



Development And Validation Of A UV Spectrophotometric Method For The Determination Of Lobeglitazonesulphate Using Green Analytical Chemistry Approach

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ABSTRACT

A simple, accurate, precise, and environmentally friendly UV spectrophotometric method for measuring the amount of lobeglitazone sulfate in bulk and pharmaceutical dosage forms. The drug showed the most absorbance (λ_{max}) at 250.5 nm when methanol was used as a solvent. The method worked well in the concentration range of 2 to 10 $\mu\text{g/ml}$, with a correlation coefficient (R^2) of 0.992. Validation was conducted according to ICH guidelines, yielding acceptable outcomes for accuracy (98.50–99.54% recovery), precision (%RSD < 2), and repeatability. The limits of detection (LOD) and quantification (LOQ) were 0.02 $\mu\text{g/ml}$ and 0.06 $\mu\text{g/ml}$, respectively, which shows that the test is very sensitive. The method that was created worked well for analyzing products that are already on the market. Additionally, the MoGAPI tool was used to check how green the method was, and it got a score of 72, which shows that it is good for the environment. The suggested method is dependable, affordable, and appropriate for regular quality control testing, and it also follows the principles of green analytical chemistry.

Key Word: Method development, Validation, Lobeglitazone, spectroscopy, green analytical approach

INTRODUCTION

Lobeglitazone:

Lobeglitazone is a pharmacophore which has a 2, 4-thiazolidinedione group with an ethoxy-benzyl N-methyl amino group bound to this as a connecting link. Its structural formula is C₂₄H₂₄N₄O₅S. Its synonyms include Duvie (Korea) and LOBG (India). The IUPAC 5-[4-(2-{{6-(4-Methoxy-phenoxy)-pyrimidin-4-yl]-methyl-amino}-ethoxy)-benzyl]-thiazolidine-2,4-dione hydrosulphuric acid. Its molecular weight is 480.5 g/mol., ^[1,2]

Lobeglitazone was based on modification of the rosiglitazone structure to introduce a p-methoxyphenoxy group at the 4-position of the pyrimidine moiety. This contributes to enhanced binding affinity of Lobeglitazone for PPAR γ , with docking analysis suggesting that the binding affinity of Lobeglitazone is 12 times higher than that of rosiglitazone and pioglitazone. ^[1]

According to earlier research, Lobeglitazone medication improved lipid profiles by an 8% rise from baseline high-density lipoprotein cholesterol (HDL-C) levels and a 13% reduction from baseline triglycerides levels. Both as a monotherapy and in combination with metformin, Lobeglitazone was well tolerated. Because of its greater affinity for PPAR, Lobeglitazone exhibits comparable glycaemic efficiency to pioglitazone at a lower effective dose of 0.5 mg. It is predicted that Lobeglitazone can be administered in individuals with renal insufficiency without dose reduction since it is mostly metabolized by the liver, with minor renal excretion and because it may have a lower risk of bladder cancer than other thiazolidinediones. Due to its favourable safety profile and proven ability to decrease blood sugar levels in both clinical and experimental studies, Korea has approved Lobeglitazone as an oral diabetes medication since July 2013. According to research conducted on animals, Lobeglitazone administration greatly decreased insulin resistance and enhanced the expression of GLUT4 and PPARs in mice given a high-fat diet (HFD). The prevalence of diabetes is significantly rising globally, primarily as a result of rising obesity and insulin resistance rates as well as an aging population that has an impact on mortality and quality of life. ^[2]

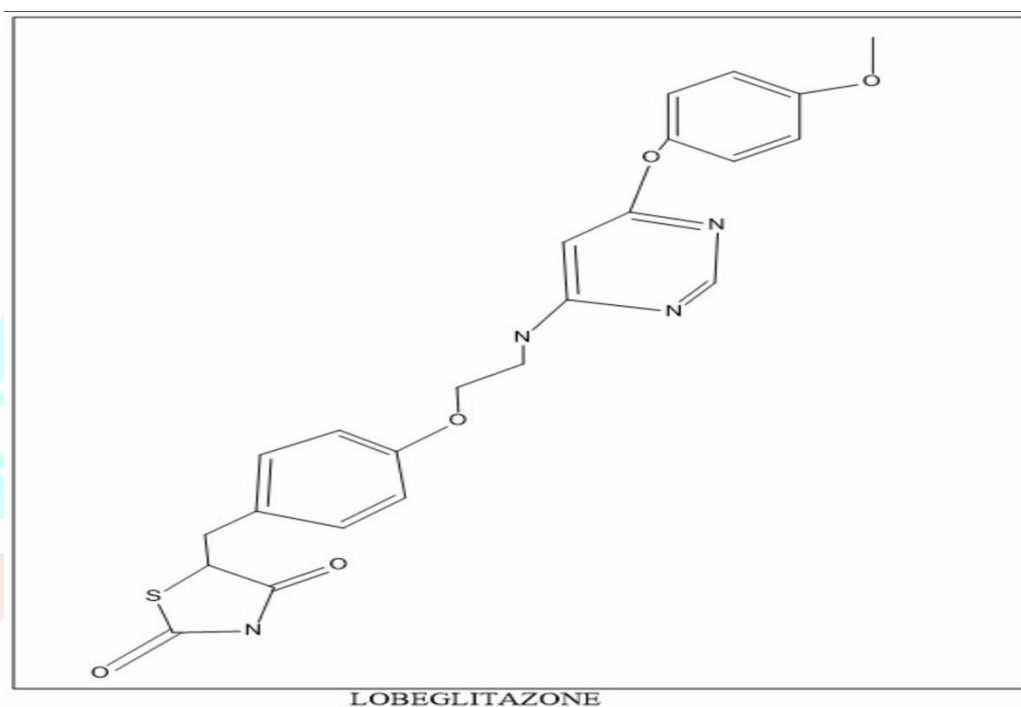
With an IC₅₀ of 20 and 18 nm, respectively, Lobeglitazone sulfate is a thiazolidinedione that works by activating PPAR- α/γ dual agonist. It was created in Korea by Chong Kun Dang Pharmaceutical to treat diabetes, and it differs significantly from the other two PPAR agonists, pioglitazone and rosiglitazone, in that it has PPAR- α activity. In South Korea and India, the medication is authorized for the management of T2DM. In a 24-week randomized, double-blind, multicenter clinical trial, Lobeglitazone sulfate 0.5

mg once daily was found to be safe and effective when compared to a placebo in treating naïve Korean people with T2D. ^[3]

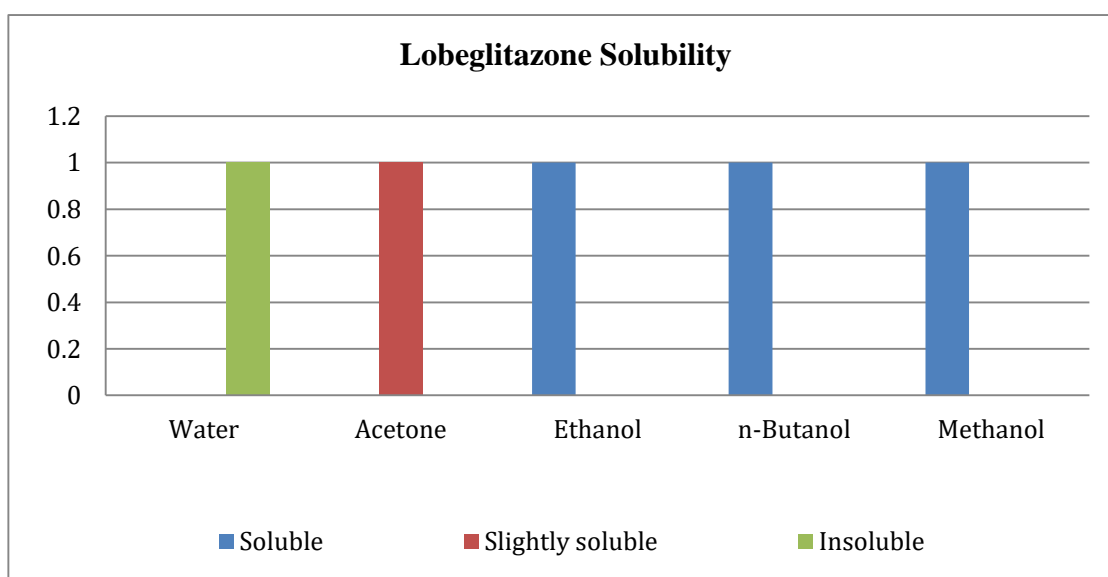
UV spectrophotometry is a popular analytical technique in pharmaceutical analysis because of its simplicity, affordability, speed, and accessibility in standard quality control labs. However, conventional analytical methods often call for hazardous organic solvents, posing environmental and

health hazards. Green analytical chemistry has gained more attention recently with the goal of minimizing the use of hazardous materials and their impact on the environment. Using methanol as a solvent, this study aims to develop and validate a simple, accurate, accurate, and eco-friendly UV spectrophotometric method for measuring lobeglitazone sulfate in bulk. The approach is verified in accordance with International Council for Harmonization guidelines, considering aspects such as robustness, linearity, accuracy, precision, and specificity.

By reducing the environmental impact and promoting safer analytical techniques, this eco-friendly method ensures accurate drug measurement while also promoting sustainable lab practices.



LOBEGLITAZONE

Figure : 1 Structure of Lobeglitazone**Figure 2: Solubility of Lobeglitazone**

Dapagliflozin and Lobeglitazone stand out as valuable oral agents for type 2 diabetes mellitus, each working through different mechanisms to deliver therapeutic benefits. Researchers have explored several methods for their analysis separately in both biological samples and pharmaceutical products but there has been no method published for combination of the both drugs. No approaches such as UV spectrophotometry, HPLC, HPTLC and LC-MS are applied for this combination and its own strengths in terms of sensitivity, accuracy. These techniques not only support everyday quality checks and pharmacokinetic evaluations but also ensure that regulatory standards are met while opening doors for future studies^[6].

MATERIALS AND METHODS

Materials

Lobeglitazone sulfate was obtained as gift sample from AmbicaPharmachem Private Limited, Ankleshwar. All the chemicals and reagents used were of analytical grade and purchased

Uv spectrophotometry :

Model	SHIMADZU UV1900i
Software	Lab Solution
Wavelength	190-1100nm

Electronic balance :

Model	MH-0095
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Preparation of standard stock solution

Weighed 10 mg of lobeglitazone sulfate was transferred to a 10 ml volumetric flask, dissolved in 10 ml of methanol with the help of sonication to obtain a stock solution of 1000 µg/ml.

From this stock solution, working solution was prepared by pipetting 1 ml into 10 ml volumetric flask and diluting upto the mark with methanol, resulting in a final concentration of 100 µg/ml.

Selection of wavelength for analysis of Lobeglitazone Sulfate

An accurate volume of 0.6 ml of the 100 µg/ml standard working solution of Lobeglitazone Sulfate was transferred into a 10 ml of volumetric flask and the volume was made up to the mark using methanol. The final concentration of 6 µg/ml was obtained and this resulting solution was scanned in the UV range 200-600 nm using methanol as blank. The spectrum of Lobeglitazone showed maximum absorbance at 250.5 nm. Then this wavelength was selected for further analysis.

Validation of the method

The validation of method was done in terms of linearity, accuracy, precision, repeatability and ruggedness.

Linearity study

Different aliquots of Lobeglitazone standard working solution (100µg/ml) in the range of 0.2-1.0 ml were transferred into a series of 10 ml volumetric flask, and the volume was made up to the mark with methanol to obtain the concentration of 2, 4, 6, 8 and 10 µg/ml respectively. The solutions were scanned in the UV range of 200-600 nm and the absorbance was recorded at 250.5 nm, which is the λ_{max} of Lobeglitazone. A calibration curve was plotted between concentration and absorbance (figure 2).

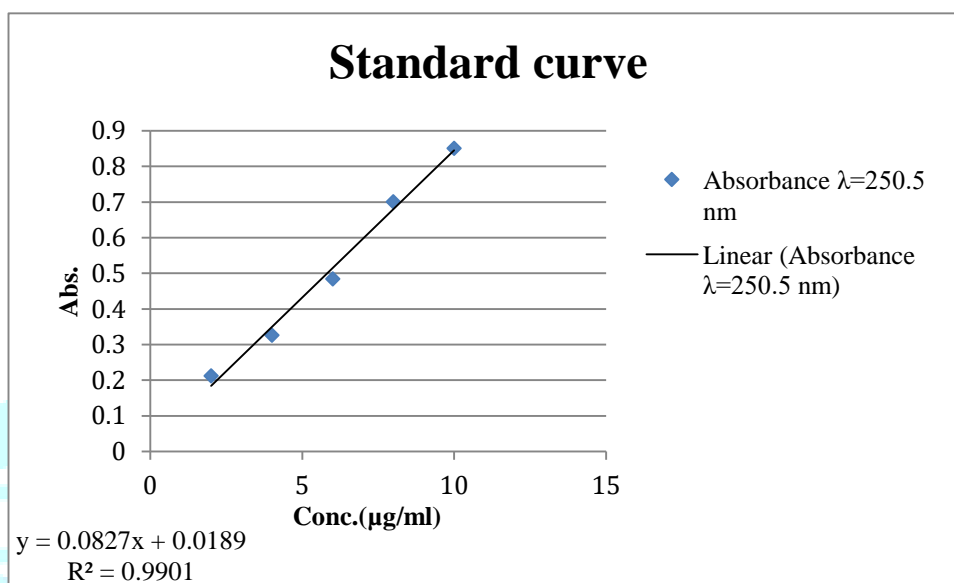


Figure 3: Calibration curve of Lobeglitazone at 250.5 nm

Accuracy

A known quantity of standard stock solution was added to the preanalyzed sample solutions at three distinct levels: 80%, 100%, and 120%. The suggested approach was used to reanalyze the solutions.

Precision

The method's precision was tested by looking at how much it changed from day to day and from hour to hour. Three times on the same day, we looked at solutions of lobeglitazone sulfate with 2, 4, 6, 8, and 10 µg/ml to see how precise they were within the same day. By measuring the same concentration levels every day for three days in a row under the same experimental conditions, we could find out how precise the measurements were from day to day. We measured the solutions' absorbance at 250.5 nm and used the mean and percentage relative standard deviation (%RSD) to show the data.

Repeatability

Repeatability was determined by analysing 6 µg/ml concentration of Lobeglitazone sulfate solution for seven times.

Sensitivity

The sensitivity of the developed analytical method for Lobeglitazone was assessed by determining the limit of detection (LOD) and limit of quantification (LOQ). These values were calculated using the expressions $LOD = 3.3 \times (N/B)$ and $LOQ = 10 \times (N/B)$, where N denotes the standard deviation of peak areas obtained from three replicate measurements and B represents the slope of the calibration curve.

Determination of Lobeglitazone Sulfate in Bulk

To prepare a stock solution of 1000 µg/ml, 10 mg of lobeglitazone sulfate was accurately weighed and dissolved in methanol using sonication. To create a 100 µg/ml working standard solution, 1 ml of the stock solution was diluted with 10 ml of methanol. A 0.6 ml aliquot of this working solution was transferred into a 10 ml volumetric flask and diluted up to the mark with methanol to obtain a final concentration of 6 µg/ml. The prepared solution was scanned in the UV region between 200 and 600 nm, and the absorbance was measured at 250.5 nm, which corresponds to lobeglitazone sulfate's λ_{max} . The linear regression equation derived from the calibration curve was used to determine the drug concentration in the bulk sample.

Application of the proposed method for pharmaceutical formulation

For the analysis of pharmaceutical formulation, twenty tablets were taken (Lobula), accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of lobeglitazone sulfate was transferred into a 10 ml volumetric flask and dissolved in methanol with the help of sonication to obtain a stock solution of 1000 µg/ml. The solution was filtered if necessary to remove insoluble excipients. From this stock solution, 1 ml was diluted to 10 ml with methanol to obtain a working solution of 100 µg/ml. Further, 0.6 ml of this solution was transferred into a 10 ml volumetric flask and diluted up to the mark with methanol to obtain a final concentration of 6 µg/ml. The absorbance was measured at 250.5 nm after the solution was scanned in the UV range of 200–600 nm. The calibration curve was used to determine the amount of lobeglitazone sulfate in the tablet formulation, and the results were presented as a percentage of the labeled claim.

Green Analytical Chemistry Evaluation – MoGAPI

Green Analytical Chemistry is an emerging approach in the field of analytical science that focus on designing analytical methods that minimize environmental impact while maintaining high analytical performance and reliability. It is an extension of the principles of green chemistry applied specifically to analytical processes.

The main goal of Green Analytical Chemistry is to cut down on or get rid of the use and creation of dangerous chemicals during sample collection, preparation, analysis, and disposal. It encourages the creation of methods that are safer, cleaner, more effective, and better for the environment. The method

is based on a few main ideas, such as using less samples and reagents, safer and less toxic chemicals, less energy, less waste, and more automation and miniaturized systems. It also promotes direct analysis methods that don't require complicated steps to prepare samples.

Green Analytical Chemistry stresses the use of safer solvents, like water, ethanol, or other low-toxicity solvents, instead of traditional hazardous ones. It also encourages the use of modern analytical tools like microextraction techniques, flow analysis, and hyphenated methods that make things more efficient and less harmful to the environment.

Green Analytical Chemistry gives us a way to make analytical methods that are both accurate, precise, and sensitive, and that are also good for the environment.

MoGAPI is a computer program that helps you figure out how green an analytical chemistry method is by looking at both green and white analytical chemistry principles. It gives a full evaluation by taking into account factors like safety, environmental impact, and how well the method works.

The tool looks at many different parts of an analytical procedure, such as safety risks, energy use, occupational hazards, waste production, waste treatment, solvent and reagent toxicity, instrumentation, and the ability to measure things.

Based on set standards, each parameter gets a score that shows how environmentally friendly and sustainable it is. To get an overall greenness score, these individual scores are added together. This score is usually shown on a number scale.

The final score is often shown as a visual index or color-coded chart, with red or orange zones showing lower scores that mean less sustainable methods and yellow to green zones showing higher scores that mean more environmentally friendly methods.

MoGAPI is a more complete tool than metrics that only look at one thing because it looks at environmental impact, analytical efficiency, and innovation all at the same time.

RESULTS AND DISCUSSION

Method validation

The analytical method was validated as per international Council of Harmonisation(ICH) guidelines.

Linearity studies

The analytical method demonstrated a proportional response within the concentration range of 2-10 µg/ml , confirming linearity of the calibration curve(figure 3). A plot of concentration versus absorbance was prepared and subjected to regression analysis. The obtained regression equation was: $y = 0.082x + 0.021$ ($R^2 = 0.992$).The data is expressed in Table 1.

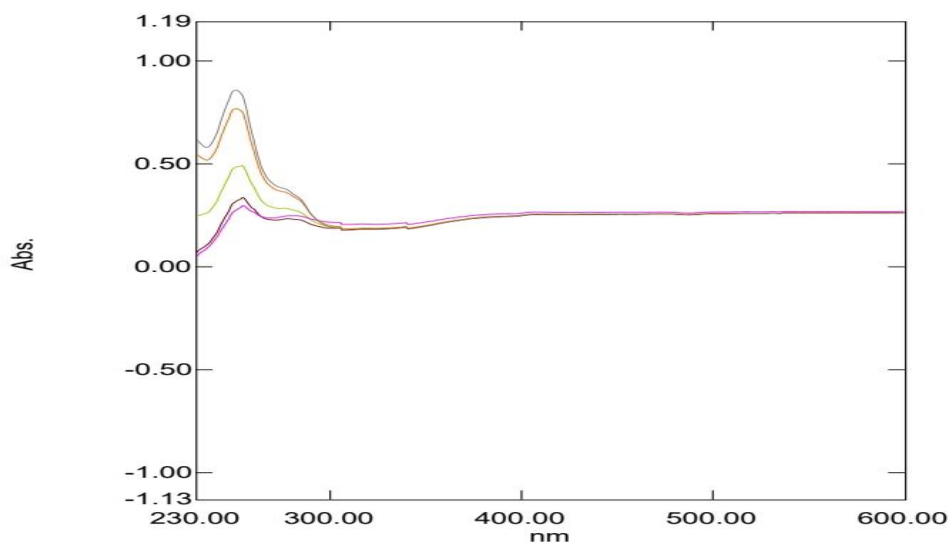


Figure 4: Spectra of Lobeglitazone (2-10 µg/ml) at 250.5 nm.

TABLE 1: Linearity study of Lobeglitazone

Concentration, µg/ml	Absorbance, mean ± SD	%RSD
2	0.212 ± 0.0021	1.90
4	0.326 ± 0.0021	0.43
6	0.484 ± 0.0005	0.42
8	0.701 ± 0.0030	0.64
10	0.851 ± 0.0056	0.99

Accuracy

The suggested method was used to reanalyze the solution; recovery study results are shown in table 2, where the percentage amount found was between 98.50% and 99.54% with %RSD >2.

TABLE 2: Recovery studies

Pre-analyzed Sample Solution (µg/ml)	Amount of Drug Added (µg/ml) (n=3)	Amount Recovered (µg/ml) (n=3)	% Recovery	% RSD
6	0	5.91	98.50	1.08
6	4.8	10.83	99.54	0.90
6	6.0	11.92	99.33	0.87
6	7.2	13.94	99.23	0.77

Precision

The precision of the method that was developed was shown by the percent relative standard deviation (%RSD). The assay can be repeated with good results, as these results show. The %RSD values were less than 2, which means that the method is accurate and dependable.

TABLE 3: Precision

Component	Concentration (µg/ml)	Intraday precision (n=3)		Interday precision (n=3)	
		Conc. found	%RSD	Conc. found	%RSD
Lobeglitazone	10	0.854	0.35	0.849	0.83
	8	0.763	0.46	0.761	0.59
	6	0.483	0.12	0.484	0.10
	4	0.316	0.18	0.317	0.31
	2	0.273	0.37	0.273	0.42

Sensitivity

Linearity was observed with the equation $Y = 0.0827X + 0.0189$, and the LOQ and LOD were found to be 0.06 µg/ml and 0.02 µg/ml, respectively.

Repeatability

Lobeglitazone sulfate solution at a concentration of 6 microgram/ml was analyzed seven times to determine repeatability. The %RSD was found to be less than 2, which complies with the acceptance criteria as per ICH [Table 4,5].

TABLE 4: Repeatability Studies

Component	Concentration	Absorbance
Lobeglitazone sulfate	6	0.484
Lobeglitazone sulfate	6	0.484
Lobeglitazone sulfate	6	0.483
Lobeglitazone sulfate	6	0.485
Lobeglitazone sulfate	6	0.483
Lobeglitazone sulfate	6	0.484



Lobeglitazone sulfate	6	0.483
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TABLE 5: Repeatability Studies

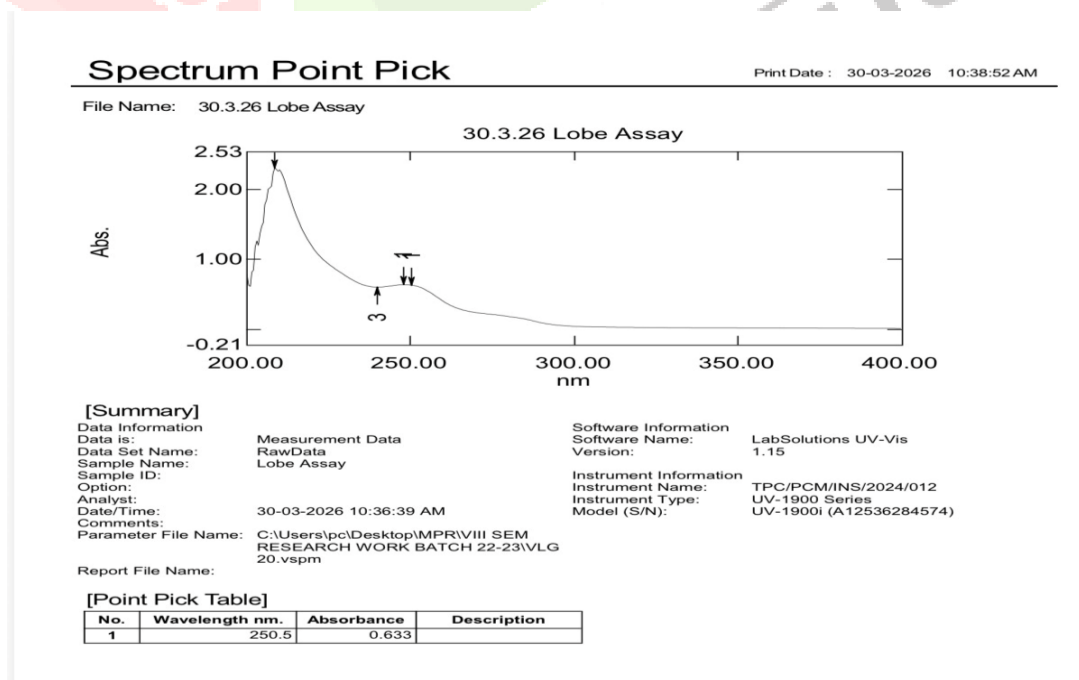
Component	Concentration	Mean Absorbance	Standard Deviation	%RSD
Lobeglitazone sulfate	6 microgram/ml	0.484 nm	0.0008	0.16%

Assay

Figure:5 Result of Assay

The assay of the given sample was carried out using a UV spectrophotometric method at a wavelength of 250.5nm. The absorbance of the sample solution was found to be 0.633 as shown in the figure 5

Figure 6: Marketed formulation of Lobeglitazone sulfate



Green Analytical Chemistry Evaluation: MoGAPI Software

The developed analytical method was evaluated using MoGAPI to assess environmental reliability of the method given as following

Questions

Sample Preparation

1. Collection : Off-line
2. Preservation : None
3. Transport : None
4. Storage : None
5. Type of method : Simple procedures, e.g., filtration, decantation
6. Scale of extraction : Not applicable
7. Solvents/ Reagents used : Green solvents/ reagents used
8. Additional treatment : Simple treatments (clean up, solvent removal, etc.)

Reagents and Solvents

9. Amount : 10-100 ml (10-100 g)
10. Health hazard : Moderately toxic; could cause temporary incapacitation; NFPA= 2/3
11. Safety hazard : Highest NFPA flammability or instability score of 2 or 3, or a special hazard is used.

Instrumentation

12. Energy : ≤ 0.1 kWh per sample
13. Occupational hazard : Emission of vapors to the atmosphere
14. Waste : 1-10 ml (1-10 g)
15. Waste treatment : No treatment
16. QUANTIFICATION : Yes

Based on MoGAPI evaluation the method achieved a final score of 72, indicating a good level of greenness. This score shows that the method follows many of the rules of Green Analytical Chemistry, especially when it comes to lowering the risk of harm, using energy wisely, and making only a little waste.

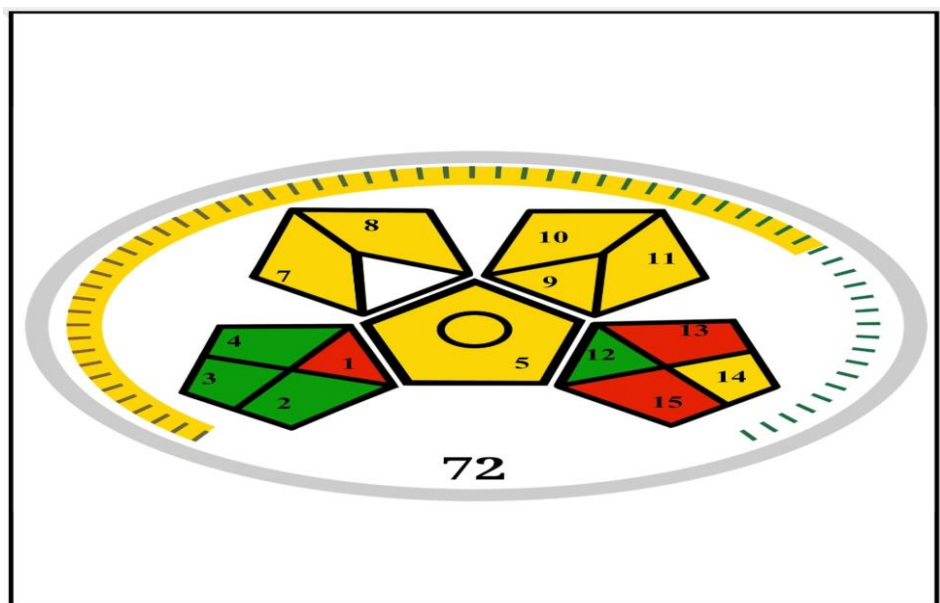


Figure 5 : MoGAPI Score

CONCLUSION

A simple, specific, accurate, precise, and eco-friendly UV spectrophotometric method has been developed and validated for the estimation of Lobeglitazone. The method successfully quantified Lobeglitazone using methanol as a suitable solvent system. Validation of the developed method was carried out in accordance with ICH (Q2 R1) guidelines, demonstrating acceptable results for parameters such as linearity, accuracy, precision. Furthermore, assessment using green analytical chemistry tools like MoGAPI confirmed that the method is environmentally friendly and sustainable.

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