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

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ABSTRACT

Background: A simple, rapid, sensitive and selective stability-indicating (RP-HPLC) method is suggested for the determination of Lobeglitazone and Metformin in pharmaceutical formulation. Lobeglitazone and Metformin was eluted from a Cosmosphere C18 (250×4.6 mm, 5 µm) column with mobile phase consisting of methanol, Acetonitrile and Potassium Dihydrogen Phosphate Buffer 2.5 pH (70:5:25 %v/v/v) pH adjusted to 2.5 with Orthophosphoric acid. The gradient was optimized with a flow rate of 0.8 mL/min and a wavelength of 250 nm.

Result: 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 0.5-0.25µg/ml µg/mL of working concentration of Lobeglitazone and 50-250µg/ml µg/mL of working concentration of Metformin with linear regression curve (R^2 = 0.999) with limits of detection (LOD) and quantitation (LOQ) being 0.0010 and 0.0031 µg/mL for Lobeglitazone and 0.30 and 0.91 µg/mL for Metformin respectively. The retention time for Lobeglitazone was 8.37 min and for Metformin was 2.83 min. The method shows good recoveries and intra-day and inter-day relative standard deviations were less than 2%. Validation parameters as ruggedness and robustness were 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.

Conclusion: The developed HPLC method could be successfully used for the estimation of Lobeglitazone and Metformin in pharmaceutical formulation. The high recovery and low relative standard deviation confirm the suitability of proposed method that can be employed for the routine analysis in bulk and pharmaceutical formulation.

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

I. BACKGROUND OF LOBEGLITAZONE AND METFORMIN

Lobeglitazone IUPAC name 5-[(4-[2-([6-(4-Methoxyphenoxy) pyrimidin-4-yl]-methylamino)ethoxy]phenyl)methyl]-1,3-thiazolidine-2,4-dione. Chemical formula C24H24N4O5S (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 hemoglobin A1C levels, and improve lipid and liver profiles. Metformin IUPAC name N, N-Dimethylimidodicarbonimidic diamide. Chemical formula $C_4H_{12}ClN_5$ (Fig.2). It reduces glucose absorption from the intestines, lowers liver glucose production, and improves insulin sensitivity. Metformin is recommended with dietary changes and exercise for better results. Managing blood sugar levels with medications like metformin can prevent complications such as kidney damage, nerve issues, blindness, amputations. This combination approved by CDSCO in the year 2022 for the treatment of Type-2 Diabetes Mellitus and available as LOBG-M in the market

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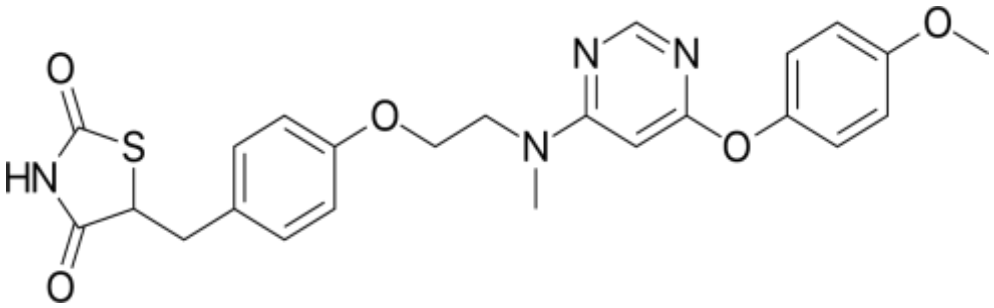

Fig.1 Chemical structure of Lobeglitazone

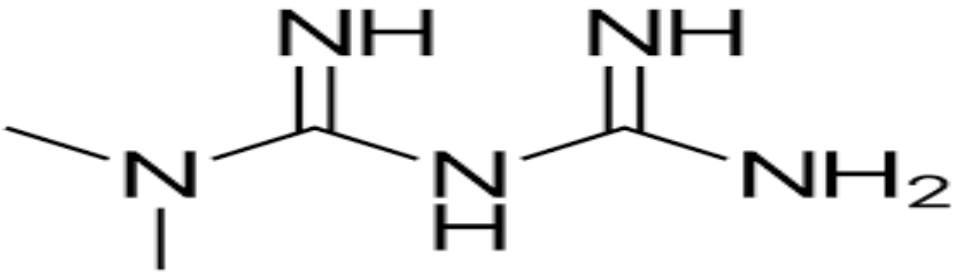

Fig.2 Chemical structure of Metformin

FORCED DEGRADATION:

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 Metformin in accordance with ICH guidelines using any of the above analytical techniques. High performance liquid chromatography (RP-HPLC) for analysis of Lobeglitazone and Metformin in pharmaceutical formulation. This paper describes an accurate, specific, repeatable, and stability-indicating method for analysis of Lobeglitazone and Metformin 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 stability indicating method can be defined as Validated Quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and drug product, and that are specific so that the content of active ingredient, degradation can be accurately measured without interference.

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 proposed storage conditions, re-analysis periods and shelf life.

METHOD DEVELOPMENT

Reagents and chemicals

Lobeglitazone was supplied as a gift sample by a Allastir Pvt. Ltd. Chennai and Metformin was by a Endoc Lifecare Pvt. Ltd. Gujarat. All the Chemicals used of (RANKEM, INDIA). Solvents and solutions were filtered through a membrane filter (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 software. The analytical column was Cosmosphere C18 (4.6 mm × 250 mm, 5 µ) and the samples were introduced through an injection valve with 10µL sample loop.

Wavelength detection

25 mg of Lobeglitazone & 25 mg of Metformin take into 25 ml of volumetric flask separately and dissolved with diluent (Stock-1 Solution) (Lobeglitazone 1000µg/ml and Metformin 1000µg/ml). From that 1ml in 10ml volumetric flask separately (Stock-2 Solution) (Lobeglitazone 100µg/ml and Metformin 100µg/ml). From that 1ml in 10ml volumetric flask separately (Working standard Solution) (Lobeglitazone 10µg/ml and Metformin 10µg/ml). UV Spectra was taken between range of 200-400nm using UV- Visible Double beam spectrometer. Absorbance of both Lobeglitazone and Metformin was observed at 250nm and 234nm respectively.

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 Metformin were injected into the HPLC system and run in different solvent system. 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, ACN and Phosphate buffer pH 2.5 gives satisfactory result. Finally, Methanol: ACN: Potassium Dihydrogen Phosphate buffer pH 2.5 (70:5:25%v/v/v) ratio was optimized as the mobile phase for the determination. pH was set by using 1% orthophosphoric acid. Injection volume was 10µL, flow rate was 0.8mL/min and the eluent were detected at 250 nm at column temperature 25 °C. These conditions showed sharp peak of Lobeglitazone and Metformin with retention time of 8.37 min and 2.83 min respectively.

Preparation of stock standard solution and sample

Stock solution: Weigh 5mg of Lobeglitazone and transferred it to 50ml of volumetric flask and makeup to the given mark with methanol (stock solution-1; 100µg/ml) Further from stock-1, take 0.5 ml in 50ml flask and makeup with methanol (Standard stock solution-2; 1µg/ml). Weigh 50mg of Metformin. Transferred it to 50ml of volumetric flask and makeup to the given mark with methanol (Standard stock solution 1000µg/ml). Take 1ml from Lobeglitazone standard stock-2 solution and 1 ml from Metformin stock solution into 10ml volumetric flask and makeup to the given mark with diluent. Lobeglitazone (0.1µg/ml) Metformin (100µg/ml). (Fig. 3)

Sample solution: (Label claim: Lobeglitazone-0.5mg; Metformin-500mg)

Twenty tablets were weighed; average weight was calculated and tablets were powdered finely. Tablet Powder equivalent to 0.5mg of Lobeglitazone and 500mg of Metformin were added into 100ml of volumetric flask Lobeglitazone (5µg/ml) and Metformin (5000µg/ml). Volume was made up to the mark with Methanol. The solution is then sonicated for 20mins and further the solution is filtrated. 1ml of each above solution of Lobeglitazone and Metformin was transferred to 10ml volumetric flask. Volume was made up to the mark with diluent, which gives Lobeglitazone and Metformin.

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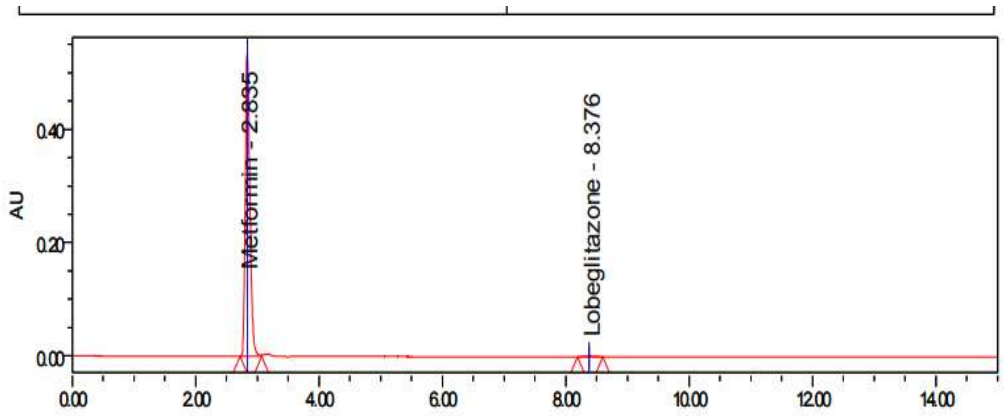


Fig. 3 Lobeglitazone (0.1µg/ml) and Metformin (100µg/ml) by using Methanol: ACN: Potassium Dihydrogen Phosphate Buffer pH 2.5 (70:5:25 % v/v/v) mobile phase

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

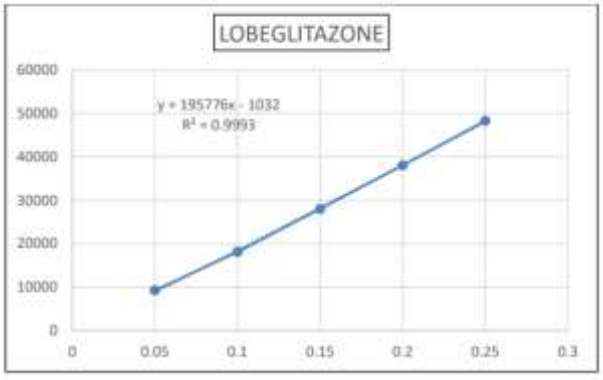
- Blank (mobile phase).
- Standard solutions Lobeglitazone and Metformin.
- Sample solution of Lobeglitazone and Metformin.

2. Linearity:

The linearity for Lobeglitazone and Metformin was assessed by analysis of standard solution in range of 0.05-0.25µg/mL for Lobeglitazone and 50 – 250µg/mL for Metformin. To obtain Lobeglitazone 0.05, 0.1, 0.15, 0.2, 0.25µg/mL; 1, 0.5, 1.5, 2, 2.5ml is pipetted out from standard stock solution(1µg/mL) into 10ml volumetric flask and further volume was adjusted with diluent to the mark. Similarly, to obtain Metformin 50, 100, 150, 200, 250µg/mL; 0.5, 1, 2, 1.5, 2.5ml is pipetted out from standard stock solution(100µg/ml) into 10ml volumetric flask and further volume was adjusted with diluent to the 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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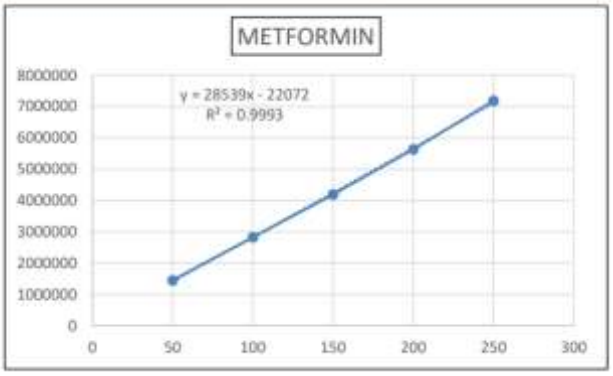

Fig.4 Calibration Curve of Lobeglitazone (0.05-0.25µg/ml) and Metformin (50-250µg/ml)

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.

A. Repeatability:

Standard solution containing Lobeglitazone and Metformin (0.1 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 Metformin (0.05, 0.1, 0.15µg /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 Metformin (0.05, 0.1, 0.15µg /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 Metformin:** Accurately weighed Lobeglitazone (5mg) was transferred into 50ml of volumetric flask and make up to the mark with diluent (Lobeglitazone 100µg/ml). From this, transfer 1ml into 10 ml volumetric flask and make up to mark with diluent (Lobeglitazone 1µg/ml). Accurately weighed Metformin (50mg) was transferred into 50ml of volumetric flask and make up to the mark with diluent. (Metformin 1000µg/ml)
-
- **Preparation of Working Standard of Lobeglitazone and Metformin:**

From the above prepared solutions; take 1ml of Lobeglitazone stock solution and 1ml of Metformin stock solution in 10ml of volumetric flask and make up to the mark with diluent. (Lobeglitazone 0.1µg/ml and Metformin 100µg/ml).

- **Preparation of Sample for Recovery:**

Lobeglitazone and Metformin (0.1µg/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 Metformin 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;

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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 Metformin (0.1 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: ±0.1

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; Metformin - 500mg)

Twenty tablets were weighed; average weight was calculated and tablets were powdered finely. Tablet Powder equivalent to 0.5mg of Lobeglitazone and 500mg of Metformin were added into 100ml of volumetric flask Lobeglitazone (5µg/ml) and Metformin (5000µg/ml). Volume was made up to the mark with Methanol. The solution is then sonicated for 20mins and further the solution is filtrated. 1ml of each above solution of Lobeglitazone and Metformin was transferred to 10ml volumetric flask. Volume was made up to the mark with diluent, which gives Lobeglitazone (0.1µg/ml) and Metformin (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 up to 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 up to the mark with water and mixed well.
- **3% H2O2 Solution:** 3ml of the 30% H2O2 solution was taken in 100ml volumetric flask and volume was made up to the mark with water and mixed well.

Acid Degradation:

- Transferring 1ml of stock solution of Lobeglitazone and Metformin 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 0.1µg/ml and Metformin 100µg/ml).

Base Degradation:

- Transferring 1ml of stock solution of Lobeglitazone and Metformin into 10ml of volumetric flask.
- Add 2ml of 0.1N NaOH solution
- Mixed well and kept for 1 hour at RT (25°C).

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- 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 0.1µg/ml and Metformin 100µg/ml).

Oxidative Degradation:

- Transferring 1ml of stock solution of Lobeglitazone and Metformin into 10ml of volumetric flask.
- Add 2ml of 3% H2O2 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 0.1µg/ml and Metformin 100µg/ml).

Photo Degradation:

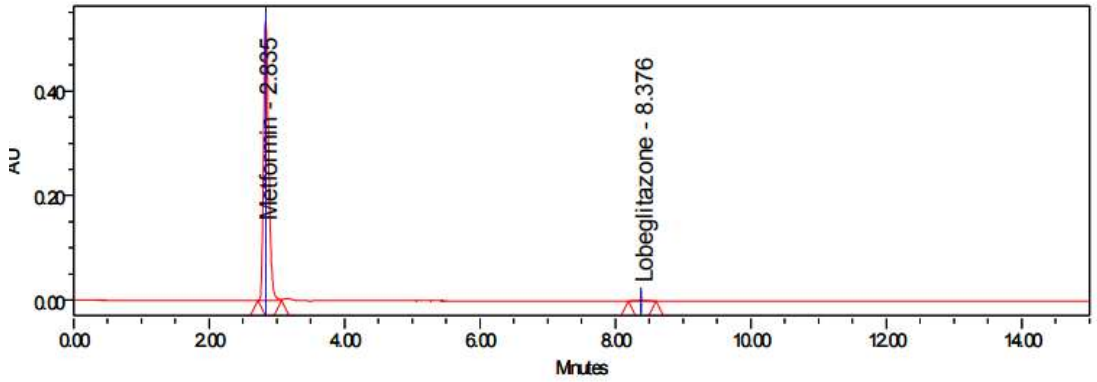
- Transferring 1ml of stock solution of Lobeglitazone and Metformin into petri dish.
- Then it was kept in UV chamber for 3 Days under 1.2 million lux h for visible light.
- Then the volume was adjusted and then diluted with the diluent to get working solution concentration (Lobeglitazone 0.1µg/ml and Metformin 100µg/ml).

Thermal Degradation:

- Lobeglitazone (25 mg) and Metformin (50mg) were taken in 50ml of volumetric flask and was kept in oven for 2 hours 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 up to mark.
- 1ml of this solution was transferred in 10ml volumetric flask.
- Volume was made up with mobile phase to get working solution concentration (Lobeglitazone 0.1µg/ml and Metformin 100µg/ml).

II. RESULTS

To develop an accurate, precise and specific stability indicating RP-HPLC method for estimation of Lobeglitazone and Metformin using stressed samples, various mobile phases with different composition and flow rate were tried. After several compositions and permutations, chromatographic conditions were optimized and established. Satisfactory estimation of MUP with good peak symmetry and steady baseline was obtained with the mobile phase Methanol: ACN: Potassium Dihydrogen Phosphate buffer pH 2.5 (70:5:25 %v/v/v) at a flow rate of 0.8 mL/min. These conditions showed sharp peak of Lobeglitazone and Metformin with retention time of 8.37 min and 2.83 min respectively and all the system suitability parameters meet with the criteria table-1.



Peak Results

	Name	RT	Area	Height	USP Tailing	USP Plate Count	Symmetry Factor	Purity1 Threshold	Purity1 Angle	Resolution
1	Metformin	2.835	2841062	536083	1.33	6679	1.33	0.637	0.219	
2	Lobeglitazone	8.376	16784	1370	1.06	10154	1.06	3.014	2.397	23.33

Table 1 System Suitability Parameters

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METHOD VALIDATION SUMMARY

Parameters		Lobeglitazone	Metformin
Specificity		Specific	
Linearity		0.05-0.25 µg/ml	50 – 250 µg/ml
Precision (RSD)	**Repeatability**	1.35	0.73
	Intraday	0.56-1.29	0.51-0.94
	Interday	0.51-1.29	0.53-0.92
Accuracy	**50%**	100-101.06	98.37-99.55
	100%	98.44-100.25	98.93-99.18
	150%	100.46-101.65	99.80-101.03
Robustness		The system suitability parameters were found well within the acceptance criteria as per system suitability.	
Limit of Detection		0.0010µg/ml	0.3019µg/ml
Limit of Quantitation		0.0031µg/ml	0.9151µg/ml
% Assay		99.41 %	100.40 %

Degradation Studies

The chromatograms obtained from samples exposed to acidic, alkaline, oxidative and photo degradation depicted well-separated peaks of pure Lobeglitazone and Metformin having tR 8.21 min and 2.89 min respectively also some additional peaks at different values. The % of degradation products with their tR values is listed in Table 12 and Figure 5,6,7,8,9.

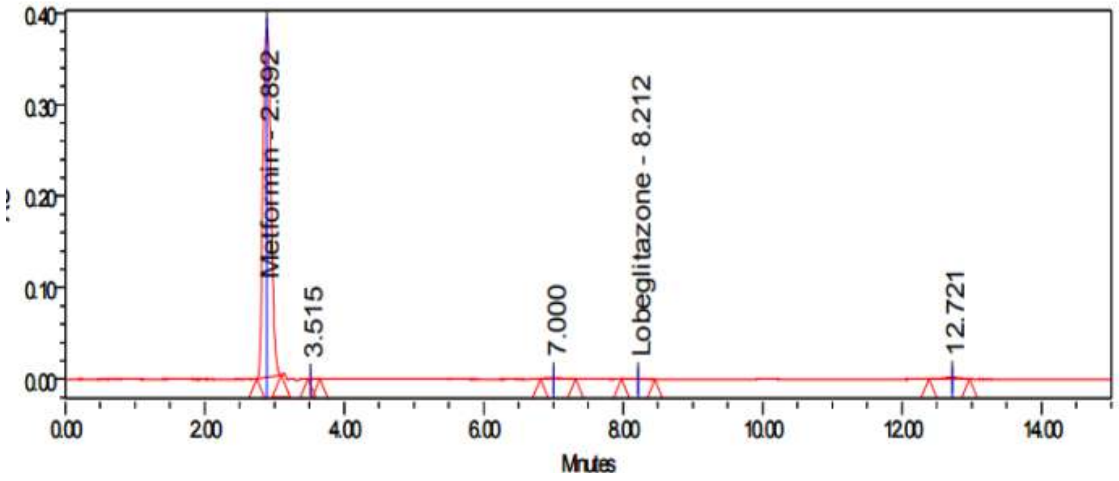

Fig.5. Chromatogram of Standard Lobeglitazone (0.1µg/ml) and Metformin(100µg/ml) for Acid Degradation

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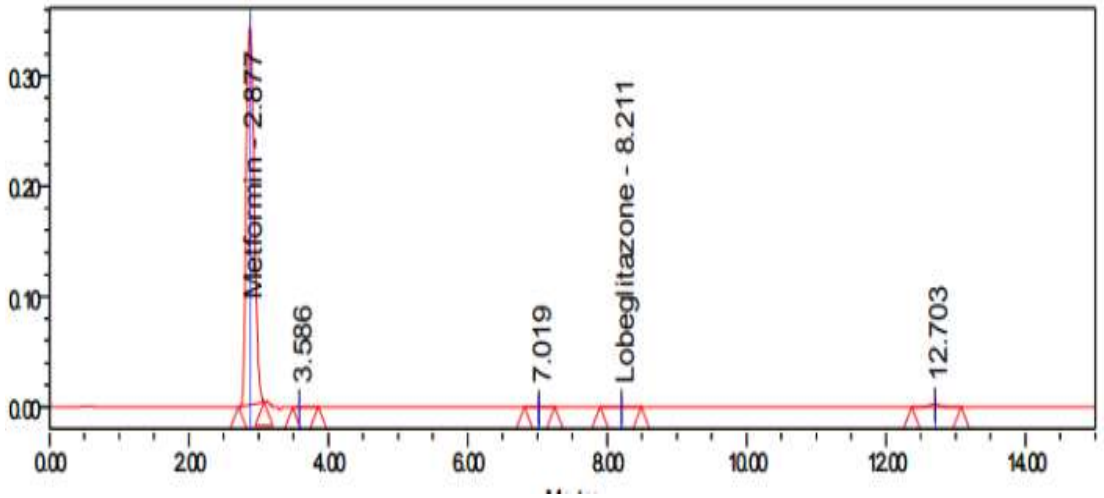

Fig. 6. Chromatogram of Standard Lobeglitazone(0.1µg/ml) and Metformin(100µg/ml) for Base Degradation

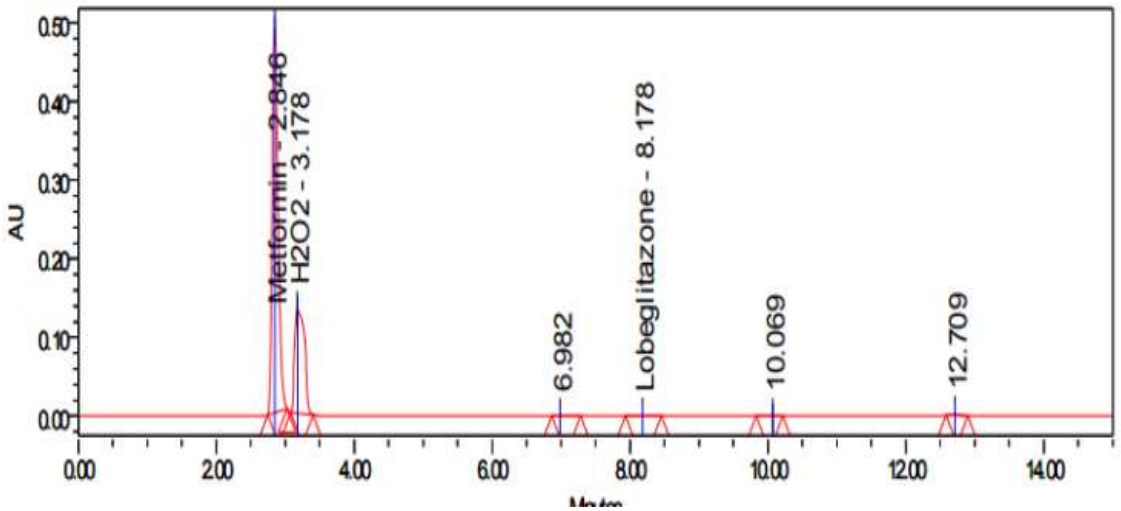

Fig. 7. Chromatogram of Standard Lobeglitazone(0.1µg/ml) and Metformin(100µg/ml) for Oxidative Degradation

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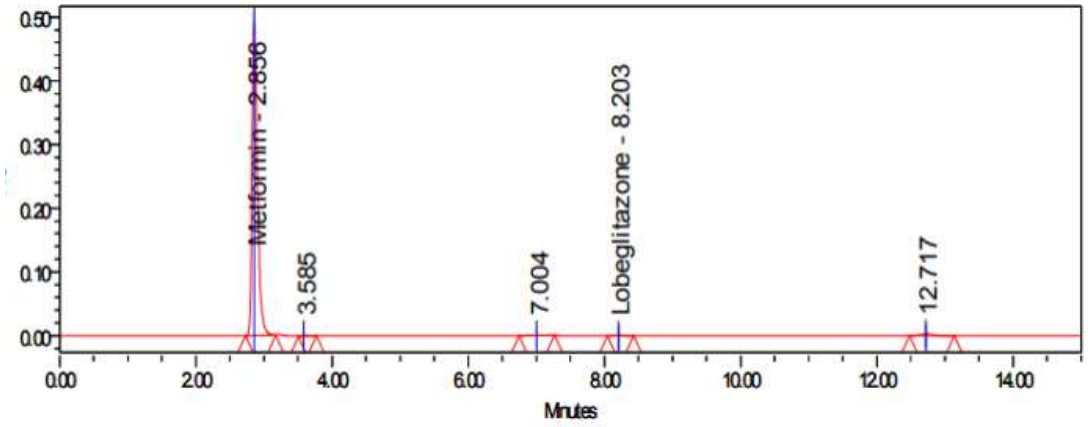

Fig. 8. Chromatogram of Standard Lobeglitazone(0.1µg/ml) and Metformin(100µg/ml) for Photo Degradation

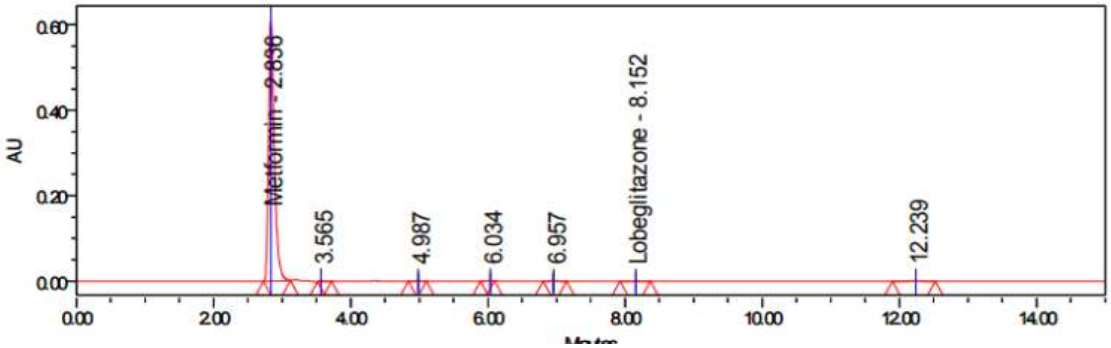

Fig. 9. Chromatogram of standard Lobeglitazone(0.1µg/ml) and Metformin(100µg/ml) for Thermal Degradation

SUMMARY OF FORCED DEGRADATION STUDIES

Sr. No.	Types of Degradation	Condition	Duration	Solution	Area	%Degradation
1	Acid Degradation	0.1 N HCL	1 Hour	Lobeglitazone	5882	18.03
				Metformin	3010166	22.51
2	Base Degradation	0.1 N NaOH	1 Hour	Lobeglitazone	5871	17.19
				Metformin	2747457	29.97
3	Oxidative Degradation	3% H_2O_2	1 Hour	Lobeglitazone	5191	26.78
				Metformin	2995794	22.88
4	Photo Degradation	-	18 Hours	Lobeglitazone	6117	13.72
				Metformin	2767531	28.75
5	Thermal Degradation	-	2 Hour	Lobeglitazone	5800	18.19
				Metformin	3421937	11.91

III. SUMMARY:

The combination of Lobeglitazone and Metformin has been approved by CDSCO on 30 December 2022.

Glenmark Pharmaceuticals has launched tablet formulation with combination of two drugs Lobeglitazone and Metformin under the brand name “LOBG-M” for treatment of Type-2 Diabetes Mellitus.

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Lobeglitazone is not official in any Pharmacopoeia and Metformin 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 Metformin 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 Cosmosil C18 RP column, 250 mm × 4.6 mm, 5 µm, using a mobile phase Methanol: ACN: Phosphate Buffer pH2.5 (70:5:25 v/v/v), Adjust pH 2.5 using 1% OPA at a flow rate of 0.8 ml/min and UV detection was carried out at 250 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 was found to be 0.999 and 0.999 for Lobeglitazone and Metformin 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 Metformin were found to be 99.54-101.25 % and 100.82- 101.096% 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 1.35 and 0.73 for Lobeglitazone and Metformin 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 0.56-1.29 and 0.51-0.94 for Lobeglitazone and Metformin respectively. RSD for Interday precision were found to be in the range of 0.51-1.29 and 0.53-0.92 for Lobeglitazone and Metformin 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 Metformin was found to be 0.076µg/ml and 0.021µg/ml respectively. Similarly, LOQ for Lobeglitazone and Metformin was found to be 0.20µg/ml and 0.63µg/ml respectively. The % assay results of 99.41 % for Lobeglitazone and 100.40% for Metformin indicate that the developed method was successfully utilized for the estimation of Lobeglitazone and Metformin 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 flow rate, pH, and so on. 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 Metformin, 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 Metformin 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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