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DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RPHPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF LOBEGLITAZONE AND METFORMIN IN TABLET DOSAGE FORMS

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Abstract

Background: A stability-indicating RP-HPLC method is proposed for the rapid, sensitive, and selective determination of Lobeglitazone and Metformin in pharmaceutical formulations. Lobeglitazone and Metformin were separated on a Cosmosphere C18 column (250×4.6 mm, 5 µm) using a mobile phase comprising methanol, acetonitrile, and potassium dihydrogen phosphate buffer adjusted to pH 2.5 with orthophosphoric acid (70:5:25 %v/v/v). The gradient elution was optimized at a flow rate of 0.8 mL/min and detection wavelength of 250 nm. **Results**: The complete method validation was conducted according to ICH guidelines. The recovery study yielded results between 98% and 102% over a concentration range of 50% to 150% of the working concentration. The method demonstrated linearity within the range of 0.5-0.25 µg/mL for Lobeglitazone and 50-250 µg/mL for Metformin, with a linear regression curve (R^2 = 0.999). The limits of detection (LOD) and quantitation (LOQ) were found to be 0.0010 and 0.0031 µg/mL for Lobeglitazone, and 0.30 and 0.91 µg/mL for Metformin, respectively. The retention times were 8.37 min for Lobeglitazone and 2.83 min for Metformin. The method exhibited good recoveries, and intra-day and inter-day relative standard deviations were below 2%. Ruggedness and robustness were evaluated and found satisfactory as per ICH guidelines. Stability studies under various stress conditions (acid, base, oxidation, thermal, and sunlight) were conducted as recommended by ICH guidelines. **Conclusion:** The developed RP-HPLC method is suitable for the accurate estimation of Lobeglitazone and Metformin in pharmaceutical formulations. The high recovery rates and low relative standard deviations validate the suitability of the proposed method for routine analysis in bulk and pharmaceutical formulations.

Key Words: Lobeglitazone, Metformin, RPHPLC, Stability, Validation

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Introduction

Background of Lobeglitazone and Metformin

Lobeglitazone, with the IUPAC name 5-[(4-[2-([6-(4-Methoxyphenoxy)pyrimidin-4-yl]methylamino)ethoxy]phenyl)methyl]-1,3-thiazolidine-2,4-dione and chemical formula C24H24N4O5S (Fig.1), belongs to the thiazolidinedione class of anti-diabetic drugs. It functions primarily as an insulin sensitizer by binding and activating Peroxisome Proliferator-Activated Receptors (PPAR) gamma in fat cells. PPAR is a transcription factor that regulates metabolism. Lobeglitazone promotes insulin binding in fat cells, thereby reducing blood sugar levels, lowering hemoglobin A1C levels, and improving lipid and liver profiles[1].

Metformin, with the IUPAC name N,N-Dimethylimidodicarbonimidic diamide and chemical formula C4H12ClN5 (Fig.2), reduces glucose absorption from the intestines, decreases liver glucose production, and enhances insulin sensitivity. It is typically recommended alongside dietary modifications and exercise for optimal results in managing blood sugar levels. Medications like metformin are crucial in preventing complications such as kidney damage, nerve issues, blindness, and amputations associated with Type 2 Diabetes Mellitus. This combination, approved by CDSCO in 2022, is marketed as LOBG-M for treating Type 2 Diabetes Mellitus [2].

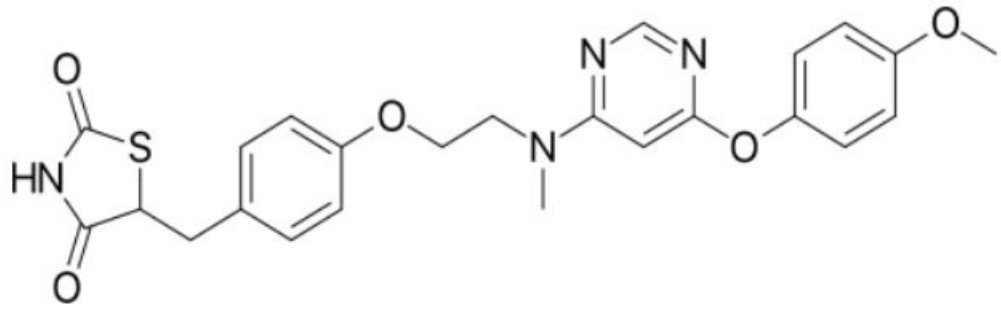

Fig.1 Chemical structure of Lobeglitazone

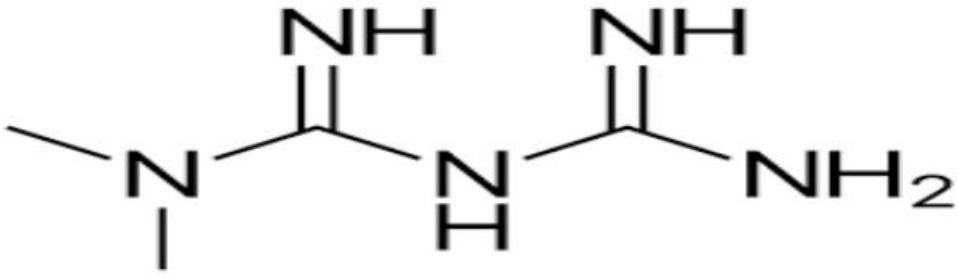

Fig.2 Chemical structure of Metformin

Forced Degradation:

Forced degradation studies are essential in the development of analytical methodologies to gain deeper insights into the stability of active pharmaceutical ingredients (APIs) and drug products. These experiments help identify degradation pathways and products, crucial for understanding the substance's stability under different conditions. Currently, there is no existing literature on stress degradation profiles of Lobeglitazone and Metformin according to ICH guidelines using the aforementioned analytical techniques. High-performance liquid chromatography (RP-HPLC) is employed for the analysis of Lobeglitazone and Metformin in

pharmaceutical formulations[3]. This study presents a precise, specific, reproducible, and stability-indicating method for their analysis, validated in accordance with International Conference on Harmonization (ICH) guidelines.

Importance and Necessity of Stability-Indicating Methods:

Stability studies aim to monitor changes in substances or materials over time and under various storage conditions. Factors influencing stability include production timelines, batch variations, process parameters, excipient efficiency, and environmental factors such as temperature and humidity.[4]

A stability-indicating method is a validated quantitative analytical technique capable of detecting changes in the chemical, physical, or microbiological properties of drug substances and products over time. It ensures specific measurement of active ingredient content and degradation without interference[5].

Precision in stability methods involves identifying potential impurities in drug materials and components through forced degradation (FD) studies, which accelerate impurity generation. This approach aids formulation scientists in developing consistent formulations efficiently[6].

Good Manufacturing Practices (GMP) incorporate a structured stability monitoring program to determine storage conditions, expiration dates, and the use of accurate test procedures. Such data are essential for assessing, confirming, or extending retest cycles and expiration dates for drug substances[7].

Preparation of Stock Standard Solution and Sample:

Stock Solution: To prepare the stock standard solutions:

- **Lobeglitazone**: Weigh 5 mg of Lobeglitazone and transfer it to a 50 mL volumetric flask. Add methanol up to the mark (Stock Solution-1; 100 µg/mL). From Stock Solution-1, take 0.5 mL and transfer it to another 50 mL flask. Make up the volume with methanol (Standard Stock Solution-2; 1 µg/mL).
- **Metformin**: Weigh 50 mg of Metformin and transfer it to a 50 mL volumetric flask. Add methanol up to the mark (Standard Stock Solution; 1000 µg/mL).

For the sample preparation:

- Take 1 mL from Lobeglitazone Standard Stock Solution-2 (1 µg/mL) and 1 mL from Metformin Stock Solution (1000 µg/mL) into a 10 mL volumetric flask. Make up the volume to the mark with diluent.
- This results in a solution containing Lobeglitazone at 0.1 µg/mL and Metformin at 100 µg/mL (Figure 3).

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

- Weigh twenty tablets, calculate the average weight, and finely powder them. Add tablet powder equivalent to 0.5 mg of Lobeglitazone and 500 mg of Metformin into a 100 mL volumetric flask.
- Add methanol up to the mark, resulting in a solution containing Lobeglitazone at 5 µg/mL and Metformin at 5000 µg/mL.
- Sonicate the solution for 20 minutes and filter it. Transfer 1 mL of each solution (Lobeglitazone and Metformin) into separate 10 mL volumetric flasks.
- Make up the volume to the mark with diluent, resulting in solutions containing Lobeglitazone and Metformin as specified.

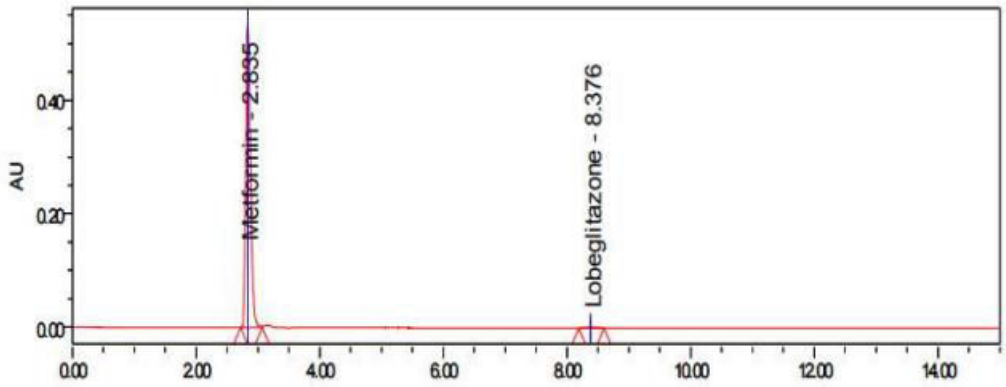
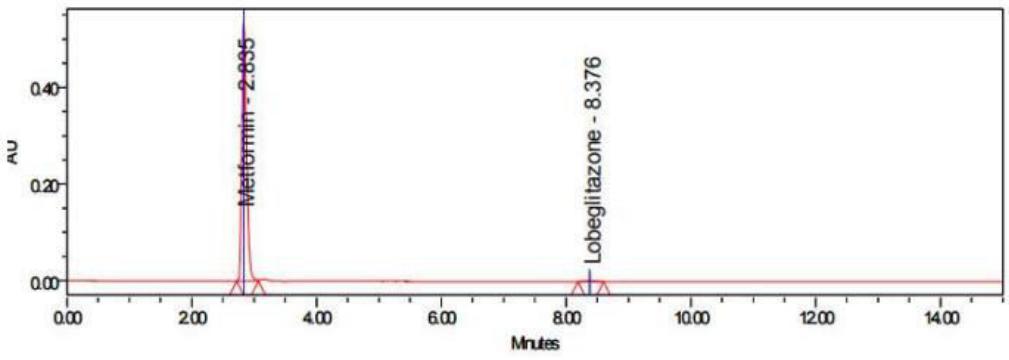


Fig. 3 Lobeglitazone (0.1µg/ml) and Metformin (100µg/ml) by using Methanol: ACN: Potassium Dihydrogen Phosphate Buffer pH 2.5 (70:5:25 % v/v/v) mobile phase

RESULTS

To develop a reliable RP-HPLC method for accurately estimating Lobeglitazone and Metformin using stressed samples, various combinations of mobile phases were tested with different compositions and flow rates. After multiple trials and optimizations, chromatographic conditions were refined and finalized. The best results were achieved using a mobile phase consisting of Methanol, Acetonitrile, and Potassium Dihydrogen Phosphate buffer adjusted to pH 2.5 (70:5:25 %v/v/v), flowing at a rate of 0.8 mL/min. These conditions produced well-defined peaks for Lobeglitazone and Metformin with retention times of 8.37 min and 2.83 min, respectively, while maintaining good peak symmetry and a stable baseline. All system suitability parameters met the criteria specified in Table 1[8].



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

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 from samples subjected to acidic, alkaline, oxidative, and photodegradation revealed distinct peaks of pure Lobeglitazone and Metformin with retention times (tR) of 8.21 min and 2.89 min, respectively. Additionally, several new peaks were observed at different retention times. Table 12 and Figures 5, 6, 7, 8, and 9 list the percentages of degradation products along with their respective retention times.

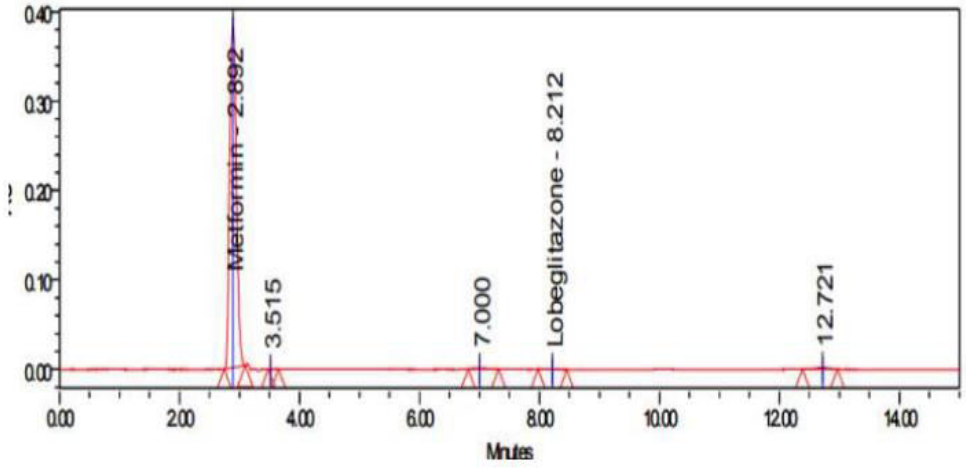


Fig.5. Chromatogram of Standard Lobeglitazone (0.1µg/ml) and Metformin(100µg/ml) for Acid Degradation

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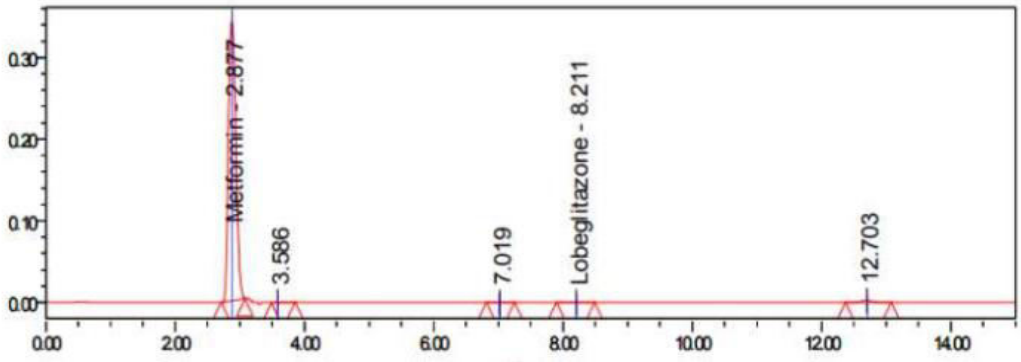

Fig. 6. Chromatogram of Standard Lobeglitazone(0.1µg/ml) and Metformin(100µg/ml) for Base Degradation

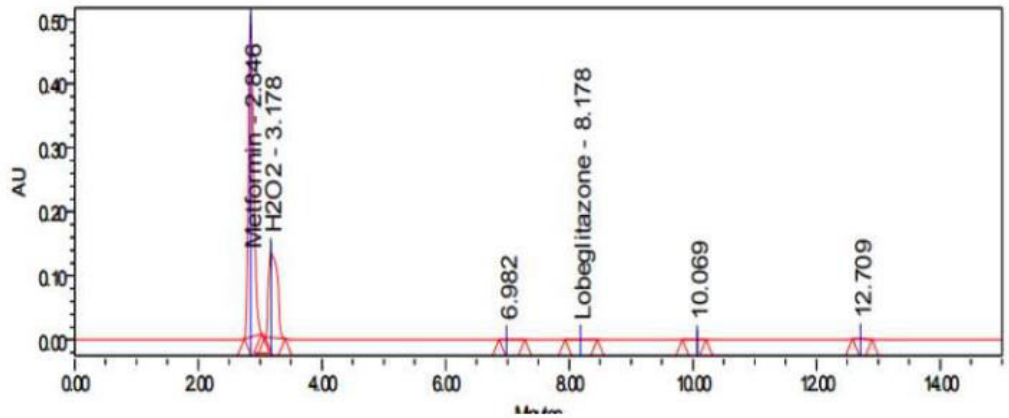

Fig. 7. Chromatogram of Standard Lobeglitazone (0.1µg/ml) and Metformin(100µg/ml) for Oxidative Degradation

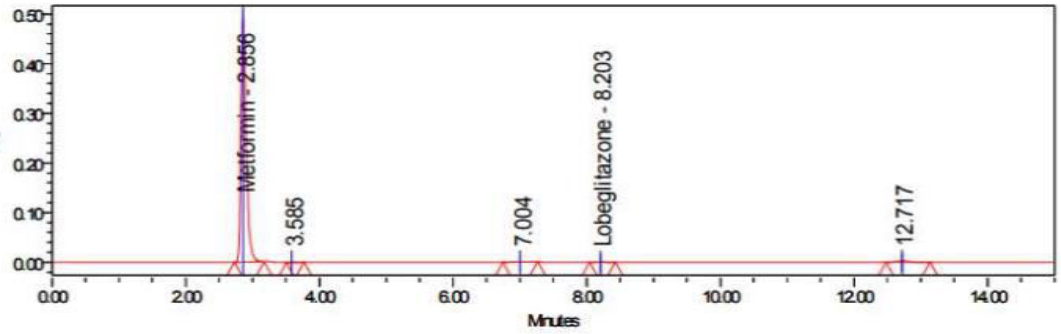

Fig. 8. Chromatogram of Standard Lobeglitazone (0.1µg/ml) and Metformin(100µg/ml) for Photo Degradation

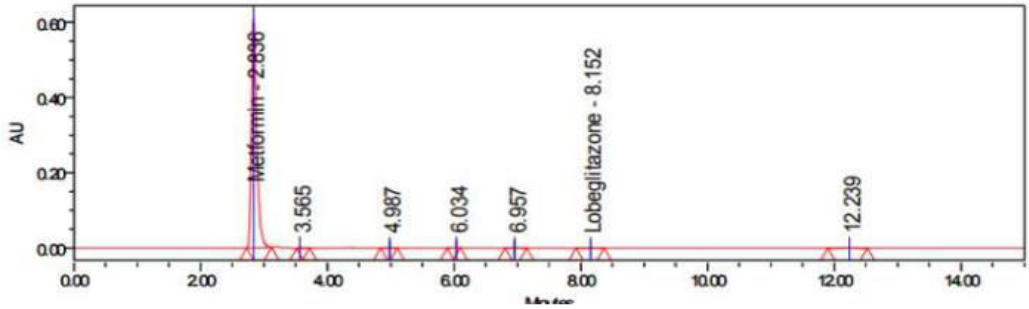

Fig. 9. Chromatogram of standard Lobeglitazone(0.1µg/ml) and Metformin(100µg/ml) for Thermal Degradation

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CONCLUSION

Based on the above observations, it can be concluded that the developed RP-HPLC method for the stability indicating and validation of Lobeglitazone and Metformin in tablets is specific, linear, accurate, precise, and robust. Therefore, this RP-HPLC method is suitable for routine analysis

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