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# QBD- Guided RP-HPLC Method Development and validation of Lobeglitazone Sulphate

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## Abstract

This study focused on developing and validating a method for analyzing Lobeglitazone using RP-HPLC based on Quality by Design (QbD). A 2FI factorial design identified and optimized key variables, including the mobile phase composition (% Acetonitrile) and flow rate. Water and acetonitrile were used as the mobile phase in an isocratic separation on a C18 column. We set the detection wavelength for medication at 248 nm. During the validation process, we examined parameters such as specificity, linearity, precision, accuracy, robustness, LOD, and LOQ, following ICH Q2(R1) guidelines. The method produced a clear, well-defined peak for Lobeglitazone, with a retention time within acceptable limits. Linearity was confirmed over a concentration range of 5-25 µg/ml for LBZ analysis, with correlation coefficients ($R^2$) of 0.999 for both compounds, indicating a strong linear response. The method showed high accuracy with recovery rates between 98 and 102%, while precision displayed %RSD values below 2%. The LOD and LOQ values confirmed the method's sensitivity. ANOVA showed that both the mobile phase and flow rate significantly affected retention time, theoretical plates, and tailing factors. We observed no interference in blank chromatograms, ensuring specificity. The method remained robust against minor intentional changes in chromatographic conditions. This research developed a QbD-based RP-HPLC method that is straightforward, accurate, precise, and reliable for simultaneously estimating LBZ. The method is suitable for routine quality control and stability testing of pharmaceutical products.

**Keywords:** RP-HPLC; QbD; Diabetes mellitus; Lobeglitazone

## 1. Introduction

Any sample that can dissolve in a solvent can have its constituent compounds separated, identified, and quantified using the chromatography process[1]. The development and validation of methods using HPLC are essential for discovering, developing, and manufacturing pharmaceutical drugs. In RP-HPLC, the stationary phase is non-polar or hydrophobic, typically consisting of silica particles bonded with long-chain hydrocarbons such as C18 or C8, while the mobile phase is relatively polar, usually a mixture of water with organic solvents like methanol or Acetonitrile. Separation occurs based on differences in hydrophobic interactions between analyte and the stationary phaseQbD, is the process of achieving a specific, predictable quality with predefined, desired specifications. An extremely helpful aspect of QbD is the comprehension of factors and the effects of their interactions through a desired set of experiments[2].

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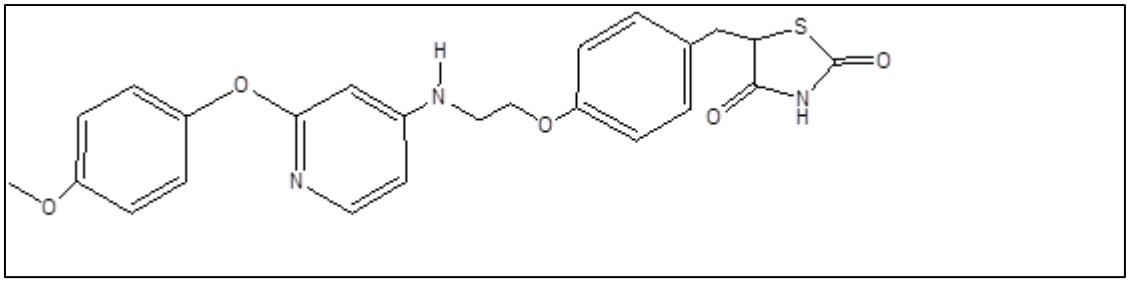


**Figure 1** Lobeglitazone (LGL)

Lobeglitazone (LGL) is a new member of the thiazolidinediones (TZDs) class. It mainly helps improve glycemic control in patients with Type 2 diabetes mellitus (T2DM). As a PPARγ agonist, LGL enhances insulin sensitivity in fat, muscle, and liver tissues. It has garnered attention for its potentially favorable safety and efficacy profile. It offers effective glycemic control with fewer side effects, especially cardiovascular risks, compared to other TZDs like rosiglitazone and pioglitazone. Quality by Design (QBD) is a systematic approach to developing Linagliptin is a medication taken by mouth that inhibits dipeptidyl peptidase 4 (DPP)-2. It was initially approved in the US, Europe, Japan, and several other regions in 2011 for enhancing blood sugar regulation in adults diagnosed with type 2 diabetes mellitus[3].Like other DPP-4 inhibitors, linagliptin is a low-risk oral antidiabetic medication that does not cause weight gain and has a minimal risk of hypoglycemia[4].In 2014, the oral hypoglycemic medication empagliflozin was approved and put into clinical use. It is classified as an inhibitor of sodium-glucose cotransporter 2 (SGLT2), which was developed to treat type 2 diabetes mellitus (T2DM). SGLT2 is a new target for T2DM treatment since it is the primary transporter responsible for glucose reabsorption from glomerular filtrate[5].

A literature survey revealed various methods has been developed for the determination of Lobeglitazone HPLC, UV-Spectrophotometr, UHPLC-QTOF-MS, HPLC-UV and Quadrupole Time-of-Flight Mass Spectrometry. Although these techniques illustrate the viability of estimating drug separately and in combination, most have been created using traditional trial-and-error methods which may not include systematic risk evaluations studies. In the current regulatory landscape, there is an increasing focus on analytical techniques that are not just accurate and precise, but also robust, reproducible, and capable of delivering consistent performance across varying conditions. This highlights the significance of applying the QbD approach. Consequently, even with existing methods for Lobeglitazone pursuing the development of a QbD-based RP-HPLC method is warranted, as it provides a more reliable, robust, and lifecycle-manageable analytical tool that aids in effective product development, quality control, and regulatory compliance.

## 2. Methods

The chromatographic separation used a Waters 2690/5 series Method HPLC system. This system included a Rheodyne manual injector (model 7725i) with a fixed loop volume of 20 µL. The setup also had a quaternary gradient pump and a programmable UV/VIS detector (SPD-10AVP) for detection. Data compilation and analysis were performed using Empower software, version 3. We achieved separation with an Inertsil C18 ODS column measuring 250 mm × 4.6 mm, with a particle size of 5 µm. This ensured effective resolution and retention for the analyte.

### 2.1. Chemicals and Reagents

All chemicals and solvents used in this research were analytical grade and met the required standards for HPLC and analytical applications. The reliability, accuracy, and precision of HPLC analysis depend on the purity and quality of the reagents, solvents, and filtration materials used during sample preparation and analysis. To maintain data integrity and reproducibility, we used only high-purity solvents and reagents throughout method development and validation.

### 2.2. Preparation of the standard solution

10 mg of Lobeglitazone was accurately measured and placed into two separate 10 mL volumetric flasks. About 7 mL of Acetonitrile was added to each flask, and the mixtures were sonicated for 10 minutes to ensure complete dissolution. The volume was then adjusted to the mark with the same mobile phase, creating 1000 ppm (1000 µg/mL) standard stock solutions for each drug.

### 2.3. Selection of wavelength

Lobeglitazone was dissolved in an acetonitrile to prepare a dilute standard solution (1000 µg/mL), ensuring compliance with Beer's law. This solution was used for further dilutions. The UV spectrum was scanned from 200-400 nm against a solvent blank (Acetonitrile) using a UV-Vis spectrophotometer. λ max was identified from the absorbance peak at 248 nm.

### 2.4. HPLC experimental conditions

We conducted several trials for HPLC method development using the Waters Model 2690/5 series HPLC system, along with a programmable variable wavelength UV/VIS detector (SPD-10AVP). Separation was achieved with an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm particle size) at ambient temperature. The flow rate was set at 1.0 mL/min, and the detection wavelength was set at 248 nm. Several trials used mobile phases of Water: Acetonitrile (90:10 v/v) and Water: Methanol (45:55 v/v). However, the peaks were partially merged and unsatisfactory. The peak shapes were not optimal, requiring further improvement for better separation and retention times. Based on these initial trials, we systematically evaluated and refined the method, focusing on key parameters. A $3^2$ full factorial experimental design was employed to examine the effects of mobile phase composition (Acetonitrile) and flow rate on retention time, theoretical plate count, and tailing factor.

### 2.5. HPLC Method Development Using QbD Approach

The HPLC method was developed using a QbD framework. This approach included a systematic assessment of risks, factorial design experiments, and statistical analysis through ANOVA and surface plots. These techniques helped identify and improve key method parameters, ensuring reliability, precision, and adherence to regulatory standards.

#### *2.5.1. Factorial Design*

To improve the RP-HPLC technique for analyzing Lobeglitazone, we applied a $3^2$ full factorial design. This focused on two independent variables: the concentration of acetonitrile in the mobile phase and the flow rate. We tested methanol concentration at three different levels: 60%, 70%, and 80%. The flow rate was examined at 0.8, 1.0, and 1.2 mL/min (table 1). This design allowed for nine experimental runs, providing a thorough analysis of how changes in acetonitrile concentration and flow rate affected important chromatographic responses, such as retention time, theoretical plate count, and tailing factor. The factorial design helped identify the best chromatographic parameters and illustrated how the two variables interacted. We analyzed the experimental data using surface plots and ANOVA, which helped visualize the interaction effects of acetonitrile concentration and flow rate, as well as assess the statistical significance of each factor regarding the chromatographic responses.

**Table 1** Factors for independent variables

| Factor | Name | Level | Low Level | Medium Level | High Level |
|---|---|---|---|---|---|
| A | % ACN | 3 | 65 | 70 | 75 |
| B | Flow Rate | 3 | 0.8 | 1 | 1.2 |

### 2.6. Evaluation of Experimental Results

In evaluating the experimental results, we identified retention time, theoretical plates, and tailing factor as key parameters to assess the effectiveness and performance of the developed HPLC method. Retention time helped us observe the elution characteristics and separation of Lobeglitazone, ensuring that both analytes were properly resolved within a suitable run duration. Theoretical plates served as an indicator of column efficiency. A higher number of plates showed improved separation performance and reduced band broadening. We examined the tailing factor to evaluate peak symmetry. Values close to 1.0 indicate sharp and symmetrical peaks, which are crucial for accurate quantification. During the factorial trials, we assessed these responses using ANOVA and surface plots to study the impact of acetonitrile concentration and flow rate.

### 2.7. Method Validation

We validated the analytical method for estimating Lobeglitazone by following standard validation parameters. We created calibration curves using standard solutions of Lobeglitazone at 10-50µg/ml to analyze the linear relationship between peak area and analyte concentration. This showed a strong correlation. We evaluated precision through studies on repeatability and intermediate precision, which demonstrated consistent results with low %RSD values. We

confirmed accuracy through recovery studies where we added 80%, 100%, and 120% of the standard to the sample matrix. The recoveries ranged from 98% to 102%, proving the method's reliability. We established specificity by comparing the retention times of the sample peaks with those of the standard solutions. This ensured that the method could accurately differentiate Lobeglitazone from other components in the formulation.

## 3. Results and discussion

### 3.1. Factorial design

Table 2 summarizes the results of a $3^2$ full factorial design used to improve chromatographic conditions for estimating Lobeglitazone by RP-HPLC. The two independent variables examined were the percentage of acetonitrile in the mobile phase (65%, 70%, and 75%) and the flow rate (0.8, 1.0, and 1.2 mL/min). The dependent variables measured included retention time (RT), theoretical plate count (TPC), and tailing factor (TF) for both analytes. The results showed that increasing the acetonitrile concentration and flow rate reduced retention times for both drugs, leading to faster elution. Theoretical plate count, which indicates column efficiency, varied across experiments. Higher values were found under optimized conditions, particularly at 65-75% acetonitrile and a 1.0 mL/min flow rate. Tailing factors for Lobeglitazone were mostly around 1, indicating symmetrical peaks. Among all the experimental runs, the combination of 75 and a 1.0 mL/min flow rate (Run 1) showed the best overall chromatographic performance. This run produced sharp, well-resolved peaks, suitable retention times (2.9 min for Lobeglitazone), high theoretical plate counts (7536.83), and minimal tailing (1.063). Thus, this condition was selected as the optimized method for further validation.

**Table 2** Optimization of parameters for analysis of Lobeglitazone using $3^2$ full factorial designs

| Run | Factor 1 A:Mobile Phase (% Acetonitrile) | Factor 2 B:Flow Rate | Response 1 RT (L) | Response 2 TPC (L) | Response 3 TF (L) |
|---|---|---|---|---|---|
| 1 | 65 | 0.8 | 3.455 | 2298 | 1.0 |
| 2 | 70 | 1 | 3.104 | 2580 | 1.13 |
| 3 | 65 | 1 | 3.299 | 2387 | 1.07 |
| 4 | 70 | 1.2 | 2.880 | 2700 | 1.16 |
| 5 | 65 | 1.2 | 2.999 | 2344 | 1.09 |
| 6 | 70 | 0.8 | 3.356 | 2298 | 1.10 |
| 7 | 75 | 1 | 2.97 | 2455 | 1.2 |
| 8 | 75 | 0.8 | 3.45 | 2400 | 1.19 |
| 9 | 75 | 1.2 | 2.711 | 2876 | 1.27 |

#### *3.1.1. Optimized Condition Obtained*

Table 2 presents the optimized chromatographic conditions identified using a $3^2$ full factorial design to yield the best results for estimating Lobeglitazone. The main goals were to maximize theoretical plate count, minimize tailing factor, and ensure appropriate retention times for the analyte, all measured using a desirability function. The ideal conditions included 75% Acetonitrile with a flow rate of 1 mL/min, resulting in a retention time of 2.911 minutes for Lobeglitazone. The theoretical plate count was 7255.947, indicating high column efficiency. The tailing factor was 1.080 for Lobeglitazone, showing symmetrical peak shapes, which are crucial for accurate quantification. A desirability score of 1.000 confirms that these conditions achieve the best balance among all response variables.

The contour plot of desirability illustrates how various combinations of Acetonitrile concentration and flow rate impact the overall performance of the method. The darkest area represents the optimal zone, indicating the highest desirability. This structured optimization method, based on Quality by Design, ensures the robustness, reliability, and adherence to regulatory standards for the developed RP-HPLC method.

### 3.2. The effect of Retention Time of Lobeglitazone

The composition of the mobile phase (% Acetonitrile) and the flow rate (mL/min) influence the retention time of Lobeglitazone. It indicates that increasing the flow rate decreases retention time, suggesting that the analyte travels through the column faster at higher flow rates. Additionally, a rise in the Acetonitrile percentage in the mobile phase also shortens retention time. This occurs because higher Acetonitrile concentration enhances elution capacity, which reduces Lobeglitazone's interaction with the stationary phase. The color gradient in the plot ranges from blue, reflecting lower retention times around 4.08 minutes, to red, showing higher retention times up to 5.21 minutes. This demonstrates how different experimental conditions affect the results. The red and pink points on the graph represent the design points from experiments either above or below the predicted surface, highlighting the model's reliability. In summary, the plot shows that both Acetonitrile concentration and flow rate greatly affect the retention time of Lobeglitazone. Shorter retention times occur at higher Acetonitrile concentrations and flow rates, which is essential for optimizing methods in chromatographic analysis.

The ANOVA analysis for the 2FI model concerning the Retention Time of Lobeglitazone shows that the overall model is statistically significant (F-value = 56.75, p = 0.0003). Both the mobile phase (% Acetonitrile) and flow rate are key elements affecting retention time, with p-values of 0.0019 and <0.0001, respectively. The low residual error supports the reliability of the model.

### 3.3. The effect of Theoretical plate of Lobeglitazone

The composition of the mobile phase (% Acetonitrile) and the flow rate influence the theoretical plate count of Lobeglitazone, serving as an indicator of column efficiency in chromatographic separation. It is evident that both factors significantly impact the theoretical plate count. Increasing the Acetonitrile percentage while decreasing flow rate causes a noticeable increase in the theoretical plate count, suggesting improved separation efficiency. The plot transitions from blue, indicating lower plate counts around 7350, to red, indicating higher plate counts peaking at about 8675. This illustrates the range of efficiency achieved under different conditions. Design points positioned above and below the surface, represented by red and pink spheres, respectively, emphasize the model's predictive accuracy. In summary, the figure confirms that optimal chromatographic performance for Lobeglitazone occurs with higher Acetonitrile concentrations and lower flow rates, as these conditions enhance the interaction between the analyte and the stationary phase, resulting in sharper and clearer peaks.

The ANOVA for the 2FI model indicates that the model is statistically significant (F = 17.28, p = 0.0045), showing its ability to reliably explain variations in theoretical plate count. Among the factors studied, only the mobile phase composition (% Acetonitrile) is significantly important (p = 0.0008), demonstrating a strong effect on the response variable. In contrast, the flow rate (p = 0.9837) and its interaction with Acetonitrile (p = 1.0000) are not significant, indicating they have little to no effect. Therefore, it may be beneficial to refine the model by removing non-significant terms while maintaining the hierarchy. .

### 3.4. The effect of Tailing Factor of Lobeglitazone

The data clearly indicates that both parameters have a slight impact on the tailing factor. Increasing the flow rate and the percentage of Acetonitrile tends to cause a minor increase in the tailing factor. This is shown by the gradual color change from blue, which indicates lower tailing, to red, which indicates higher tailing. However, the variation remains limited, ranging from 1.01 to 1.1. This suggests that the peak shape remains relatively symmetrical under the tested conditions. The design points represented in red, above the surface, and pink, below the surface, further validate the model's predictive ability. Overall, the tailing factor remains within acceptable limits across the different chromatographic conditions examined, indicating a robust method in terms of peak symmetry.

The ANOVA results reveal that the overall model is statistically significant, with an F-value of 56.52 and a p-value of 0.0003. This implies only a 0.03% chance that such a high F-value could arise from random variation. For the individual factors, both A (Mobile Phase % Acetonitrile) and B (Flow Rate) show strong significance with p-values less than 0.05, indicating they significantly affect the tailing factor of Lobeglitazone. .

### 3.5. Analytical Method Validation

#### *3.5.1. System suitability*

The collected information confirms that the HPLC system operated effectively and produced reliable results. All system suitability parameters listed in Table 4 met the predetermined acceptance criteria. This demonstrates the method's reliability and the instrument's good performance throughout the examination.

**Table 3** System suitability for Lobeglitazone Sulphate

| Sr. No. | Parameter | Lobeglitazone |
|---|---|---|
| 1. | Theoretical plates (N) | 2770.85 |
| 2. | Retention Time (RT) | 2.951 |
| 3. | Tailing factor (T) | 1.555 |

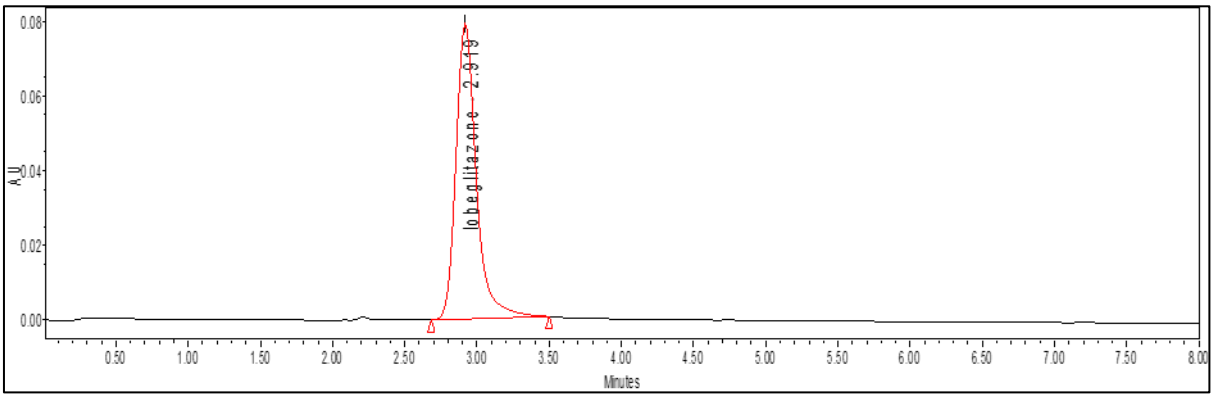


**Figure 2** Suitability of the Device Standard Chromatogram

*3.5.2. Specificity*

The specificity study showed that the chromatograms for the Lobeglitazone samples displayed a clear and positive response when compared to the reference standards. In contrast, the blank diluent showed no response or interference at all. This confirms the method's accuracy and selectivity. Figures 10 and 11 illustrate the chromatograms of the standard and blank injections, respectively.

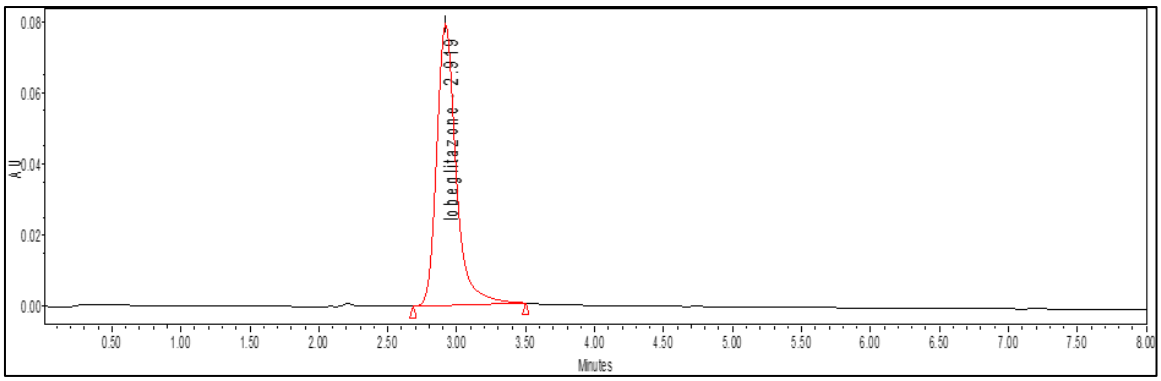


**Figure 3** Chromatogram for Standard

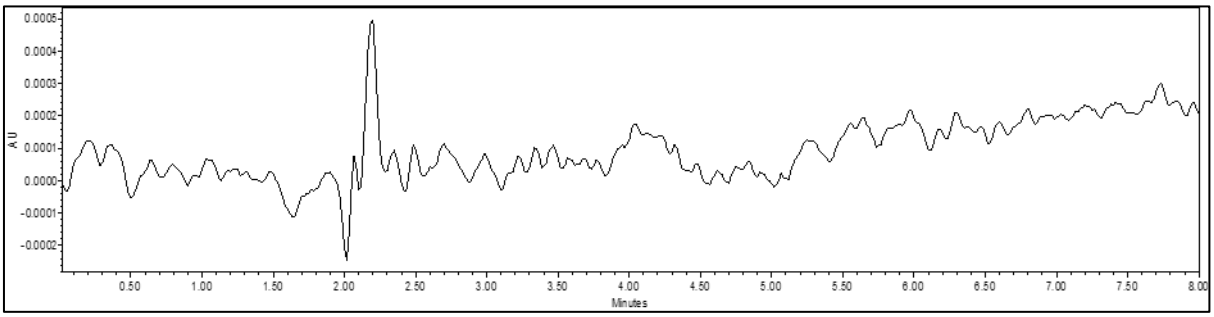


**Figure 4** Chromatogram for blank injection.

*3.5.3. Linearity*

The developed HPLC method showed linearity for Lobeglitazone within the concentration range of 5-25 µg/mL. The drug was analyzed at five different concentrations, and their peak areas were plotted against the corresponding concentrations. The calibration curves for both compounds displayed a strong linear relationship with a regression coefficient ($R^2$) of 0.999. This indicates that the method is highly linear and suitable for accurately measuring Lobeglitazone concentrations.

**Table 4** Linearity results for Lobeglitazone

| Conc | Area | | | Average |
|---|---|---|---|---|
| 5 | 366606 | 365509 | 359182 | 363765.7 |
| 10 | 788207 | 791010 | 779006 | 786074.3 |
| 15 | 1231130 | 1249110 | 1199038 | 1226426 |
| 20 | 1612290 | 1792222 | 1599835 | 1668116 |
| 25 | 2064075 | 2055563 | 2100091 | 2073243 |

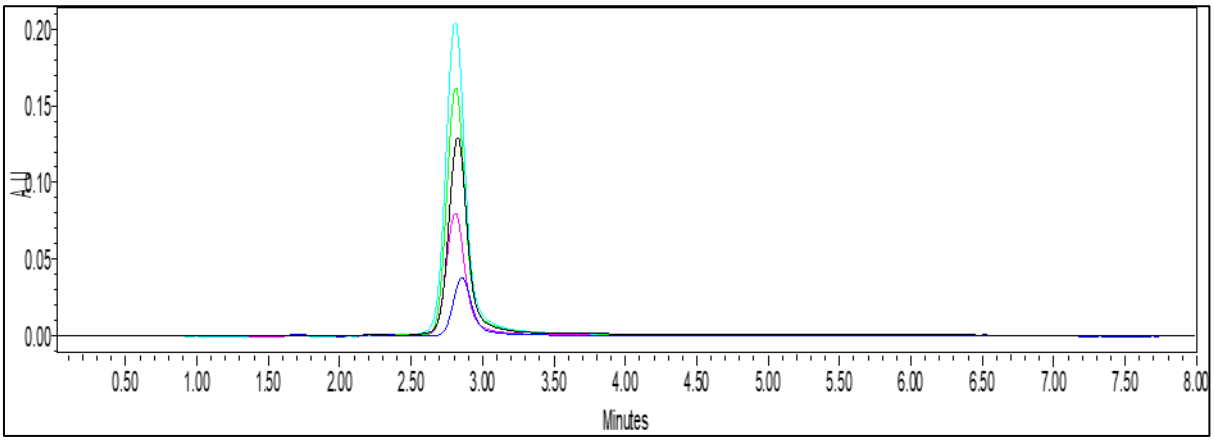


**Figure 5** Linearity graph of Lobeglitazone.

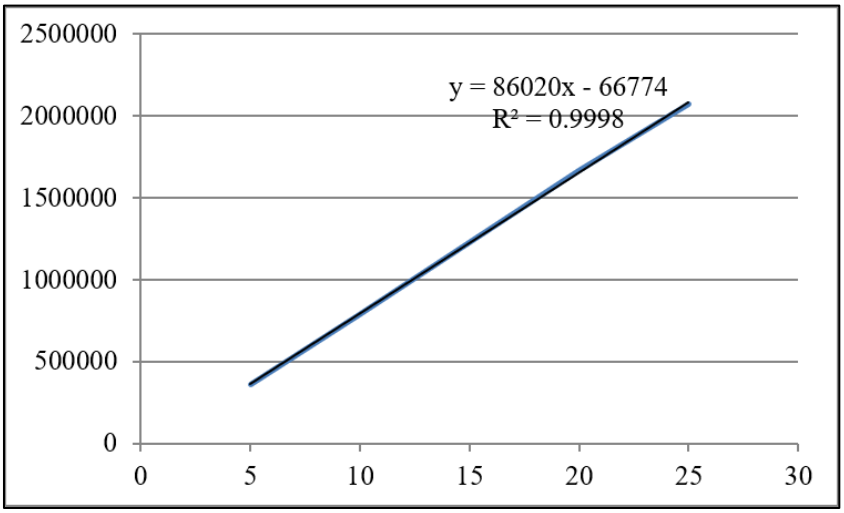


**Figure 6** Calibration curve Area vs Conc.

*3.5.4. Accuracy*

The accuracy of the method was assessed through recovery studies conducted at three different concentration levels, typically 80%, 100%, and 120% of the target concentration. The average recovery values for Lobeglitazone fell within the acceptable range of 98% to 102%. This confirms that the method is both accurate and reliable for measuring the amounts of both drugs in pharmaceutical formulations.

**Table 5** Accuracy study for Lobeglitazone

| **Lobeglitazone** | | | |
|---|---|---|---|
| **Concentration % of the spiked level** | **Amount added (ppm)** | **Amount found (ppm)** | **% Recovery** |
| 80% | 8ppm | 17.99 | 99.96 |
| | | 17.87 | 99.92 |
| | | 17.95 | 99.94 |
| 100% | 10ppm | 20.08 | 100.42 |
| | | 19.99 | 99.96 |
| | | 19.98 | 99.95 |
| 120% | 12ppm | 22.01 | 100.06 |
| | | 21.99 | 99.96 |
| | | 21.98 | 99.95 |

*3.5.5. Precision:*

The relative standard deviation (RSD) for the repeatability of the standard preparation was determined to be under 2%, demonstrating excellent reliability. This low RSD value verifies that the HPLC system produces dependable and consistent results with repeated injections of standard solutions. Consequently, the established HPLC method is appropriate and robust for the routine quantitative analysis of Lobeglitazone in various sample types (Table 7).

**Table 6** Repeatability data for Lobeglitazone

| **Sr. No** | **Lobeglitazone** | |
|---|---|---|
| | **Area** | **% Assay** |
| 1. | 806555 | 101.53 |
| 2. | 788571 | 99.44 |
| 3. | 788207 | 99.39 |
| 4. | 771822 | 97.49 |
| 5. | 780213 | 98.46 |
| 6. | 780213 | 98.46 |
| Mean | 785930.1667 | 99.129 |
| SD | 11848.663 | 1.377 |
| %RSD | 1.508 | 1.390 |

*3.5.6. Intermediate precision*

The data displaying the % RSD values are shown in Table 8 and 9.

**Table 7** Intermediated precision study for Lobeglitazone

| Sr. No | Conc. (µg/ml) | Area | mean peak area | SD(±) | %RSD |
|---|---|---|---|---|---|
| | 10 | 774146 | 773217 | 1230.086 | 0.159087 |
| 1 | | 771822 | | | |
| | | 773683 | | | |
| 2 | 15 | 12341130 | 1250529 | 22492.51 | 1.79864 |
| | | 1245272 | | | |
| | | 1275184 | | | |
| | 20 | 1643259 | 1620623 | 20976.5 | 1.294348 |
| 3 | | 1601840 | | | |
| | | 1616771 | | | |

*3.5.7. Intraday*

**Table 8** Intraday precision study for Lobeglitazone

| Sr. no. | Day | Conc. (µg/ml) | Peak Area | Mean Peak Area | SD(±) | %RSD |
|---|---|---|---|---|---|---|
| 1 | Day 1 | 10 | 774042 | 773647.3 | 555.5766 | 0.071813 |
| | Day 2 | | 773888 | | | |
| | Day 3 | | 773012 | | | |
| 2 | Day 1 | 15 | 1230111 | 1232282 | 5127.631 | 0.416109 |
| | Day 2 | | 1238138 | | | |
| | Day 3 | | 1228597 | | | |
| 3 | Day 1 | 20 | 1610759 | 1610549 | 8598.417 | 0.533881 |
| | Day 2 | | 1601848 | | | |
| | Day 3 | | 1619041 | | | |

*3.5.8. Sensitivity:*

Both Lobeglitazone demonstrated good sensitivity according to the method, as the LOD and LOQ values indicate the method's appropriateness for trace-level detection and quantification (table 11).

**Table 9** LOD and LOQ values

| Parameter | LBZ |
|---|---|
| LOD (ug/ml) | **1.2809** |
| LOQ (ug/ml) | **3.8816** |

## 4. Conclusion

A QbD-driven analytical technique was successfully developed and validated to measure Lobeglitazone using RP-HPLC. To ensure the method's robustness and dependability, a DoE approach modified key parameters, such as the mobile phase composition and flow rate. The technique met all ICH validation requirements and showed excellent linearity, accuracy, precision, specificity, and sensitivity. Its sensitivity was confirmed by the low LOD and LOQ, while accuracy and reliability were supported by recovery and repeatability studies. This technique is dependable and suitable for regular quality control analysis of pharmaceutical `dosage forms and bulk forms of Lobeglitazone.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The Authors declare no conflict of interest.

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