RESEARCH ARTICLE

Method Development and Validation for the Simultaneous Estimation of Lobeglitazone Sulphate and Glimepiride by HPLC Method

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ABSTRACT

A simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of lobeglitazone sulfate and glimepiride in bulk and tablet dosage forms. The method involved selecting various chromatographic parameters. Specifically, a new method was developed using a 250 mm × 4.6 mm (HSS) reverse-phase C18 column with 5 µm particle size (Shim-pack C18, 250 × 4.6 mm; 5 µm). The mobile phase consisted of 40 volumes of phosphate buffer (pH 3) and 60 volumes of acetonitrile, with methanol as the diluent, running as an isocratic elution. The flow rate was set at 1.0 mL/min, and UV detection was performed at 254 nm. The injection volume was 2 µL, and the total runtime was 10 minutes. The recoveries for lobeglitazone sulfate were found to be in the range of 101% to 100.6%, while those for glimepiride were in the range of 101.5% to 100%. The method's accuracy is evident from these results. The inter-day and intra-day precision of the new method were both below the maximum allowable limit (RSD% ≤ 2.0), as per International Council on Harmonisation (Q2 R1) guidelines. The method exhibited a linear response, with correlation coefficient (r2) values of 0.9972% for pioglitazone and 0.998 for glimepiride. The validation parameters, such as accuracy, precision, linearity, specificity, stability in the analytical solution and robustness, met the acceptance criteria. Hence, this method can be effectively used for the simultaneous estimation of lobeglitazone sulfate and glimepiride in both bulk and pharmaceutical dosage forms.

Keywords: Reverse phase high-performance liquid chromatography, Lobeglitazone sulphate, Glimepiride, Method development, Analytical method validation.

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INTRODUCTION

Lobeglitazone is a thiazolidinedione (TZD) developed by Chong Kun Dang Pharmaceutical Corporation in Seoul, Korea. It was designed to provide an efficient and secure TZD option for the treatment of type 2 diabetes mellitus (T2DM). The development program for lobeglitazone commenced in May 2000, and it received approval from the Ministry of Food and Drug Safety in Korea in July 2013 for the treatment of T2DM.[1]

Lobeglitazone sulfate belongs to the thiazolidinedione class of antidiabetic medications and IUPAC name is 5-4-(2-{[6-(4-Methoxyphenoxy)-4-pyrimidinylamino} ethoxy) benzyl]-1, 3-thiazolidine-2, 4-dione sulfate, as depicted in Figure 1. The empirical formula for lobeglitazone sulfate is $C_{24}H_{26}N_4O_9S_2$, with a molecular weight of 578.6 g/mol.[2]

The primary role of lobeglitazone involves enhancing insulin sensitivity by activating peroxisome proliferator-activated receptors (PPAR) gamma located within adipocytes. This activation facilitates insulin binding in adipose tissue, resulting in demonstrated reductions in blood glucose levels, decreased hemoglobin A1C (HbA1C) levels, and improvements in lipid and hepatic profiles. Unlike pioglitazone, which acts as a dual PPAR agonist targeting both PPAR-alpha and PPAR-gamma, lobeglitazone functions solely as a PPAR-alpha agonist.[3]Lobeglitazone demonstrates good glycaemic efficacy at a lower effective dose, along with favorable safety results. Additionally, it exhibits pleiotropic effects in both preclinical and clinical studies.[4] T2DM is characterized by insulin resistance and dysfunction of β-cells. Among oral antidiabetic medications, thiazolidinediones (TZDs) uniquely target insulin resistance by activating peroxisome proliferator-activated receptor γ, thereby enhancing insulin sensitivity.[5]

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proliferator-activated receptor γ, thereby enhancing insulin sensitivity. Glimepiride (GLI) is a sulfonylurea antidiabetic agent. Chemically, it is 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea. It is a third-generation sulfonylurea derivative commonly used in the treatment of non-insulin-dependent Type 2 diabetes mellitus.[6,7] It is an oral antidiabetic drug with prolonged effects, maintaining a more physiological regulation of insulin secretion. It acts as a secretagogue and has medium to long duration of action.[8]

It is used to lower blood glucose levels, and primarily works by stimulating insulin release from the pancreatic cells. It achieves this by binding to ATP-sensitive potassium channel receptors on the cell surface. This binding reduces potassium conductance, leading to membrane depolarization. As a result, voltage-sensitive calcium channels open, allowing an influx of calcium ions. The increased intracellular calcium concentration triggers insulin secretion. It can be used alongside metformin, thiazolidinedione, insulin, and alpha-glucosidase inhibitors for treating type 2 (non-insulin-dependent) diabetes mellitus. When taken orally, it is fully absorbed from the gastrointestinal tract. Possible side effects include severe hypoglycemic reactions, which can lead to coma, seizures, or other neurological impairments. The other reported side effects of sulfonylureas include clolestatic jaundice, nausea and vomiting, aplastic and hemolytic anemias, agranulocytosis, generalized hypersensitivity reactions, and rashes.[9,10] The molecular formula of glimepiride is $C_{24}H_{34}N_40_5S$ with a molecular mass of about 490.617 g/mol.[11]It is administered orally, insoluble in water, slightly soluble in methylene chloride(Dichloromethane), very slightly soluble in methanol and soluble in DMSO.[10]It can be taken alone or in combination with atorvastatin, metformin, rosiglitazone, and pioglitazone.

The development and validation of analytical techniques for estimating Glimepiride and Lobeglitazone sulfate have been thoroughly reviewed, forming the primary focus of the literature review. Extensive method development and validation efforts have been undertaken for glimepiride due to its diverse therapeutic potential. UV-visible spectrophotometric and RP-HPLC estimation methods have been reported in formulations, with RP-HPLC being commonly employed.[12] Various mobile phase compositions and column types have been tested to achieve optimal separation and quantification. Validation studies covering accuracy, precision, linearity, and robustness have demonstrated the reliability of these methods.[13]

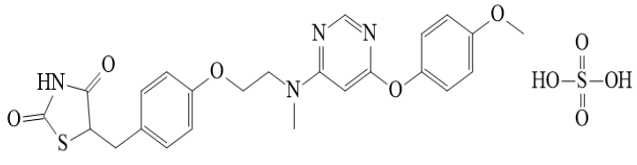

Figure 1: Structure of lobeglitazone sulfate

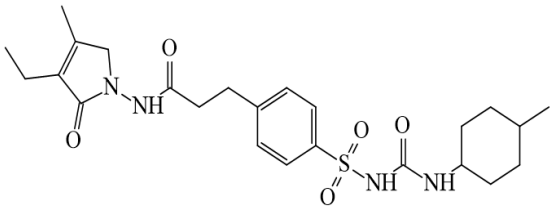

Figure 2: Structure of glimepiride

MATERIALS AND METHODS

Materials and Reagents

Lobeglitazone sulfate and glimepiride were obtained as a gift sample from Akums Drugs and Pharmaceuticals Ltd., located in Haridwar, Uttarakhand, India. Lobeglitazone sulfate and glimepiride tablet formulation in combined dosage form were procured from the local drug store. Analytical grade Phosphate buffer, Potassium dihydrogen orthophosphate, trimethylamine and orthophosphoric acid were used. The solvents were methanol, acetonitrile and Water of HPLC grade obtained from Avantor Performance Materials India Pvt. Ltd.

Instrumentation

Spectrophotometric method development was performed on Shimadzu model (UV 1900i) used for the selection of detection wavelength with UV Probe 2.71 software. An infrared spectroscopy study of a standard drug was carried out on Bruker Optics. The analysis was performed by using the HPLC system, specifically the Shimadzu (i-series) HPLC model containing the LC-2050 pump and equipped with a Photo Diode Array detector, automatic sample injector and column thermostat.

Preparation of buffer (pH 3.0) and Mobile phase

Buffer was prepared by dissolving 0.68 g of potassium dihydrogen orthophosphate in 500 mL of water and add 1-mL of trimethylamine, adjusting the pH 3.0 using 1% o-phosphoric acid solution, then filtering followed by the degassing of the solution

HPLC grade Acetonitrile and buffer solution (pH 3.0) was taken in the ratio 60:40 (v/v). The mobile phase was filtered with 0.45 µ membrane filter and was sonicated for 20 minutes.

Preparation of standard stock solution

Accurately weighed 100 mg of lobeglitazone sulfate was transferred into a calibrated volumetric flask and dissolved using 100 mL of methanol to achieve a stock solution of 1000 µg/mL. (Stock I) 5 mL of solution was taken from stock I in a calibrated volumetric flask and volume was made up to 100 mL with methanol to achieve a solution of 50 µg/mL (Stock II).

Accurately weighed 100 mg of glimepiride was transferred into a calibrated volumetric flask and dissolved using 100 mL of methanol to achieve a stock solution of 1000 µg/mL. (Stock I)10 mL of solution was taken from stock I in a calibrated volumetric flask and volume was made up to 100 mL with methanol to achieve a solution of 100 µg/mL. (Stock II)

Accurately measured 5 mL of lobeglitazone sulfate and 10 mL of glimepiride was taken and transferred it to a 100 mL volumetric flask. The drug was dissolved in methanol to obtain a solution with a concentration of 50 and 100 µg/mL, respectively. The above prepared solution of lobeglitazone sulfate and glimepiride were used as the standard stock solution.

Preparation of working standard stock solution

One mL of lobeglitazone sulfate and glimepiride working standards were accurately measured and transferred from 50 and 100 ppm solution, respectively into a 10 mL volumetric flask and dissolved in methanol and made up to the volume with the same solvent to produce 10 µg/mL of lobeglitazone sulfate and glimepiride mixture.

Sample solution preparation

Twenty tablets were weighed, made into fine powder in a mortar with a pestle and the average weight was taken. Accurately weighed powder equivalent to the average weight (0.267 mg) of each tablet were taken in a 100 mL volumetric flask and methanol was added and sonicated for 30 minutes to dissolve it completely and makeup to the mark with same diluent, and then the solution was filtered through a 0.45 µm membrane filter. The final volume was adjusted with mobile phase to get the sample solution. (100 µg/mL)

Method Development

In order to enhance system resolution and efficiency, various chromatographic conditions were explored. These conditions included optimizing parameters such as mobile phase composition, detection wavelength, column type, column temperature, and mobile phase pH, based on relevant 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 acetonitrile, ultimately selecting methanol as the solvent for this study. Based on UV-visible spectrophotometric results, a detection wavelength of 247 nm was chosen for Lobeglitazone sulfate, while 226 nm was selected for glimepiride due to their maximum absorbance at these wavelengths. Additionally, a common wavelength of 230 nm was used for the simultaneous estimation of both drugs, as they elute in the same mobile phase at maximum absorbance. Furthermore, a chromatogram was observed at 254 nm using a PDA detector.

Chromatographic Conditions

The chromatogram obtained under chromatographic conditions is depicted in Figure 3.

According to ICH guidelines, the method validation parameters assessed include system suitability, specificity, precision, accuracy, linearity, robustness, limit of detection, and limit of quantification.

Method Validation

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

Table 1: Optimized Chromatographic conditions of Lobeglitazone sulfate and glimepiride

Parameters	*Method*
Stationary phase (column)	shim-pack 5 µm C_{18} column (4.6×250 nm)
Mobile Phase	Acetonitrile: Phosphate Buffer pH 3.0 (70:30%v/v)
Flow rate (mL/min)	1.0
Run time (minutes)	10
Column temperature (°C)	40
Volume of injection loop (µL)	2

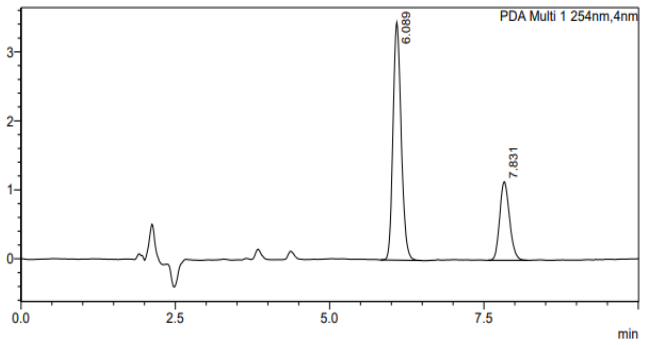

Figure 3: optimized chromatogram of Lobeglitazone sulfate and glimepiride

Linearity and Range

The linearity of the detector response was established by plotting a graph of concentration versus area for lobeglitazone sulfate and glimepiride standards and determining the correlation coefficient. A series of solutions were prepared, with lobeglitazone sulfate concentrations ranging from 10 to 50 µg/mL and glimepiride concentrations ranging from 20 to 100 µg/mL, respectively. These solutions were then injected into the HPLC system for analysis.

System Suitability

Standard solutions of lobeglitazone sulfate and glimepiride were prepared following the procedure and injected six times into the HPLC system. System suitability parameters were assessed from the standard chromatograms by calculating the %RSD (Relative Standard Deviation) of retention times, tailing factor, theoretical plates, and peak areas based on five replicate injections.

Precision

The system precision of the test method was evaluated by injecting three replicate determinations (n = 3) of the test sample against a qualified reference standard. The %RSD was calculated for both interday and intraday precision, with the %RSD not exceeding 2%.

Accuracy

Accuracy was assessed using tablet samples with known concentrations of the drugs (50, 100, and 150%). Each

concentration was injected six times, and the assay was conducted according to the developed method. From this, % recovery and the amount present (or) recovery were calculated. The outcomes of the recovery investigation are presented in Table 6.

Limit of Detection (LoD)

The limit of detection (LoD) for lobeglitazone sulfate was calculated using the following equation.

$$LoD = 3.3 \times \sigma/S$$

This equation is often used for methods employing a calibration curve approach, such as in chromatography or spectroscopy.

Where, σ= Standard deviation of the Response of Y-intercepts

S= Slope of the Calibration Curve

Limit of Quantification (LoQ)

The formula for calculating the limit of quantification (LoQ) is similar to that of the limit of detection (LoD). It's commonly expressed as:

$$LoQ = 10x\sigma/S$$

Where, S= slope of the Calibration Curve
σ= Standard deviation of the Response Y-intercepts[15]

Robustness

To evaluate the method's robustness, experimental conditions such as the mobile phase composition, pH, and flow rate were deliberately modified, and the resulting chromatographic parameters were assessed. Table 2 outlines the specific changes made to these conditions.

RESULTS AND DISCUSSION

Solubility

Solubility

Lobeglitazone sulfate exhibits solubility in methanol and DMSO, with limited solubility in acetone. On the other hand, glimepiride demonstrates very slight solubility in methanol, solubility in DMSO, and insolubility in acetonitrile. Both compounds share solubility in methanol.

Ir Identification

Fourier transform infrared (FTIR) study

- *lobeglitazone sulfate*

The FTIR absorption spectrum of sample obtained for lobeglitazone sulfate. By the interpretation of the spectra Peak at 1646 cm^{-1}attribution to C=H stretching vibration, peak at 3000 cm^{-1} was assigned to C-S stretching, peak at 1214 $cm^{-1}$was assigned C-N, Stretching and peak at 1152 $cm^{-1}$was assigned S=O Stretching which conform the structure of lobeglitazone sulfate.

- *Glimepiride*

By the interpretation of the spectra Peak at 3368 cm^{-1} attribution to N-H stretching vibration, peak at 1671 $cm^{-1}$was assigned to C=O stretching, peak at 1540 $cm^{-1}$was assigned C=C Stretching, peak at 3006 cm^{-1} was assigned C-H, peak at 1273 cm^{-1} was assigned S=O, peak at 1152 cm^{-1} was assigned C-N Stretching which conform the structure of glimepiride

Selection Of Wavelength (Λ Max)

Lobeglitazone sulfate

For the selection of analytical wavelength range for method 10 µg/mL lobeglitazone sulfate was scanned in the spectrum mode from 200 to 400 nm against methanol as blank. From the above scan selected wavelength maxima was 247 nm.

Glimepiride

For the selection of analytical wavelength range for method 10 µg/mL metformin was scanned in the spectrum mode from 200 to 400 nm against methanol as blank. From the above scan selected wavelength maxima was 226 nm.

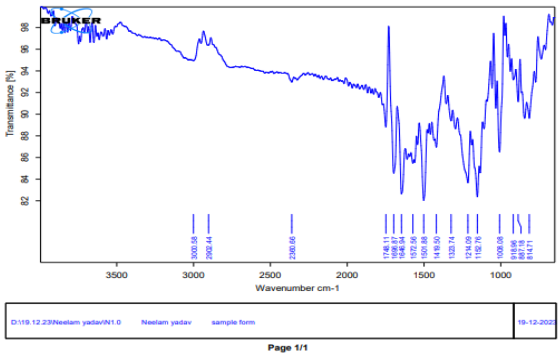

Figure 4: FTIR of lobeglitazone sulfate

Table 2: Results of solubility for lobeglitazone sulfate and glimepiride

Characteristics	*Lobeglitazone sulphate*		*Glimepiride*	
Appearance	Solid white powder		White powder	
Melting range	104–108°C		201–205°C	
Solubility	Methanol	Very soluble	Methanol	Very slightly soluble
	DMSO	Very soluble	DMSO	Soluble
	Acetone	Sparingly soluble	Acetonitrile	Insoluble

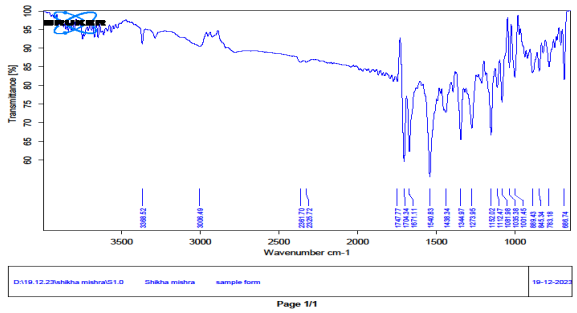

Figure 5: FTIR of glimepiride

Table 3: Calibration and regression analysis data for lobeglitazone sulfate and glimepiride

Conc. (µg/mL)	*Area mean (n = 3)*	*SD*	*%RSD*
10	171212.67	1170.29	0.68
20	305281.66	15.27	0.005
30	422677.33	29.73	0.007
40	578426.33	578.22	0.099
50	681576.33	1158.45	0.169
Range		10–50 µg/mL	
Regression equation (y = mx + c)		12939x + 43673	
Slope (m)		12939	
Intercept		43673	
Correlation coefficient		0.9972	

Conc. (µg/mL)	*Area Mean (n = 3)*	*SD*	*%RSD*
20	71535	625.05	0.87
40	261727	464.64	0.17
60	457803.66	1470.88	0.32
80	686425.33	2665.03	0.38
100	914404.33	3539.76	0.38
Range (µg/mL)		20–100	
Regression equation(y=mx+c)		10552x - 154752	
Slope (m)		10552	
Intercept		154752	
Correlation coefficient		0.9988	

Table 4: Results system suitability for lobeglitazone sulfate and glimepiride.

Parameters	*Proposed method*		*Standard value*
	Glimepiride	*Lobeglitazone*	
Theoretical plates (N)	8992 ± 1.923538	10670 ± 40693.2	Should be > 2000
Tailing factor (T)	1.179 ± 0.002739	1.161 ± 0.003347	T < 1.5
Peak Area	31826	12596	--

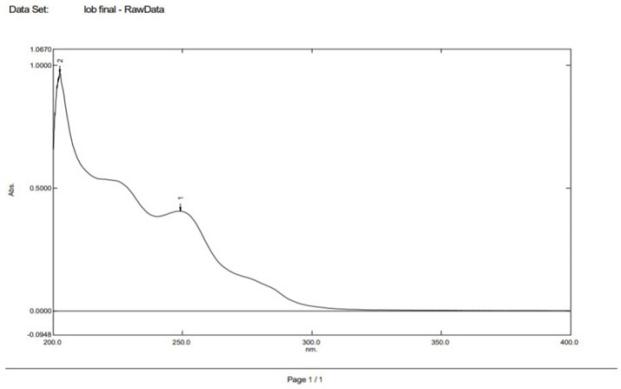

Figure 6: UV spectra of lobeglitazone sulfate

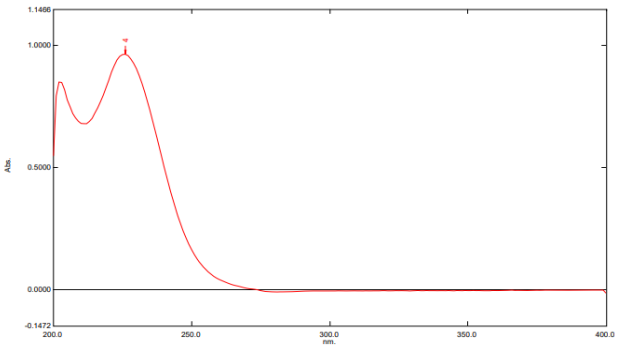

Figure 7: UV spectra of glimepiride

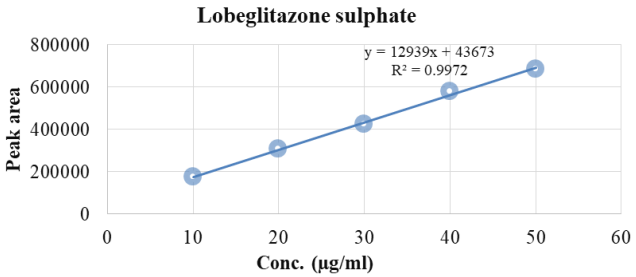

Figure 8: Calibration curve of lobeglitazone sulfate

Table 5: Result Repeatability for lobeglitazone sulfate and Glimepiride

Lobeglitazone sulfate			*Glimepiride*		
Conc. (µg/mL)	*Area (n = 6)*	*RT (minutes)*	*Conc (µg/mL)*	*Area*	*RT (minutes)*
30	12596	4.231	60	31826	6.089
30	12586	4.23	60	31829	6.084
30	12593	4.23	60	31834	6.081
30	12587	4.232	60	31828	6.087
30	12599	4.231	60	31836	6.085
30	12593	4.23	60	31829	6.082
Mean	12592.33	4.230667	Mean	31830.33	6.084667
SD	5.046451	0.000816	SD	3.829708	0.003011
%RSD	0.040076	0.019299	%RSD	0.012032	0.049487

Table 6: Method precision parameter results for glimepiride and lobeglitazone sulfate

Lobeglitazone sulfate				
Intraday precision			*Interday precision*	
Conc. µg/mL	*Peak area ± SD (n = 3)*	*%RSD*	*peak area ± SD*	*%RSD*
10	171029 ± 599.5	0.35	176660.3 ± 1654.9	0.936
30	424064.3 ± 2366.3	0.558	423845.7 ± 1013.9	0.239
50	681852.3 ± 1031.6	0.151	678720.7 ± 5311.2	0.782
Glimepiride				
Intraday precision			*Interday precision*	
Conc. µg/mL	*Peak area ± SD (n=3)*	*%RSD*	*Peak area ± SD*	*%RSD*
20	71535 ± 625.0	0.873	716495 ± 3296.1	0.46
60	456606.667 ± 461.0	0.100	458570.3 ± 1534.9	0.33
100	914856 ± 6891.5	0.753	913933 ± 2268.5	0.24

Table 7: Accuracy data of Lobeglitazone sulfate and glimepiride

% Level	*Sample (µg/mL)*	*Standard (µg/mL)*	*Spiked amount*	*Area (n=3)*	*Amount recovered*	*% recovery*	*Mean (n=3) ± SD*
Lobeglitazone sulfate							
				426359	29.58	98.59	
50%		10	30	431206	29.95	99.84	99.29 ± 0.638
				429654	29.83	99.44	
				548569	39.02	97.55	
100%	20	20	40	557564	39.72	99.29	98.38 ± 0.876
				552506	39.33	98.31	
				677896	49.02	98.03	
150%		30	50	685241	49.58	99.17	98.81 ± 0.676
				679203	49.12	98.23	
Glimepiride							
				483436	60.48	100.80	
50%		20	60	485237	60.65	101.08	100.96 ± 0.14
				484740	60.60	101.01	
				699534	80.96	101.20	
100%	40	40	80	681023	79.21	99.01	99.75 ± 1.25
				681405	79.24	99.05	
				917534	101.62	101.62	
150%		60	100	895620	99.54	99.54	100.25 ± 1.17
				896405	99.62	99.62	

Validation of Hplc

Linearity and range

A strong linear correlation between concentration and peak areas was observed over a concentration range of 10 to 50 µg/mL for lobeglitazone sulfate and 20 to 100 µg/mL for glimepiride. The correlation coefficients obtained were 0.9972 for lobeglitazone sulfate and 0.9988 for glimepiride, both exceeding 0.999, indicating excellent linearity.

Table 8: LoD & LoQ for lobeglitazone sulfate and glimepiride

Parameter	*LoD*	*LoQ*
Lobeglitazone sulfate	6.348 µg/mL	9.821 µg/mL
Glimepiride	11.923 µg/mL	20.012 µg/mL

System suitability test

Five replicate injections of a single standard solution were performed on an HPLC system to assess parameters, including

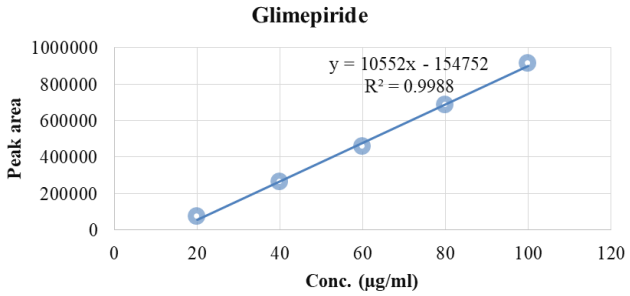

Figure 9: Calibration curve of glimepiride

the number of theoretical plates, peak tailing, and retention time. The outcomes are depicted in Table 4.

Repeatability

Six replicate injections at identical concentrations were analysed within the same day, and the %relative standard deviation (% RSD) was computed, as demonstrated in Table 5.

Precision

The %relative standard deviation for lobeglitazone Sulfate and glimepiride assay during method precision ranged from 0.936 to 0.782% and 0.46 to 0.24%, respectively. These results demonstrate excellent precision of the method.

Accuracy

The percent recovery of Lobeglitazone Sulfate ranged from 99.29% to 98.21%, while for Glimepiride samples, it ranged from 100.96% to 100.25%. These findings indicate the good

Table 9: Results Robustness of parameter for Lobeglitazone sulfate and glimepiride

S. No	*Parameters*	*Conditions*	*RT (min)*		*Area*					
			GLI	*LOBG GLI*	*%RSD*				*%RSD*	
					LOBG	*GLI*	*LOBG*	*GLI*	*LOBG*	
1.	Flow rate (mL/min)	0.9	6.083	7.817	0.092	0.092	31832	125914	0.035	0.235
		1.1	6.091	7.294			31848	125495		
2.	Mobile phase (% v/v)	65.35.00	6.094	7.425	0.058	0.058	31814	129495	0.024	1.101
		75.25.00	6.089	7.785			31825	127494		
3.	Temperature (°C)	35	6.985	7.659	0.263	0.263	31829	125954	0.146	0.514
		45	6.959	7.817			31895	125040		

Table 10: Summary of validation parameters by HPLC method which results indicate the validity of the method

S. No	*Parameters*	*Result*		
		Lobeglitazone sulfate	*Glimepiride*	
1	Linearity and Range	10-50(µg/mL)	20-100 (µg/mL)	
2	Precision			
	Intraday 10 (µg/mL)	171029 ± 599.54	Interday: 20 (µg/mL)	71535 ± 625.0528
	30 (µg/mL)	424064.333 ± 2366.34	60 (µg/mL)	456606.667 ± 461.061095
	50 (µg/mL)	681852.3 ± 1031.65	100 (µg/mL)	914856 ± 6891.52748
	Intermediate 10 (µg/mL)	176660.3 ± 1654.94	Interday 20(µg/mL)	716495 ± 3296.166
	30 (µg/mL)	423845.7 ± 1013.97	60 (µg/mL)	458570.3 ± 1534935
	50 (µg/mL)	678720.7 ± 5311.28	100 (µg/mL)	913933 ± 2268.539
3.	Accuracy			
	50%	99.29 ± 0.638	100.96 ± 0.145	
	100%	98.38 ± 0.876	99.75 ± 1.253	
	150%	98.81 ± 0.676	100.25 ± 1.178	
4.	Limit of detection	6.348µg/mL	11.923µg/mL	
5.	Limit of Quantification	11.923µg/mL	20.012µg/mL	
6.	Robustness	Robust	Robust	

accuracy of the method. Detailed results are provided in Table 7

Limit of detection (LOD) and Limit of quantification (LOQ)

The results indicated that the Limit of Detection (LoD) for glimepiride was 1.841 µg/mL, while for Lobeglitazone Sulfate, it was 1.320 µg/mL. Furthermore, it was determined that the Limit of Quantification (LoQ) for glimepiride was 61.38109 µg/mL and for Lobeglitazone Sulfate was 4.402 µg/mL.

Robustness

To assess the robustness of the method, variations were introduced in experimental conditions such as the composition and pH of the mobile phase, as well as the flow rate. Subsequent evaluation of chromatographic characteristics revealed no significant changes under altered conditions.

CONCLUSION

A reversed-phase high-performance liquid chromatographic method, designed to be simple, rapid, reliable, robust, and optimized, was successfully developed and validated in accordance with the International Conference on Harmonization guidelines for the estimation of Lobeglitazone Sulfate and Glimepiride. Hence the developed RP-HPLC method for the simultaneous determination of Pioglitazone and Glimepiride can be used for routine analysis of both these components in combined dosage form. The method underwent validation for accuracy, repeatability, and precision. Lobeglitazone Sulfate and Glimepiride displayed a strong linear relationship within their respective concentration ranges of 10-50µg/mL and 20-100µg/mL, with correlation coefficients of 0.9972 and 0.9988, respectively. Precision assessment demonstrated the method's reproducibility. Assay experiments verified the presence of Lobeglitazone Sulfate and Glimepiride in the tablet dosage form without interference from excipients

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