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Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Lobeglitazone and Glimepiride in Tablet Dosage Form

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ABSTRACT
Stability indicating RP-HPLC method was developed for Simultaneous estimation of Lobeglitazone and Glimepiride in tablets under different stability environments of acid, base, thermal, oxidation, and photolytic degradation. Separation was achieved on Ultrasphere-C18, (250 mm x 4.6 mm) 5.0 µm column by using a mobile phase (Methanol : Potassium Dihydrogen Phosphate buffer pH 3.5) (70:30) with Isocratic flow rate of 1.0 ml/min and detection was at 243 nm.
The complete analytical method validation was successfully carried out as per ICH guidelines. The retrieval study was carried out at 50% to 150% level of working concentration, and results were in the range of 98 to 102%. The linearity was proven in range of 6.25–125 µg/mL of working concentration of Lobeglitazone and 12.25 – 250 µg/mL of working concentration of Glimepiride with linear regression curve (R^2=0.999) with limits of detection (LOD) and quantitation (LOQ) being 0.099 and 0.300 µg/mL for Lobeglitazone and 0.053 and 0.161 µg/mL for Glimepiride respectively. The retention time for Lobeglitazone was 7.86 min and for Glimepiride was 10.03 min. The method shows good recoveries and intra-day and inter-day relative standard deviations were less than 2%. Validation parameters as robustness was also determined as per ICH guidelines and were found to be satisfactory. For stability study, the drug was exposed to various stress conditions such as acid, base, oxidation, Thermal and sunlight as per recommendations of ICH guidelines.Hence, the method will be usefull for routine quality control analysis.

Key Words: Lobeglitazone, Glimepiride, RP-HPLC, Stability, Validation

I. INTRODUCTION

Lobeglitazone IUPAC name 5-[(4-[2-([6-(4-Methoxyphenoxy)pyrimidin-4-yl]-methylamino)ethoxy]phenyl)methyl]-1,3-thiazolidine-2,4-dione. Chemical formula $C_{24}H_{24}N_4O_5S$ (Fig.1).It is an anti-diabetic drug in the thiazolidinedione class of drugs. It primarily function as an insulin sensitizer by binding and activating Peroxisome Proliferator-Activated Receptors (PPAR) gamma within fat cells. PPAR is a transcription factor that plays a role in regulating metabolism. By promoting the binding of insulin at fat cells, lobeglitazone has been shown to reduce blood sugar levels, lower haemoglobin A1C levels, and improve lipid and liver profiles. Glimepiride IUPAC name 3-Ethyl-4-methyl-N-[2-(4-{[(trans-4 methylcyclohexyl)carbamoyl]sulfamoyl}phenyl)et hyl]-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide. Chemical formula $C_{24}H_{34}N_4O_5S$(Fig.2). It lowering blood glucose level which depend on stimulating the release of insulin from pancreatic beta cells. This is supported by both preclinical and clinical studies demonstrating that Glimepiride administration can lead to increased sensitivity of peripheral tissues to insulin. This combination approved by CDSCO in the year 2023 for the treatment of Type-2 Diabetes Mellitus and available as LOBG-G1 in the market

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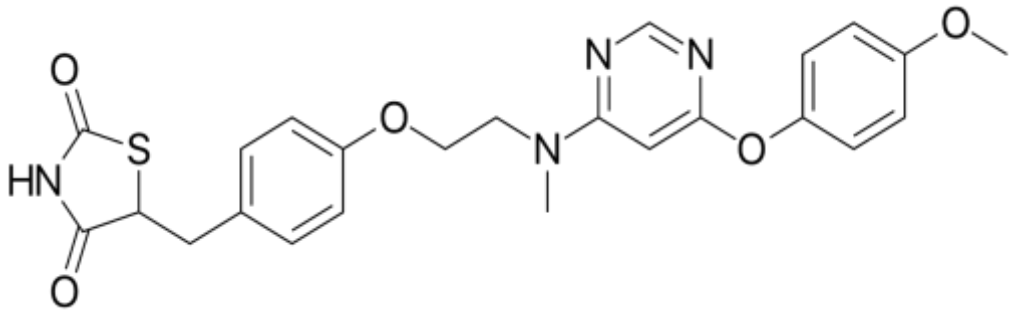

Fig.1 Chemical structure of Lobeglitazone

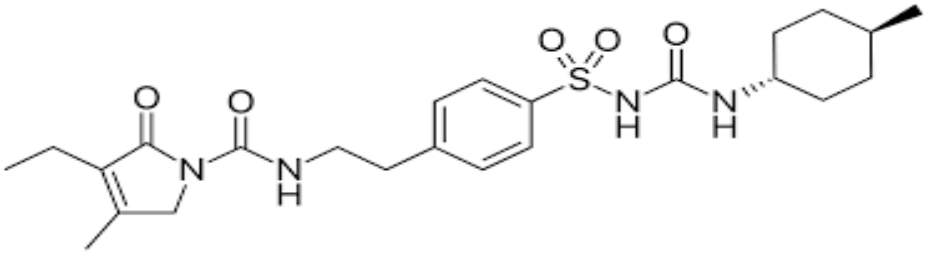

Fig.2 Chemical structure of Glimepiride

Forced degradation experiments are used to relieve the development of analytical methodology, to achieve better insightful of the stability of the active pharmaceutical ingredient (API) and the drug product, and to provide information about degradation pathways and degradation products. However, no literature is available for which deals with the stress degradation profile of Lobeglitazone and Glimepiridein accordance with ICH guidelines using any of the above analytical techniques. High performance liquid chromatography (RP-HPLC) for analysis of of Lobeglitazone and Glimepiride in pharmaceutical formulation. Tis paper describes an accurate, specific, repeatable, and stability-indicating method for analysis of of Lobeglitazone and Glimepiride in the presence of its degradation products. The method was validated in accordance with the guidelines of International Conference on Harmonization (ICH).

Necessity and importance of stability-indicating method

The goal of the stabilization studies is to track potential improvements to a substance or material over time and under various storage conditions. The factors and parameters that affect the stability are production timeframe, batch factors along with process parameters, excipients efficiency, and environmental conditions like temperature and humidity.

The access to appropriate deteriorated samples for method production assistance is a major challenge when designing a stability indicator method (SIM). Such deteriorated samples in a perfect environment must be realtime stability samples containing all applicable degradant as well as those degradant develop during ordinary storage conditions. For this cause, pharmacists must use forced degradation samples to create SIMs. Many experiments have explored the potential of forced deterioration studies to predict real-time degradation.

The precision of the stability methods showing potential impurities of the drug material and of drug components is demonstrated by forced degradation (FD). Stress experiments help to generate impurities in a much shorter period. The formulations scientist will then generate consistent formulations in less time. FD studies now include the completion of the file and the comprehension of the drug production mechanism for global controlled markets.

GMP includes a structured written monitoring program for stability, the results of which can be used to specify the storage requirements, the expiry dates and the use of accurate, meaningful and precise test procedures. If there is an effort to document drug product stability, the use of such approaches is acceptable. These data are being used to assess, conform or expand retest cycles or expiration date for the drug substance.

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The rationale for the stability studies research is to provide data as to how the consistency of the substance varies over the time under the control of a multiplicity of ecological variables, such as humidity, temperature and light, allows the proposedstorage conditions, re-analysis periods and shelf life.

Methods

Reagents and chemicals

Lobeglitazone was supplied as a gift sample by aAllastir Pvt. Ltd. Chennai and Glimepiride was by a EndocLifecare Pvt. Ltd. Gujarat. All the Chemicals used of (RANKEM,INDIA). Solvents and solutions were fltered through a membrane flter (0.45 µm pore size) and degassed by sonication before use.

Instrumentation

The chromatographic analysis was performed on Waters Alliance HPLC system equipped with PDA detector. The output signals were monitored and processed using LC Solution

Chromatographic conditions

Mobile phase selection involved selection of solvent, selection of buffer, pH of buffer and ratio of buffer and solvent. The standard solutions of Lobeglitazone and Glimepiride were injected into the HPLC system and run in different solventsystem. Various ratios of mobile phase containing Methanol: Water, ACN: Water, Phosphate Buffer pH 4.0: Methanol, Phosphate Buffer pH 6.0: Methanol were tried in order to find the best conditions for the separation of both drugs. It was found that Methanol and Phosphate buffer pH 3.5 gives satisfactory result. Finally, Methanol: Potassium Dihydrogen Phosphate buffer pH 3.5 (70:30 %v/v) ratio was optimized as the mobile phase for the determination.pH was set by using 1% orthophosphoric acid.Injection volume was 20 µL , flow rate was 1.0 mL/min and the eluent was detected at 243 nm at column temperature 25 °C. These conditions showed sharp peak of Lobeglitazone and Glimepiride with retention time of 7.86 min and 10.03 min respectively.

software. The analytical column was Ultrasphere C18 (4.6 mm × 250 mm, 5 µ) and the samples were introduced through a injection valve with 20 µL sample loop.

Wavelength detection

25 mg of Lobeglitazone & 25 mg of Glimepiride take into 25 ml of volumetric flask separately and dissolved with diluent (Stock-1 Solution) (Lobeglitazone 1000µg/ml and Glimepiride 1000µg/ml). From that 1ml in 10ml volumetric flask separately (Stock-2 Solution) (Lobeglitazone 100µg/ml and Glimepiride 100µg/ml). From that 1ml in 10ml volumetric flask separately (Working standard Solution) (Lobeglitazone 10µg/ml and Glimepiride 10µg/ml). UV Spectra was taken between range of 200-400nm using UV-Visible Double beam spectrometer.Absorbance of both Lobeglitazone and Glimepiride was observed at 249nm and 230nm respectively.

Preparation of stock standard solution and sample

Stock solution: Weigh 25mg of Lobeglitazone and 50mg of Glimepiride. Transferred it to 2 different 50ml of volumetric flask and volume was made upto mark with diluent.[Standard stock solution of Lobeglitazone (500µg/ml) and Glimepiride (1000µg/ml)]. Further Dilution From each flask transfer 1ml solution into 10ml volumetric flask and dilute to the mark with diluent (Working Standard of Lobeglitazone 50µg/ml and Glimepiride 100µg/ml)(Fig. 3).

Sample solution: (Label claim: Lobeglitazone-0.5mg; Glimepiride-1mg)

Twenty tablets were weighed; average weight was calculated and tablets were powdered finely.Tablet Powder equivalent to 10 mg of Lobeglitazone and 20mg of Glimepiride were added into 20ml of volumetric flask Lobeglitazone (500µg/ml) and Glimepiride (1000µg/ml).Volume was made up to the mark with diluent.1ml of this solution was transferred to 10ml volumetric flask.Volume was made up to the mark with diluent, which gives Lobeglitazone (50µg/ml) and Glimepiride (100µg/ml).

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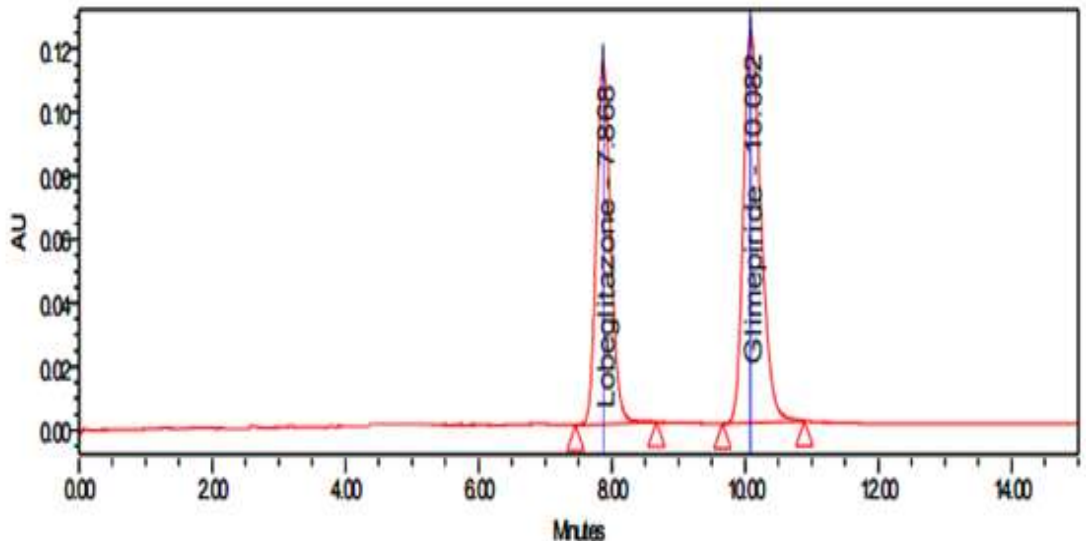

Fig. 3 Sharp peak of Lobeglitazone (50 µg/ml) and Glimepiride (100 µg/ml) by using Methanol: Potassium DihydrogenPhosphate Buffer pH 3.5 (70:30 % v/v) mobile phase

Analytical method validation

1. Specificity:

Demonstration of specificity is required to show that the procedure is unaffected by the presence of impurities or excipients. Specificity of an analytical method indicates that the analytical method is its able to measure accurately and specifically the analyte of interest without any interference from blank. So here, the specificity was determined by the comparison of the chromatograms of

- Blank (mobile phase).
- Standard solutions Lobeglitazone and Glimepiride.
- Sample solution of Lobeglitazone and Glimepiride.

2. Linearity:

The linearity for Lobeglitazone and Glimepiride was assessed by analysis of standard solution in range of 6.25-125µg/mL for Lobeglitazoneand 12.25-250µg/ml for Glimepiride. To obtain 6.25, 12.5, 25µg/mL of Lobeglitazone solution and 12.5, 25, 50µg/mL of Glimepiride solution pipetted out 1 ml from 500µg/mL of Lobeglitazone and 1 ml from 1000µg/mL of Glimepiride solution in 10 ml volumetric flask and make up with methanol uptomark.labelled this solution as solution 2 and from this solution pipetted out 1.25, 2.5, 5 ml in 10 ml volumetric flask and make up with methanol upto mark. To obtain 50, 70, 100, 125 µg/mL of Lobeglitazone and 100, 150, 200, 250µg/mL of Glimepiride pipetted out 1, 1.5, 2, 2.5 ml from 500µg/mL of Lobeglitazone and 1000µg/mL of Glimepiride solution in 10 ml volumetric flask and make up with methanol upto mark.

In term of slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted.(Fig. 4)

Acceptance criteria: value of r^2 should be nearer to 1 or 0.999.

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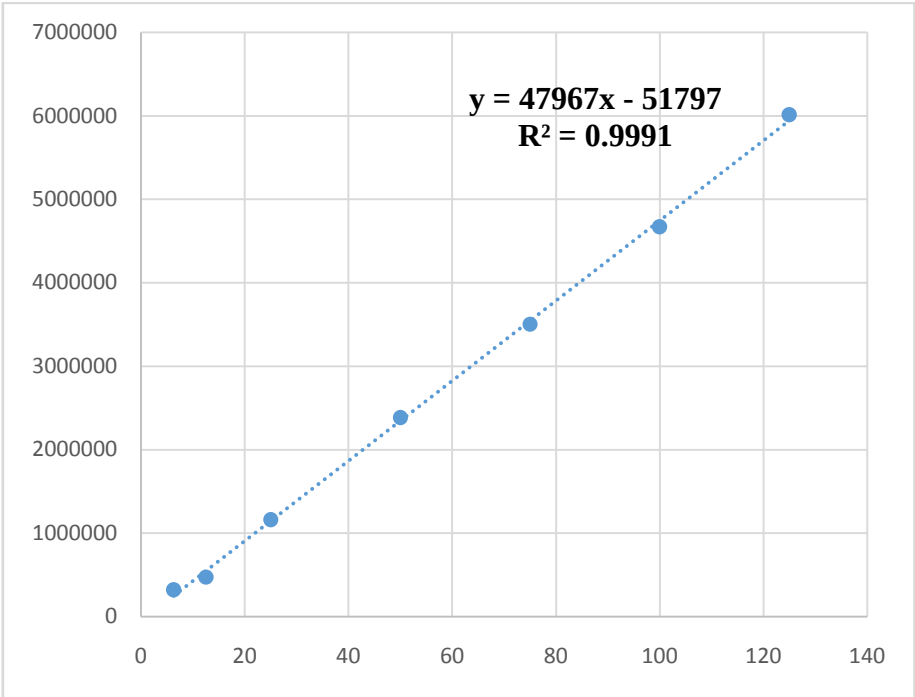

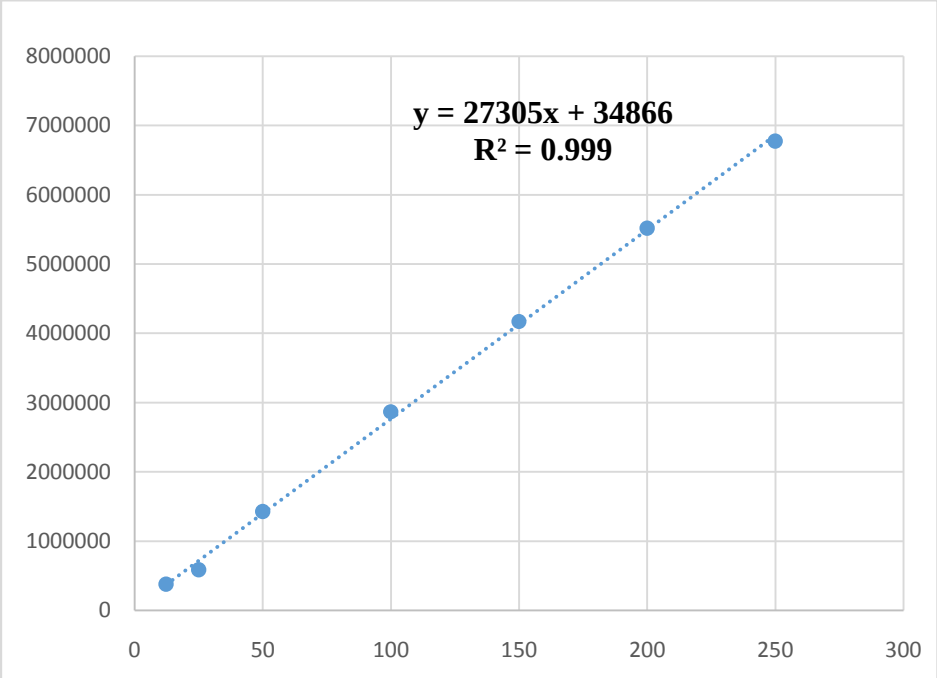

Fig.4 Calibration Curve of Lobeglitazone(50µg/ml) and Glimepiride (100µg/ml)

3. Precision:

Precision can be performed at two different levels: repeatability and intermediate precision. Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the samples during the same day. Repeatability was carried out using six replicates of the sample injection. Intra-day precision was determined by analyzing, the three different concentrations for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three consecutive days for inter-day precision. Results should be expressed as Relative standard deviation (RSD) or co-efficient of variance.

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A. Repeatability:

Standard solution containing Lobeglitazone and Glimepiride (50 and 100µg/ml respectively) was injected six times and areas of peaks were measured and RSD was calculated.

B. Interday Precision:

Standard solution containing Lobeglitazone and Glimepiride(25, 50, 75µg /ml) and 50, 100, 150µg/ml respectively) were injected three times in same day and areas of peaks were measured and RSD was calculated.

C. Intraday Precision:

Standard solution containing Lobeglitazone and Glimepiride (25, 50, 75µg /ml) and 50, 100, 150µg/ml respectively) were injected three times in different days and areas of peaks were measured and RSD was calculated.

Acceptance criteria: RSD of area should not be more than 2.0%.

4. Accuracy:

- **Preparation of Standard Stock Solution of Lobeglitazone and Glimepiride:**

Accurately weighed Lobeglitazone (25mg) was transferred into 50ml of volumetric flask and make upto the mark with diluent. (Lobeglitazone500µg/ml) Accurately weighed Glimepiride(50mg) was transferred into 50ml of volumetric flask and make upto the mark with diluent. (Glimepiride 1000µg/ml).

- **Preparation of Working Standard of Lobeglitazone and Glimepiride:**

From the above prepared solutions take 1 ml of Lobeglitazone stock solution and 1ml of Glimepiride stock solution in 10ml of volumetric flask and make upto the mark with diluent. (Lobeglitazone 50µg/ml and Glimepiride 100µg/ml).

- **Preparation of Sample for Recovery:**

Lobeglitazone and Glimepiride (50µg/ml and 100µg/ml respectively) drug solution was taken in three different flask labeled A, B and C. Spiked 50%, 100%, 150% of working standard solution in it and diluted up to 10ml. The area of each solution peak was measured.

The amount of Lobeglitazone and Glimepiride was calculated at each level and % recoveries were calculated.

5. Limit of detection (LOD) and limit of quantitation (LOQ)

Sensitivity of the proposed method was estimated in terms of LOD and LOQ. LOD is the lowest concentration in a sample that can be detected, but not necessarily quantified; under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision. In order to determine LOD and LOQ,

- The LOD was estimated from the set of 3 calibration curves used to determination linearity.

The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where, SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

- The LOQ was estimated from the set of 3 calibration curves used to determine linearity.

The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

6. Robustness:

Robustness of the method was studied by making small deliberate changes in few parameters.

- Lobeglitazone and Glimepiride (50 and 100µg/ml respectively) drug solution was taken and injected by applying little deliberate changes of the following method conditions and evaluated by RSD.

i. Column Temperature: ±1 °C
ii. Flow rate: ±0.1 ml/min
iii. Mobile phase pH : ±0.2

- **Acceptance criteria:**

- Number of theoretical plates for the analyte peak should not be less than 2000.
- Asymmetry value for the analyte peak should not be more than 2.0.
- RSD for the analyte peak should not be more than 2.0%.

7. Application of Method on Marketed Product:

- (Label claim: Lobeglitazone – 0.5mg; Glimepiride - 1mg)

Twenty tablets were weighed; average weight was calculated and tablets were powdered finely. Tablet Powder equivalent to 10mg of Lobeglitazone and 20 mg Glimepiride were added into 20 ml of volumetric flask. Volume was made up to the mark with diluent. 1 ml of this solution

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was transferred to 10 ml volumetric flask and volume was made up to the mark by diluent, which gives Lobeglitazone (50 µg/ml) and Glimepiride (100µg/ml). The quantification was carried out by keeping these values to be straight line equation of calibration curve.

8. System suitability test

System suitability testing is essential for the assurance of the quality performance of chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

FORCED DEGRADATION STUDIES

Degradation conditions

1) Hydrolysis- (a) Acid Hydrolysis -(b) Base Hydrolysis
2) Oxidative
3) Photolytic
4) Thermal

Preparation of Reagent:

- **0.1 N HCl Solution:** 0.85ml conc. Hydrochloric acid was taken in 100ml volumetric flask and volume was made upto the mark with water and mixed well.
- **0.1 N NaOH Solution:** 0.4gm of NaOH pellets were taken in 100ml volumetric flask and volume was made upto the mark with water and mixed well.
- **3% H_2O_2 Solution:**3ml of the 30% H_2O_2 solution was taken in 100ml volumetric flask and volume was made upto the mark with water and mixed well.

Acid Degradation:

- Transferring 1ml of stock solution of Lobeglitazone and Glimepiride into 10ml of volumetric flask.
- Add 2ml of 0.1N HCl solution
- Mixed well and kept for 1 hour at RT (25°C).
- The solution was neutralized with 2ml of 0.1N NaOH solution.
- Then the volume was adjusted with the diluent to get sample solution concentration (Lobeglitazone 50µg/ml and Glimepiride 100µg/ml).

Base Degradation:

- Transferring 1ml of stock solution of Lobeglitazone and Glimepirideinto 10ml of volumetric flask.
- Add 2ml of 0.1N NaOH solution
- Mixed well and kept for 1 hour at RT (25°C).
- The solution was neutralized with 2ml of 0.1N HCL solution.
- Then the volume was adjusted with the diluent to get sample solution concentration (Lobeglitazone 50µg/ml and Glimepiride100µg/ml).

Oxidative Degradation:

- Transferring 1ml of stock solution of Lobeglitazone and Glimepirideinto 10ml of volumetric flask.
- Add 2ml of 3% H_2O_2 solution
- Mixed well and kept for 1 hour at RT (25°C).
- Then the volume was adjusted with the diluent to get sample solution concentration (Lobeglitazone 50µg/ml and Glimepiride 100µg/ml).

Photo Degradation:

- Transferring 1ml of stock solution of Lobeglitazone and Glimepiride into petri dish.
- Then it was kept in UV chamber for 3 Days under 1.2 million lux h for visible light.
- Then the volume was adjusted and then diluted with the diluent to get working solution concentration (Lobeglitazone 50µg/ml and Glimepiride 100µg/ml).

Thermal Degradation:

- Lobeglitazone (25 mg) and Glimepiride (50mg) were taken in 50ml of volumetric flask and was kept in oven for 2 hour at 105°C temperature.
- Then after volumetric flask was removed and cooled down to room temp.
- Mobile phase was added to dissolve the drug and volume was made up with the diluent upto mark.
- 1ml of this solution was transferred in 10ml volumetric flask.
- Volume was made up with mobile phase to get working solution concentration (Lobeglitazone 50µg/ml and Glimepiride 100µg/ml).

II. RESULTS

To develop an accurate, precise and specific stabilityindicating RP-HPLC method for estimation of Lobeglitazone and Glimepiride using stressed samples, various mobile phases with different composition and flow rate were tried. After several compositions and permutations, chromatographic conditions were optimized and

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established. Satisfactory estimation of MUP with good peak symmetry and steady baseline was obtained with the mobile phase Methanol: Potassium Dihydrogen Phosphate buffer pH 3.5 (70:30 %v/v) at a flow rate of 1.0 mL/min. These conditions showed sharp peak of Lobeglitazone and Glimepiride with retention time of 7.86 min and 10.03 min respectively and all the system suitability parameters meet with the criteria table 1.

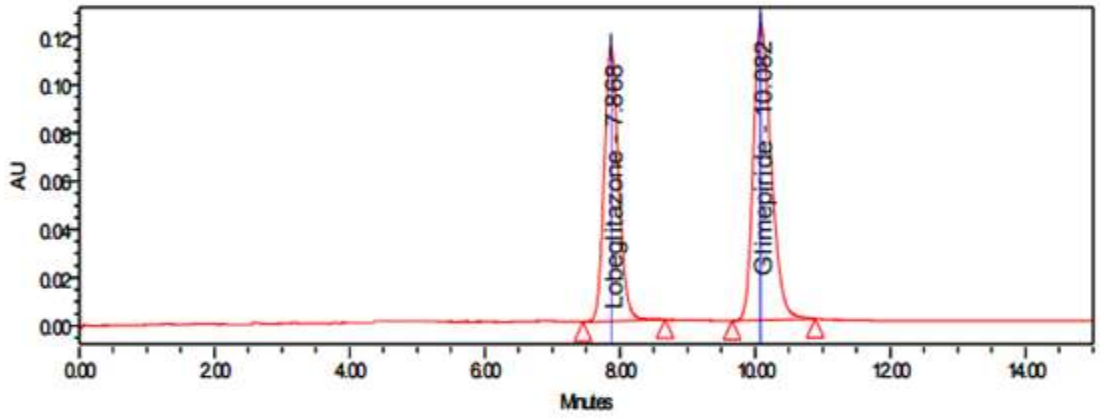

Table 1 System suitability parameters

Peak Name	Retention Time (min)	Area	Asymmetry	Theoretical Plate	Resolution	Purity angle	Purity Threshold
Lobeglitazone	7.868	2352486	1.14	6593	-	0.115	0.267
Glimepiride	10.082	2818534	1.30	7030	5.16	0.260	0.490

Linearity

The standard curve for Lobeglitazone and Glimepiride were linear over the investigated concentration range 6.25-125 µg/mL for Lobeglitazone and 12.25-250 µg/mL for Glimepiride with a percent relative standard deviation (% RSD) of not more than 2 based on seven successive readings. Correlation coefficient value should not be less than 0.995 for given range. Correlation coefficient value were found to be 0.9991 and 0.999 for Lobeglitazone and Glimepiride respectively, which is greater than 0.995. Hence, the method is linear within the range.

Precision

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision studies of proposed method were determined by repeatability, intra-day and inter-day precision. For the repeatability, RSD of the assay of six sample preparations should not be more than 2%. The obtained RSD was found to be 0.60 % and 1.16 % for Lobeglitazone and Glimepiride respectively which are well within the limit of acceptance criteria. While for the intermediate precision of the method, the same procedure was followed on a same day at specific interval and on different day. RSD for intraday precision were found to be in the range of 0.24-0.43 % and 0.28-0.57 % for Lobeglitazone and Glimepiride respectively. RSD for interday precision were found to be in the range of 0.19-0.40% and 0.25-0.60% for Lobeglitazone and Glimepiride respectively which also well within the limit of acceptance criteria and absolute difference between mean assay value of method precision and intermediate precision was found to be less than 2.0 % which is within the limit of acceptance criteria. Hence, the method can be termed as precise(table - 2,3,4,5).

Accuracy

The result of this study was found to be within the acceptance criteria of method validation (i.e. the recovery is 98% - 102% and the RSD is NMT 2.0%), this proves that the test method is

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accurate for the estimation of Lobeglitazone and Glimepiride (table-6,7).

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD for Lobeglitazone and Glimepiride was found to be 0.099µg/ml and 0.053µg/ml respectively. Similarly LOQ for Lobeglitazone and Glimepiride was found to be 0.300µg/ml and 0.161µg/ml respectively. The %assay results of 100.24% for Lobeglitazone and 101.94% for Glimepiride indicate that the developed method was successfully utilised for the estimation of Lobeglitazone and Glimepiride in their Tablet Formulation(table-8,9).

Robustness

The robustness study is used to demonstrate the method's efficiency in the face of purposeful changes in conventional method factors, such as Column temp. , flow rate, pH. The assay obtained following the changes suggested was compared to the assay obtained under normal conditions. The test difference should not be greater than 2%, according to the approval requirements. The gained outcomes are well within the acceptable ranges. As a result, the approach may be described as robust(Table- 10,11).

Assay

By taking the mean of three determinations, By RP-HPLC method %assay was found 100.24% and 101.94% for Lobeglitazone and Glimepiride respectively. So the developed method can be used for routine analysis, %RSD of drug was found to be within the limits. Thus, it can be concluded that there is no interference of the excipients.

Table 2 Repeatability data of Lobeglitazone

Lobeglitazone(50 µg/ml)				
Sr. No.	**Conc. (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**RSD**
1	50	2413275	2399463 ± 14528.02	0.60 %
		2399241		
		2418082		
		2388500		
		2379568		
		2398110		

Table 3 Repeatability data of Glimepiride

Glimepiride(100 µg/ml)				
Sr. No.	**Conc. (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**RSD**
1	100	2968996	2937239 ± 34214.75	1.16 %
		2973772		
		2953493		
		2916442		
		2885598		
		2925132		

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Ⅱ. Intraday Precision:

Table 4. Intraday Data for Lobeglitazone and Glimepiride

Lobeglitazone				Glimepiride		
SR.NO	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**RSD**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**RSD**
1	25	1159505± 3203.089	0.27	50	1391093 ± 6802.894	0.49
2	50	2375351± 10323.4	0.43	100	2821272 ± 7903.846	0.28
3	75	3535741± 8550.462	0.24	150	4151678 ± 23701.18	0.57

III.Interday Precision:

Table 5. Interday Data for Lobeglitazone and Glimepiride

Lobeglitazone				Glimepiride		
SR.NO	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**RSD**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**RSD**
1	25	1158264± 2758.516	0.24	50	1394480 ± 8423.605	0.60
2	50	2372958± 9527.132	0.40	100	2821428 ± 7279.185	0.26
3	75	3532484± 6755.312	0.19	150	4151047 ± 19765.39	0.47

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Accuracy:

Table 6. Recovery data for Lobeglitazone

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	RSD
1	50	50	25	25.356	100.47	0.61
2		50	25	24.498	99.33	
3		50	25	24.686	99.58	
1	100	50	50	50.696	100.69	0.27
2		50	50	50.765	100.76	
3		50	50	51.196	101.19	
1	150	50	75	74.889	99.91	1.56
2		50	75	73.113	98.49	
3		50	75	76.998	101.59	

Table 7 Recovery data for Glimepiride

SR. NO.	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	RSD
1	50	100	50	52.796	101.86	0.53
2		100	50	51.179	100.78	
3		100	50	52.255	101.50	
1	100	100	100	100.533	100.26	1.03
2		100	100	102.721	101.36	
3		100	100	104.746	102.37	
1	150	100	150	150.667	100.26	1.01
2		100	150	146.412	98.56	
3		100	150	150.890	100.35	

LOD AND LOQ:

Table 8. Limit of detection data for Lobeglitazone and Glimepiride

Lobeglitazone	Glimepiride
LOD = 3.3 x (SD / Slope) = 3.3 x (1442.126/47967) = 0.099µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (441.2282/27305) = 0.053µg/ml

Table 9. Limit of Quantitation data for Lobeglitazone and Glimepiride

Lobeglitazone	Glimepiride
LOQ = 10 x (SD / Slope) =10 x (1442.126/47967) = 0.300µg/ml	LOQ = 10 x (SD / Slope) =10 x (441.2282/27305) = 0.161µg/ml

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Robustness:

Table 10. Robustness data for Lobeglitazone

SR NO.	Area at Column Temp. -1 °C	Area at Column Temp. +1 °C	Area at Flow rate (-0.1 ml/min	Area at Flow rate (+0.1ml/min)	Area at pH (-0.2)	Area at pH (+0.2)
1	2414285	2344807	2451486	2338001	2448148	2353379
2	2454586	2325919	2441110	2337680	2458651	2350803
3	2462157	2382145	2411536	2362459	2422986	2341107
AVG. Area	2443676	2350957	2434711	2346047	2443262	2348430
SD	25733.31	28613.07	20729.55	14214.4	18327.72	6471.092
RSD	1.05	1.21	0.85	0.60	0.75	0.27

Condition		Mean Area	Mean	SD	RSD
Column Temp.	24	2443676			
	25	2393427			
	26	2350957	2396020	46413.86475	1.93
Flow rate (ml/min)	0.9	2434711			
	1	2393427			
	1.1	2346047	2391395	44366.91	1.85
pH of Mobile phase	.3	2443262			
	3.5	2393427			
	3.7	2348430	2395039	47436.56	1.98

Table 11. Robustness data for Glimepiride

SR NO.	Area at Column Temp. -1 °C	Area at Column Temp. +1 °C	Area at Flow rate (-0.1 ml/min	Area at Flow rate (+0.1ml/min)	Area at pH (-0.2)	Area at pH (+0.2)
1	2939101	2835521	2928827	2838025	2945675	2830657
2	2926405	2836622	2914399	2819311	2932564	2834752
3	2961110	2835528	2967662	2819566	2952648	2850635
AVG. Area	2942205	2835890	2936963	2825634	2943629	2838681
SD	17559.53	633.6516	27547.75	10731.68	10197.12	10552.72
RSD	0.59	0.02	0.93	0.37	0.34	0.37

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Condition		Mean Area	Mean	SD	RSD
Column Temp.	24	2942205			
	25	2868470			
	26	2835890	2882189	54468.97173	1.88
Flow rate (ml/min)	0.9	2936963			
	1	2868470			
	1.1	2825634	2877022	56154.91	1.95
pH of Mobile phase	.3	2943629			
	3.5	2868470			
	3.7	2838681	2883593	54083.66	1.87

Degradation studies

The chromatograms obtained from samples exposed to acidic, alkaline, oxidative and photodegradation depicted well-separated peaks of pure Lobeglitazone and Glimepiride having t_R 7.796 min and 10.512 min respectively also some additional peaks at different values. The % of degradation products with their t_R values is listed in Table 12 and Figure 4,5,6,7,8.

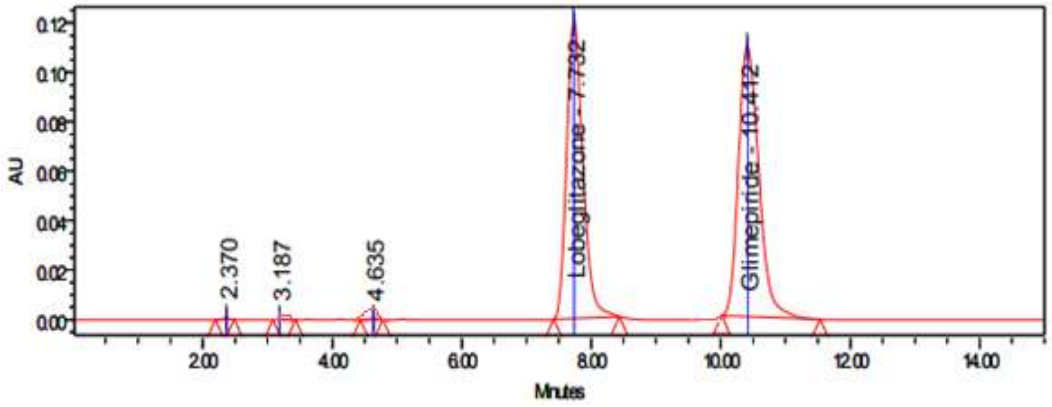

Fig.4. Chromatogram of Standard Lobeglitazone (50µg/ml) and Glimepiride(100µg/ml) for Acid Degradation

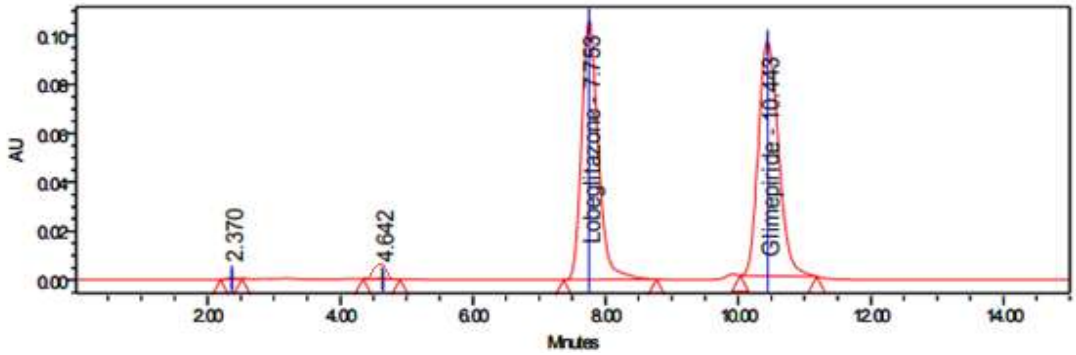

Fig. 5. Chromatogram of Standard Lobeglitazone(50µg/ml) and Glimepiride(100µg/ml) for Base Degradation

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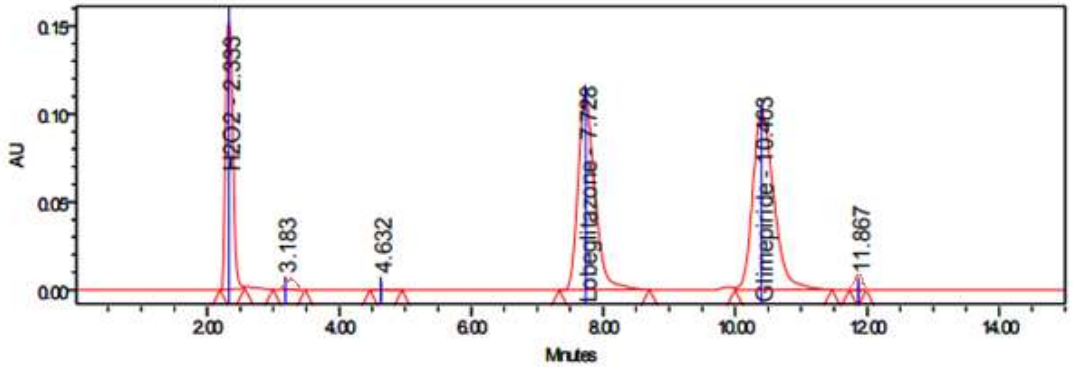

Fig. 6. Chromatogram of Standard Lobeglitazone(50µg/ml) and Glimepiride(100µg/ml) for Oxidative Degradation

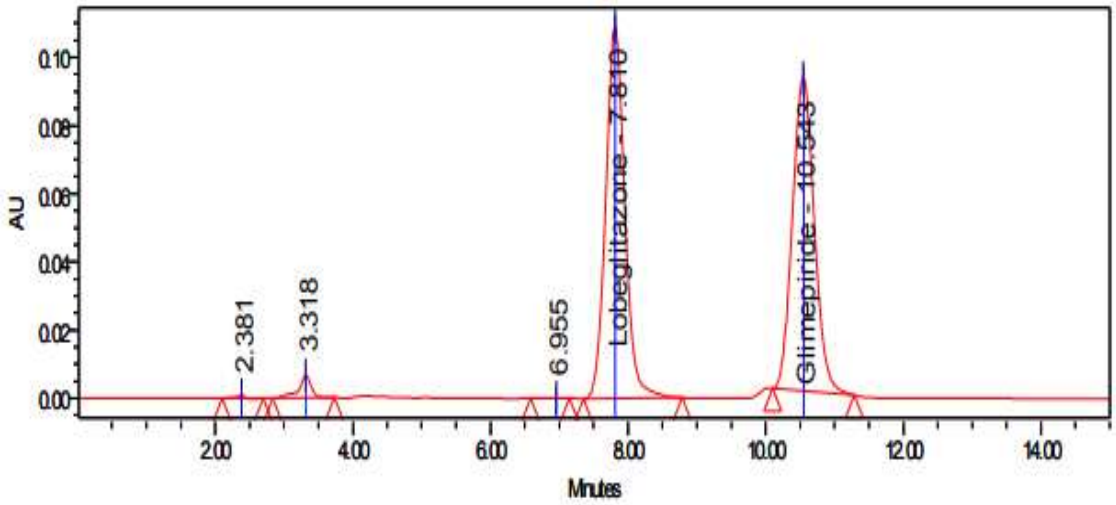

Fig. 7.Chromatogram of Standard Lobeglitazone(20µg/ml) and Glimepiride(40µg/ml) for Photo Degradation

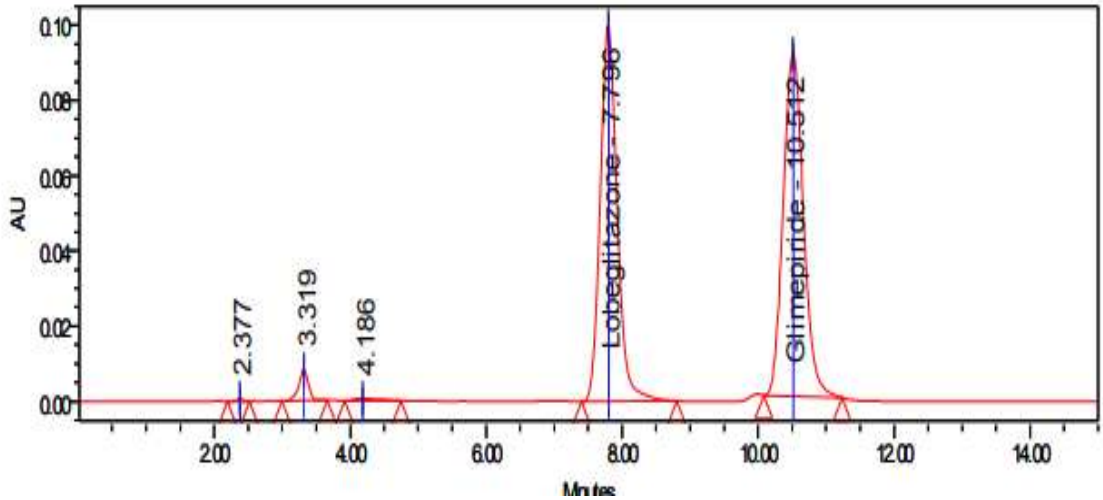

Fig. 8. Chromatogram of standard Lobeglitazone(50µg/ml) and Glimepiride(100µg/ml) for Thermal Degradation

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Table 12.Summary of Forced Degradation of Standard

Sr. No.	Types of Degradation	Condition	Duration	Solution	Area	%Degradation
1	Acid Degradation	0.1 N HCL	1 Hour	Lobeglitazone	2045185	12.11
				Glimepiride	2325421	12.02
2	Base Degradation	0.1 N NaOH	1 Hour	Lobeglitazone	1854661	20.30
				Glimepiride	2114870	19.99
3	Oxidative Degradation	3% H_2O_2	1 Hour	Lobeglitazone	1956547	15.92
				Glimepiride	2235178	15.43
4	Photo Degradation	-	7 Days	Lobeglitazone	2090974	10.14
				Glimepiride	2132925	19.30
5	Thermal Degradation	105 °C	2 Hour	Lobeglitazone	1732673	25.54
				Glimepiride	1901009	28.08

III. SUMMARY:

The combination of Lobeglitazone and Glimepiride has been approved by CDSCO on 23 May 2023.

Glenmark Pharmaceuticals has launched tablet formulation with combination of two drugs Lobeglitazone and Glimepiride under the brand name "LOBG-G1" for treatment of Type-2 Diabetes Mellitus.

Lobeglitazone is not official in any Pharmacopoeia and Glimepiride is official in Indian, United states, British and European Pharmacopoeia. An approach of forced degradation study was successfully applied for the development of stability indicating assay method for simultaneous estimation of Lobeglitazone and Glimepiride combined dosage form in presence of its degradation products. The method has shown adequate separation of main peaks from their associated degradation products. Separation was achieved on Ultrasphere-C18 RP column, 250 mm × 4.6 mm, 5 µm, using a mobile phase Methanol : Potassium Dihydrogen Phosphate Buffer pH 3.5 (70:30 %v/v), Adjust pH 3.5 using 1%OPA at a flow rate of 1 ml/min and UV detection was carried out at 243 nm.

In the present study, comprehensive stress testing of both drug in combined dosage form was carried out according to ICH guideline Q1A (R2). The specificity of the method was determined by assessing interference from blank and by forced degradation.

Specificity of the method was established by determining that peaks are separated well so there is no co-elution of any degradation products with main peaks and the results obtained were found within the acceptance criteria. Hence, the method can be termed as specific.

For the linearity, correlation coefficient value should not be less than 0.995 for given range. Correlation coefficient value were found to be 0.9991 and 0.999 for Lobeglitazone and Glimepiride respectively, which is greater than 0.995. Hence, the method is linear within the range.

Accuracy was determined over the range from lowest sample concentration to highest concentration (i.e. at 50%, 100% and 150%). According to acceptance criteria individual % recovery should be in the range of 98-102%. The results show that the % recoveries for Lobeglitazone and Glimepiride were found to be 99.79-100.00 % and 99.72-101.38 % respectively which is well within the acceptance criteria. Hence, the method can be termed as accurate.

In order to show the precision of the method, repeatability and intermediate precision were carried out. For the repeatability, RSD of the assay of six sample preparations should not be more than 2%. The obtained RSD was found to be 0.60 % and 1.16 % for Lobeglitazone and Glimepiride respectively which are well within the limit of acceptance criteria. While for the intermediate precision of the method, the same procedure was followed on a same day at specific interval and on different day. RSD for intraday precision were found to be in the range of 0.24-0.43 % and 0.28-0.57 % for Lobeglitazone and Glimepiride respectively. RSD for interday precision were found to be in the range of 0.19-0.40% and 0.25-0.60% for Lobeglitazone and Glimepiride respectively which also well within the limit of acceptance criteria and absolute difference between mean assay value of method precision and intermediate precision was found to be less than 2.0 % which is within the limit of acceptance criteria. Hence, the method can be termed as precise.

The LOD for Lobeglitazone and Glimepiride was found to be 0.099µg/ml and 0.053µg/ml respectively. Similarly LOQ for Lobeglitazone and Glimepiride was found to be

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0.300µg/ml and 0.161µg/ml respectively. The %assay results of 100.24% for Lobeglitazone and 101.94% for Glimepiride indicate that the developed method was successfully utilised for the estimation of Lobeglitazone and Glimepiride in their Tablet Formulation.

The robustness study is used to demonstrate the method's efficiency in the face of purposeful changes in conventional method factors, such as Column temp. , flow rate, pH. The assay obtained following the changes suggested was compared to the assay obtained under normal conditions. The test difference should not be greater than 2%, according to the approval requirements. The gained outcomes are well within the acceptable ranges. As a result, the approach may be described as robust.

As its results for all validation parameters are well within the limit of acceptance criteria, the technique may be regarded validated as suitable for intended purpose.

So, During stability studies on Lobeglitazone and Glimepiride, the suggested stability indicating RP-HPLC method was effectively employed for the simultaneous assessment of both drugs in combination dosage form in the presence of degradation products.

IV. CONCLUSION:

From above observations, it can be concluded that developed Stability indicating method and validation of Lobeglitazone and Glimepiride in tablets by RP-HPLC is, specific, linear, accurate, precise and robust. Thus above developed RP-HPLC method can be applied for routine analysis.

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LIST OF ABBREVATION AND ACRONYMS

Symbols	
λmax	Wavelength of maximum absorbance
r 2	Correlation coefficient
°C	Degree Celsius
g	Gram
µg	Microgram
mg	Miligram
ml	Mililiter
cm	Centimeter
mm	Milimeter
nm	Nano meter
min.	Minute
%	Percentage
±	Plus or Minus
<	Less than
>	Greater than
≥	Greater than or Equal to
≤	Less than or Equal to
OTHERS	
UV	Ultraviolet
DM	Diabetes Mellitus
HPLC	High Performance Liquid Chromatography

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RP-HPLC	Reverse Phase High Performance Liquid Chromatography
Tr	Retention time
K’	Capacity Factor
Vr	Retention Volume
V0	Void Volume
Rs	Resolution
N	Theoretical Plates
SIM	Stablilty Indicating Method
API	Active Pharmaceutical Ingredients
FDA	Food and Drug Administration
CDSCO	Central Drug Standard Control Organisation
ICH	International Conference of Harmonisation
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide
H2O2	Hydrogen Peroxide
%RSD	Relavative Standard Deviation
SD	Standard Deviation
LOD	Limit of Detection
LOQ	Limit of Quanititation
IUPAC	International Union of Pure & Applied Chemistry
ODS	Octadecyl Silica
MP-A	Mobile Phase-A
MP-B	Mobile Phase-B
ACN	Acetonitrile
CAS	Chemical Abstract Number
pKa	Ionization Constant
%v/v	% Volume by Volume
Log P	Partition Co-efficient
WS	Working Standard
IR	Infrarred
Pvt. Ltd	Private Limited
N	Normality
RT	Room Temperature