JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

REVERSE PHASE HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LOBEGLITAZONE SULFATE AND GLIMEPIRIDE IN PHARMACEUTICAL DOSAGE FORM.

Deepshree J Bumtariya[1]*, Mahida Rajvi.[2]

Research Student[1*], Assistant Professor[2]

Department of Pharmaceutical Quality Assurance

ROFEL Shri G.M. Bilakhia College of Pharmacy, Vapi, Gujarat,396191

Abstract:

A simple, rapid, precise, accurate, economical and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of Lobeglitazone Sulfate (LOB) and Glimepiride (GLM) which was used in combined ratio of (0.5:1) for treatment of patient suffering with Diabetes.[1] The separation was achieved on a Shim-pack solar C18 (250 mm × 4.6 mm, particle size of 5 µ) using a mobile phase [Acetonitrile: Water (pH 3.0 adjusted with 1% (OPA) Orthophosphoric Acid)] (65:35 %v/v). HPLC separation of LOB and GLM was carried out detection of at 224 nm. The mobile phase was pumped at a flow rate of 1ml/min. The retention times of LOB and GLM were found to be 4.472 min and 6.089 min respectively. Excellent linearity range was found between 2-6 µg/ml for LOB and 4-12 µg/ml for GLM. The method was validated with respect to linearity, precision, accuracy, specificity and ruggedness. Method was successfully applied for the simultaneous determination of Lobeglitazone Sulfate (LOB) and Glimepiride (GLM) in pharmaceutical dosage form.

Keywords: Lobeglitazone Sulfate , Glimepiride, RP-HPLC ,Method validation

I. INTRODUCTION

Diabetes mellitus is characterized by high levels of sugar (glucose) in the blood. After consuming a meal, when glucose levels rise, the pancreas secretes insulin, prompting muscle and fat cells to absorb glucose from the blood while encouraging the liver to metabolize it, thereby restoring blood sugar to normal levels. However, individuals with diabetes experience persistently high blood sugar levels due to either inadequate insulin production, insufficient insulin secretion, or reduced insulin effectiveness.[2]

Lobeglitazone Sulfate (LOB):

Lobeglitazone is an antidiabetic medication from the thiazolidinedione class of drugs. It primarily functions as an insulin sensitizer by binding and activating Peroxisome Proliferator-Activated Receptors (PPAR) gamma within fat cells. By activating PPAR-gamma and promoting the binding of insulin at fat cells, lobeglitazone thereby has been shown to reduce blood sugar levels, lower haemoglobin A1C (HbA1C) levels, and improve lipid and liver profiles.[3,4]

Glimepiride (GLM):

Glimepiride blocks the ATP-sensitive potassium channel by binding non-specifically to the B sites of both sulfonylurea receptor-1 (SUR1) and sulfonylurea receptor-2A (SUR2A) subunits as well as the A site of SUR1 subunit of the channel to promote insulin secretion from the beta cell.[5,6]

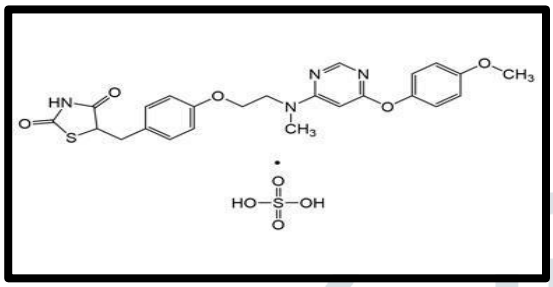

Fig. 1: Chemical Structure of LOB

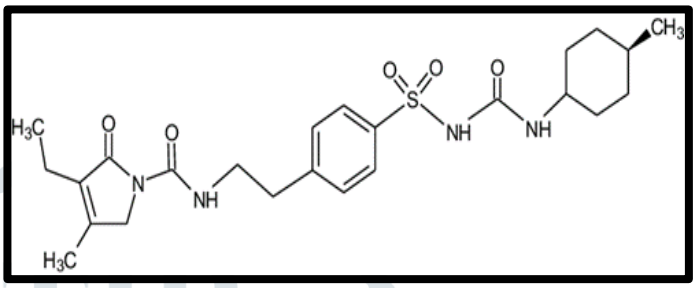

Fig. 2: Chemical Structure of GLM

A fixed dose combination of Lobeglitazone Sulfate (0.5 mg) and Glimepiride (1 mg) is used in treatment of diabetes. Literature review reveals that there are many methods available for single Lobeglitazone Sulfate and Glimepiride but there are no any RP-HPLC method reported for Lobeglitazone Sulfate and Glimepiride in combined dosage form so it was thought of interest to develop a simple, accurate, precise and rapid RP-HPLC for analysis of Lobeglitazone Sulfate and Glimepiride for pharmaceutical dosage form according to ICH Q2(R2) specifications.[7]

INTRODUCTION TO THE HPLC METHOD

High-performance liquid chromatography, also known as high-performance liquid chromatography, high-speed liquid chromatography (HSLC), high-efficiency liquid chromatography (HELC). Liquid chromatography is an analytical chromatography technique useful for separating ions or molecules dissolved in a solvent.

Principle: Adsorption is the basic principle of HPLC. The mixture and components move through the HPLC column according to their relative affinity towards the stationary phase. Components with a lower affinity for the stationary phase move faster, while those with a higher affinity for the adsorbent move more slowly. Components are separated if two components do not have the same affinity for the stationary phase.[8,9,10]

MATERIALS AND METHODS:

Apparatus and Instrument

- Model: SHIMADZU LC-2010 CHT
- Column: Shim-pack solar C18 (250 mm × 4.6 mm, 5 µm)
- Injector: Auto injector
- Detector: UV Detector
- Software: LC solution
- Electronic analytical balance (SHIMADZU 0.1 mg)
- Digital pH meter (Systronic pH system)

- Ultrasonic cleaner (Athena Technology)
- Filter paper: Vacuum filter: Membrane filter 0.45 micron
- Syringe filter: Membrane filter 0.27 micron

Chemicals:

- Lobeglitazone sulfate was obtained as a gift sample from Akums Pharmaceutical, Haridwar.
- Glimepiride was obtained as a gift sample from Exemed Pharmaceutical, Vapi, Gujarat.
- Acetonitrile (HPLC grade)- Ranken , Methanol (HPLC grade)- Ranken ,Water (HPLC grade)- Rankem
- Ortho Phosphoric Acid (HPLC grade)- Thomas baker

Instrumentation and Chromatographic Conditions:

SHIMADZU LC-2010 CHT attached to PDA detector, which is having an Auto injector and autosampler opted for chromatography. A degasser to remove the dissolved air and column oven to maintain the desired temperature is also available in the system. Operation data acquisition and analysis were performed by using LC Solution software. The mobile phase [Acetonitrile: Water (pH 3.0 adjusted with 1% (OPA) Orthophosphoric Acid)] (65:35 %v/v). HPLC separation of LOB and GLM was carried out at detection wavelength of 224 nm, the mobile phase is in low pressure gradient mode, at a flow rate of 1.0 ml/min. Stationary phase is Shim-pack solar C18 (250 mm × 4.6 mm, 5 µm). A Flow rate of the mobile phase was 1.0 ml/min and all chromatographic experiments were performed at room temperature (25 ºC ± 2 ºC). The detector wavelength was fixed at 224 nm.

Spectrophotometric Conditions:

Mode: Scan
Scan speed: Medium
Wavelength range: 200 - 400 nm
Scale of Absorbance: 0.00 - 2.00 Aº
Baseline Correction: Methanol

Selection of wavelength

Aliquots of 0.2 ml from working stock solution of LOB (100 µg/ml) and 0.4 ml from working stock solution of GLM (100 µg/ml) were pipette out and taken into two separate volumetric flasks of 10 ml and volume was made up to mark with methanol to give a solution containing 2 µg/ml and 4 µg/ml of LOB and GLM. Each solution was scanned between 200-400 nm using UV-Visible Spectrophotometer. Wavelength was selected from the overlay spectra of above solution.

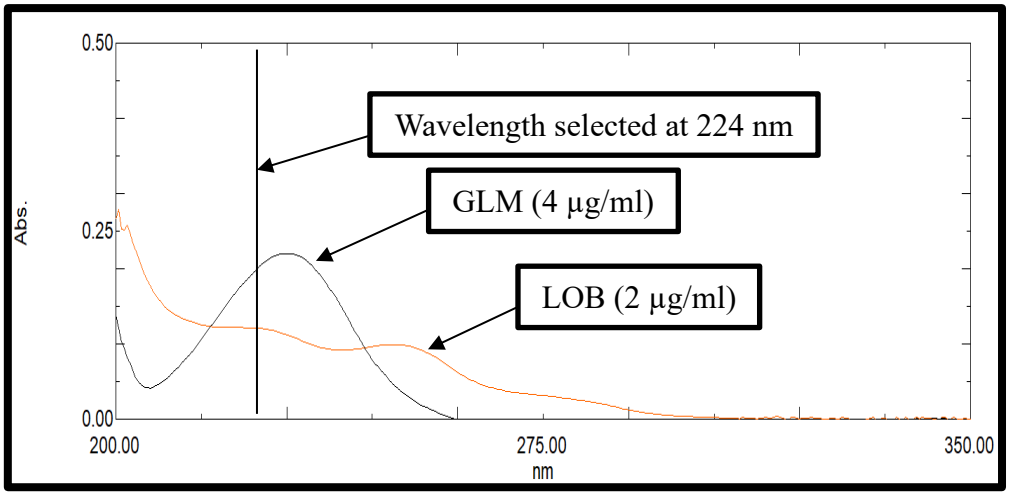

Fig. 3: Overlay UV spectrum of LOB (2 µg/ml) and GLM (4 µg/ml) showing selection of wavelength detection at 224 nm

PREPARATION OF STANDARD SOLUTION:

1. Preparation of LOB standard stock solution (1000 µg/ml):

10 mg of LOB was weighed and transferred to 10 ml volumetric flask. It was dissolved in methanol and volume was made upto the mark with methanol to give a solution containing 1000 µg/ml.

2. Preparation of LOB working stock solution (100 µg/ml):

Aliquot of 1 ml from above standard stock solution was pipetted out into 10 ml of volumetric flask and volume was made upto the mark with methanol to give a solution containing 100 µg/ml.

3. Preparation of GLM standard stock solution (1000 µg/ml):

10 mg of GLM was weighed and transferred to 10 ml volumetric flask. It was dissolved in methanol and volume was made upto the mark with methanol to give a solution containing 1000 µg/ml.

4. Preparation of GLM working stock solution (100 µg/ml):

Aliquot of 1 ml from above standard stock solution was pipetted out into 10 ml of volumetric flask and volume was made upto the mark with methanol to give a solution containing 100 µg/ml.

5. Preparation of Binary mixture of LOB and GLM:

Aliquots of 0.2 ml from working solution of LOB (100 µg/ml) and 0.4 ml from working solution of GLM (100 µg/ml) were taken into common volumetric flask and diluted upto 10 ml with methanol to make final concentration LOB (2 µg/ml) and GLM (4 µg/ml)

Preparation of Calibration Curves

I. Calibration curve for LOB

Calibration curve for LOB consisted of five different concentrations solution ranging from 2-6 µg/ml. Calibration curve of Peak area vs Conc. was plotted and regression equation was determined.

II. Calibration curve for GLM

Calibration curve for GLM consisted of five different concentrations solution ranging from 4-12 µg/ml. Calibration curve of Peak area vs Conc. was plotted and regression equation was determined.

PREPARATION OF ASSAY SAMPLE:

Twenty tablets of LOB and GLM were accurately weighed and triturated in mortar pestle to obtain fine powder. Weigh fine tablet powder and it is equivalent to about 0.5 mg of LOB and 1mg of GLM was transferred to 10 ml volumetric flask and sonicated for 15 min and was makeup upto the mark with methanol. The solution was filtered through Whatmann filter paper no. 41. Thus, resulting solution gave 50 µg/ml of LOB and 100 µg/ml of GLM respectively.

From the above solution, 0.8 ml was pipette out and transferred to 10 ml volumetric flask and volume was made upto mark with methanol in order to give a solution containing LOB (4 µg/ml) + GLM (8 µg/ml).

PREPARATION OF SOLUTIONS FOR ANALYTICAL METHOD VALIDATION:

1. System Suitability studies

The system suitability assessment involved analyzing six replicates of a mixture containing 6 µg/ml of LOB and 12 µg/ml of GLM. Parameters such as column efficiency, peak asymmetry, and resolution were calculated for each replicate to evaluate the system's performance.

2. Specificity

Specificity entails the accurate detection of an analyte in the presence of other components typically found in the sample matrix. To verify the specificity of the developed method, LOB and GLM were spiked into a hypothetical placebo containing potential interfering components. It was confirmed that the peaks corresponding to the analytes were not affected by the presence of excipients, thus demonstrating the method's specificity.

3. Linearity

The linearity response was determined by analyzing 5 independent levels of concentration in the range of 2-6 µg/ml and 4-12 µg/ml for LOB and GLM respectively.

4. Precision

a) Repeatability

Repeatability of the developed method was assessed by analysing samples from the same batch 6 times with standard solutions containing concentrations 4 µg/ml for LOB and 8 µg/ml for GLM and % R.S.D. was calculated.

b) Intraday precision

It was assessed by analyzing samples from the same batch with three standard solutions containing concentrations 3, 4, 5 µg/ml for LOB and 6, 8, 10 µg/ml for GLM. Solutions were analyzed thrice (n=3) on the same day within short interval of time and % R.S.D. was calculated.

c) Interday precision

It was assessed by analyzing samples from the same batch with three standard solutions containing concentrations 3, 4, 5 µg/ml for LOB and 6, 8, 10 µg/ml for GLM. Solutions were analyzed thrice (n=3) on the three different day and % R.S.D. was calculated.

5. Accuracy

A) Preparation of sample solution for LOB

Mixture solution X: LOB (50 µg/ml) + GLM (100 µg/ml)

Table 1: Steps for Accuracy measurement for LOB

Sr. No.	Step 1	Step 2	Step 3	Total LOB Conc.(µg/ml)
1.	Take 0.4 ml of solution X	-	Make up the volume to 10 ml with methanol	2 µg/ml
2.	Take 0.4 ml of solution X	Add 0.16 ml of solution Y	Make up the volume to 10 ml with methanol	3.6 µg/ml
3.	Take 0.4 ml of solution X	Add 0.2 ml of solution Y	Make up the volume to 10 ml with methanol	4 µg/ml
4.	Take 0.4 ml of solution X	Add 0.24 ml of solution Y	Make up the volume to 10 ml with methanol	4.4 µg/ml

B) Preparation of sample solution for GLM

Mixture solution X: LOB (50 µg/ml) + GLM (100 µg/ml)

Solution Y: GLM (100 µg/ml)

Table 2: Steps for Accuracy measurement for GLM

Sr. No.	Step 1	Step 2	Step 3	Total GLM Conc.(µg/ml)
1.	Take 0.4 ml of solution X	-	Make up the volume to 10 ml with methanol	4 µg/ml
2.	Take 0.4 ml of solution X	Add 0.32 ml of solution Y	Make up the volume to10 ml with methanol	7.2 µg/ml
3.	Take 0.4 ml of solution X	Add 0.4 ml of solution Y	Make up the volume to 10 ml with methanol	8 µg/ml
4.	Take 0.4 mlof solution X	Add 0.48 ml ofsolution Y	Make up the volume to10 ml with methanol	8.8 µg/ml

Each solution was scanned from 200-400 nm against methanol as a blank. Absorbance of solution was measured at selected wavelengths for LOB and GLM. The amount of LOB and GLM was calculated at each level (80%, 100% and 120%) and % recoveries were computed.

6. LOD and LOQ: The LOD (Limit of Detection) was estimated from the set of 5 calibration curves that were used to determine linearity of the method. The LOD was calculated by using the formula: **LOD: 3.3 x S.D. / Slope**

The LOQ (Limit of Quantitation) was estimated from the set of 5 calibration curves that were used to determine linearity of the method. The LOQ was calculated by using the formula: **LOQ: 10 x S.D. / Slope**

Where, S.D. = Standard deviation of the Y – intercepts of 5 calibration curves Slope = Mean slope of 5 calibration curves

7. Robustness: Robustness of the method was determined by subjecting the method to slight change in the method conditions like, Mobile Phase Ratio and Flow rate.

Three replicates were prepared for the same of concentration 6 µg/ml for LOB and 12 µg/ml for GLM and % R.S.D. was calculated.

RESULT AND DISCUSSION:

OPTIMIZED CHROMATOGRAPHIC CONDITION:

After Optimization of mobile phase, a well resolved peak of LOB and GLM was observed at 4.472 and 6.089 minutes respectively., final chromatographic condition is given in the table.

Table 3: Optimized Chromatographic Condition

Sr. No.	Parameters	Condition
1.	Mobile Phase	ACN: Water (65:35 %v/v) (pH-3 adjusted with 1% OPA)
2.	Flow Rate	1 ml/min
3.	Run time	10 min
4.	Volume of Injection	10 µL
5.	Detection Wavelength	224 nm
6.	Diluent	Methanol

METHOD VALIDATION:

1. SYSTEM SUITABILITY DATA:

Table 4: System Suitability Data for DAP and MET

Drugs	Parameters	Mean ± S.D. (n=6)	%R.S.D
LOB	**Retention Time**	4.472 ± 0.02854	0.6381
	Theoretical Plate	59008.07 ± 450.291	0.7631
	Tailing Factor	1.051 ± 0.005822	0.5539
GLM	**Retention Time**	6.089 ± 0.03634	0.5968
	Theoretical Plate	70790.37 ±352.821	0.4984
	Tailing Factor	0.997 ± 0.003617	0.3627
	Resolution	7.442 ± 0.04925	0.6617

2. Specificity

The method's specificity was assessed by analyzing standard drugs along with samples of LOB and GLM. The results indicated that the proposed method is indeed specific, as the presence of excipients in the marketed formulation did not impact the results. Chromatograms obtained from running only with the mobile phase, placebo, and after injection of the sample are provided in the figures below.

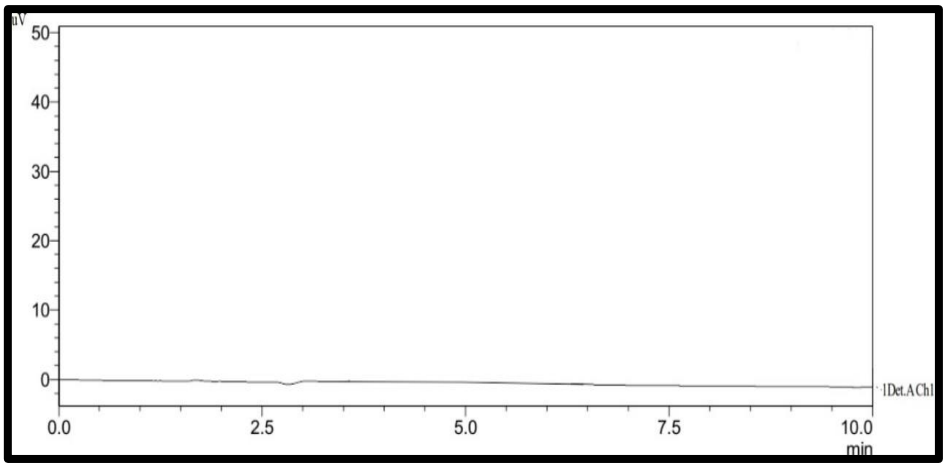

Fig. 4: Chromatogram of Placebo

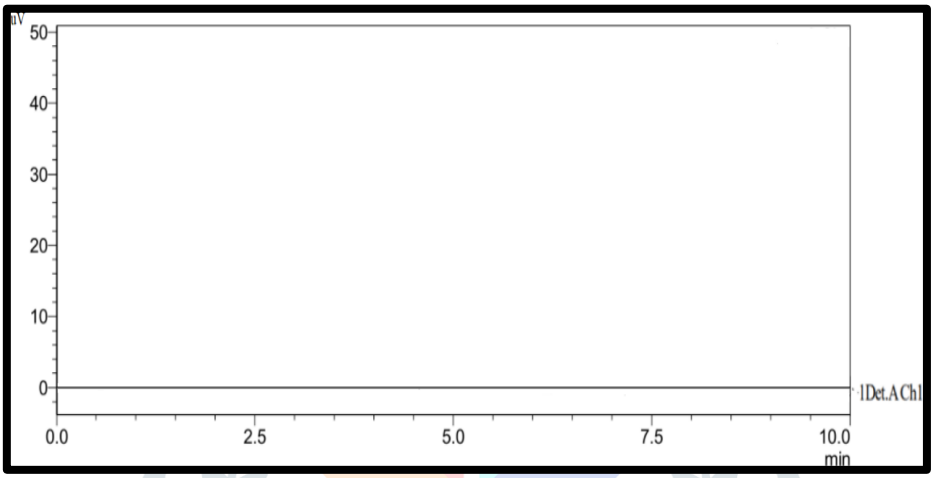

Fig. 5: Chromatogram of Mobile Phase

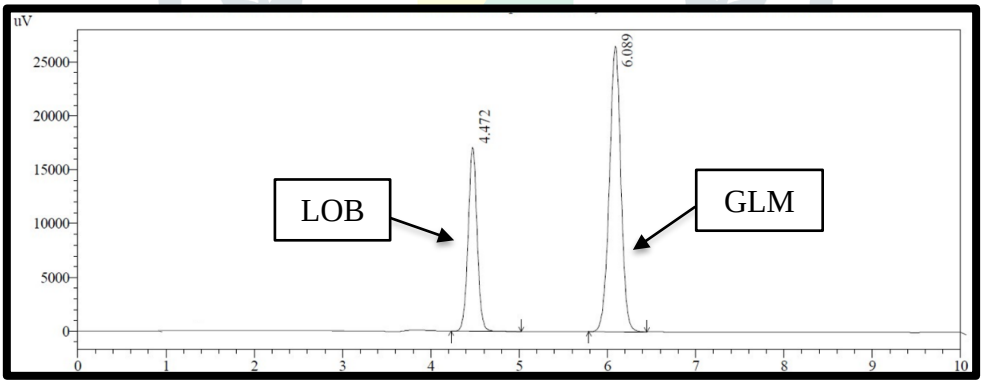

Fig. 6: Chromatogram of LOB (6 µg/ml) and GLM (12 µg/ml)

3. LINEARITY:

The linearity study was carried out for both drugs at five different concentration levels. The linearity of LOB and GLM was in the range of 2-6 µg/ml and 4-12 µg/ml are depicted in table.

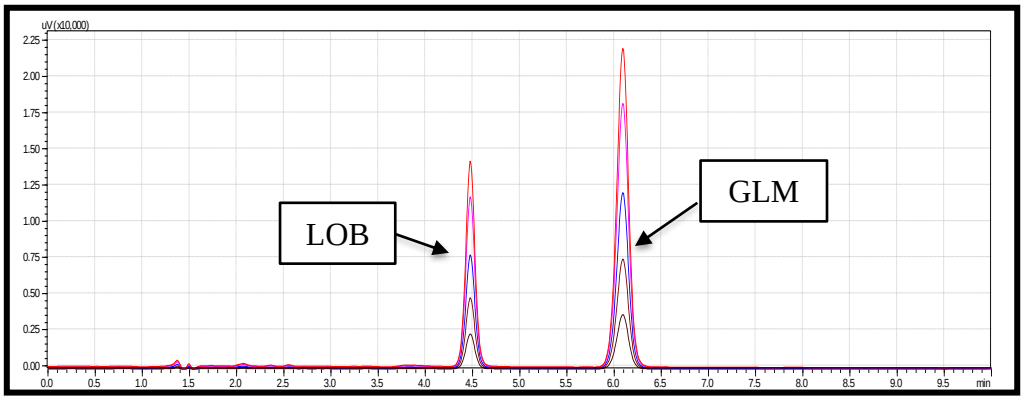

Fig. 7: Overlain Chromatogram of LOB (2-6 µg/ml) and GLM (4-12 µg/ml)

Table 5: Linearity Data of LOB

Sr.No.	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=6)	%R.S.D
1.	2	13499 ± 98.760	0.7316
2.	3	36149 ± 230.01	0.6362
3.	4	60788 ± 352.09	0.5792
4.	5	86992 ± 403.47	0.4638
5.	6	114004 ± 418.63	0.3672

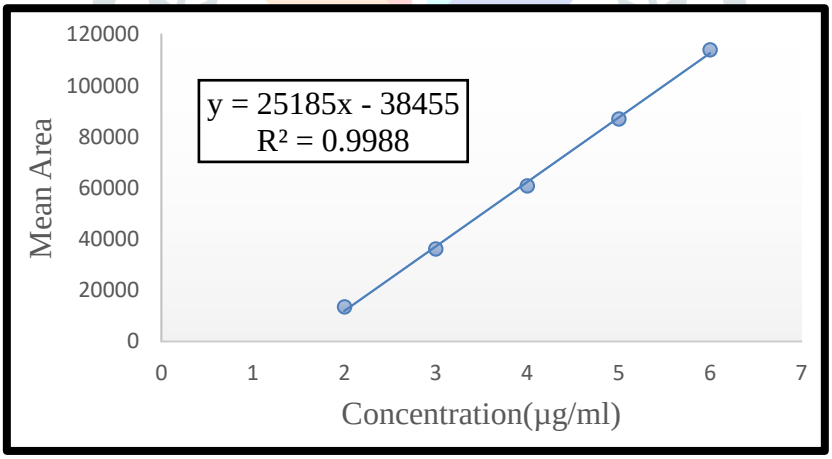

Fig. 8: Calibration Curve of LOB

Table 6: Linearity Data of GLM

Sr.No.	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=6)	%R.S.D
1.	4	31422 ± 255.43	0.8129
2.	6	71025 ± 520.26	0.7325
3.	8	123452 ± 797.01	0.6456
4.	10	176771 ± 910.73	0.5152
5.	12	231040 ± 1084.2	0.4692

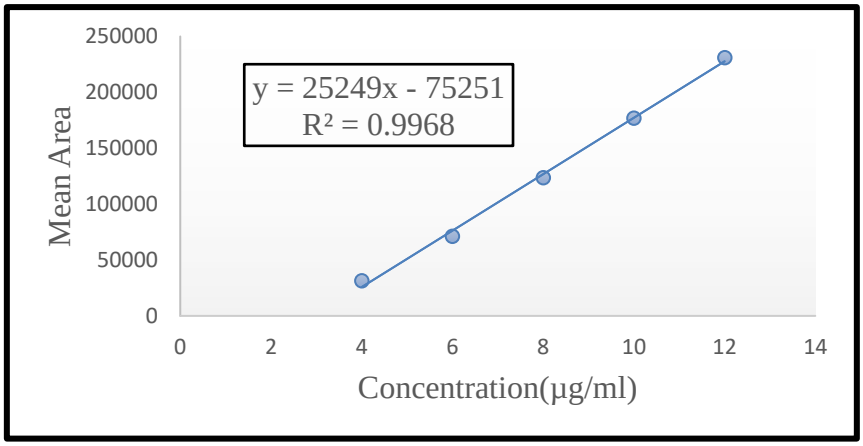

Fig. 9: Calibration Curve of GLM

4. PRECISION

A) Repeatability

The data of Repeatability for LOB and GLM is shown in table.

Table 7: Repeatability Data for LOB and GLM

Sr.No.	Drugs	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=6)	%R.S.D
1.	LOB	4	60791 ± 352.28	0.5795
2.	GLM	8	123458 ± 797.41	0.6458

B) Intraday Precision

The data of Intraday Precision for LOB and GLM is shown in table.

Table 8: Intraday Precision for LOB and GLM

Drugs	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=3)	%R.S.D
LOB	3	36152 ± 230.17	0.6366
	4	60793 ± 352.47	0.5797
	5	86996 ± 403.74	0.4641
GLM	6	71028 ± 520.56	0.7328
	8	123455 ± 797.88	0.6462
	10	176774 ± 911.44	0.5155

C) Interday Precision (n=3):

The data of Interday Precision for LOB and GLM is shown in table.

Table 9: Interday Precision for LOB and GLM

Drugs	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=3)	%R.S.D
LOB	3	36156 ± 230.63	0.6377
	4	60802 ± 353.19	0.5808
	5	87002 ± 404.91	0.4654
GLM	6	71037 ± 521.48	0.7340
	8	123468 ± 799.45	0.6474
	10	176782 ± 913.60	0.5167

5. ACCURACY

The % Recovery study was performed by the Standard Addition Method. Known amounts of standard solutions of LOB and GLM were added at 80%, 100% and 120% level to pre-quantified sample solutions of LOB (2 µg/ml) and GLM (4 µg/ml). The amounts of LOB and GLM were estimated by using regression equation of the calibration curve. The low value of standard deviation indicates that the proposedmethod is accurate. Results of recovery studies are shown in table.

Table 10: Accuracy Data for LOB and GLM

Drugs	Level	Amountof sample (µg/ml)	Amountof std. spiked (µg/ml)	Total amount (µg/ml)	Mean PeakArea ± S.D. (n=3)	Amount of sample found (µg/ml)	Mean % Recovery ±S.D. (n=3)
LOB	0%	2	0	2	14544 ± 105.72	1.988	99.40
	80%	2	1.6	3.6	55444 ± 320.80	3.586	99.61
	100%	2	2	4	61593 ± 347.51	3.992	99.80
	120%	2	2.4	4.4	67772 ± 378.13	4.431	100.70
GLM	0%	4	0	4	32448 ± 258.97	3.980	99.50
	80%	4	3.2	7.2	121015 ± 790.95	7.172	99.61
	100%	4	4	8	134472 ± 848.25	7.979	99.73
	120%	4	4.8	8.8	147919 ± 926.12	8.813	100.14

6. LOD and LOQ

The LOD for LOB and GLM was found to be 0.2765 µg/ml and 0.1938 µg/mlrespectively.

The LOQ for LOB and GLM was found to be 0.8381 µg/ml and 0.5874 µg/mlrespectively.

7. ROBUSTNESS

The robustness of the method was established by making deliberate minor variations inthe following method parameters.

Flow rate: ± 0.2 units

Mobile Phase Ratio: ± 2.0 ml

Table 11: Robustness Data for LOB

Parameters	Level	Mean Peak Area ± S.D. (n=3)	%R.S.D	Rt ± S.D. (n=3)	%R.S.D
Mobile Phase (ACN:Water) (65:35% v/v)	67:33 v/v	114538 ± 1026.60	0.8962	4.656 ± 0.03846	0.8260
	63:37 v/v	114339 ± 883.96	0.7731	4.512 ± 0.02772	0.6143
Flow Rate (1.0 ml/min)	0.8 ml/min	113994 ± 796.81	0.6989	4.592 ± 0.03558	0.7748
	1.2 ml/min	114272 ± 814.98	0.7131	4.350 ± 0.02646	0.6082

Table 12: Robustness Data for GLM

Parameters	Level	Mean Peak Area ± S.D. (n=3)	%R.S.D	Rt ± S.D. (n=3)	%R.S.D
Mobile Phase (ACN:Water) (65:35% v/v)	67:33 v/v	231682 ± 1766.14	0.7623	6.280 ± 0.04856	0.7732
	63:37 v/v	231394 ± 1670.48	0.7219	6.122 ± 0.04152	0.6782
Flow Rate (1.0 ml/min)	0.8 ml/min	230974 ± 1613.59	0.6986	6.154 ± 0.04503	0.7316
	1.2 ml/min	231150 ± 1748.21	0.7563	6.018 ± 0.04094	0.6802

8. APPLICABILITY OF PROPOSED METHOD:

Table 13: Determination of Assay of LOB and GLM

	Actual concentration (mg/tablet)		Amount obtained (mg/tablet)		LOB %Purity ± S.D. (n=5)	GLM %Purity ± S.D. (n=5)
Tablet (LOBG-G1)	LOB	GLM	LOB	GLM	99.85 ± 0.38386	99.78 ± 0.38775
	4	8	3.994 ± 0.01535	7.983 ± 0.03102		

SUMMARY OF VALIDATION PARAMETERS FOR PROPOSED METHOD:

Table 14: Summary of RP-HPLC Method

Parameters	LOB	GLM
Linearity (µg/ml) (n=5)	2-6 µg/ml	4-12 µg/ml
Regression equation (y = mx + c)	y = 25185x - 38455	y = 25249x - 75251
Regression coefficient (R^2)	0.9988	0.9968
Correlation coefficient (r)	0.9993	0.9983
Repeatability (n=6) (%R.S.D)	0.5795	0.6458
Intraday precision (n=3) (%R.S.D)	0.4641-0.6366	0.5155-0.7328
Interday precision (n=3) (%R.S.D)	0.4654-0.6377	0.5167-0.7340
LOD (µg/ml)	0.1938	0.2765
LOQ (µg/ml)	0.5874	0.8581
% Recovery (n=3)	99.40-100.70	99.50-100.14
% Assay ± S.D. (n=5)	99.85 ± 0.38386	99.78 ± 0.38775

SUMMARY AND CONCLUSION:

The proposed RP-HPLC Method were developed for simultaneous estimation of Lobeglitazone Sulfate (LOB) and Glimepiride (GLM) in the pharmaceutical dosage form are simple, precise, accurate, and sensitive. These methods have wider range with good accuracy and precision. They can be used for the routine analysis of both drugs in pharmaceutical formulations.

The method was developed and validated as per **ICH guidelines Q2(R2).**

The precision of the developed methods was confirmed by intra-day and inter-day analysis and the accuracy of the developed method was confirmed by the recovery study. The **%RSD** was found to be **<2.0%.** It indicates that the method has good precision and accuracy.

Hence the developed RP-HPLC method is simple, rapid, precise and accurate.

AKNOWLEDGEMENT:

The authors are thankful to Akums Pharmaceutical Pvt. Ltd., Haridwar. And Exemed Pharmaceutical for providing the Lobeglitazone Sulfate and Glimepiride API as a gift sample also thankful to the principal of ROFEL Shri G.M. Bilakhia College of Pharmacy, Vapi for providing necessary facilities, equipment and chemicals for doing research work.

REFERENCES

1. Drug Information, "Central Drugs Standard Control Organisation Directorate General Of Health Services Ministry of Health & Family Welfare, Government of India", https://www.cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2018/UploadCTApprovals/79%20Glenmark%20Lobeglitazone%20CT-06.pdf

2. "Introduction to diabetes", December 2023, https://www.ncbi.nlm.nih.gov/books/NBK1671/

3. Drug Profile, "LOBEGLITAZONE SULFATE ",December 2023, https://go.drugbank.com/salts/DBSALT002555

4. Drug Profile, "LOBEGLITAZONE SULFATE ",December 2023, https://pubchem.ncbi.nlm.nih.gov/compound/Lobeglitazone-sulfate

5. Drug Profile, "GLIMEPIRIDE ", December 2023, https://go.drugbank.com/drugs/DB00222

6. Drug Profile, "GLIMEPIRIDE ", December 2023, https://pubchem.ncbi.nlm.nih.gov/compound/Glimepiride

7. ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2 (R1); International Conference on Harmonization, Geneva,Switzerland, 2005, pp 4-13.

8. Shethi PD. HPLC-Quantitative Analysis of Pharmaceutical Formulations;vol.2, CBS publisher & distributors, New Delhi, 2007,pp 507-508.

9. Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis; 6th Edn;Cengage learning, Delhi, 2017, pp 806 - 835.

10. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis; 5th Edn; Himalaya Publishing House, New Delhi, 2002, pp 2.167- 2.172.

11. Gulhane D. P, Jawarkar G.S., "Bioanalytical method development and validation for determination of lobeglitazone

in human plasma",*IRJMETS.* **2023**, 5*(5)*, 8103-8116.

12. Sai K. E, Srinivas M, etal., "Development and Validation of an HPLC Method for the Determination of Lobeglitazone in Bulk and in Tablet Formulation", *Int.J.Pharm.Investig*. **2024,** 14 (*1*),204-211.

13. Karajgi S, Mali S, Kotnal R, ''Novel First Order Derivative UV Spectrophotometric Method for the determination of Glimepiride in solid dosage forms.'' *IJDDT*. **2018**, 8*(3)*, 121-126.

14. Alhadad KS, H. N. K. AL-Salman, ''Development of an RP-HPLC Method to estimation Glimepiride in Bulk and Solid Dosage Form.'' *J Popul Ther Clin Pharmacol*. **2023**, 30*(5)*, 148–155.

15. Pawar J, Sonawane S, Chhajed S, Kshirsagar S, Wagh M, ''Development and Validation of RP-HPLC Method for Simultaneous Estimation of Pioglitazone HCl and Glimepiride in Tablets.'' *IJPRS*. **2016**, 5*(2)*, 167-172.

16. Pokharkar D, Tyagi CK, ''Stress Stability Study Showing Effect of Acid, Base, H2o2 and Dry Heat on Glimepiride by HPTLC Method.''*World J. Pharm. Res.***2021** ,10*(7)*, 804-813.

17. Sen AK, Sarkar T, Sen DB, etal., "Quantitative determination of Lobeglitazone sulfate and glimepiride in combined tablet by robust high-performance thin layer chromatographic method" *Sep.Sci.Plus,***2024,** *2400059*.