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Stability Indicating Method Development and Validation of Lobeglitazone Sulfate by RP-HPLC Method

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ABSTRACT
In the present work, a new validated stability indicating RP-HPLC method for quantitative determination of Lobeglitazone sulfate (LOB) in tablet formulation was developed. The column was Phenomenex Luna C_{18} column (250 mm × 4.6 mm id; 5µm particle size) and the mobile phase was composed of Acetonitrile: Methanol: Water (70:20:10v/v/v) with a flow rate 1 ml/min. Eluents were monitored by UV detector at 248 nm. Calibration curve was linear in the concentration ranges 2–10µg/ml (R^2 value is 0.9994) for LOB. The sample (LOB) was subjected to stress conditions like acidic, alkaline, oxidation, photolysis and thermal degradation. The developed method was found to give good separation between pure drug and degraded products. The proposed method was successfully applied for the stability assay of LOB in tablet formulation and validated as per ICH guidelines.

KEY WORDS: Lobeglitazone sulfate, Forced degradation, RP-HPLC, method validation.

I. INTRODUCTION

[1-3].Lobeglitazone (Fig.1) is chemically a,5-{[4-(2-{[6-(4-methoxyphenoxy)pyrimidin-4-yl](methyl)amino}ethoxy)phenyl]methyl}-1,3-thiazolidine-2,4-dione that acts acts as an insulin sensitizer by binding and activating Peroxisome Proliferator-Activated Receptors (PPAR) gamma within fat cells. By promoting the binding of insulin at fat cells, lobeglitazone has been shown to reduce On literature survey, no methods were reported for the estimation of LOB ,So we have developed a novel, simple, rapid, accurate, precise and highly sensitive RP-HPLCmethods for estimation of LOB in bulk and tablet dosage form and validated according to ICH guidelines.

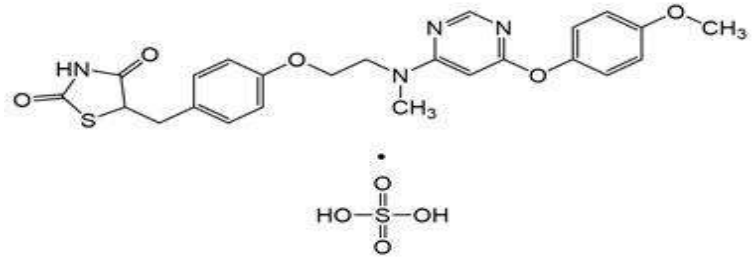

Figure 1:Structure of Lobeglitazone sulfate

II. MATERIALS AND METHODS

Reagents and Chemicals

Analytically pure sample of LOB was procured from Akums Drugs and Pharmaceuticals, Haridwar, New Delhi, Pvt. Ltd. (India). The Pharmaceutical dosage form used in the study labelled (LOBG) contains 0.5 mg of LOB was purchased from Local Pharmacy. All chemicals and reagents were of HPLC grade (Loba Chemie Pvt.

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Ltd.) and were purchased from Sudhagar Biological and Chemicals Pvt. Ltd., Chennai, India.
The instrument and chromatographic conditions

Shimadzu HPLC system, Prominence-i LC-2030 Plus (Shimadzu corporation Kyoto, Japan) consisted of a pump (LC - 2030 Plus Parallel type double Plunger, SPD-20A UV-Visible detector) run under Lab solutions software, with automating injecting facility programmed at 20 µL capacity per injection was used. The column used was Phenomenex Luna C_{18} (250 mm × 4.6 mm, 5.0 µm particle size). Different mobile phases were tested in order to find the best condition for separation of LOB. The mobile phase contained Acetonitrile: Methanol: Water in 0.1% Triethylamine (70:20:10, v/v/v) and the flow rate was maintained at 1.0 ml/min. UV detection was carried out at 248 nm. The mobile phase and samples were filtered through a 0.45 µm membrane filter. Mobile phase was degassed by Sonica ultrasonic cleaner (model 2200 MH) prior to use. The other instrument used are hot air oven.

Preparation of standard and sample solution
Diluent
Mobile phase was used as the diluent in the ratio of 1:1

Mobile phase
Acetonitrile: Methanol: Water in 0.1% Triethylamine (70:20:10, v/v/v) is programmed as RP HPLC method.

Preparation of stock standard solutions

Stock standard solution of LOB (500 μg/ml) was prepared by dissolving 25 mg of LOB in 50 ml of diluent in 50 ml volumetric flask with vigorous shaking (Stock-I), From this stock solution (20 μg/ml)1 ml was pipetted and diluted to 25 ml with methanol (Stock-II).

Preparation of working standard solution

From above standard stock solution of (Stock-II) 2 ml of LOB solution was taken into 10 ml volumetric flask, separately and was made to the mark with the mobile phase to get 4 μg/ml of LOB.

Preparation of sample stock solution

The average weight of 20 tablets was determined. Sample stock solution was prepared by dissolving tablet powder equivalent to 0.5 mg of LOB and was transferred to a 25 ml volumetric flask. Then 15 ml diluent was added and sonicated for 10 mins to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluent. Filter the sample stock solution with Whatman filter paper and 2ml of the solution was further diluted to 10 ml to get final concentration.

Preparation of sample solution

From above sample stock solution of LOB, 2 ml was withdrawn and diluted to 10 ml using diluent to get concentration of 4 μg/ml of LOB.

[5].Validation
The proposed method was validated as per ICH guidelines.

Linearity

Different aliquots of standard stock solutions 1 - 5 ml of LOB was transferred into series of 10 ml volumetric flasks separately, and the volume was made up to the mark with diluent to get concentrations such as 2, 4, 6, 8, 10 µg/ml.

Accuracy

To the pre-analyzed sample solution, a known amount of standard stock solution was added at different levels i.e. 50, 100 and 150%. The solutions were reanalyzed by proposed method.

Precision

The reproducibility of this method was determined by analyzing tablet at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision).

[4].FORCED DEGRADATION STUDIES
Hydrolytic degradation under acidic condition

Pipetted out 2 ml of sample stock solution into a 10 ml volumetric flask and added 2 ml of 0.1 N HCl. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and made up to 10 ml with diluent.

Hydrolytic degradation under alkaline condition

Pipetted out 2 ml of sample stock solution into a 10 ml volumetric flask and added 2 ml of 0.1 N NaOH. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N HCl and made up to 10 ml with diluent.

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Oxidative degradation

Pipetted out 2 ml of sample stock solution into a 10 ml volumetric flask and added 2ml of 0.1% w/v of hydrogen peroxide. Then, the volumetric flask was kept at 60ºC for 24 hours and then the volume was made up to the mark with diluent.

Photo degradation

Pipetted out 2 ml of sample stock solution into a 10 ml volumetric flask and expose to sunlight for 24 hrs and the volume was made up to the mark with diluent.

Thermal induced degradation

LOB sample was taken in petri dish and kept in Hot air oven at 60 °C for 24 hours. Then the sample was taken and diluted with diluent and injected into HPLC and analyzed.

III. RESULTS AND DISSCUSION

Method development and optimization

The HPLC procedure was optimized with a view to develop a suitable LC method for the determination of LOB in tablet dosage form. Initially, acetonitrile, buffer and water in different ratios were attempted. But LOB gave tailed peak. So, 0.1% of Triethylamine was added in water and mixtures of acetonitrile and the different ratios of acetonitrile, methanol and water were attempted. It was found that acetonitrile: methanol: water in 0.1% of Triethylamine in the ratio of 70:20:10 (v/v/v) gave acceptable retention times (2.426 min of LOB) with flow rate of 1.0 ml/min as shown in figure 3 and also performed mobile phase blank as shown in figure 2.

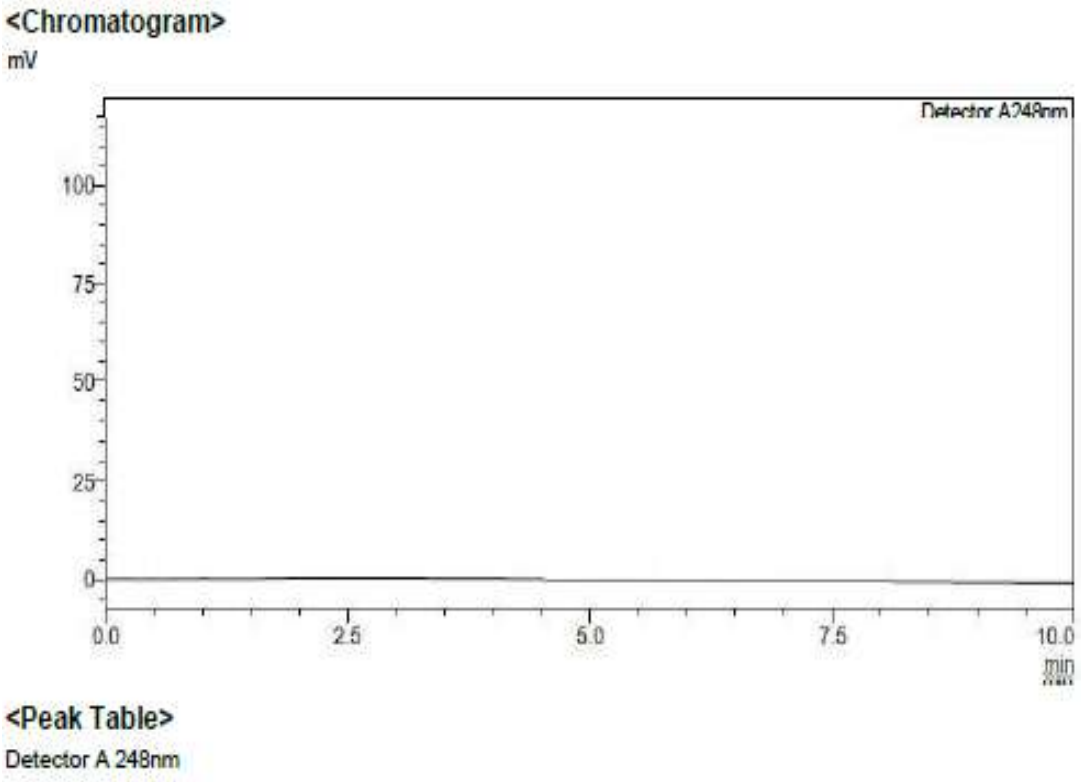

Figure 2. Blank for Optimized Mobile Phase

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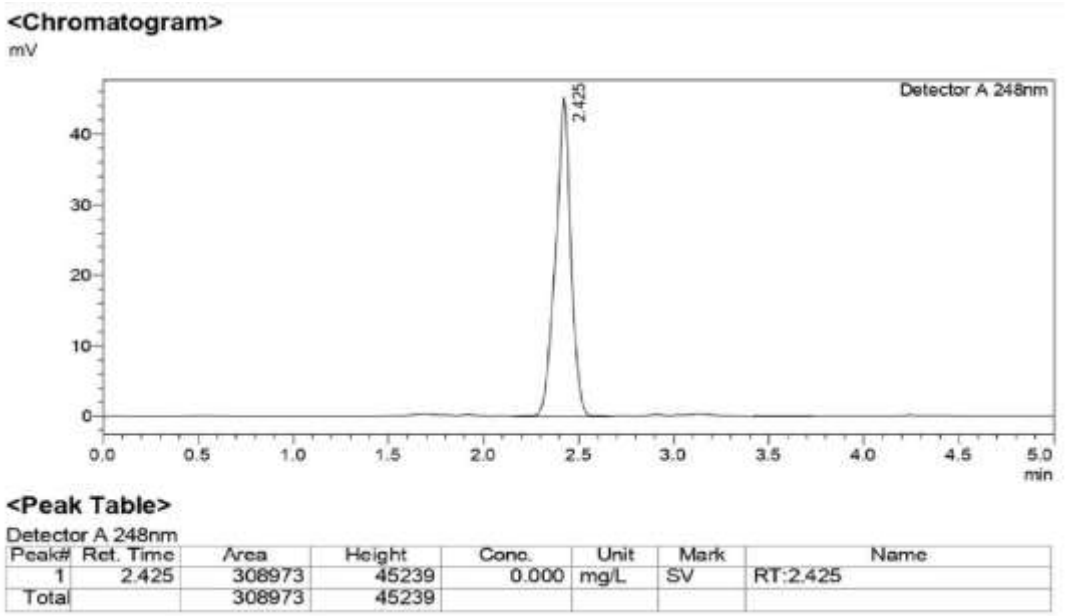

<Peak Table>
Detector A 248nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.425	308973	45239	0.000	mg/L	SV	RT:2.425
Total		308973	45239				

Figure 3. Optimized Chromatogram for Lobeglitazone sulfate

Method validation

The described method has been validated which include parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

System suitability

System suitability and chromatographic parameters were validated such as tailing factor and theoretical plates was calculated. The results are given in table 1.

Table 1: System suitability parameters.

Parameters	Lobeglitazone
Retention time	2.426
Peak area	308973
Tailing factor (T)	0.724
Theoretical plate (N)	4235

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of LOB at different concentrations in the range of 2–10 µg/ml with correlation coefficient (r^2) of 0.9994. Results are given in table 2.

Table 2: Linearity data for lobeglitazone sulfate

Drug	Concentration (µg/ml)	Area
LOB	2	169867
	4	308973
	6	472205
	8	612086
	10	768237

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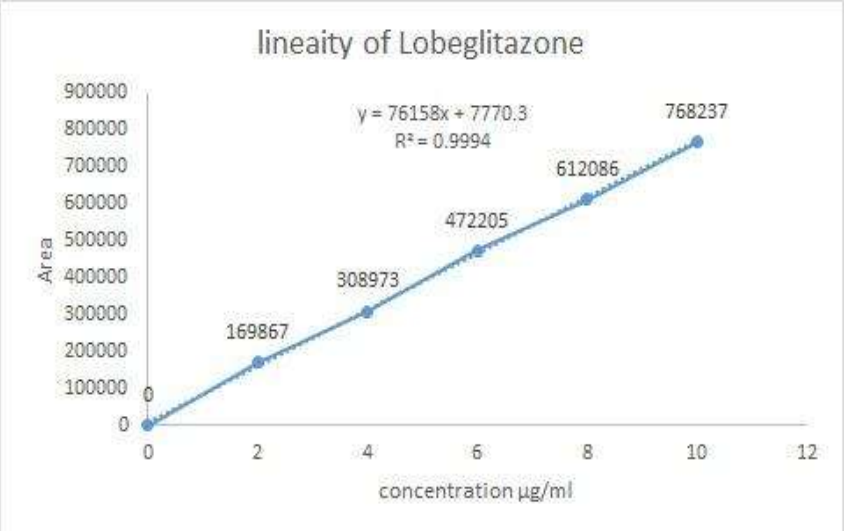

Accuracy

Accuracy of the proposed method was determined by performing the recovery experiment. The recovery experiment was studied by adding known amount of standard LOB and to the Pharmaceutical Product and calculating the recovered standard amount. At 50%, 100% and 150% standard addition level mean recovery of were found to be 100.22%, 100.05% and 100.05% for LOB. The results of recovery experiment are given in table 3.

Table 3: Accuracy for Lobeglitazone sulfate

Drug	percentage	Amount present* µg/ml	Amount Added* µg/ml	Amount Estimated*	Amount recovered	%recovery	SD	%RSD	SE
LOB	50%	4	2	6.001	2.001	100.22	0.1855	0.1851	0.107
	100%	4	4	8.004	4.004	100.05	0.090	0.081	0.05
	150%	4	6	9.999	5.999	100.05	0.090	0.081	0.05

Precision

Precision was evaluated at the repeatability and intermediate precision levels. For repeatability analysis, six independent portions of a tablet dosage form were processed through the full analytical method and results was evaluated obtained by % RSD values of 0.3704 for LOB as shown in table.4.

Table 4: Precision result for the proposed method

Sample No.	Lobeglitazone sulfate	
	Peak area response	Assay (%)
1	395464	100.23
2	396892	100.60
3	395938	100.35
4	396706	100.54
5	396794	100.56
Average	399673	101.30
% RSD	0.3704	

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Robustness

Robustness study was conducted by deliberate changes in mobile phase composition and flowrate, revealed that there was no significant variation in % assay as shown in table 5.

Table 5: Robustness of the study

Percent assay of the drug	Mobile phase, Acetonitrile: Methanol: Water in 0.1% Triethylamine		Flow rate, ml/min	
LOB	29:71 (v/v)	31: 69 (v/v)	0.9	1.1
	100.56%	100.25%	100.26%	100.10%

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

The LOD and LOQ was found to be 0.4065 (µg/ml) and 1.2195 (µg/ml) for LOB estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration. The results are given in table 6.

Table 6: LOD and LOQ data for Lobeglitazone sulfate

Drug	LOD	LOQ
LOB	0.342 (µg/ml)	1.037(µg/ml)

Forced degradation studies

Results are tabulated in table 7.

Table 7: Summary of forced degradation study.

Stress conditions	Time (hr)	Comment
0.1M HCl	24	Degraded (100%)
0.1M NaOH	24	Degraded (100%)
0.1 % H_2O_2	24	Degraded (54.79%)
Thermal	24	Not degraded
Photo	24	Not degraded

When stress conditions were applied to LOB, the HPLC results showed that there was no degradation occurs in photolysis and thermal. The drug was degraded in the acidic, basic, oxidative which shows that the LOB was sensitive to the acid, base, oxidative agent 5-9.

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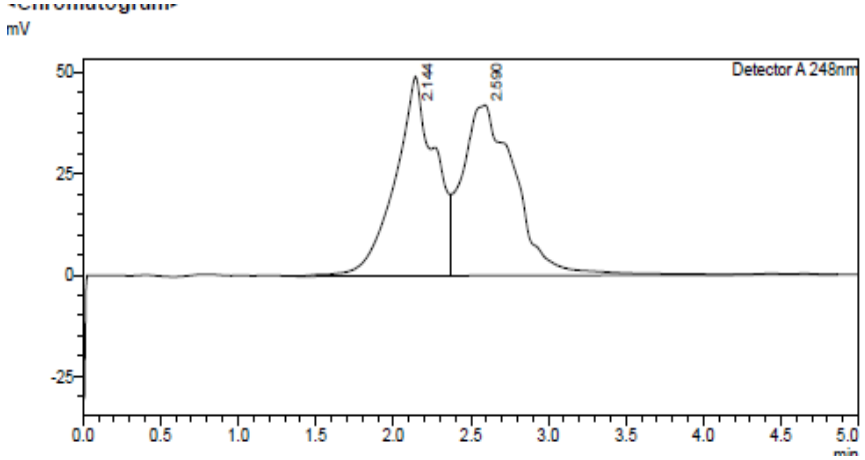

Figure 5 Typical Chromatogram of LOB under forced degradation study-Acid Degradation

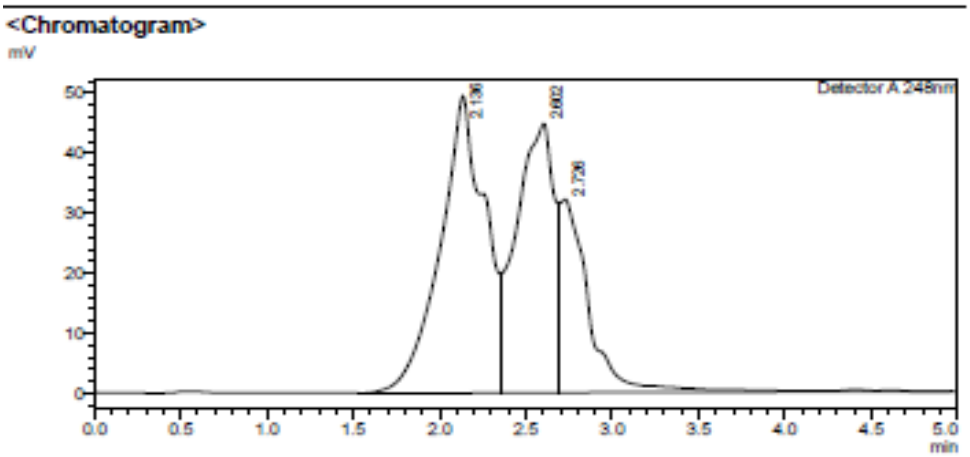

Figure 6 Typical Chromatogram of LOB under forced degradation - Base degradation

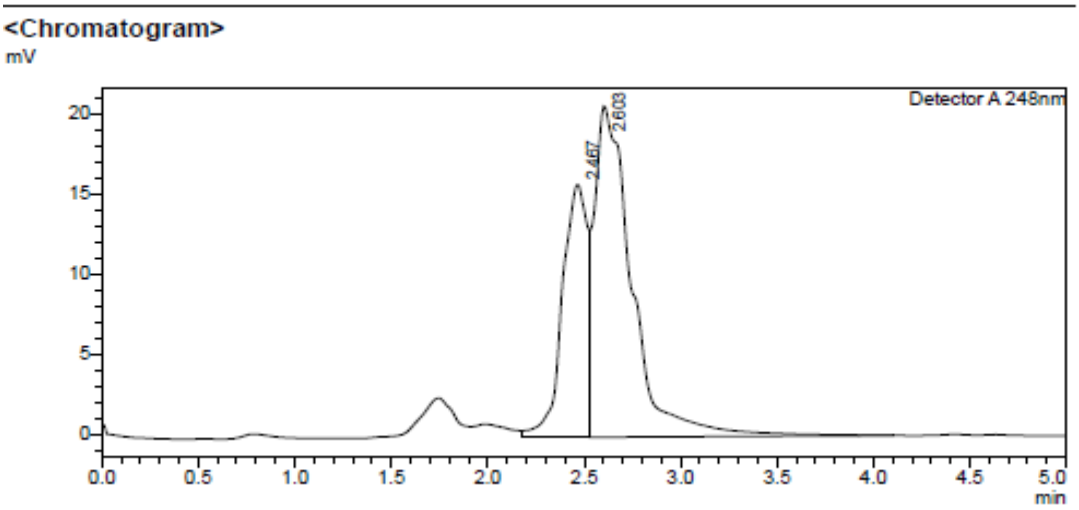

Figure 7 Typical Chromatogram of LOB under forced degradation - Oxidative degradation

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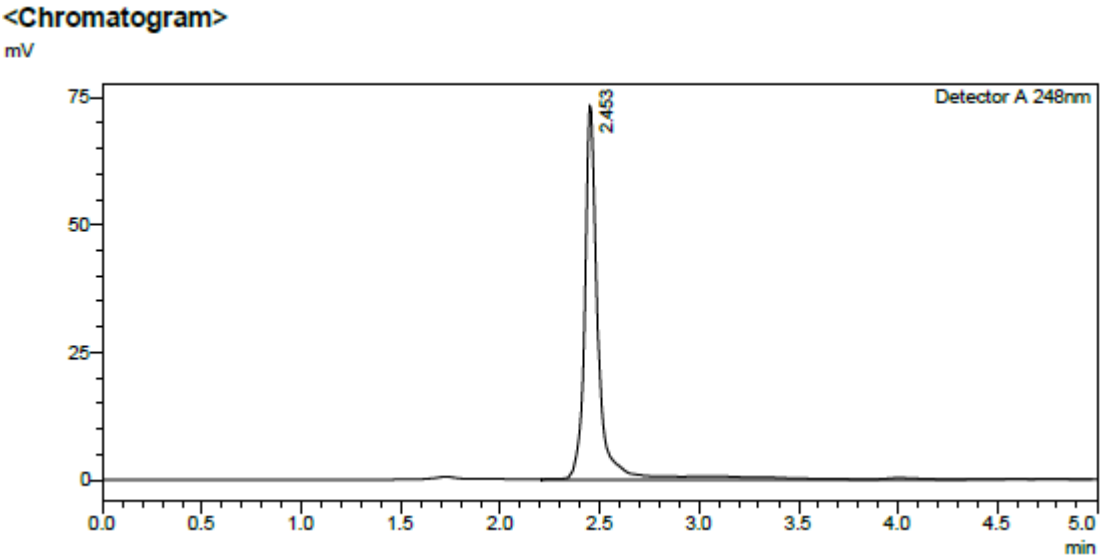

Figure 8 Typical Chromatogram of LOB under forced degradation - Thermal degradation

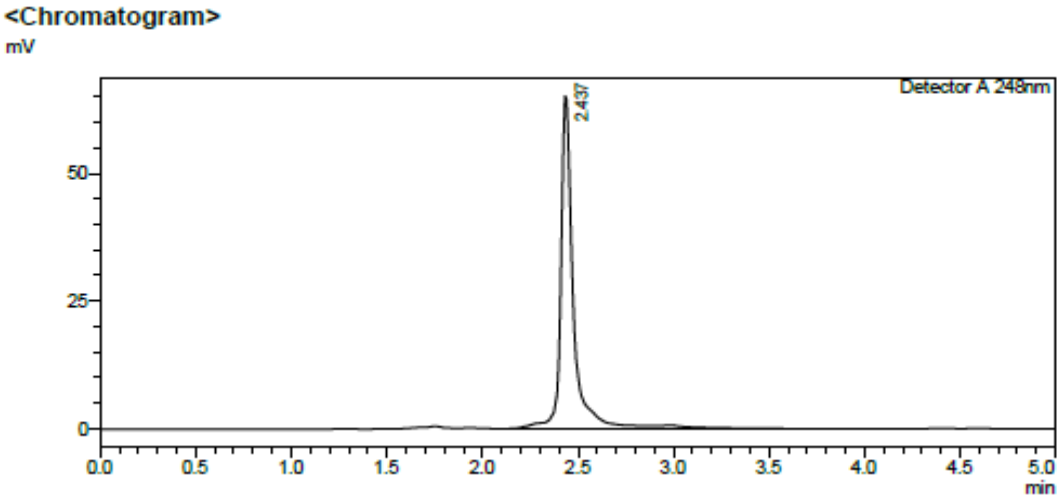

Figure 9 Typical Chromatogram of LOB under forced degradation - Photolytic degradation

IV. CONCLUSIONS

The stability-indicating RP–HPLC method was developed and validated according to ICH guidelines and applied for the determination of LOB in tablet formulation. The results obtained from validation studies revealed that, the developed method was found to be rapid, simple, accurate, precise, specific, selective and economical. Results obtained from the force degradation, indicates that there was no degradation occurs in thermal and photolysis except acid, base, oxidative. The proposed method has the ability to estimate the drug in tablet dosage form and also used for stability-indicating method to estimate of LOB in bulk powder and tablet formulation.

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Ethical committee approval: The proposed research does not require permission from an ethics commission. In this research, no matrices including humans or animals were used.

Informed Consent: Not applicable **Peer-review**: Externally peer-reviewed Authorship Contributions

Concept: Data collection or Processing, Writing, and analysis or interpretation.

Conflict of Interest: The authors declared no conflicts of interest.

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