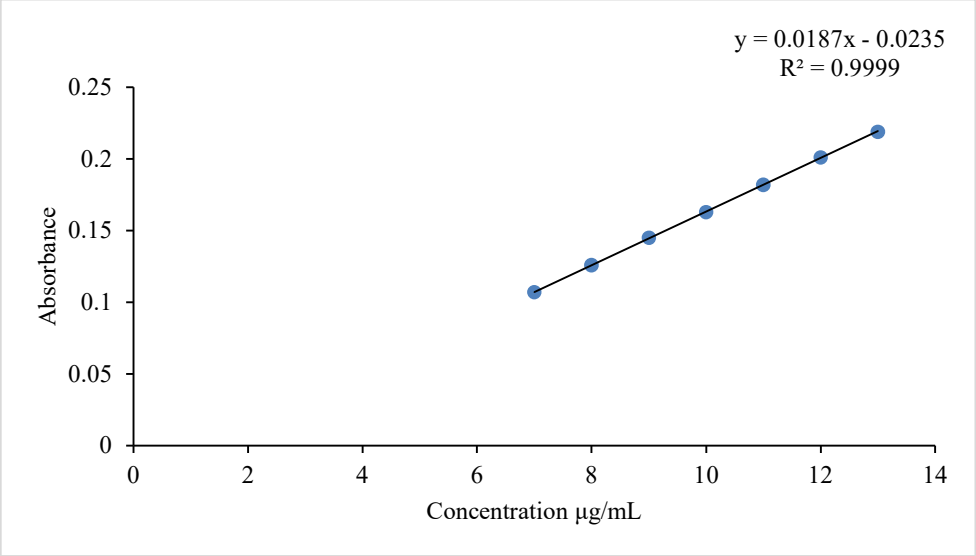

Figure 8: Calibration curve of lobeglitazone at 258nm

Table 2: The statistical parameters at the chosen wavelengths are displayed by linearity data

Wavelength (nm)	Regression equation	Slope	Intercept	R^2	LOD (µg/mL)	LOQ (µg/mL)
238	**y = 0.0544x - 0.0203**	**0.05420**	**- 0.0239**	**0.9999**	**0.0341**	**0.1034**
243	**y = 0.0555x - 0.0229**	**0.05560**	**-0.0249**	**0.9999**	**0.0372**	**0.1127**
248	**y = 0.0579x - 0.0259**	**0.05785**	**-0.025**	**0.9999**	**0.0319**	**0.0968**
253	**y = 0.0522x - 0.0156**	**0.05260**	**-0.0255**	**0.9999**	**0.0331**	**0.1003**
258	**y = 0.0406x - 0.0116**	**0.00404**	**-0.0235**	**0.9999**	**0.0331**	**0.1003**

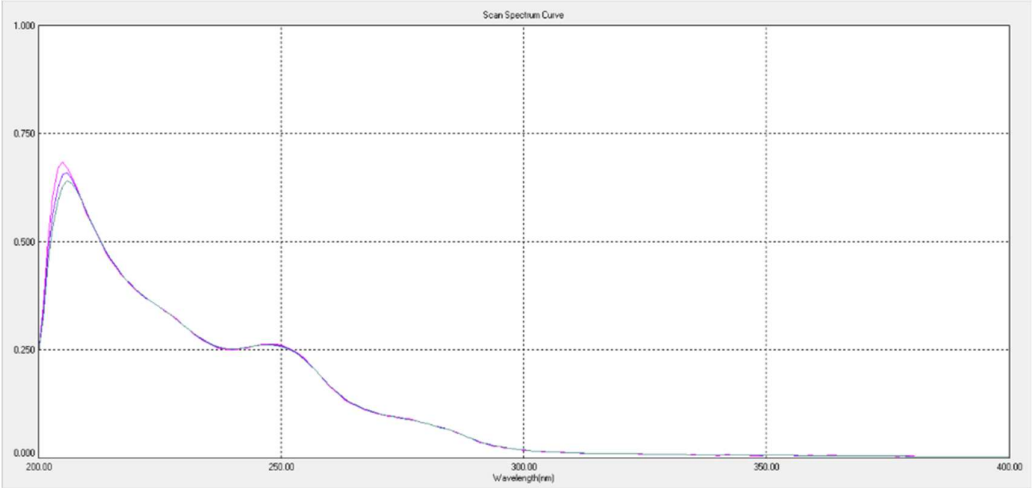

Figure 9: Lobeglitazone overlay spectra with system precision

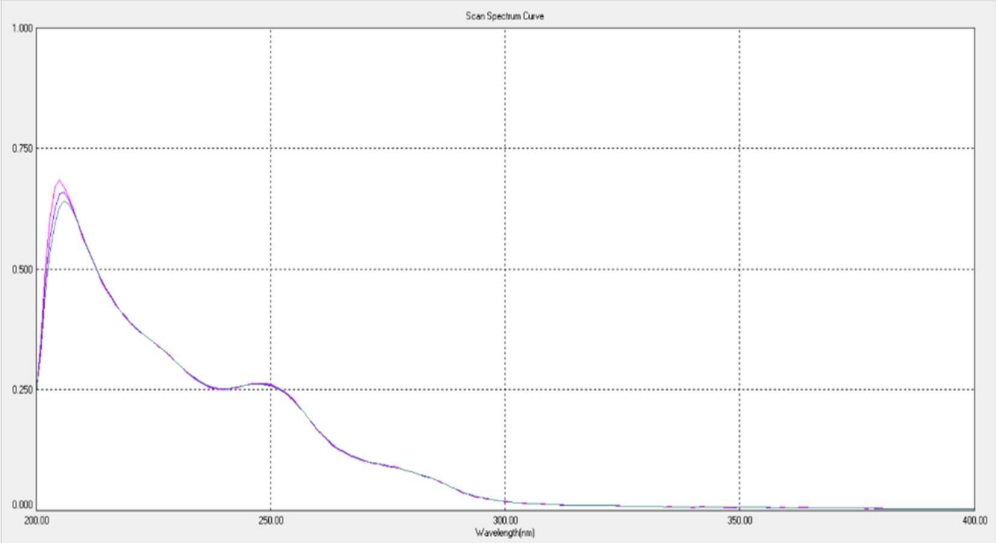

Figure 10: overlay spectrum of lobeglitazone with interday Precision

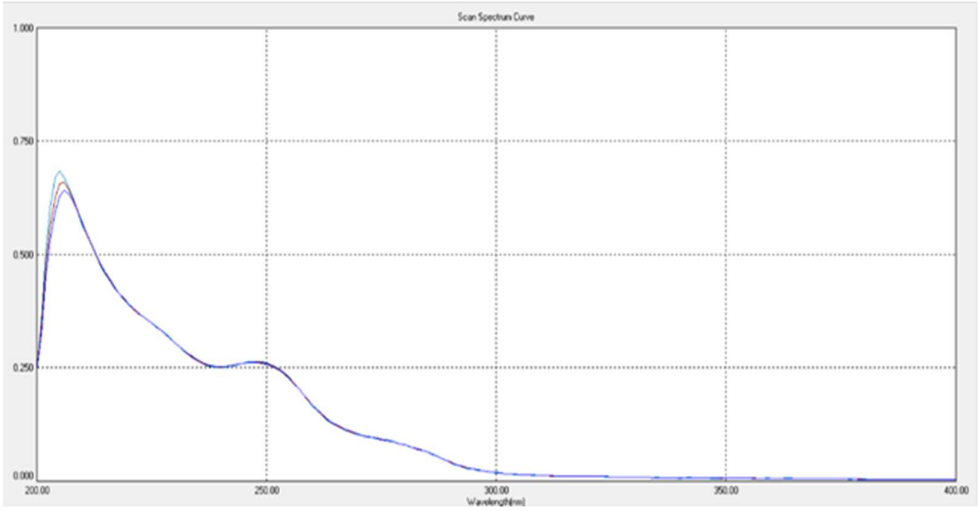

Figure 11: overlay spectrum of lobeglitazone with intraday Precision

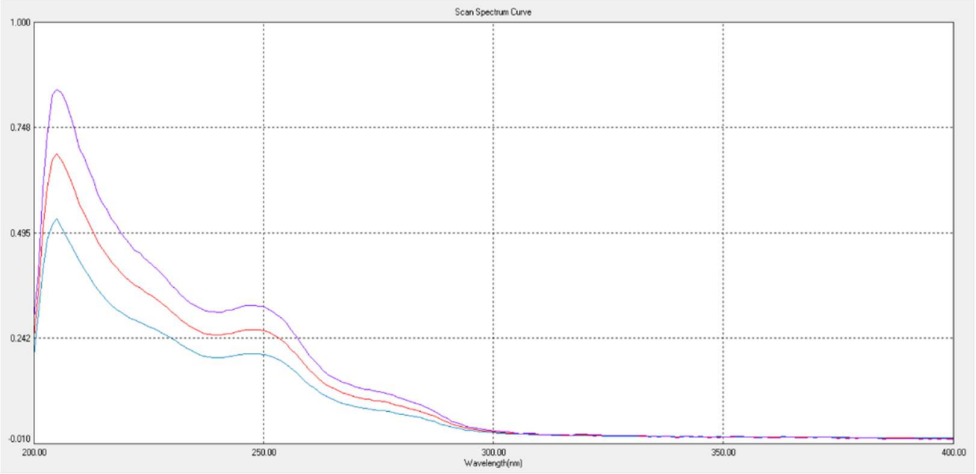

Figure 12: Overlay spectra of lobeglitazone (80%, 100%, and 120% accuracy)

Table 3: Recovery Studies

wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Absorbance	Amount recovered (µg/mL)	% Recovery
238	2.5	2	0.224	4.49	99.78
		2.5	0.252	4.98	99.60
		3	0.279	5.497	99.95
243	2.5	2	0.227	4.52	100.44
		2.5	0.255	4.99	99.80
		3	0.282	5.489	99.80
248	2.5	2	0.234	4.51	100.22
		2.5	0.263	4.96	99.20
		3	0.292	5.49	99.82
253	2.5	2	0.219	4.498	99.96
		2.5	0.246	4.98	99.60
		3	0.272	5.49	99.82
258	2.5	2	0.171	4.498	99.96
		2.5	0.191	4.98	99.60
		3	0.212	5.463	99.33

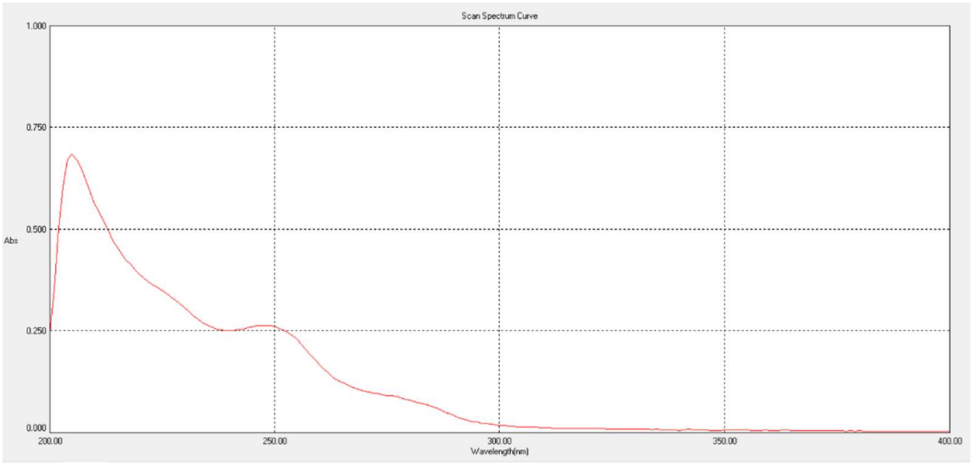

Figure 13: Standard UV spectrum of lobeglitazone (5 µg/mL) using methanol as blank

Table 4: Assay of lobeglitazone

Label claim (mg)	Amount estimated (mg)	% Assay
0.5	0.506	101.20
0.5	0.498	99.60
0.5	0.498	99.60
Average	0.50	100.13
SD		0.9238
% RSD		0.9225

Table 6: Eco-scale penalty point summary for the suggested approach

Description	Penalty points	Total Penalty Points	Score
Water	0	3	97
Instrument	0		
Occupational hazard	0		
Waste	3		

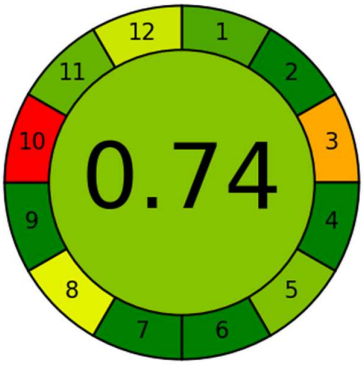

Figure 15: Output of AGREE metrics for suggested method

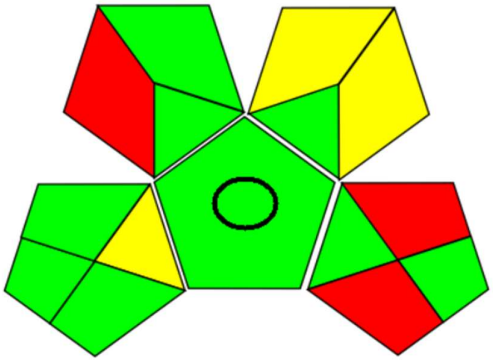

Figure 14: GAPI Pictogram of suggested method

CONCLUSION

The recently developed spectrophotometric approach for measuring lobeglitazone was validated by evaluating multiple validation criteria, and it was determined to be within acceptable ranges in compliance with ICH guidelines. Using the described approach, it was demonstrated that the lobeglitazone measurement in its tablet formulation was repeatable, sensitive, accurate, and precise. Since the suggested methodology includes a method with simple mathematical components and is more accurate than current UV spectrophotometric approaches, we strongly recommend utilising it for routine research on lobeglitazone in pharmaceutical formulations.

STATEMENT OF ETHICS

There are no animal or human participants used in this study's trials.

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DISPUTE OF INTEREST

There are no financial interests that could be at odds with this content.

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