RESEARCH PAPER

"Analytical Method Development and Validation of Glimepiride and Lobeglitazone Sulfate by Stability Indicating RP-HPLC Method"

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Abstract

The present study involves an analytical method development and validation for Glimepiride and Lobeglitazone Sulfate by Stability Indicating RP-HPLC Method in pharmaceutical dosage form. The experimental work includes preliminary characterization of the drugs, selection of appropriate solvents, determination of solubility, and identification of an optimal analytical wavelength (244 nm). Methanol was used as the diluent, and various chromatographic trials were conducted using different mobile phases to achieve optimal resolution. The optimized chromatographic conditions consisted of an Agilent Eclipse plus id C18 (4.6×250 mm) column with a mobile phase of Methanol :0.1% citric acid (60:40 v/v), a flow rate of 1ml/min, and UV detection at 244 nm. Validation of the method was performed as per ICH guidelines, covering parameters such as system suitability, specificity, linearity, accuracy, precision, robustness, ruggedness, LOD, LOQ. The method showed acceptable accuracy, precision, and linearity within the specified range. For stability study, the drug was exposed to various stress conditions such as acid, base, oxidation and sunlight as per recommendations of ICH guidelines. Hence, the method will be useful for routine quality control and was successfully applied for the analysis of marketed formulations containing both drugs.

Keywords: Lobeglitazone, Glimepiride, RP-HPLC, Stability, ICH guidelines

How to cite this article: Khairnar R, Kadam D, Borse L. Analytical Method Development and Validation of Glimepiride and Lobeglitazone Sulfate by Stability Indicating RP-HPLC Method. Int J Drug Deliv Technol. 2026;16(71s): 35-37. DOI: 10.25258/ijddt.16.71s.5

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia, which results from either insufficient insulin production by the pancreas or the body's inability to effectively use the insulin that is produced. [1] (T2DM) is a multifaceted disease associated with insulin resistance and a lowered in insulin secretion by B-cells of pancreas, leading to an increased in glucose levels in blood and the onset of diabetes-related complications. As a progressive condition characterized by diminishing B-cell function, T2DM frequently cannot be effectively controlled with monotherapy alone, necessitating additional treatments to achieve adequate glycemic control over time.[2]

High-Performance Liquid Chromatography (HPLC) stands as the most widely used and adaptable chromatographic method. In an actual separation process during the liquid phase, which entails the transfer between the stationary phase (a solid sorbent housed within a column) and the mobile phase (a moving liquid), the sample is separated into its distinct components. [3][4]

Lobeglitazone, a newly identified thiazolidinedione, presents a more effective and enhanced alternative in comparison to existing TZDs. [5] The antidiabetic agent Lobeglitazone is classified within the thiazolidinedione class of drugs and is marketed under the brand names Duvie and Chong Kun Dang. By interacting with peroxisome proliferator-activated receptors (PPARs) located in adipose tissue, this compound improves cellular responsiveness to insulin. It functions as an agonist for both PPARα and PPARγ, thus playing a significant role in its insulin-sensitizing properties. Lobeglitazone is prescribed to patients with type 2 diabetes mellitus to assist in the regulation of blood glucose levels.[6][7][8] By enhancing insulin binding in

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fat cells, Lobeglitazone has demonstrated efficacy in lowering blood glucose levels, decreasing hemoglobin AIC (HbA1C) levels, and improving both lipid and liver profiles. The drug exhibits solubility characteristics of being insoluble in water, while being soluble in DMSO (dimethyl sulfoxide) and methanol. [9] [10] [Fig. 1] Lobeglitazone sulfate is classified as a thiazolidinedione antidiabetic medication, and its IUPAC name is 5-ethoxy) benzyl]-1, 3-4-(2-{[6-(4-Methoxyphenoxy)-4-pyrimidinylamino] thiazolidine-2, 4-dione sulfate. The molecular formula for this compound is C24H26N40952, and it has a molecular weight of 578.6 g/mol. [11]

Glimepiride, similar to glyburide and glipizide, is classified as a "second-generation" sulfonylurea agent. [Fig 2] It is utilized in conjunction with dietary measures to reduce blood glucose levels by enhancing insulin secretion from the pancreas and improving the sensitivity of peripheral tissues to insulin. The mechanism by which glimepiride lowers blood glucose levels seems to rely on the stimulation of insulin release from active pancreatic beta cells, as well as enhancing the sensitivity of peripheral tissues to insulin. Glimepiride is thought to attach to ATP-sensitive potassium channel receptors located on the surface of pancreatic cells, which decreases potassium conductance and leads to membrane depolarization. This depolarization of the membrane triggers an influx of calcium ions through voltage-sensitive calcium channels. The rise in intracellular calcium ion concentration subsequently promotes the secretion of insulin. [12] [13] Glimepiride (GLM) is classified as a sulfonylurea antidiabetic agent. In terms of its chemical structure, it is identified as 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl)-3-(trans-4-methylcyclohexyl) urea with the molecular formula C24H34N405S and have molecular mass of about 490.617 g/mol. It is administered orally, insoluble in water, slightly soluble in methanol. This medication is a third-generation. derivative of sulfonylureas, frequently prescribed for the management of non-insulin-dependent Type 2 diabetes mellitus. [14]

Successful glycemic control is important to decrease the risk of micro- and macrovascular complications. Pharmacotherapy of T2DM has evolved considerably, with a broad spectrum of oral and injectable agents that act through distinct mechanisms to reduce glucose levels in blood and address underlying pathophysiology.[15] Its etiology is multifactorial, with obesity, physical inactivity, and genetic predisposition being key contributors. T2DM is associated with an increased risk of microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (cardiovascular disease) complications, significantly impacting morbidity and mortality.[16] Novel therapeutic approaches, such as sodium-glucose co-transporter 2 (SGLT2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and incretin-based therapies, provide enhanced metabolic results along with possible cardiovascular and renal advantages.[17][18][19]

On the basis of literature review, High-performance liquid chromatography (HPLC) techniques have been established for LBG in tablet formulations while stability-indicating RP-HPLC methods have been documented for GLM in tablet forms.[10][20] Considering the rising incidence of type 2 diabetes and the necessity for combination therapy to effectively control hyperglycemia, the simultaneous administration of LBG and GLM in a unified dosage form offers a promising therapeutic approach.[21]

Forced degradation experiments are conducted to facilitate the advancement of analytical methodologies, to gain a deeper understanding of the stability of the active pharmaceutical ingredient (API) and the drug product, and to offer insights into degradation pathways and degradation products. [22]

The developed UPLC method will be validated in accordance with the International Council for Harmonization (ICH) guidelines to ensure its accuracy, precision, robustness, and linearity. This approach not only addresses the analytical challenges associated with the simultaneous estimation of glimepiride. and Lobeglitazone sulfate in the commercially available formulation LOBG-GI but also contributes to the advancement of sustainable pharmaceutical analysis.

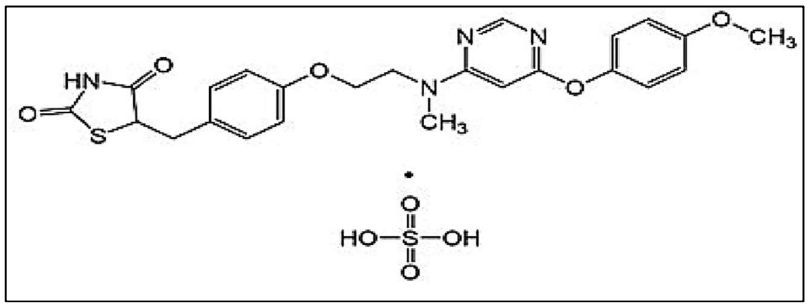

Fig.1 Structure of Lobeglitazone sulfate [22]

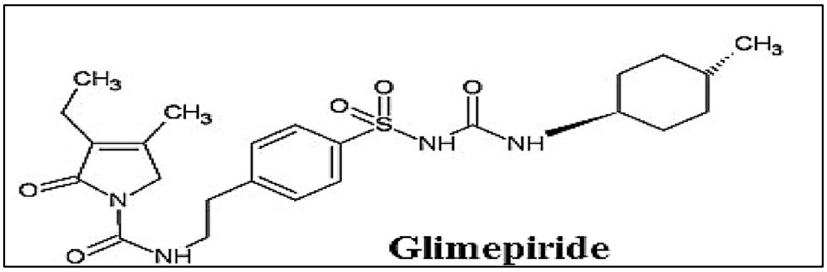

Fig.2 Structure of Glimepiride [22]

2. Materials And Methods

Materials and Reagents

Lobeglitazone sulfate and Glimepiride were obtained from Swapnaroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra, India. The tablet formulation of Lobeglitazone sulfate and glimepiride in a combined dosage form was obtained from the local pharmacy store. Also, methanol and citric acid were obtained from Merck Life Science Pvt. Ltd. Mumbai, India 400079.

Instrumentation

The instrumentation used for the present research work included a High-Performance Liquid Chromatography (HPLC) system consisting of an Agilent 1100 Gradient system. Chromatographic separation was achieved using an Agilent Eclipse Plus C18 column (4.6× 250 mm, 5 µm particle size) and equipped with a Diode Array detector. Data acquisition and analysis were performed using Agilent ChemStation software. A UV-Visible spectrophotometer (Spectro UV 2080 / UV 2080+, Double Beam UV/Vis Spectrophotometer) manufactured by Analytical Technologies Limited was also employed for analytical measurements.

Preparation of mobile phase

A 0.1% (w/v) citric acid solution was prepared by dissolving 3.202 g of citric acid in 500 mL of HPLC-grade water, and the pH was adjusted to 2.8. The mobile phase was prepared by mixing methanol and 0.1% citric acid solution in the ratio of 60:40 (v/v). The prepared mobile phase was filtered through a 0.45 µm membrane filter and sonicated for 20 minutes prior to use.

Preparation of standard Stock Solution

An accurately weighed quantity of 6 mg of glimepiride was transferred into a 10 mL volumetric flask, dissolved in methanol, and the volume was made up to the mark with methanol. The solution was sonicated for 10 minutes to ensure complete dissolution, resulting in a standard stock solution of 600 ppm.

Similarly, 3 mg of Lobeglitazone sulfate was accurately weighed and transferred into a 10 mL volumetric flask. Methanol was added, and the volume was adjusted to the mark with the same solvent. The solution was sonicated for 10 minutes to obtain a clear solution, yielding a standard stock solution of 300 ppm. Both standard stock solutions were then mixed in appropriate proportions to prepare the mixed standard stock solution.

Preparation of working standard stock solution

From the mixed standard stock solution, 0.1 mL was pipetted into a 10 mL volumetric flask and diluted to volume with the mobile phase to obtain a working standard solution containing 6 µg/mL of glimepiride and 3 µg/mL of lobeglitazone sulfate.

Preparation of Sample solution

Twenty tablets (LOBG-G1) were individually weighed, and the average tablet weight was calculated. The tablets were then finely powdered. An amount of tablet powder equivalent to 3 mg of lobeglitazone (LBG) and 6 mg of glimepiride (GLM) was accurately weighed and transferred into a 10 mL volumetric flask. Approximately 7 mL of diluent was added to the flask to dissolve the drug content and obtain a stock solution equivalent to 600 ppm of glimepiride and 300 ppm of lobeglitazone. The mixture was sonicated for 15 minutes to ensure complete extraction of the drugs, and the volume was then made up to the mark with the same diluent. The resulting solution was filtered through a 0.45 µm membrane filter.

Further, 0.1 mL of the filtrate was pipetted into a 10 mL volumetric flask and diluted to volume with the diluent to obtain a final sample solution containing (6 µg/mL) of glimepiride and (3 µg/mL) of lobeglitazone.

Method Development

To improve the resolution and efficiency of the system, a range of chromatographic conditions were investigated. These conditions involved the optimization of parameters including the composition of the mobile phase, the detection wavelength, the type of column, the temperature of the column, and the pH of the mobile phase, as informed by pertinent literature.

Lobeglitazone sulfate and glimepiride exhibit high solubility in methanol and Dimethyl sulfoxide (DMSO). The solubility of these compounds was evaluated using various dilutions of methanol and citric acid, ultimately selecting methanol as the solvent for this study. In this study, a stability-indicating RP-HPLC method was developed and validated for the simultaneous quantification of LBG and GLM in combined pharmaceutical dosage forms. The chromatographic conditions, using an Agilent C18 column with a mobile

phase of (Methanol:0.1% citric acid) (60:40 v/v), provided sharp and resolved peaks for LBG and GLM
Based on UV-visible spectrophotometric results, scan of the individual solutions Glimepiride and lobeglitazone taken separately in the range 200-400 nm using a UV Spectrophotometer. A common wavelength of 244 nm (Isosbestic point) was used for the simultaneous estimation of both drugs, as they elute in the same mobile phase at maximum absorbance.

Chromatographic Conditions

The chromatogram acquired under the specified chromatographic conditions is illustrated in (Fig.3). In accordance with ICH guidelines, the parameters for method validation that were evaluated encompass system suitability, specificity, precision, accuracy, linearity, robustness, limit of detection, limit of quantification, and ruggedness.

Table 1: Optimized Chromatographic conditions of Lobeglitazone sulfate and glimepiride

Sr. No.	Parameters	Specification
1	Detector	U.V. Detector
2	Column	Agilent C18 (4.6×250 mm)
3	Column Dimension	(4.6×250 mm)
4	Temperature	25 °C
5	Injection Volume	20 µl
6	Wavelength	244nm
7	Mobile Phase	Methanol :0.1% citric acid (60:40 v/v)
8	Flow Rate	1 ml/min
9	Run Time	10 Minutes

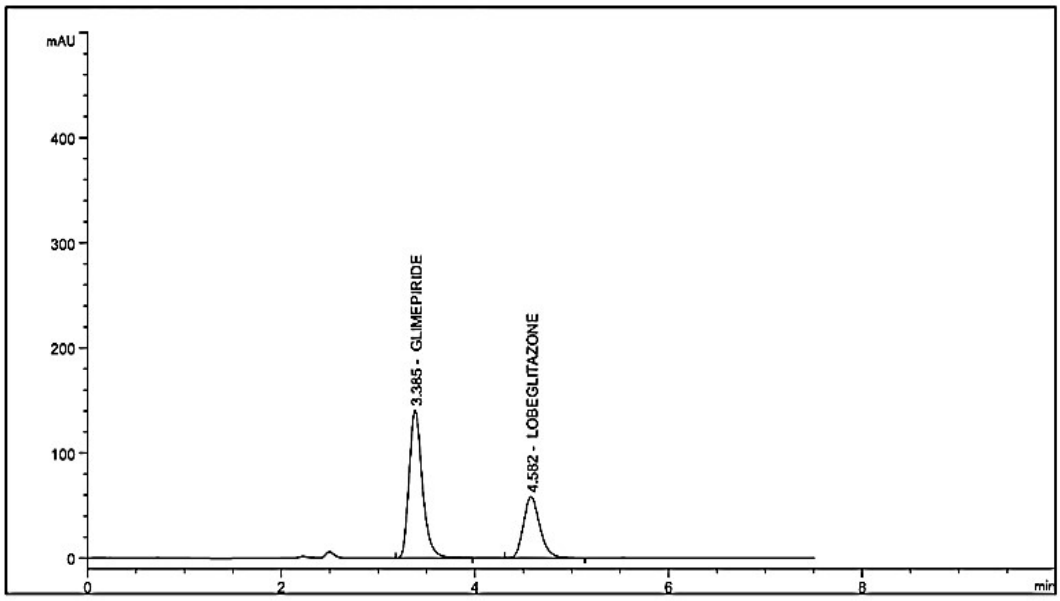

Fig. 3: optimized chromatogram of Lobeglitazone sulfate and glimepiride

Method Validation

The proposed method was validated according to ICH guidelines Q2 (R1).

1. System suitability

The tests were performed by collecting data from five replicate injection of standard drug solution containing LBG (12mu*g / m * L) and GLM (24mu*g / m * L) Method parameters namely mean area, number of theoretical plates, %RSD (Relative Standard Deviation) of retention times, tailing factor were checked.

2. Specificity

A solution containing Blank (mobile phase as a diluent) and standard solution should be injected in order to illustrate the specificity of the entire process. The ability of an analytical method to measure the analyte (LBG and GLM) accurately in the presence of other components such as impurities, degradation products, excipients, or matrix components. Chromatograms were evaluated to

ensure that the peaks of degradation products or impurities did not interfere with the peaks of the analytes.

3. Linearity

Linearity was assessed by injecting standard solutions containing LBG (3 - 15mu*g / m * L) and GLM (6 - 30mu*g / m * L) 2 times. A calibration curve was obtained by plotting the average peak area vs. concentration and the regression coefficients (R') for both drugs were calculated to determine the linear relationship between peak area and concentration.

4. Accuracy

The accuracy of the method was determined by performing recovery studies at three concentration levels (80%, 100%, and 120%) of the test concentration. Known amounts of LBG and GLM (4.8, 6, 7.2mu*g / m * L and 9.6, 12, 14.4mu*g / m * L_{1} respectively). The solutions were analysed in triplicate and mean % recovery and % RSD were determined for each concentration level.

5. Precision

Precision of method was evaluated by determining both intra-day precision and inter-day precision. For intraday, six injections of sample solution containing LBG (6, 9, 12 µg/mL) and GLM (12, 18, 24 µg/mL) were analyzed on the same day under identical conditions. For inter-day precision, the analysis was performed on 2 different days. The results were expressed as the percentage relative standard deviation (% RSD).

6. Limit of Detection and Limit of Quantification (LOD and LOQ)

As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and LOQ by using following formula:

LOD 3.3x avg SD / Slope LOQ 10 avg SD / Slope

7. Robustness

Robustness was evaluated by deliberately varying certain chromatographic conditions, such as the wavelength (±1nm) and the mobile phase composition (±1% v/v). The solution containing LBG (9 µg/mL) and GLM (18 µg/mL) was injected. The method was considered robust if the results remained within acceptable limits under these altered conditions, with % RSD values below 2%.

8. Ruggedness

The ruggedness of the proposed analytical method was evaluated by analyzing replicate samples under varied conditions using two different analysts. The results obtained for peak area, amount found, and percentage label claim were compared. For GLM, Analyst I and Analyst II showed % label claim values, and % RSD values.

3. Forced Degradation Studies

Degradation conditions

1. Acid Hydrolysis
2. Base Hydrolysis
3. Neutral
4. Oxidative Degradation
5. Photolytic Degradation

Preparation of Reagent:

a. 1 N HCI Solution: 0.85 ml of concentrated hydrochloric acid was measured into a 100 ml volumetric flask, and the volume was adjusted to the mark with water, followed by thorough mixing.

b. 1 N NaOH Solution: 0.4 g of sodium hydroxide pellets were placed in a 100 ml. volumetric flask, and the volume was adjusted to the mark with water, ensuring proper mixing.

c. 3% H202 Solution: 3 ml of the 30% hydrogen peroxide solution was added to a 100 ml volumetric flask, and the volume was brought up to the mark with water, followed by adequate mixing.

Acid hydrolysis: Sample Preparation

0.1 N HCL Take 0.5 ml Sample from Stock (API) add 0.5 ml 0.1N HCL and Make up volume with Diluent after 30, 60 min,24 hr. takes HPLC reading (Before injecting neutral sample).

Base hydrolysis: Sample Preparation

0.1 N NaOH Take 0.5 ml Sample from Stock (API) add 0.5 ml 0.IN HCL and Make up volume with Diluent after 60, 120 min take HPLC reading (Before injecting neutral sample).

Neutral degradation: Sample Preparation

Take 0.5 ml Sample from Stock (API) add 0.5 ml Water and Make up volume with Diluent after 60, 120 min take HPLC reading

Oxidative degradation: Sample Preparation

3% H2O2 Take 0.5 ml Sample from Stock (API)add 0.5 ml 0.1N HCL and Make up volume with Diluent after 60, 120 min take HPLC reading (Before inject neutral sample.

Photolytic degradation:

Sample Preparation

Pipette 0.5 mL of stock solution into a 10 mL volumetric flask. Dilute with methanol or mobile phase. Transfer the solution into a clear glass container or Petri dish. Expose to UV light (254 nm or 365 nm) or direct sunlight for 24 hours. After exposure, transfer the solution back to a 10 mL volumetric flask if needed. Make up the volume with mobile phase. Inject the prepared sample into RP -HPLC and record the chromatogram to observe degradation peaks.

4. Results And Discussion

1. Selection of Wavelength

Lobeglitazone and Glimepiride standard solution was scanned from 400 nm to 200 nm. Absorption maxima were determined for both drugs. Lobeglitazone and Glimepiride shows isosbestic point at 244 nm.

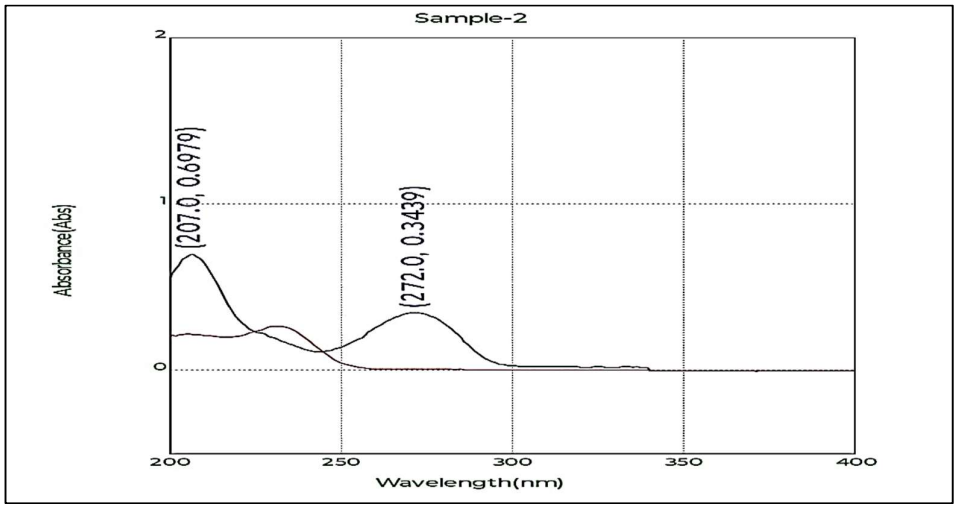

Fig. 4: UV Spectrum of Glimepiride and Lobeglitazone Sulfate (Isosbestic Point)

2. Validation of HPLC

System suitability: Systern suitability tests verify that the chromatographic system is adequate for analysis before or during sample analysis (Fig 4). The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable. The results are illustrated in Table 2.

Table 2: Result of System Suitability Test of Glimepiride and Lobeglitazone

Sr. No.	Standard Solution	Area of Glimepiride (24 µg/mL)	Area of Lobeglitazone
1	Standard_1	2734.248	1305.327
2	Standard_2	2766.574	1304.897
3	Standard_3	2736.8	1305.221
4	Standard_4	2713.1	1305.107
5	Standard_5	2676.73	1283.00
Mean		2750.41	1305.11
Theoretical plates		3088	3601
Amt. found		23.67	11.97
% Amt. found		98.61%	99.74%
SD		22.86	0.30
%RSD		0.83	0.02

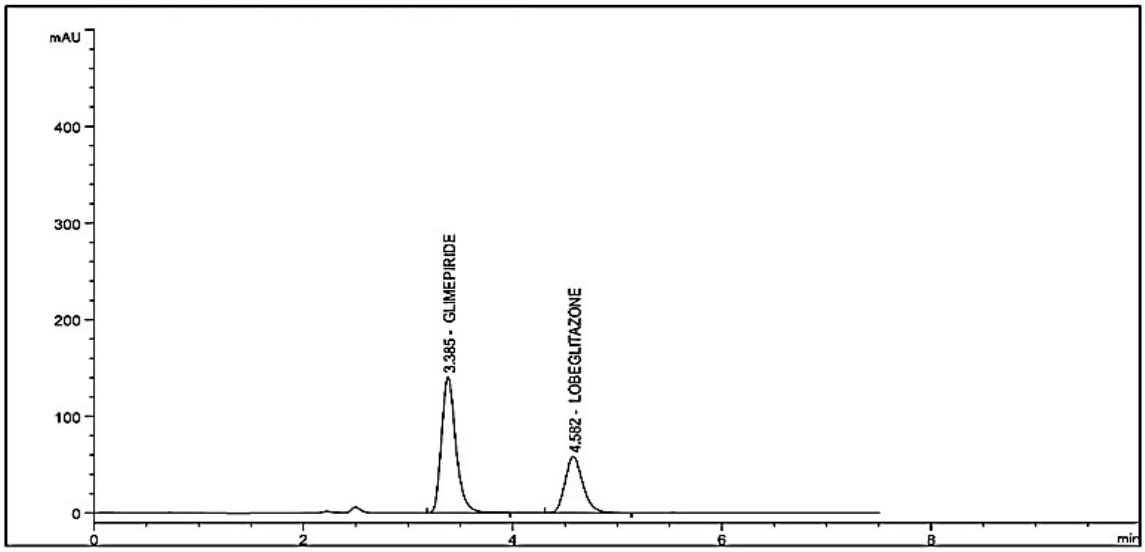

Fig. 4: Typical Chromatogram of System Suitability Test of GLM and LBG

Specificity: A solution containing Blank (mobile phase as a diluent) and standard solution should be injected in order to illustrate the specificity of the entire process. Data demonstrate that, no interference from blank, impurities, or degradation products at the retention time of the analyte. The analyte peak should be well resolved and pure. The data obtained is summarized in Table 3.

Table 3: Results of Specificity

Description	Observation
Blank	No Interference at R.T. of Glimepiride and Lobeglitazone due to Blank
Standard solution	Glimepiride R.T = 3.414 Lobeglitazone R.T = 4.630

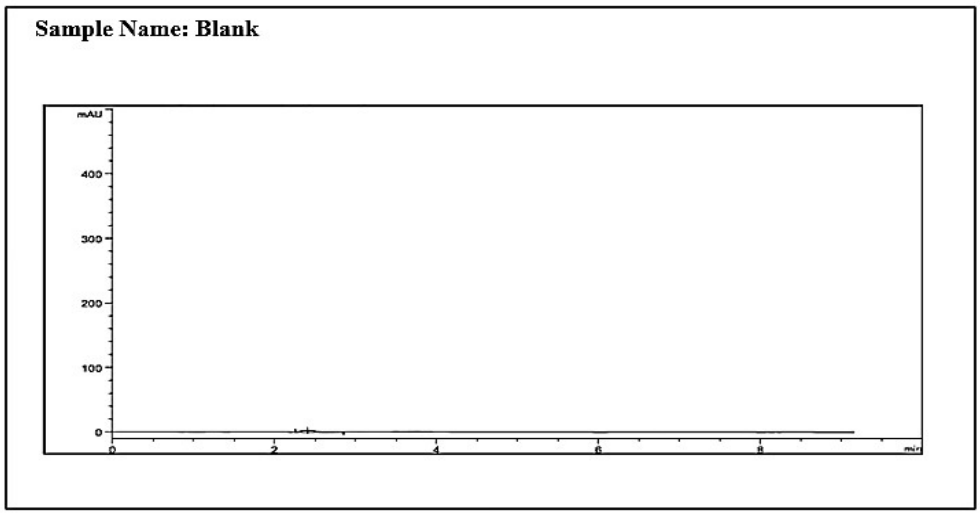

Fig. 5: Typical Chromatogram of Blank Solution

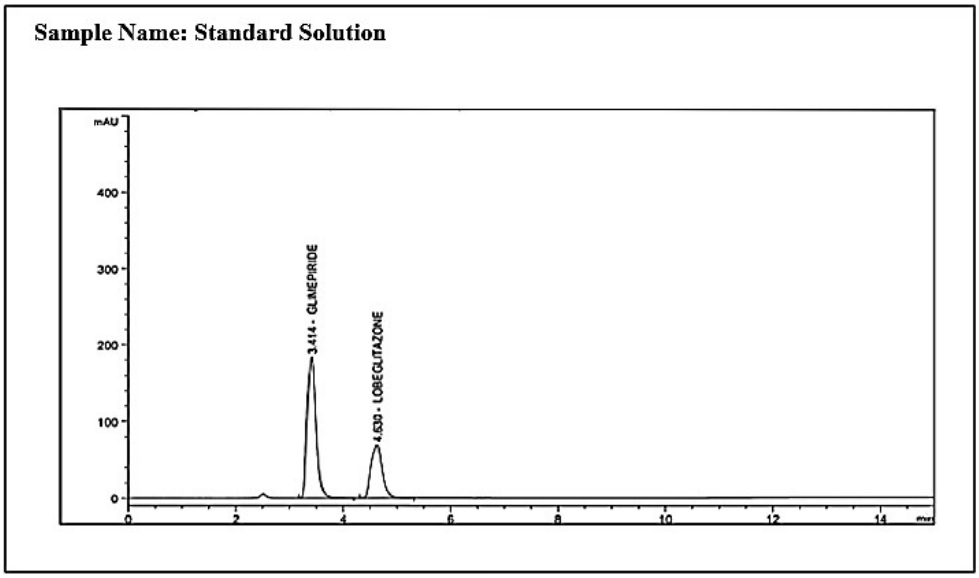

Fig. 6: Typical Chromatogram of Standard Solution

Linearity: Linearity was assessed by injecting standard solutions containing LBG (3-15 µg/mL) and GLM (6-30 µg/mL) 2 times. A calibration curve was obtained by plotting the average peak area vs. concentration Fig.7 and Fig.8. The correlation coefficients obtained were 0.9998 for lobeglitazone sulfate and 0.9998 for glimepiride, both indicating excellent linearity. The data is given in Table 4.

Table 4: Results Linearity for Glimepiride and Lobeglitazone sulfate

Sr. No.	Concentration of GLM	Mean Peak Area of GLM± S.D	% RSD	Concentration of LBG	Mean peak area of LBG±S.D	% RSD
1	6	745..58 ± 6.79	0.91	3	343..85 ± 5.08	1.48
2	12	143.8.18 ± 0.32	0.02	6	674..76 ± 0.89	0.13
3	18	212.4.04 ± 3.04	0.14	9	985..61 ± 5.65	0.57
4	24	276.6.03 ± 0.20	0.01	12	130.6.03 ± 4.15	0.32
5	30	347.5.18 ± 0.89	0.03	15	164.2.17 ± 0.25	0.01

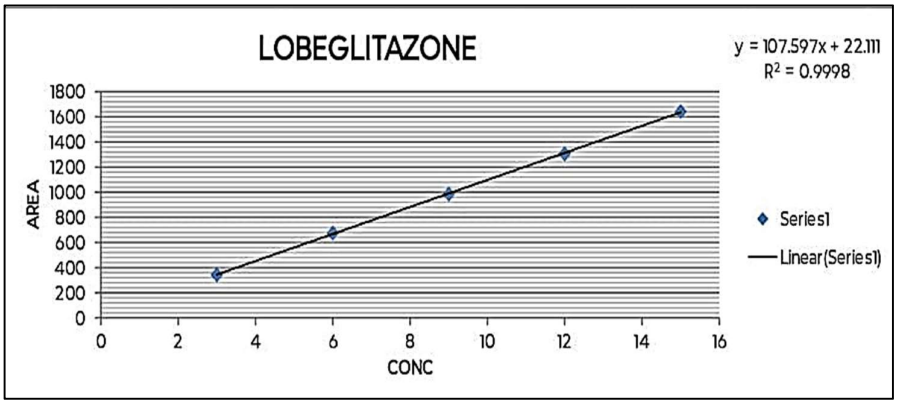

Fig. 7: Calibration Curve of Lobeglitazone

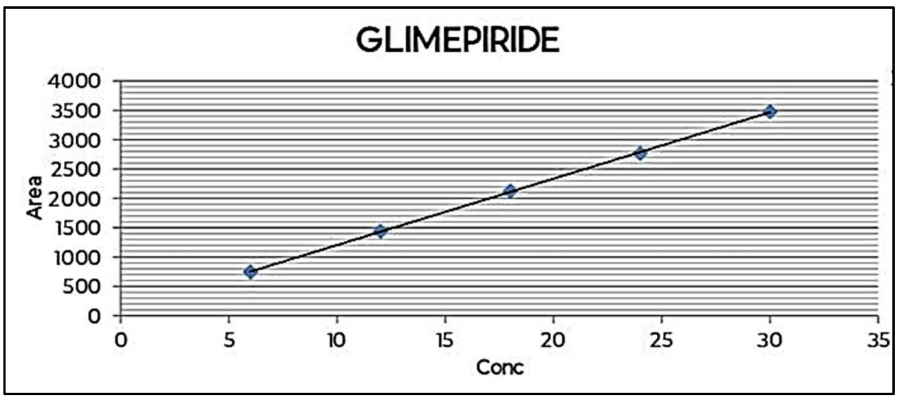

Fig. 8: Calibration Curve of Glimepiride

Accuracy: The percent recovery of Lobeglitazone Sulfate ranged from 98.46% to 100.74%, while for Glimepiride samples, it ranged from 98.96% to 99.44%. These findings indicate the good accuracy of the method. Detailed results are provided in Table 5.

Table 5: Accuracy data of Lobeglitazone sulfate and glimepiride

%Level	Amount Added µg/ml		Amt. found	Mean % Recovery
Glimepiride				
80% Level	1	9.6	21.49	98.96
	2	9.6	21.51	
100% Level	1	12	24.03	100.29
	2	12	24.02	
120% Level	1	14.4	26.34	99.44
	2	14.4	26.28	
Lobeglitazone sulfate				
80% Level	1	4.8	10.74	98.46
	2	4.8	10.71	
100% Level	1	6	11.99	99.60
	2	6	11.96	
120% Level	1	7.2	13.27	100.74
	2	7.2	13.22	

Precision: The precision study showed low %RSD values for both GLM and LBG in intraday and interday analysis, all within acceptable limits. This indicates that the developed method is precise, repeatable, and reliable for simultaneous estimation of the drugs. The outcomes are depicted in Table 6

Table 6: Precision results for glimepiride and lobeglitazone

Drug	Concentration µg/ml	Intraday % RSD	Interday % RSD
GLM	12	0.05	3.14
	18	0.27	0.42
	24	0.16	0.02
LBG	6	0.00	0.78
	9	0.19	0.61
	12	0.38	0.12

Limit of Detection and Limit of Quantification (LOD and LOQ): The results. indicated that the Limit of Detection (LOD) for glimepiride was 0.0656 µg/mL, while for Lobeglitazone Sulfate, it was 0.0986 µg/mL. Furthermore, it was determined that the Limit of Quantification (LOQ) for glimepiride was 0.1989µg/mL and for Lobeglitazone Sulfate was 0.2988 µg/mL.

Robustness: The robustness of the method was evaluated by deliberate variation in mobile phase composition (±1%) and detection wavelength (±1 nm). The %RSD values obtained under all modified conditions were found to be less than 2%, indicating that the method is robust and not significantly affected by small changes in chromatographic parameters.

Table 7: Robustness results for glimepiride and lobeglitazone sulfate

Drug	Parameter	Condition	Concentration µg/ml	Injection 1 Area	Injection 2 Area	% RSD
GLM	Mobile phase composition	39:61 (MeOH)	18	2150	2150	0.04
	Mobile phase composition	41:59 (MeOH)	18	2140	2140	0.01
	Wavelength	243nm	18	2160	2160	0.16
	Wavelength	245nm	18	3020	3020	0.01
LBG	Mobile phase composition	39:61 (MeOH)	9	1029.43	1025.05	0.30
	Mobile phase composition	41:59 (MeOH)	9	1024.64	1024.18	0.03
	Wavelength	243nm	9	1008.53	1033.20	1.71
	Wavelength	245nm	9	314.00	317.44	0.77

Ruggedness: The ruggedness of the proposed analytical method was evaluated by analyzing replicate samples under varied conditions. The %RSD of peak area, amount found, and % label claim was found to be well within acceptable limits, indicating that the method is rugged. Detailed results are provided in Table 8.

Table 8: Result and Statistical Data of Ruggedness

Drug	Analyst	Peak area	Amt. found (mg)	% Label claim	% RSD
GLM	Analyst I	2125.366	18.140	100.78	0.016
	Analyst II	2125.845	18.144	100.80	
LBG	Analyst I	982.232	8.956	99.52	0.085
	Analyst II	983.381	8.967	99.63	

3. Forced Degradation Studies

The forced degradation study of glimepiride and lobeglitazone was carried out under acidic (0.1N HCI), alkaline (0.1N NaOH), oxidative (3% H_2O_2), neutral, and photolytic (UV and visible light) conditions for different time intervals. Both drugs showed gradual degradation with increase in exposure time under acid, base, and oxidative conditions, while comparatively lower degradation was observed under neutral and photolytic conditions. Glimepiride exhibited slightly higher degradation compared to lobeglitazone under most stress conditions. The method successfully separated the drug peaks from their degradation products, confirming that the developed RP-HPLC method is stability-indicating and suitable for routine stability analysis.

Table 9: Results Forced Degradation Studies

Sr. No	Degradation	Duration	Solution	Area of Standard	Area of Degraded Sample	Degraded up to %	Actual % Degradation
1	Acid Degradation, (0.1N HCL)	1hr	Glimepiride	2121.88	1983.71	93.49	6.51
			Lobeglitazone	989.601	973.28	98.35	1.65
		2hr	Glimepiride	2121.88	1977.79	93.21	6.79
			Lobeglitazone	989.601	951.68	96.17	3.83
		24hr	Glimepiride	2121.88	1917.74	90.38	9.62
			Lobeglitazone	989.601	919.42	92.91	7.09
2	Base Degradation, (0.1N NaOH)	1hr	Glimepiride	2121.88	1979.48	93.29	6.71
			Lobeglitazone	989.601	953.62	96.36	3.64
		2hr	Glimepiride	2121.88	1976.52	93.15	6.85
			Lobeglitazone	989.601	950.30	96.03	3.97

		24hr	Glimepiride	2121.88	1959.70	92.36	7.64
			Lobeglitazone	989.601	922.53	93.22	6.78
3	Oxidative Degradation, (3% H_2O_2)	1hr	Glimepiride	2121.88	1991.78	93.87	6.13
			Lobeglitazone	989.601	975.51	98.58	1.42
		2hr	Glimepiride	2121.88	1973.59	93.01	6.99
			Lobeglitazone	989.601	944.95	95.49	4.51
		24hr	Glimepiride	2121.88	1931.08	91.01	8.99
			Lobeglitazone	989.601	917.17	92.68	7.32
4	Neutral	24hr	Glimepiride	2121.88	1926.185	90.78	9.22
			Lobeglitazone	989.601	921.61	93.13	6.87
5	Photo Degradation, (UV and Visible light)	24hr	Glimepiride	2121.88	2083.814	98.21	1.79
			Lobeglitazone	989.601	973.30	98.35	1.65

5. Conclusion

The developed RP-HPLC method for the simultaneous estimation of glimepiride and lobeglitazone was successfully developed and validated as per ICH guidelines. The method was found to be simple, precise, accurate, robust, and specific. Linearity was observed within the selected concentration range, and the RSD values for precision studies were within acceptable limits. The robustness study demonstrated that small deliberate variations inchromatographic conditions did not significantly affect the results. Forced degradation studies confirmed that the method is stability-indicating, as it effectively separated the drugs from their degradation products. Therefore, the proposed method is suitable for routine quality control analysis of glimepiride and lobeglitazone in pharmaceutical dosage forms.

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